SECOND EDITION

# Sterile Insect Technique

## Principles and Practice in Area-Wide Integrated Pest Management



Edited by
V. A. Dyck • J. Hendrichs
A. S. Robinson

CRC Press
Taylor & Francis Group

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# PREFACE

The second edition of the Sterile Insect Technique brings together all updated and relevant information about the sterile insect technique and its application in area-wide integrated pest management programmes. Following the same thematic style used in the first edition, the second edition includes a large amount of new information that has become available since the first edition was published in 2005.

In addition to updating and expanding the original 28 chapters, six new chapters have been added, covering topics that have been developed recently. These chapters discuss managing pathogens in insect mass-rearing, using symbionts and modern molecular technologies in support of the SIT, applying post-factory nutritional, hormonal and semiochemical treatments, applying the SIT to eradicating outbreaks of invasive pests, and using the SIT against mosquito vectors of disease.

In the first edition, each chapter was peer-reviewed, and in the second edition, the new chapters were peer-reviewed. We thank the reviewers for helping to make the book accurate, complete, up-to-date, and generic in content. All chapters in the second edition were thoroughly reviewed and edited by the editors. Uniform Resource Locator (URL) addresses of recent publications have been included in the lists of references, enabling readers using an electronic format to access those publications easily.

Since SIT practitioners tend to operate in the context of only one pest species, it was a challenge for authors to develop and write their chapters from generic and global points of view, stressing the principles of the technology, and including examples from a range of pest species. We are grateful to the many authors, from all over the world, for writing the chapters without financial compensation; authors who are retired, or worked on their own time, deserve special commendation.

In the past 15 years the value of the first edition has been demonstrated over and over again, and it is expected that the second edition will serve the scientific community for many more years.

The Editors
June 2020



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# FOREWORD

For several major insect pests, the environment-friendly sterile insect technique (SIT) is being applied as a component of area-wide integrated pest management (AW-IPM) programmes. This technology, using radiation to sterilize insects, was first developed in the USA, and is currently applied on six continents. For almost six decades it has been a major subject for research and development in the Joint FAO/IAEA Programme on Nuclear Techniques in Food and Agriculture, involving both research and the transfer of this technology to Member States so that they can benefit from improved plant, animal and human health, cleaner environments, increased production of plants and animals in agricultural systems, and accelerated economic development. The socio-economic impacts of AW-IPM programmes that integrate the SIT have confirmed the usefulness of this technology.

Numerous publications related to the integration of the SIT in pest management programmes, arising from research, coordinated research projects, field projects, symposia, meetings, and training activities have already provided much information to researchers, pest-control practitioners, programme managers, plant protection and animal health officers, and policy makers. However, by bringing together and presenting in a generic fashion the principles, practice, and global application of the SIT, this book serves as a major reference source for all current and future users of the technology, and also serves as a textbook for academic courses on integrated pest management.



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# INTRODUCTORY REMARKS

As evidenced by the successful area-wide insect pest control programmes described in this book, the sterile insect technique (SIT), a component of these programmes, has come of age. The technology has expanded rapidly — additional target species, new rearing techniques, better understanding of genetics and insect behaviour, and especially integration into operational area-wide integrated pest management (AW-IPM) programmes. The SIT has matured; this critical overview of its principles and practice has already greatly facilitated further research, development, and application in the field. The second edition will only accelerate such objectives.

The SIT was among the first biological insect control methods designed for area-wide application. While the SIT gained its reputation in insect eradication programmes, it is essential that the scientific community now recognizes its potential as a part of IPM strategies for the area-wide suppression, containment, prevention and, where advisable, eradication of pest populations.

Insect control methods in the first 70 years of the 20$^{th}$ century were based largely on chemical insecticides; this was especially so after the Second World War with the introduction of synthetic insecticides. The concept of IPM gradually became popular after 1970, and a more selective use of insecticides was emphasized. Attempts to significantly reduce insecticide applications have only gradually become more prominent. Biological control of pest insects, together with the breeding of insect-tolerant or resistant plants, is probably now receiving the major emphasis in IPM programmes. According to an international standard under the International Plant Protection Convention (IPPC), the SIT is now officially considered as one type of biological control, and it is ideally suited for incorporation into AW-IPM programmes.

The scientific underpinning of SIT programmes has broadened as new areas of science have developed, e.g. insect mass-production and quality, geographic information systems and data management systems, genetics and molecular biology, symbionts and pathogens, insect behaviour, aerial release systems for sterile insects, and modelling of AW-IPM. The practical success of a programme incorporating the SIT requires a holistic and multidisciplinary approach, and effective management, since in the last analysis programmes must produce substantial economic benefits. This is clearly evident in the major successes using the SIT against screwworms, fruit flies, and moths.

In spite of documented successes, many colleagues in the scientific community are only partially or inadequately informed on the application and importance of this powerful addition to the biological weapons that can be used against insect pests that are economically important or a threat to human health. The credibility and impact of the technology needs to be described in an objective, comprehensive, and balanced fashion, and in an accessible format. New insect pest problems, new restrictive legislation, as well as older problems such as insecticide resistance and maximum residue levels, require new solutions. There is a real need, and an increasing demand, for information on the SIT so that its potential for addressing some of these problems can be assessed.



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The chapters have been written by well-known experts on the SIT and other technologies that are integrated into IPM systems. Worldwide in scope, this book provides an in-depth resource for the whole range of documented scientific information about the SIT. The target audience of the book is the scientific community worldwide. It will assist animal and public health and plant protection practitioners, as well as students, teachers, and researchers, in understanding and applying the SIT. It is anticipated that the book will continue to have a considerable impact on the science and practice of pest control systems.

Research workers new to this field have difficulty accessing the literature — it tends to be widely scattered in multiple publications (some with very limited distribution), in conference proceedings, and in unpublished programme reports. To further the science and application of the SIT, the accumulated knowledge and experience needs to be integrated and synthesized from a generic standpoint. The consolidation of comprehensive information into one volume, with references to the large amount of previous work, is a major resource for the technology. Such a consolidation will facilitate the application of the SIT to those pest problems for which it is appropriate. It will also lay the groundwork for future applications. The present book is uniquely designed to fill this gap. The strengths and weaknesses, and successes and failures, of the SIT have rarely been evaluated openly and fairly from a scientific perspective.

This second edition will help develop further the use of the SIT for pest suppression, and where desirable and feasible, eradication. It will be a gold mine for graduate students who want to learn about the history, accomplishments, problems, and promises of the SIT. As an “autocidal” biological control method, it addresses present-day concerns regarding human health and the environment. There is great potential for significant advances that will make the SIT more effective and economically viable, such as commercializing the different components, developing genetic sexing strains that permit the release of only males, exposing sterile insects to nutritional, hormonal and semiochemical treatments to increase their quality and competitiveness, releasing insects from improved aerial systems, and using modern biotechnology.

It is an honour to have been asked to write these introductory remarks. The developments in this technology are exciting, and I will always remain a part of them.

Maurice Fried
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# CHAPTER 1.1.

# HISTORY OF THE STERILE INSECT TECHNIQUE

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## SUMMARY

During the 1930s and 1940s the idea of releasing insects of pest species to introduce sterility (sterile insect technique or SIT) into wild populations, and thus control them, was independently conceived in three extremely diverse intellectual environments. The key researchers were A. S. Serebrovskii at Moscow State University, F. L. Vanderplank at a tsetse field research station in rural Tanganyika (now Tanzania), and E. F. Knipling of the United States Department of Agriculture. Serebrovskii's work on chromosomal translocations for pest population suppression could not succeed in view of T. D. Lysenko's opposition to Mendelian genetics and the catastrophic conditions in the USSR during World War II, after which he died. Vanderplank used hybrid sterility to suppress a tsetse population in a large field experiment, but lacked the resources to develop this method further. Knipling and his team exploited H. J. Muller's discovery that ionizing radiation can induce dominant lethal mutations, and after World War II this approach was applied on an area-wide basis to eradicate the New World screwworm *Cochliomyia hominivorax* (Coquerel) in the USA, Mexico, and Central America. Since then very effective programmes integrating the SIT have been mounted against a number of tropical tephritid fruit fly species, some species of tsetse flies *Glossina* spp., the pink bollworm *Pectinophora gossypiella* (Saunders), the codling moth *Cydia pomonella* (L.), the cactus moth *Cactoblastis cactorum* (Berg), the painted apple moth *Teia anartoides* Walker, and the false codling moth *Thaumatotibia leucotreta* (Meyrick). In non-isolated onion fields in the Netherlands, the onion maggot *Delia antiqua* (Meigen) has since 1981 been suppressed by the SIT. In the 1970s there was much research conducted on mosquito SIT, which then went into "eclipse", but has been reviving during the last decade; a number of pilot trials are ongoing or in preparation, in conjunction with the *Wolbachia*-based incompatible insect technique (IIT) against mosquitoes, including *Aedes aegypti* (L.), *Ae. albopictus* (Skuse), *Ae. polynesiensis* Marks, and *Anopheles arabiensis* Patton. Development of the SIT for use against the boll weevil *Anthonomus grandis grandis* Boheman, the horn fly *Haematobia irritans* (L.) (Eschle et al. 1973), and the gypsy moth *Lymantria dispar* (L.) has ended, but it is in progress for two sweetpotato weevil species, *Cylas formicarius* (F.) and *Euscepes postfasciatus* (Fairmaire), the small hive beetle *Aethina tumida* Murray, the brown marmorated stink bug *Halyomorpha halys* Stål (Welsh et al. 2017), the navel orangeworm *Amyelois transitella* (Walker), the African sugarcane borer *Eldana saccharina* Walker, the European grapevine moth *Lobesia botrana* (Denis and Schiffermüller), the carob moth *Ectomyelois ceratoniae* (Zeller), the leafminer *Liriomyza bryoniae* (Kaltenbach) (Walker 2012) and other greenhouse pests, the Old World screwworm *Chrysomya bezziana* (Villeneuve), additional *Glossina* spp., other *Anastrepha* spp. and *Bactrocera* spp. fruit flies, and other pests. New technologies and continuous research support for ongoing programmes, including molecular, microbial and information technology approaches, are resulting in improved methodologies and processes, and thus in enhanced cost-effectiveness for all aspects of SIT application.

## 1. PROLOGUE

When using the sterile insect technique (SIT), it is applied usually as a component of area-wide integrated pest management (AW-IPM) (Hendrichs et al. 2007; Klassen and Vreysen, this volume). The density of the target insect pest population is

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initially reduced, eliminating already mated females, with auxiliary control methods (Mangan and Bouyer, this volume). Then the SIT imposes birth control on the population to further reduce its numbers (Klassen and Vreysen, this volume).

The SIT involves rearing large numbers of the target species, exposing them mainly to gamma rays (but increasingly also to X-rays to avoid the transport, security, and regulatory issues related to radioactive sources (Bakri et al., this volume)) to induce sexual sterility (Bakri et al., this volume; Robinson, this volume), and then releasing them into the target population. The released sterile males mate with wild females to prevent them from reproducing (Knipling 1955).

Runner (1916) found that large doses of X-rays applied to the cigarette beetle *Lasioderma serricorne* (F.) rendered it incapable of reproduction. Soon afterwards H. J. Muller (1927) showed that ionizing radiation induced visible mutations in *Drosophila*, and also a much larger number of dominant lethal mutations, which were expressed through a reduction in the hatch of eggs laid by treated females or fathered by treated males. In 1946 he received the Nobel Prize in Physiology or Medicine (Fig. 1); however, only after 1950, when Muller made a special effort to publicize the biological effects of radiation, did economic entomologists become aware that, through gamma or X-ray irradiation, sexual sterility in male insects was quite easily achieved (Bakri et al., this volume).

The fact that chemicals could also produce genetic changes in the same way as radiation was only discovered during the Second World War by Charlotte Auerbach, a German-Jewish zoologist and geneticist, who fled to the UK and had been introduced to mutation research by H. J. Muller. She found in 1941 that mustard gas was mutagenic in *Drosophila*, and also affected fertility (Auerbach et al. 1947). Most of the dominant lethal mutation-producing chemicals are alkylating agents, producing carbonium ions which in living tissues combine with the nucleic acids of cells, causing point mutations and chromosome breakage (LaChance 1967). Nevertheless, the potential of alkylating agents as chemosterilants was not recognized for at least another ten years (Davidson 1974). Then, in view of their low cost compared with radiation sources, much research was done on over 125 pest species of public health and agricultural importance (Knipling 1968; LaBrecque and Smith 1968; Davidson 1974).

The great disadvantage of alkylating agents is that their sterilizing and mutagenic effects extend to higher animals, including humans. Attempts to find safer chemosterilants resulted in the discovery of non-alkylating chemicals. Nevertheless, methods to apply them uniformly were largely impractical for large-scale conditions. Furthermore, the residues on insect bodies, resulting from chemosterilization, can result in their bioaccumulation in natural food chains (Nagel and Peveling, this volume). As a result, their application was largely discontinued in the 1970–80s, although the use of juvenile hormone analogues and other compounds in autosterilization devices in the field, to attract and sterilize individuals that then disseminate these chemosterilants to other individuals of the target population, is being reconsidered (Bouyer and Lefrançois 2014; Baxter 2016).

Nevertheless, besides the means of achieving sterilization in insects, the idea of releasing pest insects to introduce sterility into wild populations, and thus control them, had already been conceived independently in the 1930s and 1940s by A. S. Serebrovskii at Moscow State University, F. L. Vanderplank at a tsetse field

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research station in rural Tanganyika (now Tanzania), and E. F. Knipling of the United States Department of Agriculture (USDA) (Fig. 1). However, Serebrovskii and Vanderplank both sought to achieve pest control through the sterility that arises when different species or genetic strains are hybridized (Robinson, this volume).

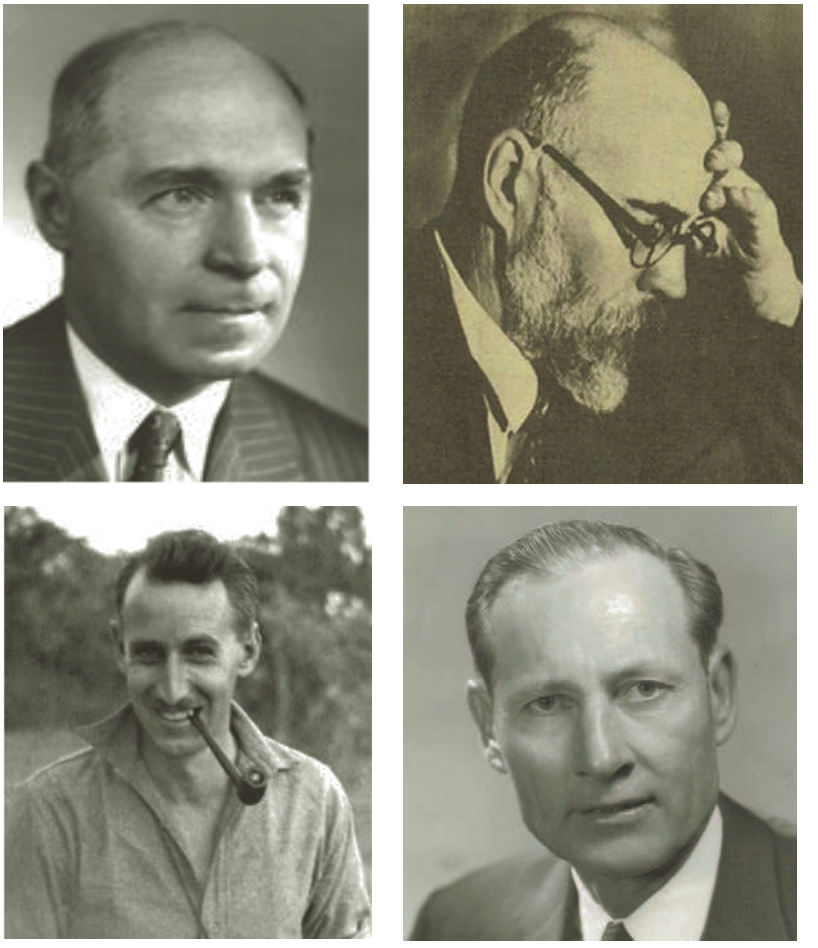


*Figure 1. Upper left: Hermann J. Muller; upper right: Aleksandr S. Serebrovskii; lower left: Frederic L. Vanderplank; lower right: Edward F. Knipling. Photo Credits: Muller – Nobel 2018; Serebrovskii and Vanderplank – Gould et al. 2006; Knipling – World Food Prize Foundation.*

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## 2. SEREBROVSKII AND POSSIBLE USE OF CHROMOSOMAL TRANSLOCATIONS TO CAUSE INHERITED PARTIAL STERILITY

Beginning in 1922, Muller encouraged and assisted Serebrovskii's genetic studies on *Drosophila*. In 1933, Muller became the director of a genetics laboratory, a position created for him by N. I. Vavilov, head of the Lenin All-Union Academy of Agricultural Sciences. Serebrovskii became embroiled in the fierce controversy with T. D. Lysenko about the validity and usefulness of Mendelian genetics in advancing Soviet agriculture (Medvedev 1969), whether genes exist, and whether the Lamarckian concept of inheritance of acquired traits is correct. Lysenko had gained the support of Stalin, and he attempted to force Vavilov, Serebrovskii, Muller, and other geneticists to recant their adherence to Mendelian genetics. In December 1936, exponents of the two trends in Soviet biology confronted each other at a special session of the Lenin All-Union Academy of Agricultural Sciences, and the geneticists vigorously defended their science. Subsequently several prominent geneticists were arrested. Probably Serebrovskii was motivated to develop the concept of using chromosomal translocations for pest population suppression as a means to deflect Lysenko's strident criticism that research in genetics was devoid of promise to benefit Soviet agriculture (Carlson 1981).

Serebrovskii (1940) noted that it was already well known in 1940 that a translocation of segments between two chromosomes caused an abnormal association of four chromosomes during meiosis in heterozygotes, resulting in the formation of gametes with lethal genetic duplications and deficiencies. These abnormalities manifested themselves as partial sterility in the translocation heterozygote. Such partial sterility tended to be passed on from one generation to the next (Curtis 1985; Marec et al., this volume). Those translocations that were viable in the homozygous state had normal meiotic pairing, and were fully fertile. Serebrovskii appreciated that, in such conditions of negative heterosis (or underdominance as it has more recently been called (Davis et al. 2001)), natural selection would favour whichever chromosome type was initially in the majority, with a point of unstable equilibrium, which would be at a frequency of 50% if the viability of the two homozygous karyotypes were equal. At a frequency of 50%, the proportion of heterozygotes, and hence of sterility in the population, would be maximal.

On the basis of Mendelian principles, Serebrovskii worked out: (1) the extent to which sterility would continue to appear in a population in the generations after a single release of translocation homozygotes, (2) ways of enhancing levels of sterility by using several different translocations, and (3) the effects of releasing only males to avoid a temporary increase in the breeding population. A single release into a wild population of strains each with two translocations would cause 93.75% of the embryos to die; correspondingly, three translocations would cause 98.4% of the embryos to die. Years later the alternative possibility was proposed — the deliberate release of a majority of insects with translocations as a means of "driving" into a vector population a gene that would render it harmless to man, e.g. a gene for inability to transmit disease (Curtis 1968, 1985).

Serebrovskii (1940) started practical work on translocations in *Musca domestica* L. and *Calandra granaria* L., but presumably it was impossible to continue it in the

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catastrophic conditions in the USSR during World War II. Unlike some other opponents of Lysenko, Serebrovskii was not arrested, but he died of natural causes in 1948. Before his death he expanded his ideas in a book (Serebrovskii 1971), but which could not be published until after the fall from power of N. S. Khruschev and of Lysenko (whom Khruschev also supported).

## 3. VANDERPLANK AND USE OF HYBRID STERILITY TO COMBAT TSETSE FLIES

In the 1930s and 1940s, Vanderplank and his colleagues developed and field-tested an entirely different system of insect control, based on sterility from species crosses and in the hybrids from such crosses. Based on field studies on the *Glossina morsitans* Westwood group of tsetse flies in East Africa, they had discovered the subtle, but unequivocal, differences between *G. morsitans sensu stricto* and *G. swynnertoni* Austen. Laboratory crosses between *G. morsitans* and *G. swynnertoni* were made by Corson (1932), Potts (1944), and Vanderplank (1944, 1947, 1948), but the cross-matings had low fertility. Vanderplank (1947) reported that the genitalia of the hybrids were distinguishable from both parent species, the hybrid males were sterile, and the female hybrids partially sterile. Hybrid sterility in tsetse flies has been studied further by Curtis (1971), and extensively by Gooding (1985, 1993).

Vanderplank (1944) proposed that sterility from crosses could be used for tsetse control, and Jackson (1945) showed that there was random mating between the two species in the field. On this basis, Vanderplank organized the mass-collection of *G. morsitans* pupae and then released the flies in a 26-km$^2$ area occupied only by *G. swynnertoni*. This habitat was separated by at least 19 km from other tsetse populations, and was considered too arid for *G. morsitans* to establish itself permanently.

Vanderplank (1947) briefly described the success of this experiment, noting that the initial effects were as theoretically expected. Surprisingly, he never published the detailed results, but he kindly gave them to C. F. Curtis. After F. L. Vanderplank's death, his son, R. J. R. Vanderplank, gave permission that these remarkable data be published (Table 1). The releases of *G. morsitans* did indeed virtually eliminate the less numerous *G. swynnertoni*, and there was a period in which hybrids could be identified, before they also declined in numbers.

Finally, the predicted decline of *G. morsitans* also occurred, presumably because of its lower tolerance of aridity than that of *G. swynnertoni*. When the density of tsetse flies had been reduced to a low level, local people moved into the area, and apparently completed tsetse eradication by bush clearance. It is unfortunate that the details of this remarkable trial have remained almost unknown for so long, and were not followed up.

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*Table 1. Effect of releasing* G. morsitans *pupae into a 26-km*$^2$ G. swynnertoni *habitat in Tanzania on the density of these two species and of the interspecific hybrids (*G. morsitans *habitat separated from other tsetse habitats by at least 19 km) (data from F. L. Vanderplank and C. H. N. Jackson, 1944–1946, reproduced with permission)*

| Date | *G. morsitans* released (number) | Average catch of old males (per 5 hours of catching) | | |
|---|---|---|---|---|
| | | *G. morsitans* | *G. swynnertoni* | Hybrids |
| June 1944 | 0 | 0 | 54 | 0 |
| July | 0 | 0 | 69 | 0 |
| August | 27000 | 64 | 50 | 0 |
| September | 25000 | 138 | 25 | 0 |
| October | 26000 | 169 | 16 | 5[1] |
| November | 11000 | 54 | 15 | 7[1] |
| December | 4500 | 39 | 12 | 11[1] |
| January 1945 | 5200 | 49 | 9 | 19 |
| February | 2300 | 68 | 5 | 21 |
| March | 0 | 40 | 4 | 28 |
| April | 0 | 22 | 4 | 20 |
| May | 0 | 17 | 3 | 10 |
| June | 0 | 16 | 1.2 | 9 |
| July | 0 | 13 | 1.1 | 7 |
| August | 0 | 15 | 0 | 4 |
| September | 0 | 11 | 0.2 | 2.4 |
| October | 0 | 7 | 0.1 | 2.1 |
| November 1945 – March 1946 | No surveys: local inhabitants now grazing cattle in the area | | | |
| April 1946 | 0 | 0.6 | 0.4 | 0.8 |
| After April 1946 | Area given over to local inhabitants who cut down most of the bush | | | |

[1]In this period, not all males were examined under the microscope, so the numbers recorded as hybrids were possibly inaccurate.

## 4. KNIPLING AND USE OF STERILITY INDUCED BY IONIZING RADIATION

### *4.1. New World Screwworm*

The debut of the development and practical application of the SIT occurred in the 1950s in the USA under Knipling's leadership. It culminated in the most successful AW-IPM programme integrating the SIT to date. It was started in 1958 to rid the south-eastern USA of the New World screwworm, a deadly parasite of livestock. During the next 48 years the technique was used to eradicate this pest from the USA, Mexico, and Central America to Panama (Vargas-Terán et al., this volume).

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*4.1.1. Early Attempts at Control, and Importance of Correct Identification*

Since ancient times, the tropical and semi-tropical New World screwworm has been a serious enemy of warm-blooded animals, including humans, in an area extending from Argentina to the southern USA. Descendants of European settlers learned to manage their herds and flocks so that the birth of most calves and lambs, as well as castration, branding, and dehorning operations, occurred only during months when screwworms were scarce (Cushing and Parish 1938). Each animal was checked for wounds at least twice per week, and each wound was treated with an insecticidal "smear" (Knipling 1985).

The correct identity of the insect concerned was established in 1858 by French medical doctor and entomologist C. Coquerel who collected larvae in the frontal sinuses of a convict, who later died, in the French penal colony of Cayenne (Devil's Island), off the coast of French Guiana. He published an accurate description of the New World screwworm in the Annals of the Entomological Society of France (Coquerel 1858) where he reported similar human cases with high mortality, describing science as powerless to prevent "these terrible ravages". Coquerel assigned the name *Lucilia hominivorax* Coquerel to this parasite; "hominivorax" literally means "man eater".

North American entomologists were, unfortunately, unaware of Coquerel's paper. Indeed, until 1933, North Americans confused the identity of the New World screwworm with the more abundant scavenger of dead carcasses, *Cochliomyia macellaria* (F.). Due to this inability to recognize that a different species was involved, livestock producers wasted much energy in burying or burning the carcasses of dead animals, and trapping adult flies, in the vain hope of reducing the population of what was believed to be the myiasis-causing screwworm.

E. C. Cushing, under the guidance of W. S. Patton of the Liverpool School of Tropical Medicine, discovered that the genitalia of adult flies that had developed in carrion were different from those of most flies collected from wound-reared specimens, and named the latter species *Cochliomyia americana* (Cushing and Patton 1933). Later this species was found to be the *C. hominivorax* described 75 years earlier by Coquerel (Laake et al. 1936). *C. hominivorax* is now referred to as the New World screwworm, and the scavenger *C. macellaria* (F.) as the secondary screwworm. As soon as the true identity of *C. hominivorax* had been clarified, Knipling and his colleagues made a concerted effort to elucidate its biology and ecology. They concluded that the number of screwworm flies that survives the winter as pupae in the soil was very low, perhaps only 40–80 per $km^2$ (Cushing and Parish 1938; Lindquist 1955; Meyer and Simpson 1995).

*4.1.2. Studies on Reared Screwworms in the 1930s, and Conception of the SIT*

*C. hominivorax* was the first obligate insect parasite to be reared on an artificial diet (Melvin and Bushland 1936), and this enabled very large numbers of screwworms to be available for study. Knipling observed the extreme sexual aggressiveness of male screwworms, as well as the refusal of females to mate more than once, and he realized that, if sexual sterility could be induced in males, and if vast numbers could be sterilized and released in the field, then the screwworm population would be suppressed. He also realized that, if releases continued for several successive

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generations, and the wild population density decreased, the ratio of the number of sterile males to that of fertile wild males would increase sharply. Provided that the target wild population was isolated, the sterile:fertile ratio would become so great that probably not even a single fertile mating would occur, and thus the wild population would be eradicated (Knipling 1955, 1985). Knipling introduced simple mathematical models to assess the effects of the SIT and the integration with insecticides and other measures on the dynamics of screwworm populations (Barclay, this volume; Klassen and Vreysen, this volume).

The idea of the SIT may well have been triggered in part by the observation of monogamy in female screwworms (Knipling 1955). Thus, initially, there was the widespread belief that the SIT could only be applied to pest species in which the females mated only once. Consequently, there was a certain reluctance to consider the method for pest control of other species. However, Knipling (1959a, b) reconsidered that a monogamous mating system may not always be required, and von Borstel and Knipling (1960) described in detail why, independent of how many times the female mates, it is the competition between normal and lethal sperm that counts. Therefore, as long as irradiated sperm is competitive and can still penetrate eggs, female mating frequency is unimportant for effective SIT application (Bushland 1960; Knipling 1979; Lance and McInnis, this volume; Whitten and Mahon, this volume).

Nevertheless, in the 1930s, mass-rearing was not developed, and no method to induce sexual sterility was known. For a decade, the paramount urgency of World War II prevented Knipling from pursuing this sterile-male concept (Klassen 2003), but R. C. Bushland made a few attempts to induce sterility using chemicals.

### *4.1.3. Sterility Based on Radiation-Induced Dominant Lethal Mutations*

In 1946, after H. J. Muller was awarded the Nobel Prize in Physiology or Medicine for his discovery of induced mutagenesis, he used his prestige to lead a vigorous campaign against the atmospheric testing of atomic weapons. He wrote a popular article in the American Scientist in which he used tombstones as symbols to depict graphically the dead progeny from matings of irradiated *Drosophila* (Muller 1950). A. W. Lindquist recognized that Muller had developed a means of sexually sterilizing insects, and drew Knipling's attention to this paper (Lindquist 1955).

Knipling wrote to Muller, asking if ionizing radiation could be used to induce sexual sterility in the New World screwworm. Upon receiving Muller's confident assurance, Bushland and D. E. Hopkins used the X-Ray Therapy Section of Brooke Army Hospital to conduct the first screwworm irradiations in 1950. They found that, when 6-day-old pupae were exposed to 50 Gy, the adults that emerged appeared to be normal. However, when irradiated males were mated with untreated females, none of the eggs hatched. Females that had been irradiated and mated to untreated males produced almost no eggs, and none hatched. When untreated and irradiated males were caged together with untreated females, the irradiated males competed about equally with untreated males (in accordance with Knipling's model) (Bushland and Hopkins 1953).

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*4.1.4. Sanibel Island Field Evaluation Pilot Test*

Sanibel Island (47 km$^2$), 4 km from the Gulf coast of Florida, was selected for a release-recapture experiment (Bushland 1960; Itô et al., this volume) using $^{32}$P-labelled flies. In addition, the ratio of radioactive egg masses to non-radioactive masses was assessed. The release in 1951 of approximately 39 sterile male flies per km$^2$ per week for several weeks resulted in up to 100% sterility of the egg masses in wounded goats, and it greatly reduced the wild population. However, eradication was not achieved, apparently because wild fertile flies were flying to the island from the nearby mainland (Baumhover 2002).

*4.1.5. Curaçao Eradication Trial — Proof of Concept*

In 1954, Knipling was informed that screwworms were causing severe damage to the dairy industry on the island of Curaçao, 65 km from Venezuela, with an area of only 435 km$^2$. For the eradication trial, flies were reared in Orlando, Florida, and irradiated pupae were packaged in paper bags, air freighted to Curaçao, and released by air twice per week. On Sanibel, the release of 39 sterile males per km$^2$ per week had been effective, but on Curaçao this rate caused only 15% sterility of egg masses, and it had little effect on the incidence of myiasis cases due to the presence of thousands of unattended goats and sheep. Since wounds on these animals were not treated, they supported a high screwworm population. The release rate was increased to about 155 sterile males per km$^2$ per week, whereupon egg sterility increased to 69%, and then to 100% by the time two generations had elapsed. Subsequently, only two more fertile egg masses were found, and so sterile-fly releases were continued for another 8 weeks. Evidently eradication had been accomplished within 14 weeks, and the releases were halted after 22 weeks (Baumhover et al. 1955).

*4.1.6. Florida Eradication Programme*

At a meeting of the Florida Livestock Association in 1956, A. H. Baumhover suggested that eradication of the screwworm in Florida might eventually be possible, and he outlined a plan that called for the release of 50 million sterile flies per week. However, Knipling was reluctant to implement immediately a high-risk USD 10-million programme on the mainland — there were too many unknown factors, with problems in mass-rearing and distribution requiring several more years of applied research (which might reduce the eventual cost of the programme by USD 2 million). However, the governor T. L. Collins noted that the agricultural economy of Florida was losing more than USD 20 million per year due to the screwworm, and he pressed for immediate implementation. Nevertheless, to upgrade the rearing and release methodology, a further trial was conducted in 5000 km$^2$ along the Atlantic coast (Baumhover et al. 1959; Graham and Dudley 1959). Meanwhile, in July 1957, the Florida Legislature appropriated USD 3 million to match federal funds for an operational programme.

A mass-rearing facility was constructed at an Air Force Base at Sebring, Florida, with the production capacity of 60 million flies per week, and the programme was scheduled to begin in July 1958 (Scruggs 1975; Meyer and Simpson 1995). However, it was decided to accelerate this schedule following the unusually cold

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winter of 1957–1958, which eliminated all screwworms in the south-eastern states, except for the southern one-third of Florida. To contain the surviving screwworm population, the production was rapidly increased in the research facilities at Orlando and Bithlo, from 2 to 14 million sterile flies per week, and by May 1958 sterile flies were being distributed north of the infested area to the border with Georgia using 10 aircraft. The programme also established a quarantine line across central Florida to prevent the shipment of any infested livestock out of southern Florida, and any localized concentrations of screwworm cases in northern Florida were quickly eliminated by treating infested wounds, spraying herds, and releasing large numbers of sterile flies.

The mass-rearing facility at Sebring reached full production in August 1958, and 20 aircraft were used to distribute sterile flies throughout Florida and parts of neighbouring states. The Florida Cooperative Extension Service conducted a public information programme, and trained county agents to educate livestock producers. Field inspectors assisted and trained producers in treating cases and submitting larvae from wounds for identification at eradication headquarters in Sebring. Cases of myiasis in each county were plotted each day. The number of sterile flies released per $km^2$ per week was increased at persistent "hot spots" from about 155 to 1160. The last autochthonous case occurred on 19 February 1959 (Baumhover 2002). All sterile fly releases were terminated in November 1959. The total cost of the programme was USD 11 million, about 50% of the annual losses in Florida (Meadows 1985).

*4.1.7. Eradication and Area-Wide Population Management in South-Western USA*

The Florida programme aroused the interest of cattle producers in Texas, the western states, and Mexico. In 1959 the presidents of the USA (D. D. Eisenhower) and Mexico (A. López Mateos) agreed to a feasibility study to eradicate the New World screwworm in Mexico.

The strategy for dealing with the USA south-west was a by-product of the use of the 160-km-wide sterile-fly barrier across Florida, with the release of sterile flies only in the overwintering area, and to let the cold weather destroy the screwworms to the north of this area. Following eradication from that area, sterile flies would be deployed to create a barrier zone along the US-Mexico border to protect against reinvasion (Bushland 1985).

A mass-rearing facility was established at a former air base converted to an insectary, fly sterilization and dispersal centre, near Mission, Texas, and releases began in 1962. By 1964 no screwworms were found in Texas or New Mexico for a period of two or three generations, and USDA officials declared the screwworm eradicated from these states. In 1965, the programme was extended to the Pacific, and in 1966 the entire USA was declared free of screwworms; the federal government took full responsibility to maintain the barrier zone from the Gulf of Mexico to the Pacific. However, no agreement with Mexico to proceed southward had been reached, and the USA remained highly vulnerable to the influx of screwworms from Mexico. At this point in time, the goal of the programme was no longer eradication in the true sense, but had become population containment (Klassen 1989, 2000; Hendrichs, Vreysen et al., this volume).

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*4.1.8. Managing Screwworm Population along the US-Mexico Border*

Both US and Mexican cattle producers were anxious to push the screwworm population south to the narrow Isthmus of Tehuantepec, where a barrier of only 220 km would be needed. In 1972 the Mexico-United States Screwworm Eradication Agreement was signed, with the aim of eradicating the screwworm to the north of the Isthmus of Tehuantepec, and to establish a sterile-fly barrier there.

In the meantime, many difficulties arose. Screwworm cases occurred as much as 480 km north of the US-Mexico border. In 1968 almost 10 000 cases were recorded in the USA, and in 1972 such cases rose to 95 000. Knipling (1979) noted that, before the programme began, the maximum flight range of screwworm adults was estimated at 80 km. It was planned that the width of the sterile-fly barrier be twice this figure. However, Hightower et al. (1965) demonstrated that natural fly movement can occur up to at least 290 km, and the pattern of screwworm movement during the spring indicated that dispersal in a single generation was up to 480 km. In addition, Knipling (1979) concluded that the main reasons for the "breakdown" of the containment programme in 1972 were the unusually favourable conditions for winter survival of screwworms, the abandonment by ranchers of traditional animal husbandry practices needed to counter screwworm infestations (including the twice-weekly inspection and treatment of animals), and the explosion of the population of white-tailed deer as a result of almost no screwworm-induced mortality during the previous decade (Nagel and Peveling, this volume).

Critics of the programme postulated changes in the behaviour of the native population through inadvertent genetic selection during mass-rearing, making wild adults prone to avoid matings with the released strain, and the existence of cryptic species in the field (Bush et al. 1976; Richardson et al. 1982). However, no data were generated to support these views (Krafsur 1998; Krafsur and Ouma, this volume), which were strongly rebutted (LaChance et al. 1982). Another important factor was the unwise attempt to reduce sterile fly distribution costs by releasing flies on parallel flight lanes spaced 8 or 16 km apart, and this failed to deliver adequate numbers of sterile flies to all locations where wild virgin females were present (Krafsur 1978; Hofmann 1985).

The Mexico-United States Screwworm Eradication Commission began field operations in Mexico in 1974. A mass-rearing facility, with a capacity of 500 million sterile flies per week, was built at Tuxtla Gutiérrez, Chiapas (south of the Isthmus of Tehuantepec), and it reached full production in January 1977. Nevertheless, as late as 1976, almost 30 000 cases occurred in the USA. Major relief came when fly production at Mission, Texas, was supplemented from the new factory in Mexico (Meyer and Simpson 1995). Subsequently, the need for the Mission facility diminished rapidly, and in 1981 it was closed. The last autochthonous case in the USA occurred in August 1982.

Knipling (1979) stated:

> Had scientists known of the long flight range of the insect, they would not have recommended a sterile fly release programme in the south-west. This would have been unfortunate. By taking this gamble, up to a billion [1000 million] dollars [USD] have been saved. We have learned that despite the long-range movement of the insect, a high degree of pest population suppression can be achieved even against non-isolated populations.

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*4.1.9. Programmes in Central America: the Drive to Panama*

By 1984 the Commission had achieved the goal of eradicating the screwworm to Mexico's Isthmus of Tehuantepec (Peneda-Vargas 1985). Nevertheless, at the request of ranchers in southern Mexico and Central America, in 1986 operations were extended to the Yucatán Peninsula and the countries bordering Mexico (Irastorza et al. 1993). Eradication was declared as follows: Mexico 1991, Belize and Guatemala 1994, El Salvador and Honduras 1996, Nicaragua and Costa Rica 1999, and Panama 2006 where since 2004 a permanent sterile-fly barrier is being maintained in the Darien Gap along the border with Colombia (Wyss 2000; Maxwell et al. 2017; Skoda et al. 2017; Vargas-Terán et al., this volume). A smaller mass-rearing facility was constructed in the early 2000s at Pacora, Panama, and the larger facility at Tuxtla Gutiérrez was closed in 2013.

*4.1.10. Screwworm Eradication Programme in North Africa*

In 1988, the New World screwworm was discovered at Tripoli, Libya, where it rapidly spread over 28 000 $km^2$. Many feared that the insect would spread throughout North Africa, the Middle East and southern Europe, and migrate up the Nile River to sub-Saharan Africa, with serious consequences for the African people, livestock, and the already endangered large mammals.

In 1989, the Government of Libya asked the Food and Agriculture Organization of the United Nations (FAO) for assistance in eradicating the screwworm. The operational programme was planned in detail by consultants assembled by the Joint FAO/IAEA Division, and Libya and various donor countries provided the funding (FAO 1992; Vargas-Terán et al., this volume).

The infested area was partially isolated by the Mediterranean Sea, desert to the south, and barren areas with few livestock to the east and west. One hundred teams, each consisting of two individuals equipped with a jeep, inspected all livestock every 21–28 days, applied insecticide to every wound, and sprayed many of the animals. About 80 swormlure-baited wing traps were deployed across the lines of flight of the aircraft from which the sterile screwworms were dropped.

Sterile screwworms were supplied from Mexico — mating studies showed that the factory-strain flies were sexually compatible with the Libyan strain. Some differences in the mitochondrial DNA were observed, but they were not considered to indicate a barrier to applying the SIT (FAO 1992). Each weekly flight from Tuxtla Gutiérrez to Tripoli carried 40 million sterile screwworm pupae. In Tripoli, adult emergence was controlled to allow two early morning releases per week.

The attack on the pest population was planned for the early winter of 1990, since by then cool weather would have greatly reduced the density and the reproductive capacity of this insect. Also, cool weather would synchronize the life stages, and eliminate generation overlap. The number of sterile flies released was quickly increased to the maximum to saturate all suitable niches with sterile males. Thus, from the time that indigenous females emerged from under the soil, they would be in the company of sexually sterile males.

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The impact of this strategy was dramatic. Only six instances of wounds infested with screwworm larvae were found in 1991, compared with more than 12 000 cases in 1990. Releases of sterile flies were continued until October 1991, and surveillance of all livestock until June 1992 (Lindquist et al. 1993). Eradication was declared in June 1992 (FAO 1992). As previously in Texas, where critics cast doubt on the effectiveness of the sterile insect technique, claiming that eradication was caused principally by cold winters and hot, dry summers (Richardson 1978; Readshaw 1986, 1987, 1989), there were again voices that affirmed that weather, instead of sterile male releases, had been decisive in screwworm elimination from Libya. These arguments were clearly rebutted, not only for Texas (Krafsur et al. 1986; Krafsur 1987), but also for Libya, where it was shown that all of the conditions for successful screwworm overwintering and dispersal existed in a 15–25-km-wide zone along the Mediterranean coast (Krafsur and Lindquist 1996; Kouba 2004).

#### *4.1.11. Screwworm Programmes in the Caribbean*

By 1975 the screwworm had been eradicated from Puerto Rico and the United States Virgin Islands. In 1999, the Government of Jamaica initiated a programme to eradicate the screwworm that was terminated without reaching eradication (Vreysen et al. 2007; Dyck, Reyes Flores et al., this volume; Vargas-Terán et al., this volume).

No eradication programmes have been initiated on Cuba and Hispaniola, even though they can easily be sources of *C. hominivorax* reintroduction into areas that have been cleared of this pest (Vargas-Terán et al., this volume). This threat has been confirmed by multiple interceptions of screwworm-infested animals at ports of entry, as well as significant outbreaks in Aruba, Curaçao, Mexico, and most recently in Florida, that were efficiently eliminated applying the SIT (Skoda et al. 2018; Vargas-Terán et al., this volume).

### *4.2. Tephritid Fruit Flies*

Currently, the SIT is most widely applied against tephritid fruit flies (Enkerlin, this volume). Already in 1955, immediately after the successful screwworm eradication trial in Curaçao, investigations were initiated by the USDA in Hawaii into the possibility of developing and integrating the SIT to eradicate populations of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), melon fly *Zeugodacus* (*Bactrocera) cucurbitae* (Coquillett), and oriental fruit fly *Bactrocera dorsalis* (Hendel) (Steiner and Christenson 1956; Steiner et al. 1970). Also, prior to 1960, pioneering investigations were underway on the Queensland fruit fly *Bactrocera tryoni* (Froggatt) in Australia, and on the Mexican fruit fly *Anastrepha ludens* (Loew) in Mexico and the USA (Klassen et al. 1994; Enkerlin, this volume). Furthermore, extensive research and development was started in the early 1960s at the FAO/IAEA Laboratory in Seibersdorf, Austria, to develop the SIT for the Mediterranean fruit fly and other fruit fly pests (Klassen et al. 1994).

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The first successful use of the SIT against an insect pest, other than the screwworm fly, was the eradication in 1963 of the melon fly from Rota in the Mariana Islands (Steiner et al. 1965). The first large-scale programme, established by Mexico and the USA in the late 1970s, stopped the invasion of the Mediterranean fruit fly from Central America into southern Mexico (Hendrichs et al. 1983). In Japan, the SIT was employed in the 1980s and 1990s to eradicate the melon fly in Okinawa and all of Japan's south-western islands, permitting access for fruits and vegetables produced in these islands to the main markets in the Japanese mainland (Kuba et al. 1996). In Chile, the SIT was used to rid the north of the country of the Mediterranean fruit fly; thus by 1995 the entire country had become a fly-free zone, and a joint programme with Peru operates in northern Chile and southern Peru. Since then Chilean fruits have entered the US market in huge volumes without the need for any quarantine treatment, providing a major benefit to the Chilean economy (Enkerlin, this volume). Argentina also has developed significant SIT Mediterranean fruit fly programmes in several fruit-producing provinces, some of which have succeeded in establishing pest free areas. Mexico has also applied the SIT to get rid of various *Anastrepha* species from northern Mexico (Enkerlin, this volume).

The SIT is increasingly being applied with the objective to reduce losses and pesticide use rather than fruit fly eradication, with suppression programmes ongoing in Brazil, Croatia, Israel, South Africa, Spain and Thailand, and in preparation in Morocco and Vietnam. To prevent Mediterranean fruit fly establishment in the continental USA through infested imported (smuggled) fruit, sterile males are being released preventively in the Los Angeles Basin, Tampa, and Miami. Consequently, there is no longer a need to spray these urban areas with malathion insecticide to suppress and eliminate incipient pest populations each time there are outbreaks.

Several species of tropical fruit flies are extremely destructive pests of fruits and vegetables. Tephritid fruit flies are major economic pests because they have:

- A multivoltine life cycle with an explosive reproductive capacity,
- A polyphagous behaviour with the capacity to exploit a large number of hosts,
- The ability to disperse widely as adults or to be moved in fruit as larvae,
- The ability (adults) to survive several months of inclement weather.

Tropical fruit flies not only cause great losses in fruit and vegetable production, but they also seriously impede international trade because of quarantine regulations designed to avoid cross-border introductions. Consequently, efforts to remove, suppress, or exclude these pests have been made in at least 32 countries (Klassen et al. 1994; Hendrichs 2001; Enkerlin, this volume).

The mating behaviours of tropical fruit flies are very different from the aggressive behaviour of male screwworms, involving complex courtship behaviours with males aggregating in mating arenas or leks, and where receptive females determine mate choice (Hendrichs et al. 2002; Robinson et al. 2002). Thus, special attention in terms of product quality control must be given to the effects of colony-holding conditions, artificial diets, irradiation, and handling procedures on the acceptability to wild females of released sterile males (Cayol 2000; Hendrichs et al. 2002).

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*4.2.1. Mexican and Queensland Fruit Flies*

In 1964, the SIT was used to eradicate the Mexican fruit fly from outbreaks in southern California, and as a containment measure to prevent the pest from re-entering California from Baja California Norte in Mexico, and a decade later to exclude the pest from the Rio Grande Valley of Texas. Both SIT containment programmes have continued since then, but the programme on the California-Mexico border was terminated after the Mexican states of Baja California Norte, Baja California Sur, Chihuahua, and Sonora, following successful SIT projects in the 1990s against *A. ludens* and the West Indian fruit fly *Anastrepha obliqua* (Macquart), were converted into fruit fly-free zones from which citrus, stone fruits, apples, and vegetables are now being exported without any postharvest treatment (Reyes F. et al. 2000; Enkerlin, this volume).

Field trials of the SIT against the Queensland fruit fly began in 1962 in New South Wales, Australia. Although the population was suppressed strongly, it could not be eradicated because of long-range immigrants. Since the mid-1990s, an SIT containment programme has tried to protect a "Fruit Fly Exclusion Zone" comprising the "Tri-State" major fruit production area (southern New South Wales, northern Victoria, and eastern South Australia), although the Queensland fruit fly has been increasingly extending its range southward into this free area (Capon et al. 2017). In 1990, use of the SIT resulted in the eradication of this pest from an incipient infestation in 125 $km^2$ at Perth, Western Australia (Fisher 1996). Also, the SIT was used to eradicate the Mediterranean fruit fly in Carnarvon in Western Australia (Fisher et al. 1985), and is now used to eradicate recurrent outbreaks of this pest in South Australia (Smallridge et al. 2002).

*4.2.2. Moscamed*

In 1955, the Mediterranean fruit fly was found in Costa Rica. After the pest had established a small foothold in Nicaragua, and a pilot programme conducted in 1967 (Rhode 1970), an operational programme to contain this pest was initiated to prevent it from invading countries to the north (Rhode et al. 1971). However, very unfortunately, a review team concluded that the Mediterranean fruit fly is not economically important to Central America, and recommended that the programme be terminated (Rhode 1976; Dyck, Reyes Flores et al., this volume). Thus, by 1976 the pest had expanded its range into Honduras, El Salvador, and Guatemala, and by 1979 it already occupied 15 000 $km^2$ in southern Mexico.

In the meantime, to meet this emergency, the Government of Mexico entered into cooperative agreements with Guatemala and the USA to establish the first large-scale fruit fly AW-IPM programme using the SIT. Construction of a rearing facility at Metapa, Mexico, to produce 500 million sterile flies per week, began in 1977 and initiated production in early 1979 (Schwarz et al. 1985). By 1982, pest eradication in the infested area of Mexico was achieved (Hendrichs et al. 1983), and a containment barrier was created through Guatemala (Villaseñor et al. 2000; Enkerlin, this volume). For approximately 40 years, this programme has kept Mexico, the USA, and half of Guatemala free of the Mediterranean fruit fly, allowing Mexico over this period to significantly expand its fruit and vegetable exports, mainly to the USA (Enkerlin et al. 2015, 2017). Mexican horticulture export earnings since 1994 have

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tripled to more than USD 9000 million per year (Enkerlin, this volume). In the meantime, the production capacity of the Moscamed programme has increased to over 4000 million sterile males per week, the majority of which are produced at the El Pino facility in Guatemala (Rendón et al. 2004).

*4.2.3. Melon Fly Eradication in the South-Western Islands of Japan*

Between 1919 and 1970, the melon fly gradually invaded most island groups in the south of Japan, including Okinawa. The shipment of fruits and vegetables to markets in mainland Japan was strictly forbidden. Consequently, the Japanese National Government assisted the Prefectural Governments of Kagoshima and Okinawa to conduct two separate programmes to eradicate the melon fly from all of the south-western islands.

A pilot eradication experiment on small Kume Island (60 $km^2$) began in 1972, and eradication was declared in 1978. In 1984, an operational programme was undertaken in the Miyako Islands. The capacity of the rearing facility was 30 million flies per week. Since the wild population was estimated at 34.4 million, male annihilation (using cotton strings impregnated with cuelure and insecticide) was used to reduce it to 5% of its original level. The production of high-quality flies, and supplementary releases in high-density areas, were critically important (Yamagishi et al. 1993; Kakinohana 1994). By 1986 the production capacity had been expanded to almost 200 million sterile flies, and the programme gradually moved from island group to island group until eradication of the melon fly from all of Japan was achieved in 1993 (Kuba et al. 1996; Koyama et al. 2004).

*4.2.4. Mediterranean Fruit Fly Genetic Sexing Strains*

In the early 1960s, the Citrus Marketing Board of Israel developed an insecticide-based area-wide programme against the Mediterranean fruit fly that was able to meet the certified quarantine security requirements of fruit importing countries (Cohen and Cohen 1967). Bisexual releases of sterile flies were avoided for this programme because sexually sterile female Mediterranean fruit flies can cause (with their ovipositors) cosmetic damage in some varieties. However, in work with the Australian sheep blow fly *Lucilia cuprina* (Wiedemann), Whitten (1969) found that male and female pupae of a strain, in which the segment of the autosome bearing a gene for black puparium is translocated to the Y chromosome, could be separated mechanically, as all males are brown, and all females black.

This encouraged Rössler (1979) to construct a similar strain of the Mediterranean fruit fly in which male pupae (brown) could be separated from female pupae (white). This special strain was mass-reared and pupae sorted by a seed-sorter at the FAO/IAEA Seibersdorf Laboratory in Austria; it performed well in large-scale tests in Israel (Franz et al., this volume).

Subsequently, this laboratory developed a genetic sexing strain in which a segment of an autosome bearing the dominant wild type allele of a temperature-sensitive lethal (*tsl*) mutant was translocated to the Y chromosome (Franz and Kerremans 1994; Caceres et al. 2004). This enabled the elimination of females at the

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egg stage, thereby saving significant costs during mass-rearing, handling, and release, and increasing sterile male production in the same facility.

In addition, this laboratory developed a "filter rearing system" to maintain strain stability in the mass-rearing of such genetic sexing strains (Fisher and Caceres 2000; Franz et al., this volume; Parker, Mamai et al., this volume).

#### *4.2.5. Sterile "Genetic Sexing Strain" Males Alleviate Mediterranean Fruit Fly Crises in California and Florida, USA*

Until 1980, the Mediterranean fruit fly invaded California and Florida at only infrequent intervals. Outbreaks, occurring usually in urban areas, were eliminated mainly by applying malathion-bait sprays. It cost more than USD 100 million to eradicate the infestations in California detected in 1980 and 1982.

In the decade 1987–1997, multiple new infestations were encountered annually in California and Florida. Since each outbreak was addressed independently, this non-area-wide approach often resulted in continuous new satellite infestations, and there was a real threat that the pest would become established. Therefore, in 1994, an area-wide SIT eradication programme was initiated, with twice-weekly releases of sterile Mediterranean fruit flies over the entire Los Angeles Basin. This programme was so successful and cost-effective that, in view of the many introductions, in 1996 a permanent preventive release programme was established over this area (Dowell et al. 2000; Barry et al. 2004). The same preventive approach has also been followed since 1998 in three Florida counties that are at high-risk in terms of introductions.

These preventive release programmes have now been in operation for over 20 years, maintaining the Mediterranean fruit fly-free status of the United States (although this is disputed by Carey et al. 2017, but see rebuttals by McInnis et al. (2017) and Shelly et al. (2017)). Sterile males of the *tsl* sexing strain VIENNA 7 or VIENNA 8, mainly produced by the Moscamed programme in Guatemala, are used for these preventive release programmes. Sexing strains are also used in most Mediterranean fruit fly suppression, containment, or eradication programmes (Caceres et al. 2004; Franz et al., this volume; Augustinos et al. 2017).

#### *4.2.6. Jordan-Israel-Palestine Mediterranean Fruit Fly Programme*

The signing of the Oslo Peace Accord created an opportunity for the international community to assist Middle East countries, notably the Hashemite Kingdom of Jordan, the Palestinian Authority, and Israel, to undertake joint projects that would foster cooperation. An economic analysis of three area-wide programme alternatives was conducted (with the support of the FAO/IAEA) (Enkerlin and Mumford 1997). In the operational programme, initially focused on the Arava/Araba region between Israel and Jordan, the genetic sexing strain VIENNA 7 (Franz et al., this volume) has been used for population suppression rather than eradication (at present, there is no intention to establish disruptive quarantines along a major highway). As a result of this suppression programme, the export of fresh vegetables from the Arava region has reached more than USD 30 million per year (Cayol et al. 2004; Enkerlin, this volume). Initially, the sterile pupae for this programme were shipped from the

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Moscamed facility in El Pino, Guatemala, but then BioBee (the largest producer of biological control agents in Israel) constructed a commercial mass-rearing facility, with the goal of expanding suppression to other fruit and vegetable production areas in Israel, West Bank, and Jordan, and to sell sterile flies internationally, for example, to the Mediterranean fruit fly suppression programme in the Neretva Valley in Croatia (Bassi et al. 2007; Bjeliš et al. 2016).

#### *4.2.7. Trend is Suppression, not Eradication, of Fruit Flies*

For technical and economic reasons, including the undesirability of establishing quarantines that interfere with trade, or difficulties in maintaining them effectively, today many fruit fly AW-IPM programmes that integrate the SIT aim to suppress the pest populations (Mumford 2004; Enkerlin, this volume; Hendrichs, Vreysen et al., this volume; Mumford, this volume). Examples of suppression programmes using sterile flies as an additional IPM tool are:

- Mediterranean fruit fly
  - Costa Rica (Reyes et al. 2007)
  - Arava, Israel (Cayol et al. 2004)
  - Hex River Valley, South Africa (Barnes et al. 2004; Venter et al. 2021)
  - San Francisco Valley, Bahia, Brazil (Malavasi et al. 2007)
  - Valencia, Spain (Generalitat Valenciana 2003)
  - Neretva Valley, Croatia (Bjeliš et al. 2016)
  - Souss Valley, Agadir, Morocco (Enkerlin, this volume)
  - Southern Brazil, Brazil (Kovaleski and Mastrangelo 2021)
- Mexican fruit fly
  - Mexico (Orozco-Dávila et al. 2017)
- Oriental fruit fly and *Bactrocera correcta* (Bezzi)
  - Ratchaburi Province, Thailand (Sutantawong et al. 2004).

### *4.3. Onion Maggot*

Since 1981, the SIT has been applied by a private firm (now de Groene Vlieg Bio Control b.v.) in The Netherlands to control the onion maggot *Delia antiqua* (Meigen) on an aggregate area of ca. 10 000 hectares (Loosjes 2000; Everaarts 2016). The flies are reared year-round, and stockpiled in diapause for release during the onion-growing season. Individual farmers contract for the service of population monitoring and releasing the sterile flies independently of their neighbours, many of whom use chemical control (the relevant insecticides were eventually banned by the European Union in the 2000s but the industry lobby managed to get exemptions for the onion crop). As a result of this uncoordinated approach, much efficiency is lost because the sterile flies are not applied on an area-wide basis (protected fields do not form a contiguous block). Some growers in the general area of sterile-fly releases benefit from them, but do not contribute to the programme (free-riders). Nevertheless, the programme has been able to expand to ca. 40% of the onion production area.

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### *4.4. Tsetse Flies*

Tsetse flies are confined to sub-Saharan Africa where they transmit the disease trypanosomosis [trypanosomiasis] to humans (sleeping sickness) and livestock (nagana). They are regarded as a major cause of rural poverty because they prevent mixed farming. Crops have to be cultivated manually with hoes because nagana kills draught animals. The surviving cattle produce little milk, and manure is not available to fertilize the worn-out soils. The conquest of sleeping sickness and nagana would be of immense benefit to rural development in sub-Saharan Africa.

Understanding this fundamental role of tsetse at the root of rural poverty, it was E. F. Knipling who in the early 1960s assigned D. A. Dame from the USDA to lead a research effort in Southern Rhodesia (Zimbabwe), in collaboration with the University of Rhodesia, to determine the feasibility of applying the SIT against tsetse flies and to develop mass-rearing technology. Also, the FAO/IAEA, and other institutions from the previous colonial nations, initiated studies to assess the potential of the SIT for tsetse species. Of the over 30 tsetse species, only some (6 or 7) are of economic importance, and since then have been the subject of SIT development (Feldmann et al., this volume).

Tsetse flies are unique among pest insects in being larviparous, i.e. females do not lay eggs but gestate a larva in a uterus (one larva at a time), with a gestation period of about 9 days. Thus, these flies have extraordinarily low rates of reproduction. Therefore, relatively low release rates should be sufficient, compared with those required for highly fertile oviparous pests (Hendrichs, Vreysen et al., this volume). However, rearing tsetse flies is relatively laborious and expensive because adults of both sexes require frequent blood feeding. The development in the 1980s at the FAO/IAEA Laboratory in Seibersdorf, Austria, of an *in vitro* rearing system with membranes and heated abattoir-collected blood (that is previously irradiated to eliminate any microbial contamination) to replace the use of live animals, represented a major breakthrough that enabled the upscaling of mass-rearing (Parker, Mamai et al., this volume).

Table 2 summarizes the SIT trials that have been conducted on tsetse flies. (Data from the trial by Vanderplank are shown above in Table 1.) In a trial on *G. m. morsitans* in 1969 on an island (5 $km^2$) in Lake Kariba, Zimbabwe, pupae collected in the field were chemosterilized in the laboratory, and then returned to the field to permit adult flies to emerge. The sterile flies were fully competitive, but adult flies that were sterilized after emergence and held in captivity suffered an 80% loss in field competitiveness. These studies were followed in 1977–1978 by a larger-scale (195 $km^2$) trial in Tanzania using mass-reared *G. m. morsitans* fed on live animals, which demonstrated full sterile-fly competitiveness following irradiation and release in the pupal stage.

Among the other releases (conducted in the 1970s and 1980s) were several that successfully integrated releasing sterile males with deploying recently developed attractant traps and insecticide-treated targets. Three tsetse species were eradicated simultaneously in 3000 km2 in Burkina Faso (Politzar and Cuisance 1984), and one species in 1500 km2 area in Nigeria (Takken et al. 1986). The technology was successfully applied, but unfortunately the programmes were not conducted area-wide and thus the pest free status of the areas was not sustainable.

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*Table 2. Summary of SIT trials with tsetse flies* Glossina *spp.*

| Species, habitat, and location | Method | Outcome and objectives | References |
|---|---|---|---|
| *Glossina swynnnertoni*, savannah, north-western Tanzania | Release of *G. morsitans*, which mated with *G. swynnertoni* | 99% suppression in 256 km$^2$, permitted development of the area for agricultural production | Vanderplank 1947, and hitherto unpublished data shown in Table 1 |
| *G. morsitans morsitans,* savannah, Lake Kariba, Zimbabwe | Insecticidal suppression followed by release of chemically sterilized pupae | > 99% suppression on 5-km$^2$ island, feasibility study | Dame and Schmidt 1970; Dame et al. 1981 |
| *G. tachinoides* Westwood, riverine, Chad | Radiation-sterilized, transport from France, ground release sterile ♂ | Feasibility study, sterilization, transport, release | Cuisance and Itard 1973 |
| *G. palpalis gambiensis* Vanderplank, riverine, Burkina Faso | Suppression by aerial insecticide treatment, ground release sterile ♂ | Feasibility study (16 linear km) to control sleeping sickness | Van der Vloedt et al. 1980 |
| *G. palpalis palpalis* Robineau-Desvoidy with *G. tachinoides* as a control, riverine, Lafia, Nigeria | Suppression with traps and targets followed by ground release of radiation-sterilized adults | Eradication of *G. p. palpalis* in 1500 km$^2$ | Takken et al. 1986; Oladunmade et al. 1990 |
| *G. morsitans morsitans*, savannah, Tanzania | Insecticidal suppression followed by ground release of radiation-sterilized pupae | 90% suppression (195 km$^2$), feasibility study | Dame et al. 1975; Williamson et al. 1983 |
| *G. morsitans morsitans,* and *G. pallidipes* Austen*,* savannah, Lake Kariba, Zimbabwe | Autosterilization of wild flies with pyriproxyfen | Suppression (12 km$^2$), feasibility study | Hargrove and Langley 1990 |
| *G. morsitans submorsitans* Newstead*, G. palpalis gambiensis, G. palpalis palpalis, G. tachinoides*, riverine and savannah, Burkina Faso, Nigeria | Insecticide application and trapping suppression followed by ground release of radiation-sterilized adults | Eradication (3000 km$^2$ - Burkina Faso, 1500 km$^2$ - Nigeria) | Politzar and Cuisance 1984; Takken et al. 1986 |
| *G. austeni* Newstead, bushland and forest, Unguja, Zanzibar, Tanzania | Suppression with insecticide on livestock and attractive devices followed by aerial release of radiation-sterilized adults | Eradication (1650 km$^2$), trypanosomosis transmission ceased | Msangi et al. 2000; Vreysen et al. 2000; Feldmann et al., this volume |
| *G. fuscipes fuscipes* Newstead, forest, Buvuma Islands, Uganda | Autosterilization of wild flies with triflumuron vs. insecticide-impregnated traps | Suppression (5 km$^2$), abandoned because of funding shortfall | Oloo et al. 2000 |

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Traps were also used on a small island (12 km$^2$) in Lake Kariba, Zimbabwe, to attract wild flies that were autosterilized by coming into contact with the traps, and then departed (Hargrove and Langley 1990). This, and another failed eradication trial, also in a small island in a Ugandan lake, are almost the only attempts to date to apply the autosterilization principle which avoids or minimizes the need for a rearing facility.

The eradication in 1997 of the tsetse fly *G. austeni* in Unguja Island, Zanzibar, Tanzania, confirmed the feasibility of the area-wide integration of aerial sterile-male releases with other suppression methods to create sustainable tsetse-free areas (Vreysen et al. 2000). This successful programme freed cattle from the burden of nagana, resulting in major socio-economic benefits (Msangi et al. 2000; Feldmann and Jannin 2001; Feldmann et al., this volume). In response, in 2001, the African Heads of State and Government committed their countries to rid Africa of this disease (Feldmann and Jannin 2001). However, the dream to conquer nagana will require many decades of concerted effort. Furthermore, there is a debate about the desirability of using the SIT with other approaches to eradicate populations of major tsetse species from large areas of the African mainland (DFID 2002; Hargrove 2003; Nagel and Peveling, this volume), and the perceived high cost of applying the technique.

Currently ongoing programmes to free selected areas from tsetse are under way against *G. pallidipes* and *G. fuscipes* in the Deme valley in southern Ethiopia, and against *Glossina palpalis gambiensis* in the Niayes area of Senegal (Zerihun 2017; Vreysen et al. 2021; Feldmann et al., this volume).

### *4.5. Mosquitoes*

Mosquito-borne diseases (malaria, dengue, filariasis, yellow fever, chikungunya, Zika) annually cause severe mortality and morbidity. For mosquito SIT, since females bite, blood-feed and may transmit major human pathogens, it is essential that releases of female mosquitoes be reduced to an absolute minimum. Therefore, efficient sexing is needed on a large scale, and genetic sexing systems are the preferred approach (Gilles et al. 2014; Franz et al., this volume). Naturally occurring pupal size difference between males and females, for example in some *Aedes* mosquitoes, are currently exploited for mechanical sexing, although it is difficult to achieve a 100% pure separation under operational conditions and is very labour-intensive when applied on a large scale.

As shown in Table 3 and in Benedict and Robinson (2003), in the 1960s and 1970s there was considerable interest and activity developing the SIT for mosquitoes, and several release trials were conducted, some of them integrating the *Wolbachia*-based incompatible insect technique (IIT), based on the ground-breaking work by Hannes Laven on cytoplasmic incompatibility (CI) (Laven 1958, 1967). The largest-scale trials were conducted in El Salvador and India. Unfortunately, in both countries, political factors in the mid-1970s interrupted further work — civil war in El Salvador, and in India false accusations that the project was intended to collect data on biological warfare (Nature 1975; WHO 1976).

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*Table 3. Summary of release trials with sterile or semi-sterile male mosquitoes*

| Target species | Location | Sterilization and sex-separation method | Outcome | References |
|---|---|---|---|---|
| *Anopheles quadrimaculatus* Say | Lakes in Florida, USA | Pupal irradiation, adult release, sex separation by pupal size | Poor competitiveness of colonized males for wild females, which may have been mismatched for sibling species | Weidhaas et al. 1962; Dame et al. 1964 |
| *Culex quinquefasciatus* Say | Okpo, Myanmar | Cytoplasmic incompatibility | Eradication of small village population | Laven 1967 |
| *Anopheles gambiae s.s.* Giles | Pala, Burkina Faso | *An. melas* Theobald x *An. arabiensis* Patton cross yielding sterile hybrid males and few females | Poor competitiveness of hybrid males | Davidson et al. 1970 |
| *Culex quinquefasciatus* | Sea Horse Key, Florida, USA | Chemosterilization with thiotepa | Sterilization of small island population, moderate competitiveness | Patterson et al. 1970 |
| *Culex pipiens* L. | Village near Montpellier, France | Chromosome translocations | Persistent semi-sterility in wild population | Laven 1972; Cousserans and Guille 1974 |
| *Culex quinquefasciatus* | Villages near Delhi, India | Thiotepa sterilization or cytoplasmic incompatibility plus translocations, sex separation by pupal size | 300 000 released per day, 99.8% male, adequate competitiveness in the field for females of wild origin, but high egg sterility not achieved by mass-release due to immigration | Sharma et al. 1972; Singh et al. 1975; Curtis 1976; Grover et al. 1976a; Yasuno et al. 1978; Curtis et al. 1982 |
| *Aedes aegypti* (L.) | Urban areas in or near Delhi, India | Thiotepa sterilization or sex-ratio distorter plus translocations, sex separation by pupal size | Rearing and sex separation as for *Culex quinquefasciatus* above, high competitiveness in the field for females of wild origin | Curtis et al. 1976; Grover et al. 1976b; Ansari et al. 1977; Suguna et al. 1977 |
| *Aedes aegypti* | Mombasa, Kenya | Chromosome translocations | Partial sterility detected in wild population | McDonald et al. 1977 |

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*Table 3. Continued*

| Target species | Location | Sterilization and sex-separation method | Outcome | References |
|---|---|---|---|---|
| *Anopheles albimanus* Wiedemann | Lake Apastapeque, El Salvador | Chemosterilization of pupae with bisazir, inaccurate sex separation based on pupal size | 100% sterility induced in wild population, which fell below detection level after 5 months | Lofgren et al. 1974 |
| *Anopheles albimanus* | Pacific coast of El Salvador | Bisazir sterilization, sex separation originally by pupal size + feeding on malathion-treated blood, later by a Y chromosome propoxur-resistance translocation inversion (MACHO strain) | Eventually 1 million MACHO released per day and found competitive, a natural population increase was suppressed, but eradication prevented by immigration in spite of a barrier zone | Seawright et al. 1978; Dame et al. 1981 |
| *Culex tritaeniorhynchus* Giles | Village near Lahore, Pakistan | Pericentric inversion plus translocation, sex separation by temperature-sensitive lethal | Assortative mating — competed for females of colony origin but not for females of wild origin | Baker et al. 1978, 1979 |
| *Anopheles culicifacies* Giles | Village near Lahore, Pakistan | Bisazir sterilization, sex separation by Y chromosome dieldrin translocation | Released males behaved normally in the field but showed subnormal mating competitiveness | Baker et al. 1980, 1981; Reisen et al. 1981 |
| *Culex tarsalis* Coquillett | Kern County, California, USA | Adult irradiation after separation of males by hand | Partial assortative mating — reduced competitiveness for wild females, but supercompetitiveness for colony females | Reisen 1982 |

The work in India showed that two important vector species of culicine mosquitoes could be mass-reared, and the sexes separated (according to pupal size) to ensure that 99.8% of the released mosquitoes were males. Males were chemosterilized in the pupal stage, or by male-linked chromosome translocations combined either with cytoplasmic incompatibility or sex-ratio distortion due to meiotic drive. Field tests showed that the mating competitiveness of the males of both species was acceptable. However, the mass release of *Culex quinquefasciatus* males in villages achieved only limited levels of sterility in eggs laid by wild females. This was attributed to the influx of already-mated females from outside the target release area. A planned mass release of sterile male *Aedes aegypti*, aimed at

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the eradication of this urban mosquito from a whole town, was prevented by the political problem mentioned above.

In El Salvador the target was the malaria vector *Anopheles albimanus*. It was multi-resistant to insecticides (partly due to the agricultural use of insecticides), and therefore difficult to control by conventional means. In the initial study, releases during 5 months around Lake Apastapeque were successful in inducing 100% sterility in eggs laid by wild females (Lofgren et al. 1974; Weidhaas 1974). Sex separation in that trial was based on pupal size differentiation; however, it was very imperfect, yielding 15% females among the released males. In a second larger trial, a second separation was added; adults were offered malathion-laced blood through a membrane. This effectively eliminated most of the females, but the males were debilitated from being caged and handled. Therefore, a genetic sexing strain was developed — a chromosome translocation was induced, linking a propoxur-resistance gene to the Y chromosome, and this was combined with a crossover-suppressing chromosome inversion. Propoxur treatment at the egg stage selectively eliminated all but 0.2% of females, thereby allowing a doubling of the male production for release (Seawright et al. 1978). By eliminating the handling losses in the adult stage, the net release was increased from 200 000 males per day to over 1 million, and these males, when released as sterile pupae, were almost fully competitive in the field. Compared with the seasonal upward trend of the untreated population, the releases reduced the target field population by more than 97% (Dame et al. 1981; Benedict and Robinson 2003). However, complete control was thwarted by immigration, and political unrest and war caused the trials to be terminated.

As a result of the increase in the global burden of mosquito-borne diseases, the unsustainability of relying on insecticides for vector control due to increasing insecticide resistance, and the absence of vaccines and efficient, safe and inexpensive drugs to combat malaria, dengue, chikungunya, Zika, and other emerging diseases, the idea of genetic control of mosquitoes is now undergoing a revival (Häcker et al., this volume; Lees et al., this volume).

During the last 15 years considerable progress has been made in developing the SIT as a potential new tool in the arsenal to manage mosquitoes. Recent developments enable efficient mass-rearing and irradiation for male release (Lees et al. 2015; FAO/IAEA 2017b, c, 2018). Equipment and protocols have been developed and validated for efficient mass-rearing, irradiation and release of Aedines and Anophelines. Assessment of male quality is becoming more sophisticated, showing that mosquitoes are not different in their radiation-sensitivity, a common misconception. For example, the radiation doses to sterilize male *Aedes albopictus* (Skuse) and *Aedes aegypti* range between 35–40 Gy and 60–80 Gy, respectively, and have a negligible effect on the biological quality of the mosquito (Bourtzis et al. 2016).

Several institutions and governments are currently evaluating the feasibility of mosquito SIT in various pilot settings and stages of development, including in Brazil, China, Cuba, French Polynesia, Italy, Mauritius, Mexico, Reunion, Singapore, Spain, Sudan, Thailand, and USA (Lees et al., this volume).

Until perfect sexing mechanisms exist, the SIT in combination with *Wolbachia*-induced cytoplasmic incompatibility and pathogen interference is considered the safest solution for *population suppression* (Zhang et al. 2015; Bourtzis et al. 2016;

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Bown 2019; Zheng et al. 2019). Being *Wolbachia*-infected, any accidently released females cannot result in population establishment because they are irradiated and therefore sterile**,** but at the same time because of *Wolbachia* they will also be unable to transmit human pathogens. This is not the case with transgenic or only symbiont-based approaches that are also under development (Thomas et al. 2000; Alphey and Andreasen 2002; Itô et al. 2002; Hoffmann et al. 2011; Bourtzis et al. 2014; Carvalho et al. 2015; Häcker et al., this volume), where released females can either vector disease (will result in *population replacement*) or leave an "ecological footprint" (Evans et al. 2019). For such approaches, issues such as stability, sustainability, and biosecurity need to be addressed. Regulatory issues and those relating to intellectual property and the economic cost of application must also be overcome (Bourtzis et al. 2016).

### *4.6. Coleoptera*

#### *4.6.1. Field Cockchafer*

Cockchafers (Scarabaeidae) are important pests of root vegetables. The flight period can be accurately forecast, and is restricted to a few weeks every third year, with males emerging before females. In 1959 and 1962, Horber (1963) conducted two field trials on *Melolontha vulgaris* F. in 30 hectares of agricultural land in Switzerland. He collected adult males in light traps, and gave them a sterilizing dose of 33 Gy. In the two trials, 3109 and 8594 sterile males were released, and the wild populations were reduced by 80% and to eradication, respectively.

Incidentally, in another family of beetles, the SIT is under development for application against the small hive beetle *Aethina tumida* Murray (Coleoptera: Nitidulidae), a serious threat to bee-keeping (Downey et al. 2015).

#### *4.6.2. Sweetpotato Weevils*

Two weevil species are found in Okinawa Prefecture in south-western Japan — sweetpotato weevil *Cylas formicarius* (F.) and West Indian sweetpotato weevil *Euscepes postfasciatus* (Fairmaire) (Yasuda 2000). These are invasive alien pests that threaten agricultural production, and have been designated as plant quarantine pests. Consequently, the transport of fresh sweet potatoes from Okinawa to mainland Japan is prohibited. Currently, both species are the targets of pilot AW-IPM eradication trials integrating the SIT on Kume Island.

Radiation has detrimental effects on these weevils. Digestive obstruction following the collapse of the epithelial tissue of the midgut has been suggested as the cause of the short lifespan of irradiated adults. Nevertheless, mitigation measures, including partial sterility and fractionated-dose irradiation at low temperature, successfully overcame these effects (Kumano et al. 2010, 2012).

From 1994 to 1999, the male annihilation technique (MAT), using synthetic sex pheromone and insecticide in wood fibreboard squares, was applied. The population of *C. formicarius* was suppressed by about 90%, and the plant infestation level dropped from 9.5 to less than 1%. Subsequently, sterile weevils were aerially released, and "hot spots" were treated with additional ground releases (Kohama et

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al. 2003). In 2013 *C. formicarius* was declared eradicated from Kume Island (Haraguchi et al. 2014).

Following a successful SIT trial in 1995, and using weevils reared on artificial diet (Shimoji and Miyatake 2002; Shimoji and Yamagishi 2004), an island-wide AW-IPM programme integrating the SIT against *E. postfasciatus* is ongoing on Kume Island.

### *4.7. Lepidoptera*

Throughout the world, lepidopteran larvae cause immense damage to food and forage crops, forests, and stored products. However, there are several problems when applying the SIT against moths (Bakri et al., this volume; Lance and McInnis, this volume; Marec et al., this volume; Simmons et al., this volume):

- Some tolerance to dominant lethal induction by ionizing radiation, because of the holokinetic chromosomes of Lepidoptera,
- Reductions in ability to mate when inducing full sterility,
- Production of eupyrene and apyrene sperm,
- Spermatophore formation,
- Complex sperm transfer.

For species (such as the codling moth) that undergo diapause, the potential exists to mass-rear year-round and stockpile the pupae, and then activate them later in the spring for release in synchrony with the wild population (Parker, Mamai et al., this volume). However, on the whole, the large-scale rearing of moths is more difficult than that of most flies. For these reasons, the development of the SIT against moths has lagged behind work with flies. Nevertheless, the potential to use the SIT against lepidopteran pests appears to be very great (Marec et al., this volume), and its application is increasing (Simmons et al., this volume).

M. D. Proverbs (1962, 1982) in British Columbia, Canada, developed the SIT to suppress the codling moth, an economic pest of apples and pears. He found that very high and debilitating doses of ionizing radiation (about 400 Gy) are required to induce 100% sterility. However, considerably lower and less debilitating doses induce inherited sterility (IS), and the level of sterility of the $F_1$ progeny is greater than that of the irradiated parent (North 1975). Simulation models showed that released males with IS would suppress the wild population to a greater extent than the release of equal numbers of fully sterile males (Marec et al., this volume). In 1994, releases of irradiated codling moths were initiated in the Okanagan Valley of British Columbia, and continue with the objective of permanent area-wide population suppression (Dyck et al. 1993; Bloem and Bloem 2000; Simmons et al., this volume). Overall, codling moth populations have been reduced by 94% relative to pre-programme levels and damage to less than 0.2% of fruit in more than 90% of the orchards in the programme area (Nelson et al. 2021).

Another AW-IPM programme integrating the SIT, which has been implemented as a large-scale operational programme, is for the pink bollworm. Since 1967 sterile moths have been released in 0.4 million hectares of cotton fields in the San Joaquin Valley of California to prevent the establishment of this pest by moths migrating from southern California (Staten et al. 1993; Walters et al. 2000). This programme,

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largely funded by growers, has been key to the survival of cotton production in California. More recently, in conjunction with the extensive planting of *Bt*-cotton in the mid-2000s, the programme succeeded in eliminating this pest from south-west USA and northern Mexico (Staten and Walters 2021; Simmons et al., this volume).

The SIT is also being applied for continuous suppression of the false codling moth in citrus orchards in South Africa (Boersma 2021). It has also been applied successfully to eradicate outbreaks of invasive moth pests (Hendrichs, Enkerlin et al., this volume) such as the painted apple moth in New Zealand (Suckling et al. 2007) and the cactus moth in Mexico (Bello-Rivera et al. 2021). Extensive SIT/IS application on the gypsy moth was made in the past (Simmons et al., this volume). Furthermore, it is under development for application against a number of other moth pests (Vreysen et al. 2016; Simmons et al., this volume) including the African sugarcane borer in South Africa (Conlong and Rutherford 2017), the navel orangeworm in California, the European grapevine moth in Chile, and the carob moth in North Africa.

## 5. TRANSBOUNDARY SHIPMENT AND INTERNATIONAL RECOGNITION OF THE SIT

Over the years, the transboundary shipment of sterile insects across borders increased significantly, with cumulative numbers shipped reaching approximately 600 thousand million pupae by 2015 (see Appendix 3 on History of Transboundary Shipments of Sterile Tephritid Fruit Flies in FAO/IAEA 2017a). Also, mass-production of sterile insects has become semi-commercial or fully commercial in several situations (Loosjes 2000; Barnes 2007; Bassi et al. 2007; Everaarts 2016). Consequently, there were increasing demands for guarantees that sterile insects can be safely and legally shipped internationally, as well as for harmonized international regulations to facilitate this trade in order to reduce the need for independent development of national regulations that often hinder insect control programmes. However, even though sterile insects are non-invasive agents that leave no "ecological footprint" because of their sterility (contrary to insecticides or other measures that are toxic or pathogenic, or to most other biological agents whose establishment is irreversible once they are released), they were not formally accepted as biological control agents by the International Plant Protection Convention (IPPC) because they did not fit the IPPC definition of "reproducing" biological control agents (IPPC 1995).

In response to these requests, the FAO/IAEA Division organized a consultant's meeting to quantify transboundary movement of sterile insects and to assess their risks (they were found to be negligible) (see Appendix 4 on Transboundary Shipment of Sterile Insects in FAO/IAEA 2017a). Therefore, at the request of the FAO/IAEA, when the original International Standard for Phytosanitary Measures Number 3 (ISPM 3) was revised by the IPPC in 2005, sterile insects were included in the updated standard (IPPC 2017a). Consequently, as a result of the significant international recognition, positive track record, and innocuous character of the SIT, sterile insects were officially categorized among *beneficial organisms* by the IPPC to which most countries are parties. Furthermore, the terms "sterile insect" and

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"sterile insect technique" were formally included in the IPPC Glossary (IPPC 2017b):

> *Sterile insect*: An insect that, as a result of a specific treatment, is unable to reproduce [ISPM 3, IPPC 2017a]
>
> *Sterile insect technique*: Method of pest control using area-wide inundative release of sterile insects to reduce reproduction in a field population of the same species [ISPM 3, IPPC 2017a].

## 6. EPILOGUE

Krafsur (1998) noted that many scientists view the SIT and related genetic methods negatively (Whitten and Mahon, this volume). For example, Hargrove (2003) cast doubt on the potential value of the SIT for tsetse eradication. Especially academic researchers tend to be opposed to programmes they perceive as costly, and would rather see instead more funding of research at universities and research institutions. They are also critical of the scarcity of data clearly linking releases with induced sterility in wild females and population suppression. Krafsur (1998) identified the fundamental problem as follows:

> There is a paucity of published data that relate sterile male releases to population suppression. It would lend much credibility to the efficacy of SIT if sterile mating frequencies were estimated in challenged populations. Numerous models have been constructed that relate sterility and genetic deaths to population density but few were tested with field data. Studies in which target population dynamics are evaluated in terms of sterile mating rates and other covariates are badly needed. In this way, useful mathematical models could be made and tested with actual data.

Nevertheless, in the screwworm programmes, sterile and fertile egg masses were collected routinely in the Sanibel Island, Curaçao, and Florida programmes, and these data were related to target population decline (Baumhover 2002). Vreysen et al. (2000, 2018) described a clear connection between the release of sterile tsetse males and induced sterility in wild females, and between induced sterility and subsequent population decline in the field. Also Rendón et al. (2004) established this relationship on a large scale for the Mediterranean fruit fly.

Regarding lepidopteran pests, North and Snow (1978) confirmed the interaction of released irradiated moths and the wild population by capturing wild females, allowing them to oviposit, dissecting them to determine whether or not the spermatophores were coloured with the dye from the rearing medium, and examining their progeny cytogenetically for chromosomal aberrations. Others have adopted this approach for other species (Simmons et al., this volume; Marec et al., this volume; Vreysen, this volume). All these data confirm the relationship between sterility and pest suppression, yet much remains to be done to further address the paucity of field data identified by Krafsur.

AW-IPM programmes that integrate the SIT serve a very useful role in focusing attention on the need for area-wide strategies in managing major pest problems (Klassen and Vreysen, this volume). They are difficult to manage (Dyck, Reyes Flores et al., this volume), but those that exist have shown that the conventional IPM

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programmes tend to be conducted on too small a scale for optimum efficiency, and often employ insecticides in a way that destroys natural enemies.

Progress in developing and implementing the SIT for AW-IPM programmes has been slow in some cases but rapid for others (such as fruit fly pests). The SIT is being or has been practised on an industrial and area-wide scale against the New World screwworm, Mediterranean fruit fly (and other fruit fly species, e.g. *Anastrepha ludens, A. obliqua, Zeugodacus cucurbitae, B. dorsalis, B. tryoni*), pink bollworm, false codling moth and codling moth (Hendrichs 2000; Hendrichs and Robinson 2009). Mass-rearing facilities in several countries produce several thousand million sterile Mediterranean fruit flies per week. Currently, the infrastructure to rear the pink bollworm is being adapted for the production of the navel orangeworm for the suppression of this pest in almond and pistachio orchards in California. The codling moth programme in Canada has weathered major concerns (Myers et al. 1998), and has significantly reduced insecticide use (local pesticide sales indicate a 96% reduction since 1991 in the amount of active ingredient used against the codling moth) (Nelson et al. 2021). The small onion maggot programme releasing sterile flies in The Netherlands survives, even without authority for a mandatory area-wide application, and the citrus production areas in South Africa that are under the false codling moth programme continue to expand. The African Union is attempting to facilitate the integrated use of the SIT to establish tsetse-free zones in selected areas (Feldmann et al., this volume). The SIT has been shown to be valuable in dealing with certain transboundary pest problems, and in eliminating outbreaks of invasive pests (Hendrichs, Enkerlin et al., this volume), as demonstrated again by the recent eradication of a large Mediterranean fruit fly outbreak in the Dominican Republic (Zavala-López et al. 2021).

Programmes continue to receive research support, resulting in improving technologies and thus in enhanced cost-effectiveness for all aspects of the SIT (Abd-Alla et al. 2013; Beier et al. 2014; Bourtzis and Hendrichs 2014; Ready and Feldmann 2014; Vreysen et al. 2014, 2016; Scott et al. 2017). This includes improved colony management, strains and sexing systems (Augustinos et al. 2017; Sánchez-Rosario et al. 2017; Franz et al., this volume), and better mass-rearing, sterilization, shipment, holding and release systems (Bakri et al., this volume; Dowell et al., this volume; Parker, Mamai et al., this volume), a better understanding and management of pathogens and symbionts in mass-rearing (Abd-Alla et al., this volume; Augustinos et al., this volume), post-factory treatments to improve sterile male performance based on the integration of semiochemicals, hormones and symbionts (Pereira et al. 2013; Pereira et al., this volume), and the incorporation of population genetics, GIS, satellite imagery, information technologies and habitat analysis in project planning and implementation (Bouyer et al., this volume).

Finally, the accelerating development and application of modern biotechnology tools are being exploited to develop self-sustaining as well as self-limiting approaches. While the former aim at population replacement strategies using gene drive or *Wolbachia*-based approaches, SIT-based control systems are self-limiting strategies that aim at leaving no “ecological footprint” (Häcker et al., this volume).

Molecular technologies have already been applied to create sterility and sexing strains, and to introduce stable markers for population monitoring purposes (Bourtzis and Hendrichs 2014). Several such strains have undergone an initial

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evaluation under mass-rearing scenarios or in open-field trials, although their wider adoption has so far been slow because of low public acceptance of transgenic approaches, and the regulatory requirements and approvals that are required in most countries for their application (Häcker et al., this volume).

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# CHAPTER 1.2.

# MISCONCEPTIONS AND CONSTRAINTS DRIVING OPPORTUNITIES

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## SUMMARY

In theory, the sterile insect technique (SIT) is applicable to a wide variety of invertebrate pests. However, in practice, the approach has been applied successfully to only a few major pests. Chapters in this volume address possible reasons for this discrepancy, e.g. Klassen et al., Lance and McInnis, and Hendrichs and Robinson. The shortfall between theory and practice is partly due to the persistence of some common misconceptions, but it is mainly due to one constraint, or a combination of constraints, that are biological, financial, social or political in nature. This chapter's goal is to dispel some major misconceptions, and view the constraints as challenges to overcome, seeing them as opportunities to exploit. Some of the common misconceptions include: (1) released insects retain residual radiation, (2) females must be monogamous, (3) released males must be fully sterile, (4) eradication is the only goal, (5) the SIT is too sophisticated for developing countries, and (6) the SIT is not a component of an area-wide integrated pest management (AW-IPM) strategy. The more obvious constraints are the perceived high costs of the SIT, and the low competitiveness of released sterile males. The perceived high up-front costs of the SIT, their visibility, and the limited private investment (compared with alternative suppression measures) emerge as serious constraints. Area-wide SIT application is management intensive, requiring effective and dedicated management; insufficiently trained, funded or committed managers can negatively affect successful implementation. Failure to appreciate the true nature of genetic approaches, such as the SIT, may pose a significant constraint to the wider adoption of the SIT and other genetically-based methods, e.g. transgenic genetically modified organisms (GMOs). Limited support for the necessary underpinning strategic research also appears to be an important constraint. Hence the case for extensive strategic research in ecology, population dynamics, genetics, and insect behaviour and nutrition is a compelling one. Raising the competitiveness of released sterile males remains the major research objective of the SIT.

## 1. INTRODUCTION: CHALLENGE OF A NOVEL METHOD FOR AREA-WIDE INTEGRATED PEST MANAGEMENT

The 20th century witnessed at least four major advances in methods of suppressing pestiferous invertebrates. The two most ubiquitous of these, synthetic pesticides and biological control, did not require any prior conceptual revolution in scientific thinking for their development and adoption. Both approaches witnessed significant technical innovations, but, conceptually, each was essentially "more of the same". In contrast, the other two important approaches to pest management, autocidal/genetic control (including the sterile insect technique (SIT)) and, more recently, genetically modified organisms (GMOs), have emerged from a totally new worldview as to how each generation passes on the "instructions of life". No language could even come close to explaining these genetic approaches before the 20th century and the influence of Darwin and Mendel. They were simply beyond anticipation (Whitten 1985).

The existence of DNA, and just how these giant molecules might carry the heredity blueprints from one generation to the next as cryptic coded information, was completely outside the thinking of 19th century scientists. Advances in the discipline of genetics — the study of change during an individual's development, and variation between populations over space and time — became the hallmarks of 20th century biology. Not surprisingly, genetics and evolutionary concepts now impinge on many aspects of pest management. They shape our understanding of pesticide resistance and its management (McKenzie 1996), help us determine the specificity and safety of biocontrol agents (Whitten and Hoy 2000), and facilitate the design of novel genetic tactics for pest suppression such as the SIT (Knipling 1955, 1979) and chromosomal rearrangements (Serebrovskii 1940; Curtis 1985; Whitten 1985). They have also enabled researchers to identify toxin-producing, pesticide- and disease-resistant, and

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other relevant genes, along with the means to transfer such information to unrelated species, whether crop plants or insects (Häcker et al., this volume).

Unlike biological control or pesticides, both household concepts, an important corollary of the genetics revolution has been a need for the broader community to understand the nature of the hereditary material, and how we can manipulate it, to appreciate the risks and benefits of genetics-based approaches to pest control. Even then, some understanding of ecology and economics is required to put the basket of tactics into a realistic context. In comparison with other pest control tactics, it is difficult for stakeholders, without a basic level of knowledge, to make informed decisions about whether to support or oppose the SIT and transgenic GMOs. Herein lies the biggest constraint to the successful application of the SIT and other genetic means of pest suppression.

It is important that scientists and their institutions enter into meaningful dialogue with the ultimate beneficiaries of genetic approaches to pest control, particularly farmers and communities affected by insect pests and insect-borne diseases, but also the wider public. This is especially true in developing country-generated initiatives; and formal requests for area-wide integrated pest management (AW-IPM) programmes that integrate the SIT are often unrealistic, their implementation is poorly managed (including corruption in some cases), and they lack close interaction with local communities and other stakeholders (Dyck, Regidor Fernández et al., this volume; Dyck, Reyes Flores et al., this volume) (Box 1).

*Box 1. Unsuccessful Attempts to Deploy the SIT and Genetic Control of Mosquito-Borne Disease Vectors in India*

The value of achieving a joint understanding and ownership of genetic control tactics can be illustrated by reference to the unsuccessful attempts of the World Health Organization (WHO) and the Indian Council of Medical Research (ICMR) to develop novel genetic control tactics for three mosquito vectors of disease (malaria, yellow fever and filariasis) in rural India during the 1970s. This ambitious programme evaluated three approaches to genetic control, namely the SIT using either irradiation or chemosterilants, and chromosomal rearrangements (Davidson 1974). Initially, some common ground for this pioneering project was created when the general approach was described to rural communities as family planning for mosquitoes — a concept widely promoted for human population control by the Indira Ghandi Government. Unfortunately, the mosquito programme floundered when journalists, exploiting ignorance and prejudice, persuaded Indian politicians that the project was designed to serve foreign interests and not the national well-being. The programme was abruptly abandoned in 1975, to the chagrin and embarrassment of the WHO and ICMR, to the bewilderment of many, and, undoubtedly, to the lasting detriment for rural communities scourged by mosquito-borne diseases (Nature 1975; WHO 1976; Dyck, Regidor Fernández et al., this volume). There may be some parallels between the attempts for genetic control of mosquito populations in India and those for the eradication of some tsetse *Glossina* populations in Africa. These include the risks of using foreign aid to implement sophisticated technologies not adequately understood by the intended beneficiaries, with the expectation of eliminating a suite of disease vectors on a large area-wide basis covering different political entities and diverse ecologies.

A study on the efficacy, economics and social impact of genetically engineered *Bt*-cotton in China illustrates well the importance of technology users having a reasonable understanding of the available pest control tactics (Pemsl et al. 2003). A similar conclusion can be drawn about the evolution of resistance and its management, whether we are talking about pesticides, GMOs, or the SIT. Not surprisingly, in very few countries is the concept of resistance management understood and effectively

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applied (McKenzie 1996). Without doubt, a major challenge for proponents of genetic-based interventions is to secure from participating countries, their communities (especially in rural areas), and relevant stakeholders the prior informed consent, commitment and genuine ownership of the proposed programme (Dyck, Reyes Flores et al., this volume). Otherwise, misconceptions will arise, and serious constraints can emerge unnecessarily.

Finally, a brief case study of tsetse suppression/eradication in Africa illustrates how misconception and constraint can interplay to influence decision-makers on the feasibility of pest control options surrounding the SIT. Ironically, tsetse fly suppression/eradication, using sterile hybrids (Vanderplank 1947), is often cited as the first convincing field demonstration of autocidal control of an insect pest (Krafsur 1998; Klassen et al., this volume; Robinson, this volume). Yet, subsequent attempts at area-wide eradication of tsetse populations, first with chemicals, and then integrating the SIT, have achieved some success (Vreysen et al. 2000), but have also attracted controversy (Linear 1985; Rogers and Randolph 2002). For example, it is claimed that the pesticide campaign of the Food and Agriculture Organization of the United Nations (FAO), costing about USD 1000 million by 1985, increased rather than reduced the distribution of tsetse (Linear 1985). Important ecological aspects of the debate, such as the role of tsetse in "protecting" vast tracts of natural habitat from occupation by human populations with environmentally damaging cattle ranching, are not directly germane to this discussion. Nevertheless, Nagel and Peveling (this volume) state that new areas are opened up to cultivation as a result of rapidly increasing human population pressure in sub-Saharan Africa, independent of tsetse presence or absence. However, other criticisms levelled by Linear, and partly revived more recently (Rogers and Randolph 2002), at SIT-based tsetse programmes, are more pertinent (Box 2).

## 2. SOME COMMON MISCONCEPTIONS

### *2.1. Are Irradiated Insects Radioactive?*

During the early development of the SIT, chemosterilants were assessed but abandoned, mainly because of concern over the release of sterile insects with residues of non-specific chemosterilants into the environment (Lance and McInnis, this volume; Robinson, this volume). All current and proposed programmes that release sterile insects use Co-60, Cs-137, or X-rays as the irradiation source (Bakri et al., this volume). Is there a similar risk that irradiated insects contain harmful residual radiation?

A study of the irradiation of foodstuffs, where irradiation levels are much higher, dispels this fear. The conclusion of all the studies on irradiated foods for human or animal consumption indicates that there are no levels of residual radiation to give cause for concern. The issue of greater concern was related to the production of biologically active compounds that could be harmful. Again, this concern has been dismissed as insignificant.

Is irradiated food radioactive? The process of irradiating food is regulated and designed to ensure that that is not the case. The radiation sources used to irradiate food

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and agricultural products (including insects) are restricted to: gamma rays from the radionuclides Co-60 and Cs-137, and accelerator-generated radiation either as 10 million electron volt (MeV) electrons or 5 MeV X-rays (FAO 2003). Also, it is important to realize that food (non-irradiated), and any natural substance, contain natural radioactivity that can be measurable. Therefore, it is important to put in perspective the issue of possible induced radioactivity in food and natural radioactivity in food. In a document published by the IAEA (2002a), all relevant data generated over the last 40–50 years, and various nuclear mechanisms that can produce radioactivity, were analysed, and it was concluded that:

> . . . the increase in radiation background dose from consumption of food irradiated to an average dose below 60 kGy with gamma rays from Co-60 or Cs-137, with 10 MeV electrons, or with X-rays produced by electron beams, with energy below 5 MeV, is insignificant. It is best characterized as zero.

*Box 2. Can Tsetse Species be Suppressed/Eradicated by the SIT?*

The debate on the potential of the SIT to suppress/eradicate large tsetse populations indicates a mix of misconceptions, and possibly some significant constraints. These include:

- The SIT is highly technological (a misconception driven by inadequate understanding, but to the extent that this is true, the quality of its implementation, which is essential for successful SIT application, can be negatively affected by insufficiently trained or paid managers).
- At least 31 species and subspecies of Glossina are disease vectors, with various mating barriers between taxa. (In fact, most tsetse taxa are not economically important, and are restricted to rain forests, having little contact with humans or domestic animals. In Kenya, for example, elimination of populations of G. pallidipes Austen would eliminate the entire agricultural problem. In Ethiopia, two species are important — G. pallidipes and G. morsitans ssp.).
- The complexity and difficulty of the problem — with 10 million km2, more than 30 countries infested, and multiple taxa — pose immense logistical, financial and coordination problems, especially if eradication is the objective. In fact, only selected populations in priority areas at the edge of tsetse belts are currently being targeted (Feldmann et al., this volume).
- The possibility exists that a neighbouring non-target tsetse species would invade an empty niche following the application of the SIT. This could apply to G. pallidipes and G. morsitans, which are sometimes sympatric.
- Expensive pre-release field studies are required to determine species presence, distribution and abundance (part misconception, and probably an overstated constraint).
- Target tsetse populations are often remote from human population centres (a logistical challenge?).
- Multiple-mating of females, thereby possibly reducing the effective impact of sterile males, is largely a misconception (section 2.3.).
- Sterile tsetse males can act as disease vectors, and the argument of "net benefits" may not suffice (in fact, steps are routinely taken to prevent males from acting as disease vectors (Feldmann et al., this volume; Nagel and Peveling, this volume).
- Pre-release tsetse population reduction measures, e.g. pesticides and trapping, are required (integration with other pest management approaches could be seen as a strength).
- The SIT can be prohibitively slow and costly, and large up-front costs presupposing eradication represent a constraint. However, the low economic returns from animal traction, livestock production and other benefits resulting from eradication are a misconception as shown by the major benefits deriving from the eradication of Glossina austeni Newstead from the island of Unguja in Zanzibar (Feldmann et al., this volume).
- Failure to eradicate could represent a zero return on a substantial investment, although temporary suppression is also the goal of other tactics.
- Technological complexities and poor economics create difficulties in obtaining sponsorship (a constraint).

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In recent years the use of irradiation for protecting foodstuffs to meet quarantine and sanitation requirements has become more mainstream without adverse incident (Heather and Hallman 2008). For example, for years all guava exported from Mexico to the USA is irradiated (The Packer 2011), and honeybee hives, infected with bacterial diseases or arthropod pests, are routinely commercially decontaminated with gamma radiation, reducing the need to destroy valuable equipment (Titěra 2009; Queensland Government 2018).

The above facts about food irradiation and related applications can be extended to insect sterilization procedures, since the same sources of irradiation are used (Co-60, Cs-137, electrons or X-rays). Furthermore, insect sterilization for sterile insect releases requires a much lower radiation dose than for food irradiation. In food irradiation, the dose varies from about 100 Gy to 60 kGy, depending on the end objectives. In insect sterilization, the dose is in the range of only 100–300 Gy, depending on the insect and irradiation conditions (Hallman 2000; Bakri et al. 2005; IDIDAS 2018; Bakri et al., this volume). Thus, the induction of radioactivity in insects irradiated for programmes that release sterile insects can also be "best characterized as zero" (Elias and Cohen 1977; Terry and McColl 1992; IAEA 2002a, b).

Given the low levels of irradiation given to insects, the small biomass of the released insects and their wide dispersal, there are no plausible grounds for concern about residual radioactivity, or radiation-induced toxins, in released insects. Conversely, one might argue that the SIT is more environment-friendly than some alternative suppression tactics such as synthetic pesticides or augmentative or classical biological control. Pesticide contamination of foodstuffs, exposure and poisoning of workers, and environmental damage are often very real issues, particularly in developing countries (EJF 2002, 2003). The absence of any collateral damage following directly from the release of radiation-sterilized insects into the environment is indeed one of the strengths of the SIT, compared with chemical control, biocontrol or even GMOs. As with GMOs, concern about the process, and not the product itself, is sometimes the basis for apprehension. It would be very unfortunate if this misconception about the SIT ever became the reason for an otherwise viable AW-IPM programme not being implemented.

### *2.2. In What Sense Are "Sterile Insects" Sterile? Is This a Misnomer or a Misconception?*

In terms of physiology and behaviour, male insects "sterilized" by gamma radiation are not sterile (Robinson, this volume). Indeed, they are expected to generate viable and functional sperm capable of fertilization, to produce a full complement of seminal fluids, and also to enjoy undiminished libido. The population-suppressing impact of released sterile males is realized via radiation-induced dominant lethals that are lethal in the next or subsequent generations. By way of contrast, irradiated females may produce fewer or no eggs, and their behaviour may well differ in other critical ways from normal non-irradiated females. Thus "conventional" sterility is often induced in females, but that is of no consequence in many programmes that release only sterile males.

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In species where females multiple-mate and store sperm in spermathecae, it is important that irradiated sperm from sterile males are competitive with non-irradiated sperm in fertilizing eggs. In species where female receptivity is normally terminated after the first mating, again it is important that sterile males induce this behaviour. In species where it is predominantly sperm from the last mating that fertilize egg batches, it is important that sterile males are equally capable of maintaining this outcome.

The above considerations have become important since recent advances in biotechnology and molecular biology have suggested alternative means of sterilizing males (Häcker et al., this volume). For example, the use of gene-silencing techniques (Nowak 2003) to "switch off" a gene essential for sperm development could well induce true sterility. However, unless the male accessory gland product that "switches off" female receptivity is transferred during mating, these techniques would only serve to create a situation where the released males had little or no impact on the target population (Hendrichs and Robinson, this volume).

By way of contrast, Gould and Schliekelman (2004) suggested a range of novel molecular approaches to sterility that cause lethality in the progeny of released males. Such approaches are unlikely to encounter problems in generating the envisaged impacts. However, as stressed by Gould and Schliekelman, it is important that molecular biologists remain in dialogue with field entomologists so that practical outcomes are optimized. These authors noted that closer collaboration existed during the early period of SIT and autocidal programmes than often exists today between laboratory-based researchers and field ecologists, especially with the latter now an "endangered species" in many countries. Burt (2003) discussed some recent advances in molecular biology, with far-reaching and possibly profound implications for genetic engineering or suppression of natural populations. These revolutionary ideas address the potential of removing the need to mass-rear or irradiate insects before releasing engineered individuals into target populations. On the other hand, such self-sustaining approaches have the disadvantage that they aim at leaving an irretrievable "ecological footprint", require regulatory approvals, and often face public opposition to the release of transgenic insects (Häcker et al., this volume). Furthermore, unlike radiation, which is a random physical process, molecular approaches include the likelihood of resistance development (Hendrichs and Robinson, this volume).

### *2.3. Is It Essential That Females Mate Only Once?*

Conceptually and operationally, it is simpler if females of the target species mate only once. Initially, E. F. Knipling thought that monogamy was desirable, but later realized that it was not of central importance (Knipling 1955, 1979; Klassen and Vreysen, this volume; Lance and McInnis, this volume). Actually, monogamy can be a significant constraint where migration introduces inseminated females from outside the sterile-male-release area (Barclay, this volume).

In species where females are monogamous, it is essential that sterilized males evoke the same response as normal males during mating to terminate further receptivity by the inseminated female. The competitiveness of the released male is then measured by the difference between the two ratios — sterilized males to normal males versus field females that have been inseminated by the two classes of males.

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Multiple matings complicate the situation, but do not disqualify a pest from being a candidate for the SIT. In the latter case, the issue centres around sperm viability and competitiveness in fertilizing eggs, and the competitiveness of sterile males for second or subsequent matings. However, for tsetse flies *Glossina* spp. (Curtis 1968), females mated first to an irradiated male, and then to a non-irradiated male, were less than 50% fertile, just as the reverse order of mating produced greater than 50% fertility. Curtis also addressed another special problem with a viviparous female containing a mix of dominant lethal and normal sperm — an embryonic death might affect the time of the next ovulation. It turned out that this is only a minor problem, and, in the event of an early embryonic death emptying the uterus, the time before the next ovulation is advanced only from the normal 9 to 7 days.

### *2.4. Should Released Insects Be Fully Sterile?*

There is little doubt that released females should be fully sterile — not so much for ecological or operational reasons, but for good public relations and to avoid potential litigation or compensation claims. Fortunately, for the majority of species of Diptera against which the SIT is deployed, females require a lower dose than males for complete sterilization, although for a few groups of insects this is not true (Bakri et al. 2005). However, where suppression and not eradication is the objective, males need not be as sterile, especially if the higher dose required for complete sterility significantly reduces competitiveness (Toledo et al 2004; Bakri et al., this volume; Lance and McInnis, this volume; Marec et al., this volume; Robinson, this volume). The objective of the SIT, like any genetic control stratagem, is to impart a genetic load to the target population. If that can be imparted more efficiently through the release of fewer but more competitive males, that are not fully sterile, then that must be the favoured strategy.

### *2.5. Does SIT Application Require Isolated Populations or an Area-Wide Approach?*

The SIT, and genetic control methods (as well as pheromone-based mating disruption), are generally sensitive to the effects of immigration, particularly the incursion of females already mated to wild-type males, e.g. studies on compound chromosome strains of *Drosophila* sp. (McKenzie 1976, 1977). In this situation, monogamy can be disadvantageous since an immigrant inseminated female, immune to the abundance of sterile males, can produce fertile offspring (Barclay, this volume). Apparently, the immigration of inseminated females was the cause of only limited success in a large-scale trial on *Culex quinquefasciatus* Say (then known as *C. fatigans* Wiedemann) conducted by the WHO/ICMR in villages in India during the 1970s (Curtis 1976). Immigration was also a negative factor during trials against *Anopheles albimanus* Wiedemann in El Salvador. In one trial an isolated population was eliminated (Lofgren et al. 1974), but in a larger trial immigration reduced the efficacy (Dame et al. 1981), and suppression was achieved only after increasing the release rate of sterile males (Benedict and Robinson 2003).

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The negative impact of immigration on the efficacy of the SIT can be countered by applying the technique on an area-wide basis (Hendrichs et al. 2007; Klassen and Vreysen, this volume). For a newly established pest population, such as a recent introduction of an exotic pest, the target area is much less extensive than for an endemic and widespread pest. However, the releases need to extend beyond the target area, taking into account known distribution, habitat suitability, and the pest's dispersal capacity (Barclay et al. 2011).

There would be very few situations where it would be appropriate for a single beneficiary, e.g. owner of a single orchard or farm, to use the SIT independently (Klassen and Vreysen, this volume). Area-wide approaches require organization, coordination, and importantly, financial backing. To provide those resources, the imposition of a levy on stakeholders/producers may be considered (Dyck, Reyes Flores et al., this volume). However, in many situations, some individuals would prefer not to contribute to area-wide control, either because of a perceived loss of independence or a preference for alternative measures (Krafsur 1998). Often the actual or potential beneficiaries of the SIT are farmers, a group that traditionally is independent-minded; and it is inevitable that some would robustly resist making mandatory payments.

It is self-evident that populations of organisms on "islands", either physical (surrounded by water or mountains) or ecological (a patchy distribution of suitable habitat), are isolated to varying degrees from gene flow (Krafsur and Ouma, this volume). The "island" concept might include urban mosquito populations (where the target species is absent in the surrounding countryside) as a form of ecological island population; their suppression or eradication would benefit many people (Curtis 1976). Such situations present attractive targets. Isolation ensures that the method is at its most economical. However, while long-distance dispersal of the target pest is a disadvantage in any programme releasing sterile insects, imposing an area-wide approach and "barrier zones" can, in suppression programmes, accommodate certain levels of immigration (Hendrichs, Vreysen et al., this volume). If barrier zones are "reasonable", and the costs and benefits remain favourable, physical barriers to gene flow have not proved to be absolutely necessary.

In terms of an area-wide approach to release strategies and impact, an interesting contrast can be made between biological control and the SIT (Hendrichs, Enkerlin et al., this volume). Box 3 illustrates the enormous regenerative powers of some insect species when colonizing a vacant niche.

### *2.6. Is Eradication the Only Objective When Using the SIT?*

The term "eradication" may be interpreted in a variety of ways. For example, for the World Health Organization, it necessitates the removal of every last individual of a species on the earth (Heymann 2006). Range-wide species extinctions are a natural, indeed inevitable, consequence of the evolutionary process (Wilson 1992). Mankind has been responsible for many accidental, but few deliberate, "eradications".

On the other hand, eradication at the population level, such as presented by Newsom (1978), is commonly used in plant protection:

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> Eradication is the destruction of every individual of a species from an area surrounded by naturally occurring or man-made barriers sufficiently effective to prevent reinvasion of the area except through the intervention of man.

Newsom (1978) argued (with some cause given the containment mode of the programme at the Mexico-USA border at the time) that eradication of the New World screwworm *Cochliomyia hominivorax* (Coquerel) in the USA using the SIT had been an "abysmal failure". However, there should be little argument now, using Newsom's own definition, that populations of the New World screwworm have indeed been eradicated, not only from the USA, but also from Mexico, and all of Central America (Hendrichs, Enkerlin et al., this volume; Hendrichs, Vreysen et al., this volume; Vargas-Terán et al., this volume).

*Box 3. Comparison between the SIT and Classical Biological Control for Area-Wide Control of a Pest*

Both pest management tactics require an area-wide impact to be practical, but operationally, the procedures to achieve this outcome can be quite different. For example, in August 1996 the South African tephritid bitou bush seed fly *Mesoclanis polana* (Munro) was released at two sites, some 300 km apart, along the eastern seaboard of Australia to control seed production by the invasive weed, bitou bush *Chrysanthemoides monilifera rotundata* (L.) (Edwards et al. 1999, 2009). In total, 124 adults of mixed sexes were liberated (67 at one release site, 57 at another). By October 1998, the species had occupied virtually the entire range of bitou bush, a distance of over 1200 km (Edwards et al. 1999), establishing vast populations in the process. Thus, within 2 years, these two miniscule founding populations displayed exceptional rates of dispersal, located and colonized scattered and quite isolated pockets of bitou bush, and in the process traversed vast stretches, even cities like Brisbane, that contain no host plants. In May 2004, a survey of six sites, along 700 km of the New South Wales coastline, revealed that 98% of all flowerheads were attacked by *M. polana*, with a mean of 12 eggs laid in each (P. B. Edwards, personal communication). It would be a sobering thought to contemplate the effort and efficacy of rearing and releasing sufficient sterile males of *M. polana* along the eastern coastline of Australia to reverse the phenomenal outcome achieved by the bitou bush seed fly following its release. Yet, we have no reason to doubt a similar or even greater dispersal and reproductive capacity of an invading pestiferous tephritid such as the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) which, unlike the bitou bush seed fly, is polyphagous with an enormous host range. One is a beneficial insect, the other a pest, but only because of the economic status bestowed by their hosts.

Where repeated immigration of fertile females occurs, either natural or human-made, eradication may need to be followed by preventive releases in areas of high risk of pest incursions (Hendrichs, Vreysen et al., this volume). The recurrent invasions of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) into California represent such a situation (Krafsur 1998). The preventive release for 25 years of sterile flies over the Los Angeles basin, where most incursions occur, has avoided the establishment of the Mediterranean fruit fly in the face of repeated human-assisted incursions (Dowell et al. 2000). Although Carey et al. (2017) claim that there are established Mediterranean fruit fly populations at "undetectable levels" in California, the validity of the concept that populations with a high reproductive rate can remain undetectable for years under favourable host and climatic conditions has not been accepted by trading partners, and has been challenged by McInnis et al. (2017) and Shelly et al. (2017). Thus, in such situations, the SIT is an effective preventive, rather than an eradication, tool to maintain the pest free status (Dowell et al. 2000; Hendrichs, Enkerlin et al., this volume).

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A final example of accommodating the objectives of an AW-IPM programme integrating the SIT, by recognizing immigration, is the pink bollworm *Pectinophora gossypiella* (Saunders). The cotton-growing areas of the San Joaquin Valley, California, were protected for decades from infestation by the pink bollworm through the implementation of an AW-IPM programme in which the SIT was a key component in containing the pest. The SIT also played a key role in the subsequent eradication of the pink bollworm from the USA and northern Mexico (in combination with growing transgenic cotton and the use of pheromones for mating disruption) (Staten and Walters 2021).

The eradication of an established population of a pest species is a challenging objective. Even deciding whether or not to attempt eradication is not a simple matter. Myers et al. (2002) stated that:

> . . . evaluation of the ecological and economic costs and benefits of removing an exotic species is difficult, and often the benefits are exaggerated and the costs are understated.

Nevertheless, there is no doubt that, in the case of incipient outbreaks of invading non-native pests, eradication is the most cost-effective alternative when compared with allowing the permanent establishment of a new pest that will have to be suppressed continuously. Furthermore, in view of its inverse density-dependence, the SIT has been shown to be particularly suited to eliminate invasive pest populations in an environment-friendly way (Hendrichs, Enkerlin et al., this volume).

While the SIT does become more effective as the target population dwindles and the ratio of sterile to fertile males increases, this positive attribute of the SIT should not condemn it to be exclusively an eradication option. Krafsur (1998) noted:

> . . . because SIT can lead to eradication, anything less may come to be judged a failure.

In this sense, the necessity of using the SIT only for eradication is clearly a misconception.

Frequently, other strategic options, such as containment, prevention and suppression, rather than eradication, are the objective of applying the SIT (Hendrichs, Vreysen et al., this volume). The objective of suppression, instead of eradication, is often necessitated by the undesirability of establishing quarantines, the lack of isolation resulting in unacceptable levels of immigration, as well as other factors. In this common situation, the integration of the SIT into AW-IPM programmes, such as those for several moths and fruit flies, demonstrates that the SIT can be an important component of such suppression programmes (Hendrichs, Vreysen et al., this volume).

### *2.7. Is the SIT a Stand-Alone Technology or Part of an Area-Wide IPM Strategy?*

The SIT is not a stand-alone technology, and thus it has to be integrated in various degrees with other control tactics (Simmons et al., this volume; Mangan and Bouyer, this volume). Even in the North American New World screwworm regional eradication programme, there has been some integration with other suppression measures, such as dressing infested wounds with larvicides, mobilizing ranchers to report cases of infestation, as well as cultural and quarantine activities (Klassen et al., this volume; Vargas-Terán et al., this volume). In contrast, all the various fruit fly

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programmes that release sterile flies range from area-wide suppression to eradication; the SIT for fruit flies is integrated with baits, lures, and cultural practices (Enkerlin, this volume; Klassen et al., this volume; Mangan and Bouyer, this volume).

One impressive example of integrating the SIT into AW-IPM, where the goal is sustained suppression, is the Okanagan-Kootenay Sterile Insect Release (OKSIR) Program (codling moth *Cydia pomonella* (L.)) in British Colombia, Canada; it uses a "knowledge-based" approach (Simmons et al., this volume). This programme requires the cooperation of commercial fruit growers and property owners. The programme is funded through local taxation; it obtains 60% of its funding from property owners in the community and 40% from commercial growers (OKSIR 2018; Seymour 2018; Nelson et al. 2021). At various times the OKSIR has applied the following tactics (*Bt* sprays were deemed to be ineffective):

- Various tree-husbandry practices and field hygiene
- Legal authority to enter orchards and residential properties to inspect for infestation and issue control orders for fruit stripping and tree removal
- Initial chemical sprays to suppress a high pest population
- Initial sex pheromone mating disruption in some orchards
- Sex pheromone traps used for monitoring (about one trap per hectare)
- Twice-weekly sterile moth releases
- End-of-season banding of host trees (to trap mature larvae) in some orchards; later the strips are removed and destroyed).

Although eradication was the initial objective, no quarantines were implemented, and thus this is no longer deemed practical as a short-term solution. Instead, a sustainable programme has evolved, which has successfully implemented the revised objective to:

> . . . substantially reduce the amount of toxic pesticides currently used to control [suppress] codling moth, and that means a more competitive fruit industry and healthier local environment for everyone.

From 1991 to 2016 there was an estimated 96% sustainable reduction in insecticides used against the codling moth in the target areas (Nelson et al. 2021). Two important take-home messages emerge from the Canadian codling moth OKSIR Program: informed stakeholders are not hampered by misconceptions, and constraints are viewed as challenges to be met and overcome.

### *2.8. Could Eradication Have Deleterious/Unanticipated Consequences?*

Eradication of a pest population, through whatever means, could conceivably have unexpected consequences. If the species is exotic to the area, and the incursion is small, eliminating the invasive population will re-establish the original situation. However, at the other end of the scale, eradication of a widespread indigenous species, such as a tsetse or screwworm species, may lead to undesirable impacts that need to be considered (Nagel and Peveling, this volume). Myers et al. (2000) provided examples of "secondary" consequences of eradication, following the eradication of exotics from islands, such as the removal of vertebrates, rats, and rabbits. These authors point out that reversing the changes to native communities following the eradication of exotics

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is not a trivial undertaking, and requires a sophisticated ecological understanding. In general, however, the negative externalities of the SIT are less than those of most alternative pest suppression tactics, especially pesticides (Nagel and Peveling, this volume).

## 3. SOME CONSTRAINTS ON THE SIT

### *3.1. Changing Contextual Framework for the SIT*

In spite of the successes of significant AW-IPM programmes integrating the SIT against various moth and fruit fly species (Simmons et al., this volume; Enkerlin, this volume), the successful eradication of the New World screwworm in North and Central America (Vargas-Terán et al., this volume) remains a primary model, in terms of its size, economic importance and durability, for most SIT endeavours. Nevertheless, several changed economic and social circumstances, especially over the past 40 years, suggest that even this highly successful programme would encounter difficulties if it were launched today. Changing social, economic, and political circumstances have probably reduced the feasibility of implementing similar-sized programmes elsewhere in the world. In recent times, perhaps of greater relevance as successful SIT models, are the bi-national USA-Mexico pink bollworm programme, and the on-going Mediterranean fruit fly AW-IPM programmes in Latin America.

The successful North American screwworm programme has been characterized as having a pragmatic and opportunistic "can do" approach. For example, it adapted a "mothballed" hangar at a well-sited but unused air force base in southern Texas. This facility did not demand the construction and safety standards currently required of a similar facility. Today, in most countries, occupational health and safety issues, and general worker conditions, are more stringent, and remuneration is more substantial, than in the 1960s. Public liability, insurance premiums, and tendencies to litigate have increased enormously in the past 40 years. Such factors mean that the initial outlay costs for constructing and operating a mass-rearing facility are now much higher. Nevertheless, a facility to rear the New World screwworm has been built in Panama, and several fruit fly rearing facilities are being constructed.

The decline in primary-producer contribution to the gross domestic product (GDP) of many developed nations (now often less than 3%, according to the World Bank) has reduced the political influence of farmers (Europe, Japan, and the USA remain exceptions to this trend). This decline, together with a greater emphasis on the "user-pays principle", aggravates the situation. In cases where pest eradication is the principal objective, reinvasions due to the increased global movement of people, livestock and materials, and threats of bio-terrorism, all serve to reduce the prospects of a sound economic return on the not-insignificant initial outlay. Also, as mentioned earlier, large up-front costs, and the need for stringent quarantine measures that can interfere with trade and the movement of goods, may be serious disincentives to invest in the SIT for eradication of large, established populations. However, this is not the case for the application of the SIT to eliminate outbreaks of invasive pests (Kean et al. 2018; Hendrichs, Enkerlin et al., this volume).

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A general decline in financial support for relevant research and development (R and D), compared with that available to the US screwworm programme during the 1960s and earlier (Krafsur 1998), linked to some of the factors listed above, will often mean that less is known about future target populations.

The construction of multi-purpose mass-rearing facilities could lessen the impact of some of the above negativities, and provide significant economies (Fisher 2002). However, in turn, such centralized facilities create new problems, such as increased complexity in management and greater technical challenges relating to the competitiveness of released males. The logistics of material supplies for mass-rearing, and transport of sterile insects to release sites, are also likely to represent greater challenges. Certainly many of the above considerations would impact on risk-adverse decision-makers in Australia, New Zealand and Canada, and probably the European Union, regarding the wisdom of conducting area-wide eradication programmes based on the SIT (Vargas-Terán et al., this volume).

Realistically, if an area-wide campaign were to cover transboundary populations in diverse countries in a region like South-East Asia or Africa, the political, logistical, and ecological challenges would be significant. Such considerations have meant that, in Australia (for area-wide eradication of an existing pest such as the Australian sheep blow fly *Lucilia cuprina* (Wiedemann), or pre-emptive construction of a mass-rearing capacity to cope with an intrusion of the Old World screwworm *Chrysomya bezziana* (Villeneuve) (Vargas-Terán et al., this volume)), the SIT is effectively not on the agenda, in spite of policies to the contrary. Nevertheless, the SIT continues to be used as a rational approach to suppress several fruit fly related problems in Australia and elsewhere (Fisher 1996; Meats et al. 2003; SITplus Partnership 2018).

Compared with most other pest management tactics, the SIT is uniquely affected by another constraint — limited independent and robust experimental field data to evaluate the short- and long-term impact of released sterile insects on population dynamics. Information is often inadequate on key issues such as competitiveness, dispersal rates, and density dependence (Itô et al., this volume); however, data obtained on the Mediterranean fruit fly in Guatemala provide insight into pest population suppression and competitiveness in large-scale field programmes (Rendón et al. 2000, 2004). As Krafsur (1998) noted, many experimental SIT trials have striven to isolate and reduce a target population. A combination of suppression measures are often integrated to achieve this outcome (Mangan and Bouyer, this volume; Nagel and Peveling, this volume). Thus, frequently, according to Krafsur (1998):

> . . . the effects of sterile male challenge are confounded by other treatments.

In this sense, AW-IPM programmes that integrate the SIT, even highly successful ones like the New World screwworm programme in North America, are usually not designed to test hypotheses, but to achieve specific operational outcomes. While this strategy is understandable, it has a serious downside — sometimes little is learned from SIT successes (and failures) that could be applied to other pests.

In spite of the limitations imposed by changes in attitude and resources in the world, the SIT flourishes for species for which the technology is well established, such as the New World screwworm, and various moth and fruit fly pests.

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Some encouraging new developments include a revival of interest in the application of the SIT for *Aedes* spp. and *Anopheles* spp. mosquitoes (Touré et al. 2004; Bourtzis et al. 2016; Lees et al., this volume), and in addition two species of tsetse, *Glossina austeni* Newstead and *Glossina palpalis gambiensis* Vanderplank, have been added to the list of targets against which the SIT has been successfully applied (Vreysen et al. 2000, 2021). Also significant is the exploration of opportunities for commercially viable sterile insect production facilities (FAO/IAEA 2008) that hopefully will serve as examples and, in time, provide alternatives to government-funded and government-controlled programmes.

### *3.2. Is the SIT Too Expensive, or Does It Fail the "User-Pays" Principle?*

When control options in AW-IPM programmes are being assessed, whether to suppress or eradicate an existing pest population or deal with an incursion, the question of cost is often foremost. The cost of SIT strategies generally will include the following: expense for monitoring and some other form of control to reduce an initial high target population, and cost of purchasing and releasing the sterile insects. In many programmes, the cost of providing and releasing sterile insects comprises a high proportion of the costs. However, model inputs show that other costs may outweigh the cost of sterile insects (Mumford, this volume). For example, in the case of an eradication programme, proof of pest free status required by a trade partner may drive up trapping costs significantly.

In cases where a reliable and economic supply of sterile insects does not exist, the "deal breaker" need not be just the total cost of the sterile insects, but the substantial upfront investment needed to construct and equip a new production facility. The start-up time for establishing a colony and reaching sufficiently high production is also a factor. However, the risk can be somewhat spread at each step in the SIT-development pathway by collecting evidence to assess the merits of further investment so that it becomes an incremental process: if progress is not promising, withdrawal is possible along the way (FAO/IAEA 2008). For example, at first, small pilot projects are carried out to assess the feasibility of the approach and to validate the technology. Moreover, when planning a mass-rearing facility, this can follow a modular design as described by Tween (1987), where construction of new rearing modules around a central core only proceeds if the demand for sterile flies gradually expands.

Increasingly, the SIT has been used in suppression or seasonal control programmes in a manner similar to pesticides, e.g. codling moth management in Canada, Mediterranean fruit fly in Croatia or Israel, false codling moth *Thaumatotibia leucotreta* (Meyrick) in South Africa, and onion maggot *Delia antiqua* (Meigen) in The Netherlands. The onion maggot control programme is the only example of an ongoing long-term (over 30 years) suppression programme where growers pay a private company to monitor as well as produce and apply the sterile insects. This programme faced the challenge of those who would turn to the SIT only when other options had failed, and therefore the population of the pest was too high to achieve good results using the SIT (Loosjes 2000). Also, due to rotation of crops and some "free-riders", there is no coordination among growers or area-wide approach, and thus no accumulated benefit to the users of the SIT, only a season's benefit. Furthermore,

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early support from the Dutch government, in recognition of the enterprise as an environmental business, was terminated due to a change in policy. The loss of this funding eliminated any recognition of public benefits from the reduced use of pesticides, and also caused some users to wonder if the pull-out of the government reflected on the overall product or concept. In spite of these setbacks, the private supply of sterile onion maggots has been maintained, the surface treated has gradually increased to an aggregate area of ca. 10 000 hectares (40% of the onion production), and the programme continues to be successful (Everaarts 2016).

Several large-scale eradication programmes have been cost-effective. Without doubt, successful programmes such as the North and Central American screwworm programmes have proved to be enormous economic successes (Vargas-Terán et al., this volume). The same applies to the Mediterranean fruit fly containment programme that, for 40 years, has maintained Belize, Mexico, USA and ca. half of Guatemala free of this pest, with annual USD multi-thousand-million [multi-billion] benefits to the respective horticultural industries (Enkerlin et al. 2017). These successes have served to ensure a serious evaluation of other potential programmes. However, the problem of cost is more complex than just raising the substantial resources to construct and operate a mass-rearing facility, and conduct the release and monitoring activities. The risk of an eradication venture failing, with no return on investment, can daunt all but the hardiest of optimists. However, a suppression strategy can represent a viable fall-back position, as shown by the Canadian codling moth programme, and some other moth and fruit fly programmes.

One must keep in mind, in any case, that since the SIT does not entail any intellectual property rights (IPR) at this stage, there is little incentive for private enterprise to participate, other than as a "hired hand". Profits must come from the sale of services and sterile flies, as there is little scope for sale of assets of such a company compared with other types of businesses (Barnes 2007; FAO/IAEA 2008). However, in the case of long-term suppression, preventive or containment programmes, that continuously require sterile insects, the financial risk to private investors would be lower than in eradication programmes, where the demand terminates as soon as eradication has been achieved (FAO/IAEA 2008; Simmons et al., this volume; Enkerlin, this volume; Hendrichs, Vreysen et al., this volume).

It can be concluded, in terms of cost-effectiveness, that area-wide application of the SIT is not too expensive when all benefits are quantified, and beneficiaries better understand the technology and participate in the programme (Kruger 2021). In an increasingly "user-pays world", it is equally important to convince those who will capture the benefits to contribute resources in proportion to their benefits. Nevertheless, the most vexatious financial issue is ensuring that all those who capture the benefits pay equitably (Mankad et al. 2021). In suppression programmes, the beneficiaries often contribute to programme costs, e.g. the codling moth OKSIR Program in Canada, where growers and property owners cover all of them (Nelson et al. 2021; Simmons et al., this volume), or the Mediterranean fruit fly programmes in Latin America and South Africa where they pay part of them (Dyck, Reyes Flores et al., this volume; Enkerlin, this volume).

The actual public benefits have usually not been well-measured to date, with some exceptions such as the evaluation in Madeira of indirect impacts on reduction of pesticide use, preservation of cultural heritage, and support of tourism (IAEA 2005).

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Unintentional subsidies by government for the production of sterile insects used in third-party programmes will certainly discourage private investment in the sterile insect production sector (Bassi et al. 2007; FAO/IAEA 2008). This could lead to further use of government funds for the SIT, even in those cases where stakeholders are willing to pay their fair share of programme costs.

Much more problematic is the task of identifying the appropriate financial resources, and securing the necessary budget, for programmes that have wider benefits or where the beneficiaries could not afford the cost. For eradication programmes, often the only realistic source is probably the public purse — governments in developed countries, and support from international aid agencies in less-developed countries (Dyck, Reyes Flores et al., this volume). Even when the identity of beneficiaries is clear, if the benefits are not sufficiently appreciated, users are unlikely to pay. For example, in Australia, various attempts to develop a genetic control programme for the Australian sheep blow fly (Foster et al. 1993; Krafsur 1998; Klassen and Vreysen, this volume), either on the mainland or the island of Tasmania, were abandoned, either because the cost was deemed to be too high (Waterhouse 1962), or because it was difficult to obtain the necessary financial backing (King et al. 1992).

In conclusion, AW-IPM programmes that release sterile insects are often susceptible to the "user-pays principle", but many cases fall outside this approach. It will be just as important to establish the "public good" in privately funded programmes, and obtain partial support for that benefit in the future, as in the past it has been to identify and engage private beneficiaries in government-led programmes.

### *3.3. Adverse Population, Ecological, and Behavioural Parameters*

The suppression effects of flooding a natural insect population with sterile males depend on a suite of factors. Difficulty in adequately satisfying some of these could amount to a serious constraint in successfully applying the SIT to a particular pest. However, articulating such issues can sometimes transform a constraint into a challenge, and ultimately into an opportunity. Constraints and complementary opportunities can include:

- Large size of the natural target population versus methods of suppressing numbers before releases by other means (e.g. pesticides, mating disruption, sanitation and other cultural interventions), or simply exploiting seasonal fluctuations (Mangan and Bouyer, this volume)
- Migration from non-target populations versus choice of target area to maximize effective immigration barriers (Itô et al., this volume; Hendrichs, Vreysen et al., this volume)
- Low competitiveness of released insects (due to initial laboratory colonization, artificial diets, radiation and shipment damage, artificial colony management conditions over many generations, especially for adults and mature larval stages which may normally enjoy solitary conditions in the field) versus emphasis on quality control (and more natural colony holding, diet and rearing conditions), strain selection or regular injection of new genetic field material into mass-reared colonies, improving sterile male performance, and using probiotics and symbionts (Liedo et al. 2007; Pereira et al. 2013; Sánchez-Rosario et al. 2017; Augustinos et

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al., this volume; Bakri et al., this volume; Lance and McInnis, this volume; Parker, Mamai et al., this volume; Parker, Vreysen et al., this volume)

- Adverse impact of released females (sting damage from some fruit flies or biting behaviour of blood-feeding pests, or animal or plant disease-transmitting vectors) (Lees et al., this volume; Nagel and Peveling, this volume) versus genetic means of removing females, preferably early in the life cycle (Franz et al., this volume)
- Frequency-dependent factors affecting competitiveness (i.e. ratio of released to field males) versus release ratios that minimize this effect (Vreysen, this volume)
- Increased ability of field females to discriminate against released males versus exploration of colony management and mass-rearing conditions that mitigate this selection (Miyatake 1996, 1998), and regular strain replacement to minimize risk of discrimination (Hoffmann and Ross 2018; Lance and McInnis, this volume).

It is one thing to identify constraints and the complementary opportunities, but to turn these to our advantage requires long-term research. As noted by Krafsur (1998) and Gould and Schliekelman (2004), conditions for the necessary underpinning strategic research, both in terms of resources, expertise, and collaboration, were more ideal during the 1960s and 1970s. Resources and creative ideas are abundant in molecular genetics (Burt 2003; Häcker et al., this volume), but unless these are linked to field-oriented research in ecology and behaviour, the advantages are unlikely to be captured (Gould and Schliekelman 2004; Itô et al., this volume).

### *3.4. Mating Barriers — Serious Constraint on Programmes that Release Sterile Insects?*

For the SIT to work, sterile males must be competitive with the field males of the target population(s) of a pest in seeking mates. If we are dealing with a group of distinct species, or even subspecies with limited interbreeding, it is clear that each taxon will have to be tackled separately for progress to be made. This is especially true if the species in the complex are ecologically equivalent, and where a neighbouring taxon can be expected to occupy the vacated niche (Box 2). If the pestiferous species, perhaps vectoring the same disease, is already coexisting, then there is little to be gained by targeting one taxon with the SIT. In such cases, the specificity of the SIT could be a disadvantage.

The above constraint applies equally to cryptic species, where the situation could be more insidious if the mating barriers are undetected because the relevant biological knowledge is simply unavailable. Even supposedly well-known entities such as major malaria vectors, e.g. *Anopheles gambiae s.l.* Giles (White 1974), were found to harbour morphologically indistinguishable, but genetically distinct, species with important consequences for insecticidal suppression programmes (Paterson 1963). Conversely, raising taxa that are not genetically isolated to species status could unnecessarily discourage SIT practitioners (Hendrichs et al. 2015). To avoid both types of problem, it would be prudent to conduct the definitive test — determine if sterile males compete effectively with field males in the target populations (Parker, Vreysen et al., this volume). In his hybridization experiments, Vanderplank (1947) confirmed that this was the case in his pioneering work on autocidal control of tsetse

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(Klassen et al., this volume). Below several situations are described where this critical behavioural test was conducted, demonstrating that the SIT should, and did, work.

Based on morphological, cytological, and allozyme differences, Richardson et al. (1982) postulated that the New World screwworm in Mexico and the USA consisted of a number of sympatric, and generally non-interbreeding, populations. The existence of these "gamodemes" was hotly debated (LaChance et al. 1982), and the subsequent eradication of the pest from these regions argues strongly against the Richardson scenario. The question has been rendered irrelevant, now that all screwworm populations north of the Panama-Colombian border have been eliminated (Vargas-Terán et al., this volume). However, what is relevant is that the Richardson analysis could have been used to stop the successful expansion of the screwworm programme, and thereby denying farmers in North and Central America the enormous economic benefits that the SIT actually provided.

Equally important has been the direct-mating test in northern Africa, following the introduction of the New World screwworm into Libya in the late 1980s). Taylor et al. (1991) examined the "reproductive compatibility" of Libyan flies and the mass-reared strain. They also examined genetic variability (chromosome morphology, homolog pairing and m-DNA restriction-site analyses) of populations from Libya, the mass-reared strain, and extant Central American, Mexican, and Caribbean populations. Their analyses indicated that mating barriers should not prevent the eradication of the Libyan populations using "Mexican" sterile flies. Indeed, the successful programme proved to be one of the landmark achievements of the SIT (FAO 1992; Lindquist et al. 1992; Krafsur and Lindquist 1996).

A second example of research being conducted to ensure better mating and genetic compatibility (Feldmann and Ready 2014; Parker, Vreysen et al., this volume), and thus success of the SIT, is provided by the Old World screwworm. Given that the species is found over a large geographical range, possible incursions of this fly might be refractory to the SIT when using a colony established from a distant population. In response, samples of screwworms from southern Africa, the Middle East, and South-East Asia were subjected to various analyses — allozyme (Strong and Mahon 1991), cytological (Bedo et al. 1994), cuticular hydrocarbons (Brown et al. 1998), and laboratory hybridization tests (Mahon 2002a; J. P. Spradbery, unpublished data). No indication of discontinuities, that might indicate the presence of sibling species, was found. Subsequently, analyses of mitochondrial DNA (Hall et al. 2001) resulted in a similar conclusion. However, with the increased resolution of the technology employed, these authors detected the existence of two mitochondrial "races", one in sub-Saharan Africa and the other extending from the Persian Gulf to South-East Asia (Feldmann and Ready 2014). Thus, if the SIT were to be implemented in Australia, it would be prudent to establish a colony from either the introduced flies or the source population.

The absence of mating barriers could reasonably be assumed where the evidence suggests recent movements of an insect species around the globe, including the Australian sheep blow fly, Mediterranean fruit fly (Cayol 2000), the oriental fruit fly *Bactrocera dorsalis* (Hendel) (Hendrichs et al. 2015), and the codling moth (Taret et al. 2010).

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### *3.5. Evolution of Resistance to the SIT?*

Insects have shown a remarkable propensity to evolve resistance to chemical challenges used by man in efforts to suppress them. Some species, such as the house fly *Musca domestica* L., certain mosquitoes and whiteflies, are recidivists in that they have evolved resistance to a wide range of insecticides. With this background, it is not surprising that the question is sometimes asked, "Can insects evolve resistance to the SIT?"

Certainly, a species could "escape" if it was able to adopt asexuality. Perhaps only those species that had a pre-existing mix of sexual and asexual modes of reproduction could respond rapidly enough to make use of this mechanism. A sexual species would more likely be driven to extinction before the drastic genetic changes necessary for this process could occur.

A possible caveat to the belief, that an asexual response from an SIT-targeted population represents only a minor threat, is if the cytoplasmic symbiont *Wolbachia* sp. is already present in the species, and plays a role in the determination of sex and the sex ratio. *Wolbachia* is a rickettsia-like bacterium that is found in up to 76% of arthropod species (O'Neill et al. 1997). The presence of these organisms produces a wide range of effects, including conversion of genetic males into functional females, male-killing, cytoplasmic incompatibility, thelytoky, and also may have effects on viability (Bourtzis 2008). Since the effects of an infection with these cytoplasmically-inherited organisms can be so comprehensive, e.g. thelytoky in hymenopteran species (parthenogenesis where the population consists only of females), the implementation of a programme releasing sterile insects could cause the conversion of an apparently sexual species into an asexual one through the elimination of the uninfected, i.e. the sexual, portion of the population.

The dramatic effects of *Wolbachia* vary between groups of species, with perhaps conversion to thelytoky being an extreme effect. Thus, while consideration should be given to the possibility of a programme being thwarted by the effects of *Wolbachia* or similar symbionts, existing programmes have not encountered such problems. More often, *Wolbachia*-induced cytoplasmic incompatibility is being applied as a tool for vector population suppression in a self-limiting way, analogous to the SIT (known as the incompatible insect technique (IIT)) (Lees et al. 2015). However, female removal is critical for such *Wolbachia*-based IIT strategies to be sustainable, but existing mechanical separation is labour-intensive and does not result in the required complete elimination of females, and is therefore not practical for application on a large scale. On the other hand, combining the SIT and the IIT against vectors such as mosquitoes, where male-only releases are an absolute prerequisite, assures that any accidentally released females are sterile, while simultaneously benefitting from the pathogen interference properties of *Wolbachia*, thus avoiding any possibilities of disease transmission by such females (Bourtzis et al. 2016). Alternatively, *Wolbachia* can be used in a self-sustaining way as a means of "driving" desirable genotypes into populations, although such a population replacement is irreversible (Turelli and Hoffman 1999; Bourtzis et al. 2016; Robinson, this volume).

While an asexual response is considered unlikely, and there are certainly no examples of it, the form of resistance to the SIT more commonly considered (at least to be a possibility) is that females of the target population could evolve behavioural

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mechanisms to avoid mating with mass-reared sterile males. The argument for such a possibility has been put forward most strongly by Boesiger (1972), who considered it almost inevitable that, while early releases of sterile males might be highly efficacious, the survivors would be enriched for genotypes capable of avoiding sterile males, and thus selection for this ability (McInnis et al. 1996).

Through genetic divergence, or physiological changes associated with mass-rearing, there should be ample "markers" for field insects to discriminate against mass-reared insects, but the question remains, "Is it likely?" The likely outcome is that in eradication programmes the population will become extinct before resistance has evolved. On the other hand, in suppression or containment programmes, such selection is usually countered by certain levels of immigration of already mated females. Indeed, AW-IPM programmes integrating the SIT have been active for decades against both the Mediterranean fruit fly and the New World screwworm. To date there is no evidence that resistance has developed, and the SIT remains effective for both species (Krafsur 1998). Similarly, the SIT has been deployed against other species as well, and again there is no evidence to suggest the evolution of resistance to the SIT (but note comments by Hendrichs and Robinson, this volume; Lance and McInnis, this volume).

A possible exception is a study on the melon fly *Zeugodacus (Bactrocera) cucurbitae* (Coquillett). Hibino and Iwahashi (1991) examined the mating behaviour of the melon fly using males from a long-term colony, and females from two islands in the Ryukyu Archipelago of Japan. Females from Okinawa Island (where SIT releases had been ongoing for some time) discriminated against mass-reared males, whereas females from Ishigaki Island, not yet exposed to SIT releases, did not. This finding was contrary to an earlier study performed before implementing the SIT, wherein Koyama et al. (1986) found that the mass-reared strain and those from Okinawa mated at random. Hibino and Iwahashi (1991) interpreted this as evidence that Okinawa females had "evolved sexual isolating barriers" to the mass-reared males (note comments by Itô et al., this volume).

Clearly, care should be taken to document any changes in behaviour and mating compatibility during the course of a programme releasing sterile insects. However, perhaps it is more appropriate simply to avoid the problem by maintaining the genotypic variation within the mass-reared colony as similar as possible to that of the target population (Hoffmann and Ross 2018). This can be achieved by "refreshing" the genetic variability within the mass-reared strain, such as the regular replacement of the mass-reared New World screwworm strain with field-derived colonies (Mangan 1992), or the regular infusion of genetic material from the target population into the culture (Parker, Mamai et al., this volume).

Recent advances in our ability to "cryopreserve" the embryos of recently colonized strains of species of economic importance where the SIT is either ongoing or could potentially be employed (Leopold et al. 2001), should facilitate this process. Mahon and Leopold (2002) reviewed the potential uses of this technique within programmes that integrate the SIT, one of which would be to prevent the "deterioration" of colonies through genetic changes brought about by long-term selection in mass-rearing. The suggested procedure is to cryopreserve batches of embryos as soon as a newly established colony becomes sufficiently laboratory adapted to be suitable for mass-rearing. After some generations, the mass-reared colony could be discarded and a

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fresh batch of embryos revived to re-establish the colony (Parker, Mamai et al., this volume).

### *3.6. Poor Competitiveness of Released Insects Can Be a Major Constraint*

In this volume, many chapters address aspects of "competitiveness": Bakri et al., Barclay, Hendrichs and Robinson, Itô et al., Klassen et al., Lance and McInnis, Lees et al., Marec et al., Parker, Vreysen et al., Pereira et al., Robinson, and Vreysen.

Competitiveness is an important feature of released insects. Poor competitiveness, when it occurs, contributes to an increased cost of the SIT, and cost is perhaps the major constraint to a more general application of the technique. Superficially, many of the costs of any successful AW-IPM programme that releases sterile insects may appear to be fixed, but the size of the required mass-rearing facility, and the costs of the diet and insect release operations, are all dependent on the competitiveness of the sterile males. Reduced competitiveness inflates the cost if, as occurs often, mass-reared and sterilized insects perform poorly in the field in terms of longevity, flight behaviour or ability to compete for mates. Consequently, the programme must commit to produce and release more insects than would be required if released insects were equal to field (wild) insects in their mating propensity and capability.

Lance and McInnis (this volume) suggest that the competitiveness (as defined by Haisch 1970 and Fried 1971) of Mediterranean fruit fly sterile males may in very extreme cases be less than 1%. This implies that more than 100 sterile males are required to equate to the mating behaviour of a single wild (fertile) male. Clearly, a slight improvement in competitiveness would have a dramatic effect on the costs and benefits of the SIT. Progress in developing semiochemical, nutritional, hormonal and other post-factory treatments to improve sterile male performance has been essential in addressing this major constraint (Pereira et al. 2013).

The low tolerance to fruit damaged by Mediterranean fruit flies, and the relatively low cost of insect production for the SIT, provide positive benefit/cost ratios for this species. However, for other species, this may not be the case. For example, Australian authorities are re-evaluating appropriate preparedness strategies to respond to a potential future incursion of the Old World screwworm (Vargas-Terán et al., this volume). In an SIT trial conducted in Malaysia (Mahon 2002b), the competitiveness of mass-reared and irradiated Old World screwworms was approximately 4%. A re-analysis of a similar trial in Papua New Guinea performed by Spradbery et al. (1989) yielded a similar value (R. J. Mahon, unpublished data). If competitiveness is calculated in this way, the merits of SIT integration could be questioned, but much depends on the programme costs and benefits in relation to alternative tactics that can similarly achieve eradication of the invasive population. Further work must be done to improve competitiveness, thereby ensuring that the costs and benefits of the SIT for this species remain positive (Parker, Vreysen et al., this volume). Unfortunately, increasing the number of sterile insects released (and hence the release:wild ratio) may not be the simple remedy for low competitiveness, especially if there is a negative relationship between the ratio of released:wild males and mating success, as suggested in field trials of sterile tsetse *Glossina palpalis gambiensis* (Rogers and Randolph 1985) and translocation-bearing males of the Australian sheep blow fly (Mahon 1996).

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## 4. CONCLUSIONS

The SIT has proved to be a robust and "green and clean" technology, yet the range of species to which it is presently being applied is relatively limited. The outstanding successes of the SIT with the New World screwworm in North and Central America, and in Libya (Vargas-Terán et al., this volume), as well as with the Mediterranean fruit fly and other species of fruit flies and moths in a number of regions of the world (for suppression, eradication, containment, or prevention) (Enkerlin, this volume; Hendrichs, Vreysen et al., this volume), raise questions about why the SIT has fallen short of the high expectations for the technology.

Some misconceptions are partly to blame but, in general, these seem to be more like irritants than real impediments. Two strengths of the SIT are its specificity, and the absence of negative externalities that haunt other pest management tactics such as synthetic pesticides and, to a lesser extent, classical and augmentative biological control. Concern about residual irradiation, or the production of toxic metabolites, is discussed primarily to dismiss this issue as lacking foundation. Other common misconceptions identified include: the need for monogamous females and for released males to be fully sterile; maintaining that eradication is the only possible goal; the SIT is too sophisticated for developing countries; and failure to recognize the SIT as a valuable component of area-wide IPM strategies. In each case, we concede that these warrant analysis, but conclude that each is not an impediment for a successful programme that releases sterile insects.

The identified constraints pose more serious threats to extending the SIT to other insect pests. In particular, we recognize that the SIT and other genetic methods of pest control are knowledge-based technologies. Thus, SIT application is complex and management intensive, requiring dedicated and effective managers to be successful (Vreysen et al. 2007; Dyck, Reyes Flores et al., this volume). Failure of the public to understand the nature of genetic material, and how interventions to disrupt the orderly inheritance or expression of this genetic information can be achieved, can introduce fear of the unknown. Financial considerations rank high on the list of constraints. The need for large upfront expenditure, risk aversion (failure to get any return on a failed eradication campaign), and rigid application of the beneficiary-pays principle in some countries without establishing the "public good" of such programmes, have proved to be serious disincentives for some potential SIT eradication projects that otherwise appeared to be feasible technically.

In the technical arena, the low competitiveness of released males is a serious constraint, especially if not given top priority by managers. Increasingly, detailed quality control manuals are available to assess carefully sterile male behaviour and performance in field cages and the field; also molecular tools and other markers have been developed that allow quantifying sterile and wild sperm presence in females of the target population (Juan-Blasco et al. 2013; FAO/IAEA/USDA 2019; Johnson et al. 2017).

Lack of support for the necessary underpinning strategic research also appears to be an important constraint. Hence, the case for extensive strategic research in ecology, population dynamics, density-dependent regulation, genetics, insect behaviour, and insect nutrition is a compelling one. Raising the competitiveness of released males remains the major research objective for the SIT.

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Exciting new developments in molecular biology are being exploited to develop entirely novel genetic tactics, both in support of self-limiting, such as the SIT, as well as self-sustaining approaches that aim at population replacement strategies using gene drives or *Wolbachia* systems (Häcker et al., this volume). Even if the latter do not depend on the release of large numbers of mass-reared and sterilized insects, with all the associated challenges, they have a higher likelihood of resistance development, require regulatory approvals, often face public opposition, and leave an ecological footprint.

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# CHAPTER 2.1.

# AREA-WIDE INTEGRATED PEST MANAGEMENT AND THE STERILE INSECT TECHNIQUE

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SUMMARY

Area-wide integrated pest management (AW-IPM) focuses on the preventive management of pest populations throughout the ecosystem. It seeks to treat all habitats of the pest population in space and time so that none produces migrants to re-establish significant infestations in areas of concern. In contrast, the conventional strategy focuses narrowly on defending the valued entity (crop, livestock, people, buildings, etc.) from direct attack by pests. AW-IPM requires multi-year planning and commitment, coordination among stakeholders, and an organization dedicated exclusively to its implementation, whereas conventional pest management involves minimal forward planning, tends to be reactive, and is implemented independently by individual producers, businesses, or households. AW-IPM tends to integrate advanced technologies, whereas the conventional strategy tends to rely on traditional tactics and tools. The sterile insect technique (SIT) is a species-specific form of birth control imposed to suppress or contain target pest populations, although it is a very powerful tool for "mopping up" sparse and isolated pest populations. On environmental, economic and biological grounds, in many situations the case for the SIT is compelling. While only some area-wide programmes integrate the SIT, deployment as part of an area-wide management system is essential for the SIT to be effective.

## 1. AREA-WIDE INTEGRATED PEST MANAGEMENT STRATEGY

The area-wide concept is a modernizing strategy of providing services efficiently wherever needed. Area-wide management takes advantage of the power of organization and coordination intelligently applied. In older societies, many services were provided on an individual basis by the family, e.g. collecting water and fuel, protection of the family, disposal of waste and sewage, etc., and independently of the activities of the neighbours. These services proved to be rather unreliable, ineffective, and were obtained at great individual expense. Reduced costs, better physical protection, and increased effectiveness were the drivers towards more effective delivery services. As a result, many of these services evolved from an individual to area-wide provision (Hendrichs et al. 2007). Today, many of these services, such as police protection, potable water, garbage and sewage removal, mail service, retailing, ambulance, fire protection, public health, electrical supply, high-speed transport, telephone, and internet services are being provided on an area-wide basis (Lindquist 2001; Hendrichs et al. 2007).

Area-wide approaches to deal with insect pests have ancient roots in coping with locust plagues and vector-borne diseases (Klassen 2000; Faust 2008). By systematic use of quarantines, Venice and Milan in 1347–1350 contained bubonic plague transmitted by the oriental rat flea *Xenopsylla cheopis* (Rothschild), and this approach was gradually adopted throughout Europe.

Area-wide integrated pest management (AW-IPM) came into a degree of prominence in the late 19$^{th}$ and early 20$^{th}$ centuries with the development of classical biological control (1888) (Caltagirone 1981), the use of pest-resistant plants (such as the grafting of all European grapes on phylloxera-resistant American rootstocks in the 1870s) (NAS 1969), campaigns to eradicate the gypsy moth *Lymantria dispar* (L.) in Massachusetts (1890–1901) (Liebhold et al. 2021) and *Boophilus* spp. cattle ticks from the southern USA (1906–1943) (Rutherford 2007), the suppression of yellow fever and malaria vectors in Havana, Cuba (1898) (Lechuga and Castro 2016) and in the Panama Canal Zone (1904) (Stern 2005), and the organization of mosquito-abatement districts, first in West Africa and then in several continents (1900) (AMCA 2004; Foley IV et al. 2021).

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The area-wide approach is one of several major strategies for coping with pest problems (Kogan 2000). Various pest management strategies have evolved in response to differences in the challenges presented by various pests (Hendrichs, Vreysen et al., this volume). The strategy for coping locally with a pest problem on an empirical basis gives only temporary alleviation. As methods of control, and guidelines for their use, are developed and refined, they form the basis of the conventional strategy of localized management of only fractions of pest populations. This is the most widely used strategy, and individual producers, businesses and households practice it independently and with no coordination with other stakeholders. However, in response to individual producers or households not being able to adequately meet the challenge of certain very mobile and dangerous pests, the need for total population management or an AW-IPM strategy emerged (Rabb 1972). Mobile pests include not only those that fly long distances, but also those transported passively on wind, water, or animal hosts, or in infested commodities traded locally or internationally.

Edward F. Knipling, the "father" of the sterile insect technique (SIT), was a strong proponent of the area-wide concept to manage many of the major pest problems, independent of the SIT (Klassen 2003). Therefore, area-wide management is often incorrectly associated with the SIT. However, while area-wide management is essential for effectiveness of SIT use, it applies equally to the effective implementation of other control tactics against many other pest insects that are not the target of SIT application. Table 1 lists examples of area-wide programmes not involving the integration of the SIT.

### 1.1. D*efining Area-Wide Integrated Pest Management*

For the purposes of this book, AW-IPM is defined as:

> IPM applied against an entire pest population within a delimited geographic area, with a minimum size large enough or protected by a buffer zone so that natural dispersal of the population occurs only within this area.

The common thread that runs through all AW-IPM programmes is the strong emphasis on the preventive control over space and time of foci of infestation from which recruits emerge to re-establish damaging densities of the pest population in areas of concern.

A few scientists have attempted to define the AW-IPM strategy. Knipling, as cited by Dickerson et al. (2001), stated:

> Area-wide pest management is the systematic reduction of a target key pest(s) to predetermined population levels through the use of uniformly applied control measures over large geographical areas clearly defined by biologically based criteria.

Lindquist (2000) wrote:

> An area-wide insect control programme is a long-term planned campaign against a pest insect population in a relatively large predefined area with the objective of reducing the insect population to a non-economic status.

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*Table 1. Examples of area-wide programmes to suppress or eradicate insect pests without integration of the SIT*

| Programme | References |
|---|---|
| Preventive anti-locust programmes in Africa, Asia and China — some coordinated by the FAO | Showler 1997, 2002; van Huis 2007; Cressman 2021 |
| Area-wide biocontrol of the cassava mealybug *Phenacoccus manihoti* Matile-Ferrero with the parasitoid *Epidinocarsis lopezi* (De Santis) in 38 countries of sub-Saharan Africa | Herren and Neuenschwander 1991 |
| Area-wide biocontrol of the pink hibiscus mealybug *Maconellicoccus hirsutus* (Green) with two parasitoid species in the Caribbean Basin, Florida and California | Meyerdirk 1999 |
| Area-wide control of the brown planthopper *Nilaparvata lugens* (Stål) and rice stem borers in China, Japan and South Asia by asynchronous rice planting and the conservation of the natural enemy fauna through landscape engineering | Oka 1991; Ives and Settle 1997; Kiritani 2007; Zhu et al. 2007; Heong et al. 2021 |
| *Dendroctonus* pine bark beetles: long-term landscape level management aimed at age and species mosaics unfavourable for large outbreaks in western USA and Canada | Keen 1952; Carroll 2007 |
| Global Malaria Eradication Campaign, initiated by the WHO in 1955, but disintegrated in 1969; malaria was eradicated in 37 countries, and in the end 74% of the people at risk were protected | Wright et al. 1972; Nájera et al. 2011 |
| Mosquito control districts first implemented in West Africa in the 1890s against malaria vectors; ≈260 districts in USA, many in other countries | AMCA 2004 |
| Area-wide management of silverleaf whitefly *Bemisia tabaci* (Gennadius) in Australia based on elimination of weed hosts, preservation of natural enemies, and tight-planting windows | Schellhorn et al. 2008 |
| Area-wide management and containment of the gypsy moth *Lymantria dispar* (L.) in the north-eastern USA -- *Bt* sprays and mating disruption. | Liebhold et al. 2021 |
| African Programme for Onchocerciasis Control to eliminate river blindness; insecticides used to kill *Simulium* spp. larvae in rivers, and ivermectin to treat infections | WHO 1994; Hougard 2000; Tekle et al. 2016 |
| Caribbean *Amblyomma* Programme to eradicate the tropical bont tick *Amblyomma variegatum* (F.), Bridgetown, Barbados; treatment of all ruminant livestock with pour-on Bayticol$^{R}$ | Pegram et al. 2000, 2007 |
| Buffalo flies *Haematobia exigua* de Meijere in Australia | James et al. 2021 |
| Chagas' disease, reduviid vectors in Latin America; spray infested homes, eliminate habitat, screen blood banks for trypanosomes | Schofield 2000; Hashimoto and Schofield 2012 |
| Boll weevil *Anthonomus grandis grandis* Boheman eradication; pheromone trapping, insecticide treatment, and cultural control | Dickerson et al. 2001; El-Lissy and Grefenstette 2007; Allen 2008 |
| Corn rootworm (*Diabrotica* spp.) management programmes in the US; *Bt* hybrid corn planting with resistance management, and local soil insecticide or foliar sprays applications in high-rootworm-density fields | Chandler 2002; Chandler et al. 2008; Quinn 2018 |
| Area-wide biointensive management plan for the brown marmorated stink bug *Halyomorpha halys* (Stål) | Leskey et al. 2016 |
| Codling moth *Cydia pomonella* (L.) suppression through the area-wide use of pheromone-mediated mating disruption in Washington State, Oregon and California | Calkins et al. 2000; Coop et al. 2000 |

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*Table 1. Continued*

| Programme | References |
|---|---|
| Codling moth eradication by integrating area-wide male annihilation and removal of all host and potential host trees in urban areas | Kovaleski and Mumford 2007 |
| Fruit fly AW-IPM in dragon fruit in Binh Thuan Province, Viet Nam | Hien et al. 2020 |
| Area-wide use of encapsulated semiochemicals in lure-and-kill sprays against the olive fruit fly *Bactrocera oleae* (Rossi) in Greece and Spain | Jones and Casagrande 2000 |
| Eradication of the oriental fruit fly *Bactrocera dorsalis* (Hendel) from Mauritius using male annihilation and bait application technique | Seewooruthun et al. 2000 |
| Eradication of *Bactrocera papayae* Drew and Hancock from northern Queensland, Australia, by male annihilation and strategic foliage baiting | Hancock et al. 2000 |
| Area-wide treatment of all properties to suppress Formosan subterranean termite *Coptotermes formosanus* Shiraki infestations of structures | Lax et al. 2007 |
| Eradication of the red palm weevil *Rhynchophorus ferrugineus* (Olivier) in the Canaries, Spain -- strict quarantines, palm treatment, and removals | Fajardo et al. 2021 |
| Regional management of *Helicoverpa armigera* (Hübner) in China | Wu 2007 |
| Eradication of *Hypoderma* spp. warbles from livestock in France using pour-on insecticides | Amouroux 2000 |
| Management of Russian wheat aphid *Diuraphis noxia* (Kurdjumov) and the greenbug *Schizaphis graminum* (Rondani) on wheat in the US Great Plains based on cultural practices, pest-resistant cultivars, and biological control agents | Keenan et at. 2007; Giles et al. 2008 |
| Area-wide augmentative biological control of the green peach aphid *Myzus persicae* (Sulzer) on tobacco in China | Yu et al. 2021 |
| Area-wide suppression of invasive *Solenopsis* spp. fire ants using insecticide-baits and self-sustaining biological control agents | Vander Meer et al. 2007; Aubuchon and Vander Meer 2008 |
| Stored-grain insect area-wide integrated pest management based on systematic sampling and decision-support software to guide fumigations | Flinn et al. 2007; Hagstrum et al. 2008 |
| Grape moths *Lobesia botrana* (Denis and Schiffermüller), and *Eupoecilia ambiguella* Hübner, area-wide pest management in Italy based on pheromone mating disruption | Ioriatti et al. 2008 |
| Area-wide application cultural method for the control of western tarnished plant bug *Lygus hesperus* Knight in cotton | Carrière et al. 2006; Abel et al. 2007 |
| Area-wide mosquito abatement or management districts to suppress mosquito populations by integrating physical and biological management, with larviciding, and adulticiding | Fonseca et al. 2013; Foley IV et al. 2021 |
| Cattle fever tick eradication programme operating since 1906, first to eradicate *Rhipicephalus annulatus* (Say) and *Rhipicephalus microplus* (Canestrini) from the USA (1943), and since then to contain incursions along the border with Mexico | George 1989; Rutherford 2007 |
| Area-wide suppression of the Asian citrus psyllid *Diaphorina citri* Kuwayama in Florida based on coordination of insecticide sprays to protect against this vector of devastating citrus greening (huanglongbing) | NAS 2010; Singerman et al. 2017 |
| Navel orangeworm *Amyelois transitella* (Walker) area-wide management in California almonds, pistachios and walnuts through sanitation, mating disruption and integration of selective pesticides and/or biological agents | USDA/ARS 2018 |
| Glassy-winged sharpshooter *Homalodisca vitripennis* (Germar) area-wide management in California based on coordinated insecticide sprays | USDA/APHIS 2015 |

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Both of these definitions have considerable merit, and they fit the majority of area-wide programmes. However, slightly different definitions may be needed to describe programmes on the conservation of natural enemies (Schellhorn et al. 2015; Heong et al. 2021), and on classical biological control where the adaptation of the introduced biological agent to all new environments cannot be known in advance of release (Knipling 1992a; Wyckhuys et al. 2021). Also, it may not be possible to clearly define the boundary of the pest population. In a similar vein, Showler (2002) stated:

> Locust swarms can be highly variable, influenced by many factors, including geography, vegetative conditions, land-use patterns, environmental sensitivity, availability of resources and tactics, prevailing winds, insecure areas, and rainfall patterns. Reliance on a single control strategy is therefore unrealistic [Showler 1997]. A more appropriate approach would be to develop specific strategies that will fit with projected scenarios, mostly by harmonizing them with national contingency plans.

### *1.2. Characteristics of Area-Wide Integrated Pest Management*

It is intuitively obvious that the immigration of individuals of a target pest into a managed ecosystem prevents the effective suppression or eradication of a population of that pest. Indeed, the dispersal and migration of pests, usually from small untreated foci into a managed area, have a tremendous impact, and is often underestimated (Byrne 2008). For example, very few codling moths develop in well-managed commercial orchards. Butt et al. (1973) found that the number of codling moths that overwintered in the Wenas Valley of Washington State, USA, dropped by 96% when a few abandoned orchards and neglected non-commercial apple trees were either removed or sprayed with insecticide. This study indicated that most of the moths originated from untreated trees that in aggregate were less than 5% of the host resources of the moth. Similarly, experiences in coping with the pink bollworm *Pectinophora gossypiella* (Saunders) and the boll weevil have shown that the few growers (who do not destroy crop residues immediately after harvest) provide food for these pests to reproduce and to enter diapause (Allen 2008; Staten and Walters 2021). This lapse in field sanitation can be the direct cause of devastating levels of these pests appearing in the following season on neighbouring farms.

Knipling (1972) graphically pointed out the drastic implications of allowing a small fraction of a population of a major pest to reproduce without control (Fig. 1). Knipling showed that more pest individuals would be produced if 1% of the total population was allowed to reproduce without control, while 100% control was applied to 99% of the population, than if only 90% control was imposed uniformly on the total population. Thus Knipling (1972) elaborated the basic principle of total population suppression:

> Uniform suppressive pressure applied against the total population of the pest over a period of generations will achieve greater suppression than a higher level of control on most, but not all, of the population, each generation.

Currently, for the most part, individual producers, who often rely heavily on the use of insecticides, carry out defensively the control of highly mobile and very destructive pests. Although other control technologies are often incorporated into the

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producer's IPM system, these technologies are also usually applied by the individual producer independently of other producers. This conventional uncoordinated and localized farm-by-farm or field-by-field strategy provides opportunities for the pest population to build-up and to infest well-managed neighbouring fields. Consequently, on most farms, insect pest populations increase to damaging levels each year, and the farmer is forced to apply broad-spectrum fast-acting insecticides as a rescue treatment. This defeats the primary goal of the IPM system, which is to take maximum advantage of naturally occurring biological control agents (Rohwer and Knipling 1992; Heong et al. 2021). Moreover, the application of insecticides, when an insect pest population reaches the economic threshold, does not prevent the losses that occur before the threshold has been reached. For commodities that are planted on vast areas, such losses are immense. For example, the world production of maize (corn) was roughly 1134 million metric tons in 2017 (World Data Atlas 2017). Avoidance of a loss of only 3% would make available 34 million metric tons, which could be a major factor in alleviating hunger.

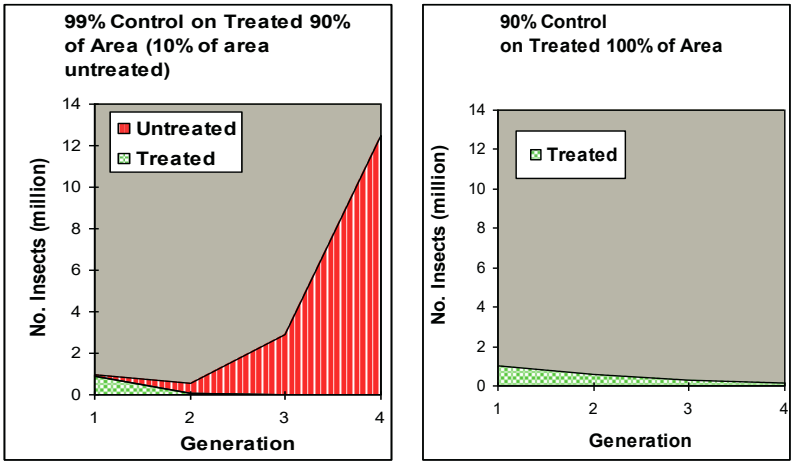



*Figure 1. Results of a model that shows the outcome of neglecting to suppress a small fraction of a pest population in an agroecosystem versus the effect of uniformly suppressing the entire pest population. Left: 10% of the population is untreated, and in four generations it produces a large number of individuals, while the 90% of the population that is treated declines. Right: Entire pest population in the agroecosystem is suppressed uniformly, and its numbers decline from generation to generation. (Figure adapted from Knipling 1972, reproduced with permission.)*

AW-IPM differs from conventional pest management of local pest populations in several important ways (Lindquist 2000; Koul et al. 2008):

1. It focuses on the preventive management of pest populations throughout the ecosystem, while the conventional strategy focuses narrowly on defending a valued entity (crop, livestock, people, buildings, etc.) from direct attack by pests.
2. It requires multi-year planning and commitment, and an organization dedicated exclusively to implementing the strategy; the conventional strategy involves only

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minimal forward planning, tends to be reactive, and is implemented independently by individual producers, businesses, or households.
3. It tends to utilize advanced "high-tech" technologies, whereas the conventional strategy tends to rely on traditional "low-tech" tactics and tools that can be reliably implemented by non-specialists (Lindquist 2000, 2001).

Community-based control can be considered as a special case of localized control whereby local communities are responsible not only for the implementation of the control activities but are also involved in their development and management (Vreysen 2001). There have been many examples of community-based control in Africa against tsetse fly populations, and these have shown that these operations can be successful in small areas and when the farmers have access to expertise and initial "seed money" (Dransfield et al. 1991). However, none of these community-based control activities has proven to be sustainable as exemplified by such efforts against the vectors of human sleeping sickness in Ivory Coast (Laveissière et al. 1985) and Uganda (Lancien 1991), or the vectors of nagana in Kenya (Dransfield et al. 1991). The reasons for the unsustainability of this approach are multiple, e.g. lack of scale and central coordination, various sociological factors (e.g. theft of the suppression devices, sustaining the interest of the farmers, willingness to contribute, etc.), inaccessibility of some habitats, and the need to deploy the suppression tools at high densities in some habitats (Vreysen 2006). Despite the quick localized benefits these approaches can bring, they cannot be sustained indefinitely by communities, becoming ineffective in the long run (Vreysen 2001).

Indeed, it is the use of organizations dedicated to coordinate and conduct area-wide programmes that provides the opportunities to employ sophisticated technologies and professional management. Computer-based models are utilized in planning and management (Bouyer et al., this volume), and satellite imagery is used to identify localities of alternate hosts that can be treated to reduce the number of migrants that cause the damage in commercial production areas. Area-wide programmes acquire or develop highly sensitive detection systems, use spatial tools such as the global positioning system (GPS), geographic information systems (GIS), and satellite remote sensing (RS), and employ specialised software for data management to ensure efficient implementation of these programmes.

Also, they may implement area-wide approaches to resistance management to prevent or retard the development of resistance to insecticides or the loss of host-plant resistance (Prasad et al. 2009). Computer programmes and real-time environmental data to predict insect populations can be used effectively in an area-wide programme, but usually not on an individual farm basis. Thus, modelling of pest distribution and immigration patterns, analysis of weather to predict increases or decreases of populations, genetic analysis to determine and forecast resistance levels, or to assess the level of isolation of targeted insect pest populations, etc., are utilized in area-wide programmes (Elliott et al. 2008; Bakhoum et al. 2021; Bouyer et al., this volume).

Most insect populations are not distributed uniformly in time and space (Vreysen, this volume). Metapopulation and landscape ecology are approaches that have been used to study or characterize spatially structured insect populations, and the basic concept of these dynamics is the heterogeneity of the environment (Levins 1969; Clark 2010). This heterogeneity results in discrete patches that are suitable or

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unsuitable, as well as persistent or unstable, for inhabitation of the pest insect (Elliott et al. 2008). The main difference between the two approaches involves the role of the matrix in the dynamics of the population patches, i.e. uninhabitable but consistent effects on the dynamics of the population in the metapopulation approach versus the matrix having varying effects on the local populations in the landscape ecology approach, which can influence within-patch dynamics (Hanski 1998). These approaches are important predictive modelling tools for a better understanding of the dynamics of the pest populations and, hence, allow for better planning of AW-IPM programmes.

Efficient implementation of AW-IPM requires defining the temporal and spatial scales that must account for the behaviour and longevity of the target pest. The minimum time-unit for analysis is likely to be the generation time of the target insect, whereas the minimal spatial unit should be the average two- or three-dimensional scale that a target insect can disperse within a generation (Elliott et al. 2008), and should be large enough to encompass all normal movement of the average individual insect (Schneider 1989; Barclay et al. 2011).

Finally, area-wide programmes can take advantage of the power and selectivity of specialized methods to manage insect pests that, for the most part, are not effective when used on a farm-by-farm basis. These include the SIT, inundative releases of parasitoids, the use of semiochemicals, mating inhibitors, large-scale trap cropping, treatment of hosts on public lands and in private gardens, etc.

### *1.3. Benefits of Area-Wide Integrated Pest Management*

Suppressing highly mobile pests on an area-wide basis is usually more benign environmentally, more effective, and more profitable, than on a farm-by-farm basis because of economies of scale (Carlson and Wetzstein 1993; Keenan and Burgener 2008). Also, AW-IPM is better at capturing the benefits of mobile natural enemies (Knipling 1992a; Ives and Settle 1997).

Area-wide programmes enable many producers and other stakeholders to pool resources to utilize technologies and expertise that are too expensive for individual producers. These may include mass-rearing facilities, aircraft services, information technologies, and highly trained specialists. In addition, a coordinated area-wide programme can avoid or internalize external costs. External costs (externalities) are the harmful effects arising from pest control operations, which affect parties other than the pest control decision-maker, but for which no compensation is paid to the persons harmed (Reichelderfer et al. 1984). Spray drift onto neighbouring properties frequently provokes disputes. Also, insecticide use to protect agricultural crops has caused resistance to develop in other insects, including vectors of disease. This has been an important factor in the resurgence of malaria.

AW-IPM is more likely to succeed when the number of farmers involved is small and the crops are similar. In addition, the suppression of the pest under the leadership of a third party makes it easier for a heterogeneous group of farmers to collaborate. Farmers who cultivate crops with a high economic value and low-pest tolerance suffer greater losses than farmers who cultivate a crop of lower economic value but with a higher pest tolerance (Yu and Leung 2006). Therefore, it is much

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easier to encourage the former to participate in an area-wide programme as compared with the latter (Stonehouse 2007).

AW-IPM provides imperfect public goods, and is prone to free-riding, and therefore most successful programmes depend on government funding, coordination, and management (Hendrichs et al. 2007). However, joint funding by federal/state governments and growers can enhance implementation because growers are stakeholders (Ervin and Frisvold 2016). The contributions of the producers and/or the general public (Halasa et al. 2012; Mumford, this volume) are often obtained by levying taxes, e.g. the codling moth programme in Canada (Nelson et al. 2021), or through contracts between farmers and a private entity, e.g. the false codling moth *Thaumatotibia leucotreta* (Meyrick) programme in South Africa (Boersma 2021). Sometimes, unexpected economic outcomes are obtained, e.g. results from an AW-IPM programme against the Queensland fruit fly *Bactrocera tryoni* (Froggatt) in Australia indicated that returns from tighter roadblocks were greater than returns from increased surveillance or enhanced eradication capacity (Florec et al. 2013).

Benefit/cost analyses have become important and valuable tools to assess the direct and indirect benefits of AW-IPM programmes; they provide consistency, flexibility, objectivity, and comprehensiveness at the decision-making stage (Enkerlin et al. 2007; Quinlan and Larcher-Carvahlo 2007; Mumford, this volume). Direct benefits are easy to assess because they result from increased crop yield and crop quality, increased livestock productivity, and reduced insecticide use and control costs. Indirect benefits are more difficult to quantify because they often relate to reduced environmental or human-health costs (Quinlan and Larcher-Carvahlo 2007; Enkerlin, this volume).

As evidenced by the few examples below, the benefits of properly executed AW-IPM programmes can be enormous:

1. The boll weevil, since its introduction into the USA in the 1890s, has cost the cotton industry more than USD 2300 million in economic losses. As exemplified in Georgia, its eradication provided significant benefits to growers; the average gross crop revenues increased from USD 70 million per year (prior to eradication) to USD 400 million per year (afterwards) (USDA/APHIS 2013).
2. The eradication of the New World screwworm *Cochliomyia hominivorax* (Coquerel) in North and Central America resulted in annual benefits to livestock keepers amounting to USD 1300 million, despite an investment of the same amount to eradicate the pest over 45 years. The eradication of the same pest when it invaded Libya resulted in a benefit/cost ratio of 5:1 for the infested area and 10:1 for the whole of Libya (Vargas-Terán et al., this volume).
3. A recent benefit/cost analysis of the Moscamed containment programme, assuming that the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) is eradicated in Guatemala and the containment barrier is moved to the border with El Salvador and Honduras, indicated a benefit/cost ratio of 140:1 for Belize, 16:1 for Guatemala, and 127:1 for Mexico (Enkerlin et al. 2017).
4. An economic analysis of an AW-IPM programme against the Asian tiger mosquito *Aedes albopictus* (Skuse) in New Jersey, USA (based on adulticiding, larviciding, and resource reduction) indicated that the incremental cost of the programme was USD 41 per person per year. Over a period of 3 years, a benefit/cost ratio of 8.6:1 was obtained, indicating that for every USD spent, the

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programme gave adults additional time outdoors worth USD 8 (Shepard et al. 2014).

Finally, economies of scale can be captured in area-wide programmes, although complex trade-offs may be involved. The more mobile the pest, and the more uniform the damage caused by the pest, the larger the area under coordinated management should be (Barclay et al. 2011). The total cost of pest detection, monitoring, and suppression per hectare of crop usually declines as the size of the managed area increases. However, as the project size increases, the per-hectare organizational cost usually increases because of the increased need for coordination and other communication costs. For these reasons, in very large programmes such as the effort to eradicate the boll weevil or the pink bollworm in the USA, the vast area was subdivided into several operational zones (Allen 2008; Staten and Walters 2021). Also, considerable organizational cost savings may be realized in instances where towns, municipalities, or cooperatives already have structures in place for decision-making and communication.

### *1.4. Contingencies Often Dictate Changes in Strategy*

Contingencies often arise that require replacement of one strategy with another (Geier 1970; Hendrichs, Vreysen et al., this volume). For example, at various times during the 45-year campaign to remove the New World screwworm from the USA, Mexico and Central America, different pest management strategies had to be selected. This programme began when an unusual series of frosts, beginning early in December 1957, killed all screwworms in the south-eastern USA north of a line from Tampa to Vero Beach in southern Florida. Sexually sterile flies, from a culture in a research laboratory, were released in a broad band north of this line to contain the pest population. In the summer of 1958, this exclusion or containment strategy was replaced by the strategy of eradication; the sterile flies were derived from a recently constructed mass-rearing facility that was able to produce 50 million sterile flies per week. Eradication was accomplished in 1959; in 1966 the New World screwworm was officially declared eradicated in the USA. However, it was obvious that, unless the insect was removed from northern Mexico, it would reinvade the USA. Nevertheless, the programme to eradicate the screwworm from northern Mexico could not commence immediately because, until 1972, there was no agreement between the Governments of the USA and Mexico. Therefore, in 1966, the programme strategy had to change from eradication to exclusion in the form of a sterile-insect barrier along the entire Mexico-USA border. Sterile flies were released in a zone that was about 130-km wide. Unfortunately, this barrier proved to be too narrow to exclude female screwworms from entering the USA and causing many cases of screwworm myiases (Graham 1985). Finally, operations began in Mexico in 1974. The last screwworm case in the USA occurred in 1982 (Vargas-Terán et al., this volume).

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### *1.5. Legal Authority for Area-Wide Integrated Pest Management*

Legal authority to implement area-wide and other regulatory programmes is absolutely essential, but is still evolving (Dyck, Reyes Flores et al., this volume). Without explicit legal authority, the organization implementing an area-wide programme would not be able to conduct operations on private property, nor operate quarantines.

In about 1860 the grape phylloxera *Daktulosphaira vitifoliae* (Fitch) was introduced into France from the USA. Within 25 years of its arrival, this insect had destroyed one million ha of vineyards (fully one-third of the production capacity). To protect the German wine industry, in 1873 the Government of Germany passed the first law that provided for quarantines and regulatory control of agricultural pests (NAS 1969). Other governments quickly followed the example, and in 1881 representatives of many European countries together developed a set of regulations governing the movement of grape-propagating material.

In about 1880 the establishment of the San Jose scale *Quadraspidiotus perniciosus* Comstock in California, its rapid spread on nursery stock throughout the country, and the failure of a programme to eradicate it, caused Canada, Germany, and Austria-Hungary in 1898 to prohibit the importation of American fruit and living plants (Klassen 1989). This crisis in the USA led to the passage in 1905 of federal legislation on quarantine and the regulation of interstate shipments (Plant Quarantine Act) (Womach 2005).

Indeed, now many countries have legislation on: (1) prevention of the introduction of new pests from foreign countries, (2) prevention of the spread of established pests within the country or state, and (3) enforcement of the application of control measures against exotic pests to prevent their introduction and establishment, eradicate their outbreaks, retard their advance or prevent damage by them. In some countries the law allows people, who wish to organize a programme against a pest, to hold a referendum. If the referendum passes by a certain margin (usually 67% in US counties), then all parties at interest must cooperate in the venture (Boll Weevil Eradication Law 1992; Grefenstette et al. 2009; Ervin and Frisvold 2016).

The programme to eradicate the citrus canker disease in Florida was initiated in 2000 but was hampered for 4 years because of insufficient legal authority. This pathogen is carried considerable distances on driving rain, and to achieve eradication the Division of Plant Industry decided to destroy all citrus trees within a radius of 578 m from an infected tree. Homeowners in urban areas, who did not understand the need for such drastic action, felt that their rights were violated by workers who, as part of the eradication programme, entered residential yards and destroyed citrus trees. Thus, the Broward County Circuit Court ruled that programme employees must have a separate court-issued warrant to enter each privately-owned property. The need to apply for tens of thousands of warrants prompted the Florida Department of Agriculture and Consumer Services to appeal this ruling; in 2004 the Florida Supreme Court overturned it. Nevertheless, as a result of all these delays, during which three major hurricanes traversed infected areas, the disease was spread widely, and the mandatory eradication programme had to be abandoned in January 2006 (Centner and Ferreira 2012).

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### *1.6. Apathy, Outrage, and Area-Wide Integrated Pest Management*

The strategy of eradication emerged just over a century ago as the brainchild of C. H. Fernald of the University of Massachusetts, USA. Under Fernald's leadership, Massachusetts attempted to eradicate an introduced pest, the gypsy moth, in an 11-year campaign from 1890 to 1901. Initially, the primary eradicant was Paris green, but this insecticide, of only modest efficacy and phytotoxicity, had to be abandoned because of adverse public reaction. Forbush and Fernald (1896) noted:

> Considerable opposition to the use of Paris green for spraying was manifested by many people living in the infested towns. A mass-meeting of opponents of the spraying was held in Medford. One citizen, who attempted to cut the hose attached to one of the spraying tanks, and threatened with violence the employees of the Board who had entered upon his land, was arrested and fined. Others neutralized the effects of the spraying by turning the garden hose upon trees and shrubs that had been sprayed, and washing off the solution. The opposition to the spraying affected the results of the work unfavourably to a considerable extent.

Clearly, apathy by many members of the public had turned into outrage.

If not concerned primarily with the economic dimension of the pest problem, stakeholders tend to be highly concerned with ecological, environmental, social, and human-health implications of area-wide programmes (Rabb 1972; Dreistadt 1983; Scribner 1983; Myers et al. 1998). Therefore, leaders of AW-IPM programmes need to be very sensitive to the perceptions and attitudes of the public toward certain programme operations (Dyck, Regidor Fernández et al., this volume). Often, eradication efforts must be conducted by the ground rules of the urban, rather than rural, setting. In programmes to eradicate outbreaks of the Mediterranean fruit fly in California and Florida, members of the urban public strongly protested against the aerial malathion bait-spray applications (Scribner 1989), even though the same insecticide was used without dissent for mosquito abatement. On the other hand, the same public has usually applauded the release of sexually sterile male flies.

As explained by Sandman (1987), the public is usually apathetic towards technological programmes. However, certain factors inherent in programmes, and in the manner in which they are managed, can precipitate an almost irreversible shift in the public's attitude from apathy to outrage. A sense of outrage can be evoked by involuntary exposure to pesticide residues, imposed levies of fees, quarantines, right of trespass, unfair and inequitable sharing of risks, costs and benefits, temporary loss of control of one's property or field operations, the perception that endangered species may be harmed, etc. Starr (1985) asserted that:

> Public acceptance of risk is more dependent on public confidence in risk management than on the quantitative estimates of risk consequences, probabilities and magnitudes.

He noted that, when a zoo wishes to acquire a tiger, the public does not demand a refined assessment of the risk that the tiger might escape and kill someone. Instead, the public wants assurance that the zookeeper can be trusted to prevent the tiger from escaping, and that, if the tiger should escape, the zoo is fully prepared to implement an emergency plan to meet this contingency. Similarly, the public's confidence in the management of an area-wide programme is of paramount importance.

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In each area-wide programme, a special effort must be made to anticipate and identify those factors that may be emotionally upsetting to various stakeholders, and take pre-emptive actions to avoid or mitigate adverse reactions. Public officials must be kept apprised, public education materials produced (FAO/IAEA 2017), fora must be created for effective two-way communication with the public, surrogates of the public must be included in oversight and decision-making processes, and referenda may have to be conducted to secure support and funding for the programme (Batra and Klassen 1987; Klassen 1989; Dyck, Regidor Fernández et al., this volume).

### *1.7. Invasive Pests, Global Trade, and Area-Wide Integrated Pest Management*

The rapid globalization of trade in agricultural products, and increasing tourism, have dramatically increased the spread of invasive harmful organisms (Klassen et al. 2002; Hendrichs, Enkerlin et al., this volume). We have entered an era of an unprecedented level of travel by exotic invasive organisms. Native flora and fauna on islands are sustaining great harm from non-indigenous invasive organisms, and major pests are becoming established with increasing frequency on all continents, except Antarctica.

For about a century many countries relied on the inspection of arriving cargo and passengers at the port of entry as the primary exclusion strategy. However, the volume of arriving cargo is doubling every 5 or 6 years (Zadig 1999), and it is not possible to increase similarly the human and other resources devoted to inspection at ports of entry. Clearly exclusion at the port of entry is no longer sufficient to protect plant resources, even though a number of emerging technologies are likely to facilitate safeguarding activities (Batkin 1999). Thus, to stem the influx of exotic pests into the USA, the National Plant Board asserted that the most important change needed in the US safeguarding system is to shift primary reliance from exclusion at the port of entry to offshore actions, i.e. pest-risk mitigation in the areas of production, certification at the point of origin, and pre-clearance at the port of export (NPB 1999).

An important approach to offshore risk mitigation is the creation of pest free areas. Indeed, countries that export raw agricultural commodities can effectively remove the threat of exotic pests to the importing country by creating and maintaining pest free areas (Rohwer 1992; Malavasi et al. 1994; Dalal et al. 2017; Enkerlin, this volume; Hendrichs, Vreysen et al., this volume). A pest free area is an area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained (FAO 2019). There are two kinds of pest free areas: (1) pest free zones — large geographic areas, including entire countries such as Chile, Japan or New Zealand — that are certified free of tropical fruit flies of economic importance, and (2) pest free production fields or areas that require demonstration of non-host-status or the demonstrated suppression of quarantine pests to non-detectable levels. In addition, there are low-prevalence areas that are established by means of a systems approach through the application of a series of preharvest and postharvest suppression and mitigating measures.

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According to Griffin (2000), both the International Plant Protection Convention (IPPC) and the World Trade Organization (WTO) Agreement on Sanitary and Phytosanitary Measures (Devorshak 2007):

> … are structured to accept and encourage area-wide pest management as a tool for promoting safe trade and contributing as much as possible to the complementary goals of food security and economic security for all countries.

The first US-recognized pest free area was established in 1988 in Sonora, Mexico (SARH/DGSV-USDA/APHIS 1990), and since then Mexico has been ridding large additional sections of its territory of all fruit fly species of economic importance (CNCMF 2002). The Mexican states of Baja California, Chihuahua, and Sonora have been freed of all economically important species of fruit flies. Citrus, stone fruits, apples, and vegetables are being exported from these states without any suppression or postharvest treatment. In other parts of Mexico, low-prevalence fruit fly areas are being established by means of a systems approach (Reyes F. et al. 2000; Enkerlin, this volume; Hendrichs, Vreysen et al., this volume). Chile rid the entire country of the invasive Mediterranean fruit fly by 1995 (SAG 1995), and since then large volumes of Chilean fruits have been exported to the USA and other markets without the need for any quarantine treatments. Also, other countries have created fruit fly-free areas (Rendón and Enkerlin 2021; Enkerlin, this volume).

Other examples of successfully preventing the establishment of invasive insect pests are the eradication of outbreaks of the European grapevine moth *L. botrana* in California (Simmons et al. 2021), the red palm weevil *R. ferrugineus* in the Canary Islands (Fajardo et al. 2021), the painted apple moth *Teia anartoides* Walker in New Zealand, and the cactus moth *Cactoblastis cactorum* (Berg) in Mexico (Hendrichs, Enkerlin et al., this volume; Simmons et al., this volume).

An area of low pest prevalence is an area (whether the entire country, part of a country, or all or parts of several countries), identified as such by the competent authorities, in which a specific pest occurs at low levels, and which is subject to effective surveillance, suppression or eradication measures (FAO 2019; Hendrichs, Vreysen et al., this volume). Requirements to establish low-prevalence areas include a sensitive detection programme, suppression of the quarantine-significant pest to non-detectable-levels, strict sanitation and control of the fields, and safeguards to prevent infestation during packing and transit to the port of export (Riherd 1993; Simpson 1993; Malavasi et al. 1994; Riherd et al. 1994).

Florida is able to export grapefruit to Japan by creating pest free grapefruit groves in about 22 counties. Regulatory experts from Japan inspect the entire process of production, packing, and transit. Official protocols for pest free fields can be found in Gomez (1999). Similarly, fruit groves free of the South American cucurbit fruit fly *Anastrepha grandis* (Macquart) have been created in Mossoro, Brazil, and Guayaquil, Ecuador, through demonstration of non-host status under appropriate crop management (Malavasi et al. 1994).

The concept of low prevalence, using bait sprays and the SIT, was pioneered during the early 1960s against the Mexican fruit fly *Anastrepha ludens* (Loew) along the Mexico-USA border (Knipling 1979). Also, in the early 1960s, the Citrus Marketing Board of Israel developed a bait-spray-based area-wide programme

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against the Mediterranean fruit fly that has been able to meet the certified quarantine security requirements of fruit-importing countries (Cohen and Cohen 1967).

A significant development has been a preventive approach involving the continuous area-wide release of sexually sterile male Mediterranean fruit flies in the Los Angeles Basin (Dowell et al. 2000; USDA/APHIS 2004; Enkerlin, this volume), and around high-risk ports in southern Florida, to prevent Mediterranean fruit fly establishment resulting from recurrent introductions in ports of entry (USDA/APHIS 1998; Hendrichs, Enkerlin et al., this volume).

### *1.8. Area-Wide Integrated Pest Management – Future Directions*

Despite the fact that AW-IPM programmes are technically complex and managerially challenging (Vreysen et al. 2007), this strategic approach bears great promise for the future. Over the last two decades, interest in this strategy has been mounting, especially considering the substantial benefits obtained when area-wide programmes are properly executed. However, it should be emphasized that these programmes require long-term planning, i.e. from stakeholder commitment to feasibility studies, pilot trials, and pre-operational and operational phases (Vreysen et al. 2007; Bouyer et al. 2020; Vreysen et al. 2021; Vreysen, this volume); they also need close collaboration among researchers, farmers, extension workers, community leaders, local and regional governments, and the general public (Liu and Yan 1998).

AW-IPM is probably most appropriate for high-value crops, key livestock pests, and major human-disease vectors; it is also appropriate for those rural areas where the number of farmers is small, agroecological heterogeneity is low, and only a few key pests need to be addressed (Hendrichs et al. 2007). However, the approach may not be applicable to very mobile or migratory insects or to all animal- or human-disease vectors.

A major obstacle to the successful implementation of AW-IPM is the lack of trained extension workers -- that may lead to even increased insecticide inputs. In Asia, many state-employed extension workers are also involved in marketing insecticides, and their advice to farmers becomes biased against IPM approaches (Koul and Cuperus 2008; Heong et al. 2021). Better education of farmers and the general public will remain key to better implementation of AW-IPM programmes, and also to better understand economic trade-offs among treatment alternatives.

Another major limitation in implementing AW-IPM, as advocated by E. F. Knipling, is the lack of sufficient knowledge about the spatial and temporal dynamics of the target pest insects (Levandowsky and White 1977). Recent advances in landscape ecology (Pickett and Cadenasso 1995) and ecological modelling (Carrière et al. 2006) can help explore the temporal and spatial dynamics of the pest insect. In addition, landscape genetics, an innovative emerging approach (that associates population genetics of the target insect with spatial tools) enables an understanding of how geographic and environmental features structure genetic variations at the population level (Bakhoum et al. 2021). This approach not only increases ecological knowledge but also explains spatial genetic patterns, isolation by distance, genetic boundaries to gene flow, etc. (Manel et al. 2003). All of these new tools and approaches can facilitate conceptualizing AW-IPM programmes.

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Improved lifestyles and higher gross domestic products will result in increased aversion to insecticide use, and consumers will be increasingly prepared to pay a higher price for clean biological commodities. Major opportunities to promote AW-IPM will be closely linked with consumers' demand for food safety, and with increased support for research, extension services, and legislation (Koul and Cuperus 2008).

## 2. BASIC ELEMENTS OF THE SIT

The SIT is an autocidal genetic control tactic that interferes with the reproductive potential of a target pest population. It can be considered as a form of birth control imposed on an insect pest population to reduce its numbers. The SIT harnesses the sex drive of insects. According to the International Plant Protection Convention (IPPC) (FAO 2019), a sterile insect is defined as:

> An insect that, as a result of a specific treatment, is unable to reproduce.

and the SIT is defined as:

> Method of pest control using area-wide inundative release of sterile insects to reduce reproduction in a field population of the same species.

The SIT requires the rearing of large numbers of the target pest species, exposing them to ionizing radiation to induce sexual sterility, and releasing them into the target pest population on an area-wide basis. A derivative of the SIT avoids the need to mass-rear the target insect pest species by attracting individuals of the wild populations to a chemosterilant, e.g. Mediterranean fruit fly (Navarro-Llopis et al. 2004) and *Glossina* spp. tsetse flies (Hargrove and Langley 1990; Langley 1999). The latter approach, however, has only been used on an experimental basis. Thus, treated insects are prevented from reproducing; they act as biological agents that nullify the biological potential of untreated individuals with which they mate. Both kinds of SIT are effective only against pest species that reproduce sexually (Lance and McInnis, this volume).

### *2.1. How Sterile Males Suppress Populations: Numbers Game*

Knipling (1968) recognized that the level of suppression required to stabilize the density of a population depends on its intrinsic rate of increase (Table 2).

Knipling (1968) estimated that an overwintering screwworm population typically increases approximately five-fold for the next two or three generations. For example, if in an area of 100 000 $km^2$ 1 000 000 screwworms overwinter, this population will increase to 5 000 000, 25 000 000, and 125 000 000 in the $F_1$, $F_2$, and $F_3$ generations, respectively. To calculate the consequences of releasing sterile screwworms into a population of fertile screwworms, Knipling listed the various types of matings, calculated the frequency of each type of mating, and assigned ten progeny to each mating of an untreated female (UF) with an untreated male (UM). When both sterile males (SM) and sterile females (SF) are released, there are four types of matings possible. Thus the wild population has 500 000 untreated males [UM] and 500 000

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untreated females [UF], and 4 500 000 sterile males [SM] and 4 500 000 sterile females [SF] are released. The frequencies of the various matings, and the number of progeny produced, are shown in Table 3. Using this method, Knipling calculated the trend of this hypothetical screwworm population (Table 4).

*Table 2. Rates of mortality and survival required to maintain a stable pest population*

| Intrinsic rate of increase between generations | Number of progeny per female | Number (fraction) that must survive to prevent population from declining. | To prevent population increase | | |
|---|---|---|---|---|---|
| | | | Number (fraction) that must die | Percentage that must die | Percentage that may survive |
| 2-fold | 4 | 2 (1/2) | 2 (1/2) | 50 | 50 |
| 3-fold | 6 | 2 (1/3) | 4 (2/3) | 67 | 33 |
| 4-fold | 8 | 2 (1/4) | 6 (3/4) | 75 | 25 |
| 5-fold | 10 | 2 (1/5) | 8 (4/5) | 80 | 20 |
| 10-fold | 20 | 2 (1/10) | 18 (9/10) | 90 | 10 |
| 20-fold | 40 | 2 (1/20) | 38 (19/20) | 95 | 5 |

Knipling realized that, to obtain a reduction of the wild population, the degree of sterility introduced into the wild population by releasing sterile males must be sufficiently high to overcome the rate of increase (reproductive success) of the wild females. Knipling (1968) stated:

> If we assume that a given insect has a net capacity to increase five-fold each generation, the ratio of fully competitive sterile to fertile insects will have to be 4:1 to keep the natural population stable. Theoretically, an initial ratio as low as five sterile to one fertile will be adequate to start a downward trend in the natural population when the net increase rate is only five-fold. Actually, starting with this lower overflooding ratio, theoretical elimination of the population will be achieved with fewer insects than will be required with a 9:1 ratio. However, in actual practice, the density of insects will vary in different parts of the environment. Moreover the distribution of sterile insects will never be uniform. Therefore, in control operations, the initial ratio should be sufficiently high to be certain that an overall reduction in the population will occur in all parts of the environment from the start. In some instances, the sterility procedures might reduce the vigour and competitiveness of the organism. Allowance must be made for this factor.

As shown in Table 4, the ratio of sterile to fertile insects increases asymptotically as the density of the wild population declines to low levels. Thus, to take advantage of the tremendous power of the SIT against sparse populations of pests, Knipling advocated that the release of sterile insects should be initiated when the wild population was at a seasonal low or immediately after its decimation by adverse weather events, such as freezes and hurricanes. In addition, Knipling (1966, 1979, 1992a) designed pest management systems in which insecticides, biocontrol agents, etc., were used to reduce the density of the target population to a level at which the SIT could manifest its great suppressive power (Fig. 2).

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*Table 3. Method of calculating frequencies of various types of matings and resultant progeny when sterile males and females are released into a population of untreated males and females (text provides details)*

| Type of mating | Number of matings | Number of progeny/ mating | Number of progeny |
|---|---|---|---|
| UF X UM | $\frac{500\,000 \text{ X } 500\,000}{5\,000\,000} = 50\,000$ | 10 | 500 000 |
| UF X SM | $\frac{500\,000 \text{ X } 4\,500\,000}{5\,000\,000} = 450\,000$ | 0 | 0 |
| SF X UM | $\frac{4\,500\,000 \text{ X } 500\,000}{5\,000\,000} = 450\,000$ | 0 | 0 |
| SF X SM | $\frac{4\,500\,000 \text{ X } 4\,500\,000}{5\,000\,000} = 4\,050\,000$ | 0 | 0 |

*Table 4. Trend of an insect population subjected to sterile insect releases when the normal increase rate is five-fold*

| Generation | Uncontrolled natural population (5 X increase rate) | Controlled population | | Ratio of sterile to fertile |
|---|---|---|---|---|
| | | Natural population | Sterile population | |
| 1 | 1 000 000 | 1 000 000 | 9 000 000 | 9:1 |
| 2 | 5 000 000 | 500 000 | 9 000 000 | 18:1 |
| 3 | 25 000 000 | 131 625 | 9 000 000 | 68:1 |
| 4 | 125 000 000 | 9535 | 9 000 000 | 942:1 |
| 5 | 625 000 000 | 50 | 9 000 000 | 180 000:1 |

## 3. USING THE SIT TO IMPLEMENT PEST MANAGEMENT STRATEGIES

The major pest management strategic options or objectives are: (1) suppression, (2) eradication of well-established pest populations, (3) containment (exclusion) and (4) prevention of invasive pest establishment (Hendrichs, Vreysen et al., this volume).

The SIT is a pest-specific tactic that can play a role in implementing all of these area-wide strategies. In this sense, a "tactic" is a method for detecting, monitoring or controlling a pest. A "system" is an assemblage of tactics that are applied simultaneously or sequentially so that the effects of individual tactics on the pest population are either additive or mutually potentiating, and counter-productive

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(negative) interactions are avoided or minimized. A pest management "strategy" is a broad overall plan that aims to achieve the specific strategic objective. The merits of a pest management strategy may be judged by its short- and long-term ecological, economic, sociological and political impacts (Rabb 1972; Enkerlin 2003; Enkerlin et al. 2003; Hendrichs, Vreysen et al., this volume).

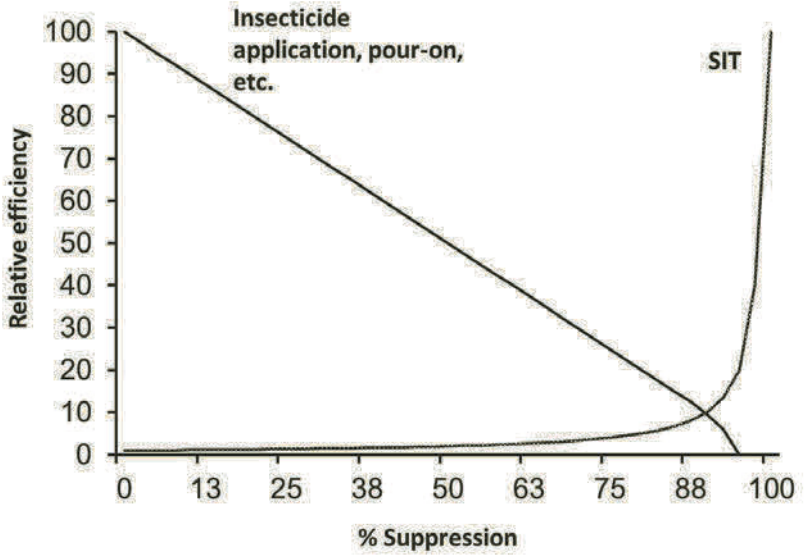



*Figure 2. Schematic representation of the sequential use of methods of suppression that efficiently decimate a pest population followed by using the SIT, which becomes progressively more suppressive as the population declines. The term "efficiency" refers to the ease in further suppressing the population, and not to the cost of eliminating an individual pest (cost per kill). (Figure adapted from Feldmann and Hendrichs 2001.)*

To implement a strategy effectively and efficiently, each control tactic has characteristics that need to be considered in the design of a system. The relationship of the density of the pest to the efficiency of the control tactic is of paramount importance. Some tactics (such as insecticide spraying) are most useful only against dense and moderately dense populations, while others, such as the SIT and sex pheromones, are effective only against sparse populations. Also, ecological selectivity is important to avoid the destruction of natural enemies needed to prevent the resurgence of the pest population following a control operation, and to prevent environmental damage. Most conventional insecticides are non-selective, whereas the SIT and other genetic techniques, pheromones, pest-resistant crop varieties, certain insect pathogens, parasitoids and predators, and certain artificial and naturally occurring attractants, are highly selective tactics. Light traps, some attractant baits, "general" predators, parasitoids and pathogens, and certain insecticides, are only moderately selective.

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### *3.1. Principles of Designing Pest Management Systems*

The design of a pest management system requires information on the selectivity and efficiency of available tactics. When the suppression of populations to a very low level is required, methods that are effective against high populations, and methods that are effective against low populations, should be integrated so that the actions of the former potentiate those of the latter (Fig. 2) (Mangan and Bouyer, this volume). For example, the use of a selective insecticide potentiates the SIT by increasing the sterile:fertile ratio. Moreover, the release of both a pest-specific parasitoid and sterile insects is likely to be mutually potentiating (Simmons et al., this volume). When the economic threshold of the pest is moderately high, several tactics that have additive effects against dense populations may be combined to give much more reliable suppression than from a single method. Knipling (1979) discussed these principles thoroughly (Hendrichs, Vreysen et al., this volume).

### *3.2. SIT and Local Suppression of Portions of Populations*

Since 1981, the SIT has been used commercially to suppress locally the onion fly *Delia antiqua* (Meigen) on an area of ca. 10 000 ha in The Netherlands (Loosjes 2000; Everaarts 2016). The flies are reared year-round and stockpiled for release during the onion-growing season. To protect against immigrant flies, sterile flies are constantly maintained in the fields. Individual farmers contract for the SIT independently of their neighbours, many of whom use chemical control. Free-riders (growers in the release area who benefit but do not pay) weaken this local suppression programme, which has only been able to expand gradually over the years to 40% of the onion-producing area. In addition, further efficiency is lost because the onion fields receiving sterile flies do not form a contiguous block (Everaarts 2016).

### *3.3. SIT and "Total" Population Suppression*

The SIT can be applied for population suppression rather than eradication (Hendrichs, Vreysen et al., this volume). As a sequel to the 1993/1995 Oslo Accords, AW-IPM programmes using the SIT against the Mediterranean fruit fly were established in some fruit-producing areas of Israel, Jordan, and the Territories under the Jurisdiction of the Palestinian Authority. Enkerlin and Mumford (1997) concluded that, over a 9-year time frame, the net economic benefits from this SIT suppression programme would be greater than from an SIT-based eradication programme, or from an area-wide bait-spray programme. Assuming that sufficient sterile flies can be purchased from existing rearing facilities, SIT suppression is profitable beginning in the first year. In contrast, eradication would have a payback period of 4 years, because of the need for an upfront investment in a rearing facility, high quarantine costs, and the need for more intensive monitoring. Similar SIT suppression programmes are ongoing in the Western Cape of South Africa (Venter et al. 2021), and Valencia, Spain, and Neretva, Croatia, in southern Europe (Bjeliš et al. 2016; Enkerlin, this volume).

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The SIT suppression programme against the codling moth in Canada substantially reduced populations of the pest and also the number of insecticidal sprays. Similar success against the false codling moth in South Africa was achieved (Bloem et al. 2001; Boersma 2021; Nelson et al. 2021; Simmons et al., this volume).

### *3.4. SIT and Eradication of Well-Established Populations*

Eradication of a pest population is the sustainable removal of every individual of the pest species in an area surrounded by natural or man-made barriers sufficiently effective to prevent reinvasion except through the intervention of man (Newsom 1978; Hendrichs, Vreysen et al., this volume). Although the eradication of a population may not endure forever, the elimination of a pest from a defined area, followed soon after by reinvasion, is not considered to be eradication.

Through an unrelenting effort from 1957 to 2000, the SIT, as part of an AW-IPM strategy, was used to eradicate the New World screwworm in its native range in the southern USA, Mexico, and Central America (Vargas-Terán et al., this volume). An area-wide programme was essential because the screwworm infested wild as well as domestic animals. Similarly, the SIT was an important component of the AW programmes that eradicated the pink bollworm in southern USA and northern Mexico (Staten and Walters 2021), as well as the Mediterranean fruit fly in areas of Argentina, Chile, USA, and Mexico, and the melon fly *Zeugodacus cucurbitae* (Coquillett) in the Okinawa archipelago of Japan (Kuba et al. 1996; Enkerlin, this volume).

The SIT was used as part of an integrated approach to eradicate tsetse populations in Nigeria and Burkina Faso in West Africa, but as the approach was not area-wide, the eradication status could not be sustained (Cuisance et al. 1986; Oladunmade et al. 1990). An AW-IPM approach that included an SIT component was used to permanently remove a tsetse population from the Island of Unguja, Zanzibar (Vreysen et al. 2000). A similar approach has been used in the last decade in the Niayes area of Senegal (Vreysen et al. 2021). However, the goal of creating a number of tsetse-free zones in Africa (Feldmann et al., this volume) will require significant improvements in mass-rearing technology, an in-depth knowledge of the behaviour of released and wild flies, and a careful consideration of the design of pest suppression systems. In addition, besides technical concerns, considerable development of programme management, and political and financial support, is essential for tsetse eradication programmes (Vreysen et al. 2007; Dyck, Reyes Flores et al., this volume).

### *3.5. SIT and Containment and Prevention of Pest Populations*

As already noted, the SIT and quarantines are major tactics in systems used to implement the containment (exclusion) and the prevention strategies. Examples of the containment strategy include Panama, where a barrier of sterile New World screwworm flies is maintained to contain the screwworm at the Panama-Columbia border (Vargas-Terán et al., this volume), and a barrier of sterile Mediterranean fruit

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flies is maintained to exclude this pest from Mexico (Villaseñor et al. 2000; Enkerlin et al. 2017).

For the prevention strategy, examples include releases of sterile pink bollworms to prevent the pest from establishing on cotton in California's San Joaquin Valley (Staten et al. 1993), preventive releases of the male-only strain of the Mediterranean fruit fly over the Los Angeles Basin in California and major metropolitan areas in Florida, and releases of sterile Mexican fruit flies to prevent immigrant flies from crossing over from Mexico into the lower Rio Grande Valley of Texas (Hendrichs, Enkerlin et al., this volume). The SIT and quarantine stations were used, initially, to prevent the spread of the New World screwworm throughout Africa, and then to eradicate it in the coastal region of Libya (FAO 1992; Lindquist et al. 1993).

### *3.6. Lessons Learned from Using the SIT to Achieve AW-IPM*

In most AW-IPM programmes that integrate the SIT, the goal of containment, suppression or eradication has been threatened by the existence of untreated or inadequately treated refugia or microhabitats unusually favourable for the pest, i.e. "hot spots", from which recruits could come to reinfest cleared areas. In the New World screwworm campaigns, some hot spots were the ranches where livestock wounds were not being treated. Breaches of quarantine lines were very troublesome. The overarching lesson was that absolutely all segments of the population, i.e. the total population, must be suppressed.

More specifically, with respect to the SIT, the following conclusions can be drawn:

- An extended lag period may occur between the initiation of the release of sterile insects and a noticeable effect on the density of the pest. This is inevitable as the wild population will include many already mated females, sexually immature females, pupae, larvae, and eggs. The released sterile males can only prevent the reproduction of unmated females. As the immature forms mature, they become subject to the impact of the sterile flies. However, the time of one generation will pass before the progeny of previously mated females can be affected. Consequently, the release ratio must be sustained over a period of time equivalent to several generations.
- If a high proportion of the wild population is present as eggs and larvae when the release of sterile insects begins, then the wild population will even increase for a time in spite of the releases of sterile insects. However, this period can be shortened if an insecticide application (or any other control tactic that is effective against high-density wild populations) is made to kill females previously mated to wild males.
- Severe weather events, such as periods of cold weather, may reduce the density of the pest population, and also synchronize the development of the population by halting reproduction and killing exposed life stages. In this way the generation overlap may be eliminated.
- An influx of pests into the target area, even a few mated females, will greatly prolong an eradication programme. Great care must be taken not to underestimate the flight range of the pest. For most pest insects one can assume

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that an immigrant female will produce 10–20 adult progeny in a small area. However, the progeny may disperse and be thinly distributed, and thus vulnerable to the SIT.

Krafsur (1998) asserted that the SIT is an underutilized and widely misunderstood technology (Whitten and Mahon, this volume). He refuted several misconceptions concerning the evolutionary responses to the SIT, the role of weather in AW-IPM programmes using the SIT, and the occurrence of undetected populations where eradication had been claimed prematurely.

## 4. REQUIREMENTS AND RESEARCH NEEDED TO ACHIEVE AW-IPM

The requirements for implementing AW-IPM are complex and sophisticated. Of paramount importance is the recognition that AW-IPM must be preventive, offensive, and planned on a multi-year basis (Lindquist 2001; Dyck, Reyes Flores et al., this volume). Requirements include the following (Vreysen et al. 2007):

- The biology, ecology, distribution, population dynamics, etc. of the pest in the target area must be thoroughly understood.
- There must be political support, as well as strong stakeholder cohesiveness and commitment to the campaign (Dyck, Regidor Fernández et al., this volume).
- Adequate funding needs to be available to cover all aspects of the programme.
- An effective and knowledgeable programme leader, supported by an effective organization, preferably independent from the day-to-day government bureaucracy, is needed (Vreysen et al. 2021; Dyck, Reyes Flores et al., this volume). This team must formulate and continuously update both technical and business plans.
- Staff with the proper qualifications, expertise and experience need to be hired for the AW-IPM programme; they should have no other job.
- Relevant monitoring data need to be collected before, during, and after the programme for the managers to be able to make informed decisions. Data need to be analysed properly, and in short intervals, to allow mitigating actions in case of a problem.
- There must be a system of programme review, including by external and independent experts.
- Legal authority is required to execute all aspects of the programme, e.g. conduct operations on private properties, and operate quarantines (section 1.5.).
- Since losses caused by emerging pest situations rapidly mount with the passage of time, the speed required to organize and implement a programme is critically important, especially when it is financed in part by an industry under economic stress (Kruger 2021).

Applied research and methods-development support is essential to backstop ongoing AW-IPM programmes. In addition, there are a number of general research needs that require more attention to further increase applicability, effectiveness, and efficiency of area-wide programmes:

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- Apply the integration of species distribution modelling and landscape genetics to facilitate and optimize the management of target populations (Bakhoum et al. 2021).
- Further improve the use of Geographic Information Systems (GIS), Remote Sensing (RS), mathematical modelling, and data bases to facilitate decision-making in operational programmes, and to better manage large volumes of data sets (Barclay, this volume; Bouyer et al., this volume).
- Study the detection and sampling of very sparse populations. Disagreement (on the interpretation of sample data) enveloped area-wide programmes against the boll weevil in an especially bitter and costly controversy (NRC 1975). Similar difficulties have been encountered in programmes against tsetse flies *Glossina* spp., screwworms, the gypsy moth, and fruit flies (McInnis et al. 2017; Shelly et al. 2017).
- Automate the collection of field data, resulting in substantial cost savings in AW-IPM programmes, e.g. insect traps (baited with a species-specific attractant) that send a radio signal when a catch is made (Schellhorn and Jones 2021).
- Identify sociological barriers, and opportunities to surmount them, in implementing AW-IPM programmes (Kruger 2021; Mankad et al. 2021).
- Encourage the investigation of socio-economic impact to assess benefit/cost ratios and other economic indicators of AW-IPM programmes (Bouyer et al. 2014; Singerman et al. 2017).
- Encourage the implementation of environmental-impact studies to accompany AW-IPM programmes to assess the impact of the programme on the ecosystem (USDA/APHIS 2015).

## 5. KNIPLING'S IMPERATIVE

When the World Food Prize was awarded to E. F. Knipling and R. C. Bushland, Knipling (1992b) stated:

> If major advances are to be made in coping with most of the major arthropod pest problems, then the tactics and strategies for managing such insects, ticks and mites must change. They must change from the current, limited scale, reactive, broad-spectrum measures to preventive measures that are target-pest specific and rigidly applied on an area-wide basis.

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# CHAPTER 2.2.

# BIOLOGICAL BASIS OF THE STERILE INSECT TECHNIQUE

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## SUMMARY

In principle, the sterile insect technique (SIT) is applicable to controlling a wide variety of sexually reproducing insect pests, but biological factors, interacting with socio-economic and political forces, restrict its practical use to a narrower set of pest species and situations. This chapter reviews how the biology and ecology of a given pest affect the feasibility and logistics of developing and using the SIT against that pest insect. The subjects of pest abundance, distribution, and population dynamics are discussed in relation to producing and delivering sufficient sterile insects to control target populations. Pest movement and distribution are considered as factors that influence the feasibility and design of SIT projects, including the need for population- or area-wide management approaches. Biological characteristics, that affect the ability of sterile insects to interact with wild populations, are presented, including the nature of mating systems of pests, behavioural and physiological consequences of mass production and sterilization, and mechanisms that males use to block a female's acquisition and/or use of sperm from other males. An adequate knowledge of the biology of the pest species and potential target populations is needed, both for making sound decisions on the suitability of integrating the SIT into an area-wide integrated pest management (AW-IPM) programme, and on the efficiency and effectiveness of applying the technique.

## 1. INTRODUCTION

In principle, the sterile insect technique (SIT) is simple — the release of a large number of reproductively sterile male insects into a wild population of the same species so that they mate with, and block the reproduction of, wild females (Knipling 1955). Sterility is most often induced by exposing insects to ionizing radiation (Bakri et al., this volume); release of insects with conditionally expressed dominant lethal alleles is increasingly being considered as an alternative and is addressed elsewhere in this volume (Häcker et al., this volume; Lees et al., this volume). Released insects are most often completely sterile (or nearly so), but inherited ($F_1$) sterility (IS) is an option with species (primarily Lepidoptera) in which appropriate substerilizing doses of radiation produce males that are partially sterile but sire completely sterile offspring (Marec et al., this volume).

The successful application of the SIT requires: (1) the ability to rear, sterilize, and distribute the number of insects that will achieve a sufficiently high "overflooding" (sterile:wild insect) ratio in the field, and (2) that the sterile males can successfully compete and mate with their wild counterparts. Although the concept of the SIT is simple, the implementation is complex (Seawright 1988). Insects are mass-produced in an artificial environment and, after being irradiated, are often densely packed and shipped to a distant facility. Their subsequent release into the field can involve procedures such as immobilization, chilling, and ejection from flying aircraft (Dowell et al., this volume). Through all of this the insects must remain "competitive", i.e. able to survive and perform behaviours that allow them to mate successfully with wild insects (Parker, Vreysen et al., this volume).

Several key biological questions must be considered when deciding whether the use of the SIT would be warranted in a given pest situation (Table 1). Although economic and political considerations may drive decisions on when and where the technique is developed and deployed, these biological issues ultimately determine

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both the logistical feasibility and economics of whether the SIT can contribute to the suppression of a given pest population. Understanding a pest's biology also allows programmes to optimize procedures and avoid pitfalls that could make the SIT impractical or ineffective.

*Table 1. Key biological considerations affecting the decision to use the SIT*

| Question | Biological consideration |
|---|---|
| Is the pest an appropriate target for the SIT? | Role of pest in agroecosystems<br>Existence of negating or complicating trait(s)<br>Potential of integration into (typically area-wide) pest management system |
| Can adequate sterile:wild ratios be achieved? | Pest ecology and population dynamics<br>Biological factors affecting production, distribution, and release<br>Integration with other suppression methods |
| Will released sterile males be able to compete effectively against wild males in target populations? | Effects of mass-rearing and sterilization on insect behaviour and physiology<br>Evolution and the SIT<br>Mating systems<br>Post-copulatory factors<br>Potential for enhancing sterile insect competitiveness |

## 2. ECOSYSTEM, AGRONOMIC, LIFE HISTORY, AND BIONOMIC CONSIDERATIONS

### *2.1. Role of Pest in Agroecosystems*

#### *2.1.1. Pest Status*

The SIT is mostly used when the selective removal of, or a great reduction in, a population of an individual species would have significant benefit. Examples (not inclusive) of applicable pest situations are shown in Box 1. Alternatively, the SIT would generally not be warranted if the suppression or elimination of a single pest population would not substantially reduce overall management costs or efforts. For example, Ankersmit et al. (1977) concluded that the use of the SIT against the summer fruit tortrix *Adoxophyes orana* (Fischer von Röslerstamm) in The Netherlands would do little to reduce the number of required insecticide sprays in apple orchards because other tortricid pests are present.

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### *2.1.2. Pest Stage Producing Damage*

In his original treatise on the SIT, Knipling (1955) suggested that:

> It probably would be impractical to release insects which are highly destructive in the adult stage.

The theoretical basis of the SIT is largely unaffected by which stage(s) produces damage, but sterile insects themselves can be nuisances, disease vectors, or agricultural pests (Nagel and Peveling, this volume). For example, in the case of blood-feeding horn flies *Haematobia irritans* (L.) (Patterson and Miller 1982), large releases of sterile insects that would affect livestock may preclude the use of the SIT. If the damage from sterile insects is done primarily by females, e.g. mosquitoes, the development of genetic sexing strains can allow the SIT to be used with few or no negative consequences (Seawright 1988; Franz et al., this volume).

*Box 1. Examples of Pest Situations Where the SIT Could be Considered for Control*

- Incipient population of an non-native pest that, if established, would severely impact agricultural or environmental ecosystems, e.g. eradication of the Mexican fruit fly *Anastrepha ludens* (Loew) in California, USA (Enkerlin, this volume; Hendrichs, Enkerlin et al., this volume; Hendrichs, Vreysen et al., this volume)
- Vector of a serious disease (plant or animal), e.g. tsetse fly *Glossina* spp. eradication programmes (Feldmann et al., this volume)
- Presence of a "key pest" that greatly increases management costs and/or is quarantined in potential export markets, e.g. New World screwworm *Cochliomyia hominivorax* (Coquerel) in North America; *Bactrocera* spp. in Okinawa (Kuba et al. 1996)
- Alternate methods of controlling a pest disrupt ecological processes that regulate populations of other pests, e.g. chemical control of the boll weevil *Anthonomus grandis* Boheman disrupts the biological control of noctuids such as *Helicoverpa* sp.
- Preventing establishment of an important pest by maintaining a continuous population of sterile insects in an area of high risk of introduction, e.g. releases of sterile Mediterranean fruit flies *Ceratitis capitata* (Wiedemann) in Los Angeles, California, USA (Lance et al. 2014).

### *2.1.3. Plant Part Attacked*

In the case of plant pests, the SIT has most often been deployed against insects that attack marketable, especially fruiting, tissues. Small numbers of pests can cause substantial economic damage by attacking these high-value substrates, and, as a technology, the SIT is best used to drive small populations to very low levels or even to local extinction. Similarly, relatively few vectors of plant or animal diseases can be highly damaging. In contrast, many agricultural crops can sustain modest levels of damage to vegetative tissues such as leaves or roots with little or no economic loss, and populations of pests attacking such tissues can, in some cases, be very large (Sutter et al. 1998), and hence the SIT is less appropriate for such pest situations.

## *2.2. Reproductive/Life History Strategy*

Since the SIT relies on sterile males mating with wild females, most sexually reproducing insects are at least potential targets of the technique. Beyond that, various aspects of a species' basic biology tend to make that species relatively

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amenable to (Box 2), or a poor candidate for (Box 3), the SIT. Natural parthenogenesis, even at low levels, is a potential pitfall of the SIT, which could theoretically select for parthenogenesis in wild populations (Templeton 1978). Nevertheless, use of the SIT has been considered against facultatively parthenogenetic aphids (Steffan and Kloft 1973). Controlling eusocial insects, such as termites (diplodiploid), with the SIT, while not theoretically impossible, would also present immense challenges in mass-rearing, sterilization, and release. Haplodiploidy, common in Hymenoptera, could make classical deployment of the SIT (rear, sterilize, release) problematic, but could open possibilities for simplified male-only release strategies. Broad taxonomically based generalizations about the applicability of the SIT are risky except, perhaps, that the technique may often be simpler with holometabolous than hemimetabolous insects (Box 2). The presence of the quiescent pupal stage tends to facilitate the harvest, sterilization, and transport of mass-reared insects. Also, larvae of many holometabolous insects have limited mobility and, in some cases, feed gregariously within a restricted unit of habitat such as a fruit. These habits tend to facilitate mass-rearing, whereas in other cases containerization is required to avoid cannibalism. However, the historical bias of area-wide integrated pest management (AW-IPM) programmes integrating the SIT toward Diptera, Lepidoptera, and to a lesser extent Coleoptera, is arguably, as much as anything, a reflection of the large numbers of pest species in these orders. In particular, they contain high percentages of regulatory pests – plant pests and, in the case of Diptera, pests of public and veterinary health concern. Such pests can be prime targets for government-sponsored development of the SIT.

*Box 2. Examples of Biological Characteristics that Allow, or Increase the Feasibility of, Using the SIT*

- Sexual reproduction (exclusively)
- Methods of mass-rearing are available, or can be developed
- Species is holometabolous (quiescent pupal stage facilitates sterilization and handling)
- Males exposed to sterilizing doses of ionizing radiation can compete with wild males for mates
- Methods are available to monitor released sterile and wild populations
- Low intrinsic rate of increase.

*Box 3. Examples of Biological Characteristics that Could Negate or Severely Complicate Using the SIT*

- Parthenogenesis
- Highly synchronous, aggregated, ephemeral mating system (found in many eusocial insects and other groups such as some Ephemeroptera)
- Extended life cycle, e.g. typical of many cicadas
- Sterile insects themselves are a serious pest, disease vector, or nuisance pest, such as horn flies, locusts, house flies, cockroaches or female mosquitoes
- Migratory behaviour involving long-distance flight and/or movement along weather fronts, as in various moths, locusts, planthoppers and stable flies.

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### *2.3. Potential of Integrating the SIT with Other Control Strategies*

In most applications of the SIT, it is a key component of AW-IPM programmes (Klassen and Vreysen, this volume). As such, the SIT is commonly integrated with other control methods, most often: (1) following pre-release suppression of the target population (often involving insecticides) to the point where the SIT becomes more effective, less costly, or even feasible at all (Knipling 1979), (2) during simultaneous suppression using SIT-compatible controls (for example, the use of larvicides against mosquitoes or screwworms), or (3) exploiting the specificity of the SIT to provide control without disrupting biological control of the target population and/or other species in the area (Knipling 1998). Thus, the potential for area-wide integration of the SIT depends on a species' basic biology, the specific population situation, and the availability of effective and/or compatible suppression tools. Mangan and Bouyer (this volume) discuss supplementary control tactics used in AW-IPM programmes that release sterile insects.

## 3. PEST ECOLOGY AND THE SIT

The successful application of the SIT requires knowledge of the target population's ecology, including estimates of the absolute density of the adult population, and how that density changes over time (Lindquist 1969; Lindquist et al. 1974; Knipling 1979; Itô et al., this volume). For example, the number of insects needed for a given programme depends on the size of the target population, the area covered by the programme, the goal of the programme (Hendrichs, Vreysen et al., this volume), and the required ratio of sterile:wild insects in the field. When Knipling (1955) initially developed the theoretical basis of the SIT, he used a simple mathematical model to demonstrate that a wild population of 1 000 000 insects could be driven to extinction in 4 or 5 generations by maintaining a level of 2 000 000 sterile insects within the area (an initial 2:1 overflooding ratio). This model assumed that the wild population was stable, i.e. the average female produced one female offspring that survived to reproduce, and that sterile males were equivalent to wild males in their ability to mate with wild females. In practice, these assumptions are rarely true.

Subsequent models, which contain parameters that incorporate behavioural and ecological information, provide more realistic estimates of overflooding ratios needed for desired levels of suppression (Knipling 1968; Barclay, this volume; Klassen and Vreysen, this volume). In practice, when high rates of increase are involved, the required overflooding ratios can be quite high. Using empirical data, Brower and Tilton (1975) calculated an optimal sterile:wild ratio of about 100:1 for the almond moth *Cadra cautella* (Walker). In some programmes, ratios greater than 100:1 have not controlled rapidly increasing populations (Vargas et al. 1994; Rendón et al. 2004; Shelly and McInnis 2016).

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### *3.1. Abundance of Pest*

#### *3.1.1. Numerical Size of Population*

The need to produce enough sterile insects to overflood a wild population places practical limits on the size of the target population that can be suppressed or eradicated, and has led to the assertion that the SIT is best applied against relatively small numbers of insects (Knipling 1955, 1979). This can include pests that are widely distributed but tend to occur at low densities, such as the New World screwworm (Knipling 1968), and others that occur in higher densities but exist (at least in the programme area) in relatively small and somewhat isolated patches of habitat. An example of the latter would be populations of Mediterranean fruit flies as they exist in some Middle Eastern areas (Rossler et al. 2000). The SIT has also been used to eradicate highly isolated populations such as the melon fly *Zeugodacus cucurbitae* (Coquillett) in Okinawa (Kuba et al. 1996), and incipient populations of the Mediterranean fruit fly and the Mexican fruit fly in the United States (Penrose 1996; Lance et al. 2014). For the latter types of programmes, pest surveys must be sensitive enough to detect and delimit a population before it grows beyond the capacity of the system and resources available for eradication (Lance 2014).

#### *3.1.2. Pest Population Dynamics*

Since the SIT interacts with a pest population at the point of reproduction, overflooding ratios must account for any tendency of the population to increase. As a simple demonstration, Knipling (1968) extended his 1955 model to show that, if a 2:1 overflooding ratio could reduce or eliminate a "stable" pest population, a ratio of 9:1 or 10:1, i.e. 2:1 X 5, would be needed for a population that was increasing 5X per generation. Such rates of increase are not uncommon among insects. For example, Bartlett and Butler (1979) documented a 10-fold increase per generation in pink bollworm *Pectinophora gossypiella* (Saunders) populations, and Cirio et al. (1972) reported generation-to-generation increases of greater than 40-fold in the Mediterranean fruit fly. Since rates of increase in the field are difficult to predict, operational programmes should be monitored carefully for effectiveness and to ensure that proper overflooding ratios are being maintained (Knipling 1979). Relationships of pest population dynamics to the SIT are discussed by Barclay (this volume) and Itô et al. (this volume).

#### *3.1.3. Seasonality and Voltinism*

Pest populations do not continuously increase at high rates. In warmer regions, many insects breed year-round, or at least go through multiple generations annually, but the populations cycle in response to factors such as the abundance of food, e.g. host plants, weather (temperature, wet/dry cycles), and cropping cycles (Adkisson 1971; Wong et al. 1983). Conceptually, seasonal periods of low and declining pest numbers provide opportunities to effectively apply the SIT against pest populations that are too large during other seasons (Knipling 1968; Lindquist 1969; Adkisson 1971; Hendrichs, Vreysen et al., this volume); empirical data support this concept

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(Iwahashi 1976; Baumhover 2002). However, following reductions in target population numbers, maintaining adequate "pressure" from sterile insects on the pest population can be difficult when resources subsequently become abundant and pest populations rapidly increase in size. For example, Carpenter and Gross (1993) were not able to stop season-to-season increases in populations of the corn earworm *Helicoverpa zea* (Boddie) with releases of substerile males, although they consistently delayed or reduced the extent of those increases. If releasing sufficient sterile insects becomes impractical during some portions of the year, then alternating sequences of the SIT with other methods of pest management, e.g. mass-trapping or cultural control, may prove more cost-effective than continual releases of sterile insects (Cirio 1974; Thomas and Mangan 2005).

Many insect species, especially in temperate areas, have a dormancy period. Dormancy may involve diapause, induced by environmental factors such as photoperiod or temperature, and be either facultative or obligate (Leopold 2007). Temperate species are frequently univoltine (one generation per year); in some, a single generation requires two or more years. Mating, then, is restricted to specific periods within the year, and the production and release of sterile insects must be properly timed to ensure the maximum benefit (Mastro and Schwalbe 1988). If partial sterility carries across generations, conditions that initiate and break diapause in sterile insects must be appropriate, or sterile insects may not be present when needed. As examples, relatively normal diapause characteristics were observed in *H. zea* with inherited ($F_1$) sterility and in sterile backcross hybrids of *Heliothis subflexa* (Guenée) x *Heliothis virescens* (F.) (tobacco budworm), allowing appropriately timed activity and/or survival over winter (Stadelbacher and Martin 1981; Carpenter and Gross 1989). In multivoltine species with facultative diapause, competitiveness can vary between insects that were reared under diapause-inducing versus non-diapause conditions (Bloem et al. 2006; Sarvary et al. 2008).

### *3.2. Dispersion and Dispersal of Wild and Sterile Populations*

Programmes that release sterile insects can be strongly affected by both the dispersion (distribution of organisms over an area) and the dispersal (movement, or displacement, of individuals) of wild and sterile populations. These two parameters are influenced by a variety of ecological and behavioural factors intrinsic to a species' basic biology, and that tend to make that pest species more or less amenable to the implementation of the SIT.

#### *3.2.1. Dispersion*

Most insect populations have clumped distributions with areas of relatively high density amid regions of substantially lower density. This clumping is often related to the distribution of resources, such as host plants, and may vary seasonally (Shiga 1986). For example, Nakamori and Shiga (1993) outlined zones of *Z. cucurbitae* density in Okinawa based on the seasonal availability of host fruits, and identified "hot spots" where host fruits were abundant year-round. In such local areas with high densities of wild insects, the overflooding ratio is substantially lower than the

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overall ratio of sterile to wild insects throughout the programme area. As a result, other things being equal, the effectiveness of sterile insect releases would decrease as the degree of clumping in the target population increases (Sawyer et al. 1987; Barclay 1992). This effect will be overcome to the degree that the sterile insects are distributed, or redistribute themselves, to mirror the distribution of the wild insects. The degree to which sterile insects actually do concentrate themselves in areas of high wild insect density has reportedly varied from high (Knipling 1979; Gavriel et al. 2012; Ageep et al. 2014) to low (Shiga 1986; Hendricks et al. 1973; Meats 2007) across diverse taxa. In any case, the distributions of wild and sterile populations must be understood to be able to allocate and distribute sterile insects optimally (Lindquist et al. 1974; Itô et al., this volume). Geographic information (GIS) and database systems can facilitate the identification, monitoring, and differential treatment of population foci on relatively broad spatial scales (Bouyer et al., this volume). However, if the aggregations of target populations occur on finer spatial scales, or are not predictable, the overall release rates may have to be adjusted upwards to ensure that the local sterile:wild ratios are high enough to achieve the desired level of sterility.

### *3.2.2. Host Specificity*

The distribution of an insect population will, of course, be influenced by the distribution of its hosts and, as a result, by the pest's degree of monophagy or polyphagy. Plant pests targeted in AW-IPM programmes that use the SIT have ranged from relatively monophagous or oligophagous pests such as the pink bollworm to highly polyphagous pests such as *H. virescens* and *C. capitata* (Proshold et al. 1983; Staten et al. 1999; Liquido et al. 2015; Staten and Walters 2021). Monophagy should tend to simplify the application of the SIT, especially if host plants are restricted to discrete patches. For polyphagous pests, the widespread presence of alternate hosts can increase the area, logistical complexity, and costs required for effective control (Vargas et al. 1995). Movement of insects to and from sites of adult food, or other ecological requisites, can also influence pest distribution (Hendrichs and Hendrichs 1990). For example, adult male New World screwworms, when waiting for potential mates, will often perch near sources of adult food (nectar) rather than near the animals that are the larval hosts (Guillot et al. 1978).

### *3.2.3. Dispersal*

The ability of wild insects to move within and between habitat patches influences the required size of release areas, the need for and size of barrier or buffer areas, and the pattern of insect release (Knipling 1979). The immigration of small numbers of mated females or large numbers of males into an SIT release area can potentially disrupt a programme (Prout 1978; Barclay, this volume). The magnitude of the impact from immigration depends on pest biology and on programme goals, with less isolation being required where moderate suppression rather than eradication is desired. For example, Ankersmit et al. (1977) reported that the SIT appeared to be capable of suppressing populations of the summer fruit tortrix in small orchards with a modest degree of isolation, even though the efficacy obtained was not sufficient

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for eradication. The dispersal capacity of a pest species determines the need to isolate treatment areas from immigration, and is the primary factor dictating a population-wide approach to AW-IPM programmes integrating the SIT (Hendrichs, Vreysen et al., this volume; Klassen and Vreysen, this volume).

In eradication programmes, the potential for reinvasion also needs to be considered. The melon fly was eradicated from subtropical Japan (Kuba et al. 1996), but continuous surveys are now needed in the region, with preventive sterile fly releases in the southernmost islands, because this species is capable of flying in from Taiwan (Koyama and Tanaka 1984; Kohama and Kuba 1996). The New World screwworm was eliminated from North America but, because the flies are capable of dispersing more than 280 km, the eradication campaign had to be extended into northern Mexico, covering simultaneously a large area to protect the southern USA. Eventually, these large eradication blocks were progressively moved southward into Central America, where the areas to be covered became gradually narrower, reducing the size and cost of the programme (Lindquist 1969; Jones et al. 1999; McGraw 2001; Vargas-Terán et al., this volume). A wide band of sterile-fly releases across eastern Panama has been in place since the early 2000s to prevent reinvasion from South America. Conversely, when the SIT is used in too small an area against an incipient, isolated infestation, undetected dispersal away from the site of the initial introduction can foil eradication efforts by producing satellite infestations beyond the SIT release zone (Penrose 1996).

Pilot-scale testing of the SIT also requires relatively isolated venues such as islands or oases (Proshold et al. 1983; Cayol and Zarai 1999; Baumhover 2002; Bellini et al. 2013) or, alternately, plots that are buffered with wide barrier or treatment zones (Rendón et al. 2004). Such tests tend to be relatively large-scale, and can produce the added benefit of detecting logistical or biological problems that would not arise in smaller-scale studies (Seawright 1988; McInnis et al. 1996). However, large-scale tests are expensive, and logistical issues often force limits on size and/or replication. As a result, field data on relationships between sterile:wild ratios, sterile insect competitiveness and level of sterility, and effects on wild populations, are minimal in many cases (Krafsur 1998; Vreysen, this volume). In some instances, the initial stages of an operational programme have to function as a feasibility study (Lindquist et al. 1974).

Although long-distance movement can create problems for programmes that apply the SIT, dispersal on a more local scale is essential to the technique's effectiveness. Modelling studies indicate that a moderately high dispersal capability may tend to facilitate the SIT by reducing spatial heterogeneity in the pest population (Barclay and Vreysen 2011; Barclay, this volume). Hence, arthropods that are largely sedentary, such as ticks and mites, as well as various homopterans, are much less amenable to the SIT. Moreover, released sterile males must move sufficiently to locate resources such as food, mating arenas, and/or mates (Parker, Vreysen et al., this volume; Vreysen, this volume). The dispersal capability of sterile males is a primary consideration when designing release methods and protocols for a specific pest species, since it is critical that sterile males are distributed throughout the release area, at least in habitats where wild insects may occur (Andress et al. 2013; Dowell et al., this volume).

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### *3.3. Chemical Ecology*

Chemical communication is often involved in mating, feeding, or other key ecological interactions of insects (Matthews and Matthews 2009). Accordingly, an insect's chemical ecology can have important implications for the SIT (Table 2). In particular, the common involvement of semiochemicals in intraspecific finding and/or recognizing mates means that sterile males must respond to, and in some cases produce, semiochemicals appropriately for the SIT to be effective (Table 2). In addition, an insect's chemical ecology can often be exploited to benefit AW-IPM programmes that integrate the SIT. Long-range attractants are very useful for assessing the distribution of wild and sterile insects (section 3.2.1.), monitoring overflooding ratios (Vreysen, this volume), and evaluating specific aspects of sterile male quality (Parker, Vreysen et al., this volume). In addition, males of species that use a long-range female-produced sex attractant are normally mobile enough to disperse well throughout the release area (sections 3.2.1. and 3.2.3.).

In some species, feeding, or otherwise exposing sterile males to an appropriate compound, can optimize mating competitiveness (Pereira et al. 2013; Pereira et al., this volume). Sometimes these chemicals are components (or precursors of components) of male-produced pheromones, which may be long-range sex attractants or close-range "aphrodisiacs" (Boppre 1990; Nishida et al. 1997; Shelly et al. 2010; McInnis et al. 2011). In other cases, reasons for the enhancement are not clear; e.g. mating competitiveness is improved when sterile Mediterranean fruit fly males are exposed to the parapheromone α-copaene (Shelly and McInnis 2001).

*Table 2. Examples of types of semiochemicals utilized by insects, and their potential implications for programmes releasing sterile insects*

| Type of semiochemical | Implication for programmes | Reference |
|---|---|---|
| Sex attractant pheromone (female-produced) | Used for monitoring or evaluating programmes that apply the SIT<br>Used for assessing sterile male quality | Bloem et al. 1998<br>Staten et al. 1999<br>Staten and Walters 2021 |
| Sex attractant or aggregation pheromone (male-produced) | May be critical component of sterile male competitiveness (section 5) | Heath et al. 1994<br>Bosa et al. 2016 |
| Parapheromone (such as the "male attractants" of tephritids) or aggregation pheromone | Used for monitoring or evaluating programmes that apply the SIT<br>Used for assessing sterile male quality (section 5) | McInnis and Cunningham 1986<br>Andress et al. 2013<br>FAO/IAEA/USDA 2019 |
| Aphrodisiac and/or contact recognition pheromone | May play important role in mating process and affect mating competitiveness (section 5) | Carlson et al. 2007 |
| Host-plant or other food-related kairomones | Used for monitoring programmes that apply the SIT<br>May play critical role in mating system<br>May need to be provided in diet as precursor of pheromone component | Dominiak and Nicol 2010<br>Shelly et al. 2010<br>McInnis et al. 2011<br>Benelli et al. 2014 |

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### *3.4. Sterile Insect Longevity*

The frequency of sterile insect releases will depend on each species, and varies according to the average longevity. Preferably, sterile insects will survive in the field as long as their wild counterparts. If the longevity of sterile insects declines, the frequency of releases, and numbers of insects released, must be increased to maintain the desired overflooding ratio (Dowell et al., this volume; Vreysen, this volume). A reduction in longevity can be a side effect of mass-rearing, strain genetics, sterilization, or handling and release methods (Fay and Meats 1987; Meats 1998). Assessment of the longevity of sterile insects has largely been conducted in the laboratory (Meats 1998; Thomas and Loera Gallardo 1998), but over the past decade there has been increasing recognition that survival in the field is critical and is influenced by factors beyond the scope of laboratory tests. For example, released insects can suffer proportionately higher predation than wild insects if release methods concentrate or temporarily immobilize insects, or if mass production alters normal predator-avoidance behaviour (Iwahashi 1976; Hendrichs and Hendrichs 1998; Hendrichs et al. 2007; González-López et al. 2016). The survival of sterile insects to reproductive age is especially critical, but in many programmes immature adults that suffer significant mortality are released before reaching sexual maturity. Ideally, they should not be released into the field until they are sexually mature (McInnis et al. 2013), or at least have acquired nutritional reserves (Dowell et al., this volume), although this increases the required holding capacity. Many SIT programs now recommend periodically measuring survival of released insects in the field as part of their standard quality control protocols (FAO/IAEA/USDA 2019; Parker, Vreysen et al., this volume; Vreysen, this volume).

## 4. BIOLOGY AND STERILE INSECT PRODUCTION

### *4.1. Feasibility of Rearing and Cost of Production*

Technical issues surrounding the production of sterile insects are reviewed elsewhere (Parker, Mamai et al., this volume), but some biological factors that influence the feasibility of mass-rearing bear mention here. As noted above, sterile insect production should generally be easier with holometabolous than with hemimetabolous species. Also, dormancy periods must be taken into account when designing rearing systems (Parker, Mamai et al., this volume), and, in some cases, they can be exploited to "stockpile" sterile insects (Mastro and Schwalbe 1988; Bloem et al. 2000; Leopold 2007). In some insects, developing specific components of rearing systems can prove intractable, e.g. the continued lack of useful artificial diets for rearing larvae of some bark beetles (Mattanovich et al. 1999), root-feeding beetles (Branson et al. 1988; Klein and Allsopp 1994), or parasites of mammals such as *Dermatobia hominis* (L., Jr.) (Banegas et al. 1967; Arce 1968; Borja 2002).

The cost of rearing insects, in particular, affects the economic feasibility of the SIT (Mumford, this volume), and is influenced by basic biological characteristics. Small insects with rapid life cycles can often be reared relatively cheaply. For example, Mediterranean fruit flies develop from the egg to prepupal stage in 6–9

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days within large trays of relatively inexpensive diet, and several thousand sterile flies can be produced for USD 1 (Hendrichs et al. 2002). At that rate, tens of thousands of Mediterranean fruit flies can be produced for the current cost of rearing a single individual of the Asian longhorned beetle *Anoplophora glabripennis* (Motschulsky), a 3-cm-long cerambycid with a 10-month larval period (Dubois et al. 2002). Developmental and operational costs of mass-rearing can also increase with cannibalism, which can require individual containerization, provision of refugia and/or reduced rearing density (Da Silva and Parra 2013; Fontenot et al. 2015). Some insects also need specialized environments, such as artificial streams for simuliid larvae (Edman and Simmons 1985). In many cases, innovation and automation help to reduce costs and other problems of mass-production (Parker, Mamai et al., this volume). Finally, while lower reproductive rates generally reduce overall SIT costs by requiring lower overflooding ratios in the field, they tend to increase the unit rearing cost because facilities needed to maintain such colonies (tsetse species are an extreme case) are large relative to production output (Hendrichs, Vreysen et al., this volume).

### *4.2. Mass-Rearing and Competitiveness*

A sterile male's ability to compete for mates against wild males is a function of its phenotype, which, as in all living organisms, is determined by the expression of its genotype within its environment. Insects in mass-rearing facilities typically experience biotic and physical environments that clearly are very different from those in which wild insects develop. These environmental differences can influence the phenotype of sterile insects both directly and, along with other factors, by inadvertently selecting for genetic differences between laboratory and wild populations. Careful monitoring of phenotype (manifest as sterile insect quality) is critical to the success of programmes that use the SIT (Parker, Vreysen et al., this volume; Vreysen, this volume).

Many aspects of mass-rearing environments directly affect sterile insect quality. Characteristics of artificial larval or adult diets, such as nutritive elements, contaminants, microbial load (including probiotics), moisture, texture, and pH can influence body size, survival, longevity, flight ability, mating ability, and responsiveness to light (Economopoulos et al. 1990; Villavaso et al. 1998; Chang et al. 2000; Damiens et al. 2012; Yuval et al. 2013); in some cases effects can extend to the following generation (Keena et al. 1998). Handling methods and environmental conditions that optimize, or are simply convenient for, mass-rearing do not always produce the most competitive insect. A classic example is the "droopy-wing syndrome" and poor flight ability that were found to occur in tephritid flies when pupae were sifted from the pupation medium during the time of flight muscle development (Ozaki and Kobayashi 1981). Lance et al. (1988) reported another example where holding *Lymantria dispar* (L.) pupae at typically warm laboratory temperatures "programmed" sterile males with inappropriately timed mating activity. Conversely, temperature preconditioning (cold-conditioning) enhanced the survival and mating success of sterile male Queensland fruit flies *Bactrocera tryoni*

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(Froggatt) (Fay and Meats 1987). More details on rearing methodology and insect quality are presented by Parker, Mamai et al. (this volume) and Parker, Vreysen et al. (this volume).

Reproductive sterility is typically induced by exposure to X-rays, electron beams, or gamma rays from a $^{60}Co$ or $^{137}Cs$ source (LaChance 1975; Bakri et al., this volume; Robinson, this volume), which all cause chromosomal damage. Sterility is usually permanent, although irradiated males of some species may, over time, regain at least partial fertility, especially following multiple mating (Brower 1976). Nevertheless the irradiation process typically reduces insect quality in some measurable way. Various strategies minimize somatic damage and thus preserve quality: irradiating insects near, or after the completion of, adult development, i.e. late-stage pupae or adults, irradiation in a reduced-oxygen atmosphere, "fractionating" the dose into several smaller doses, and using irradiators with small maximum to minimum dose ratios (which allow the minimum sterilizing dose to be achieved without substantially overdosing a large proportion of the insects) (Economopoulos 1977; ISO/ASTM 2013; Bakri et al., this volume; Parker, Vreysen et al., this volume).

Radiation doses that sterilize males typically kill oogonial cells, but the radiation sensitivity of oocytes varies with such factors as maturity and meiotic stage. As a result, females of some species may retain a degree of fertility after irradiation, especially when treated late in development. For example, in *C. capitata,* careful monitoring is needed to ensure that pupae are not irradiated too early, resulting in poor quality, or too late, leaving some females with residual fertility (Williamson et al. 1985). Some insect species that are irradiated as adults, such as the boll weevil, require alternative or augmentative sterilization strategies (McKibben et al. 2001).

Chemical sterilization, symbiont-based cytoplasmic incompatibility (CI), and incorporation of conditionally lethal genes are among the alternative methods of generating mass-reared insects for genetic control programs (Augustinos et al., this volume; Häcker et al., this volume; Hendrichs and Robinson, this volume; Robinson, this volume). In particular, advances in molecular biology have facilitated the production of strains for release of insects carrying a dominant lethal (RIDL), which can potentially be applied using either a bisexual or self-perpetuating sex-linked (males only) approach (Hendrichs and Robinson, this volume). RIDL, as well as *Wolbachia*-based CI, have been advanced furthest for mosquitos (Alphey 2014; Augustinos et al., this volume; Lees et al., this volume), but both have been under development for other taxa as well (Zabalou et al. 2009; Morrison et al. 2012; Leftwich et al. 2014; Häcker et al., this volume).

Chemosterilization was considered a viable alternative to radiation early in the development of the SIT (LaBrecque and Smith 1968), but was largely abandoned due to the potential of chemical residues on the sterile insects that can have mutagenic effects on non-targets (including humans). Much safer insect chemosterilants are now available but their use to date has been proposed primarily for field application (autodissemination) rather than SIT production (e.g. Moya et al. 2010).

Mass-rearing can also produce genetic differences between wild and laboratory populations (Cayol 2000). Indeed, dramatic shifts in strain genetics, including reductions in diversity (heterozygosity), can be observed within a relatively few

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generations under laboratory conditions (Zygouridis et al. 2014). Genetic changes in rearing colonies have been cited as the likely causes of shifts in such traits as flight ability, mating age, age at first reproduction, cuticular hydrocarbons, and adult longevity (Spates and Hightower 1970; Itô and Koyama 1982; Pomonis and Mackley 1985; Hammack 1987; Mangan 1991; Miyatake and Shimizu 1999; Suenaga et al. 2000; Meats et al. 2004). Mating arenas, in particular, may differ greatly between field and laboratory environments, and inadvertent selection of inappropriate mating behaviours in mass-reared colonies could be especially detrimental to the SIT (Edman and Simmons 1985; Briceño and Eberhard 1998; Shelly 2012). Strategies for maintaining the competitiveness of mass-reared strains include holding colonies under "relaxed" conditions to minimize selection of undesirable traits, regular replacement of mass-reared strains, and active selection for desirable traits such as mating compatibility with wild strains (Leppla et al. 1983; McInnis et al. 1985; McInnis et al. 2002; Orozco-Davila et al. 2014; Bosa et al. 2016; Quintero-Fong et al. 2016). Of course, suboptimal genetics in mass-reared strains can result from factors other than inadvertent selection, such as genetic drift associated with "bottlenecking" or even less-than-ideal traits in the insects that were used to found the colony.

## 5. MATING SYSTEMS

Given that population suppression by the SIT is overwhelmingly a function of matings between sterile males and wild females (McInnis et al. 1994), the ability of released sterile males to compete for mates is critical. The mating competitiveness of sterile males is a function of their mating propensity and mating compatibility. Mating propensity, the tendency to locate a mate, copulate and inseminate, is primarily of concern as a component of sterile insect quality (Parker, Vreysen et al., this volume). Mating compatibility is a relative measure of how readily two populations of insects are reproductively compatible, and, in relation to the SIT, most often refers specifically to matings of sterile males with wild females (FAO/IAEA/USDA 2019). Parker, Vreysen et al. (this volume) describe methods of assessing and quantifying compatibility. In programmes that release sterile insects, it is necessary to ensure that those insects are compatible with the target insect population (FAO 1992; Cayol et al. 2002).

Insect mating systems are almost as diverse as the insects themselves, and have been categorized in a variety of ways, such as their relation to ecological resources (Hendrichs et al. 2002), type or degree of aggregation, the type or extent of male-male competition (Robacker et al. 1991; Hendrichs et al. 2002), means by which females select mates (Eberhard 1996), or the involvement and type of semiochemicals. Insects use a variety of sensory modalities to locate, identify, and evaluate potential mates and related resources, including vision, sound, odours, contact chemoreception, and "touch" (Matthews and Matthews 2009).

For the SIT, the modalities used in an insect's mating system have to be understood. Sterile males must be competent in their ability to communicate with females, as receiver and/or sender of signals, to be fully competitive (Table 3). Most

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mating systems, in themselves, do not preclude the use of the SIT, but they influence the efficiency and logistical difficulty of the technique. In general, greater levels of complexity in the role of the male in mating will require more effort in tracking male behaviour as a part of product quality control (Hendrichs et al. 2002; Parker, Mamai et al., this volume), and will lower expectations of high mating competitiveness of mass-produced sterile males (Shelly and McInnis 2016).

*Table 3. Characteristics of insect mating systems that are favourable or less favourable for the development and operation of programmes releasing sterile insects*

| Characteristic of mating system | Favourable | Less favourable |
|---|---|---|
| Behavioural role of male, including any courtship ritual | Simple | Complex |
| Female choice of mates | Passive (accepts first male) | Active (chooses among males) |
| Sex pheromone | Female-produced, simple (1- or 2-component), long-range | Male-produced, complex |
| Characteristics of adult male | Long-lived, active disperser | Short-lived, sedentary |
| Male-male competition | Indirect (scramble for mates) | Contest for mates or resources |
| Mating in time and space | Distributed throughout habitat, asynchronous | Highly aggregated, e.g. termite swarms |

### *5.1. Simple Mating Systems*

Relatively simple mating systems often involve scramble competition for females. For example, adult female gypsy moths emerge essentially mature, do not feed, and begin "calling" (releasing a single-component sex attractant) near their pupation sites, which are spread throughout their habitat. To mate successfully, a male moth must be active at the time of day when females begin calling (Lance et al. 1988), be capable of locating the source of the pheromone (before another male finds it), and then recognize and attempt copulation with a female when he, literally, steps on her (Charlton and Cardé 1990). Mating is slightly more complex in the New World screwworm where male flies must locate and perch in sites where they can encounter flying females (Guillot et al. 1978). The males dart out and grab at small objects flying by, recognizing and attempting to mate with females if a contact pheromone is present (Hammack 1992). Such relatively simple mating systems are amenable to the SIT, and can lead to the production of highly competitive sterile males. Sterile male gypsy moths are typically nearly 100% competitive, based on their ability to locate pheromone sources (Mastro 1978), and on the relationship of induced egg sterility to the ratios of sterile:wild males trapped during pilot tests

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(Mastro and Schwalbe 1988; Simmons et al., this volume). One downside of simple mating systems, especially those with pure scramble competition, is that they are often associated with short adult lifespans and compressed mating periods (sections 3.4. and 3.1.3.).

### *5.2. Complex Mating Systems*

The difficulty of producing highly competitive sterile males will, as a general rule, increase with the complexity of the males' mating-related behaviours (Shelly and McInnis 2016). For example, Mediterranean fruit fly males attract females by releasing a very complex pheromone (Jang et al. 1989) from an appropriate microhabitat, which typically, but not always, is the underside of a sun-lit leaf. Males often call near other calling males at locations that have been referred to as leks (Prokopy and Hendrichs 1979). When a female approaches a male, he initiates a complex courtship ritual and, if the female remains to the end of the display, he attempts to mount her. Following mounting the female can mate, or reject the male by dropping from the leaf (Lance et al. 2000). Given the complexity of male behaviour, differences in pheromone composition and sexual behaviour between wild and sterile male Mediterranean fruit flies are not unexpected (section 4.2.), and in fact have been quantified (Heath et al. 1994; Briceño and Eberhard 1998; Shelly 2012; Vaníčková et al. 2012). Accordingly, in small-scale mating assays through pilot-scale tests, sterile male Mediterranean fruit flies have usually been less than fully competitive, and in very extreme cases less than 1% competitive (Wong et al. 1986; McInnis et al. 1994, 1996; Rendón et al. 2004). Such lapses in mating competitiveness can increase costs, and compromise the effectiveness of programmes that release sterile insects (Parker, Vreysen et al., this volume; Vreysen, this volume; Whitten and Mahon, this volume). In spite of this, the SIT is increasingly being used against the Mediterranean fruit fly and other tephritids in suppression, eradication, containment and prevention contexts (Rossler et al. 2000; Lance et al. 2014; Enkerlin, this volume; Hendrichs, Vreysen et al., this volume). Several potential methods of enhancing the competitiveness of sterile Mediterranean fruit flies have been investigated, such as optimizing pre-release feeding regimes (Yuval et al. 2007, 2013) and hormonal treatments (Pereira et al. 2013). "Aromatherapy", the pre-release treatment of adults with semiochemicals (section 3.3.), has been shown consistently to enhance Mediterranean fruit fly mating competitiveness, and is now an integral part of the operating procedures at most fly emergence and release facilities (Shelly and McInnis 2001; Dowell et al., this volume; Pereira et al., this volume).

The mating behaviour of sterile males becomes especially critical when females, as in the example above, actively choose among mates based on male phenotype. In these insects, seemingly minor differences in behaviour between wild and sterile insects can translate into poor competitiveness (Lance et al. 2000). In addition, selection can potentially favour wild females that are adept at identifying and rejecting sterile males, resulting in wild populations that are "behaviourally resistant" to the SIT (Itô et al., this volume; Whitten and Mahon, this volume).

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Apparently, this occurred after several years of sterile insect releases against *C. capitata* on the island of Kauai, Hawaii, USA — the percentage of successful courtships dropped from about 10% to 1 or 2% for interactions between sterile males and wild females; however, the compatibility of the sterile males with wild females from other islands was unaffected (McInnis et al. 1996; Lance et al. 2000). A similar erosion of mating compatibility had previously been noted and overcome during the SIT-based successful eradication of *Z. cucurbitae* in Japan (Hibino and Iwahashi 1991).

## 6. POST-COPULATORY FACTORS

The effectiveness of mating between sterile males and wild females can be lost partially or entirely if the females also mate with wild males, and preferentially use sperm from the latter for fertilization. To determine if the SIT is appropriate for use against an insect species, Knipling (1955) proposed that one principle to consider was:

> Females must normally mate only once.

This assertion is still occasionally voiced dogmatically, at least as a question, although polyandry does not negate the basic principles of the technique (Barclay, this volume; Whitten and Mahon, this volume). However Knipling (1955) continued:

> If females of a species mate more frequently, the sperms from irradiated (sterile) males must be produced in essentially the same number and compete with sperms from fertile males.

Indeed, all else being equal, the overall sterility induced into a population of ten females by a sterile:wild overflooding ratio of 9:1 should be the same whether each female mates once — nine with a sterile male and one with a wild male — or each female mates ten times — nine times with sterile males and once with a wild male. This issue is broader than Knipling's assertions, but regardless of the number of times that a female "normally" mates, the competitiveness of sterile males will be influenced by post-mating factors, including the ability to induce mating refractoriness in females, sperm competition, and/or sperm precedence, depending on the species.

Patterns of female receptivity include variations on three basic themes: (1) monogamy, (2) females cycle through periods where they alternately are or are not receptive, and (3) continuous receptivity (Ringo 1996). In most insects, female receptiveness drops sharply after mating, typically due to a physiological response to materials passed from male to female during copulation (LaChance 1975; Eberhard 1996; Ringo 1996; Kraaijeveld et al. 2005). In many species the sperm or spermatophores themselves produce the effect, but in others (best documented among dipterans) an "anti-aphrodisiac" in the seminal fluid — often an accessory-gland protein — produces the change (Ringo 1996). An accessory-gland factor has been documented in *C. capitata*, and males from sterile and wild strains were found

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equally competent at inducing females to shift from mate-seeking to oviposition behaviours (Jang et al. 1998); similarly, sterile and wild *Anastrepha fraterculus* (Wiedemann) were found to be comparable in rendering females refractory to further mating (Abraham et al. 2013). Female *Z. cucurbitae* became unreceptive after mating either with virgin or with "spermless" (sperm-depleted) males (Kuba and Itô 1993), and female *Anopheles gambiae* Giles became refractory after mating with males that were rendered spermless by RNAi silencing of a germ cell differentiation gene (Thailayil et al. 2011).

In other species, the transfer of a full complement of sperm appears to be the critical factor that turns off female receptiveness. For programmes for these other species, this requires that sterility be based on dominant lethal mutations rather than, for example, on the elimination of sperm production (LaChance 1975). Male lepidopterans produce sperm that are apyrene (anucleate) as well as eupyrene (functional), and in particular the presence of eupyrene sperm appears to be important in shutting off receptivity (LaChance 1975). Radiation doses that cause reproductive sterility can also reduce the quantity and/or quality of a male's sperm (North et al. 1975; LaChance et al. 1979; Proshold et al. 1993). Also, sperm are often depleted faster (after fewer matings) in radiation-sterilized males than in unirradiated males (Haynes and Mitchell 1977). The $F_1$ sterile progeny of substerilized males may also transfer less than a full complement of sperm (Carpenter et al. 1987; Proshold et al. 1993; Marec et al., this volume). Sterilization-related reductions in the amount of sperm transferred to females can reduce sterile male competitiveness by increasing the incidence of remating among females that mate with both sterile and wild males (Haynes and Mitchell 1977; Carpenter et al. 1987).

When females mate with both sterile and wild males, the proportion of eggs fertilized by the sperm of sterile males can be influenced by the species' patterns of sperm precedence and/or the competitiveness of the males' ejaculates. In many species sperm from recent matings takes precedence over sperm from earlier matings (Brower 1975; Etman and Hooper 1979), although other species show first-mating (El Agoze et al. 1995) or variable sperm precedence (Conner 1995; LaMunyon and Huffman 2001). In some species sperm precedence is complete, or nearly so (Brower 1975; Etman and Hooper 1979), and specialized mechanisms exist to expel or otherwise inactivate sperm from previous matings (Waage 1979). However, often sperm from different matings mix to various degrees, and the proportion of offspring a male sires will depend at least in part on the competitiveness of his ejaculate. The Mediterranean fruit fly shows a general sperm precedence for more recent matings (Saul and McCombs 1993), but sperm from earlier matings is conserved and may become more available as fresher sperm is depleted (Scolari et al. 2014).

Ejaculate competitiveness can potentially be related to a variety of factors such as male age (LaMunyon 2001), but often the determinant is simply the quantity or quality of sperm transferred (Saul and McCombs 1993; Alyokhin and Ferro 1999; LaMunyon and Huffman 2001). The proportion of a multiple-mated female's eggs that any given male fertilizes can be reduced by sterilization procedures (LaMunyon 2001). This effect has been shown to be dose-dependent in the fall armyworm

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*Spodoptera frugiperda* (J.E. Smith) (Carpenter et al. 1997), and influenced by the age at irradiation in the boll weevil (Villavaso et al. 1998).

## 7. CONCLUSIONS

No insect is a "perfect" target for the SIT. Sterile Mediterranean fruit flies can be produced in large numbers at a reasonable cost, but the high release rates required, and the complex role of the male in mating, can create problems for operational programmes. New World screwworm flies have a relatively simple mating system but are not easy to rear, and in a mass-production situation the quality of a colony tends to degrade rapidly (Mangan 1991). Although numbers of tsetse required for release are much lower than for other species, it is also problematic to rear them in sufficient numbers (Opiyo et al. 2000). In spite of these problems, the SIT is being used successfully against all of these insects in AW-IPM programmes that, in some cases, are among the most extensive insect management programmes ever undertaken.

For other insects, such as the boll weevil and gypsy moth, functional SIT technology has been developed but is not being used (at least not on a significant scale) because simpler or more cost-effective alternative control methods are available. Agronomic, socio-economic, and biological factors must be weighed when deciding whether the SIT is an appropriate tool for managing a given pest. One generalization that probably can be made regarding pest biology and the SIT is this: when considering, developing, or conducting an AW-IPM programme integrating the SIT, an understanding of the pest's biology is critical to making appropriate decisions and to the overall success of the programme.

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# CHAPTER 2.3.

# GENETIC BASIS OF THE STERILE INSECT TECHNIQUE

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## SUMMARY

The use of the sterile insect technique (SIT) for insect control relies on the introduction of sterility in the females of the wild population. This sterility is produced following the mating of these females with released males carrying, in their sperm, dominant lethal mutations that have been induced by ionizing radiation. The reasons why the SIT can only be effective when the induced sterility in the released males is in the form of dominant lethal mutations, and not some form of sperm inactivation, are discussed, together with the

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relationship of dominant lethal mutations to dose, sex, developmental stage and the particular species. The combination of genetic sterility with that induced by radiation is also discussed in relation to the use of genetic sexing strains of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) in area-wide integrated pest management (AW-IPM) programmes that integrate the SIT. A case is made to lower the radiation dose used in such programmes so as to produce a more competitive sterile insect. Increased competitiveness can also be achieved by using different radiation environments. As well as radiation-induced sterility, natural mechanisms can be recruited, especially the use of hybrid sterility exemplified by a successful field trial with tsetse flies *Glossina* spp. in the 1940s. Modern biotechnologies are having some impact on the SIT, especially regarding the introduction of markers for released flies and the construction of genetic sexing strains, as well as other applications such as molecular sterility. Nevertheless, it is concluded that using a physical process, such as radiation, will always have significant advantages over genetic and other methods of sterilization for the large-scale application of the SIT.

## 1. INTRODUCTION

E. F. Knipling realized in the 1930s (Lindquist 1955) that, if male insects could be sterilized genetically without affecting their ability to mate, then they could be used to introduce a genetic load into a wild population in the field that would lead to its suppression or even eradication. For some time, geneticists were aware that X-rays could induce mutations in insects (Runner 1916; Muller 1927), but it was not until A. W. Lindquist showed a publication by H. J. Muller (1950) to Knipling that applied entomologists realized the great potential it offered (Baumhover 2001, 2002). The results from the first experiments to sterilize the New World screwworm *Cochliomyia hominivorax* (Coquerel) were published in 1951 (Bushland and Hopkins 1951). This demonstration, that X-rays could indeed induce sterility, was the first small step on the way to the eradication of this important livestock pest in the southern states of the USA, and then in Mexico and all the countries of Central America, as well as Panama (Vargas-Terán et al., this volume). A permanent barrier of sterile insects has been established in eastern Panama to prevent the reinvasion of the pest from South America. Baumhover (2001, 2002) provided a historical account of the early days of the screwworm eradication programme, and Klassen et al. (this volume) describe the sterile insect technique (SIT), in general, from an historical perspective.

During the first field trials of sterile screwworms in Curaçao, the genetic basis of sterility was poorly understood, but it was realized that sterility resulted from the induction of dominant lethal mutations in the irradiated sperm (Bushland and Hopkins 1951; LaChance et al. 1967). At that time the level of understanding of the genetics of the screwworm led Bushland to comment that (quoted by LaChance 1979):

> . . . we eradicated screwworms from Curaçao and the south-eastern United States without knowing how many chromosomes it had.

Prior to the adoption of radiation to sterilize insects, and following the discovery in the early years of the Second World War that mustard gas and other chemicals can produce mutations and affect fertility in *Drosophila* (Oehlkers 1943; Beale 1993), chemical mutagens were extensively evaluated (Borkovec 1966). However, difficulties relating to toxicity, handling, and residues were considerable, and so radiation has usually been the method of choice. Even though field trials with chemosterilized *Anopheles albimanus* Wiedemann mosquitoes in El Salvador were successful (Breland et al. 1974), it is unlikely that today such releases could be carried out.

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## 2. STERILITY REQUIREMENTS FOR SIT APPLICATION

It is very important that the word "sterility" be precisely understood in terms of its use in the SIT. The word "sterility" describes one of many possible end points of the reproductive process, but it can cover a multitude of causal factors. The following definitions of sterility were taken at random from three biological dictionaries:

- Structural or functional inability to reproduce,
- Involuntary total inability to reproduce,
- Any complete or partial failure to produce functional gametes or viable zygotes.

These definitions cover genetic, physiological, morphological or even "psychological" factors, which can lead to a final end point of sterility, and clearly many of these manifestations would not be useful for sterility in area-wide integrated pest management (AW-IPM) programmes. For the SIT to be effective, females of the wild population in the field have to be permanently prevented from reproducing, and any factor(s) transferred by the released male that accomplishes this would, in fact, be sufficient. True genetic sterility in released male insects requires: (1) production of viable sperm, (2) their transfer to the wild female during mating, (3) their use in fertilization of eggs, and (4) the inability of the fertilized zygote to complete development to a fertile adult. Thus, an irradiated male insect must be able to carry out all the functions of a normal fertile insect — it must produce fully functional sperm that succeed in fertilizing eggs and initiating the development of fertilized eggs.

In the SIT, the radiation-induced sterility is actually produced in the generation following the release of the males, i.e. with the death of the embryo, larva, pupa or adult, or the production of $F_1$ adults that themselves produce gametes that result in zygotes that do not develop. A male insect that cannot mate, is aspermic, or that transfers non-functional sperm, could be classed as sterile, but males with any of these defects would probably not be effective for the SIT.

The International Plant Protection Convention (IPPC 2017) adopted the following definition of a sterile insect:

> An insect that, as a result of a specific treatment, is unable to reproduce.

and the following definition of the SIT:

> Method of pest control using area-wide inundative release of sterile insects to reduce reproduction in a field population of the same species.

Irradiated males must also be able to transfer the appropriate accessory gland fluids during mating, ensuring that female behaviour corresponds to that following mating with a fertile male. In some insects, this female post-mating response involves temporary or permanent refractoriness to further mating, and a change in female behaviour. In *Drosophila* sp. the peptides transferred in the accessory fluid, that are involved in the female post-mating behavioural changes, have been well studied (Chen 1996), and it has even been possible to sterilize females by the ectopic expression (i.e. gene expressed in all tissues) of a transgene which codes for the sex peptide (Aigaki et al. 1991). In fact, a male that only transferred accessory gland fluids, and which could elicit the correct post-mating female response, could theoretically "sterilize" the female. In the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), it has been shown that irradiated sterile males produce the same kind of change in wild female

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behaviour, from mating to oviposition, as do fertile males (Jang et al. 1998; Jang 2002).

In species where females remate (von Borstel and Knipling 1960), sterility for use in the SIT must be efficiently induced in sperm without affecting sperm function and its capacity to compete with other sperm, and exert its effect only after fertilization of the female egg. Dominant lethal mutations are such sterility factors. They are readily induced in all chromosomes by irradiation, and they have little effect on the phenotype of the sperm, at least at the doses usually used for the SIT (Bakri et al., this volume).

Lethality occurs when the haploid nucleus, carrying such a mutation or mutations, is combined with a normal haploid nucleus, resulting in the death of the early embryo at the moment when the genetic information required for normal development is absent or incorrect (Muller 1927). In addition, cell division can become asynchronous and lead to the death of the zygote.

## 3. DOMINANT LETHAL MUTATIONS

The mechanisms by which these mutations cause lethality in Diptera in the developing zygote are now well documented (LaChance 1967; Smith and von Borstel 1972); they are illustrated in Figure 1.

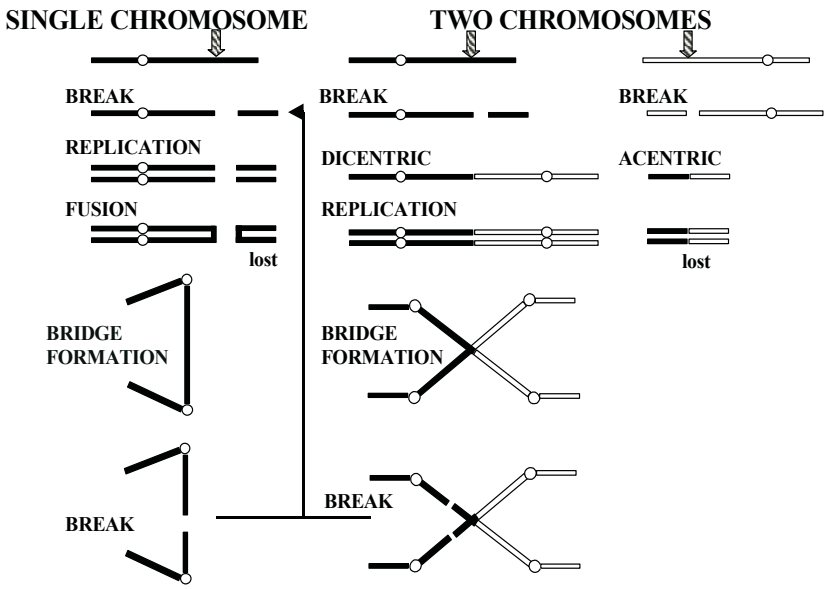



*Figure 1. Schematic representation of the fate, during cell division in the embryo, of chromosomes with radiation-induced dominant lethal mutations, leading to the accumulation of serious imbalances in the genetic information of subsequent daughter cells.*

The primary lesion leading to a dominant lethal mutation is a break in the chromosome, in this case, induced by radiation. When a break is induced in a chromosome in mature sperm, it remains in this condition until after the sperm has entered an egg. (This was shown very elegantly by mating queen bees to irradiated

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males, and then measuring fertility after storage of the sperm in the spermatheca for one year; there was no difference in the level of sterility in the same queen tested one year later (Lee 1958)). Following fusion, nuclear divisions begin, and a break in a chromosome can have drastic effects on the viability of the embryo as development proceeds. During early prophase the broken chromosome undergoes normal replication, but during metaphase the broken ends can fuse leading to the formation of a dicentric chromosome and an acentric fragment. The acentric fragment is frequently lost, while the dicentric fragment forms a bridge at anaphase leading to another chromosomal break. This whole process then repeats itself, leading to the accumulation of serious imbalances in the genetic information of the daughter cells. The accumulation of this genetic damage finally leads to the death of the zygote.

If two different chromosomes are broken they can also rejoin in the way depicted in Fig. 1. These chromosomes produce the same problems for the dividing cells as those formed by a break in a single chromosome, by undergoing incorrect fusion and leading to the *breakage-fusion-bridge cycle* (McClintock 1941). In this way dominant lethal mutations can cause cell death, and the accumulation of genetic imbalance in the developing zygote leads to lethality.

### *3.1. Dose Response for Dominant Lethals*

Dose-response curves for the induction of sterility by radiation are generally developed using measurements of hatchability of eggs, deposited either by irradiated females mated with non-irradiated males, or by non-irradiated females mated with irradiated males. The implicit assumption is that most dominant lethal mutations exert their effect during early embryonic development. Data from *Drosophila* sp., and other dipteran species containing chromosomes with monokinetic centromeres, have shown that this is the case (Demerec and Fano 1944; Catcheside and Lea 1945; Lee 1958; Tantaway et al. 1966; Franz 2000), making egg hatch an appropriate stage to evaluate. In insect species with holokinetic chromosomes, many dominant lethal mutations exert their lethal effects only at later developmental stages (Marec et al., this volume).

Dose-response curves for dominant lethal mutations can be developed by irradiating insects with increasing doses of radiation and calculating the percentage egg hatch. The curves tend to show a characteristic shape, depending on the cell type and stage irradiated. The shape of the curve can be used to infer information about the types of initial chromosomal lesion producing the lethal effect. In irradiated sperm, there is an approximately linear relationship between the dose and the induction of dominant lethals at low doses, but at higher doses there is a noticeable departure from linearity, i.e. it tends to saturate at higher doses, and approaches 100% sterility asymptotically. This is due to the induction of multiple lethal events in the same cell, but only one is needed to cause the egg to die. The shape of the curve, as well as indicating the underlying causal factors of dominant lethality, should help in selecting a dose that will be used to sterilize insects for release. Fig. 2 shows the dose-response curves for the house fly *Musca domestica* L. and the large milkweed bug *Oncopeltus fasciatus* (Dallas). The difference in the shape of the curves is due to the fact that the latter species has holokinetic chromosomes (section 3.4.), and this results in the need for much higher doses of radiation for sterilization.

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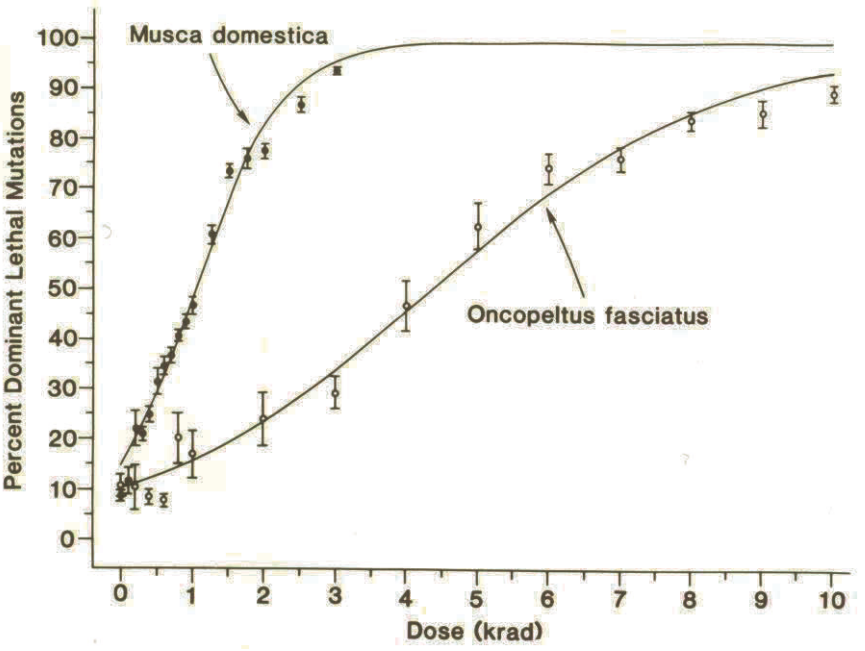



*Figure 2. Dominant lethal dose-response curves for* Musca domestica *and* Oncopeltus fasciatus*. (Figure from Lachance and Graham 1984, reproduced with permission from Elsevier.)*

Assuming that dose-response curves have been developed for both sexes, that both sexes are released, and that close to full sterility is required, then the minimum radiation dose chosen must lead to full sterility in females. Any residual fertility in released females can be extremely damaging, as this will actually contribute to the reproductive potential of the wild population. For example, a residual fertility of 2% in irradiated females, following a sterile:wild release ratio of 100:1, will actually double the number of insects in the next generation. Residual fertility in males is of less importance; it simply reduces the rate at which the population is suppressed.

These simplistic assumptions ignore the impact of any density-dependent regulation in the population. The full impact that the decision — on the dose to be used, based on the shape of the curve (Fig. 2) — has on the effectiveness of the SIT is described below (section 4.).

### *3.2. Dominant Lethals and Sex*

Male and female insects normally differ considerably in their sterility response to radiation, primarily because sperm are haploid post-meiotic cells, whereas mature eggs are pre-meiotic. Eggs usually develop in the female to metaphase 1, and then are arrested until oviposition when meiosis is completed with the production of a single

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pro-nucleus and three polar bodies. Mature eggs in the female are much more tolerant of radiation than are the earlier oocytes, with the consequence that a radiation dose which induces full sterility in mature eggs often leads to the cessation of oogenesis at a later date. This can lead to an increase in the lifespan of females as energy is redirected away from reproduction to survival (White and Hutt 1970; Hafez and Shoukry 1972). In most cases this is of no consequence, but it can be important if the sterile females that are released are themselves damaging in some way. In tsetse, for example, it is essential to remove sterile females from the released insects as their increased lifespan might enable them to be efficient vectors of disease (Dean and Clements 1969; Abd-Alla et al. 2013).

In addition to this basic difference between male and female germ cells, there are also species-related differences in the overall sensitivity of male and female insects to radiation sterilization. In most species, females are more sensitive than males to radiation sterilization, and therefore it is possible to identify the sterilizing dose for the SIT based on the acceptable dose for males (section 4); use of this dose will ensure that all released females are fully sterile. In the minority of species where the female is more tolerant (Haynes et al. 1977; Crystal 1979), the sterilizing dose for females must be used, and this can compromise the competitiveness of the sterilized males. These patterns of differential sensitivity of germ cells in males and females are important to investigate when the radiation dose for the SIT is identified.

### *3.3. Dominant Lethals and Developmental Stage*

To maximize genetic damage to mature germ cells and minimize somatic damage, insects should be irradiated as late as possible in the development pathway, ideally as fully differentiated adults. In the adult stage, insects are most tolerant to somatic damage because somatic cell division is at a minimum. However, even in a fully differentiated insect, certain somatic cells, especially in the gut, continue to divide, and sterilizing radiation doses can compromise viability and hence the fitness of the treated insect (Flint et al. 1966).

In spite of these biological relationships, operational considerations in implementing the SIT often play the major role in determining the stage at which insects are irradiated. The operational decision is based on identifying the most convenient stage for radiation, from the point of view of mass-rearing, handling and release protocols. In most insects, this is the pupal stage, and consequently radiation is applied to pupae as late in development as possible. In New World screwworm and Mediterranean fruit fly AW-IPM programmes applying the SIT, pupae are irradiated two days before emergence, enabling the shipment of irradiated pupae to fly emergence and release facilities far from the production plant, where adults emerge and are fed and processed for release.

In AW-IPM programmes integrating the SIT for some other species, adult insects have to be irradiated. In Lepidoptera, larvae generally pupate within a cocoon that attaches itself to a substrate, and in these cases it is impractical to irradiate this stage, and thus teneral adults are irradiated (Proverbs et al. 1975; Boersma 2021). In tsetse, before release, adult males are fed blood containing a trypanocide and irradiated at 3-4 days of age (Dyck et al. 2000; Vreysen et al. 2000). In general, operational factors tend

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to be decisive in determining the developmental stage that is irradiated (Bakri et al., this volume).

### *3.4. Dominant Lethals and Species*

The class Insecta comprises 31 orders (Wheeler et al. 2001), and pest species are found in most major orders. This very diverse class exhibits widely different sensitivities, both within and between orders, to the induction of dominant lethal mutations (Bakri et al. 2005; IDIDAS 2020), and to the somatic effects of radiation (Willard and Cherry 1975). Probably there are many factors that contribute to these differences, including the centromere structure (Smith and von Borstel 1972), the degree of chromosome condensation (Israelewski 1978), radiation repair (Beatty and Beatty 1967), and the chromosome environment (Thoday and Read 1947). This is certainly not an exhaustive list, and other factors, as yet unidentified, could still be important.

Insects can, however, be divided into two main groups which show major differences in sensitivity to the induction of dominant lethals, and for which experimental evidence is available that identifies the underlying biological and genetic factors responsible. Orders such as Diptera, Hymenoptera, and Coleoptera can be classed as radiation-sensitive, while orders such as Lepidoptera and Homoptera are more radiation-tolerant. A major difference between these two groups of insects is that the former group has a localized centromere (monokinetic), while the latter has a diffuse centromere (holokinetic) (Bauer 1967), and this difference plays a major, although not exclusive, role in radiation sensitivity (Tothová and Marec 2001). Lepidoptera also do not show the classical breakage-fusion-bridge cycle that is a characteristic of dominant lethals induced in Diptera. It appears that lepidopteran chromosomes can tolerate telomere loss without the drastic effects that this has on chromosomes in other orders. Discussions on this phenomenon are found in Bakri et al. (this volume) and Marec et al. (this volume).

## 4. APPROPRIATE RADIATION DOSE FOR THE SIT: IS 100% STERILITY REQUIRED?

The word “sterile” in the acronym “SIT” is generally perceived to imply a requirement for full sterility, and hence a radiation dose is sought that achieves 100% sterility. However, because there are varying levels of sterility that can be induced, the word “sterile” is not an absolute term, and often it is not required that released insects are fully sterile. As described above, the relationship of radiation dose to dominant lethal induction in the mature sperm of insects approximates to linearity at low radiation doses, but not at high doses, producing an asymptotic approach towards full sterility (Fig. 2). This curve is used as a decision-making tool to identify the radiation dose appropriate for a specific insect in a specific AW-IPM programme using the SIT. The fact that the curve approaches 100% sterility asymptotically makes it difficult to select the dose that gives the required full sterility. The equation used to describe the curve predicts that, in theory, given a large enough sample of eggs, it will always be possible to find one that hatches, and hence the goal of full sterility in eggs will never actually be met (although these may not reach the adult stage). However, in practical terms, a

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dose is chosen which prevents the hatch of more than 99% of eggs in a large sample. This decision is often taken solely on the basis of the dominant lethal-induction curve; the effects of radiation on somatic tissues, and hence on the final ability of the insect to introduce sterility into wild females in the field, are generally ignored.

In fact, the goal of attempting to achieve full sterility in treated insects can seriously compromise their competitiveness in the field. This follows from the shape of the dose-response curve. At high doses, increasing amounts of radiation are required for proportionally smaller increases in sterility. LaChance and Graham (1984) described the dominant lethal-induction curve for several insect species, and, using equations derived from their curves, it can be shown that, for example, to decrease egg hatchability from 2 to 1%, an 11% increase in dose is required. This marginal increase in sterility, obtained at the expense of a disproportionate increase in the radiation dose, can have major negative effects on the competitiveness of the released insects. Since the shape of the curve relating somatic damage to dose is not known, some assumptions have to be made in arriving at this conclusion.

The proportion of wild females rendered sterile by a given number of males released depends on both their sterility and their success in competing with wild males to mate with wild females. Therefore, to optimize the balance between somatic fitness and genetic sterility, it is in the interest of programmes applying the SIT to choose radiation doses that maximize the genetic load introduced into the wild populations. This means that chosen optimal radiation doses to achieve this objective may give sterility levels well below 100% (Toledo et al. 2004; Rull et al. 2012).

A word of caution is required here. It is known that, in *Drosophila* spp., males receiving substerilizing radiation doses can recover fertility over time (Luning 1952), and it is important to evaluate this aspect if lower doses of radiation are used. Nevertheless, currently all operational programmes consistently err on what may be considered the side of caution, and use radiation doses that induce almost 100% sterility in the treated insects. Unfortunately, high levels of somatic damage, and hence lower sterile-male performance, normally result from such high doses.

## 5. RADIATION IN DIFFERENT ENVIRONMENTS

Usually radiation is the final treatment that insects receive at the mass-rearing facility before being transported for release in the field. Any technique that reduces somatic damage induced by the treatment would be advantageous. Mass-produced insects are expensive, and need to function as well as possible in the field. The amount of genetic damage produced by radiation, both in reproductive and somatic tissues, is related to the environment in which the tissue finds itself, in particular, the oxygen tension. It is well known that treatment in low-oxygen tension, e.g. irradiating in nitrogen or hypoxia, reduces radiation damage (O'Brien and Wolfe 1964; Bakri et al., this volume).

However, if low-oxygen tension gives the same protective effect for somatic tissue (competitiveness) and sperm (sterility), then there would be no net gain. To achieve the same level of sterility as irradiation in air, irradiation in nitrogen would have to be at a higher dose. Nevertheless, the differential protection afforded to somatic cells by irradiation in nitrogen is related to the fact that somatic tissue is diploid and still

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undergoing cell division, whereas sperm are fully differentiated and haploid. This means that damage induced in somatic cells can manifest itself during the further life of the insect, whereas damage induced in sperm cells is only realized following fertilization of eggs in the wild females.

The positive effects on competitiveness of irradiation in nitrogen have been demonstrated for several insect species (Hooper 1971; Curtis and Langley 1972; Hallinan and Rai 1973; LaChance and Richard 1974; Economopoulos 1977). Two successful fruit fly AW-IPM programmes integrating the SIT were carried out in Australia using pupae irradiated in nitrogen (Hooper 1971; Fisher et al. 1985; Fisher 1996), and this approach is still being used for Mediterranean fruit fly programmes in that country (Fisher 1997). At present, no other operational programmes irradiate insects in nitrogen, whereas holding pupae in airtight containers to achieve hypoxia before irradiation is a common practice (Bakri et al., this volume). López-Martínez and Hahn (2012) demonstrated the benefit of this simple practice by showing that exposure to gamma radiation under anoxic conditioning results in lower lipid and protein oxidative damage in males at sexual maturity.

## 6. COMBINATION OF RADIATION AND GENETIC STERILITY

Genetic sexing strains are now being used in almost all programmes that use the SIT for the Mediterranean fruit fly (Robinson et al. 1999; Caceres et al. 2004; Franz et al., this volume). Since these strains are constructed using male-linked translocations, they are semi-sterile (Laven 1969). To obtain a more competitive insect, it has been suggested (Steffens 1982, 1983) that this genetically contrived sterility be combined with a lower dose of radiation-induced sterility. At lower doses of radiation, the overall sterility of males from a genetic sexing strain is, of course, higher than that of males from a normal strain. However, as the radiation dose increases, the contribution from genetic sterility gets progressively less, and eventually disappears as the sterility increases. For a male with a normal karyotype, and a male carrying a translocation, the radiation dose close to full sterility is the same. Nevertheless, the use of males produced from a genetic sexing strain offers the opportunity to seriously re-examine the radiation strategy of Mediterranean fruit fly programmes, at least those that aim at suppression, to maximize the genetic load introduced into wild populations.

## 7. HYBRID STERILITY

When hybrids are formed between closely related species, or even between some populations of the same species, sterility is observed. The sterility phenotype can include the total absence of viable progeny, the production of hybrids of both sexes with varying levels of sterility, or the production of a unisexual sterile $F_1$ generation that is usually male. Examples of all these are given below.

This array of different hybrid phenotypes has a corresponding wide range of underlying genetic and cytoplasmic causes, which in many cases overlap to produce a very complex phenotypic picture. The causes of hybrid sterility can be grouped roughly into those factors that act on the nuclear genome of the insects themselves, and

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those that are maternally (cytoplasmic) inherited. Many nuances and interactions are possible within these two major groups. Currently the picture is far from complete.

An early paper by Haldane (1922) reviewed cases of classical hybrid sterility in many different animal groups, including a large number of lepidopteran species. His analysis showed that there was preferential sterility or inviability in the hybrids of the heterogametic sex. This observation has become known as *Haldane's Rule*, and has largely stood the test of time (Orr 1997). For pest insects, this means that, for example in Lepidoptera where the female is heterogametic, the major hybrid effects would be seen in the resulting female hybrids, whereas in Diptera where the male is heterogametic, the $F_1$ males will be more affected.

### *7.1. Cytoplasmic Incompatibility in* Culex pipiens *L.*

Mosquitoes of the genus *Culex* are important vectors of filarial worms and some arthropod-borne viral diseases such as arboviruses. As early as 1938, Marshall (1938) showed that certain crosses of populations of *Culex pipiens* from England and France failed to produce progeny. In the 1950s Laven (1967a) carried out a worldwide survey of compatibility among many different populations of this complex. He showed that this phenomenon was cytoplasmic in origin, and that incompatibility could be uni- or bi-directional. The causative agent was a rickettsia-like bacterial symbiont; its removal by antibiotic treatment abolished the sterility phenotype (Yen and Barr 1973). The symbiont has been classified as *Wolbachia pipientis* Hertig, and is widely distributed in arthropods, with up to 76% of insect species so far examined showing evidence of infection (Jeyaprakash and Hoy 2000). It has a wide variety of effects on arthropod reproduction (Bourtzis and O'Neill 1998), and has been implicated in maintaining the viability of filarial worms that cause river blindness.

In insects, females infected with *Wolbachia* can successfully reproduce with males that are uninfected, but the reciprocal cross is sterile. This, coupled with the maternal inheritance of the infected state, enables the bacterium to spread through a population and carry with it any other factor that is exclusively maternally inherited (Pettigrew and O'Neill 1997; Curtis and Sinkins 1998). Releasing *Wolbachia*-infected males into a naive population would be equivalent to the release of radiation-sterilized males.

In 1967, an experiment to use cytoplasmic incompatibility to suppress a small isolated population of *Culex fatigans* Wiedemann was carried out in Okpo, a small village near Rangoon (Laven 1967b). Over a period of 4 months, each day about 5000 infected or incompatible males were released into a population estimated to fluctuate between 2000 and 10 000 mosquitoes. After 4 months of releases, all the remaining egg rafts collected were sterile. Unfortunately, the arrival of the monsoon season prevented any further observations. Curtis et al. (1982) carried out a much larger field trial. Although releases of incompatible males did reduce the population build-up, it was not possible to increase the percentage of sterile egg rafts above 70%. It was concluded that immigration of fertilized females from outside the village caused the stagnation in the numbers of sterile egg rafts observed.

The use of this approach for suppression requires that exclusively males of the incompatible strain be released; any females that are co-released would be compatible with the males and would lead to establishment of the *Wolbachia* strain in the wild

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population and its loss for suppression purposes (Augustinos et al., this volume; Lees et al., this volume). To solve the problem of error-free sexing, as well as escaping females, a low dose of radiation can be given since female mosquitoes are generally more sensitive than males (Arunachalam and Curtis 1985; Bourtzis et al. 2016).

### *7.2. Hybrid Sterility in Tsetse*

The first evidence that sterile hybrids are formed by crossing different tsetse species was provided by Corson (1932), who obtained progeny from mass-matings involving female *Glossina morsitans centralis* Machado and male *G. swynnertoni* Austen, but as he did not make observations on mating, he concluded that it was due to parthenogenesis. Potts (1944) repeated these experiments, and concluded that true hybrids were obtained as evidenced by the morphology of the hybrid male genitalia. He also was able to backcross $F_1$ females and produce progeny. Vanderplank (1944) confirmed these observations, but noted that, when no choice of mates was offered, inter-specific crosses took place as readily as the intra-specific crosses. By catching and identifying each member of copulating pairs, Jackson (1945) confirmed that this was true also in the field. Extensive work by Curtis (1972), Rawlings (1985), Gooding (1997a, b, 2000), and Gooding and Krafsur (2005) expanded the knowledge of tsetse hybridization phenomena, and indicated how this might, or might not, be used to develop methods of pest population suppression. Interspecific crosses in tsetse produce sterile male hybrids and partially sterile female hybrids, and there is a strong asymmetry in the sterility phenotype of the reciprocal crosses. In some crosses no $F_1$ progeny are produced. The male hybrids can copulate with and inseminate females, but the hybrid sperm cannot always succeed in fertilising eggs. For other crosses, Curtis (1972) showed that, following multiple mating of females, the two types of sperm were equally competitive. However, Gooding (1992) showed that, in multiple-mated females of the *G. morsitans* subspecies, the sperm from the conspecific male was used preferentially. The inability of hybrid males to fertilize females, and the preferential use of conspecific sperm, would be serious handicaps to the use of hybrid sterility for pest suppression.

Hybrid sterility in tsetse appears to be mediated by both genetic and maternally inherited factors. A very complex picture has emerged, with many factors interacting, each with differing contributions to the total picture (Doudoumis et al. 2013). Apart from the expected chromosomal and genic interactions, two interesting observations have been made. Firstly, the fertility of reciprocal crosses between the different taxa show high levels of asymmetry. This is reminiscent of phenotypes induced by *Wolbachia* symbionts, and it is known that many tsetse species carry *Wolbachia* (Chen et al. 1999; Abd-Alla et al. 2013). Secondly, it appears that in some hybrid crosses there is a form of interaction between the mother and hybrid offspring that determines whether a pregnancy will be successfully completed. (Tsetse flies reproduce by adenotrophic viviparity, where the fertilized egg hatches into a larva that is fed within the female by milk glands, and a mature third-instar larva is produced every 9–10 days). Results by Olet (2001) have shown that females mated to males of a different taxon, though initially sterile, can as they get older begin to produce viable progeny.

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Using hybrid sterility for tsetse control would involve either the release of fertile males into a non-compatible population, or the release of $F_1$ sterile males into a population of either of the parental species. The use of $F_1$ males has the disadvantage that two taxa have to be maintained in the laboratory, and that males and females from the respective taxa have to be obtained to set up the appropriate cross. In addition, the release population must be sexed to prevent the release of semi-sterile $F_1$ females, and the $F_1$ males need to be good inseminators. For the release of fertile males of one taxon into a wild population of a second taxon, only the release population must be sexed (however 100% accuracy is required).

In a large field experiment, Vanderplank (1947) eliminated a population of *G. swynnertoni* from the Shinyanga area, Tanzania, by releasing into it fertile *G. m. centralis* from Kondoa-Irangi, Tanzania (Klassen et al., this volume). About 140 000 *G. m. centralis* pupae were released over a 7-month period, and the population of *G. swynnertoni* fell, presumably due to the reduced fertility of the hybridized *G. swynnertoni* females and the subsequent matings with sterile hybrid $F_1$ males. *G. swynnertoni* was eradicated, but *G. m. centralis* did not become established because of the harsh climatic conditions, and therefore the area remained tsetse-free for some time. These field trials were carried out before it was known to applied entomologists that sterility could be readily induced by radiation, and clearly demonstrated the potential of hybrid sterility to suppress tsetse flies. They were carried out before mass-rearing of these species was possible, and all the flies released, both males and females, were from field-collected pupae (there was no way to separate the sexes). Recent improvements in tsetse mass-rearing procedures and sex-separation methods (Opiyo et al. 2000; Dowell et al. 2005) should encourage a re-examination of this form of pest suppression for tsetse.

### *7.3.* Heliothis *Hybrids — an Exception to Haldane's Rule*

Laster (1972) demonstrated that, when *Heliothis subflexa* (Guenée) females and *Heliothis virescens* (F.) males are hybridized, sterile male and fertile female progeny are produced, and the hybrid females continue to produce sterile male and fertile females through many generations of backcrossing to *H. virescens* males. Since females are heterogametic, they would be expected to suffer more from hybridization, so novel factors may be involved. Hybrid males can mate and transfer a spermatophore, but no eupyrene sperm reach the female spermathecae, even though they are produced in the testis. However, as the number of backcross generations increases, the ability of the males to produce eupyrene sperm decreases. This pattern of hybrid sterility suggests a very strong maternal component, but treatment of the moths with agents known to be lethal for *Wolbachia* failed to remove the sterility syndrome (LaChance and Karpenko 1981, 1983). An analysis of mitochondrial biogenesis in hybrid males failed to identify elements of protein synthesis or transport as being the cause of the sterility, but sperm mitochondria were implicated in the phenomenon (Miller and Huettel 1986). Such hybrid males would unlikely be effective "sterile males" in the field. Nevertheless, field trials on the island of St. Croix were conducted using the release of backcross males (Proshold 1983; Proshold et al. 1983), and temporary sterility could be demonstrated in the target population.

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### *7.4.* Anopheles gambiae *Complex*

The *Anopheles gambiae* Giles complex in Africa consists of seven sibling species, and crosses between every combination led to hybrid males, with some crosses producing no females (Davidson et al. 1967). The sterility is due to chromosomal interactions, and cytoplasmic factors have not been implicated. Davidson (1969) carried out a successful series of laboratory cage experiments to evaluate the use of these sterile males, and was able to demonstrate the induction of the expected levels of sterility in target mixed-sex populations. However, a field trial (Davidson et al. 1970) failed to demonstrate any substantial degree of mating between released sterile hybrid males and wild females. Pre-mating isolation mechanisms played a decisive role in the failure, illustrating the behavioural constraints that underlie the use of hybrid sterility in the field.

## 8. TRANSFORMATION AND MOLECULAR STERILITY

The successful demonstration of transformation in *Drosophila melanogaster* Meigen (Rubin and Spradling 1982) encouraged the development of similar systems in other insects. A review by Häcker et al. (this volume) illustrates the current state of modern biotechnology for introducing foreign genes into pest insects, including classical transformation technologies via transposable elements, and site-specific genome modification techniques using recombinases. The use of molecular tools in support of the SIT involves mainly the development of marker and genetic sexing strains (Bourtzis and Hendrichs 2014; Franz et al., this volume).

Also, molecular sterility systems have been developed that might be used to produce both an elimination of females from the insects to be released, and sterility induced by matings with the released males (Heinrich and Scott 2000; Thomas et al. 2000; Häcker et al., this volume). Some of these rely on conditional lethality in $F_1$ females that are produced following the mating of released transgenic males with wild females. To date, some transgenic strains are available of several pest insects, although what is known is giving some cause for concern in two areas related to the technology itself, namely stability of the insertion and expression of the transgene. Currently, there are insufficient data to predict if transgenic strains will retain the appropriate expression patterns of the constructs under industrial mass-rearing over many generations, and following their large-scale release in the field. This will be particularly relevant for systems based on molecular sterility where permanent and absolute sterility of the system is essential. It will be difficult to predict the long-term stability of transgenes, and hence their expression in the transformed strains. This problem could be magnified once fertile laboratory transgenic strains are released into a wild population, the genomic diversity of which is unknown.

There is also the slightly disconcerting idea that a transgene, once released in a fertile insect, cannot be “recalled” or destroyed. This applies particularly to self-sustaining strategies, which leave an “ecological footprint” because they are intended to persist indefinitely in the target population and may invade other populations (Alphey 2014). The integration of transgenic technology with the SIT, as part of self-limiting strategies, would be an option to reduce risk, since radiation-induced sterility

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would prevent vertical transmission of the transgene. Public concern related to genetically modified organisms needs to be addressed, and a regulatory framework is required, so that available transgenic insect strains are properly field-tested.

## 9. CONCLUSIONS

Due to the extensive studies on the radiation biology of *Drosophila* sp. carried out in the 1950s and 1960s, there is a very good understanding of the genetic basis of radiation-induced sterility in that species. However, even though the underlying mechanisms are known and are probably of wide relevance, there still are many unexplained observations, especially in regards to the different dose-response curves found for different species, even within the same order (Bakri et al. 2005). For one type of mutation, i.e. specific locus mutations, there is a very good correlation between the dose-response curve and the DNA content of the haploid genome (Abrahamson et al. 1973); these studies ranged from bacteria to man. There is as yet insufficient information on genome size, in a large enough number of insect species, to assess whether such a simple relationship holds for dominant lethal induction. In addition, in species with holokinetic chromosomes, the mechanism(s) of dominant lethal induction has/have still to be fully explained (Marec et al., this volume).

Radiation is usually one of the last procedures that insects undergo before leaving mass-rearing facilities for release in the field, and it is important that it be applied in a way that minimizes its detrimental effects on insect competitiveness. Firstly, it is essential that the dosimetry of the radiation source be checked to ensure that all the insects receive the required minimum dose, and that none is unnecessarily overdosed (Bakri et al., this volume). Secondly, a dose should be chosen that maximizes the level of introduced sterility in the wild females in the field, i.e. both the sterility level in the released males and their competitiveness has to be taken into account. Thirdly, numerous studies have shown that irradiation in nitrogen or hypoxia can provide protection against the detrimental somatic effects of radiation.

Currently, modern biotechnology tools are applied extensively in support of the SIT to develop marker and genetic sexing strains (Häcker et al., this volume). Furthermore, the development of molecular methods to sterilize pest insects, by the release into the field of fertile insects carrying transgenes, has made considerable progress, in spite of the many unknowns inherent in such systems. Several of these strains are now available, and some have undergone an initial evaluation under mass-rearing scenarios or in open-field trials (Carvalho et al. 2015). Overall, however, their wider adoption has so far been quite limited in view of the negative public opinion on transgenic technology, and the regulatory requirements and approvals that are required in most countries for their application.

Using biological/molecular methods to sterilize insects is quite different from using a physical process, such as radiation. In the former, the essential biological interaction required to generate the sterility phenotype is subject to biological variation in both components, and is therefore unpredictable in the long term. A physical process such as radiation is not subject to this variation, and hence, in some ways, is an ideal methodology. Insects cannot become resistant to ionizing radiation as it is used in the SIT (Whitten and Mahon, this volume).

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# CHAPTER 2.4.

# INHERITED STERILITY IN INSECTS

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## SUMMARY

The unique genetic phenomena responsible for inherited sterility (IS) in Lepidoptera and some other arthropods, as compared with full sterility, provide advantages for pest control. Lepidopteran females are usually more sensitive to radiation than males of the same species. This allows the radiation dose to be adjusted to suit programme requirements. When partially sterile males mate with wild females, the radiation-induced deleterious effects are inherited by the $F_1$ generation. As a result, egg hatch is reduced and the resulting offspring are both highly sterile and predominately male. Compared with the high radiation required to achieve full sterility in Lepidoptera, the lower dose of radiation used to induce $F_1$ sterility increases the quality and competitiveness of the released insects as measured by improved dispersal after release, increased mating ability, and superior sperm competition. $F_1$ sterile progeny produced in the field enhance the efficacy of released partially sterile males, and improve compatibility with other pest control strategies. In addition, $F_1$ sterile progeny can be used to increase the production of natural enemies, and to study the potential host and geographical ranges of exotic lepidopteran pests.

## 1. INTRODUCTION

Lepidopteran species are among the most destructive pests of annual and perennial crops, forests, and stored products throughout the world. Following the successful area-wide integrated pest management (AW-IPM) programme, integrating the sterile insect technique (SIT), against the screwworm fly *Cochliomyia hominivorax* (Coquerel) (Bushland 1971), studies were conducted on the possibility of suppressing lepidopteran pest populations through the release of radiation-sterilized moths. However, because Lepidoptera are radioresistant compared with most other insects (LaChance et al. 1967; Bakri et al., this volume), a dose required to achieve full sterility is so high that it reduces the ability of sterile moths to compete with wild moths. To increase the competitiveness of irradiated Lepidoptera, Proverbs (1962) investigated the effects of substerilizing doses of radiation on the codling moth *Cydia pomonella* (L). He noted that male moths treated with substerilizing doses and then mated to fertile females produced reduced numbers of $F_1$ progeny, the majority of which were males with very low fertility. This discovery prompted numerous investigations in many lepidopteran pests. North (1975) and LaChance (1985) provide thorough reviews of the early investigations on inherited sterility (IS), and discuss potential advantages of using IS in suppressing pest populations and its possible genetic basis.

IS is also referred to as inherited partial sterility, partial sterility, delayed sterility, semi-sterility and $F_1$ sterility (Vreysen et al. 2016). Although it is difficult to find a satisfactory definition for IS, LaChance (1985) described several attributes that are common to IS in Lepidoptera: $F_1$ male and female offspring are more sterile than the irradiated parental ($P_1$) generation, and more $F_1$ male progeny than female progeny are produced. Other attributes may include longer developmental time and reduced sperm quality in the $F_1$ generation. Radiation-induced deleterious effects can be inherited for several generations; however, the majority of the inherited deleterious effects are expressed in the $F_1$ generation.

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## 2. HISTORICAL OVERVIEW

IS was first reported during studies in the Soviet Union of radiation-induced genetic anomalies in the silkworm *Bombyx mori* (L.) (Astaurov and Frolova 1935). A few years later Ostriakova-Varshaver (1937) reported IS in the greater wax moth *Galleria melonella* (L.). In North America, Proverbs (1962) was the first to describe IS in the codling moth. Within the order Hemiptera, LaChance and Degrugillier (1969) reported IS while conducting genetic studies on the large milkweed bug *Oncopeltus fasciatus* (Dallas), and Delrio and Cavalloro (1975) and Maudlin (1976) documented IS in *Gonocerus acuteangulatus* (Goeze), a coreid pest of hazelnuts, and in *Rhodnius prolixus* (Stål), a reduviid vector of Chagas' disease, respectively. IS has also been reported in mites of the family Tetranychidae (Henneberry 1964). Although Curtis (1969) and Curtis et al. (1973) found low levels of sterility (5–15%) in the $F_1$ generation of irradiated tsetse flies (Diptera: Glossinidae), this level of sterility was less than that found in the irradiated parents, and other attributes common to IS in Lepidoptera were not demonstrated in these insects.

The genetic basis for IS has been reviewed and discussed by many authors (Bauer 1967; LaChance 1967, 1974, 1985; LaChance et al. 1970; North and Holt 1970; North 1975; LaChance and Graham 1984; Anisimov et al. 1989; Marec et al. 1999; Tothová and Marec 2001). In this chapter, emphasis will be given to the more recent research findings in the order Lepidoptera. Also the use of genetic sexing, together with IS for the suppression of lepidopteran populations, will be discussed briefly. The advantages of IS as compared with full sterility, the potential for IS to suppress pest populations, and the compatibility of IS with other pest control methods, particularly with biological control, are also discussed. Table 1 provides a comprehensive list of arthropod species where IS has been documented, and includes key references that deal with radiation biology and field studies.

## 3. GENETICS AND INHERITED STERILITY

No comprehensive review of lepidopteran genetics has been published in the last 45 years. Robinson (1971) provided useful information on formal genetics (including karyology) for many species in this large order. Additional information can be extracted from published research on three economically important species: the silkworm (Tazima 1964; Goldsmith 1995; Fujii et al. 1998; Nagaraju 2000), the Mediterranean flour moth *Ephestia kuehniella* (Zeller) (Caspari and Gottlieb 1975; Leibenguth 1986), and the pink bollworm *Pectinophora gossypiella* (Saunders) (LaChance and Ruud 1980; Bartlett 1989; Bartlett and Del Fosse 1991). Recent advances in the molecular biology and genetics of established and emerging lepidopteran models have been summarized in Goldsmith and Marec (2010).

The lepidopteran genome exhibits a number of peculiarities that distinguishes it from the genomes of other insect orders, except perhaps from that of the closely related order Trichoptera. Chromosomes in Lepidoptera are usually small, numerous, and possess few differentiating features. Most species are reported to have haploid numbers close to 30 (n = 28–32); n = 31 appears to be the ancestral number (Ahola et al. 2014). However, karyological studies have identified species

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with lower or higher chromosome numbers that are probably the result of chromosome fusion or fission (Suomalainen 1969a; Robinson 1971; reviewed in Marec et al. 2010). Lepidopteran chromosomes are usually spherical and uniform in shape, and consequently not much is known about their morphology, kinetic organization and behaviour during mitotic and meiotic cell division. Lepidopteran chromosomes lack distinct primary constrictions (centromeres) and, as a result, sister chromatids separate by parallel disjunction during mitotic metaphase. Many researchers have concluded that these genomic peculiarities are an indication that lepidopteran chromosomes are holokinetic (Murakami and Imai 1974). However, ultrastructure studies suggest that lepidopteran chromosomes are intermediate between holokinetic and monocentric (Wolf 1996). Nevertheless, a recent functional analysis of the silkworm kinetochore supports the holokinetic structure, although different from the other groups of organisms with holokinetic chromosomes (Mon et al. 2014).

Two other groups of arthropods have shown IS, mites (Acari) and Hemiptera; both possess holokinetic chromosomes and are radioresistant (Brown and Nelson-Rees 1961; Hughes-Schrader and Schrader 1961; LaChance and Degrugillier 1969; Wrensch et al. 1994). Gassner and Klemetson (1974) showed that the kinetochore (centromere) in the large milkweed bug covers more than 70% of the chromosomal surface. Gonzales-Garcia et al. (1996) provided indirect evidence of the holokinetic nature of hemipteran chromosomes while working with *Graphosoma italicum* (Muller) (Pentatomidae). Nonetheless, Wolf (1996) suggests that further investigation is needed to verify the holokinetic nature of hemipteran chromosomes.

The sex chromosomes of Lepidoptera are of the WZ type, in which females are heterogametic (WZ) and males homogametic (ZZ). Lepidopteran species, where sex chromosomes have been identified, show a typical ♀WZ/♂ZZ system, or variants such as Z/ZZ, $W_1W_2Z$/ZZ or $WZ_1Z_2/Z_1Z_1Z_2Z_2$ (Suomalainen 1969b; Robinson 1971; Nilsson et al. 1988; Traut and Marec 1997; Rishi et al. 1999; reviewed in Traut et al. 2007 and Marec et al. 2010). Male chromosomes display a normal sequence of meiotic events. In contrast, female chromosomes undergo normal meiosis until they approach the pachytene stage (Fig. 1), when they pair by means of synaptonemal complexes to form bivalents, and synaptonemal complexes become visible (Marec 1996). From this point onwards, female meiosis proceeds without meiotic recombination, and is achiasmate (Traut 1977; Nokkala 1987). The synaptonemal complexes in the female transform into elimination chromatin that later detaches from the bivalents and persists during chromosome segregation in the metaphase plate (Rasmussen 1977). The female W and Z chromosomes, although often non-homologous and of different size, pair completely during meiosis and form a regular bivalent.

Another peculiarity of the lepidopteran genome is the presence of one or more heterochromatic bodies in female somatic cells during interphase. This female specific heterochromatin (also known as W- or sex-chromatin) is derived from the W chromosome. Since sex chromatin is easily identified in interphase nuclei and is especially visible in highly polyploid somatic cells, it can be used as a marker to determine the sex of embryos and larvae and also to identify sex chromosome aberrations in mutagenesis screens (Traut and Marec 1996; Fuková et al. 2009).

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*Table 1. Arthropod species, including key references of radiation biology and field studies, in which inherited sterility (IS) has been documented*

| Family, species, and common name | Key references | |
|---|---|---|
| | Radiation biology | Field studies |
| | ***Arachnida – Acari*** | |
| **Tetranychidae** | | |
| *Tetranychus urticae* Koch twospotted spider mite | Henneberry 1964 | |
| | ***Insecta – Hemiptera*** | |
| **Coreidae** | | |
| *Gonocerus acuteangulatus* (Goeze) box bug | Delrio and Cavalloro 1975 | |
| **Lygaeidae** | | |
| *Oncopeltus fasciatus* (Dallas) large milkweed bug | LaChance and Degrugillier 1969<br>LaChance et al. 1970 | |
| **Reduviidae** | | |
| *Rhodnius prolixus* Stål | Maudlin 1976 | |
| | **Insecta – Lepidoptera** | |
| **Bombycidae** | | |
| *Bombyx mori* (L.) silkworm | Sugai and Mirumachi 1973<br>Murakami 1976 | |
| **Crambidae** | | |
| *Crocidolomia binotalis* (Zeller) cabbage webworm | Sutrisno Apu and Hoedaya 1993 | Sutrisno Apu and Hoedaya 1993<br>Sutrisno Apu 2001 |
| *Diatraea saccharalis* (F.) sugarcane borer | Walker and Quintana 1968a, b<br>Walker et al. 1971<br>Sanford 1976; 1977<br>García and González 1993<br>González and García 1993 | |
| *Ostrinia furnacalis* (Guenée) Asian corn borer | Li et al. 1988<br>Zhang et al. 1993<br>Wang et al. 2001 | Wang et al. 2001 |
| *Ostrinia nubilalis* (Hübner) European corn borer | Shang and Lo 1980<br>Nabors and Pless 1981<br>Rosca and Barbulescu 1990<br>Barbulescu and Rosca 1993<br>Rosca and Barbulescu 1993 | Barbulescu and Rosca 1993<br>Rosca and Barbulescu 1993 |

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*Table 1. Continued*

| Family, species, and common name | Key references | |
|---|---|---|
| | Radiation biology | Field studies |
| **Gelechiidae** | | |
| *Pectinophora gossypiella* (Saunders) pink bollworm | Cheng and North 1972<br>Graham et al. 1972<br>LaChance et al. 1973; 1976<br>Henneberry and Clayton 1981<br>Miller et al. 1984<br>Qureshi et al. 1993a | Bariola et al. 1973<br>Flint et al. 1974<br>Qureshi et al. 1993b |
| *Phthorimaea operculella* (Zeller) potato tuberworm | Makee and Saour 1997<br>Makee et al. 2007 | |
| *Sitotroga cerealella* (Olivier) Angoumois grain moth | Cogburn et al. 1966 | |
| *Tuta absoluta* (Meyrick) tomato leafminer | Cagnotti et al. 2012<br>Carabajal Paladino et al. 2016 | Cagnotti et al. 2016 |
| **Gracillariidae** | | |
| *Conopomorpha sinensis* Bradley litchi stem-end borer | Fu et al. 2016<br>Zhang et al. 2016 | Fu et al. 2016<br>Zhang et al. 2016 |
| **Lymantriidae** | | |
| *Lymantria dispar* (L.) gypsy moth | Mastro et al. 1989<br>Proshold et al. 1993<br>Proshold 1995 | Maksimovic 1972<br>Mastro et al. 1989<br>Mastro 1993<br>Strom et al. 1996 |
| *Teia anartoides* Walker painted apple moth | Suckling et al. 2002<br>Wee et al. 2005 | Suckling et al. 2002 |
| **Noctuidae** | | |
| *Agrotis ipsilon* (Hufnagel) black cutworm | Elnagar et al. 1984 | |
| *Helicoverpa armigera* (Hübner) cotton bollworm | Saifutdinov 1989<br>Ocampo 2001<br>Sachdev et al. 2014 | |
| *Helicoverpa zea* (Boddie) corn earworm bollworm tomato fruitworm | Carpenter et al. 1987c<br>Carpenter and Gross 1989<br>Carpenter 1991; 1992<br>Carpenter and Wiseman 1992a<br>Hamm and Carpenter 1997 | North and Snow 1978<br>Carpenter et al. 1987a, b; 1989<br>Carpenter and Gross 1993<br>Mannion et al. 1994; 1995 |
| *Heliothis virescens* (F.) tobacco budworm | Proshold and Bartell 1970; 1972a, b; 1973<br>Guerra and Garcia 1976 | North and Snow 1978 |

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*Table 1. Continued*

| Family, species, and common name | Key references | |
|---|---|---|
| | Radiation biology | Field studies |
| **Noctuidae** | | |
| *Spodoptera exigua* (Hübner) beet armyworm | Debolt 1973<br>Carpenter et al. 1996 | |
| *Spodoptera frugiperda* (J. E. Smith) fall armyworm | Carpenter et al. 1983; 1986; 1997<br>Carpenter and Young 1991<br>Arthur et al. 1993<br>Hamm and Carpenter 1997 | Carpenter et al. 1985<br>Carpenter and Wiseman 1992b |
| *Spodoptera littoralis* Boisduval Egyptian cotton leafworm | Wakid and Hayo 1974<br>Sallam and Ibrahim 1993 | Sallam and Ibrahim 1993 |
| *Spodoptera litura* (F.) Oriental leafworm taro caterpillar | Seth and Sehgal 1993<br>Sutrisno Apu et al. 1993<br>Seth and Sharma 2001<br>Seth et al. 2016a, b | Seth et al. 2016b |
| *Trichoplusia ni* (Hübner) cabbage looper | North and Holt 1968; 1969<br>Ercelik and Holt 1972<br>Karpenko and North 1973 | Toba et al. 1972 |
| **Pieridae** | | |
| *Pieris brassicae* (L.) the large white | Bauer 1967 | |
| **Plutellidae** | | |
| *Plutella xylostella* (L.) diamondback moth | Omar and Mansor 1993<br>Sutrisno Apu and Hoedaya 1993<br>Sutrisno Apu et al. 1993<br>Nguyen Thi and Nguyen Thanh 2001 | Sutrisno Apu and Hoedaya 1993<br>Okine et al. 1998<br>Mitchell et al. 1999<br>Nguyen Thi and Nguyen Thanh 2001<br>Sutrisno Apu 2001 |
| **Pyralidae** | | |
| *Amyelois transitella* (Walker) navel orangeworm | Husseiny and Madsen 1964<br>Light et al. 2015 | |
| *Cactoblastis cactorum* (Berg) cactus moth | Carpenter et al. 2001b<br>López-Martínez et al. 2016 | Bloem et al. 2003a<br>Hight et al. 2005 |
| *Cadra cautella* (Walker) almond moth | Ahmed et al. 1971<br>Gonnen and Calderón 1971<br>Brower 1980; 1982<br>Al-Taweel et al. 1990<br>Makee 1993 | |

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*Table 1. Continued*

| Family, species, and common name | Key references | |
|---|---|---|
| | Radiation biology | Field studies |
| **Pyralidae** | | |
| *Corcyra cephalonica* (Stainton) rice moth | Chand and Sehgal 1982 | |
| *Eldana saccharina* Walker African sugarcane stalk borer | Mudavanhu et al. 2016<br>Walton and Conlong 2016 | |
| *Ephestia kuehniella* Zeller Mediterranean flour moth | Riemann 1973<br>Marec et al. 1999<br>Tothová and Marec 2001<br>Ayvaz and Tunçbilek 2006<br>Ayvaz et al. 2007 | |
| *Galleria mellonella* (L.) greater wax moth | Nielsen 1971<br>Nielsen and Lambremont 1976<br>Nielsen and Brister 1980 | |
| *Plodia interpunctella* (Hübner) Indian meal moth | Cogburn et al. 1966<br>Ashrafi et al. 1972<br>Ashrafi and Roppel 1973<br>Brower 1976; 1979; 1981<br>Ayvaz et al. 2008 | |
| **Sphingidae** | | |
| *Manduca sexta* (L.) tobacco hornworm | Seth and Reynolds 1993 | |
| **Tortricidae** | | |
| *Cydia pomonella* (L.) codling moth | Proverbs 1962<br>Fossati et al. 1971<br>Charmillot et al. 1973<br>Pristavko et al. 1973<br>White 1975<br>Anisimov et al. 1989<br>Bloem et al. 1999a | Charmillot et al. 1973<br>Charmillot 1977<br>Proverbs et al. 1978<br>Bloem et al. 1999b; 2001 |
| *Epiphyas postvittana* (Walker) light brown apple moth | Soopaya et al. 2011 | Woods et al. 2016 |
| *Grapholita molesta* (Busck) oriental fruit moth | Genchev 2001 | Genchev 2001 |
| *Lobesia botrana* (Denis & Schiffermüller) European grapevine moth | Steinitz et al. 2015 | Saour 2016 |
| *Thaumatotibia leucotreta* (Meyrick) false codling moth | Schwartz 1978<br>Bloem et al. 2003b | |

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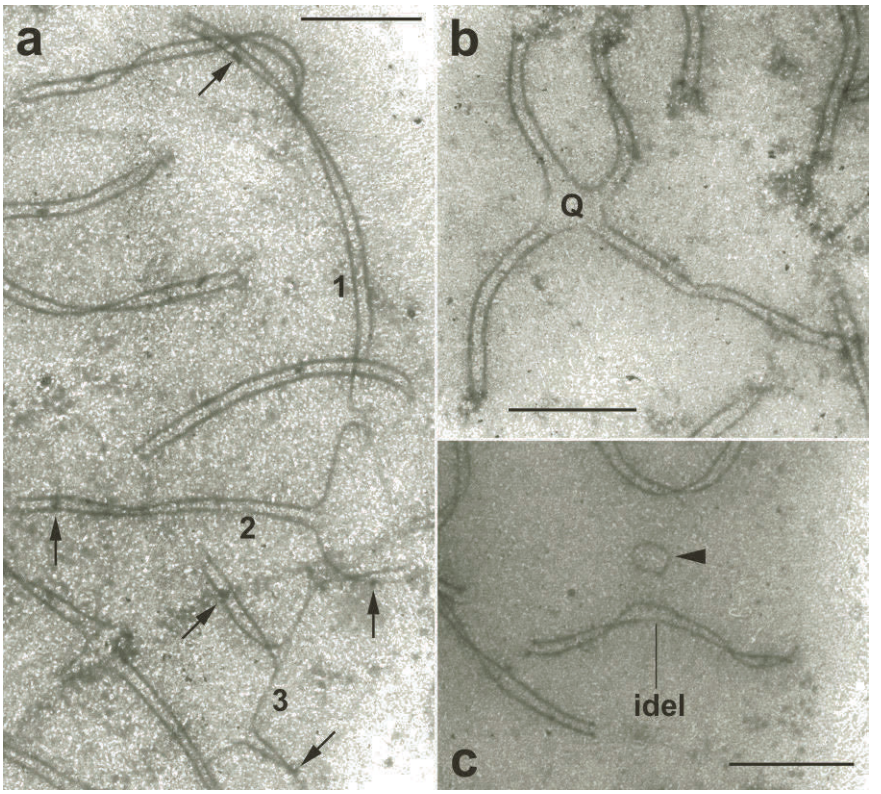



*Figure 1. Examples of pachytene configurations in aberrant chromosomes of* Ephestia kuehniella *in microspread spermatocytes of $F_1$ males after the parental male was irradiated with 150 Gy. a: Multiple chain translocation that involves 7 lateral elements, each representing the protein axis of one chromosome, where 1–3 represent structurally normal chromosomes inherited from the untreated female parent; arrows indicate recombination nodules; b: Quadrivalent (Q) typical in reciprocal translocations; c: Ring fragment (arrow) plus a bivalent with one shorter lateral element (idel) that indicates interstitial deletion. All are electron microscope (EM) micrographs stained with phosphotungstic acid. Scale = 2 μm.*

Lepidopteran males undergo two distinct modes of spermatogenesis/meiosis that result in the production of two different types of sperm (Wolf 1994; Friedländer et al. 2005): the larger, nucleate and fertile eupyrene sperm, and the smaller, anucleate and non-fertile apyrene sperm. Apyrene sperm are more abundant and contain less mitochondrial material (Kawamura et al. 1998), while eupyrene sperm are less abundant and typically comprise 10–15% of the total sperm transferred to a female during mating (Gage and Cook 1994; Cook and Wedell 1996). The role of apyrene sperm is not fully understood, although it has been suggested that they aid the transfer of eupyrene sperm to the female (Cook and Wedell 1996), have a nutritive

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function (Friedländer 1997), or may be involved in sperm competition. The latter function was studied in *Pieris napi* (L.), where the presence of apyrene sperm was shown to delay female remating (Cook and Wedell 1999).

### *3.1. Radioresistance in Lepidoptera*

A high resistance to the effects of ionizing radiation is a characteristic feature of moths and butterflies (LaChance 1985). Cultured lepidopteran cells are 50–100 times more resistant to radiation-induced death than similarly cultured mammalian cells. In contrast dipteran cells are only three to nine times more resistant than mammalian cells (Koval 1996; Chandna et al. 2004). Results of recent studies of a Sf9 cell line, originally derived from *Spodoptera frugiperda*, suggest that several intracellular mechanisms, such as an efficient antioxidant defence and a high activity of histone deacetylases, contribute to the radioresistance of lepidopteran cells (Suman et al. 2015; Sharma et al. 2016). This high radioresistance in Lepidoptera also applies to germ cells, and in particular to mature sperm. As a consequence very high doses of radiation are required to fully sterilize lepidopteran males (LaChance and Graham 1984).

LaChance and Graham (1984) and Koval (1996) suggested that possible molecular mechanisms responsible for the high radioresistance in Lepidoptera might include an inducible cell recovery system and a DNA repair process. Even though lepidopteran chromosomes are not truly holokinetic, a significant role in their radioresistance can be attributed to their holokinetic "nature" (as first suggested by LaChance et al. 1967) and to the fate of the radiation-induced chromosome fragments during mitotic cell cycles as explained below (Tothová and Marec 2001). Lepidopteran chromosomes possess a localized kinetochore plate to which the spindle microtubules attach during cell division (Gassner and Klemetson 1974; Traut 1986; Wolf and Traut 1991; Wolf et al. 1997). The kinetochore plates are large and cover a significant portion of the chromosome length (Wolf 1996), ensuring that most radiation-induced breaks will not lead to the loss of chromosome fragments as is typical in species with monocentric chromosomes. In species with large kinetochore plates, the fragments may persist for a number of mitotic cell divisions, and can even be transmitted through germ cells to the next generation (Marec and Traut 1993a; Marec et al. 2001). The plates also reduce the risk of lethality caused by the formation of dicentric chromosomes, acentric fragments, and other unstable aberrations (Tothová and Marec 2001) (Fig. 2).

### *3.2. Radioresistance in Hemiptera*

A difference in radiosensitivity between males and females has also been documented in several hemipterans including species of economically important leafhoppers (Shipp et al. 1966; Ameresekere and Georghiou 1971) and mealybugs (Brown and Nelson-Rees 1961). However, LaChance and Degrugillier (1969) were the first to document IS in the order Hemiptera when they induced chromosomal fragments and translocations in the large milkweed bug. These authors demonstrated

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that the induced fragments were both mitotically and meiotically stable, and could be transmitted through three generations of outcrosses to normal females.

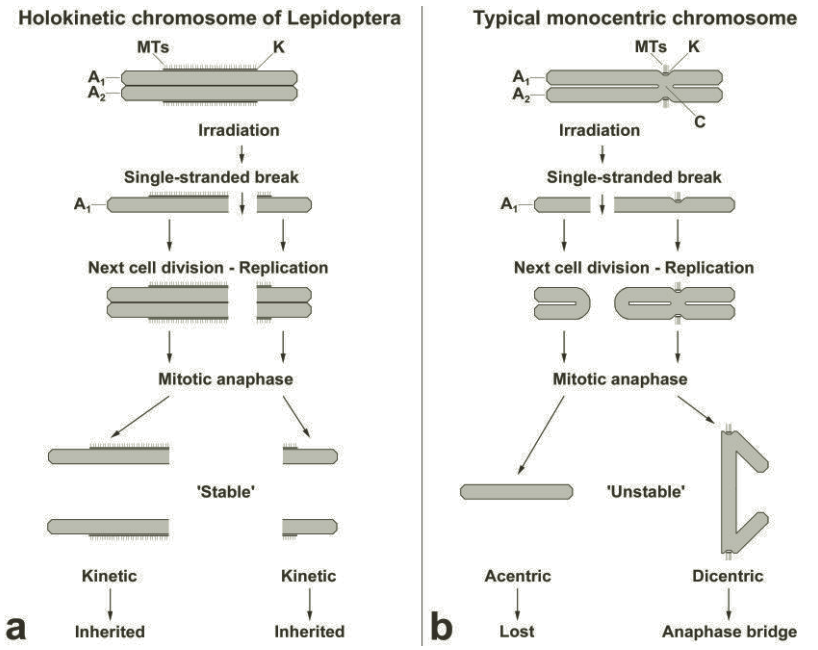



*Figure 2. Kinetic structure of lepidopteran chromosomes during mitotic metaphase, and consequences of chromosome breakage;* ***a****: Holocentric chromosome with two sister chromatids ($A_1$ and $A_2$), each with a kinetochore plate (K) covering about 50% of the chromosome surface; spindle microtubules (MTs) are attached to the kinetochore;* ***b****: Typical monocentric chromosome, where sister chromatids are joined by the centromere (C); the kinetochore is localized on the surface of the centromere.*

### *3.3. Radiation-Induced Sterility in Parental ($P_1$) Generation*

Radiation-induced sterility is generally a consequence of dominant lethal mutations (DLMs) that result in the death of the zygote or the embryo. The chromosomal damage responsible for DLMs is characterized by the formation of anaphase chromosome bridges, chromosome fragments and other abnormalities in the dividing nuclei. In most insects DLMs are expressed during early embryogenesis (LaChance 1967). In Lepidoptera, however, the frequency of DLMs is much lower than in other insects and the majority are expressed very late in embryonic development (LaChance 1974; Berg and LaChance 1976). Furthermore, no chromosomal bridges, indicating the presence of dicentric chromosomes, are evident during embryonic development in Lepidoptera (LaChance and Graham 1984) and Hemiptera studied thus far (LaChance et al. 1970; Maudlin 1976).

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For males of four insect species, LaChance and Graham (1984) constructed dose-response curves for the induction of DLMs in mature sperm. The species exhibited a wide range of radiosensitivity, with Hemiptera showing intermediate and Lepidoptera high radioresistance. When analysed mathematically, the dose-response curves approximated an S-shape (LaChance and Graham 1984; Marec et al. 1999). For highly radioresistant Lepidoptera, the curves approximated those expected for 8–16-hit kinetics while in Hemiptera the curve exhibited 4-hit kinetics. These data suggest that multiple chromosome rearrangements must be induced in lepidopteran males to be manifested as DLMs, explaining why lepidopteran males require very high radiation doses (350–500 Gy) to be fully sterilized.

Radiation-induced sterility in lepidopteran males may also have other causes. Anisimov et al. (1989) observed a dose-dependent increase in the number of matings that produced no eggs, or the number of females that laid only unembryonated eggs, following mating with irradiated male codling moths. A significant proportion of unembryonated eggs (that might represent unfertilized eggs and/or eggs with early embryonic mortality) were also observed after treated males were mated to females of *Manduca sexta* (L.) (Seth and Reynolds 1993), *E. kuehniella* (Marec et al. 1999), and *Spodoptera litura* (F.) (Seth and Sharma 2001). Furthermore, Koudelová and Cook (2001) demonstrated with *E. kuehniella* that the volume of sperm transferred during copula decreased, and mating times increased, as the dose increased. Irradiated males of *Tuta absoluta* (Meyrick) produced a significantly higher portion of deformed eupyrene sperm bundles than untreated males (Carabajal Paladino et al. 2016). Taken together, the above data suggest that an important component of male sterility can be due to physiological disruptions during copulation, including the inability to copulate and abnormal sperm transfer.

Lepidopteran females are considerably more radiosensitive than males. In a number of species, a dose of 100 Gy is sufficient to achieve almost full sterility in treated females (Anisimov et al. 1989; Marec and Mirchi 1990; Bloem et al. 1999a) (Fig. 3). It appears that this difference in radiosensitivity between males and females is related to the stage of development of the reproductive cells at the time of irradiation. Lepidoptera are usually irradiated as mature pupae or newly emerged adults. At this stage of development eupyrene spermiogenesis in the male has been completed, and the nuclei in eupyrene sperm are in interphase. In contrast, female meiosis is arrested at metaphase I in the nuclei of mature oocytes and the process does not resume until the eggs have been laid (Traut 1977). As a consequence irradiation of newly emerged females or of mature female pupae may disrupt the normal course of meiosis including chromosome segregation. Finally, various secondary detrimental effects can be expected in oocytes, which have a large amount of cytoplasm (that contains many components required for embryonic development) in comparison with the essentially cytoplasm-free sperm. Almost nothing is known about the radiosensitivity of female Hemiptera, although Delrio and Cavalloro (1975) showed that *G. acuteangulatus* females were fully sterilized at a dose of 50–60 Gy. At this dose males were about 70% sterile.

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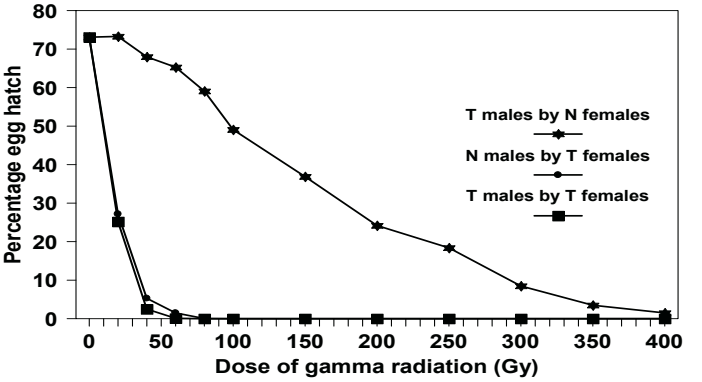



*Figure 3. Percentage egg hatch obtained when codling moth adults were treated with increasing doses of gamma radiation, and either inbred or outcrossed with fertile moths. T = Treated, N = Non-treated. (Figure from Bloem et al. 1999a.)*

### *3.4. Radiation-Induced Sterility in $F_1$ Generation*

Cytogenetic work by Tothová and Marec (2001) showed that various types of translocations (non-reciprocal, reciprocal and multiple) are responsible for the production of genetically unbalanced gametes in $F_1$ progeny and, as such, represent the main chromosomal mechanism of IS. In addition, they demonstrated that two types of intra-chromosomal aberration, fragmentation, and interstitial deletion increase the frequency of unbalanced gametes; however, their contribution to overall sterility appears to be less significant. The study also revealed that the predicted level of $F_1$ sterility, based on the observed frequency of aberrations, was much higher than the sterility observed in a previous study (Marec et al. 1999). This suggests that Lepidoptera possess a mechanism that corrects the predicted unbalanced state towards a balanced segregation of chromosomes. The authors suggested that the increased number of chiasmata might facilitate a balanced disjunction of chromosomes from translocation multivalents in $F_1$ males. The modified synaptonemal complex, which ensures regular disjunction of homologous chromosomes during female meiosis in Lepidoptera (Rasmussen 1977; Marec 1996), might play a similar role in $F_1$ females (Tothová and Marec 2001).

Several authors have documented the effects of radiation on the incidence of visible chromosomal aberrations in $F_1$ males using light microscopy (North and Snow 1978; Saifutdinov 1989; Al-Taweel et al. 1990; Carpenter 1991; Carpenter et al. 1997; Carabajal Paladino et al. 2016).

To better understand the principle of inherited sterility, Tothová and Marec (2001) used a modified micro-spreading technique, first employed by Weith and Traut (1980), to study radiation-induced chromosome aberrations in $F_1$ individuals of *E. kuehniella*. The micro-spread chromosomes were viewed with an electron microscope and several types of aberrations were documented. In $F_1$ individuals from male parents treated with 100 and 150 Gy, the overall frequency of aberrations

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varied between 4.2 and 4.8 per $F_1$ larva in both sexes. A significant increase in aberrations was found in $F_1$ males from male parents treated with 200 Gy (6.2 per male). Fragmentation and several types of translocations (non-reciprocal, reciprocal, and multiple) were the most common aberrations, while interstitial deletions and inversions were rare. Multiple translocations forming complicated configurations were found with increasing radiation dose. In males the mean number of chromosomal breaks resulting in aberrations increased linearly with dose, from 8.4 to 16.2 per nucleus. In females this value reached a maximum of 11.2 breaks per nucleus when male parents were treated with a dose of 200 Gy (Fig. 1).

### *3.5. Sex-Specific Differences in Inherited Sterility*

Two consequences of radiation-induced IS are sex-specific and positively correlated with treatment dose. First, the sex ratio of the $F_1$ generation is skewed toward males (Lepidoptera — Proverbs 1962; Hemiptera — LaChance et al. 1970) and, second, the level of IS in $F_1$ female progeny is lower than in $F_1$ males (Lepidoptera — Anisimov et al. 1989; Al-Taweel et al. 1990; Seth and Reynolds 1993; Bloem et al. 1999a).

Marec et al. (1999) suggested that the sex ratio distortion in the $F_1$ generation in Lepidoptera occurs as a result of recessive lethal mutations induced in the Z sex chromosomes of treated male parents. Since lepidopteran females are heterogametic, all female $F_1$ progeny will be hemizygous for Z and, as a consequence, any deleterious Z-linked mutations will result in $F_1$ female mortality. In contrast, the $F_1$ male progeny will inherit one Z chromosome from the treated father and the other from the mother and, as such, will be heterozygous for any Z-linked mutation.

The induction of $F_1$ sterility by transmitting complex chromosome translocations to the progeny of treated males was first suggested by North (1967) and North and Holt (1968) for Lepidoptera and by LaChance and Degrugillier (1969) for Hemiptera. Tothová and Marec (2001) suggested three factors that might explain the higher level of sterility found in $F_1$ male progeny of Lepidoptera:

- The ability of $F_1$ males to survive even though they inherit a large number of chromosome breaks. The authors found that in $F_1$ males of *E. kuehniella*, the mean frequency of chromosome breaks was positively correlated with a dose-dependent increase in sterility, whereas a clear correlation was lacking for $F_1$ females. They suggested that this difference was due to higher mortality in $F_1$ females that inherit a high number of breaks. Furthermore, at higher treatment doses, there is increased probability that the sex chromosome (Z) will be damaged and, as a consequence, any resulting recessive lethal mutation would kill the $F_1$ females but not the males. Those $F_1$ females that survive carry a smaller number of chromosome breaks and, therefore, are more fertile than the $F_1$ males.
- The occurrence of crossing-over during spermatogenesis. In $F_1$ males crossing-over during spermatogenesis might increase the number of unbalanced gametes produced, but only if it occurs at the crossover point between an aberrant chromosome (that arose by two or more breaks) and its structurally normal homologue. This situation might occur when inversions and multiple

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translocations are formed. However Tothová and Marec (2001) rarely detected inversions in their study on *E. kuehniella*. They concluded that crossing-over contributes to the sterility in $F_1$ males mostly through the formation of multiple translocations. Since female meiosis is achiasmate during oogenesis, this factor cannot play a role in the sterility level of $F_1$ females (Rasmussen 1977; Traut 1977; Nokkala 1987; Marec and Traut 1993b).

- A higher impact of radiation-induced deleterious effects on the fertility of $F_1$ males. Some studies have reported finding a higher number of $F_1$ male crosses that are fully sterile or that have resulted in the female laying a large number of unembryonated eggs, whereas most $F_1$ female crosses laid embryonated eggs (Anisimov et al. 1989; Marec et al. 1999). The data suggest that induced genetic changes impaired the fertilizing ability of some $F_1$ males while the $F_1$ females were not similarly affected. In addition, Koudelová and Cook (2001) reported great variability in the number of sperm that were transferred by $F_1$ males of *E. kuehniella*. They found that, on average, the number of eupyrene sperm decreased, whereas the number of apyrene sperm increased, resulting in an abnormally high ratio of apyrene to eupyrene sperm. The ratio fluctuated between 9.5:1 for untreated males and as high as 100:1 for treated males. These results suggest that chromosomal rearrangements in $F_1$ males may have altered the mechanisms regulating dichotomous spermiogenesis or those underlying copulation and sperm transfer.

### *3.6. Genetic Sexing in Lepidoptera*

Genetic sexing has been documented in only two lepidopteran species. Strunnikov (1975) reported that genetic sexing was possible in the silkworm, *B. mori*. About 15 years later Marec (1990, 1991) and Marec and Mirchi (1990) were successful in constructing in *E. kuehniella* a balanced lethal genetic sexing strain, according to the scheme of Strunnikov (1975). This strain, called BL-2, results in males that are trans-heterozygous for two sex-linked recessive lethal mutations, *sl-2* and *sl-15*. When BL-2 males are mated to wild-type females, the $F_1$ generation consists almost exclusively of male progeny. The $F_1$ females die during embryogenesis because they inherit one of the lethal mutations from their father, i.e., females are hemizygous for *sl-2* or *sl-15* (Fig. 4).

BL-2 males of *E. kuehniella* could be released directly into nature to introduce lethal mutations into the wild population (Marec et al. 1996), or they could be maintained in the laboratory and used to generate a male-only mutant strain that could be irradiated and released into the environment (Marec et al. 1999). Marec et al. (1999) suggested that the production of male-only colonies through the use of balanced lethal strains would reduce rearing costs and enhance population suppression when used alone or in combination with $F_1$ sterility. Furthermore, outcrosses of balanced lethal males with wild-type females one generation before irradiation and release would improve the competitiveness of males through positive heterosis. Finally, in addition to the genetic changes induced by treating the males with gamma radiation, the released mutant males would introduce sex-linked recessive lethal mutations into the wild population that would further reduce the

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number of $F_1$ females produced in the field. However, this system relies on sexing two different strains and making directed crosses, and would be impractical in an operational programme.

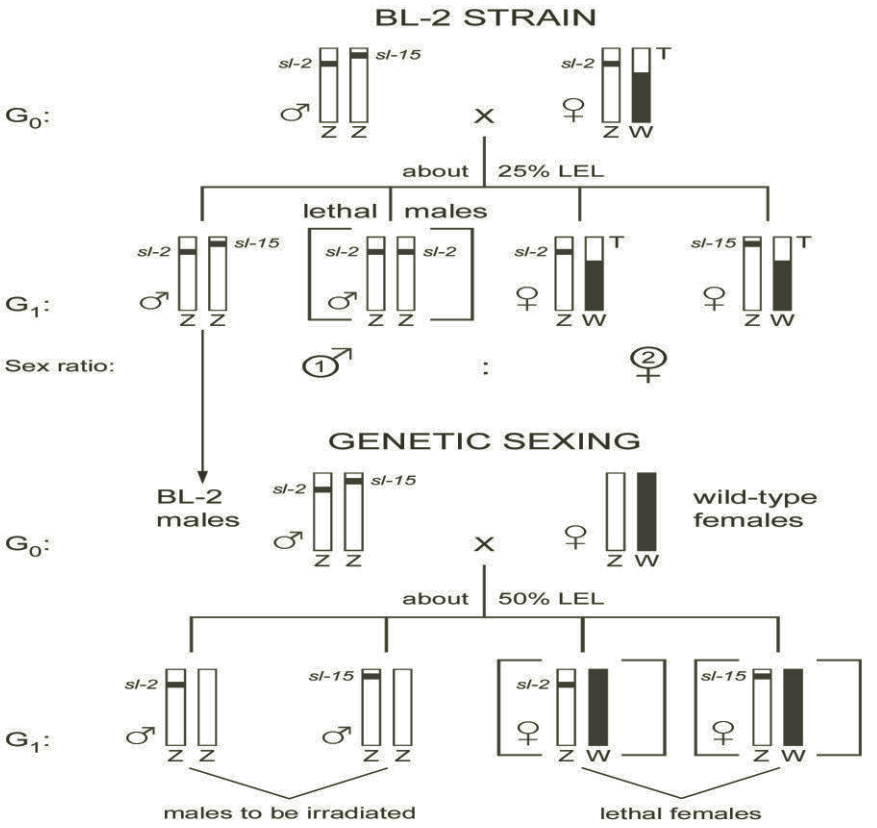



*Figure 4. Genetic sexing system developed in the Mediterranean flour moth; the cross between BL-2 males and wild-type females produces only male progeny. Z and W represent the sex chromosomes; sl-2 and sl-15 are sex-linked recessive mutations. LEL = late embryonic lethality (details in text).*

Some significant obstacles still need to be overcome before these types of genetic sexing strain can be used against pest Lepidoptera. For example suitable markers are currently lacking for the construction of similar mutant strains in other economically important species. Also the genetic sexing system requires the mass-rearing of two different colonies, a wild-type strain and a balanced lethal strain, for which sex separation somehow has to be carried out under mass-rearing conditions. Finally, the mutant strain must be routinely checked to prevent the loss of its genetic structure through genetic recombination or colony contamination (Marec 1991; Franz et al., this volume).

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Discovery of the sex-determining mechanism in Lepidoptera might facilitate the development of a more convenient and sophisticated sexing system. However, little is known about sex determination in Lepidoptera and, except for the silkworm *B. mori,* the exact locations of the primary sex-determining factors are unknown (Traut and Marec 1996; Traut et al. 2007). In this species, significant progress is being made that will undoubtedly facilitate future research on lepidopteran genetics. Ohbayashi et al. (2001) found a homologue of the *Drosophila doublesex* (*dsx*) gene in *B. mori* called *Bmdsx*. A sex-specific alternative splicing of the primary *Bmdsx* transcript, along with results of functional studies, strongly suggest that the gene controls sexual differentiation, as does the *Drosophila dsx* gene (Suzuki 2010). In the silkworm, sex determination depends on the presence or absence of the W chromosome, which carries a dominant female-determining factor (*Fem*) (Fujii and Shimada 2007). Recently, Kiuchi et al. (2014) showed that *Fem* is not a protein-coding gene as originally assumed but a W-encoded small RNA named *Fem* piRNA. The *Fem* piRNA controls female-specific splicing of the *Bmdsx* gene by downregulating expression of the Z-linked *Masculinizer* (*Masc*) gene. It is not known yet if the *Fem* piRNA-*Masc* sex-determining pathway is conserved in other Lepidoptera. Nevertheless, the *dsx* function in sexual differentiation appears to be conserved at the bottom of a sex-determining cascade in many insects including Lepidoptera (Suzuki 2010; Gempe and Beye 2011). Thus, the *dsx* gene may be a good target for the development of genetic sexing strains in pest Lepidoptera.

Advances in genetic technologies, especially in transgenesis, open up new possibilities for genetic sexing in Lepidoptera. Germ-line transformations, with a *piggyBac* transposable element, have been demonstrated — in the pink bollworm by Peloquin et al. (2000) and in the silkworm by Tamura et al. (2000). It has been suggested that genetic sexing strains based on a transgenic approach may be a possible solution to this problem (Marec et al. 2005), where the females would be transgenic but not the sterile males for release. Some progress in developing such strains has been reported in the codling moth (Marec et al. 2007) but this work has not yet been completed. Nevertheless, the feasibility of this approach has been demonstrated recently in the silkworm by the successful insertion of a transgene carrying the EGFP (enhanced green fluorescent protein) reporter into the W chromosome (Ma et al. 2013). Also, a new transgenic conditional lethal genetic sexing system has been developed in the silkworm (Tan et al. 2013) and in two pest species, the diamondback moth and the pink bollworm (Jin et al. 2013). This system is based on the tetracycline-repressible transactivator (tTAV) protein, which is expressed only in females through the alternatively spliced region of the pink bollworm *dsx* gene (*Pgdsx*).

## 4. ADVANTAGES OF INHERITED STERILITY VERSUS FULL STERILITY

The unique characteristics of IS in Lepidoptera and other arthropods provide some inherent advantages over the use of full sterility in pest control programmes (North 1975). Since lepidopteran females generally are much more radiosensitive than males of the same species, the radiation dose may be adjusted to suit programme requirements, i.e. treated females are completely sterile and males partially sterile.

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When these partially sterile males mate with fertile females the radiation-induced deleterious effects are inherited by the $F_1$ generation. As a result egg hatch is reduced and the resulting ($F_1$) offspring are both highly sterile and predominately male (Table 2). The lower dose of radiation used to induce $F_1$ sterility increases the quality and competitiveness of the released insects (North 1975) as measured by improved dispersal after release (Bloem et al. 2001), increased mating ability (Carpenter et al. 1987a), and superior sperm competitiveness (Carpenter et al. 1987a, 1997). In addition, because $F_1$ sterile progeny are produced in the field, the release of partially sterile males and fully sterile females is more compatible with other pest control mechanisms or strategies (Carpenter 1993; Cagnotti et al. 2016).

*Table 2. Typical attributes of male lepidopteran insects (and their progeny) receiving substerilizing doses of radiation*

| Dose applied to $P_1$ (Gy) | 1 Egg hatch (%) | 2 Larval mortality (%) | 3 Sex ratio ♂:♀ | 4 Egg hatch (%) | |
|---|---|---|---|---|---|
| | | | | $F_1$♂ | $F_1$♀ |
| 0 | 71.8 | 20.0 | 1.0:1 | 82.5 | 76.8 |
| 100 | 46.1 | 51.1 | 2.6:1 | 10.8 | 13.9 |
| 200 | 30.8 | 69.5 | 5.1:1 | 0.9 | 7.5 |
| 250 | 19.1 | 75.1 | 7.0:1 | 0.8 | 6.1 |

1. Reduced $F_1$ egg hatch resulting from $P_1$ (parental) generation
2. Increased mortality during $F_1$ development
3. Skewed sex ratio in favor of males in the $F_1$ generation
4. Reduced $F_2$ egg hatch resulting from $F_1$ generation; sterility in $F_2$ generation higher than in $F_1$ generation

Data for codling moth (Bloem et al. 1999a)

Knipling (1970) used a mathematical model to explore the application of IS for control of lepidopteran pests (Barclay, this volume). He found that the release of partially sterile insects offered greater suppressive potential than the release of fully sterile insects, and suggested that the partially sterile-to-wild overflooding ratio could be as low as a one-quarter of what is normally required for fully sterile insects. Population models using data collected from several pest species (Walker and Pederson 1969; Brower and Tilton 1975; Carpenter et al. 1987a; Anisimov 1993; Carpenter and Layton 1993; Kean et al. 2011) corroborate Knipling's findings.

## 5. POTENTIAL FOR INHERITED STERILITY TO SUPPRESS PEST POPULATIONS

Field releases of partially sterile insects have demonstrated the usefulness of IS to control many lepidopteran pests, including the cabbage looper (North and Holt 1969), corn earworm (Carpenter and Gross 1993), gypsy moth (Mastro 1993),

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codling moth (Proverbs et al. 1978; Bloem et al. 1999b; Bloem et al. 2001), cactus moth (Hight et al. 2005), and many others (Bloem and Carpenter 2001; Simmons et al., this volume). The effect of $F_1$ sterility to influence pest populations has been most convincing when irradiated insects have been released in the field throughout the entire growing season. Season-long releases of irradiated (100 Gy) *H. zea* in mountain valleys of North Carolina, USA, delayed and/or reduced seasonal increases of wild *H. zea* males (Carpenter and Gross 1993). The incidence of *H. zea* larvae with chromosomal aberrations indicated that irradiated males were very competitive in mating with wild females, and were successful in producing $F_1$ progeny, which further reduced the wild population. Release ratios averaged less than 5:1 overall, but reduced the wild population of *H. zea* by more than 70%. In another case, season-long field studies of the codling moth were conducted in apple orchards in Washington State, USA, that compared: (1) twice-weekly releases of partially sterile codling moths treated with either 100 or 250 Gy, and (2) combinations of mating disruption plus the release of partially sterile (100 Gy) codling moths, to control wild populations (Bloem et al. 2001). The results showed that fruit damage was significantly lower in all treatment plots when compared with control plots located outside the treatment areas.

## 6. COMPATIBILITY OF INHERITED STERILITY WITH OTHER PEST CONTROL METHODS

The success of releasing insects irradiated with substerilizing doses of radiation for the suppression of pest populations is influenced by the ability of released insects and their progeny to survive and interact with the wild population. Field survival rates for $F_1$ larvae from irradiated parents should be comparable with field survival of wild larvae because many of the deleterious effects induced by radiation are manifested and therefore eliminated during the $F_1$ egg stage (Carpenter et al. 1985). Any mortality agents such as insecticides, entomopathogens, and natural enemies (parasitoids and predators) could potentially interfere with the effectiveness of $F_1$ sterility if the agent killed a higher proportion of treated than wild larvae. Likewise host-plant resistance could potentially interfere with the effectiveness of $F_1$ sterility if the host-plant defenses somehow prevented a higher proportion of treated than wild larvae from establishing and developing on the host plant (Carpenter 1993).

The compatibility of different pest control methods with $F_1$ sterility has been investigated in both laboratory and field studies. Examples include the use of nuclear polyhedrosis viruses with $F_1$ sterility for controlling *H. zea* and *Spodoptera frugiperda* (Hamm and Carpenter 1997), host-plant resistance and $F_1$ sterility in *H. zea* and *S. frugiperda* (Carpenter and Wiseman 1992a, b), $F_1$ sterility and insecticide resistance in *S. frugiperda* (Carpenter and Young 1991), $F_1$ sterility and a commercial formulation of *Bacillus thuringiensis* in *P. opercullela*, $F_1$ sterility and synthetic pheromones to reduce wild populations of the codling moth (Bloem et al. 2001), and the use of parasitoids and $F_1$ sterility (Mannion et al. 1994, 1995; Carpenter et al. 1996; Bloem and Carpenter 2001; Cagnotti et al. 2016; Mangan and Bouyer, this volume). All studies have shown that $F_1$ sterility is compatible with other pest control methods.

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## 7. POPULATION MODELS COMBINING THE EFFECTS OF $F_1$ STERILITY WITH OTHER CONTROL METHODS

Knipling (1964), Barclay (1987), and Wong et al. (1992) recognized the potential benefit of combining sterile insects with conventional pest control methods. According to population models (Barclay 1987; Knipling 1992), combining inundative releases of natural enemies and sterile insects should yield additive or synergistic effects. Even though the SIT and natural enemies have different modes of action, the effectiveness of the SIT increases the ratio of natural enemies to adult hosts, and the effectiveness of natural enemies increases the ratio of sterile to fertile insects. Therefore, greater suppression could be expected if parasitoid releases were combined with the $F_1$ sterility technique (Carpenter 1993). Not only is $F_1$ sterility theoretically more effective than full sterility in reducing population increase (Carpenter et al. 1987a), but $F_1$ sterility results in the production of sterile $F_1$ eggs and larvae that provide an increased number of hosts for parasitoids. As a result, the number of parasitoids produced should increase even if the rate of parasitism remained the same (host-density independent), and whether or not additional parasitoids are released. Although population models (that independently consider augmentative releases of parasitoids (Knipling 1992) and $F_1$ sterility (Carpenter et al. 1987a)) suggest that both methods are highly efficacious, integrating lepidopteran $F_1$ sterility and augmentative biological control results in synergistic effects (Carpenter 1993). Therefore, the greatest impact of $F_1$ sterility and augmentative parasitoid releases as area-wide methods against lepidopteran pests can be realized only when the two methods are integrated (Barclay, this volume).

Population models also provide insight into how different control strategies could be combined for greatest efficiency. Although the effectiveness of $F_1$ sterility continues to increase as the ratio of irradiated to non-irradiated insects increases, the efficiency per released moth declines. A similar loss of efficiency occurs in parasitoid releases (Carpenter 1993). According to these models the economic benefit of combining $F_1$ sterility and parasitoid augmentation would be greatest when the ratios of irradiated to non-irradiated, and parasitoid to host, are low (i.e. equal to or less than 10:1). The model presented in Fig. 5 demonstrates that population suppression is increased when $F_1$ sterility and parasitoid releases are combined, and that the percentage reduction in population growth is greater when parasitized hosts produce adult parasitoids than when no parasitoids are produced (Carpenter 2000).

## 8. INHERITED STERILITY IN COMBINATION WITH BIOLOGICAL CONTROL

Fully successful integration of $F_1$ sterility and parasitoid augmentation into a management approach can occur only if parasitoids do not negatively impact irradiated insects and their progeny more than that of the wild population, and if $F_1$ sterility does not negatively impact the efficacy and reproduction of parasitoids. Knowledge of any negative impact of $F_1$ sterility on parasitoids would be important before implementing an AW-IPM programme using $F_1$ sterility. For example, if

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parasitoids that attack the $F_1$ sterile progeny are unable to develop normally, and most of the hosts present are $F_1$ sterile progeny, then there could be a negative impact on subsequent parasitoid populations. Conversely, if parasitoids develop normally on $F_1$ eggs, larvae, and pupae, then the greater number of hosts available would allow for an increase in the parasitoid population. Since many hosts of the $F_1$ generation would experience genetically-induced mortality before they reached the adult stage, any parasitoids able to develop on these hosts would result in a positive and synergistic increase in the efficacy of an AW-IPM programme (Carpenter 2000).

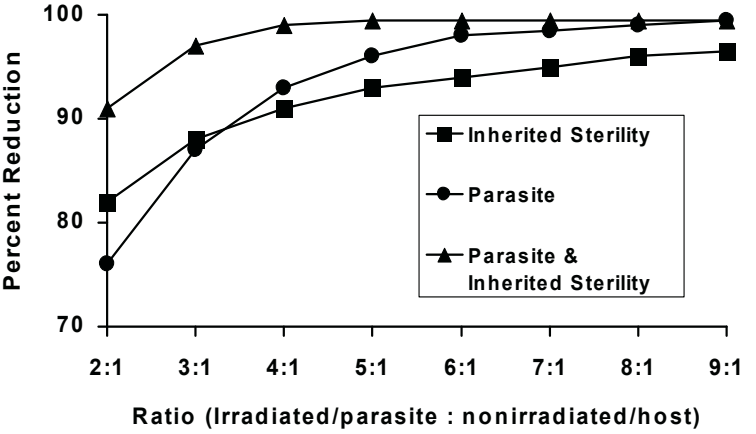



*Figure 5. Comparison of the projected reduction in normal population growth when only parasitoids [parasites] are released (Knipling 1992), when only irradiated (100 Gy) male moths are released (Carpenter et al. 1987c), and when releases are equally divided between parasitoids and irradiated male moths.*

Field, greenhouse and laboratory studies compared the acceptability and suitability of progeny from irradiated (100 Gy) and untreated *Spodoptera exigua* males as hosts for the braconid larval parasitoid *Cotesia marginiventris* (Cresson) (Carpenter et al. 1996), and progeny from irradiated (100 Gy) and untreated *H. zea* males as hosts for the tachinid *Archytas marmoratus* (Townsend) (Mannion et al. 1994, 1995). Results from these studies demonstrated that progeny of irradiated males and untreated females were acceptable and equally suitable hosts for parasitoid development. Female parasitoids showed no oviposition preference for progeny from females paired with either irradiated or untreated males. Other studies on different lepidopteran pests also have reported compatibility between the two control methods (Bloem and Carpenter 2001).

There are many different scenarios in which $F_1$ sterility could be integrated with natural enemies to suppress pest populations (Carpenter 1993; Hendrichs et al. 2009) (Box 1). The release of partially sterile males and fully sterile females would produce large numbers of $F_1$ eggs and larvae that could be field-reared on early-season host plants or crop plants that tolerate some larval feeding damage, e.g.

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whorl-stage corn. Natural enemies (native and/or released) could use the $F_1$ eggs, larvae and pupae as hosts and thereby substantially increase the natural enemy population for the next generation of the pest insect (Proshold et al. 1998). Also surviving sterile $F_1$ progeny would produce sterile adults that would negatively impact the next generation of the pest insect. If the economic injury level of cultivated host plants indicated that the additional sterile $F_1$ larvae were undesirable, then the dose of radiation could be increased to a level that would reduce or eliminate the number of progeny from irradiated females, or releases could be limited to irradiated males.

*Box 1. Opportunities for Combining Inherited Sterility with Biological Control*

For pest suppression:

- Simultaneous or sequential inundative releases of irradiated insects and natural enemies
- Irradiated insects and their sterile progeny serve as host/prey for wild natural enemies.

For research:

- Elucidation of the potential host range of exotic pests
- Prediction of the potential geographic range of exotic pests
- Delineation of the potential impact of native natural enemies on invading pests.

Although the compatibility of $F_1$ sterility with the application of synthetic organic insecticides has been demonstrated (Carpenter and Young 1991), parasitoids and/or predators generally are not compatible with these products. When insecticides are required to reduce pest infestations, insect growth regulators or other formulations that are compatible with natural enemies should be considered. Another management option would be to establish host plants for the pest in insecticide-free areas adjacent to insecticide-treated crops. Host plants could be artificially infested with pest larvae to provide natural enemies (native and/or released) with an adequate supply of hosts. If the pest larvae used in the artificial infestations (nursery crops) were sterile, i.e. the progeny of irradiated parents, then non-parasitized larvae would not contribute to the increase of the wild population but would produce sterile adults that would negatively impact the next generation of the pest insect (Okine et al. 1998; Carpenter 2000).

In addition to using $F_1$ sterility as a direct pest control method, there are opportunities to use $F_1$ sterility to facilitate the development of other pest suppression methods. For example the $F_1$ sterile progeny (eggs, larvae and pupae) of a pest may be used as hosts/prey for natural enemies that are shipped commercially, especially in quarantine-sensitive shipments (Hendrichs et al. 2009). The use of sterile insects in commercial shipments would ameliorate concerns regarding the reproductive viability of non-parasitized and non-consumed pests upon arrival at the shipment destination. Also the use of $F_1$ sterile progeny as hosts for parasitoids would eliminate the need to wait for non-parasitized pests (either eggs or pupae) to emerge before shipment of the parasitized pest (Greany and Carpenter 2000; Hendrichs et al. 2009).

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## 9. ADDITIONAL APPLICATIONS OF $F_1$ STERILITY FOR RESEARCH AND MANAGEMENT

Greany and Carpenter (2000) reported that $F_1$ sterility could provide a new risk management tool for assessing the safety of exotic lepidopterans being considered as biological control agents against invasive weeds (Moeri et al. 2009; Tate et al. 2009) (Box 1). Production of $F_1$ sterile progeny permits developmental and behavioral observations to be made under actual field conditions without concern that a breeding population would be established (Carpenter et al. 2001a). This facilitates field observations on oviposition behaviours and host associations, larval feeding preferences, and larval development and survival on both target and non-target plant species. Also, the impact that native natural enemies might have on exotic candidate species being considered as biological control agents for invasive weeds, and the ability of these candidate species to survive and overwinter under various climatic conditions could be studied in the field through this innovative application of $F_1$ sterility.

$F_1$ sterility could be used to conduct research on exotic pests that are expanding their geographical range. Strom et al. (1996) suggested that gypsy moth host preferences, or the quality of potential hosts outside the generally infested area, could be investigated using releases of $F_1$ sterile larvae of *L. dispar*. In addition to host range studies, Carpenter et al. (2001a) suggested that $F_1$ sterility could be used to predict the potential geographic range and to evaluate the potential impact of native natural enemies on the rate of spread of exotic lepidopteran pests.

## 10. CHALLENGES AND OPPORTUNITIES

Challenges are inherent in all pest management methods, and Whitten and Mahon (this volume) discuss constraints and misconceptions unique to the SIT. In addition to those in common with the SIT, the major challenge to the use of IS is the perception that sterile $F_1$ larvae cause economic damage to crops, especially high-value crops such as fruit. Consequently, low doses of radiation, which would certainly result in more competitive insects, are often avoided. For example, Proverbs et al. (1978) found that codling moths treated with 250 Gy were more competitive in the field, and provided better control, than did fully sterile moths (400 Gy). Nevertheless, in spite of these findings, Proverbs et al. (1982) continued to be concerned that $F_1$ larvae would cause economic damage, and used 350 Gy to irradiate moths released in a pilot study conducted in British Columbia, Canada, from 1976–1978. Also, in the current codling moth programme in British Columbia, Canada, which began field operations in 1994, moths treated with 350 Gy are released, even though doses as low as 100 Gy have been suggested (Anisimov 1993; Bloem et al. 1999a, 1999b). It was found in field studies that season-long releases of moths treated with 100 Gy did not cause fruit injury (Fig. 6), and moths receiving 100 Gy were more competitive than those receiving 250 Gy (Bloem et al. 2001). These studies indicate that fears of increased fruit (or plant) injury resulting from $F_1$ larvae are ill founded, especially when the radiation dose causes partial sterility in males and full sterility in females. Therefore, the production of sterile $F_1$ larvae

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should be seen not as a problem but rather an opportunity to enhance the production of natural enemies and to produce more competitive sterile moths in the field.

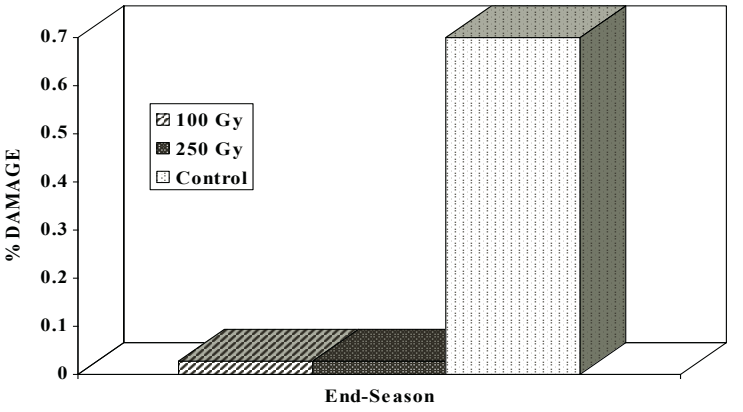



*Figure 6. Percentage fruit damage at harvest caused by the codling moth in apple orchards in Washington State, USA. One series of three plots received season-long releases of partially sterile codling moths treated with 100 Gy (female moths were 100% sterile, and males 50% sterile). A second group of plots received 250-Gy-treated moths (females 100% sterile, and males 75% sterile). The control areas were treated with six applications of azinphosmethyl insecticide. At the end of the fruiting season, 2500 fruit per treatment were sampled (Bloem et al. 2001).*

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# CHAPTER 2.5.

# MATHEMATICAL MODELS FOR USING STERILE INSECTS

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## SUMMARY

This chapter begins with a consideration of simple population models. The sterility formulation proposed by Knipling is then included in the population models, and these are elaborated in systematic fashion to include the major biological factors that will affect the success of a sterile insect technique (SIT) control programme. These factors include residual fertility, differential competitive ability of wild and sterilized males, mating patterns, immigration, and various combinations of these features. Also examined are density-dependence, age structure, population aggregation, Allee effect, biotic interactions with other species, and then integration of the SIT with other control methods. It was found that combinations of factors are often synergistic: combinations of detrimental factors such as residual fertility and inferior competitive ability put severe limits on the probable success of the control programme, while combinations of control methods are much more likely to succeed than single control methods. This is because each control method needs only to account for a smaller proportion of the total mortality when combined with other methods than when acting alone. The barrier width is computed to protect an area from pests outside the controlled area using a diffusion equation, and a case study is presented using Mediterranean fruit flies. The redistribution of sterile males released from aircraft travelling along predetermined flight lines is also addressed using a diffusion equation. To minimize costs, a short method is presented to calculate the optimal frequency of releases, distance between flight lines, and number of sterile males released on each flight. This short method is capable of calculation using a hand calculator. Tentative results are also presented on optimizing the number of sprays and time intervals between sprays of a pest population in preparation for an SIT project. The question of whether to release only sterile males or both sexes is examined in the light of results for tephritids. Parameter estimation is mentioned, and also an assessment of the models presented herein. Finally, a brief section on future needs and trends for modelling the SIT is presented.

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## 1. BRIEF OVERVIEW OF MODELLING

### *1.1. Types of Models*

Modelling is the abstraction of processes or states of being. Mathematical models involve equations, graphs or algorithms behind computer code. Virtually all models of the sterile insect technique (SIT) are population models, either analytic (just with equations) or computer models (often called numerical models and in which the equations are usually implicit, rather than being made explicit). Population models keep track of population numbers, and include various features that influence population size and trend, such as birth rate, mortality, age structure, immigration and emigration, competition, etc. Population growth can be either density-independent, in which birth rate and mortality are independent of population size, or density-dependent, in which either or both of birth rate and mortality depend on population size, usually in such a way as to eventually stabilize the population around some long-term mean value.

Mathematical models of populations are typically posed as difference equations or as differential equations. Difference equations are discrete and use some meaningful time step, such as days, years, generations, etc. These are popular with entomologists, since many insects breed seasonally, such as most temperate forest insect pests (bark beetles, budworms, tent caterpillars, etc.). Differential equations are continuous, involving an infinitesimal time step, and rely on calculus to solve them. They are useful in species that breed continuously within some period of time, such as aphids, stored products pests and animal parasites. However, difference equations do not involve calculus, and generally are easier for the non-mathematician to understand. Another dichotomy is between deterministic models, which always yield the same model result, and stochastic models, which involve randomness. Most of the models of the SIT have so far been deterministic models, involving no random elements.

In this chapter, only models that predict some aspect of system behaviour (population dynamics) will be explored. Thus, purely statistical analysis of data, however valuable and relevant that might be to a release programme, will not be considered here. Likewise the derivation of regressions for use in models will not be considered here as SIT modelling. However, a section on model parameter estimation, in which such techniques are mentioned, is included.

### *1.2. Simple Population Models*

For density-independent population growth, the simplest models are geometric growth for a species with non-overlapping generations, and its continuous counterpart, exponential growth (Box 1). A simple modification to these models, to include resource limitation, puts an upper limit on growth. Many formulations exist for limiting geometric growth; a few were provided by Hassell (1978). This small complication makes some formulations insoluble analytically, and it is a common feature of population models that non-linearity renders the models insoluble analytically; it is then necessary to resort to numerical solutions using a computer.

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*Box 1. Simple Growth Models*

*Geometric and Exponential Growth*

The geometric model is $N_{t+1}=\lambda N_t$. Here $N_t$ is the size of the population at time $t$, where $t$ is scaled to generations and $\lambda$ is the rate of increase each generation. In each generation the population size is $\lambda$ times the size it was in the previous generation. In this model, generations are discrete and non-overlapping. This model is easy to solve. At any time $t$, $N_t=N_0\lambda^t$, where $N_0$ is the size of the population at time $t$=0. The exponential growth model is $dN/dt=rN$. The solution to this model is $N=N_0\exp(rt)$, where $\exp(rt) = e^{rt}$, $e$ being the base of natural logarithms, $r$ is the instantaneous rate of growth, and $N_0$ is the initial size of the population at time zero.

*Density-Dependent Growth*

With density-dependence, the geometric model becomes $N_{t+1}=\lambda N_t\exp(-gN_t)$, in which the exponential term has no real biological meaning, and is simply a convenient device to limit population numbers. The continuous version is the logistic equation: $dN/dt=rN(K–N)/K$, where $K$ is the carrying capacity imposed by resource limitation.

### *1.3. Host-Parasitoid and Predator-Prey Models*

Modelling host-parasitoid and predator-prey systems in insect population dynamics has a long history, and the "workhorses" are the Nicholson-Bailey difference equation model and the Lotka-Volterra differential equation model (Box 2). Hassell (1978) described such models, and these models have been used in modelling sterile insect releases for populations under the influence of biotic interactions with other species.

*Box 2. Predator-Prey and Host-Parasitoid Model*

*Nicholson-Bailey Model*

Without density-dependence $N_{t+1}=\lambda N_t\exp(-aP_t)$; $P_{t+1}=\lambda N_t[1–\exp(-aP_t)]$ where $N_t$ and $P_t$ are the host and parasitoid population sizes at time $t$.

With density-dependence $N_{t+1}=\lambda N_t^{(1-b)}\exp(-aP_t)$; $P_{t+1}=\lambda N_t^{(1-b)}[1–\exp(-aP_t)]$ where $b$ is a parameter for imposing density-dependence and has no obvious biological meaning, and $\exp(-aP_t)$ is the zero term of a Poisson series, representing those hosts not found each generation by a group of randomly searching parasitoids.

*Lotka-Volterra Model*

Without density-dependence $dN/dt=rN–bNP$; $dP/dt=P(cN–e)$ in which the first equation gives the rate of change of the prey population ($N$) in terms of the intrinsic rate of increase, $r$, and a predation rate per predator, $b$; the second equation gives the rate of change of the predator population ($P$) in terms of the rate of increase per prey, $c$, and a death rate, $e$.

With density-dependence $dN/dt=rN(1–aN)–bNP$; $dP/dt=P(cN–e)$, where $a$ is a density-dependent death rate of the prey.

## 2. MODELS OF STERILE INSECT RELEASES

### *2.1. Three Kinds of Control Programmes Using Sterility*

There are three methods of using sterile insects for population control. These are: (1) the standard method of releasing sterile males (or males and females) that have been

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reared and sterilized; earlier work in modelling of sterile releases was previously summarized by Hamada and Miyai (1985); (2) the treatment of insects with sub-sterilizing doses of radiation or chemosterilants so that the matings are partially sterile, but the offspring of matings involving treated insects are sterile, called inherited sterility (Kean et al. 2007; Marec et al., this volume); and (3) the deployment of chemosterilants in field traps to sterilize insects that are attracted to the traps. It is mainly the first of these three methods that will be dealt with here. Chemosterilants are seldom used in the field because of their carcinogenic potential and the related risk of bioaccumulation in the food chain, although modelling has been done on this technique by Knipling (1960), Lawson (1967), Staley et al. (1971), Hawkes and Coaker (1977), Barclay (1981a), and Wall and Howard (1994). In addition, although the SIT is usually used for insect control, in some cases the concept can apply to other animals (Klassen et al. 2004).

### *2.2. Initial Contribution of Knipling to Modelling the SIT*

Knipling produced a simple numerical model that foreshadowed most future modelling developments (Knipling 1955, 1959). The central feature of Knipling's model, and one found in almost all subsequent models, is the ratio of fertile males to all males in the population: $(M/(S+M))$ where $M$ is the number of fertile males (or females, assuming a 1:1 sex ratio) and $S$ is the number of sterile males. This gives the proportion of the population, under ideal conditions, that results in fertile egg production as a result of some fertile females mating with fertile males. Knipling's (1955) model for the release of sterile insects was a simple modification of the geometric model in Box 1 using the sterility factor above:

$$F_{t+1}=\lambda F_t(M_t/(S+M_t)) \quad (1)$$

where $F_t$ and $M_t$ are again the population size (fertile females and males) at time $t$, $\lambda$ is the rate of increase per generation, and $S$ is the release rate of sterile males each generation. This yields a stable steady state at $F=0$ and an unstable positive steady state for $F$ when $S=S^*$, the critical release rate, where $S^*=F(\lambda-1)$, the value of sterile release rate that holds the population at the steady state (Berryman 1967). If $S>S^*$, then the pest population will collapse and be eliminated. If $S<S^*$, then the population in this model will increase indefinitely.

### *2.3. Sex Ratio*

One question asked early in work on releasing sterile insects was, "Is there an optimal sex ratio for the sterile insects being released?" It was initially thought that the release of sterile females would be counterproductive. This question was first addressed by Ailam and Galun (1967) and Lawson (1967); using probabilistic models of mating, they predicted that the release of females should never be detrimental (assuming they are all fully sterile), and in fact may assist the control programme if males are limited in their mating ability, in which case some fertile

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females might not get mated. However, for female-choice mating systems such as fruit flies the opposite was shown; in the absence of sterile females, sterile males are 3-4 times more effective because they focus only on fertile females and do not waste their limited sperm on sterile females (Hendrichs et al. 1995; Rendón et al. 2004). This was confirmed theoretically for female-choice mating systems, such as in tephritids (Vreysen et al. 2006). On the other hand, for moths (which have a very different mating system) the limited field evidence so far indicates that there is no advantage to male-only releases. In addition, for insects such as mosquitoes where females are disease vectors, it is essential not to release sterile females because that might increase the number of disease vectors.

### *2.4. Residual Fertility of "Sterile Insects"*

If some of the treated insects are not completely sterilized, then the situation becomes more complicated. Klassen and Creech (1971) constructed a simple numerical model in which a certain proportion of the released males remained fertile. They found an upper limit to this "residual fertility" that was compatible with the success of the release programme. Their model can be put into algebraic form and generalized. When there is incomplete sterilization of the released insects, a fraction, $q$, of males remains fertile. In that case, Knipling's model can be modified as in Box 3. The critical sterile release rate is then only finite for $q<1/\lambda$. If $q>1/\lambda$, then the population is not controllable by sterile releases. Thus, for example, if the rate of increase, $\lambda$, is 10, then $q$ must be less than 0.1, i.e. the released males must be greater than 90% sterile in order for control by the SIT to be possible. Also, if the residual fertility is more than about three-fourths of the limiting value, then the required rate of sterile releases has to be much higher than with complete sterility (Fig. 1).

If both males and females are released and neither sex is completely sterile, then the fertile male X fertile female matings can be modelled as in Box 3. If residual fertility exists in both sexes following release, it becomes impossible to eliminate the pest population by sterile releases alone; the best that can be done is to suppress it to a low level with continuing sterile releases. In addition, control is impossible unless $q_m<F/\lambda(F+q_fS_f)$, where $q_m$ and $q_f$ are the residual fertilities of males and females, respectively. This value of $q_m$ is smaller than that without the release of females (Fig. 2). Thus, less residual male fertility can be tolerated with the release of residually fertile females (Barclay 2001). Fortunately, in most species targeted by the SIT, females are more sensitive than males to radiation sterilization (sperm are post-meiotic cells, whereas mature eggs are pre-meiotic), and therefore it is possible to identify sterilizing doses for the SIT that assure full female sterility (Bakri et al., this volume; Robinson, this volume).

### *2.5. Competitive Ability of Males*

The ability of sterile males to compete with wild males for mates can be affected by sterilization through the debilitating effects on either sperm competition or the

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behaviour of the adults (Parker, Vreysen et al., this volume; Lance and McInnis, this volume). This problem was modelled by Berryman (1967), Bogyo et al. (1971), Berryman et al. (1973), Itô (1977), and Barclay (1982a). Their models are summarized by the model in Box 4. All of their models show that the critical release rate increases as the competitive ability of sterilized insects decreases.

*Box 3. Residual Fertility of "Sterile Insects"*

Here Knipling's model becomes: $F_{t+1}=\lambda F_t(M_t+qS)/(M_t+S)$, where it is assumed that either only males ($M_t$) are released or that released females are completely sterile and only males display residual fertility. This model has a stable steady state at $F=M=0$, and an unstable positive steady state for $F$ and $M$ when $S=S^*$, the critical release rate, where $S^*=M(\lambda-1)/(1-\lambda q)$. Here, $S^*$ is only finite for $q<1/\lambda$.

If both males and females are released and neither sex is completely sterile, then the fertile male X fertile female matings can be modelled by the equation: $F_{t+1}=\lambda(F_t+q_fS_f)(M_t+q_mS_m)/(M_t+S_m)$, in which $F_t$ is the number of wild fertile females in generation $t$, $q_m$ and $q_f$ are the proportions of treated males and females, respectively, that remain fertile, and $S_m$ and $S_f$ are the number of treated males and females, respectively, that are released each generation (Barclay 2001). So far there is no restriction on the sex ratio. Thus $q_fS_f$ released females that remain fertile are added to the wild fertile females each generation, and $q_mS_m$ released males are added to the number of wild fertile males each generation, with the assumption that treated insects are equally competitive for mates with wild insects. This model has a lower stable steady state and an upper unstable steady state for $F$ and $M>0$ when $S=S^*$, and $S^*_m=(\lambda-1)(FM+\lambda q_f S_f M)/(F(1-\lambda q_m) -\lambda q_m q_f S_f)$, and this equation is only soluble if the sex ratio is known. If we assume a one-to-one sex ratio (where $M_t=F_t$ and $S_m=S_f=S$), then we obtain a quadratic equation: $\lambda q_mq_fS^2-[1-\lambda(q_m+q_f)]FS+(\lambda-1)F^2=0$ which gives two roots when solved for either $S$ or $F$. The upper root of $F$ is unstable, and represents the size of the population before initiating sterile releases. The lower root of the equation for $F$ is stable, and is the value at which the population would be maintained by residual fertility after collapse due to suppression by sterile releases. Thus it is impossible to eliminate the pest population by sterile releases alone; also control is only possible if $q_m<F/\lambda(F+q_fS_f)$. The relationship between the maximum values of $q_m$ and $q_f$ is hyperbolic (Fig. 2).

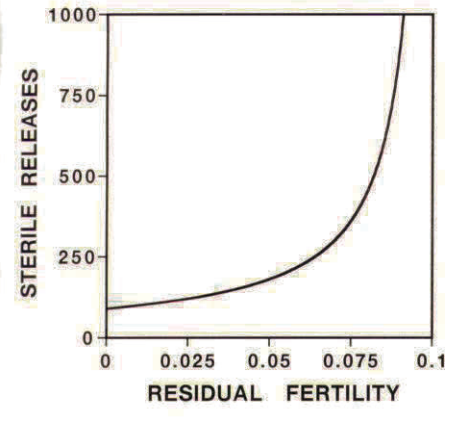



*Figure 1. The critical sterile release rate, S*, as a function of residual fertility, q. λ = 10.*

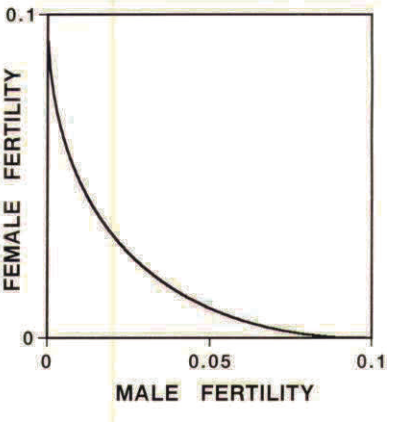



*Figure 2. Allowable residual fertility when it is in both males and females. λ = 10.*

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*Box 4. Competitive Ability of Males*

We define $c$ as a coefficient of competitive ability, with 0 being completely non-competitive and 1 being fully competitive. Then $F_{t+1} = \lambda F_t(F_t/(F_t+cS))$. This model has a stable steady state at $F=M=0$ when $S>0$. The positive (unstable) steady state for $F$ occurs when $S=S^*$, the critical value, where $S^*=(\lambda\text{-}1)F/c$, which is greater than $(\lambda\text{-}1)F$, with full competitive ability (Itô et al., this volume).

### *2.6. Interactions of Residual Fertility and Competitiveness*

In this model only males display residual fertility; females are either completely sterilized or not released.

#### *2.6.1. Residually Fertile Insects Are Fully Competitive*

Here the insects that remain fertile after treatment are fully competitive with wild insects (Box 5). The allowable residual fertility of males is an almost linear function of the competitive ability of released sterile males (Fig. 3A, solid line), unless $\lambda$ is very small. Also, for a given degree of residual fertility, $S^*$ becomes larger as $c$ becomes smaller (Fig. 3B). Thus, the extent of residual fertility, and the degree of competitive ability that is lacking, tolerable to achieve some control, each have to be more restricted in the presence of the other (Barclay 2001). In this case, each of $c$ and $q$ will be more restrictive than is allowable if only one of these limitations exists (both must be within the range that allows for control by the SIT).

*Box 5. Residual Fertility and Competitiveness*

If residual fertile insects are fully competitive, $F_{t+1}=\lambda F_t(F_t+qS)/(F_t+qS+cS(1-q))$, then this model has a stable steady state at $F=M=0$ when $S>0$. The positive (unstable) steady state occurs when $S=S^*=(\lambda\text{-}1)F/(c(1-q)-q(\lambda\text{-}1))$, and $S^*$ is finite only if $q<c/(\lambda\text{-}1+c)$.

If residual fertile insects are of reduced competitiveness, then the model becomes $F_{t+1}=\lambda F_t(F_t+cqS)/(F_t+cS)$. If there is no residual fertility, there is a stable steady state at $F=M=0$, if $S>0$, and a positive unstable steady state when $S=S^*=(\lambda\text{-}1)F/c$.

If there is both residual fertility and unequal male competitive ability, the steady state for $F$ is at $S^*=(\lambda\text{-}1)F/(c(1-q\lambda))$, and this is finite only if $q<1/\lambda$.

#### *2.6.2. Residually Fertile Insects Have Reduced Competitiveness*

Here the insects that remain fertile after treatment are not fully competitive with wild insects (Box 5). The critical sterile release rate in this case (Fig. 3C and Box 5) is not as large as the corresponding value when residually fertile insects are of full competitive ability (Fig. 3B).

### *2.7. Mating Patterns*

Another question asked early in the use of sterile releases was, “Should the females of the target species in a sterile release programme mate once or more than once?” The question has been addressed by Von Borstel and Knipling (1960) and Knipling (1964), and in the models of Berryman (1967), Lawson (1967), Zouros (1969), and

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Barclay (1984). The answer appears to be that female remating (polygamy) is quite compatible with the SIT, as long as mating is random, with sterilized male sperm being fully competitive with wild male sperm. In addition, in polygamous species, it doesn't matter whether sperm is diluted, replaced or excluded after the first mating, again as long as mating is random, and sterile males are fully competitive (Lance and McInnis, this volume).

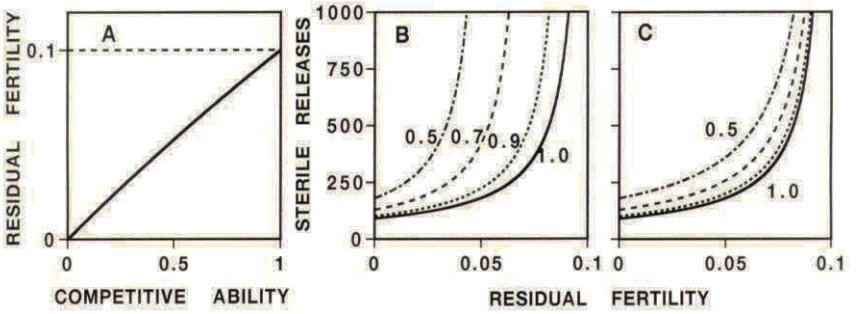



*Figure 3. A: Changes in allowable residual fertility with changes in competitive ability of sterile males. Residually fertile insects are of reduced competitive ability (broken line) or are fully competitive (solid line). B and C: Critical sterile release rates for residual fertility (0 to 0.1) and competitive ability (0.5, 0.7, 0.9, 1.0). In B, residually fertile insects are fully competitive, and in C, residually fertile insects are of reduced competitive ability. $\lambda = 10$ in all cases. (Figure from Barclay 2001, reproduced with permission.)*

### *2.7.1. Interaction of Polygamy with Competitive Ability*

If sterile males are equally competitive for mates with fertile males, sterile sperm is fully competitive with fertile sperm, and there is no residual fertility, then the effects of polygamy (multiple female mating) are simply to reshuffle the sperm at each mating, and polygamy has essentially no effect. On the other hand, if sterilization is incomplete, and/or sterilized males (or their sperm) are of inferior competitive ability, then the situation is more complex. The work of Berryman (1967) (also addressed by Zouros (1969)) is particularly insightful in this matter. If only the first mating of a female results in sperm retention, or if all of the sperm of previous matings is replaced at each successive mating, and if all matings occur before oviposition begins, then the effective number of matings is just one. If the sperm from all matings mixes and is retained, then the effect of multiple matings depends on sperm competition as well as on competition between sterile and fertile males for mates. Berryman (1967) addressed this important problem, and it is worthwhile revisiting his results, making appropriate changes in his notation to make it consistent with the development above. Berryman considered three cases, depending on sperm action (Box 6).

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*Box 6. Mating Patterns*

*Interaction of Multiple Female Mating and Competitiveness*

The probability of a fertile female mating with a sterile male is defined as $P_s=c_aS/(F_t+c_aS)$, and the probability of a fertile female mating with a fertile male as $P_f=F_t/(F_t+c_aS)=1-P_s$, where $c_a$ is the competitive ability of sterile adults (equivalent to $c$ in Box 4). Berryman (1967) considered the joint distribution of the number of matings, and the number of sterile matings, as a sequence of marginal distributions of the number of sterile matings given the number of matings. Thus a female can mate from zero to $M$ times, and for a given number, $m$, of matings the number of sterile matings is binomially distributed. If ${}_mC_n$ is defined as the number of combinations of $m$ things taken $n$ at a time ($=m!/n!(m-n)!$), then the conditional probability that a given female mates with $n$ sterile males, given that she mates $m$ times, is $P(n|m)={}_mC_nP_s^n\ P_f^{m-n}={}_mC_nP_s^n(1-P_s)^{m-n}$. This is one term of a binomial distribution that describes the number of sterile matings given the number of matings, and there will be $M+1$ such distributions, including one for no matings. Berryman considered three cases, dependent on sperm action.

*Non-Functional Sperm*

If the sperm of sterilized adults is either nonexistent or immotile, then a female mating $m$ times will only produce sterile eggs if all the matings were with sterile males. The probability of this occurring is $P_s^m$, and so the probability of at least one fertile mating is $(1-P_s^m)$. Then the probability of at least one fertile mating, over the range of mating frequencies, is $\Sigma P_m(1-P_s^m)$ for $m$ = 1,2,3, …, $M$, and so the population equation becomes $F_{t+1}=\lambda F_t\Sigma P_m(1-P_s^m)$ for $m$ = 1,2,3, …, $M$, where $P_m$ is the probability of mating $m$ times.

*Dominant Lethal Mutations*

In the binomial expansion of the probabilities of $m$ matings, where $m$ goes from 0 to $M$, each of the terms representing mixed fertile and sterile matings will be weighted by a factor, $c_s$, representing the competitive ability of sterile sperm. Thus the probability of an egg being fertilized by a sterile sperm, taken over all mating frequencies, will be $P(e)=P_s^m+c_s((m-1)/m)_mC_{m-1}\ P_s^{m-1}(1-P_s)+c_s((m-2)/m)_mC_{m-2}P_s^{m-2}(1-P_s)+....+(0/m)_mC_0(1-P_s)^m$, which can be reduced to $P_s^m+c_sP_s(1-P_s^{m-1})$, where $P_s=c_aS/(F_t+c_aS)$, as above. We can write the equation as $F_{t+1}=\lambda F_t\Sigma P_m[1-(P_s^m+c_sP_s(1-P_s^{m-1}))]$ for $m$ = 1,2,3, …, $M$. The values of the critical release rates, $S^*$, are shown in Fig. 5A and Fig. 5B against the adult sterile competitive ability, $c_a$, and for the sperm competitive ability, $c_s$ in Fig. 5C. In addition, the values of $c_a$ and $c_s$ are shown for given values of the critical release rate (250, 500), $S^*$, when it is held constant (Fig. 5C); as shown, there is a trade-off between $c_a$ and $c_s$.

### *2.7.2. Non-Functional Sperm*

If the sperm of sterilized adults is either non-existent or immotile, then a female mating $m$ times will only produce sterile eggs if all the matings were with sterile males. The resulting critical values (Box 6) of the sterile release rate, $S^*$, are shown in Fig. 4 for several values of $M$, the maximum number of matings, values of the adult competition coefficient from 0.5 to 1.0, two values of the probability of mating, and assuming a binomial distribution of mating frequencies.

### *2.7.3. Dominant Lethal Mutations with Fully Competitive Sperm*

If sterility is caused by dominant lethal mutations, and the sperm of sterilized adults is fully competitive with that of fertile adults, then it can be shown that the probability that an egg is fertilized by a sterile sperm is independent of the number of matings, and the results from section 2.5. on competitive ability still hold with polygamy, and correspond to the case of $M$=1 in Fig. 4.

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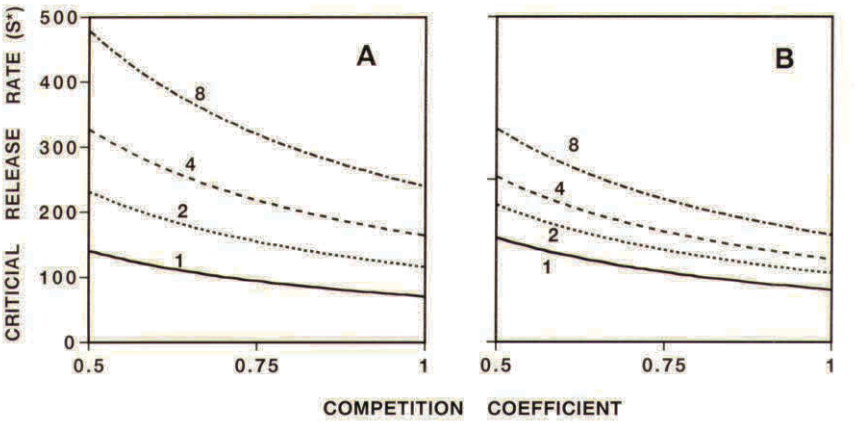



*Figure 4. Critical sterile release rates when sterile males produce non-functional sperm. The maximum possible number of matings, M, is 1, 2, 4, 8, and the probability of mating in A is 0.8, and in B is 0.9. The adult competition coefficient ranges from 0.5 to 1.0. λ=10 in all cases. (Figure generated from equations, Berryman 1967.)*

### *2.7.4. Dominant Lethal Mutations with Reduced Sperm Function*

If sterility is caused by dominant lethal mutations, and the sperm of sterilized adults is of reduced competitive ability compared with that of fertile adults, then it can be shown that the probability that an egg is fertilized by sterile sperm depends on the number of matings. The values of the critical release rates, $S^*$, in Box 6, are shown in Fig. 5A and Fig. 5B against the adult sterile competitive ability, $c_a$, and in Fig. 5C for the sperm competitive ability, $c_s$. In addition, the values of $c_a$ and $c_s$ are shown for given values of the critical release rate (250, 500), $S^*$, when the critical release rate is held constant (Fig. 5C); there is a trade-off between $c_a$ and $c_s$, the presence of each one limiting the value of the other.

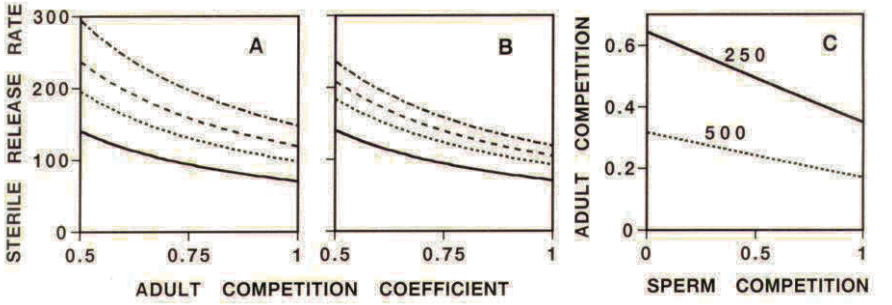



*Figure 5. A and B: Values of the critical release rates, S*, for values of adult sterile competitiveness, $c_a$, four mating frequencies (1, 2, 4, 8), and two probabilities of mating — A: 0.6, B: 0.8. C: Limits on adult sterile competitiveness compatible with control of the pest population; these are shown for a range of values of sperm competitiveness and two values of sterile releases (250, 500). (Figure generated from equations, Berryman 1967.)*

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### *2.7.5. Choice of Mates*

Mating systems of insect pests amenable to SIT application can be categorized as (1) resource defence-based mating systems, (2) non-resource-based mating systems, and (3) lek and swarm mating systems (Hendrichs et al. 2002; Lance and McInnis, this volume). Systems (1) and (2) are mostly determined by male competition, and are male-choice mating systems. System (3) is a female-choice mating system, where the female chooses a mate from among the courting males in the lek. The distinction between male- and female-choice systems, as well as the propensity of wild individuals to mate with their wild counterparts rather than sterile insects, has implications for the effectiveness of an SIT project. Using mating models, Vreysen et al. (2006) examined these questions, as well as the release of only males or of both sexes, and arrived at the following conclusions.

In a male-choice mating system, such as in the New World screwworm *Cochliomyia hominivorax* (Coquerel), overcoming a situation where there is a propensity of wild males to preferentially mate with wild females if sterile females are also being released, would require a doubling of the number of sterile males compared with male-only releases. In the model on female choice, the release of both sexes and male-only releases required the same sterile to wild male overflooding ratio. This, however, is at odds with field projects that have shown a significant benefit with male-only releases against insects which have a female-choice mating system, e.g. Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Hendrichs et al. 1995; Rendón et al. 2004). Models were constructed to assess the potential effect of sterile female presence or absence on reduced sterile sperm quantity with remating, reduced sterile sperm quality with aging, and incomplete redistribution of the sterile males with the wild insects. In all three cases, male-only releases result in relatively more efficient sterile insects compared with projects releasing both sexes (Vreysen et al. 2006).

A problem with female-choice mating systems is that any deficiency in sterile male behaviour or function can bias females against mating with them, and this can have severe consequences for the success of any SIT project.

## *2.8. Interaction of Polygamy with Residual Fertility*

A similar analysis on residual fertility (not shown) can be performed. Starting with the equation for residual fertility, and assuming that sterile sperm are fully functional, one proceeds as with the case of dominant lethal mutations and fully functional sperm. The result is that the probability that a female will mate with a sterile male is independent of the number of matings (Barclay 2001), and the probability that an egg will be fertilized by a sterile sperm is also independent of the number of matings. Thus, multiple female mating (polygamy) and residual fertility have no interaction, and the results derived above for residual fertility alone apply both to monogamy and polygamy.

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### *2.9. Stochastic Models*

Stochastic models involve the specification of certain variables as being random. If the processes involved are well known and the extent of variation is known, then stochastic models can give additional information on the expected variability of the resulting control, as well as deviations of mean values from those predicted by deterministic models. This is especially true in areas like genetics in which the mechanisms of variation, e.g. meiosis, are clear. However this information is often not well known in animal ecology, and therefore stochastic models may be of limited use. In fact, if the wrong features are allowed to vary (e.g. birth rate is variable in the model, whereas in reality it might be mortality that varies), then stochastic models can give misleading results. In addition, unless they are solved numerically, stochastic models are usually much more difficult to analyse than deterministic models, and for these reasons the history of stochastic population modelling has been rather disappointing.

Stochastic models of sterile insect releases were developed by Kojima (1971), Bogyo (1975), Costello and Taylor (1975), Taylor (1976), and Kimanani and Odhiambo (1993), and they confirmed the results of Knipling (1955) and others that used deterministic models. They also derived a threshold release rate that leads to local extinction, and showed that much greater release rates above this threshold will not result in a greatly reduced time to extinction, although Lawson (1967) and Itô and Kawamoto (1979) offered evidence to the contrary using both a deterministic model and a probabilistic model.

### *2.10. Population Movement*

The release of sterile insects, together with immigration from outside the control area, can be modelled by a simple modification of the model in Box 4 (Dietz 1976; Prout 1978). Flores (2003) has also provided a diffusion approach to the problem of immigration. Following Prout, two cases must be accommodated here: (1) immigration before mating, and (2) immigration after mating. In these two models it is assumed that all sterilized insects that are released are completely sterile.

#### *2.10.1. Immigration before Mating*

Assuming that $V$ males and $V$ females immigrate each generation prior to mating, the female immigrants are thus available for mating with the released sterile males as well as the wild males, and the male immigrants are available for competing with the sterile males. The model (Box 7) has two positive roots for $F$, with the upper one being unstable (the population as it existed just prior to sterile releases) and the lower one being stable. This lower steady state represents a population in a state of collapse due to sterile releases, but which is replenished each generation by immigrants. Note that zero is not a steady state solution here. The required sterile release rate grows rapidly with $V$, but there is no value of immigration that disallows control by sterile releases. The values of $S^*$ depend only modestly on $V$, if the immigration rate each generation is only a small proportion of the total population.

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*Box 7. Immigration*

*Immigration before Mating*

If we include immigration into the model, we obtain $F_{t+1}=\lambda(F_t+V)(F_t+V)/(F_t+S+V)$. Solving for steady state, we obtain the quadratic: $(\lambda\text{-}1)F^2\text{-}[S\text{-}(2\lambda\text{-}1)V]F+V^2=0$ which has two positive roots for $F$, with the upper one being unstable and the lower one being stable. Note that zero is not a steady state solution here.

The critical release rate is $S^*=[(\lambda\text{-}1)F+\lambda V\,](F+V)/F$.

*Immigration after Mating*

The equation here is $F_{t+1}=[\lambda F_t^2/(F_t+S)]+\lambda V$. Note that, if the wild population is reduced to zero, it will be reconstituted the following generation, as then $F_{t+1}=\lambda V$. Again solving for steady state, we obtain $(\lambda\text{-}1)F^2\text{-}[S\text{-}\lambda V]F+\lambda VS=0$.

The critical sterile release rate is given by $S^*=F[(\lambda\text{-}1)F+\lambda V]/(F\text{-}\lambda V)$, and sterile releases can only control the population if $V<F/\lambda$.

For a given immigration rate, the required sterile release rate is much higher if immigration occurs after mating rather than before mating.

### *2.10.2. Immigration after Mating*

In this case it is assumed that $V$ males and $V$ females immigrate each generation after mating. The female immigrants are thus not available for mating with the released sterile males or the wild males, however the male immigrants are available for competing with the sterile males. Thus, immigrating females remain fully fertile (unless there is some remating). Note that, if the wild population is reduced to zero, it will be reconstituted the following generation, as $F_{t+1}=\lambda V$. For a given value of the immigration rate, the required sterile release rate, $S^*$, is much higher if immigration is after mating than before mating, due to the fully fertile nature of the immigrating females.

### *2.10.3. Combinations of Residual Fertility, Reduced Competitiveness, and Immigration*

Four models can be considered, being the four combinations of: (1) those sterilized insects that show residual fertility can be either of reduced competitive ability (reduced) or fully competitive (equal) with the wild insects, and (2) insects can immigrate either before or after mating.

Barclay (2001) provided equations for the four cases, and the values of the limiting residual fertilities are shown in Table 1 and Fig. 6. In Table 1, the allowable residual fertility for the case of residually fertile insects being of reduced competitive ability is the same as for the case involving sterile insects being fully competitive and immigration occurring. The other two cases yield more stringent limits on allowable residual fertility than with no immigration. It is apparent that there is a strong interaction among residual fertility, competitive ability, and immigration, with the feasible limits on each factor becoming much more restrictive in the presence of the other factors.

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*Table 1. Limits on residual fertility (q) when competitive ability of "sterilized but residually fertile insects" is either reduced or equal to that of wild fertile insects, and immigration is either before or after mating*

| | Reduced Competitiveness | Equal Competitiveness |
|---|---|---|
| Before | $q<F/\lambda(F+V)$ | $q<cF/[\lambda(F+V)-F(1-c)]$ |
| After | $q<(F-\lambda V)/\lambda F$ | $q<c(F-\lambda V)/[\lambda F-(1-c)(F-\lambda V)]$ |

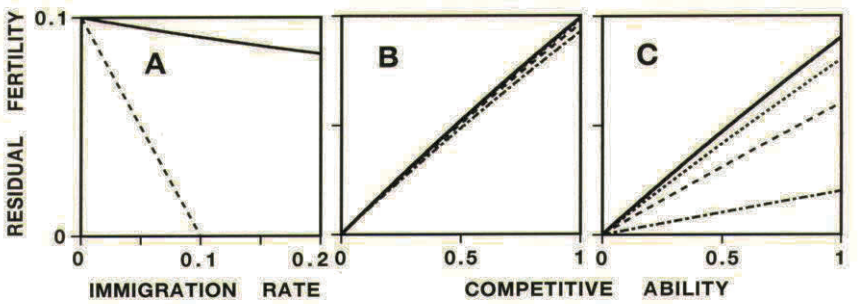



*Figure 6. A: The allowable residual fertility (q) when the released insects that are fertile are also fully competitive with wild insects. Immigration occurs before mating (_____); immigration occurs after mating (- - - - -). B and C: Allowable residual fertility as a function of competitiveness of released insects. Immigration occurs at 1% (_____), 2% (……..), 4% (- - - - ), and 8% (- . - . -.-) of the wild population size F. B: Immigration occurs before mating. C: Immigration occurs after mating. (Figure from Barclay 2001, reproduced with permission.)*

### *2.10.4. Large-Scale Population Movement*

The problem of large-scale population movement was addressed by Manoranjan and van den Driessche (1986), Lewis and van den Driessche (1993), Plant and Cunningham (1991), and Marsula and Wissel (1994). They showed, using diffusion equations, that dispersal of insects, coupled with non-linear growth terms, can result in waves of invasion or extinction. Both the velocity and direction of these waves depend critically on the rate of release of sterile insects. With low rates of release, the travelling wave advances as an invasion; when the density of sterile insects exceeds a critical density, the wave recedes, giving rise to local extinction. This is likely to have considerable relevance to programmes releasing sterile insects such as the New World screwworm eradication programme in the southern USA, Mexico and Central America, in which the pest population has been pushed back to Panama and a sterile insect buffer zone created. Matlock and associates are pursuing this approach with the screwworm, assessing the size of the buffer zone needed to ensure

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that insect invasion into the eradicated area does not occur (section 2.18.; R. B. Matlock, personal communication).

*2.10.5. Effects of Movement of Individuals on Efficiency of the SIT*

Apart from the effects of immigration by pest insects, the effect of diffusion (random non-directed movement) or redistribution of sterile insects within the control area appears to make control by SIT integration easier. Barclay and Vreysen (2013) presented a compartment model of tsetse (*Glossina palpalis gambiensis* Vanderplank) together with four control methods, one of which was the SIT. The critical sterile release rate for control increased with the degree of spatial aggregation but decreased with the extent of tsetse diffusion.

*2.10.6. Redistribution of Released Sterile Insects*

A problem that plagues many SIT projects is estimating the speed and extent of redistribution of sterile insects after aerial release, especially as sterilized insects may be less active and incur greater mortality than their wild counterparts (Parker, Vreysen et al., this volume; Dowell et al., this volume; Vreysen, this volume). Barclay et al. (2016) used diffusion equations to derive optimal spacing of flight lines and frequency of flights given the calculated sterile male population required to effectively cause population collapse. The optimization was done using a numerical treatment of the diffusion process to minimize total cost given the cost of sterile males and of flying time. They focussed on a midline between two flight lines of interest and calculated diffusive input of insects from eight flight lines on each side of the midline and from the eight previous flights. The optimization was found to depend strongly on the diffusion coefficient ($D$) and also on the daily mortality ($\mu$) of the released sterile males. Estimates of diffusion coefficients and daily mortalities were noted to vary widely among insect species, with $D$ being approximately 0.46 for tsetse flies and 0.006 for Mediterranean fruit flies, while $\mu$ varied between about 0.02 for tsetse and 0.23 for the fruit flies. At the low end of $\mu$ (0.04), longer flight intervals are suggested, and fewer sterile males need to be released; at the high end of $\mu$ (0.24), shorter flight intervals are suggested and higher numbers of sterile males need to be released.

These authors also derived a shorter approximate method of optimizing the sterile insect release based on the observation from the results of the numerical method that the optimal spacing of flight lines ($\omega$) was twice the standard deviation, $2(2D\tau)^{½}$, of the Gaussian distribution resulting from the diffusion process. Here $\tau$ is the time interval (days) between flights. Two assumptions of this method are: (1) the relationship between $\omega$ and the standard deviation of the distribution observed from the longer method is assumed to hold for other values of the parameters, and (2) the midline between flight lines being used for computations is assumed to be well within the control area so that edge effects are not evident and diffusion is assumed to be in equilibrium. This shorter method is outlined below.

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Given the values of $D$ and $\mu$, use each possible value of $\tau$, $\tau$ = 1, 2, 3....$k$. For $n$ flights and a requirement of $N_M$ sterile males alive per km$^2$, the number of sterile males to be released per km$^2$ ($N_{SK}$) is:

$$N_{SK} = N_M \frac{(exp(\mu\tau) - 1)}{(1 - exp(-n\mu\tau))} \quad (2)$$

Now, to find the optimal value of $\tau$, we must minimize the formula for total cost ($C_T$) per km$^2$ per day: cost of flying time + cost of sterile males:

$$C_T = C_H / (S\omega\tau) + (C_S / 10^6)\, N_M\, (exp(-\mu\tau) - 1) / \tau\, (1 - exp(-n\mu\tau)) \quad (3)$$

Here $C_H$ is the cost per hour of flying time, $S$ is the speed of the aircraft, and $CS$ is the cost per million sterile males. In the example below, $n$ is taken as 8 and $N_M$ is $10^6$, $C_S$ is USD 250, and $C_H$ is USD 5/km actually flown over the field, once the transit to and from the field is included.

For Mediterranean fruit flies, $D$ = 0.005, so with $n$ = 8 flights, using these dollar figures,

$$C_T(\tau) = 5 / (2(2D)^{0.5}\, \tau^{1.5}) + 250 * (exp(\mu\tau) - 1) / (\tau\, (1 - exp(-n\mu\tau))) \quad (4)$$

When $C_T$ (τ) is plotted against τ, this is a convex curve, i.e. a concave-up curve, with a minimum value that represents the optimal values of τ (Fig. 7). A hand-held graphing calculator, or graph-plotting software on a computer, can be used to obtain easily the minimum value, and the τ (which must be a positive integer) at which it occurs. When the cost starts to increase, one should stop calculating; the cost will only increase beyond this point.

As an example of the approximate method for $\mu$ = 0.24 using a basic calculator, we obtain the following: from Fig. 7, the best value of $\tau$ is 2 days. This then gives $\omega$ = 2 sqrt (2*0.005*2) = 0.283 (= 283 m). The number of sterile insects to be released per day is then 1 000 000 • (exp(0.48)-1)/(2*(1 - exp(-3.84))) = 315 000.

### *2.10.7. A Model for* Eldana saccharina *with Diffusion and Partial Sterility*

Potgieter et al. (2013) presented a stage-structured diffusion model for the African sugar cane borer *Eldana saccharina* Walker. Eleven stages were tallied. The released female moths were fully sterile while released males were partially sterile, and sterility existed in the $F_1$ generation. The habitat was heterogeneous and compartmentalized into relatively homogeneous subpopulations; diffusion provided the only movement among these subpopulations. The model was applied to an existing sugar cane field in KwaZulu-Natal in South Africa. Numerical results indicated that the optimal release distribution and release ratio depend on the dispersal capability of the insect, method of release, frequency of releases, and growth or mortality rate of the population (Potgieter et al. 2013).

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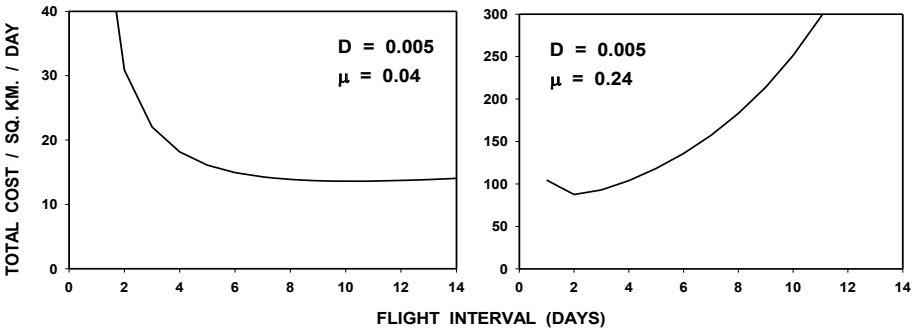



*Figure 7. Curves for optimizing τ (the time interval (days) between flights) using the approximation. In both curves the value of D is 0.005, while in the left curve μ = 0.04 and in the right curve μ = 0.24. In both cases, 8 flights have been used. The location of the optimum value of τ is well defined (τ = 2) for μ = 0.24, but not so well defined (τ = 10) for μ = 0.04. For μ = 0.24 the cost would be notably greater if τ were assigned to 1 or 3, whereas for μ = 0.04 the difference in cost would be minimal if τ was misassigned to be 8, 9, 11 or 12 when the optimum was 10.*

### *2.11. Density-Dependence in Population Regulation*

Density-dependence in a population has been shown by modelling to predispose it to control by sterile releases (Miller and Weidhaas 1974; Itô 1977; Prout 1978; Barclay and Mackauer 1980a). There are several formulations that include density-dependence in a model of a population, and all yield the same qualitative results. The two distinct ways that density-dependence assists the SIT are: (1) it reduces the effective biotic potential of a species by increasing the natural mortality at higher densities, and (2) it introduces a bifurcation (splitting of one root of the equation into two) into the model whereby the population suddenly collapses as the sterile insect release rate is increased above the level required at the bifurcation (Fig. 8). This avoids the necessity of the high levels of release needed to reduce the population below the unstable steady state in the model with no density-dependence. This bifurcation occurs in all the models involving density-dependence, and appears to result from the interaction of the depressing effects of density-dependence and the unstable equilibrium created by the SIT formulation in section 2.2., which results in the release of sterile insects being more effective at low density than at high population density, and thus the efficiency of the SIT increases as the population declines. The sudden collapse of a population under attack by sterile insect releases has indeed been observed in the programme against the melon fly *Zeugodacus cucurbitae* (Coquillett) in Okinawa, Japan (Iwahashi 1977). Thus, this predicted bifurcation appears to be a robust result, and one that apparently mimics nature.

The exact behaviour of the SIT under density-dependence appears to depend sensitively on the biology of the system. Lawson (1967) and Berryman et al. (1973) pointed out that overcrowded populations may deplete their resources sufficiently such that survival to the adult stage is low. In this case, killing some of them (or

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lowering egg production) might actually result in a higher survival to the adult stage, making the use of the SIT counterproductive in such a situation. Another situation might be encountered in the case of an insect species wherein egg production is much higher than the resource allows, e.g. the olive fruit fly *Bactrocera oleae* (Rossi), in which one egg per fruit is laid. If sterile eggs did not deter insects from laying further eggs in a fruit already containing a sterile egg, then reduction in fertile egg production would have to be substantial before any effect would be noticed.

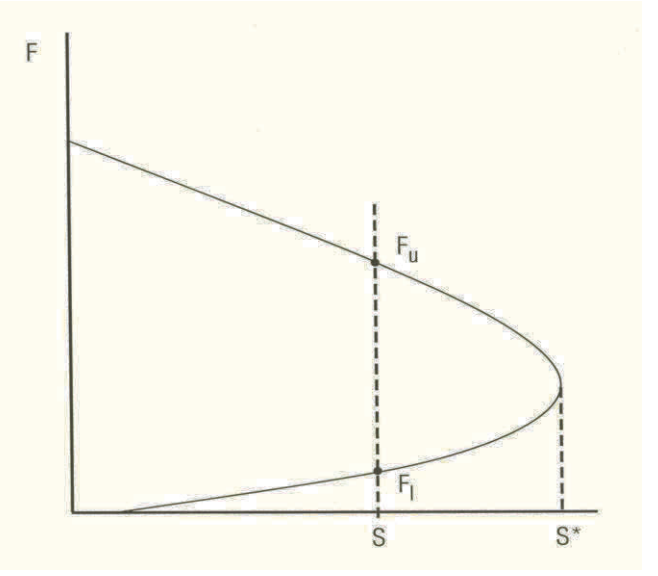



*Figure 8. Isoclines formed by setting the equations for sterile insects (S, vertical line) and fertile insects (F) to zero; where they cross are the two steady states. The upper one is stable, and the lower one unstable. As sterile releases increase, the sterile isocline moves to the right. The bifurcation is at the point where the isoclines are tangent to each other. Larger values of sterile insect releases result in a sudden collapse of the population. (Figure generated from equation 12, Barclay 2001.)*

## *2.12. Age Structure*

### *2.12.1. Two Age Classes*

The existence of two or more life stages of a species complicates the dynamic responses of a population to mortality factors, especially if the two stages are ecologically different, as they are in mosquitoes and other pest species in which the two active stages occupy different habitats. If density-dependence is strong in one stage and weak or absent in the other stage, then the density-dependent stage is strongly buffered against mortality factors and tends not to vary greatly, while the other stage may vary more but the mean size is a linear function of the buffered stage. As such, somewhat different responses to mortality affecting mainly one stage

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would be expected, and indeed this appears to be the case. In the case of the SIT, the sterile insects released always affect the adult stage, reducing fertile egg production.

Prout (1978) modelled the SIT for species with identifiable age structure and subject to immigration. His results indicated that, if the larval stage caused the pest problem, a higher level of immigration of mated adults was tolerable.

Barclay (1980b) showed that the critical release rate ($S^*$) is a larger proportion of the larval equilibrium size when density-dependence is in the larval survivorship than when density-dependence is in the adult survivorship. Thus, relative to a given larval equilibrium, the population requires fewer sterile insect releases when density-dependence is in the adult stage.

### *2.12.2. Daily Age Classes*

Barclay et al. (2014) modelled a population of a monogamous species with egg, larval, pupal and adult stages, and a daily time step. Survivorship was age-specific, and there was no density-dependent population regulation. Eggs take $ke$ days from oviposition to hatch, and there is no daily mortality. The numbers of eggs in the $i^{th}$ age class at time $t$ is $E_{i,t}$, and as there is no egg mortality, $E_{i+1,t+1} = E_{i,t}$ for $1 \leq i < ke$. Eggs of age $ke$ hatch into larvae, so that $L_{1,t+1} = E_{ke,t}$.

Larvae require $kl$ days for development, and the number of larvae in the $i^{th}$ larval age class at time $t$ is $L_{i,t}$ for $1 < i \leq kl$. Larvae survive from age $i$ to age $i$+1 at a rate $q_i$, so that $L_{i+1,t+1} = q_i\ L_{i,t}$ for $1 \leq i < kl$. Similarly, pupae require $kp$ days for development, and the number of pupae in the $i^{th}$ pupal age class at time $t$ is $P_{i,t}$ for $1 \leq i \leq kp$. Pupae survive from age $i$ to age $i$+1 at a rate $u_i$, so that $P_{i+1,t+1} = u_i\ P_{i,t}$ for $1 \leq i < kp$. Also, the first pupal age class is $P_{1,t+1} = q_{kl}\ L_{kl,t}$.

There are five categories of adults, also referred to as adult population components; at age $i$ and at time $t$ they are: virgin females ($V_{i,t}$), females mated to fertile (wild) males ($F_{i,t}$), females mated to sterile males ($G_{i,t}$), wild males ($M_{i,t}$) and sterile males ($N_{i,t}$). The virgin adult females are assumed to become receptive to male mating advances at age $\tau$ days, and all of age $\tau$ mate on that day and immediately start ovipositing. Adult virgin females, fertile-mated females and sterile-mated females all survive at a rate $w_i$ from female age $i$ to age $i$+1, except that mated females start on their day 1 at survivorship $w_{1+\tau}$ because adult emergence occurred $\tau$ days before. Wild males survive at a proportional rate $y_i$ from age $i$ to $i$+1, and sterile males correspondingly survive at a rate $z_i$. Thus, age classes $i$ at time $t$ survive as follows: $V_{i+1,t+1} = w_i\ V_{i,t}$; $F_{i+1,t+1} = w_{i+\tau}\ F_{i,t}$; $G_{i+1,t+1} = w_{i+\tau}\ G_{i,t}$; $M_{i+1,t+1} = y_i\ M_{i,t}$; $N_{i+1,t+1} = z_i\ N_{i,t}$.

If sterile males are being released, then there is a release rate, $r^*$, at which the population will not increase further and a steady state (equilibrium) will be attained. This equilibrium is unstable in the absence of density-dependent regulation, and it is of interest only inasmuch as it separates success (if the actual release rate, r, is greater than $r^*$) from failure (if the sterile release rate is less than $r^*$). This sterile male release rate, $r^*$, is called the critical release rate, and it is what is calculated in standard models of the SIT, e.g. Berryman 1967; Dietz 1976; Itô 1977; Barclay and Mackauer 1980; Barclay 2001; Barclay et al. 2014).

Two derived statistics are required to calculate fertility, and thus to calculate the size of the first egg age class, and also to determine the critical release rate; these are

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the net survivorship from eggs to adult emergence, $\gamma$, and the mean daily fertility (mdf), $\mu$ (called "mean fertile eggs per fly-day" by Carey 1989):

$$\gamma = (\Pi_1^{(kl-1)} q_i) (\Pi_1^{(kp-1)} u_i) \quad (5)$$

$$\mu = \Sigma l_x m_x h_x / \Sigma l_x \quad (6)$$

where $l_x$, $m_x$, and $h_x$ are the survivorship and fecundity of fertile-mated females and hatchability of their eggs, respectively, from age $x$ to age $x$+1 (Carey 1989); here $\mu$ (fertility) is not the same as $\mu$ in section 2.10.6. (mortality). The equation for the critical sterile release rate, $r$* (Barclay et al. 2014), is then obtained by solving the five adult equations at equilibrium simultaneously for $r$ in terms of $Fe$, the fertile-mated female subpopulation. Doing the appropriate substitutions and simplifying yields the critical value of $r$, $r$*:

$$r^* = \gamma \mu Fe\ a\ (b\ c - 1) / d \quad (7)$$

where:
$a = [\Sigma_0^{(km-1)} \Pi_0^{(i)} y_j]$, i.e. the sum of the products of fertile male survivorships;
$b = \gamma \mu [\Sigma_0^{(kf-1)} \Pi_0^{(i)} w_j]$, i.e. $\gamma \mu$ times the sum of the products of female survivorships;
$c = \Pi_0^{(\tau)} w_j)$, i.e. the survivorship of virgin females to the $\tau^{th}$ age class; and
$d = [\Sigma_0^{(km-1)} \Pi_0^{(i)} z_j]$, i.e. the sum of the products of sterile male survivorships.
Even though this appears to be rather complicated, it is easily computed if the age-specific fecundities, hatchabilities, and survivorships are known or can be estimated. Also, it is remarkably parallel to the models with no age structure, in which $\gamma \mu$ is the analogue of $\lambda$ in Knipling's model.

One further complication in calculating fertility should be mentioned. For the special case of species giving single live births of larvae at intervals of several days, such as tsetse flies, it has been common practice to calculate fertility as the reciprocal of the inter-larval period. It has been shown recently (Barclay et al. 2020) using eq. (6) above that a better estimate of fertility is about 10% greater than the reciprocal of the inter-larval period. This has implications for calculating the minimum sterile-male release rate required for control or eradication.

### *2.12.3. Overflooding Ratios and Release Ratios*

The overflooding ratio, a key parameter, has long been a measure of the ratio of sterile males to wild males in the population (Steiner et al. 1965). It is sometimes thought of as the size of sterile releases compared with the wild population; this is approximately valid in species with short reproductive seasons and non-overlapping generations. However, in species with a long reproductive period, often extending throughout the warm season in temperate climates, a surviving population of sterile males exists following several releases, and the overflooding ratio is not equal to the periodic release rate divided by the wild male population size, and must be estimated by sampling or some other means. The overflooding ratio is here called $\varphi$.

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Another ratio is the release ratio, defined as the ratio between the periodic sterile male releases and the wild male subpopulation, and here called ρ. For species with one short reproductive period each year, the two ($\varphi$ and $\rho$) are approximately equal, allowing for some mortality following release. Such populations yield the same sterile release rate required to stop population growth, and then hold the population at equilibrium, as that predicted by the model at equilibrium. In freely growing populations with overlapping generations, the release ratio and the overflooding ratio are not equal.

### *2.12.4. Stopping a Growing Population*

Barclay et al. (2014, 2016) derived critical control rates that are required to hold an age-structured population at an unstable equilibrium. This equilibrium separates success (if the control rate is above the critical rate) from failure (if the control rate is below the critical rate). The paper by Barclay (2016) builds on a previous publication (Barclay et al. 2014) on the control of age-structured populations, and shows that the equilibrium statistics derived are only a first step towards being useful. They are not sufficient to allow calculation of the appropriate values of the sterile release rates and overflooding ratios required to stop a growing age-structured population using the SIT. A measure called the "required sterile release rate", *r*', is defined as the value of the sterile male release rate, r, necessary to stop a population growing freely in a stable-age distribution, i.e. with sterile-male releases, the growth rate eventually becomes zero with $r = r$'. Such populations are largely undergoing density-independent growth, as may be the case with many spot infestations.

The values of *r*' were all moderately close to the calculated value of *r**, the critical rate, but the computed overflooding ratios were very different for a growing age-structured population than for one at equilibrium; the value of the overflooding ratio calculated for an equilibrium population, i.e. when $r = r$*, greatly underestimates the value required to stop a population in unrestricted growth. As the value of *r*' increases with population size, accurate estimation of population size is necessary to calculate realistic values of *r*'. For the Mediterranean fruit fly example that was developed (Barclay 2016), the discrepancy was huge; the overflooding ratio calculated from an equilibrium population was only about 1% that found to be required in the simulation. This is because, at equilibrium, females mated to fertile males constitute a very small part of the equilibrium population, whereas in a growing population, fertilized females constitute a much larger portion of the total population. Thus, in this situation, it is the actual critical release rate, r*, that should guide the control programme, rather than deriving the overflooding ratio from sampling the males in a freely growing population.

## *2.13. Population Aggregation*

In nature most populations are not dispersed evenly over the available habitat. Some processes, such as territoriality, result in dispersion patterns that are more regular (Fig. 9, upper left) than one would expect of a random spatial distribution (Fig. 9, upper right). However, most populations will tend to have a somewhat aggregated

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dispersion pattern. Aggregation is the most difficult pattern to deal with in making sterile insect releases, because one has to know where the clumps are located (Fig. 9, lower panels).

Modelling of spatial aggregation has been done by Wehrhahn (1973) and Barclay (1992a). Wehrhahn used a mosaic of patches, inhabited by differing numbers of insects, and compared the required release rates for various patterns of aggregation. He used Monte-Carlo simulation, which introduces random numbers to allow stochastic variation, in this case, of migration rates among patches. Wehrhahn pointed out that the control programme itself will probably change the nature of the spatial distribution. Bakhoum et al. (2021) described using species distribution modelling and landscape genetics for tsetse fly control.

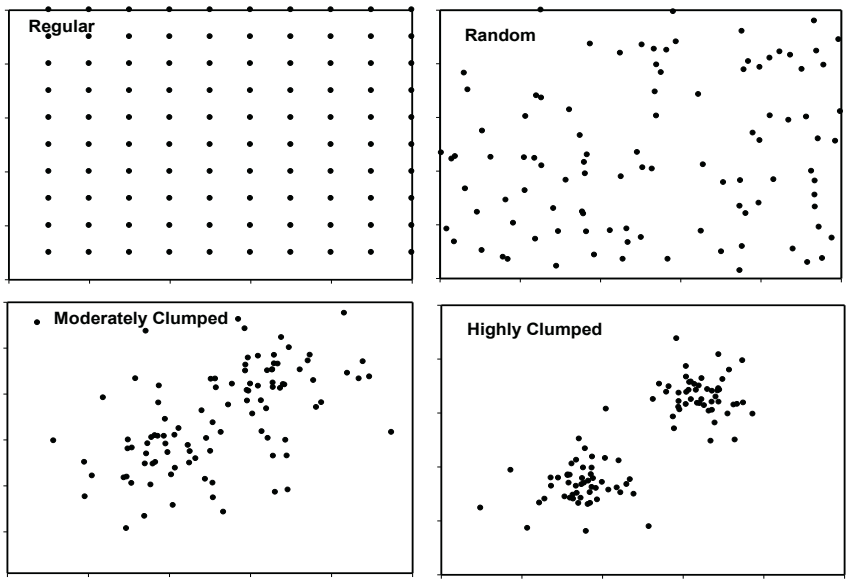



*Figure 9. Examples of spatial distributions: regular (uniform), upper left; random, upper right; moderately clumped, lower left; highly clumped, lower right (reproduced from FAO/IAEA 2016). These can be quantified by various aggregation indices (Pielou 1969; FAO/IAEA 2016).*

Another approach has used probability distributions to describe the extent of aggregation (Barclay 1992a). There is a long history of using these distributions in ecology, summarized by Pielou (1969) and Patil and Stiteler (1974). The most commonly used distribution to quantify aggregation is the Negative Binomial Distribution in which the parameter $k$ measures clumping. If aggregation is extreme, then $k$ is close to zero; as $k$ goes to infinity, the dispersion approaches a random pattern. Another approach uses $1/k$, which increases with the degree of clumping. Barclay (1992a) used the negative binomial distribution to derive required sterile insect release rates of an aggregated population as a function of the clumping

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parameter, $k$. For moderately aggregated populations ($k$=0.25), it was found that the required release rate was about four times that for a randomly dispersed population. Shiga (1986) analysed spatial distributions in the context of fruit fly eradication using male annihilation and the SIT.

Many aspects of aggregation involve behavioural components. Horng and Plant (1992) modelled the impact of lek mating on the SIT, using a Poisson binomial distribution. They found that the sterility effect, presence or absence of female mate-choice, and sterile male mating competitiveness were the most important factors in their model in determining the success of a programme releasing sterile insects.

### *2.14. Predation, Parasitism, and Competition*

The effects of predation on the efficiency of the SIT were first modelled by Knipling (1979) using a simple numerical model. His model predicted a synergistic interaction between predation and sterile insect releases, such that the net effect would be considerably greater than either alone. This would only be expected to be true if predation is random, with sterile and wild males equally able to evade predator attacks. Nevertheless, there is growing evidence that sterile males have largely lost this capacity and suffer much more predation (Hendrichs et al. 1994; Hendrichs and Hendrichs 1998; Hendrichs et al. 2007). On the other hand, the integration of augmentative parasitoid releases and inherited sterility can be highly synergistic (Barclay 1987a, b; Carpenter 2013).

Barclay and Mackauer (1980b) included sterile insect releases in the Lotka-Volterra predator-prey model, and demonstrated that, not only was the critical release rate lower with than without predators, the system was also greatly destabilized, and population collapse often occurred with release rates well below the critical value. This model was subsequently shown by Harrison et al. (1982) to have a very complicated dynamic behaviour, and this is presumably related to the inherent instability. This model was then extended, and an even greater array of dynamical behaviour was found (Barclay and van den Driessche 1990). In addition, the general features of the predator-prey system were found similar to the situation involving hosts and parasitoids (Barclay 1987a). Knipling (1998) analysed extensively the effects of augmentation of predators and parasitoids on the efficiency of the SIT.

If the pest species is in competition for resources with another species, then this is of some value to the release programme, because it reduces the initial pest population size, but apart from that there seems to be little effect of the competing species on the release programme (Barclay 1981b).

### *2.15. Stability under Various Conditions*

Stability is a very important aspect of populations, and is of special interest to pest managers. If, for any reason, the population is likely to collapse, this is important. Barclay (1982b) examined four systems or cases for their relative stability under sterile insect releases. These were all differential-equation models and represented: (1) a simple one-species model with only one identifiable life stage (adult), (2) a

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one-species model with two life stages (larval and adult), (3) a model of two competing species, one of which is the pest, and (4) a predator-prey model in which the prey is the pest. There are many possible definitions of stability in ecology, and they involve various dynamic characteristics of the system. None has yet emerged as definitive, although an extensive analysis of the topic is now available (Mueller and Joshi 2000). Barclay (1982b) examined five criteria of stability for each system, and ranked the systems. These stability criteria involved characteristics such as extinctions, time to extinction, amplitude of fluctuations, and time until return to equilibrium. The most stable was the single species – single stage model, followed by the competing species model, then the two-stage model, and by far the least stable was the predator-prey system above (section 2.14.). The existence of obligate predators (or parasitoids) both lowers the critical release rate of sterile insects and also destabilizes the system, so that it is likely to collapse even when the sterile release rate is lower than the critical rate. Unfortunately, there appears to be no experimental evidence to test these ideas.

### *2.16. Integration of Control Methods*

Since the SIT works best at low pest densities (section 2.11.), it is common practice to reduce the population with an insecticide or other control method (or to start at naturally low population levels), prior to the release of sterile insects. This brings the population down to a level at which the number of sterile insects (required to be produced by a rearing facility) is manageable. It might also be possible to combine contemporaneously the action of the SIT with other control methods to share the required mortality among two or more imposed sources, each one then having to impose only a modest level of mortality, and each one perhaps operating best under conditions not favourable to the others (Barclay 1992b; Mangan and Bouyer, this volume). On the other hand, certain combinations might interfere with each other and thus prove unsuitable (Barclay 1987c).

Knipling (1964, 1979) examined several combinations of various control methods with the SIT, using simple numerical intra-generational models. These include combinations of sterile releases with insecticides, sterilants, pheromones, parasitoids or predators. Barclay (1980b, 1987a, b) and Barclay and van den Driessche (1989) also examined some of these combinations using more general inter-generational algebraic models. They found that the results of the combined use of sterile insects and other control methods became less clear when other biotic interactions were included; for example, a three-way interaction with sterile insects, predators, and insecticides may be counterproductive because the insecticides would probably reduce the effectiveness of the predators. In general, in population dynamics, three-way interactions are much more difficult to predict and analyse than two-way interactions.

#### *2.16.1. SIT with Application of Insecticide*

It might be thought that insecticides and the SIT are incompatible because the insecticide would kill sterile, as well as fertile, insects. However, Knipling (1964,

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1979) reasoned that insecticide application would kill both sterile and fertile insects in the same proportion, and thus maintain the overflooding ratio, rendering the two control methods compatible. By numerical examples he showed that these two methods could work well together, and thus reduce both total costs and the need for excessive insecticide application. If the sterile insects were also resistant to insecticide, then the combination would be even more effective.

Barclay (1980a, b) found that, when the pest species was considered in isolation, the application of insecticide coupled with the release of sterile insects increased total mortality. However, the results of the combined use of insecticide and sterile insects became less clear when specific tritrophic biotic interactions were included, e.g. predators or parasitoids. For example, if the pest is already under considerable predation, the combination of insecticide and sterile insects might be detrimental. Therefore, when using sterile insects against species with two life stages, a larvicide appears to be more useful than an adulticide.

### *2.16.2. SIT with Pheromone Traps for Male Annihilation*

Knipling (1979) found that the combined use of sterile releases and pheromone traps was less efficient than an equivalent effort put into either method alone. This was because of the interference caused by the killing of sterile males in the pheromone traps. As a variant of this combination, Knipling proposed that releasing pheromone-treated sterile insects could enhance mate-finding, thus increasing the competitive ability of sterile insects, especially at low densities. Knipling found that, for insects in which the males produce female-attracting pheromone, such as the boll weevil *Anthonomus grandis grandis* Boheman, the release of pheromone-treated males would substantially increase the effectiveness of the control programme, assuming that the applied pheromone did not deteriorate badly. Knipling also considered the situation in which females produce male-attracting pheromone, and he modelled the release of pheromone-treated sterile females alone. These would probably be most effective if they were free-living rather than contained in traps. He again found that this method was much more effective than the use of untreated sterile females, and that control might be possible using pheromone-treated sterile females where the release of only non-treated sterile females would be hopelessly inadequate. Pheromone-treated sterile insects of a species that is easier to mass-rear might also act as vehicles for mating disruption of the wild population of a different target pest (Suckling et al. 2011).

Hamada and Miyai (1985), using a continuous model, modelled the combination of the simultaneous release of sterile insects and pheromone trapping for male annihilation (Box 8). They found that the two methods in combination required less effort for each control method than when using either method alone. Their recommendation was to use male annihilation first and then sterile releases. Although the model did not specifically explore that scenario, this approach was successfully applied in Japan against the melon fly and the oriental fruit fly *Bactrocera dorsalis* (Hendel) (Enkerlin, this volume).

Barclay and van den Driessche (1989) also modelled this combination, and found that the two methods combine synergistically, especially when the fecundity and daily survivorship are both high. When the fecundity and survivorship are low,

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the synergism disappears. For parameter values approximating those of tsetse flies *Glossina* spp., synergism is reduced.

Knipling (1979) described the interaction of methyl eugenol (ME) for male annihilation used concurrently with the release of both sterile males and females, and found no interference and a high degree of synergism. Since the potential for development of resistance to ME has been demonstrated (Shelly 1997), it might also be possible to incorporate the use of sterile insects of the resistant strain, and thus increase effectiveness even more.

In the case where the attractant is non-sex-specific, such as with food baits, Barclay and van den Driessche (1989) showed that the two methods combine synergistically, especially when sterile insects are fed before release, and fecundity and survivorship are high. For parameter values approximating those of tsetse flies, there is still some synergism.

Barclay et al. (2014) also modelled the interaction of ME for male annihilation applied simultaneously with the release of ME-fed sterile males that are non-responsive to ME male annihilation, using the same age-structured model as in section 2.12.2. They found that the two methods are synergistic. This is fortunate, as ME used alone appears not to be very useful in this context (Barclay and Hendrichs 2014), but elimination of wild males through male annihilation together with the SIT becomes very effective if the sterile males are raised with ME in their adult diet before release, after which they exhibit reduced attraction to ME traps and thus may come to dominate the male subpopulation over and above the numerical advantage they may achieve though high release rates. It appears that ME used alone can only be effective if trapping or response to male annihilation devices occurs prior to mating each day, which may or may not commonly occur in a control programme (Barclay and Hendrichs 2014). When ME is used simultaneously with the SIT, the order of mating and trapping is still important, but makes less difference than it does with ME alone.

*Box 8. Combination of Sterile Releases and Pheromone Trapping*

Miyai's model consisted of four differential equations: $dM/dt = F(a-bF) - cM - kM$; $dV/dt = F(a-bF) - cV - \alpha[\min(M+S, V)]$; $dF/dt = \alpha[\min(M+S, V)]\, M/(M+S) - cF$; $dS/dt = R - cS - kS$; where $M$, $V$, $F$ and $S$ are the numbers of males, virgin females, fertilized females, and sterile insects, respectively. The parameters are: $a$ is a density-independent fecundity, $b$ is a density-dependent fecundity, $c$ is a death rate, $k$ is the rate of trapping of males, $\alpha$ is the mating efficiency, and $R$ is the sterile insect release rate.

### *2.16.3. SIT with Release of Parasitoids*

This combination has the advantage that parasitoids work well at high host densities, while the SIT works best at low pest densities. Knipling (1979, 1998) considered the release of both sterile males and females, and also *Trichogramma* sp., an egg parasitoid. His tables showed clearly that these two methods were synergistic. A recent field study found that the two methods in combination were more efficient than either method alone (Bloem et al. 1998). Especially in the case of inherited sterility, the release of partially sterile males and sterile females provides $F_1$ eggs and larvae for oviposition by egg and larval parasitoids, further augmenting the

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parasitoid populations (Marec et al., this volume). If both sterile insects and parasitoids are released inundatively (Marec et al., this volume), then each should become more efficient as the density declines, offering a powerful source of synergism (Knipling 1998).

Barclay (1987b) modelled the interaction of the inundative release of parasitoids and sterile insects using several variations on the usual host-parasitoid equations. This combination shows a high degree of synergism in all the models investigated, and appears to be close to ideal (Fig. 10). The main problems to be anticipated probably involve dispersal and phenology.

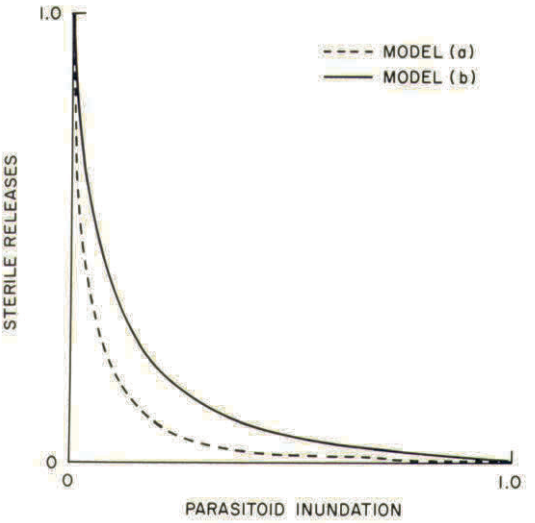



*Figure 10. Critical release rates of sterile insects and parasitoids. All points on each curve are just sufficient to eradicate the pest population. Density-dependence is in the hosts in model (a), and in the parasitoids in model (b). (Figure from Barclay 1987c, reproduced with permission.)*

### *2.16.4. SIT with Pathogens*

The combination of the SIT and male-killing bacteria has been investigated by Berec et al. (2016) for bacteria that are transmitted vertically. They found that if vertical transmission is 100%, then the SIT is not required. If transmission is less than 100%, then the bacteria and the SIT act synergistically, and the combination is more efficient than either method alone. Specifically, with a relatively high rate of transmission of the bacteria, the required level of sterile male releases is much lower than without the bacteria. They investigated the interaction of bacteria with both non-heritable sterility (the usual SIT method employed for dipterans) and inherited sterility (used with lepidopterans) (Marec et al., this volume), and found that the efficiency of this combination for inherited sterility is best at intermediate doses of radiation. In New Zealand Suckling et al. (2007) integrated the use of *Bt* with sterile releases and achieved local eradication of the painted apple moth *Teia anartoides*

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Walker. Toledo et al. (2017) used *Beauveria bassiana* with an autoinoculation device for the control of *Ceratitis capitata* with a similar synergism.

### *2.16.5. SIT with Sanitary Measures and Oviposition Traps*

If sanitation destroys oviposition habitat, and traps remove females that oviposit eggs, then there should be a complementary effect between the two, albeit moderated by density-dependence. If sterile males are also released, then the system has three sources of population reduction, none of which interferes with any other. Knipling (1979) calculated that these three should result in a highly efficient combination for control.

### *2.16.6. Synergism between the SIT and a Natural Allee Effect*

The Allee effect (Allee 1931) is the phenomenon in which density-dependence becomes negative at very low population densities as a result of both sexes having difficulty in finding mates. It has since been generalized to include other sources of negative density-dependence as well (Yamanaka and Liebhold 2009). Although the Allee effect is not really a pest control method, it represents a natural weak point in the pest population dynamics (Suckling et al. 2012). Gordillo (2015a, b) has incorporated the Allee effect into models of the SIT, and noted the synergism that increasingly results with decreasing pest population, causing local extinctions.

Conversely, it may be possible for pockets of pest persistence to exist with sufficiently high dispersal even when the sterile release rate is above the predicted requirement for extinction, especially in heterogeneous habitats in which the number of sterile males depends on the estimated pest density. This is similar to the result of Levins and Culver (1971) in which populations could persist in a patchy environment in the face of local extinctions if dispersal is high enough, because some of the pest individuals disperse into neighbouring patches before extinction occurs in the home patch. Nevertheless, the dispersing pest population needs to be sufficiently high to overcome the Allee effect.

## *2.17. Optimizing Initial Knockdown for the SIT*

Age at first oviposition is an important factor affecting spray intervals (Mangan and Bouyer, this volume). Adult insects usually take time to mature before mating and then ovipositing. If sprays targeting adults are timed so that the interval between sprays is smaller than the interval from adult emergence to first oviposition, then subsequently emerging adults will be killed before being allowed to oviposit. The number of sprays will depend on the time it takes from oviposition to adult emergence and number of generations per year or number of generations during the critical period required for population suppression. An example will illustrate this. In tsetse, the period between larviposition, pupation, and emergence of adults from the pupae is about 27 days at 27ºC, while the age to first larviposition is about 17 days following emergence of the adults. If the mean temperature is 27ºC, and the inter-spray interval is 15 days, and sprays kill 90% of the adult population, then the first spray will eliminate most of the adults, the second spray 15 days later will

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eliminate most of those adults that have emerged from the developing pupae, but before they start ovipositing, and a third spray will kill most of the adults that have emerged from the remaining pupae that were alive at the time of the first spray, again before they start ovipositing. The population is then in a suitable state to be controlled by sterile releases, as there will be little increase in the adult population for some time, long enough that the SIT should decrease the recruitment of juveniles and prevent this increase.

A model of tsetse developed by Barclay and Vreysen (2011) predicts the course of the adult population following two, three, and four applications of aerial insecticide sprays, each of which kills 98% of the adult population. For each of these three scenarios, a sterile release ratio (daily releases divided by the existing wild male population) of 0.2 was used, calculated immediately after the last spray. The tsetse example is presented because it is particularly clear. For most species, spray efficiency will be less than 100%, and additional sprays will be required.

If sprays target larvae, then the length of the larval period is the factor that determines the time between sprays (Barclay et al. 2019). If sprays are 100% efficient at killing the existing larvae, then a sufficient number of sprays to cover the entire life cycle will eliminate the population, if there is no immigration from outside the control area. If sprays are less than 100% efficient at killing larvae, which will usually be the case, then additional sprays will be required to bring the population down to a level suitable for the initiation of the SIT.

According to Barclay et al. (2019), an insecticide should be selected that will target the stage of longer length (larvae or adults), if that choice is available.

### *2.18. Exclusion of Pests from an Area: Barrier Width*

Barclay et al. (2011) constructed a model that attempts to assess the minimum dimensions of a buffer area (within which control is also imposed) to protect a pest free area of low pest prevalence. Random dispersal would result in insects entering the buffer zone from outside the control area; these insects and their progeny would ultimately disperse across the buffer and enter the area to be protected unless they were killed within a sufficiently wide buffer. Dispersal in this case is modelled by means of diffusion equations, e.g. Okubo 1980, to model a pest population that is diffusing across the barrier and being controlled within the barrier in an effort to protect the inner core area inside. Their model considers a rectangular core area, surrounded by a rectangular buffer zone, and using the SIT as the control agent within the buffer. We are interested here mainly in determining the barrier width.

They assumed that (1) the core area is already a pest free area as a result of previous control efforts; (2) the host density in all areas (the core area, the buffer zone, and outside the buffer zone) is assumed to be at equilibrium; (3) there is a constant influx of pest insects from the region outside the buffer zone; and (4) no transport of the target pest insects by wind, storms, or humans into the core area occurs. As this treatment assumes that the equilibrium has already been achieved, the barrier width calculated is that required to maintain the status quo. Dispersal describes movement of the insects across the buffer zone and will determine the

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width of the buffer zone, which results in the density of the invading population approaching zero at the edge of the barrier zone adjacent to the core area.

*2.18.1. Description of the Diffusion Model*

The simplest 2-dimensional diffusion equation is the partial differential equation (Pielou 1969):

$$\partial U(x,y,t) / \partial t = D\,(\partial^2 U(x,y,t) / \partial x^2 + \partial^2 U(x,y,t) / \partial y^2) \qquad (8)$$

(see also Edelstein-Keshet 1988). This equation is a 2-dimensional analogue of an equation that was originally developed to describe the diffusion of heat along a metal rod (Fourier 1822), but has since been used for many other purposes as well, including insect dispersal. The diffusion coefficient, $D$, is defined in units of length squared per unit time, and is usually estimated by tabulating the linear difference between the initial and final positions resulting from insect dispersal, as well as the number of movements in a given time interval, and then computing the means of the squared net distances travelled per unit of time. For a population of insects released simultaneously at a point, equation (8) predicts an expanding Gaussian distribution with variance $4Dt$ at time $t$:

$$U(x,y,t) = (1/(4\pi Dt))\,[\exp\{-((x^2+y^2)/4Dt)\}] \text{ for } t > 0 \qquad (9)$$

Although most insect motion is demonstrably non-random, diffusion equations have been successful at predicting longer-term patterns of insect movement (Kareiva 1983; Turchin 1998) because population-level averaging occurs.

*2.18.2. Width of the Buffer Zone*

The pest population will have a certain density outside the buffer zone, and will disperse from outside into the buffer zone. If control measures are imposed within the buffer zone, the density of the pest will decrease from the outer edge of the buffer to the inner edge. The width of any buffer zone around a core area should be large enough to reduce the density of the pest close to zero in the core area. The buffer zone should be wide enough to prevent a gravid female insect and any of her offspring from crossing the buffer zone. Then an appropriate model is:

$$\partial F(x,t) / \partial t = D\,(\partial^2 F(x,t) / \partial x^2 + \partial^2 F(x,t) / \partial y^2) - g\,F(x,t) \qquad (10)$$

where $g$ is the growth function, $F$ is the female population density, $x$ is a spatial coordinate, and $t$ is time. If $g$ is linear, and births and deaths can be separated and are independent of $x$ and $t$, then:

$$gF = \beta F - \delta F \qquad (11)$$

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in which $\beta F$ and $\delta F$ are the instantaneous birth and death rates. Boundary conditions are such that at the outer edge of the buffer zone, $F(0,t) = F_0$, where $F_0$ is the density of insects at the outer edge of the buffer as a result of the influx of insects, and at the inside edge of the buffer, $F(w,t)$ = a small proportion of $F_0$ (e.g. $10^{-6}$), so that almost all the insects have died before reaching the inner side of the buffer. As a result of the continuous nature of the model, we can never actually achieve a zero density, but some small density below the Allee threshold that is non-viable will suffice.

If we are manipulating the death rate within the buffer by attract and kill devices that are evenly spread out to cover the whole of the buffer region, then ($\beta F$ - $\delta F$) will be negative, because now the second term in parenthesis consists of the sum of natural ($\delta$) and imposed ($\mu$) mortality from traps or other control sources. For control by killing, the kill rate ($\mu$) may be combined multiplicatively with the natural mortality ($\delta$) by multiplying survivorships (Barclay 1992b), so that if natural daily mortality is 0.05 and 10% of the population is killed each day, then $\delta + \mu$ becomes 1.0 – (1.0-0.05) (1.0-0.10) = 0.15 – 0.005 = 0.145. The upper limit of total daily mortality is 1.0 and the net change is $\beta$ – 1.0, although this may be impossible to achieve or even approach.

If the SIT is used as the control method, then we are manipulating $\beta$, and it will decrease from its natural value as sterile male releases increase. In this case, ($\beta F$ - $\delta F$) is also negative if control is to be effective. To simplify the solution of the diffusion equation when using the SIT, it will be assumed that the release of sterile males is proportional to the size of the wild insect population at all points within the buffer. The dynamics of this situation has been described by Knipling (1960), Hawkes and Coaker (1977), and Barclay (1981a), among others. Using this simplification, the same development described below will be useful for both killing insects or reducing reproduction. For control by SIT integration, the natural fertility is multiplied by a factor equal to the fertility factor, $\sigma = F / (M + S)$ in the SIT equation. If the sex ratio is 1:1, then the fertility factor is $M / (M + S)$. Thus, if $M$ = 100 and $S$ = 200, and if natural daily fertility is 0.10, then $\beta \sigma$ becomes 0.10 [100 / (100 + 200)] = 0.033. The lower limit of suppression here occurs as $\sigma \rightarrow 0.0$, so the net change is 0.0 - $\delta$, although this also may be impossible. Even with maximal SIT action, the rate of change of population density with distance across the buffer is likely to be slow.

Since we assume that we are dealing with an equilibrium situation, in which the insects have been diffusing and the buffer has been under control for a long time, the time derivative is zero, as nothing is changing over time. Only the space derivative is still non-zero. This yields the equilibrium equation:

$$dF / dx = (\delta - \beta \sigma) F \tag{12}$$

and this has solutions proportional to $e^{-\varphi x}$, where $\varphi^2 = (\delta - \beta \sigma) / D$. Assuming the insect density gradient across the buffer is $F(x) = c\, e^{-\varphi x}$, the boundary conditions dictate that $c = F_0$ and $F_0\, e^{-\varphi w} = 10^{-6} F_0$. Taking logarithms, $-\varphi w = \ln(10^{-6}) = -13.8$. This leads to the minimum buffer width ($w$):

$$w = 13.8 / \varphi = 13.8 / [(\delta - \beta \sigma) / D]^{1/2} \tag{13}$$

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to reduce the population at the inner edge of the buffer to one millionth of that at the outer edge. If a decrease down to $10^{-6}$ of the original density outside the buffer ($F_0$) is not satisfactory, then some other small fraction can be chosen, e.g. for $10^{-5}$ the 13.8 becomes 11.5, and for $10^{-7}$ the 13.8 becomes 16.1. The units of $w$ in eq. (13) are in the units of $D$, and the units of $\beta$ and $\delta$ must be the same as those of $D$.

For the case of killing insects, (13) becomes:

$$w = 13.8 / [(\delta + \mu) - \delta \mu - \beta) / D]^{1/2} \quad (14)$$

It is clear that, for control by SIT integration, the value of $\sigma$ must be less than $\delta/\beta$ to cause a reduction in the population. Thus, $\sigma < \delta/\beta$ implies that $M/(M+S) < \delta/\beta$, and this implies that $S > M(\delta/\beta - 1)$. Graphs of $w$ vs. $\mu$ and $w$ vs. $\sigma$ are shown in Fig. 11 for two values of the diffusion coefficient, two values of $\delta - \beta$, and one value of acceptable density on the inner edge of the buffer, $10^{-6}$.

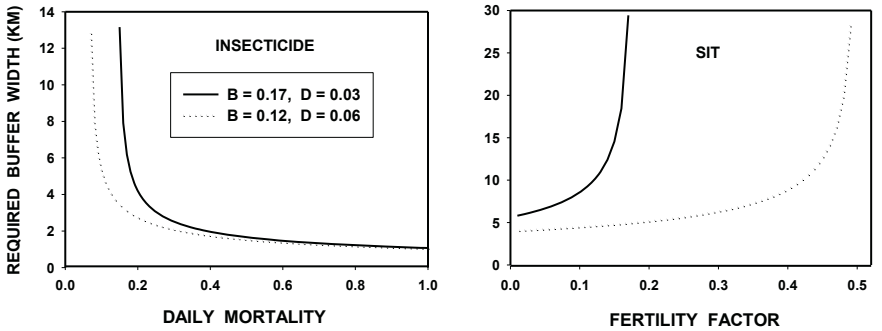



*Figure 11. Estimates of required buffer widths to reduce the population density at the inner edge of the buffer to $10^{-6}$ of that at the outer edge. The curves show buffer width as a function of daily mortality (left panel) or fertility factor using SIT application (right panel) for daily death and birth rates that might be appropriate to the Mediterranean fruit fly. The legend values apply to both panels.*

Control by the SIT and by insecticidal traps are not commensurate, as they use different mechanisms, but they can be compared by forming an equivalence in which the value of $w$ using the SIT equals the value of $w$ using traps. Equating (13) and (14) we obtain: $13.8 / [(\delta - \beta\sigma) / D]^{1/2} = 13.8 / [(\delta + \mu) - \delta\mu - \beta) / D]^{1/2}$, which simplifies to

$$\sigma = [\beta - \mu(1 - \delta)] / \beta \quad (15)$$

The parameter $\sigma$ is the fertility factor, $M / (M + S)$, so that to achieve a buffer width equal to that using a daily trapping rate of $\mu$, an overflooding ratio of $S / M = (1 - \sigma) / \sigma$ needs to be maintained. Note that, in a continuously reproducing population, the overflooding ratio is not necessarily the release ratio (section 2.12.3.). The release ratio (number of sterile males released each time period divided

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by the total number of wild males present) is related to the overflooding ratio as follows. If the daily mortality of the wild males is $q$ and that of sterile males is $r$, and if the daily recruitment rate of wild males is $m$, and the daily release rate of sterile males is $n$, and if the survivorship curves of both are exponential (geometric if measured by days), and if the lifetimes of both are unlimited, then the total number of wild males in the population at equilibrium is obtained from an infinite geometric series, $M = m/(1-q)$, and the total number of sterile males is $S = n/(1-n)$, so that the overflooding ratio is $[n/(1-r)] / [m/(1-q)] = (n/m) [(1-q)/(1-r)]$. If the daily survivorships of wild and sterile males are equal, then this reduces to an overflooding ratio of $n / m$, i.e. the quotient of the recruitments, which will also be the quotient of the wild and sterile male population sizes at equilibrium.

If neither traps nor the SIT yield reasonable buffer widths, it is possible to use both traps and the SIT in combination and further reduce the buffer width. It is true that the traps are likely to attract sterile males as well as fertile males, but if sterile and fertile males are equally attracted to the traps, the overflooding ratio will not change, but the wild population will be reduced by both control methods. In that case, the buffer width is:

$$w = 13.8 / [(\delta + \mu) - \delta \mu - \beta \sigma) / D]^{1/2} \quad (16)$$

and this is finite and positive only if $\delta + \mu - \delta \mu > \beta \sigma$, i.e. if mortality is greater than fertility.

Gordillo (2014) extended this approach to include optimization of sterile releases on the basis of the cost of sterile males for a density-dependent population, and including the possibility of variable sterile-male release rates. He used a one-dimensional diffusion equation and a numerical process called "simulated annealing", and concluded that in some cases sterile release rates should be uniformly high near the high-pest-density boundary of the buffer, and then drop sharply to much lower levels towards the core area to be protected.

### *2.18.3. Case Study: Mediterranean Fruit Fly*

Carey (1989) gave the following daily values for *C. capitata*: $\beta = 0.17$ and $\delta = 0.03$, and $r = 0.14$. The diffusion coefficient, $D$, has been estimated by Plant and Cunningham (1991) to be $D = 0.006$ km$^2$/day. It is clear that, if the kill rate or reduction in fertility is less than 0.14 per day, then the number of new daily recruits is still greater than the natural death rate. If the daily kill rate, $\mu$, is 0.20, then using eq. (14), the minimum buffer width is $w = 13.8 / \sqrt{[(0.23 - 0.17) / 0.006)]} = 4.36$ km.

If the SIT is the control method, and if the overflooding ratio is 10:1, then $\sigma = 0.091$, so the minimum buffer width is $w = 13.8 / \sqrt{[(0.03 - 0.17\ (0.09)) / 0.006]} = 8.87$ km. If both insecticidal traps and the SIT are used, then the buffer width is: $w = 13.8 / \sqrt{[(0.23 - 0.17(0.091)) / 0.006]} = 2.31$ km. This compares with the use of traps only, at twice the kill rate (0.40), which yields a buffer width of 2.10 km, or the use of the SIT at twice the overflooding ratio ($\sigma = 0.045$), which yields a buffer width of 7.29 km. Since it appears unlikely that traps can catch 40% of the insects daily, the combination of the SIT and trapping appears potentially to be useful.

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Recent studies of Mediterranean fruit fly dispersal suggest that a 2-km buffer zone is a reasonable first guess of the buffer width. Meats and Smallridge (2007) found that 90% of released flies remained within 0.4 – 0.7 km of the release point. It has been shown in Guatemala that most adult flies disperse short distances of only a few hundred metres, although some can disperse on winds up to 51 km from the release point (Villatoro 2014; Enkerlin et al. 2016), and it may be possible for flies to spread considerable distances when the food resource is depleted. Thus, a 2-km buffer might be useful if the aim of control was simply to yield a low-level pest infestation in a low-level horticultural production area. However, a much wider buffer will likely be more realistic, especially if the aim is complete exclusion of the pest from a protected area. In addition, the diffusion model used here assumes random movement, and many insects move quite non-randomly under conditions of stress or lack of food. We stress that the parameter values we have used are used for illustrative purposes only; any real control programme should use data obtained from the control area as well as any information on long-distance movement.

For the Mediterranean fruit fly example above, it would seem that control must be quite high to achieve a reasonable buffer width. This implies that a combination of synergistic controls would probably be advisable. A mitigating factor in these estimates of barrier width is that the formulas were developed assuming that the overflooding ratio would be constant across the barrier, rather than the sterile release rate being constant as the wild population decreases toward the inner edge of the barrier. This assumption would have the effect of increasing the estimate of the required barrier width. As the model appears to be intractable with a constant sterile release rate across the barrier width, a more reasonable approach might be to calculate $S$ using the population density at the uncontrolled edge of the barrier and to use that value throughout the barrier; this would reduce the required barrier width.

### *2.19. Optimization of Programme Releasing Sterile Insects*

Optimization inevitably involves economics (Mumford, this volume). Although that is somewhat outside the scope of this chapter, a beginning has been made on this topic. Geier (1969) used demographic models incorporating density-dependence to analyse the efficiency of programmes that release sterile insects, and to derive optimal strategies for control. Barclay and Li (1991) used a general treatment of combinations of pest control to determine optimal proportions of each control method. Atzeni et al. (1992) examined the situation for the Old World screwworm *Chrysomya bezzania* (Villeneuve), and included buffer width, male competitiveness, and population aggregation in their analysis. Anaman et al. (1994) performed a benefit/cost analysis with *C. bezzania,* and incorporated beef losses into the equation. Gordillo (2014) addressed the optimization question for sterile releases in a buffer zone that protects an insect-free area, and Ramirez and Gordillo (2016) used benefit/cost analysis to optimize sterile releases for insects that are long-lived but have short reproductive periods, such as some beetles.

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### *2.20. Development of Resistance*

There has been a marked tendency for insects to develop resistance to insecticides or other control methods. It is conceivable that a wild pest population could develop resistance to the use of sterile releases as a means of control (Itô et al., this volume; Lance and McInnis, this volume; Hendrichs and Robinson, this volume; Whitten and Mahon, this volume). This resistance might involve behavioural mechanisms that would preclude the sterile-fertile matings (Barclay 1990). Selection for resistance to several pest control methods operating together has been modelled by Barclay (1996), and it appears that selection for resistance to a particular control method is a linear function of the amount of mortality being inflicted by that control method, and the degree of isolation of the targeted population.

## 3. PARAMETER ESTIMATION FOR THE MODELS

Knowledge of several parameters is crucial to the success of any programme that releases sterile insects. With reference to the models outlined above, the basic parameters that will always be of interest are: $F$, the population size; $\lambda$, the potential rate of population increase each generation; $q_m$ and $q_f$, the proportions of the released males and females that remain fertile; and $c$, the competitive ability of sterile males relative to the wild fertile male population. Some of the estimations can be done using standard population biology methods. The population size can be crudely estimated from trap catches. Hargrove (1990) used mark-recapture techniques to estimate the size of tsetse fly populations (Weidhaas 1973; Itô et al., this volume). The rate of increase, $\lambda$, would normally be determined using oviposition rates. The residual fertilities, $q_m$ and $q_f$, could be determined by caging sterile males with fertile females, and fertile males with sterile females, either in groups or pairs, and noting the resulting fertile egg production. Competitive ability of sterile males, $c$, could then be determined from the laboratory, but preferably from field cage or small-scale field experiments, where immigration could be assumed to be negligible, using the equation involving competitive ability, and then solving for $c$. The information on $\lambda$, $q_m$ and $q_f$ must be determined first, or the equation becomes confounded. Alternatively, Meats (1998) used release and recapture techniques to estimate the quality of released sterile insects. Immigration into the control area could then be determined using either mark-recapture or the equation involving immigration and solving for $v$. Plant and Cunningham (1991) detailed procedures for estimating the dispersal of Mediterranean fruit flies, and estimates of immigration could be obtained from considerations of dispersal.

The determination of density-dependence is problematic; there are many models of density-dependence, and none of them is particularly mechanistic. Thus rates of oviposition and subsequent survivorship would have to be monitored at various densities to derive a function to describe the depressing effects at various levels. In many wild populations, even just detecting the existence of density-dependence is difficult, much less the quantification of depressing effects. However, in view of the potential assistance to the SIT, an estimation of the effects of density-dependence is

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worthwhile. Itô et al. (this volume) develop further the subject of parameter estimation.

## 4. ASSESSMENT OF SIT MODELLING

### *4.1. Uses of Models*

Models can be used to predict and explain the behaviour of a population. This information guides research, generates hypotheses, and aids teaching. Most models of the SIT have so far have been aimed mainly at predicting the behaviour of pest populations under various constraints, such as incomplete sterility, lack of competitive ability of sterile males, the immigration of wild insects into a control area, etc. One of the best uses of models is to generate ideas or hypotheses that are capable of experimental testing. Thus, ideally, modelling should go hand-in-hand with field and laboratory experiments to verify or falsify a model's predictions.

There have been a number of computer simulations of the SIT for the purpose of instruction in both the technique and in insect population dynamics generally. At least two have been documented and are available to the public. Both represent various elaborations on the original model of Knipling.

Sawyer et al. (1987) described a simulation that includes spatial heterogeneity, aggregation, immigration, random effects, and reduced sterile male competitiveness. This model was adapted by Flanders and Arneson (2018) under the name "*Curaçao*", and is available for downloading. The user can specify the various parameters and options, and then compare runs to draw conclusions.

Potgieter et al. (2013, 2016) aimed their models at a particular lepidopteran insect, and incorporated some of the features described above. Such models are valuable in any SIT programme against a particular insect.

### *4.2. Advantages and Limitations of Modelling*

The models of the SIT constructed thus far fall generally into three groups: (1) models that investigate processes that determine the proportion of eggs laid that are sterile, (2) models involving population dynamics and other population level phenomena, and (3) models that investigate the interactions of the SIT with other control methods, although it might be argued that the last two really belong together. The first category, including residual sterility, reduced sterile competitive ability, mating patterns, and immigration, is of crucial importance in planning and executing a programme that releases sterile insects. Unless one can accurately predict the level of sterility in eggs produced by the wild females, the programme is liable to fail. In addition, it is here that models are most likely to give realistic answers, as these processes rely mostly on determinable proportions or coefficients, rather than somewhat nebulous population processes.

Models of more general aspects of population dynamics involve many hidden factors, such as the strength of density-dependence, the functional responses of predators, synchrony of pest and predator phenologies, the degree of pest population

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aggregation, the extent to which sterile insects assume the same spatial patterns as the wild insects, etc. These are not easy to determine, and the models in the second and third categories must be taken as heuristic, rather than quantitatively predictive. They provide insights into the kinds of responses to expect, but quantitative accuracy must await species-specific simulations based on accurate and detailed biological and ecological information regarding the whole system.

### *4.3. Transient versus Equilibrium Models*

Many analytic models of the SIT are solved for equilibrium, and the results of the parameters on the equilibrium are noted. In real life, populations are almost always changing. Analysis of the equilibrium behaviour certainly has much to say about the effects of the parameters on the transient behaviour as well as on the equilibrium, but a proper analysis should include the effects of the parameters on the dynamics of transient behaviour. The problem is that there is an infinite number of trajectories that any population can follow, and to encapsulate the behaviour of these in digestible form is no small task. One criterion that can be used is that of stability of the system, in its various forms. Stability characteristics can be related to parameter ranges, and certain characteristics of the resulting transient behaviour can be inferred from them.

### *4.4. Future Directions and Information Needs*

The models reviewed in this chapter cover most of the relevant topics in the dynamics of the SIT. However, it is only a good beginning, and there is much left to do. Models that have a more realistic ecological basis will be required to suggest new hypotheses and to give more accurate predictions of behaviour. One area still largely untapped is metapopulation models — models including patches with migration among patches, local extinctions and re-establishments. A start has been made with the models including immigration, heterogeneity, and diffusion (Lewis and van den Driessche 1993; Gordillo 2015a). The next step is to tie these together into a meaningful whole.

Another area that will yield useful information is the construction of species-specific models for the SIT, including all relevant factors. Many species-specific models have been constructed, but many appear to have inadequate detailed ecological information. In addition, the area of behavioural ecology will probably emerge as being especially relevant.

Testing models, experimentally and in the field, is in its infancy. Information is needed on the effects of pest density-dependent regulation on the efficiency of the SIT, the effects of predators and parasitoids on the dynamics of the SIT, and the effects of ecosystem resilience. The simultaneous use of other control methods with the SIT is still largely hypothetical, and this potentially useful area needs considerable investigation.

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# CHAPTER 3.1.

# ROLE OF POPULATION AND BEHAVIOURAL ECOLOGY IN THE STERILE INSECT TECHNIQUE

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## SUMMARY

Important principles of population and behavioural ecology in relation to the application of the sterile insect technique (SIT) for the control of a pest are explained. These include: (1) a logistic population model for estimation of the population fluctuation of target animals and the number of sterile males to be released for successful eradication, (2) mark-recapture estimations of density and mortality rate of the target population, especially for remote areas, where repeated releases and recaptures are difficult, (3) models of dispersal to assess dispersal distance of target animals, and (4) equations for estimating the decrease of sexual competitiveness of mass-reared strains under field conditions. The method to estimate dispersal distance curves when attraction areas of traps are overlapping, and changes in mate-choice of wild females resulting from inadvertent selection when the SIT is applied, are explained. The necessity of field estimation of sexual competitiveness of released sterile males is also emphasized.

## 1. INTRODUCTION

In one of three seminal papers (Baumhover et al. 1955; Knipling 1955; Lindquist 1955) reporting the first success of the sterile insect technique (SIT) to eradicate insect pests, Knipling presented a table showing an example of model simulation for explaining the effect of sterile male releases. The model is

$$N_{g+1} = N_g RQ$$

where $N_g$ and $N_{g+1}$ are numbers of females at the $g$th and $(g+1)$th generation, and $R$ and $Q$ are rates of change in the population size per generation and the proportion of normal females (females which can lay hatchable eggs), respectively. This early model shows that population ecology theory and models have been an integral part of the SIT from the outset (Barclay, this volume; Barclay et al., this volume).

The early success in eradicating the New World screwworm *Cochliomyia hominivorax* (Coquerel), in the area-wide integrated pest management (AW-IPM) programme in Florida in 1959, was not always replicated in subsequent AW-IPM programmes integrating the SIT (Krafsur 1998; Liebhold et al. 2016). In many cases this was because government officials thought about the SIT as an established technique consisting only of releasing sterile insects, with animal and plant health workers engaged in programmes releasing sterile insects without basic ecological and behavioural studies. Some early programmes did not include first estimating the number of wild females, simulating the process based on a population model incorporating the SIT, and evaluating in the field the mating competitiveness of the released sterile males. Thus, in some of those cases, many sterile males were released but eradication failed, and it was not possible to know the major reason for failure, in

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spite of overflooding the wild population with sterile males, e.g. ratios of the number of sterile to wild males were 112:1 in a Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) programme in Nicaragua (Rhode et al. 1971), and 311:1 in another *C. capitata* programme in Procida Island (Cirio and de Murtas 1974). Without a full understanding of the population and behavioural ecology, the planning, implementation, and evaluation of programmes that release sterile insects become very difficult (FAO/IAEA 2016; Barclay, this volume; Barclay et al., this volume; Vreysen, this volume).

The goal of this chapter is to describe basic theory, models, and some mathematical techniques from population and behavioural ecology that are helpful for planning and executing the SIT component of an AW-SIT programme aimed at the control of pest insects – suppression, containment, prevention or eradication (Hendrichs, Vreysen et al., this volume).

## 2. THEORETICAL POPULATION DYNAMICS

### *2.1. Logistic Model*

The most basic model of population increase is the Hale-Malthus model:

$$dN_t/dt = rN_t \tag{1}$$

or, integrating equation 1,

$$N_t = N_0 e^{rt} \tag{2}$$

where $N_t$, $N_0$, $r$ and $e$ are number of individuals at time $t$, number at the beginning of increase, intrinsic rate of increase, and the base of natural logarithms (= 2.71828...), respectively. If population increase with discrete generations, as seen in many insects, is considered, equation 1 can be written as

$$N_{g+1} = N_g R \tag{3}$$

or

$$N_g = N_0 R^g \tag{4}$$

the equation used by Knipling (1955), where $\ln R = r$ (Begon and Mortimer 1981).

In equations 1 and 3, the population size increases indefinitely, but the large $N_t$ or $N_g$ may result in a smaller rate of increase, due to density-dependency, and the population may reach an upper limit. The most widely used model of density-dependent population increase is the logistic model,

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$$dN_t/dt = N_t(r - hN_t) \tag{5}$$

where $h$ is the suppressive effect of existence of an individual on the intrinsic rate of increase. Here $r/h$ is the upper limit of increase, and writing $r/h = K$,

$$\frac{dN_t}{dt} = rN_t\frac{K - N_t}{K} \tag{6}$$

or by integration,

$$N_t = \frac{K}{1 + e^{a-rt}} \tag{7}$$

where $a$ is a constant.

To establish a population model of the melon fly *Zeugodacus* (formerly *Bactrocera*) *cucurbitae* (Coquillett) under control by the SIT, Itô (1977) used a discrete expression of equation 7 to calculate generation-based increase of a logistic population (Fujita and Utida 1953). We have the following relation from equation 7 of $g$ generation: $e^{a-rg} = (K/N_g) - 1$. By substituting this relation for equation 7 of ($g$ + 1) generation, we obtain

$$R_g = N_{g+1}\Big/N_g = \frac{K}{1 + e^{a-rg}e^{-r}}\Big/N_g = e^r\Big/(1 + N_gB) \tag{8}$$

where $B = (e^r - 1)/K$. In place of Itô's procedure, we can use a logistic difference model, such as

$$N_{g+1} = \frac{N_gR}{(1 + cN_g)^b} \tag{9}$$

where $b$ and $c$ are constants (Hassell 1975; Begon et al. 1996), in place of equation 4 for constant increase. Fig. 1 shows examples of Hale-Malthusian (dashed) and logistic increase of density ($N_g$).

### *2.2. Dynamics of Populations under Control by the SIT*

In equations 8 or 9, the population size of the next generation can be expressed as

$$N_{g+1} = N_gR_g \tag{10}$$

and when the SIT is applied

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$$N_{g+1} = N_g R_g H_g \tag{11}$$

where $H_g$ is the proportion of fertile (hatchable) eggs, and this value indicates the suppressive effect of sterile males on population increase. Thus, $H_g$ is considered to be a function of the ratio of the number of sterile males, $N_s$, to fertile (normal) males, $N_f$, in the field, that is,

$$H_g = f(N_{s(g)}/N_{f(g)}) \tag{12}$$

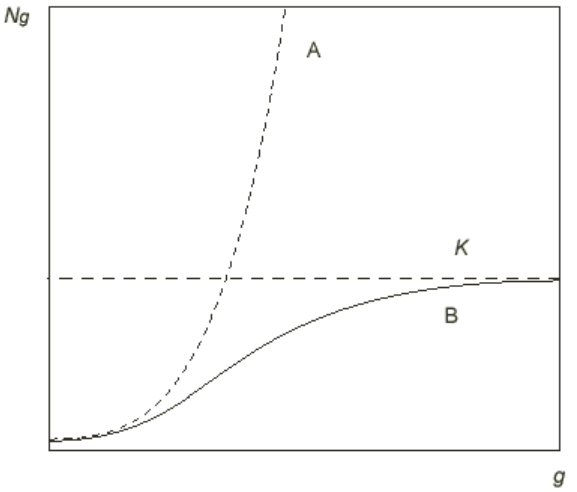



*Figure 1. Exponential (A) and sigmoidal (B) increase in the population. The equation giving a sigmoidal increase is the logistic model.*

If the number of sterile males released in each generation is the same,

$$H_g = f(N_s/N_{f(g)}) \tag{12'}$$

how can $f$ be determined? To establish a model of the SIT process for the melon fly on Kume Island, Okinawa, Itô (1977) adopted a Poisson distribution (mean number of matings = 1.61) for the frequency of matings per wild female. The probability that a female would mate with normal and/or sterile males was approximated by the binomial distribution. Based on this Poisson-binomial model, a curve showing the relationship between the expected hatchability of eggs and the $N_s/N_f$ ratio was obtained (Fig. 2). The $H_g$ values read from this graph are incorporated into equation 11 where $R_g$ is derived from equation 8.

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For the melon fly on Kume Island, Itô used the following values: $N_0$ = 125 000, $K$ = 2 700 000, $r$ = 1.2 (3.3 times increase per generation when $N_0$ is near 0), and $a$ = 3.971. $R_g$ changes in response to $N_g$, but based on the observed tendency that the number of melon flies decreases twice per year, in summer and winter in almost every year, Itô used the following $R$ values for 4 months (assuming 12 generations per year): $R_4 = R_5 = 0.5$, $R_{10} = 0.2$ and $R_{11} = 0.238$. By these decreases the population returned to the minimum density (125 000). Calculation of $N_g$ for the untreated period (before the SIT) gave a series of bimodal curves in which 125 000 and 2 621 568 were the annual minima and maxima, respectively (curve A of Fig. 3). $H_g$ values estimated from Fig. 2, using $N_g$ ($N_{f(g)}$ in equation 12) and a constant $N_s$, were used for simulation of the SIT process.

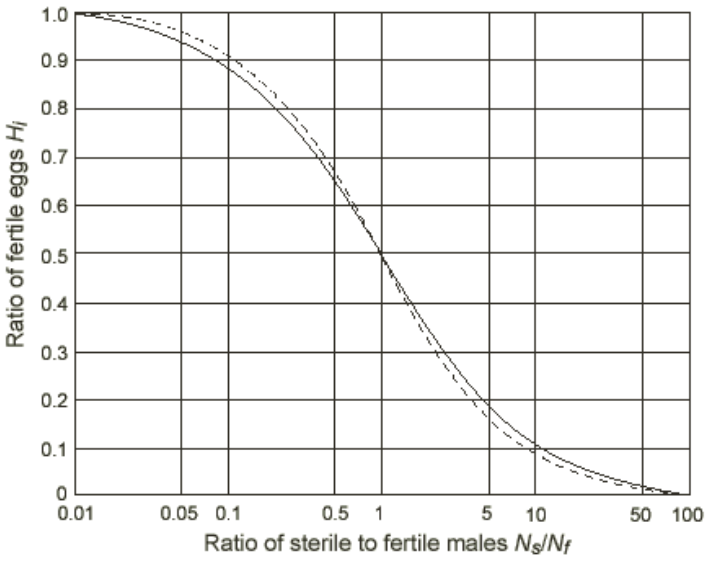



*Figure 2. Relationship between the ratio of sterile to fertile males, and the ratio of fertile to sterile eggs, in a Poisson-binomial model (solid line). The broken line depicts the relationship when the insects mate only once. (Figure from Itô 1977, reproduced with permission.)*

Curves B, C, and D of Fig. 3 are the results of simulation (initial ratios of sterile to normal males are shown in the legend). Fig. 3 indicates that, although eradication was not possible when the ratio of the number of sterile males released per month was one half of the minimum density of wild males ($N_0$ = 125 000 and $N_s$ = 62 500), eradication can be attained only during 2 years when the number of released males is the same as the minimum density (curve C). Note that the ratio of sterile males to wild ones is less than unity when a decrease of wild insects has occurred.

Itô's published result was unexpected since it shows that if the sexual competitiveness (hereafter mating competitiveness + sperm competitiveness) of

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released sterile males is the same as that of wild males, the release of much smaller numbers of sterile males than previously anticipated (e.g. 10/1 in Knipling 1955) can lead to success in an eradication programme. In the same year, using a similar logistic model for mosquito populations, Haile and Weidhaas (1977) obtained a similar result.

Once the curve of $H_g$ against $N_s/N_f$ is obtained, this curve can be used to estimate the sexual competitiveness of sterile males under field conditions. If the ratio of fertile eggs known from observation of eggs laid by wild females from the target population fell within the area above the curve in Fig. 2, this can be due to a reduction of sexual competitiveness of the released males.

The required ratio of sterile to wild males is a function of $r$ or $R$. Itô and Kawamoto (1979) attempted to simulate the population processes based on Itô's model by substituting different values of $r$. Their results show that a ratio of 10 sterile males to 1 wild male could result in the eradication of the target species within only 12 generations even when the rate of increase per generation, $R$, was 20 ($r = 3$).

These results show that the quality (sexual competitiveness) of mass-reared and sterilized males is much more important than the overflooding ratio.

Barclay and Mackauer (1980), Barclay (1982), and Itô et al. (1989) provided detailed explanations of differential equation models of the SIT.

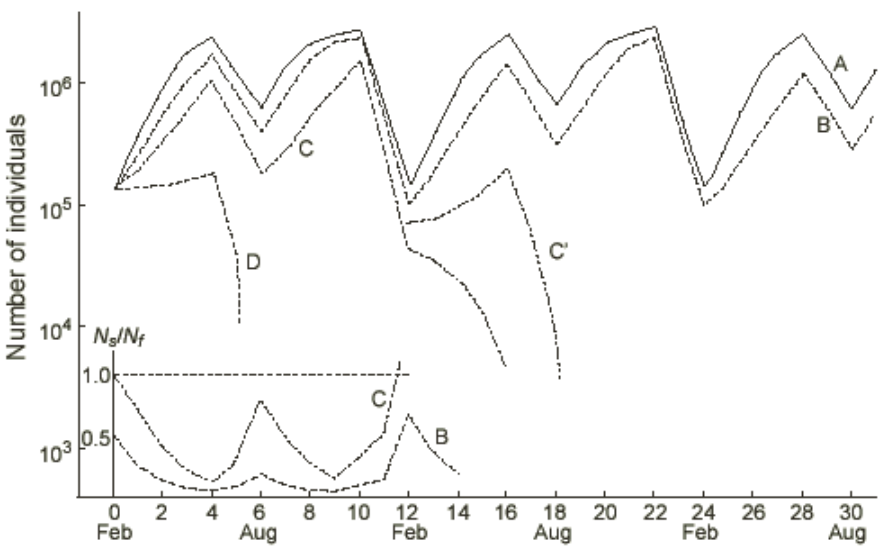



*Figure 3. Fluctuations in the number of flies in the model population without the SIT (A) and populations with the SIT. The initial ratios of sterile to normal males are 0.5/1 (number of sterile males = 62 500) in B, 1/1 (number of sterile males = 125 000) in C, and 2/1 (number of sterile males = 250 000) in D, respectively. C' is the trend when winter mortality was halved in treatment C. Seasonal trends in the ratio of sterile to fertile (wild) males ($N_s/N_f$) are shown in the lower section of the figure. (Figure from Itô 1977, reproduced with permission.)*

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### 2.2. *Spatial Considerations*

The above description of population dynamics does not consider the effect of spatial differences on population dynamics under the SIT. For programs in locations such as Kume Island, with an area of around 60 km$^2$, this is entirely appropriate and effective. However, in many continental settings, or in contexts where there is a "pest free" area with a surrounding area where the pest is established, further development is helpful.

Hendrichs, Vreysen et al. (this volume) gave a useful starting point, defining a rectangular core area where the goal is to reduce or eliminate the pest. Surrounding this core on all sides is a larger buffer zone, where the aim is to kill target species transiting toward the core area. One important question is how wide this buffer zone must be to give a reasonable certainty that the targeted pest is not able to enter the core area. Barclay et al. (2011) worked thorough an example of how to apply this model, summarized below. A simple model for the population in the buffer zone has the density of the pest increasing closer to the outer edge. If the population in the buffer has births ($\beta$) and deaths ($\delta$), and movement is modelled via simple diffusion (described in detail in section 4.1.) controlled by the diffusion coefficient $D$, then

$$\frac{dF(x,t)}{dt} = D\nabla^2 F(x,t) + \beta F(x,t) - \delta F(x,t) \tag{13}$$

Setting boundary conditions such that at the outside of the buffer area, $F(0,t) = F_0$, the density of insects is highest due to entry into the buffer of pests from the outer area, and at the inside edge of the buffer, $F(w,t)$, the density is a small proportion of $F_0$, e.g. $10^{-6}$. In this situation, almost all insects entering the buffer zone die before reaching the core area.

If either the survivorship or reproductive viability of insects in the buffer area are equal to the birth/entry rate, such that $\beta F - \delta F = 0$, then we have a simplified steady-state situation where only the space derivative is non-zero

$$D\nabla^2 F = (\delta - \beta)F \tag{14}$$

which has solutions proportional to $e^{[-(\delta-\beta)/D]x}$. Again, assuming the boundary conditions above (outer edge = $F_0$; $F_0 e^{[-(\delta-\beta)/D]x} = 10^{-6}$) taking logarithms, $\ln(10^{-6})=13.8$, and so the buffer width ($w$) is

$$w = \frac{13.8}{[(\delta-\beta)/D]^{1/2}} \tag{15}$$

Note that units need to be consistent: if $\beta$ and $\delta$ are in time units per week, then the diffusion coefficient $D$ also must be per week.

For the case of control in the buffer zone via the SIT with a release rate proportional to the ambient population, then $\delta$ can include both natural mortality and sterility from mating with a sterile male; the model is identical to the one above (Barclay, this volume).

Two other important spatial considerations are aggregation or "clumping", and

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whether the sterile insects distribute themselves similarly to the target pest. If the target is not randomly distributed, but the sterile insects generally are, then release rates might have to be increased to achieve control (FAO/IAEA 2016). A common way to assess clumping is to calculate the Coefficient of Dispersion (CD) (Southwood 1978) from the counts of insects found across samples (e.g. traps or fruit). CD is the ratio of the sample variance over the sample mean. A CD value of 1 (variance and mean are identical) suggests a random distribution in space; the sample is consistent with a Poisson distribution. A value above 1 suggests clumping or aggregation, while a value below 1 indicates a uniform distribution.

In practice, the CD statistic is less useful (has less power) if the population is sparse. An alternative is to use the negative binomial distribution. To assess clumping Barclay (1992) applied this approach (in the negative binomial distribution the parameter $k$ with values approaching zero indicate increasing aggregation, while higher values indicate random distributions). He found that, for $k = 0.25$, about four times the release rate would be needed for the same effectiveness when compared with a uniformly distributed target population (Barclay, this volume). A related practical example with field data is given in Shiga (1986).

In at least two recent studies, where the distribution of wild and released sterile insects have been examined, the two were found to be similar (Vreysen et al. 2011; Gavriel et al. 2012). Despite this, spatial aggregation remains an important factor to be considered when determining parameters for application of the SIT. It should be assessed for each target population and location, especially in determining required release rates.

## 3. ESTIMATION OF POPULATION DENSITY AND MORTALITY BY MARK-RECAPTURE

The previous section indicated that the number of sterile males to be released must be the same as, or more than, the minimum number of males in the target locality. Thus the density of target populations must be estimated to determine the appropriate number of sterile males for release. Since direct counting is not applicable to adults that move freely, mark-and-recapture methods are the only way to estimate the population size of many species that are targets of the SIT. In this section, equations of mark-and-recapture methods are briefly explained. Krebs (1989) provided a detailed explanation of equations and ways of using them. Another comprehensive source on mark-recapture calculations is Service (1993).

### *3.1. Petersen Method*

The Petersen method (also called the Lincoln Index) is the simplest way to use the mark-recapture method because it is based on a single episode of releasing marked animals and a second single episode of recapturing individuals. The equation is

$$\hat{N}_P = M_1 n_2 / m_2 \tag{16}$$

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where $\hat{N}_P$, $M_1$, $n_2$ and $m_2$ are the estimated total population, the number of individuals marked and released on the first day (day 1), the number of individuals caught on day 2, and the number of marked individuals recaptured on day 2, respectively. Variance of $\hat{N}_P$ can be estimated by

$$V(\hat{N}_P) = \frac{M_1(M_1 - m_2)n_2(n_2 - m_2)}{m_2^3} \tag{17}$$

In a release experiment to estimate population size for the SIT, usually laboratory-reared insects are released. This is especially necessary at the final stage of the SIT, because at this stage the number of wild males is small. The estimation of the number of wild individuals (unmarked individuals) is of greater interest than the total number of individuals. An estimate of the number of wild individuals, which is denoted by $\hat{U}_P$, can be obtained by

$$\hat{U}_P = M_1 u_2 / m_2 \tag{18}$$

where $u_2 = n_2 - m_2$. The variance is given by

$$V(\hat{U}_P) = \frac{\hat{U}_P(\hat{U}_P - u_2)(u_2 + m_2)}{u_2 m_2} \tag{19}$$

The following five assumptions are used for the Petersen method: (1) the population is closed, so the total number is constant, (2) all animals have the same chance of getting caught in the first sample, (3) marking individuals does not affect their catchability, (4) animals do not lose their marking(s) between the two sampling periods (Box 1), and (5) all marked individuals are reported on discovery in the second sample. Even when these assumptions hold, the Petersen method tends to overestimate the actual population, especially in small samples. For small samples (e.g. $m_2 < 10$), using the following two modified equations is recommended (for equations for variance, see Southwood 1978 and Itô and Murai 1978):

A: $$\hat{N}_{P'} = \left[ \frac{(M_1 + 1)(n_2 + 1)}{m_2 + 1} \right] - 1 \tag{20}$$

B: $$\hat{N}_{P''} = \frac{M_1(n_2 + 1)}{m_2 + 1} \tag{21}$$

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If estimated $N_P$ is very large as compared with $M_1$ and $n_2$ (e.g. $N_P > 10M_1$ or $10n_2$), equation 21 is recommended, but if $N_P$ is relatively small and/or $n_2$ animals are killed during sampling (e.g. by trapping), equation 20 is recommended (Itô and Murai 1978; Southwood 1978).

*Box 1. Method to Estimate Loss of Marking(s)*

The fourth assumption in the Petersen method, that animals do not lose marking, is a prerequisite for every model of mark-recapture estimation. Seber (1982) described a method to estimate the loss of marking.

Mark all the $n_1$ individuals in the first sample (or all the laboratory-reared animals released on day 1) in two ways, *A* and *B* (by two colours or two points on the body). Then

$\pi_x$ = Probability that a marking of type *x* is lost by the time of the second sample (*x* = *A, B*),

$\pi_{AB}$ = Probability that both markings are lost,

$m_x$ = Number of marked animals caught on day 2, with mark *x* only,

$m_{AB}$ = Number of marked animals on day 2 with both marks, and

$m_2'$ = True number of recaptures on day 2 ( $m_2' + u_2 = n_2$).

Then the maximum likelihood estimates of $m_2'$, $\pi_A$ and $\pi_B$ are:

$$\pi_A = m_B/(m_B + m_{AB}),$$

$$\pi_B = m_A/(m_A + m_{AB}) \text{ and}$$

$$m_2' = (m_A + m_{AB})(m_B + m_{AB})/m_{AB}$$

$$= c(m_A + m_B + m_{AB})$$

This means that the observed recapture ($m_A + m_B + m_{AB} = m_2$) must be corrected by a factor $c\,[= 1/(1-\pi_A)(1-\pi_B)\,]$ to give an estimate of the actual number of recaptures $m_2'$.

For large samples,

$$\hat{N}' = n_1 n_2 / m_2'$$

and, if laboratory-reared animals are released,

$$\hat{N}' = n_1 u_2 / m_2'$$

with

$$\hat{V} = \frac{N^3}{n_1 n_2}\pi_A \pi_B \left[\frac{1}{(1-\pi_A)(1-\pi_B)}\right]$$

$$+\frac{N^3}{n_1 n_2}\left[1 + \frac{2N}{n_1 n_2} + 6\left(\frac{N}{n_1 n_2}\right)^2\right]$$

When $n_1 = 500$, $n_2 = 149$, $m_{AB} = 7$, $m_A = 1$ and $m_B = 2$,

$\pi_A = 2/(2 + 7) = 0.222$, $\pi_B = 1/(1 + 7) = 0.125$

$m_2' = [(1+7) \times (2 + 7)]/7 = 10.286$

As $m_A + m_B + m_{AB} = 10$, $m_2' - m_2 < 1$, suggesting that the rate of loss of marking is negligibly small. Here

$\hat{N}' = (500 \times 149)/10 = 7450$ and

$\hat{V} = 6869641$, and s. d. = 2621

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The assumption of a closed population is usually not satisfied. For long-lived animals, mark and recapture during a short period may permit neglecting mortality, but the longevity of adult insects is often relatively short. However, Itô (1976) showed that the Petersen method gives the true population density on day 1 (before death) if only mortality exists, and gives the density on day 2 (after recruitment) if only recruitment exists. If both exist, the true density on day 1 is $N_P/(1 + B)$ or $N_P/(1 + B/S)$, where $S$ and $B$ are rates of survival and recruitment, respectively (Table 1). If there is no recruitment, the Petersen method gives a good estimate of density even when mortality and/or emigration exist. For a method to determine the existence of recruitment, see Seber (1982).

*Table 1. Effects of mortality and/or emigration and dilution (emergence and/or immigration) on the Petersen estimates*

| | $N_2$ | $M_2$ | $n_2$ | $m_2$ | $N_P$ |
|---|---|---|---|---|---|
| (1) Mortality/ emigration occurs but no dilution | $SN_1$ | $SM_1$ | $pSN_1$ | $pSM_1$ | $(pSN_1M_1)/(pSM_1) = N_1$ |
| (2) Dilution occurs but no mortality/ emigration | $N_1(1+B)$ | $M_1$ | $pN_1(1+B)$ | $pM_1$ | $[pN_1(1+B)M_1]/pM_1 = N_1(1+B)$ |
| (3) Both mortality/ emigration and dilution occur | $N_1(S+B)$ | $SM_1$ | $pN_1(S+B)$ | $pSM_1$ | $[pN_1(S+B)M_1]/pSM_1 = N_1(1+B/S)$ |
| (4) Same as in (3) but in reverse order | $SN_1(1+B)$ | $SM_1$ | $pSN_1(1+B)$ | $pSM_1$ | $[pSN_1(1+B)M_1]/pSM_1 = N_1(1+B)$ |

$N_2$, $M_2$, $n_2$ and $m_2$ are the numbers of individuals living in the target area, those marked and released, individuals caught on day 2, and recaptured on day 2, respectively.
$N_P$, $S$, $B$ and $p$ are Petersen estimates (of day 1), rates of survival and dilution from day 1 to day 2, and rate of capture, respectively.

### *3.2. Yamamura Method*

Although the Petersen method is the simplest method of estimation, the estimates are subject to large biases because the method ignores the mortality that must exist in the field. Yamamura et al. (1992) proposed a method that includes field mortality, where: (1) individuals reared in the laboratory are marked and released, and (2) traps are used for the recapture census, and all captured individuals are removed from the field population. This method requires one release procedure and two capture censuses.

Thus one more sampling census must be added to the Petersen method. There are four assumptions: (1) the wild population size is constant during the two consecutive sampling censuses (even if many wild individuals are lost by emigration from the

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study area or by artificial removal, the wild population size returns to the original level through immigration from the surrounding area), and (2) the proportion of marked individuals that survive and remain in the population between the two successive censuses, i.e. the rate of remaining, is constant. The last two assumptions are the same as (3) and (4) of the Petersen method.

If the marked individuals are released on day 1, and recaptured on day 2 and day 3, then the maximum likelihood estimates of the survival rate, $S_Y$, and the wild population size, $U_Y$, are given by

$$\hat{S}_Y = \frac{u_2 m_3}{m_2 u_3} + \frac{m_2}{M_1} \tag{22}$$

$$\hat{U}_Y = \hat{S}_Y M_1 u_2 / m_2 \tag{23}$$

### *3.3. Jolly-Seber Method*

Most populations are constantly changing in size because birth (emergence for adult populations), death, immigration, and emigration are not always balanced. Although the wild population is assumed to be nearly constant in two successive census periods in the Yamamura method, this is not always the case. The Jolly-Seber method is applicable to such a changing population. Two or more releases are required to apply the Jolly-Seber method. Recapture censuses are also required two or more times, the first recapture being conducted just before the second release. Estimates are obtained by using the following equations (for small samples, see Seber 1982 or Krebs 1989):

$$\hat{M}_i = \left(R_i Z_i / r_i\right) + m_i \qquad (i = 2,3, \ldots, s-2)$$

$$\hat{U}_{J(i)} = \hat{M}_i u_i / m_i \tag{24}$$

$$\hat{N}_{J(i)} = \hat{M}_i n_i / m_i$$

$$\hat{S}_{J(i)} = \hat{M}_{i+1} / (\hat{M}_i - m_i + R_i)$$

$$\hat{B}_{J(i)} = \hat{N}_{J(i+1)} - \hat{S}_{J(i)}(\hat{N}_{J(i)} - n_i + R_i)$$

where

$\hat{M}_i$ = Estimated number of marked individuals living in the area just before the sample $i$.

$\hat{N}_{J(i)}$ = Total number of individuals just before the sampling at time $i$.

$\hat{U}_{J(i)}$ = Number of unmarked individuals just before the sampling at time $i$.

$\hat{S}_{J(i)}$ = Survival rate during $i$ and $i$+1.

$\hat{B}_{J(i)}$ = Number of individuals that entered the population during $i$ and $i$+1.

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$n_i$ = Total number of animals caught in sample $i$ (= $m_i + u_i$).
$m_i$ = Number of marked animals caught in sample $i$.
$u_i$ = Number of unmarked animals caught in sample $i$.
$R_i$ = Total number of animals released after sample $i$.
$r_i$ = Number of the $R_i$ individuals released at sample $i$ and caught again in some later sample.
$Z_i$ = Number of individuals marked *before* sample $i$, not caught in sample $i$, but caught in some sample after time $i$. Let $m_{hj}$ be the number of marked animals caught in sample $j$ last caught in sample $h$ $(1 \le h \le j-1)$. Then we obtain

$$Z_i = \sum_{j=i+1}^{s} c_{i-1,j} \qquad \text{where} \qquad c_{i-1,j} = \sum_{h=1}^{i-1} m_{hj}$$

An example calculation is shown in Box 2. The method of calculation for larger samples, and special tables to be used for these, are shown in Seber (1982) using good numerical examples including a method to estimate variances. The variance formula for $\hat{U}_{J(i)}$ is given by Yamamura et al. (1992). Assumption (3) of the Petersen method is especially important for the Jolly-Seber method. Krebs (1989) described many methods to assess whether this assumption is satisfied or not.

### *3.4. Hamada Method (Modified Jackson Positive Method)*

The above-mentioned methods were listed in ascending order of the required amount of work. The Petersen method requires one release and one capture census, the Yamamura method one release and two capture censuses, and the Jolly-Seber method two releases and two capture censuses. The difference in requirements between the Yamamura and the Jolly-Seber methods seems to be very large, since the addition of one more release usually requires more work than that of one more capture census. Since AW-IPM programmes releasing sterile insects are often carried out in remote areas, the addition of a release is especially laborious. Therefore in many cases one release is conducted, accompanied by three or more subsequent capture censuses, resulting in a series of regression estimates (see Jackson (1939) method).

Jackson (1939) presented equations to estimate the density and survival rate in an open population by a single release and multiple recapture censuses (Jackson positive method). As a first step an index $y_i$ is calculated using the following equation:

$$y_i = 10^4 m_i / M_0 n_i \tag{25}$$

where $M_0$ is the number of individuals marked and released on the first day (day 0; here day 0 is used to show the first day in place of day 1 as in the preceding methods, for simplicity of explanation of the regression method), $y_i$ is a standardized number of

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marked insects to be recaptured on day $i$, assuming that 100 marked individuals are released on day 0 and 100 individuals are randomly caught on day $i$. Other symbols are the same as in the preceding equations. If total insect density is almost constant, and if marked individuals are returned to the field after being recaptured, by plotting $y_i$ against $i$ (Fig. 4) a survivorship curve of marked individuals in the field is obtained.

*Box 2. Jolly-Seber Three-Point Method*

As a special case of the Jolly-Seber method, an example of estimating population parameters based on two releases and two recaptures is shown. Let us consider a typical case of mass-marking experiment in which individuals reared in the laboratory are marked and released. All captured individuals are killed. On day 1, individuals are released with a red mark. On day 2, individuals are released with a blue mark. The following is the result of a release experiment of *Spodoptera litura* (F.) conducted by Wakamura et al. (1992).

| Day | $n_i$ | $R_i$ | $U_i$ | Number of recaptured individuals | |
|---|---|---|---|---|---|
| | | | | Red marked | Blue marked |
| 1 | | 1934 | | | |
| 2 | 409 | 1968 | 26 | 383 | |
| 3 | 633 | | 24 | 181 | 428 |

The Jolly-Seber method yields

$$\hat{M}_2 = R_2 Z_2 / r_2 + m_2 = (1968 \times 181/428) + 383 = 1215$$

$$\hat{S}_{J(1)} = \hat{M}_2 / R_1 = 1215/1934 = 0.63$$

$$\hat{U}_{J(2)} = \hat{M}_2 u_2 / m_2 = 1215 \times 26/383 = 83$$

For the Yamamura method, we obtain

$$\hat{S}_Y = (u_2 m_3)/(m_2 u_3) + m_2 / M_1$$
$$= (26 \times 181)/(383 \times 24) + 383/1934 = 0.71$$

$$\hat{U}_Y = \hat{S}_Y M_1 u_2 / m_2 = 0.71 \times 1934 \times 26/383 = 93$$

For the Petersen method, we obtain

$$\hat{U}_p = M_1 u_2 / m_2 = 1934 \times 26/383 = 131$$

The estimate obtained by the Petersen method is much larger than that obtained by other methods. The Yamamura method generally yields results similar to those of the Jolly-Seber method if marked individuals are sufficiently mixed with wild individuals.

If the survival rate is constant, the survival rate can be estimated using a linear regression:

$$\log y_i = \log y_0 + i \log S \tag{26}$$

where $S$ is the survival rate per unit time (as $0 \leq S \leq 1$, $\log S$ is always negative), $y_0$ is a constant representing the expected number of recaptures on the assumption that 100 marked individuals released on day 0 are instantaneously intermingled into the wild population, and that 100 specimens are randomly caught before either mortality or recruitment occurs. Then an estimate of the total number of individuals on day 0 using

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$$\hat{N}_{J+} = 10^4 / y_0 \tag{27}$$

is obtained together with the survival rate $S$ from the slope of the regression line.

In the Jackson positive method, recaptured individuals should be returned to the original population. Since, in programmes integrating the SIT, recaptures are made with traps, the survival rate decreases as the number of recaptures increases, resulting in an overestimated $y_0$ (Fig. 4). Thus $N_{J+}$ will be underestimated. Itô (1973) devised a modified equation to reduce some of this bias by using a modified index $y'_i$, such that

$$y'_i = 10^4 m_i / M'_{0(i)} n_i \tag{28}$$

where $$\hat{M}'_{0(i)} = M_0 - \sum_{j=1}^{i-1} m_j$$

then $$\log y'_i = \log y'_0 + i \log S \quad \text{and} \quad \hat{N}_I = 10^4 / y'_0$$

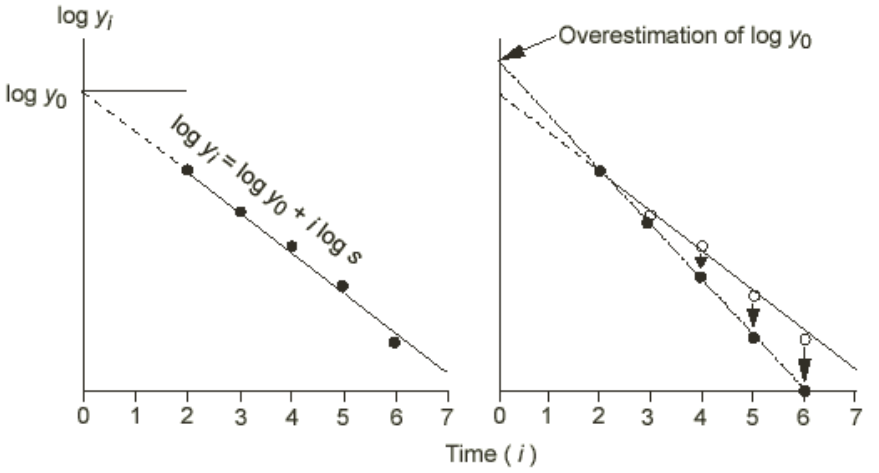



*Figure 4. Comparison of Jackson positive and Itô methods using hypothetical values. Assuming no trapping mortality, change in* $y_i$ *should parallel the survivorship curve of marked insects and, when daily survival rate is constant, the curve of* log $y_i$ *should be linear against* i *(left). However, if there is trapping mortality,* $y_i$ *should have smaller than expected values with time, and the linear regression should yield an overestimated value of* log $y_0$ *(right), which in turn results in an underestimation of* $N_{J+}$. *(Figure adapted from Itô et al. 1989, reproduced with permission from Elsevier.)*

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Hamada (1976) found that the Itô method gives an overestimated value when the number of released laboratory-reared marked males is much larger than the wild population size. He showed that a direct estimation of the number of wild males with the following equation gives the least (but not zero) stable negative bias (usually below 20%):

$$z_i = 10^4 m_i / M'_{0(i)} u_i \quad \text{and} \quad \hat{U}_H = 10^4 / z'_0 \tag{29}$$

where $u_i = n_i - m_i$ , and $\log z_i = \log z_0 + i \log \hat{S}$. The Hamada method has been applied widely to estimate the wild melon fly density in Okinawa. The Hamada method is based on a similar assumption as in the Yamamura method — the number of wild individuals is kept constant during the capture period. If the number of recapture censuses is two, the Yamamura method is preferable since it is the maximum likelihood estimate for this situation. Table 2 gives estimated densities and survival rates of male melon flies using the Petersen and Hamada methods, showing the relative stability of the estimated values.

*Table 2. Comparison of estimates of the density of male melon flies with the Petersen and Hamada methods (data from Tanaka et al. 1978)*

| Station | Date of release | $\hat{U}_P$ /ha | $\hat{U}_H$ /ha | $\hat{S}$ /day[1] |
|---|---|---|---|---|
| 1 | June 28 | 136 | 85 | 0.81 |
| | July 12 | 173 | 87 | 0.83 |
| 2 | July 22 | 166 | 109 | 0.74 |
| | August 9 | 251 | 180 | 0.77 |

$\hat{U}_P$ and $\hat{U}_H$ are the numbers of wild males estimated by the Petersen and Hamada methods, respectively. For the Petersen method, the numbers of males caught on the 4th (station 1) and 2nd (station 2) days after release are used as $n_2$ and $m_2$ (equation 16). Numbers of recaptures in the Hamada method are four in station 1, and five in station 2.

[1] Estimated by the Hamada method, using linear regression of $\log z_i$ and $\log \hat{S}$ in equation 26.

### *3.5. Jackson Negative Method*

Jackson (1939) also presented another model (Jackson negative method) to estimate the population size based on multiple-release single-recapture data. Marked individuals are released on several occasions (on days $-i$, $(-i + 1)$,... , $-1$) with different markings, and thereafter a single random catch is made on day 0. Here

$$y_{-i} = 10^4 m_{-i,0} / M_{-i} n_0 \tag{30}$$

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where $m_{-i,0}$, the number of individuals released on day $-i$ and recaptured on day 0, is expected to increase with time. If the survival rate of marked individuals is constant, one can estimate $y_0$ by the linear regression of $\log y_{-i}$ on $i$, that is,
$\log y_i = \log y_0 + i \log S$. Then, as in the positive method, $N_{J-}$ is

$$N_{J-} = 10^4/y_0 \tag{31}$$

When laboratory-reared individuals are released, the following equations are applicable:

$$y'_{-i} = 10^4 m_{-i,0} / M_{-i} u_0, \quad \text{and} \quad U_{J-} = 10^4 / y'_0 \tag{32}$$

Reisen et al. (1979) and Koyama et al. (1982) noted that the trapping mortality, which induces a negative bias in the Jackson positive method, does not cause bias in density estimates obtained by the negative method because recapture is made only once for any group of released individuals. This method was used to estimate melon fly density in mountainous parts of Okinawa. Multiple releases of marked flies were made from a helicopter, followed by a single recapture by the many persons who checked traps.

### *3.6. Fisher-Ford Method*

The Fisher-Ford (1947) "trellis" method differs from the approaches described above in that it incorporates an arbitrary number of releases and recaptures (greater than one of each) to obtain an estimate of population size; it also requires an estimate of daily survivorship that is independently derived, and that each released marked cohort of individuals be individually identifiable. However, the requirement of a daily survivorship can be used to set a range on the population size estimate as shown in the worked example below (Box 3).

Fig. 5 shows the results of a mark-recapture study with mosquitoes in Mali, West Africa via a "triangular table" or "trellis diagram", originally introduced by Fisher and Ford (1947). In these tables the dates are arranged horizontally along the top. The total captured on a given date is shown at the end of the cells running to the lower left side of the diagram, while the total number released on that date is given along the lower right. The recaptures are given in the body of the table, with each intersecting cell representing the number of insects marked and released on date $N$ that was recaptured in subsequent dates. For example: on July 3, 126 mosquitoes were released of a total of 160 captured. Following the left slanting diagonal, two mosquitoes marked on July 3 were recaptured on July 4, one was recaptured on July 5, and none after that. The distribution of release dates for recaptures on any given day is also apparent. For example: on March 19, three mosquitoes that had been released on March 18 were recaptured together with four from the release on March 17 and one from the release on March 16.

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*Box 3. Fisher-Ford Method*

Calculation of the population size is described in terms of operations on a table, following the approach used with the introduction of this method by Fisher and Ford (1947). Here we follow this practice with data from Baber et al. (2010), presented in Table A using data from Fig. 5 with an assumed daily survivorship of 0.6.

The first column gives sampling dates in reverse chronological order, while the last column gives the row dates + 1. Columns to the left of the vertical line refer to the dates in the first column, those to the right refer to the dates in the last. Working left to right, we have the number of days from the end of the experiment in column 2, and the number of marked individuals released in column 3 (these can also be read in Fig. 5). Column 4 is the expected number of marked individuals recaptured, calculated as follows: sum of row values from the bottom of the table, each value is

$$E_m = \sum_{i=n}^{l} MS^d$$

where $n$ is the current row, $l$ is the last row of the table, $M$ is the number of marked individuals released, and $S^d$ is the estimated daily survivorship raised to the number of days from the end of the sampling (column 2).

*Table A. Calculation of population size following the method of Fisher and Ford (1947) for mosquito data collected in Fourda, Mali, in 2008 by Baber et al. (2010), assuming a daily survivorship of 0.6*

| Date 1 | *d* | *M* | $E_m$ | *R* | *C* | *P* | Date 2 |
|---|---|---|---|---|---|---|---|
| 09 July | 1 | 0 | 63.7 | - | - | - | 10 July |
| 08 July | 2 | 0 | 63.7 | 1 | 505 | 53 606 | 09 July |
| 07 July | 3 | 0 | 63.7 | 2 | 335 | 29 634 | 08 July |
| 06 July | 4 | 299 | 63.7 | 5 | 235 | 13 859 | 07 July |
| 05 July | 5 | 157 | 24.9 | 5 | 385 | 37 841 | 06 July |
| 04 July | 6 | 144 | 12.7 | 5 | 187 | 11 995 | 05 July |
| 03 July | 7 | 126 | 6.0 | 6 | 166 | 7550 | 04 July |
| 02 July | 8 | 148 | 2.5 | 7 | 160 | 4910 | 03 July |

*d*: days from the end of experiment; *M*: number of individuals marked and released; $E_m$: number of marked individuals expected in the environment considering daily survival; *R*: number recaptured; *C*: number captured; *P*: estimated population size.

Values in columns 5–7 refer to dates in column 8 ("Date 2"). For each date *j*, the estimated population size *P* is calculated as follows:

$$P = \frac{E_m C}{S^d R}$$

Considering population size estimates with at least 3 releases (July 6 and later) yields an average population size estimate of 33 735 with an SE of 8284. Assuming a higher daily survivorship of 0.8 gives a mean population size estimate of 77 085 and an SE of 31 007.

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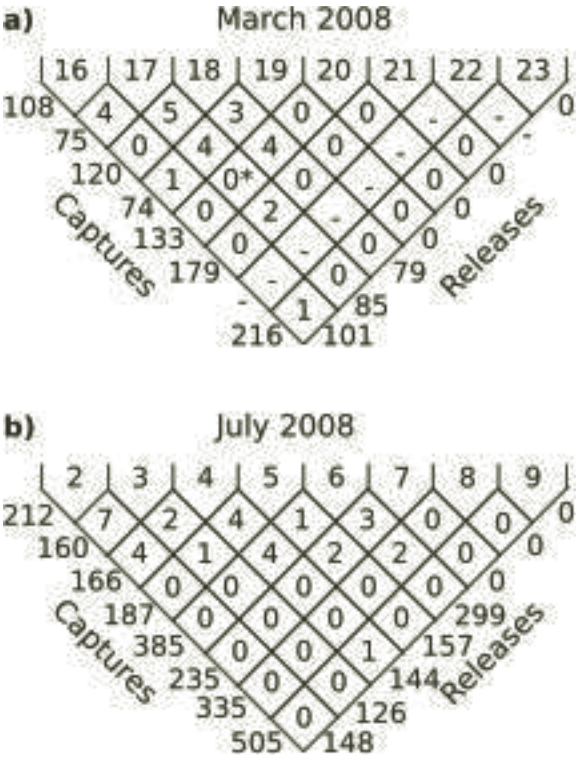



*Figure 5. Trellis diagram with results of mosquito mark-recapture experiments during the dry season (March) and wet season (July) in Fourda, Mali, from Baber et al. (2010). *: marked mosquitoes captured during night-landing survey, separate from this experiment; -: indicates no survey; see text for details.*

A survey of the literature did not reveal any examples of the Fisher-Ford method being applied in the context of SIT projects. The reason might be that most marked and released insects in the context of the SIT are mass-reared and marked with one colour only, with attendant issues of differences between them and wild stock when it comes to estimating population sizes. The Fisher-Ford method is better suited to situations where a limited number of wild insects is being captured, marked and released, or perhaps in the later stages of an SIT project when the number of wild insects is lower. However, it could still be a very helpful method that could be adapted to SIT projects because it explicitly models the survival of the released insects, and provides mean and variance of population size estimates through the approach demonstrated in Box 3.

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## 4. ESTIMATION OF DISPERSAL DISTANCE BY MARK-RECAPTURE

The immigration of wild insects into an area treated with sterile insects is one of the most important causes of failure of some AW-IPM programmes integrating the SIT (Lance and McInnis, this volume). If wild males have sufficient dispersal ability, the male sterile:fertile ratio ($N_s/N_{f(g)}$ in equation 12') will decrease, causing higher fertility in wild females. Estimation of the dispersal range is also important for estimating the $N_s/N_{f(g)}$ ratio from field data. If the dispersal range is small, the $N_s/N_{f(g)}$ ratio should be estimated using samples obtained from the small area, whereas if the dispersal range is large, samples obtained from the large area should be combined. Furthermore, if the range of dispersal is known, an optimal spatial design for the release of sterile males can be constructed. If the dispersal range is small, sterile males should be released at many spatial points to increase uniformly the male sterile:fertile ratio. In this section several techniques to estimate the dispersal range of individuals are described.

### *4.1. Diffusion Equation*

A two-dimensional simple diffusion equation will be the simplest theoretical model that can be applicable to the two-dimensional dispersal of marked individuals. Assuming that the movement of marked individuals is Brownian random motion (the rate of which is invariant in time and space), the number of marked individuals at time $t$ at coordinate ($x$, $y$), which is denoted by $m(x,y,t)$, is described by a partial differential equation (Okubo 1980, Shigesada and Kawasaki 1997):

$$\frac{\partial m(x,y,t)}{\partial t} = D\left(\frac{\partial^2 m}{\partial x^2} + \frac{\partial^2 m}{\partial y^2}\right) \tag{33}$$

where $D$ is the diffusion coefficient that measures the dispersal rate with units (distance$^2$/time). When $M_0$ individuals are released at time 0 from the origin (0, 0), the solution is given by

$$m(x,y,t) = \frac{M_0}{4\pi Dt}\exp\left[-\frac{(x^2+y^2)}{4Dt}\right] \tag{34}$$

which is a two-dimensional normal distribution with mean zero, correlation coefficient zero, and variance $2Dt$ in each dimension. If the distance from the origin is denoted by $r = \sqrt{x^2+y^2}$, then equation 34 is rewritten in a simpler form

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$$m(r,t) = \frac{M_0}{4\pi Dt}\exp\left[-\frac{r^2}{4Dt}\right] \qquad (r \geq 0) \tag{35}$$

The proportion of individuals in a circle of radius $d$ at time $t$, which is denoted by $F(d, t)$, is given by

$$F(d,t) = \frac{1}{M_0}\int_0^d 2\pi r \cdot m(r,t)dr = 1 - \exp\left[-\frac{d^2}{4Dt}\right] \tag{36}$$

Thus, the proportion of individuals in a circle of radius $\sqrt{4Dt}$ is $1-\exp(-1) = 0.63$, and that in a circle of radius $2\sqrt{4Dt}$ is $1-\exp(-4) = 0.98$. A rearrangement of the above equation yields

$$\log_e[1 - F(d, t)] = -d^2/(4Dt) \tag{37}$$

Therefore, if the distribution of marked individuals is observed at time $t$, an estimate of $D$ can be obtained by plotting the observed $\log_e[1 - F(d, t)]$ against $d^2$ and by estimating the slope $-1/(4Dt)$ using a liner regression with intercept zero (Broadbent and Kendall 1953). If the relation is not linear, it can be judged that the dispersal is not a random diffusion with a constant $D$ (e.g. Inoue 1978). The expectation of the square of the distance is given by

$$E[r^2] = \frac{1}{M_0}\int_0^\infty 2\pi r \cdot r^2 \cdot m(r,t)dr = 4Dt \tag{38}$$

Thus, the moment estimate of $D$ is given by the observed mean square of the distance divided by $4t$. If estimates of the mean square distance for several $t$ are available, the common $D$ can be estimated by plotting the mean square distance against $t$ and by estimating the slope $4D$ using the linear regression with intercept zero.

### *4.2. Random Correlated Walks*

The diffusion model described above is useful in its simplicity and mathematical tractability. In terms of an organism moving in real space with discrete time-steps, diffusion can be understood as a random walk: at each time-step the organism moves a random step-length (with given range) in a randomly chosen direction. The step direction at time $t$ is independent from the direction at $t$-1, all are equally likely including complete reversal. This "classical" random walk results in an average expected displacement of the entire population = 0, as indicated above.

Even in the absence of cues, this kind of motion is not realistic for most organisms. Forward bias is common, as are correlations between directions chosen in subsequent time-steps (Miller at al. 2015). A random correlated walk (Kitching 1971;

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Kareiva and Shigesada 1983; Byers 2001) is an extension of a classical random walk (diffusion) that restricts the range of new directions after a given step, thus resulting in more realistic dispersal.

Kareiva and Shigesada (1983) derive the relationship between an organism's movement behaviour and its expected square displacement using a random correlated walk as follows: if each move $m$ is a vector $(x_m,y_m)$ then the total displacement after $n$ moves, $R_n$, is

$$R_n = \sum_{m=1}^{n}(x_m,y_m) \tag{39}$$

the length of each move is the Euclidean distance; it and each turning angle $\theta_m$ are independent random variables with probability densities equal to $p(l)dl$ and $g(\theta)d\theta$, respectively. The expected values for these random variables are:

$$E(l) = \int_0^\infty l\, p(l)dl \tag{40}$$

$$E(l^2) = \int_0^\infty l^2\, p(l)dl \tag{41}$$

$$c = E(\cos\theta) = \int_{-\pi}^{\pi} \cos\theta\, g(\theta)d(\theta) \tag{42}$$

$$s = E(\sin\theta) = \int_{-\pi}^{\pi} \sin\theta\, g(\theta)d(\theta) \tag{43}$$

Since a set of moves can be represented by independent random draws from $p(l)$ and $g(\theta)$, the process of movement in this model is a first-order Markov chain -- where a given draw is not influenced by the previous draw. However, the probability density of values can be non-uniform. Relating the distribution of values to biological movement is straightforward: if the distribution is uniform for angles between -180 and 180 then the movement pattern will follow classical diffusion (a random walk) as described above. If the probability density is 1 for an angle of 0 and 0 for all other angles in the range, then the movement pattern is a straight line (sometimes termed a "ballistic" disperser; Miller et al. 2015). If the distribution is intermediary, then the movement is considered a random correlated walk.

A useful result from Kareiva and Shigesada (1983) is the expected squared displacement. Assuming an equal probability of right or left turns, the following equation is derived:

$$E(R_n^2) = nE(l^2) + 2E(l)^2 \frac{c}{1-c}\left(n - \frac{1-c^n}{1-c}\right) \tag{44}$$

This model enables a direct relation between behavioural changes influencing $p(l)$ or $g(\theta)$ and consequent changes in the expected net displacement.

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### *4.3. Distribution of Cumulative Recaptures*

Traps are frequently used to capture marked individuals (FAO/IAEA 2018). If traps are set for a sufficiently short period around $t$, the observed distribution of captured individuals can be used for a sample distribution of dispersing individuals at time $t$. If traps are set for a longer period, the observed distribution cannot be used for a distribution at a specific time. A trap also performs some integration of density over space as well as over time. Such a spatial integration may cause some difficulties in the estimation of dispersal distance; one such difficulty is discussed in Box 4.

In several experiments, marked individuals have been trapped until most of them died or left the study area. In these cases estimates of the distribution of the cumulative number of recaptured individuals in each trap can be obtained. The theoretical distribution of the cumulative recaptures can be described by relatively simple equations under several assumptions. Let $\delta$ be instantaneous natural mortality (or disappearance rate), and $\alpha$ trap efficiency. Assuming that the number of recaptures is sufficiently small relative to the number of total release, $M_0$, so that the mortality $\delta$ is not influenced by the mortality caused by trapping, then the cumulative number of recaptures at a trap placed at a distance $r$ is given by

$$C(r) = \frac{\alpha M_0}{2\pi D} K_0\left(\sqrt{\frac{\delta}{D}} r\right) \tag{45}$$

where $K_0$ is a zero-order modified Bessel function of the second kind (Broadbent and Kendall 1953; Williams 1961). Turchin and Thoeny (1993) used an approximation for equation 45

$$C(r) \approx \frac{A}{\sqrt{r}} \exp\left(-r\sqrt{\frac{\delta}{D}}\right) \tag{46}$$

where $A$ is a constant. This equation can be described by a linear form,

$$\log_e[C(r)] + \frac{1}{2}\log_e(r) \approx \log_e(A) - r\sqrt{\frac{\delta}{D}} \tag{47}$$

therefore $\sqrt{\delta / D}$ can be estimated by plotting the observed $\log_e[C(r)] + \frac{1}{2}\log_e(r)$ against $r$, and by estimating the slope by using a linear regression method, although such an estimation procedure is not preferred from a statistical point of view. Like Inoue's (1978) method using equation 37, equation 47 can be used to judge the randomness of dispersal. If the relation is not linear, then dispersal is not a simple random diffusion with a constant $D$ (Cronin et al. 2000). Note that $\delta$ and $D$ cannot be estimated separately since the accumulation of recaptures eliminates information on the velocity of dispersal in this estimation procedure.

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*Box 4. Overlap of Attraction Areas of Traps*

In a study of the dispersal of marked male sweetpotato weevils *Cylas formicarius* (F.), and using plastic funnel traps with 1 mg sex pheromone, Miyatake et al. (2000) placed traps in eight directions, and at distances of 10, 20, 50, 100, and 200 m, from the release point. However, Yasuda and Sugie (1990) estimated that the radius of the circular attraction area of a trap (area of 30% recapture) is about 50 m. According to Fig. 52 in Yasuda (1998), the radius of 60% recapture is less than 30 m. (The distribution of the attraction rate around a trap might be a normal distribution or a distribution with larger kurtosis. Therefore, how can the radius of the "attraction area" be determined?) If we use a trap site of 10 or 20 m from the release point, the attraction areas of traps placed by Miyatake et al. (2000) at sites near the release point are overlapping, and many males might be attracted by two or more traps. Thus such traps might attract more individuals when they are in an isolated place, leading to an overestimation of the dispersal range.

If traps were distributed in a lattice pattern, the effect of the overlap of traps can be reduced to a negligible level. If individually marked insects are released from many points of the lattice, reduction of the effect is better. Therefore it is recommended that a lattice-pattern arrangement of traps be used. However, since many data are taken if traps are arranged along four or eight directions to estimate the dispersal range, a method that reduces the effect of overlap of attraction areas is described hereafter.

Fig. A shows an example of the effect of overlap of attraction areas. Individuals in the striped area would be attracted to two traps. Therefore, we must calculate the area of half of the striped area (double stripes) and subtract this from the area of the circle. In Fig. A, the distance between the two traps, and the radius of the attraction area, are $2a$ and $R$, respectively. As $\theta = \cos^{-1}(a/R)$, $\theta$ can be calculated when $R$ is known. The area of the triangle ABC of Fig. A is $\left[2\sqrt{R^2 - a^2} \times a\right]/2$. Then half of the striped area can be calculated by $\left[\pi R^2 \times 2\theta/(2\pi)\right] - \left[\sqrt{R^2 - a^2} \times a\right]$. This area must be subtracted from the attraction area of a trap, $\pi R^2$, to obtain the true attraction area.

When this procedure is carried out for data from Miyatake et al. (2000), a smaller value for dispersal distance is obtained (Fig. B). Although Miyatake et al. (2000) set traps along eight directions on circles of which the radii were 10, 20, 50, 100, and 200 m from the single release point, data for 10 m are omitted because the attraction areas of three or more traps overlap at 10 m points.

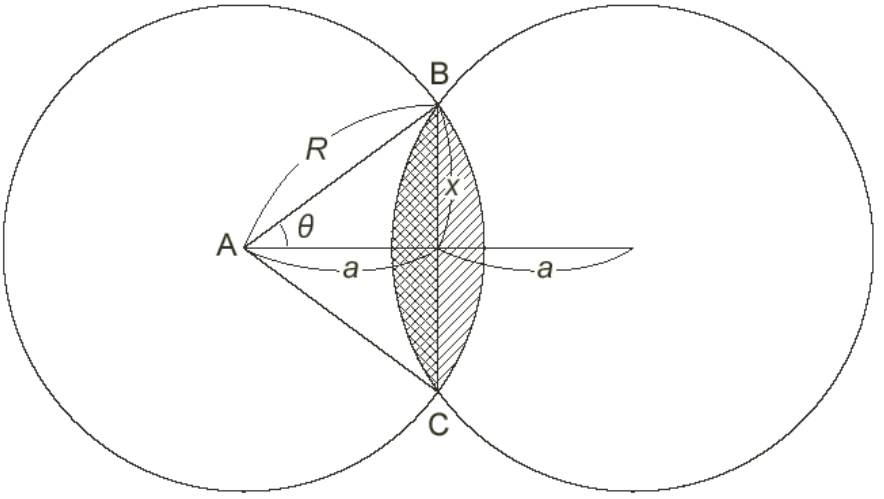



*Figure A. Method to estimate overlapping area (striped area) of attraction of two traps.*

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*Box 4. Continued*

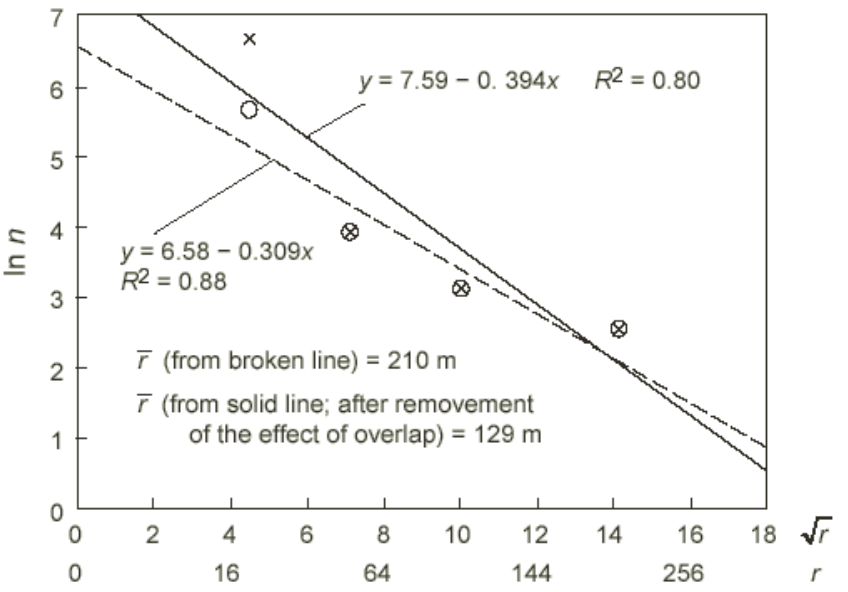



*Figure B. Recapture rate of sweetpotato weevils in traps at different distances (r) from release point (circles and broken line), and removal of effect of attraction-area overlap (radius = 10 m, crosses and solid line).*

There is a dilemma in applying these models. If trap efficiency is high, these models cannot be applied since $\delta$ is influenced by the mortality caused by traps, whereas if trap efficiency is low, the spatial distribution cannot be estimated with sufficient precision. This dilemma can be solved by a uniform placement of traps. If traps are placed uniformly in a lattice pattern, the mortality caused by traps is constant, and hence $\delta$ will be kept constant irrespective of trap efficiency.

### *4.4. Empirical Distributions*

The models described above, being based on clear assumptions such as random diffusion and constant mortality, do not always fit the data sufficiently well. The heterogeneity among individuals used to calculate the diffusion coefficient, along with the spatial and temporal heterogeneity, may be one of the causes of such discrepancy. A promising approach for incorporating the heterogeneity of the diffusion coefficient is to assume that the population consists of two groups with different $D$, as seen in Inoue (1978) and Cronin et al. (2000). An actual population may sometimes consist of many groups that have different tendencies of dispersal. At present, however, there is no simple theoretical model to describe such complicated situations. Therefore, in such cases, empirical equations will still be useful for

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describing the dispersal of individuals.

Taylor (1980) and Turchin (1998) pointed out the usefulness of an empirical equation to describe the number of recaptures, $\phi(r)$, in a trap placed at a distance $r$:

$$\phi(r) = \lambda r^{-\varepsilon} \exp\left[-(r/\beta)^{\gamma}\right] \tag{48}$$

where $\beta$, $\lambda$, $\varepsilon$, and $\gamma$ are constants. Several empirical equations are given by special cases of equation 48. If $\varepsilon = 0$ and $\gamma = 2$, we obtain a half-normal distribution (Itô and Miyashita 1965):

$$\log_e[\phi(r)] = a - br^2 \tag{49}$$

where $a = \log_e(\lambda)$ and $b = 1/\beta^2$. If $\varepsilon = 0$ and $\gamma = 1$, we obtain an exponential distribution (Kettle 1952):

$$\log_e[\phi(r)] = a - br \tag{50}$$

where $a = \log_e(\lambda)$ and $b = 1/\beta$. If $\varepsilon = 0$ and $\gamma = 0.5$, we obtain the equation used by Wallace (1966):

$$\log_e[\phi(r)] = a - b\sqrt{r} \tag{51}$$

where $a = \log_e(\lambda)$ and $b = 1/\sqrt{\beta}$. Several theoretical distributions are also described by equation 48. The instantaneous distribution of dispersing individuals, equation 35, corresponds to the case of $\gamma = 2$ and $\varepsilon = 0$. The cumulative distribution under random dispersal, equation 46, corresponds to the case of $\gamma = 1$ and $\varepsilon = 0.5$ of equation 48. Taylor (1978) compared the descriptive ability of these empirical equations, and showed that Wallace's equation (equation 51) is most preferred. Plant and Cunningham (1991), in analysing the dispersal of sterile Mediterranean fruit flies, also concluded that equation 51 is most preferred.

The parameters such as $a$ or $b$ of the above equations cannot readily be interpreted in biological terms. Several statistics will be more useful than the parameters themselves for describing the dispersal ability of individuals. Hawkes (1972) suggested that "mean dispersal distance" be used. For equation 48, the statistic is calculated by

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$$\bar{r} = \frac{\int_0^\infty 2\pi r^2 \phi(r) dr}{\int_0^\infty 2\pi r \phi(r) dr} = \beta\Gamma\left(\frac{3-\varepsilon}{\gamma}\right) \Big/ \Gamma\left(\frac{2-\varepsilon}{\gamma}\right) \tag{52}$$

where $\Gamma$ indicates the gamma function. In the case of equation 51, for example, we obtain $\bar{r} = 20/b^2$ from equation 52. The median dispersal distance, $r_{0.5}$, i.e. the radius of a circle that encloses 50% of the individuals, will be another useful statistic for the description of the dispersal ability of individuals (Turchin and Thoeny 1993). This statistic is obtained by numerically solving the equation

$$\frac{\int_0^{r_{0.5}} 2\pi r \phi(r) dr}{\int_0^\infty 2\pi r \phi(r) dr} = 0.5 \tag{53}$$

Fig. 6 is an example of fitting equation 51 to data from male sweetpotato weevils. For simplicity, linear regression was used to estimate $b$, 0.342. Hence the estimate of mean dispersal distance is obtained by $20/0.342^2$ = 171 m. The median dispersal distance was estimated by a numerical calculation to be 115.2 m. The mean dispersal distance is much larger than the median dispersal distance, since the form of $\phi(r)$ is highly leptokurtic, i.e. L-shaped. In summarizing the characteristics of the dispersal curve, the median dispersal distance will generally be preferred over the mean dispersal distance.

## 5. BEHAVIOURAL ECOLOGY: SEXUAL COMPETITIVENESS OF RELEASED STERILE MALES IN THE FIELD

The quality of sterile males to be released is the most important element in the success of an AW-IPM programme integrating the SIT. Although quality includes survival rate, dispersal ability, and other aspects that relate to the vigour of released males, the most attention must be paid to the decline of sexual competitiveness (Parker, Vreysen et al., this volume; Lance and McInnis, this volume; Vreysen, this volume).

Even when the survival rate or dispersal ability of mass-reared and sterilized males is lower than those of wild males, an increase in the frequency of mass releases or the number of release points can compensate for these deficiencies. However, if long-term mass-rearing creates a strain in which released males have a different courtship behaviour (pattern of vibration, courtship sound, etc.) than that of the wild males and therefore are not accepted by wild females, an increase in the number of released sterile males cannot compensate for this deficiency.

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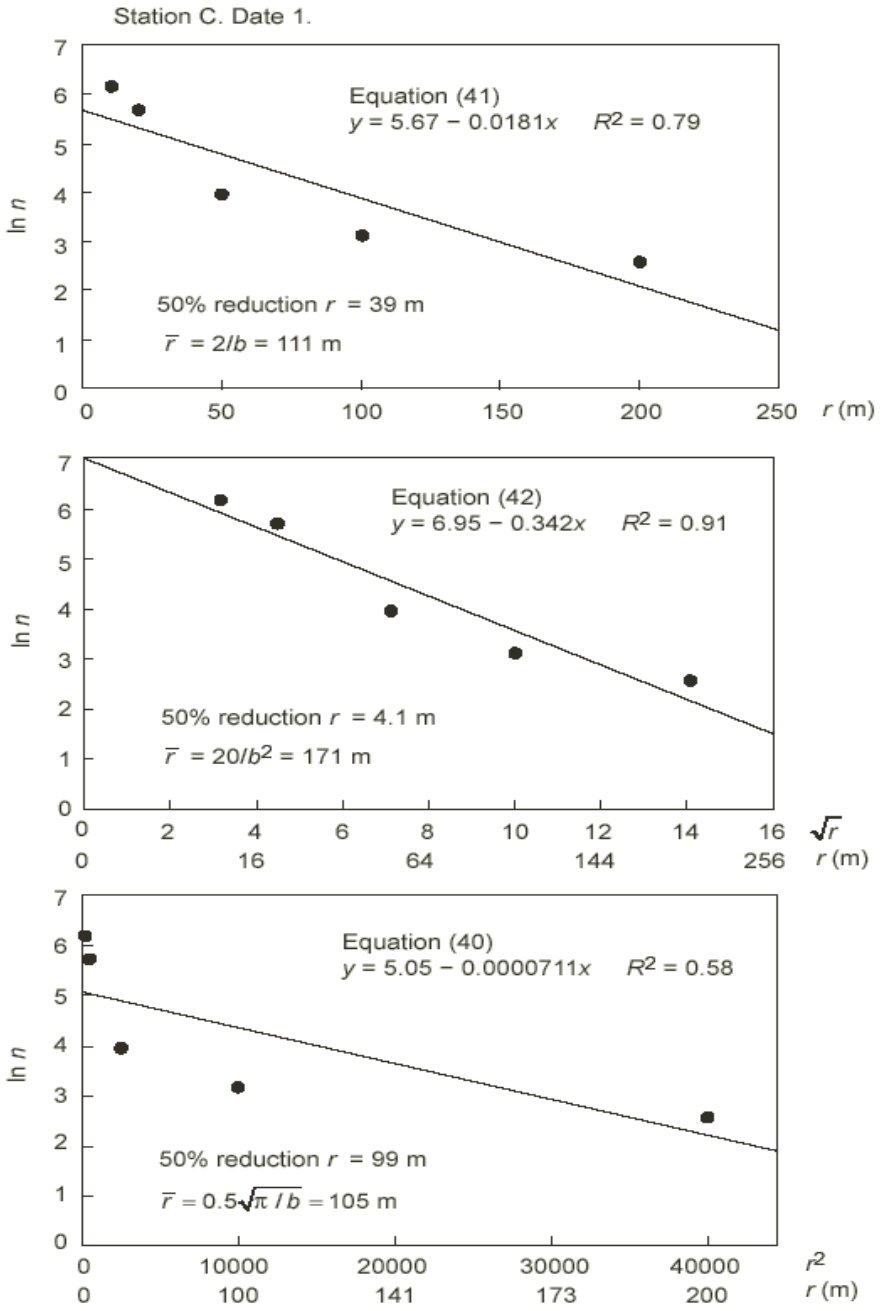



*Figure 6. Distance-density curve of number of marked adult sweetpotato weevils recaptured in traps. Equation 51 is fitted by linear regression. (Data from Miyatake et al. 2000.)*

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This is a subject of behavioural ecology or sociobiology. Although sexual behaviour and behavioural changes in mass-reared strains have been studied in several species that are targets of the SIT (e.g. Prokopy and Hendrichs 1979; Sivinski et al. 1989), probably the only AW-IPM programme releasing sterile insects, which incorporated behavioural ecology as an important aspect throughout implementation, has been the melon fly eradication programme in Okinawa, which was completely successful (Yamagishi et al. 1993).

### *5.1. Effects of Long-Term Mass-Rearing more Important than the Effects of Sterilization*

The effects of sterilization, e.g. by X- or $\gamma$-radiation, are often regarded as an important source of the decline of vigour and/or sexual competitiveness of males (Bakri et al., this volume). However, experiences accumulated during the Okinawa melon fly programme suggest that changes in sexual behaviour of released males due to long-term mass-rearing are much more important than the effects of sterilization (Itô et al. 1993; Parker, Vreysen et al., this volume). The inadvertent selection of strains with rapid development and early fecundity produces reduced longevity and other genetic changes in the selected strain (Miyatake 1996, 1998; Shimizu et al. 1997). The high-density rearing of adults in small cages can lead to the selection of strains in which reared males do not perform the same sexual behaviours as wild males in the field.

### *5.2. Competitiveness must be Measured in the Field*

In many AW-IPM programmes integrating the SIT, sexual competitiveness has been measured in the laboratory, e.g. observing wild females mating with marked wild males and mass-reared and/or sterilized males in a cage (Fried 1971; Parker, Vreysen et al., this volume). However, conditions in a laboratory cage are different from those in the field (Lance and McInnis, this volume; Vreysen, this volume). In a programme to eradicate the melon fly from Kume-zima, Okinawa (1973–1977), Iwahashi et al. (1983) measured sexual competitiveness in the field. They collected wild females from Kume-zima, the target area, and Okinawa-Hontô, the control (non-SIT) area, and examined the hatch rates of eggs laid by those females.

Haisch (1970) presented the following equation for the laboratory examination of competitiveness, $c$:

$$\hat{c} = \frac{H_n - H_c}{H_c - H_s} \cdot \frac{w}{1-w} \tag{54}$$

where
$w$ = proportion of males of wild strain among all males,
$H_n$ = percentage egg hatch in matings between normal (wild) males and females of wild strain,

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$H_C$ = percentage egg hatch in competitive matings,

$H_S$ = percentage egg hatch in matings between sterile males and normal females.

To use this equation in the field, Iwahashi et al. (1983) substituted percentage hatch of eggs laid by females collected in the target and control areas for $H_n$ and $H_c$, respectively. $H_S$ is 0 in the Okinawa melon fly programme. For comparison of $c$ values while applying the SIT, or between two or more SIT areas, an estimation of variance is necessary. Iwahashi et al. (1983) presented an equation for estimating the variance of $c$ (Box 5).

*Box 5. Variance of Haisch Index of Sexual Competitiveness of Mass-Reared/Sterilized Males*

In the final stage of an eradication programme that releases sterile insects, when most males collected are sterile males, $1 - w$ becomes near zero. In this stage, $H_C$ may also become small. As $c$ values become quite sensitive to small changes in $1 - w$ and $H_c$, estimation of variance is necessary. The following equation from Iwahashi et al. (1983) is recommended:

$$V(\hat{c}) = \left[\frac{w}{H_c(1-w)}\right]^2 \times \left[\frac{H_n(1-H_n)}{N_n} + \frac{H_n^2(1-H_c)}{H_c N_c} + \frac{(H_n - H_c)^2}{w(1-w)N_w}\right]$$

Here $N_n$ and $N_c$ are the numbers of eggs examined in the control area and the release area, respectively. $N_w$ is the number of flies examined in the release area. For other symbols, see explanation of equation 54.

Release strain sexual competitiveness decreased from about 80% in the 5th generation after the beginning of mass-rearing to 20% in the 18th generation, during the final stage of the Kume-zima programme (Fig. 7, upper). Even at this time, high sexual competitiveness in laboratory cages was still observed (see closed triangles in Fig. 7, upper).

Soemori et al. (1980) described experiments that suggest an explanation for such a discrepancy between laboratory and field data. They released individually marked flies into cages or rooms of different sizes and recorded the mating performance. Fig. 7 (lower) shows the percentage of males that mated in relation to the volume of space available per male in the experimental area. Males of the wild strain could not mate well in a small space, whereas males of the laboratory strain performed best in these conditions.

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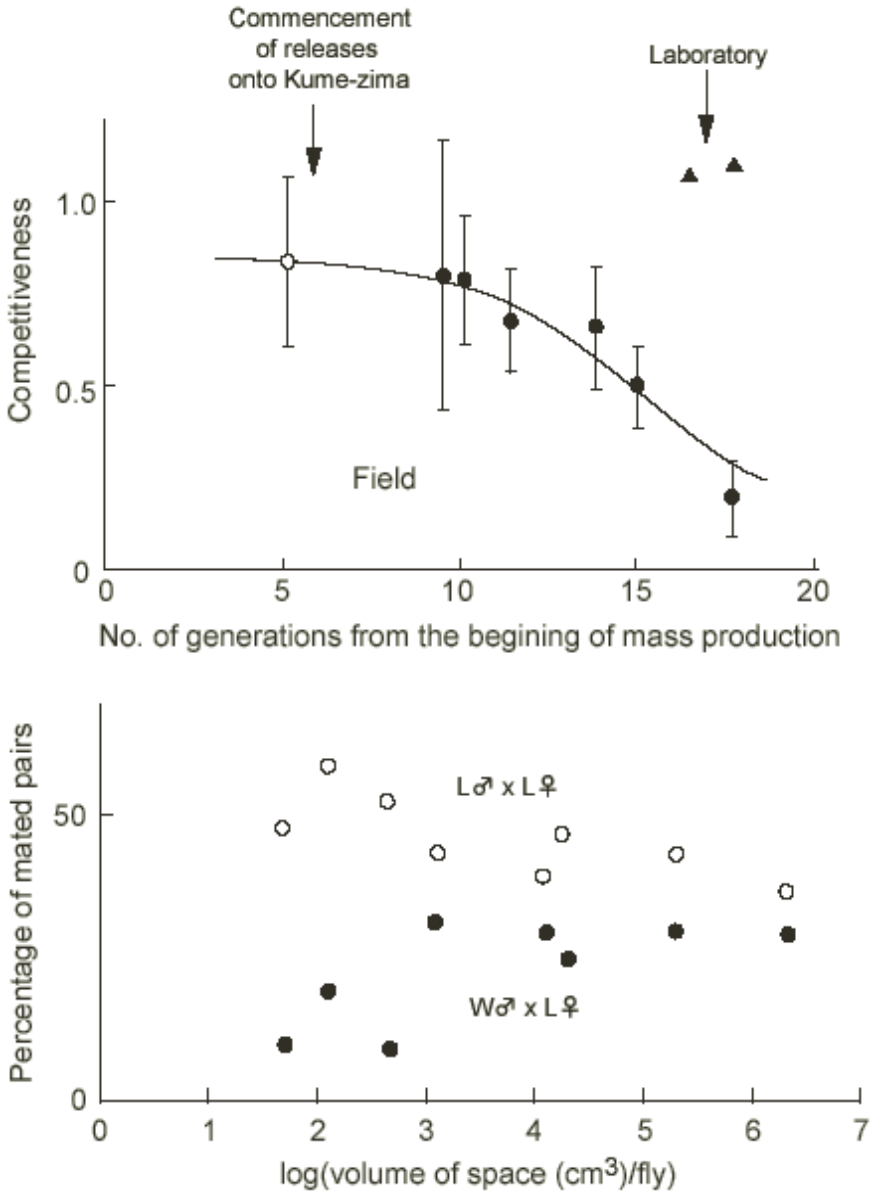



*Figure 7. Upper: Sexual competitiveness of mass-reared and sterilized male melon flies measured in the field (adapted from Iwahashi et al. 1983). Closed circles are for data from Kume-zima; the open circle is for data from another islet Kudaka-zima. Solid triangles are competitiveness values measured in laboratory cages. Vertical lines show standard deviations. (Box 5 shows the equation for variance.) Lower: Relationship between size of cage per fly and percentage of successful mating of males of mass-reared (open circles, 33–34 generations) and wild (closed circles) strains when caged together with mass-reared females. (Figure from Soemori et al. 1980, reproduced with permission.)*

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### *5.3. Inadvertent Selection of Mate-Choice when the SIT is Applied*

In a field cage, Hibino and Iwahashi (1988) compared the mating success of males of wild and mass-reared strains. One of their results, using flies of a wild strain of Okinawa Hontô (O-males and O-females), is shown in Fig. 8 (upper). Firstly, even when courted by wild males, wild females accepted copulation in only 4 of 37 courtship trials, showing strong mate-choice by females. Secondly, O-females never accepted (0/46) courtship from mass-reared (R) males. Other experiments showed similar results (Itô et al. 1993). When Hibino and Iwahashi (1988) carried out these experiments, melon flies on Okinawa Hontô, as a result of the application of the SIT, were near extinction. Therefore O-females had been subjected to strong selection pressure by the released sterile males.

Hibino and Iwahashi (1991) carried out similar experiments using wild flies taken from Ishigaki-zima, where a programme releasing sterile insects had not yet begun. Fig. 8 (lower) shows that females of Ishigaki wild flies (I-females) accepted courtship from R-males (4/57) as well as from I-males (3/51).

There are two possible explanations for the difference in behaviour between flies on the two islands: (1) O-flies and I-flies had genetically different courtship and acceptance characters, and the courtship character of R-males was more similar to that of I-males, and (2) the "SIT-resistance hypothesis" — the wild female population was initially heterogeneous and contained individuals which accepted a broad range of male courtship characters (McInnis et al. 1996; Whitten and Mahon, this volume). However, females that accepted sterilized R-male courtship could not produce progeny. Thus, under strong selection pressure from the SIT, a female genotype that accepted the courtship of mass-reared males may have become extinct. Next Hibino and Iwahashi (1991) conducted an experiment on mate choice of O-females between O- and I-males. O-females then accepted courtship by I-males (1/19) as well as by O-males (1/14), indicating that the second explanation is correct, thus demonstrating for the first time the evolution of mate-choice in insects.

### *5.4. How Can the Spread of an SIT-Resistant Strain be Overcome?*

How can the problem of an increase in the number of females that do not accept courtship from mass-reared males be overcome? Besides the obvious approach of establishing a new colony from the target population, and/or cryopreserving the embryos of recently colonized strains (Parker, Mamai et al., this volume; Whitten and Mahon, this volume), as well as improving the competitiveness of sterile males (Shelly and McInnis 2001; Pereira et al., this volume; Vreysen, this volume), an additional answer is provided by the development of the logistic population model (e.g. equation 8).

Tsubaki and Bunroongsook (1990) conducted a simulation experiment to estimate the effect of the change in mate-choice, using a logistic model (equation 8) incorporating two strains. They showed that the effect of a reduction in mating competitiveness of mass-reared and sterilized males is much more important than the effect of a change in female mate-choice. Their simulation also indicated that releases of two or three times more sterile males than would be released in a non-mate-choice

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model can eradicate a target population that has a change in female mate-choice. In the Okinawa melon fly programme integrating the SIT, an increase in the number of released sterile males over that first planned for release (based on an estimate of the wild fly density) resulted in complete eradication of the population.

The incorporation of recent ideas in behavioural ecology, as well as in population ecology, into the SIT is indispensable for its success. It is recommended that Krebs and Davies (1993) be consulted on the basic concepts of this subject.

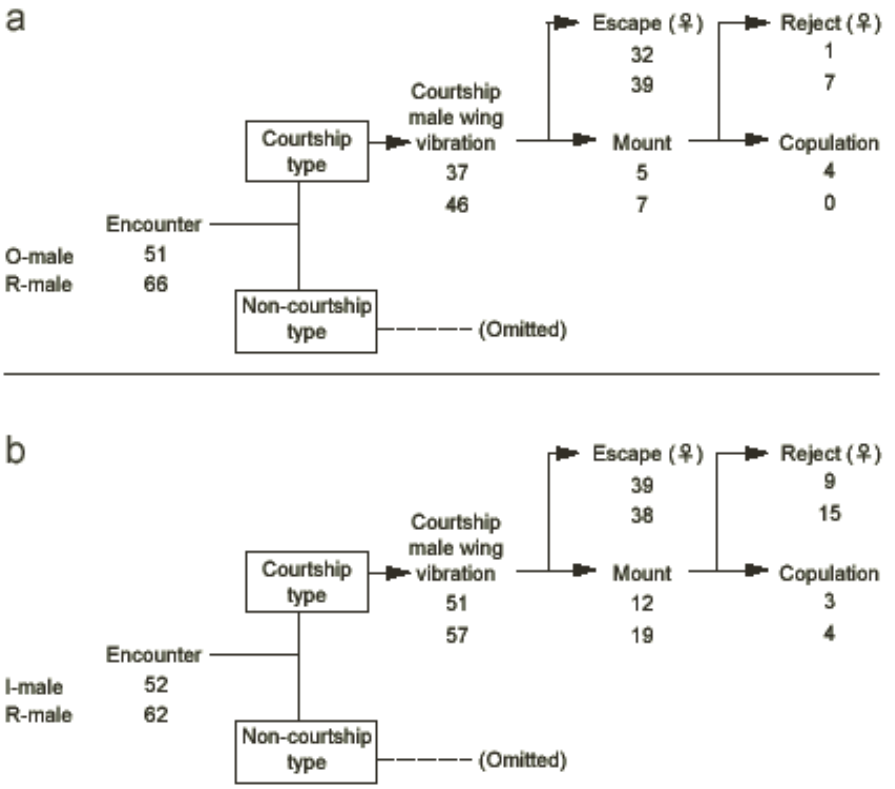



*Figure 8. Upper (a): Mate-choice by wild female melon flies collected on Okinawa Hontô (O-females) for wild males (O-males) or males of mass-reared strain (R-males). Arrows indicate the direction in which the behavioural sequence proceeds. Upper numerals indicate the frequencies of the transitions when O-females encountered O-males, lower numerals indicate cases when O-females encountered R-males (adapted from Hibino and Iwahashi 1988). Lower (b): Mate-choice by wild female melon flies collected on a non-SIT island Ishigaki-zima (I-females) for Ishigaki wild males (I-males) or mass-reared males (R-males). (Figure from Hibino and Iwahashi 1991, reproduced with permission.)*

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# CHAPTER 3.2.

# MASS-REARING FOR THE STERILE INSECT TECHNIQUE

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SUMMARY

Since the sterile insect technique (SIT) relies upon released sterile male insects efficiently competing with wild males to mate with wild females, it follows that mass-rearing of insects is one of the principal steps in the process. Mass-rearing for the SIT presents both problems and opportunities due to the increased scale involved compared with rearing insects for most other purposes. This chapter discusses facility design, environmental concerns, strain management, quality control, automation, diet, sex separation, marking, and storage in relation to rearing for the SIT. The cost of sterile insect production drops rapidly with increasing scale, but the point at which the SIT becomes economic in comparison with other methods will depend on factors such as the species concerned, local costs, and the cost of competing technologies. However, optimizing rearing for numbers without regard to quality may compromise the effectiveness of the SIT. In the future, improvements in sex-separation technologies and in understanding the impact of microbiota on insect quality may lead to significant improvements in both rearing efficiency and quality. The list of target pest species for which mass-rearing systems have been, or are being, developed continues to expand.

## 1. INTRODUCTION

The sterile insect technique (SIT) depends upon inducing a high proportion of sterile matings in a natural population that reduces reproduction to a level below population maintenance. Therefore, the production of insects, in sufficient number and of adequate quality to achieve this aim, is one of the principal requirements for the successful application of the technique. Further, since the integration of the SIT into an area-wide integrated pest management (AW-IPM) programme competes economically with other control techniques, the production of insects must be timely and cost-effective. Large numbers of insects are required to integrate the SIT into AW-IPM programmes, and therefore it is possible to take advantage of economies-of-scale in the rearing.

There are numerous published accounts of the rearing of many insect species, including general reviews (Smith 1966; King and Leppla 1984; Singh and Moore 1985; Anderson and Leppla 1992; Ochieng'-Odero 1994a; Nordlund 1999; Schneider 2009; Cohen 2015; Beukeboom 2017). The details of specific rearing systems used by the various ongoing SIT projects are described in the published literature. Some key publications for the main SIT pest target groups are:

- *Moths*: Zethner (1980), Stewart (1984), Davis (2009), Dyck (2010), Carpenter and Hight (2012), Xsit (2018), Boersma (2021),
- *Screwworms*: Brown (1984), Mahon and Ahmad (2000), Scott et al. (2017), USDA/APHIS (2017),
- *Fruit flies*: Schwarz et al. (1985), Vargas (1989), Nakamori et al. (1992), Yamagishi and Kakinohana (2000), Cáceres et al. (2014),
- *Tsetse flies*: Feldmann (1994), Gooding et al. (1997), Aksoy (2005), FAO/IAEA (2006),
- *Mosquitoes*: Gerberg et al. (1994), Balestrino et al. (2014); Carvalho et al. (2014), Zheng et al. (2015a, b), FAO/IAEA (2017a, b, 2018a), Mamai et al. (2017a, 2018), Lees et al. (this volume), and
- *Weevils*: Yamaguchi et al. (2006), Shimizu et al. (2007).

Insects are reared for many reasons — bioassays, physiological research, rearing parasitoids, postharvest treatment testing, etc. (Singh and Ashby 1985), where rearing is rarely an end in itself. For these purposes, the cost of rearing is not critical,

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e.g. the diets tend to be all inclusive, rather than minimal, and once a diet is developed that is able to maintain an adequate colony, little or no further work is done on it.

For the SIT, a rather different approach is needed, with due attention being paid to all the factors affecting quality, fecundity, and cost. Even though Singh (1985) listed more than 1300 species that have been reared on artificial diet in the laboratory for part or all of their life cycle, relatively few species have been mass-reared for the SIT (IDIDAS 2019). The details of the rearing protocol for any one species will not be discussed here, but examples to illustrate key points and issues common to the successful mass-rearing for the SIT will be given.

## 2. ECONOMICS

Mumford (this volume) discusses the economics of the SIT. However, that discussion focuses on benefit/cost analyses of overall programmes, and rearing economics appear only as a component of overall cost.

One of the main characteristics that distinguishes rearing for the SIT from other insect rearing is scale, e.g. the El Pino factory in Guatemala has the capacity to produce more than 2000 million sterile male Mediterranean fruit flies *Ceratitis capitata* (Wiedemann) per week (Figs. 1, 2).



*Figure 1. Mediterranean fruit fly production and sterilization facility, El Pino, Guatemala (2015). Recycled/processed water from the facility is retained in a pond for the production of fish. (Photo from P. Rendón, reproduced with permission.)*

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Scale brings with it problems of labour supply, automation, and diet supply, which are dealt with later in this chapter, but there is also an issue of the relative efficiency and economics of scale. In general, cost per unit reduces as scale increases, and this can be seen in the efficiency of the Guatemala facility (Hendrichs et al. 2002). At the same time, if rearing is optimized for numbers without regard to quality, there is a risk of reduced quality (Parker, Vreysen et al., this volume). The scale at which a particular programme becomes cost effective will depend on the species involved and the cost and efficiency of alternate means of control.



*Figure 2. Mass-rearing the Mediterranean fruit fly at the El Pino facility, Guatemala. Upper left: Eggs treated in a hot-water bath to kill female embryos of the temperature-sensitive lethal sexing strain; Lower left: Rearing larvae in trays, showing hanging plastic net sheets to redirect jumping mature larvae to the collection pan below; Upper right: Maturing pupae in trays stacked on trolleys; Lower right: Vertical cages for adult emergence, feeding, and egg oviposition and collection. (Photos from C. Cáceres, reproduced with permission.)*

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Unfortunately, few published data are available on the actual cost of rearing, separate from whole programme costs (IAEA 2008). The figures that are available indicate that costs have fallen dramatically, but the figures derive from different situations that are not directly comparable, and most of the change is attributable to improvements in rearing technology, rather than scale. The cost of male Mediterranean fruit fly pupae has been reduced to less than USD 300 per million in the Guatemala facility, but this cost depends on the utilization efficiency of the available capacity (Fig. 3) (Enkerlin 2003), indicating that utilization efficiency is at least as important as scale per se. Caceres et al. (2004) compared mass-rearing costs of Mediterranean fruit fly sexing and non-sexing strains; here the different characteristics of the *tsl* sexing strain lead to a small increase in rearing costs (which may be reversed with future developments), but significantly reduced post-production programme costs.

In Tanzania, with increased production, the production cost of the tsetse fly *Glossina austeni* Newstead was reduced from about USD 1 per male insect to less than USD 0.10; again, however, this is largely due to changes in procedures. The same applies to mass-rearing equipment. For example, innovations in mosquito mass-rearing techniques, including development of a low-cost adult-holding cage, resulted in a 10-fold cheaper cage (Maïga et al. 2019). Manufacturing the new mass-rearing cage locally can significantly reduce the initial investment in equipment. Also, improvements have been made in larval diets to reduce the cost 40–90% in *Anopheles arabiensis* Patton (Bimbilé Somda et al. 2017), and up to 80% in *Aedes* sp. (Bimbilé Somda et al. 2019; FAO/IAEA 2020).

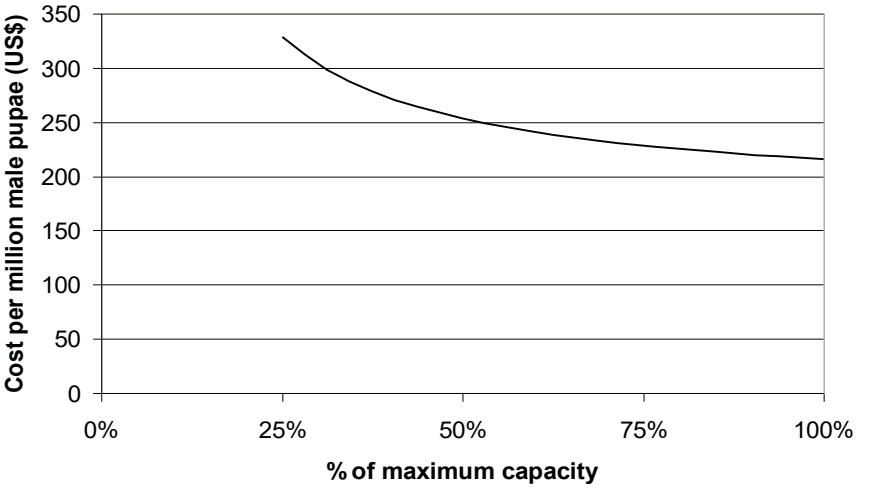



*Figure 3. Variation in Mediterranean fruit fly production cost (USD) with capacity utilization at El Pino, Guatemala. (Figure adapted from Vollmerhausen 2001.)*

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## 3. FACILITY DESIGN AND LOCATION

Facility design is a critical aspect of the rearing process (FAO/IAEA 2004, 2019b; IAEA 2008; Co 2019; NEA 2019). Poor design will lead to inefficient utilization of space and energy, increased problems with contamination, and increased risk of escape of fertile insects. Leppla and Ashley (1978), Griffin (1984), Fisher and Leppla (1985), and Fisher (2009) discuss general facility design. Specific aspects of biosecurity are discussed in USDA/APHIS/PPQ (1995), Kahn and Mathur (1999), Leppla and Eden (1999), and ACME (2003). Harrell et al. (1979), Owens (1984), Wolf (1984), Goodenough and Parnell (1985), Oborny (1998), and Schneider (2009) discuss environmental control. Tween (1987) discusses a modular approach to constructing the mass-rearing facility that has been adopted in Guatemala for the Mediterranean fruit fly; this is expanded in Cáceres et al. (2012). The modular approach has the advantage of simplifying design and expansion, but it also means that there is little economy from increased scale as each additional module costs the same as previous ones, the only saving coming from the common areas. However, it has the advantage of allowing the facility to be easily scaled to the required size, so as to maintain the utilization efficiency (Fig. 1); if demand falls, complete modules can be closed without affecting production in the remaining modules, or converted to rearing another insect.

The criteria for determining the optimum location for a mass-rearing facility must take several factors into account, both technical (Phillimore 2002; FAO/IAEA 2004) and managerial (Dyck, Reyes Flores et al., this volume). The principal factors, not in order of priority, are:

- Logistical access – ease of delivery of rearing supplies and equipment,
- Geological stability – earthquake risk, hurricanes, flooding, etc.,
- Political stability,
- Acceptance of the facility by the local community,
- Local government requirements or restrictions,
- Access to water and utilities, reliability of supplies,
- Construction and maintenance costs,
- Availability of support and repair services,
- Labour costs and availability,
- Waste disposal,
- Access to a suitable airport for rapid delivery of insects,
- Distance from release area,
- Quarantine considerations – can the insect survive in the surroundings?
- Proposed progression in case eradication is the objective – will the facility become stranded behind the eradication front?

The relative importance of a given factor will depend on the specific programme, and on factors such as labour costs and the degree of automation utilized. For highly sophisticated systems, access to support and maintenance for the equipment may be critical, as may electricity supply reliability, but for a less automated system these may be less important. Some of the factors can be accommodated within the programme itself, e.g. installing independent generation capacity and a water supply. Even though not always possible, locating the facility in an area where the insect

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cannot survive and establish, perhaps due to desert conditions or cold winters, greatly eases the quarantine considerations, and reduces costs. The problem of being stranded behind the eradication front might be addressed by developing a relocatable, containerized rearing system.

## 4. ESCAPES AND ENVIRONMENTAL CONCERNS

A problem unique to the SIT is that the pest insect itself is being reared. For other large-scale rearing, e.g. classical or augmentative biological control, the reared insect is not the pest itself, so any escape from the rearing process is unlikely to pose a threat. (An exception is where the pest must be reared as a host for a parasitoid or predator, but living hosts are increasingly being replaced by factitious hosts or artificial diets). However, when the pest is being reared, any escape of fertile material poses a risk. Usually, the rearing is done in an area where the pest is already present, but if an eradication programme is successful, the rearing facility can become stranded behind the eradication front and poses a substantial reinfestation risk. This happened to both the Mexican facility producing the New World screwworm *Cochliomyia hominivorax* (Coquerel) and the Japanese facility producing the melon fly *Zeugodacus cucurbitae* (Coquillet). Stringent containment procedures are required (see references in section 3.), usually coupled with preventive sterile insect releases and other control measures in the vicinity of the rearing facility. Following screwworm eradication in the country, the screwworm facility in Mexico operated successfully for many years (although it was eventually replaced by a new facility in Panama), employing a combination of stringent security, containment, and prophylactic sterile-male releases. An alternative is to site the facility in an area where the insect cannot, due to local environmental conditions, establish a self-sustaining population, e.g. the facility constructed in Addis Ababa, Ethiopia, to rear the tsetse fly *Glossina pallidipes* Austen. In a mosquito-rearing facility, the risk of free-flyers increases with the scale; various tools such as light, odour, and gravid traps are commonly used to reduce the number of escaped mosquitoes (Lees et al., this volume; Vreysen, this volume).

The mass-rearing process for the SIT potentially poses some environmental problems in relation to waste disposal, including preventing the accidental escape of fertile insects with the spent diet and wastewater. These waste products must be treated to ensure that no living insects in any stage remain — for the diet by steam treatment or extrusion, and for wastewater by filtering, heat treatment or their combination with biological processes. Diets should be readily biodegradable to reduce environmental concern over waste disposal, or even to allow their re-utilization for other purposes, such as feeding fish or livestock (Fig. 1). Some of the bulking agents used in diets do not readily degrade, and these should be avoided, not least because they can lead to significant waste disposal charges (Chaudhury and Alvarez 1999). Facilities are also increasingly being required to install costly wastewater treatment plants. A recent study demonstrated the potential for using recycled larval-rearing water to supplement clean dechlorinated water when rearing *Anopheles arabiensis* (Mamai et al. 2017b).

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Probably the biggest environmental concern comes from the sterilization process, which involves a radioactive source. Standard procedures for the use and eventual disposal of such sources are available and, if followed, minimize the risk (IAEA 2005). The increasing tendency to replace isotopic sources with X-ray systems greatly reduces the risks. Sterilization itself is discussed by Bakri et al. (this volume).

Insect mass-rearing can pose a significant health hazard through inhalant and contact allergies (Wirtz 1984; Wolf 1985; Bellas 1990; Kfir 1994; Myers and Barnard 2002; Reinecke 2009). Allergic reactions to mould spores, mites, and pheromones also occur. Some moths have urticating wing scales or hairs that can cause skin rashes and irritations in eyes and lungs (Moraru and Goddard II 2019). Preventing allergic reactions involves recognition and documentation of the problem (Wirtz 1980), and correction of the problem through appropriate air-handling and filtering (Owens 1984; Wolf 1984; Froehlich 1995), coupled with protective clothing and filter masks or respirators. The environmental conditions in the rearing facility may also be changed by manipulation of the diet (Vargas et al. 1984). In addition to the health hazard caused by spores and mites, they impact the rearing directly by reducing diet quality or causing disease in the insects (see sections 6., 8.4.) (Abd-Alla et al., this volume).

## 5. STRAIN MANAGEMENT

Strain management ensures that a strain continues to perform the function required of it, i.e. it survives under field conditions and remains sexually compatible with wild insects, and does not gradually deviate from wild behaviour. The strain must also maintain fecundity, and potential problems with contaminant organisms must be suppressed or eliminated. Fisher (1984), Schwalbe and Forrester (1984), Singh and Ashby (1985), and Caprio (2009) discussed strain management.

One of the most common concerns is the genetic diversity (heterozygosity) of the strain; this can be an important consideration during colony establishment. The strain must be colonized with a sufficient number of individuals from a wide genetic background. There has been much discussion, with a wide divergence of opinion, as to how many are sufficient — from many thousands to just a few tens or hundreds of females (Mackauer 1972, 1976 for parasitic wasps; Bartlett 1985 and Nunney 2002 for the SIT). It is generally agreed that the size of the founder population should depend on the degree of heterozygosity of the wild population. Usually it is stated that the founder material should come from a wide geographic range, but this is not necessarily beneficial; crossing strains from different geographic locations can break up adaptive gene combinations and render the strain less fit than the parents (Mackauer 1976). Azrag et al. (2016) compared the genetic diversity of an advanced generation of an *An. arabiensis* colony population with the field population from which the colony was derived. They found that using a small number of mosquitoes to establish the colony significantly reduced the total number of alleles, the numbers of rare and private alleles, and the fractions of heterozygote individuals at all the loci; this highlighted the need for broad sampling when colonizing a mosquito population (Benedict et al. 2009).

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Individual fecundity will also influence founder numbers. In a slow-breeding group, such as tsetse flies, each individual has to be carefully nurtured, and no one individual's or small group's progeny can dominate the next generation. The logistics of collection also play a major role in how the field population is sampled.

Insects newly collected from the field rarely thrive in the laboratory, and the first few generations usually suffer high mortality, with the colony stabilizing after about five generations (Bartlett 1984). This process involves the rapid selection of individuals better adapted to the laboratory rearing conditions (Ochieng'-Odero 1994b), resulting in a rapid decline in heterozygosity. There is considerable concern that these changes will result in insects significantly different from the wild population, and therefore non-competitive, although as yet it has not been possible to show unequivocally that a reduction in heterozygosity per se leads to a reduction in competitiveness. Leppla et al. (1983) showed that this adaptation process was not influenced by gradually introducing mass-rearing conditions.

Adverse changes resulting from long-term mass-rearing are not uncommon, with the development of strains lacking the necessary courtship behaviour and responses to pheromones, predator avoidance, host odours, environmental cues, flight range, and even vision (Boller 1972; Miyatake 1998; Cayol et al. 2002; Mudavanhu et al. 2017; Parker, Vreysen et al., this volume). During long-term mass-rearing, due to the accumulation of random mutations, heterozygosity may gradually be regained (Bartlett 1984), but the newly acquired heterozygosity will not match that of the field population. Heterozygosity can also be encouraged deliberately (Joslyn 1984). To minimize selective processes on colonized mosquito populations, the induction of hybrid vigour by crossing strains of different origins (Craig 1964; Seawright et al. 1975; Ekechukwu et al. 2015) or a regular periodic replacement of existing colonies with wild stocks (especially males) obtained directly from the field (Dyck 2010; FAO/IAEA 2017a, 2018a), have been proposed or sometimes executed (Whitten and Foster 1975; Hofmann 1985; Saul and McCombs 1993; Parker, Vreysen et al., this volume).

Potential changes in competitiveness during mass-rearing are best detected by careful quality-control monitoring (FAO/IAEA/USDA 2019). A range of parameters is monitored routinely, including mating competitiveness and flight ability (Parker, Vreysen et al., this volume). If adverse changes are observed, it may be possible to deliberately select for desirable characteristics (Collins 1984; McInnis et al. 2002; Lommen et al. 2017; Sánchez-Rosario et al. 2017), although there are some dangers associated with this approach.

A recent development in mass-rearing the Mediterranean fruit fly has a potential application to colony management in almost any insect-rearing programme. The concept, called the filter rearing system (FRS), involves maintaining a small colony at a low density, or even under semi-natural conditions, and therefore assumedly a low-selection pressure (Fig. 4) (Cáceres et al. 2000; Fisher and Caceres 2000; Hendrichs and Robinson, this volume). Surplus insects from this low-density mother stock or clean stream are fed into a high-density amplification chain, leading up to the final release numbers. The important feature is that no individuals are ever fed from the amplification stages back to the mother stock. The low-density rearing conditions of the filter can be supplemented with any further conditions deemed desirable, e.g. host plant, predators, mating competition or pheromone response, and

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non-performing individuals eliminated. Any undesirable traits selected for in the high-density amplification stages have only three or four generations to accumulate before release, and do not affect the mother stock. A further advantage is that, if it is desired to replace the strain with a new one, the new mother stock can be set up in parallel with the old one, and amplification easily switched from one to the other. Filter rearing is similar to the concept used by Bigler (1986) to rear *Trichogramma maidis* Pintureau and Voegele.

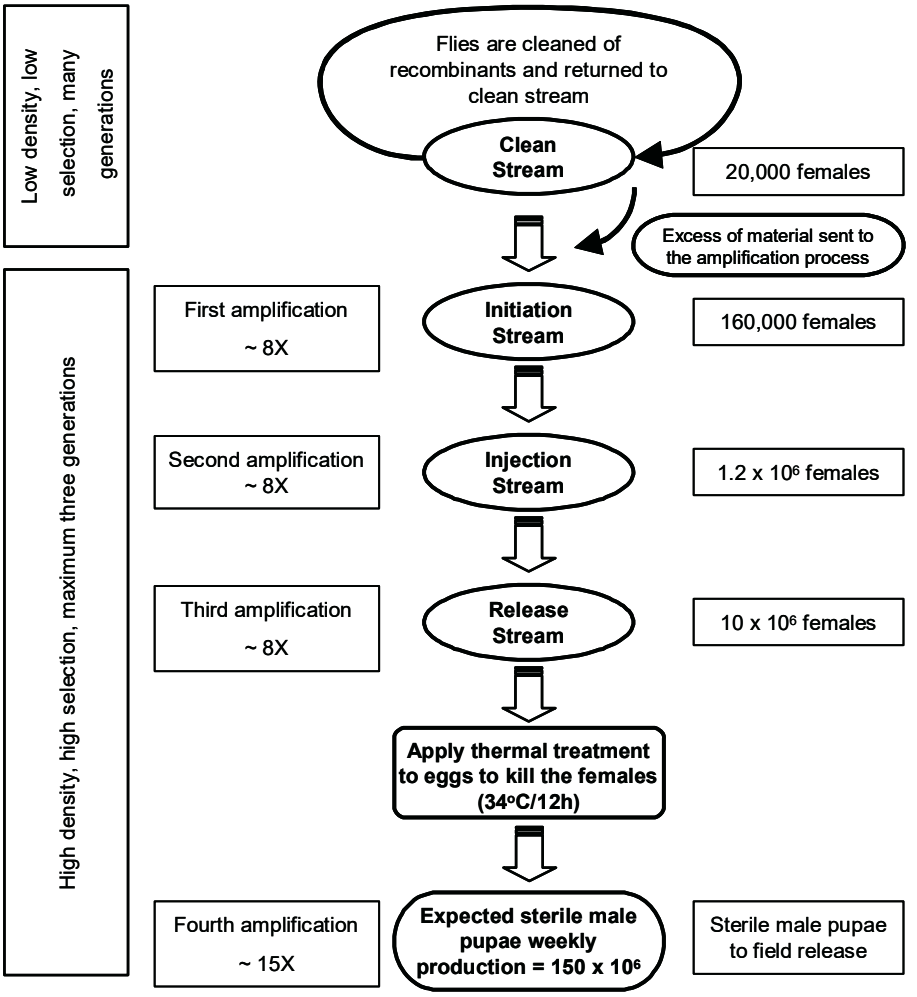



*Figure 4. Continuous filter rearing system for producing Mediterranean fruit fly sterile males in Guatemala. (Figure adapted from Cáceres et al. 2000.)*

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## 6. PRODUCTION, PROCESS, AND PRODUCT CONTROL

The concept of quality control can be divided into three areas: production control (monitoring all rearing operations in terms of personnel, materials, equipment, schedules, environment, etc.), process control (sampling immature insect stages to predict quality and determine sources of variability), and product control (both at the output from a rearing facility and in the field), according to the normal industrial definitions (Leppla 1994). All three aspects are equally important in quality control, but most work has been done on product quality control (FAO/IAEA/USDA 2019; Parker, Vreysen et al., this volume). Field quality control is a particularly neglected area (Vreysen, this volume).

In general, methods of rearing for the SIT are developed from those used to rear insects for other purposes, but on a much larger scale. The techniques are similar, but the issues of quality control require a different approach. The larger scale means larger rearing facilities, and for larger facilities production control becomes critical and potentially more difficult. There are also greater opportunities for automation, reducing labour costs, variability, and the likelihood of microbial contamination from the staff employed in the facility (Abd-Alla et al., this volume). Environmental control systems are very important, and their design must take into account the amount of insect biomass producing heat (Oborny 1998). These are also amenable to increased automation, with networked monitoring of temperature, humidity, and light as is done in the codling moth *Cydia pomonella* (L.) rearing facility in Osoyoos, Canada (Dyck 2010). In an interesting approach to process control, air space volatiles were monitored for changes that would indicate changes in the rearing parameters (Hedin et al. 1975).

The specific nature of the SIT requires that the product, i.e. reared insects, mate with the wild population, and mating behaviour tests are critical (Parker, Vreysen et al., this volume). Several of the techniques used to measure these behaviours under semi-natural conditions in tephritid fruit flies (FAO/IAEA/USDA 2019) have been adapted for tsetse flies (Mutika et al. 2001). However, monitoring itself does not correct rearing problems. Each rearing facility should develop standard operating procedures (SOPs) for rearing operations, quality-control operations, and finally responses to adverse quality-control findings (FAO/IAEA 2018b).

Effective data management is a vital component to allow managers of large-scale mass-rearing to take appropriate and timely decisions (FAO/IAEA 2018b). Akey et al. (1984) reviewed data processing, and modern computer systems have further simplified data collection and analysis. As rearing becomes commercialized, customers will require up-to-date information on insect status and quality control, and this information could be made available via the Internet.

Finally, a factor not unique to the SIT, but more prominent with increased centralization of rearing, is the issue of transboundary shipment. To date, there is no explicit regulatory framework for the transboundary shipment of sterile insects, but this issue is currently being addressed (Enkerlin and Quinlan 2004; Dowell et al., this volume; Dyck, Reyes Flores et al., this volume). In this context, the existence and careful documentation of a comprehensive quality-control system will be important (FAO/IAEA/USDA 2019).

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## 7. AUTOMATION OF REARING

The scale of rearing for the SIT naturally lends itself to some degree of automation. Rearing systems vary greatly in their sophistication, from largely manual (tsetse fly (IAEA 2003), New World screwworm (Scott et al. 2017)) to highly sophisticated (pink bollworm, melon fly) systems. The balance between labour and automation depends on many factors, but the chief one is the cost of labour. In areas with low-labour costs, the drive towards automation will be reduced, but in high-wage areas, e.g. Japan, automation is essential to reduce costs (Nakamori et al. 1992). Other reasons to automate include: (1) reduction in human error, with increased product performance and consistency, (2) reduction in microbial contamination from personnel, and (3) increased space utilization efficiency, resulting in lower costs — for building and for energy for environmental control (Cáceres et al. 2012).

Another aspect of automation is in climate-control systems. Air-handling is essential to control temperature and humidity (Schneider 2009), reduce airborne particulates and microbial contamination of the colony, and reduce medical problems of the staff (Reinecke 2009). Klassen (1978), Sikorowski et al. (1983), Harrell and Gantt (1984), Opiyo et al. (1999, 2000), Smith (1999), Smith and Nordlund (1999), Maïga et al. (2016, 2017), and Mamai et al. (2019) reviewed aspects of mass-rearing automation. For almost all stages of the process, descriptions of automated systems are available — egg collection (Leppla et al. 1974; Carlyle et al. 1975; Pearson et al. 2002), diet preparation and dispensing (Grisdale 1973; Gantt and King 1981; Miller et al. 1996), larval holding and pupal harvesting (Hartley et al. 1982), and environmental-control systems (Oborny 1998; Peng and Ohura 2000). Automation in mosquito mass-rearing is covered in more detail in Lees et al. (this volume).

## 8. DIET

Diet is probably the single most important component of rearing and, with labour, constitutes the main cost. Therefore, it is in the interest of insect-rearing programmes to improve the performance or reduce the cost of the diet. A balance has to be achieved between cost and performance of the insects; in the long run, a cheap diet that produces less competitive insects may prove more expensive. Chaudhury (2009) and Cohen (2015) reviewed all aspects of nutrition, feeding, and diet development.

Diets for many insects have been described in the literature (Smith 1966; King and Leppla 1984; Singh and Moore 1985; Gerberg et al. 1994; Ochieng'-Odero 1994a; Morales-Ramos et al. 2014; Skoda et al. 2014; Bimbilé Somda et al. 2017; FAO/IAEA 2017a, b, 2019a; Moadeli et al. 2017), with a gradual progression from natural-host materials towards synthetic and defined diets (Singh 1984; Moore 1985; Shimoji and Yamagishi 2004). A natural-host diet is limited by the availability of the host, which may be seasonal or limited in distribution, and variable in quality. It tends to be expensive, but should provide a complete diet and avoid changes in host-location behaviour. Semi-synthetic, synthetic, and defined diets offer the convenience of shelf storage and consistent product, but risk being deficient in one

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or more factors. Often natural-host material needs to be incorporated into the diet for initial colonization, but then can be removed gradually as the colony adapts. Generalist herbivore insects tend to be the easiest to feed, with specialist herbivores, predators, and parasites being more difficult. Singh (1985) worked on a range of diets suitable for several to many species, even across different orders. However, even though general diets are unlikely to provide optimum nutrition and cost, they do act as a good starting point for developing diets for newly colonized species.

Future diet development will concentrate on utilization efficiency, ease of storage, cost, and availability (Aceituno-Medina et al. 2020). Defined diets may become important, but the highly refined ingredients may be expensive, and thus semi-defined diets utilizing cheaper local raw materials are more likely to be useful.

Research on fruit flies is investigating liquid diets, which increase utilization efficiency and ease disposal problems, and offer the possibility of recycling the diet by removing waste products and replacing only the nutrients that have been consumed (Fay and Wornoayporn 2002; Chang et al. 2004, 2006). Insects such as the yellow mealworm *Tenebrio molitor* L., house fly *Musca domestica* L., and black soldier fly *Hermetia illucens* (L.) are under investigation for their potential to be used as feed for mosquitoes (Bimbilé Somda et al. 2019). Nutrigenomics offers a powerful method for determining if specific diet components are deficient or in excess (Coudron et al. 2006).

### *8.1. Diet Cost*

The first step in reducing the cost of a diet, which was not designed specifically for the species, is to remove unnecessary components. The next stage is to identify local agricultural products and industrial by-products (e.g. brewer's yeast, sugar cane bagasse) or standard commercial products that are cheap (Chaudhury et al. 1998, 2000; Cohen 2000; Shimoji and Yamagishi 2004), and provide the necessary nutrition and physical properties without adverse effects on the development, quality, and reproductive capacity of the insect (Gilles et al. 2011; Damiens et al. 2012, 2013; Khan et al. 2013; Yahouédo et al. 2014; Bimbilé Somda et al. 2017; Epopa et al. 2018). Often utilizing local components will also make the supply more secure.

Minor components of the diet frequently contribute a disproportionate amount to the cost. For example, many diets are gelled with agar, but agar is very expensive; several alternatives (as partial or complete replacements of agar) have been investigated (Leppla 1976; Spencer et al. 1976; Harris et al. 1984; Taylor et al. 1991; Honda et al. 1996; Chaudhury and Alvarez 1999). Such minor (by weight) components are important in controlling both the consistency and water retention of the diet; water is a crucial component that influences all stages of egg, larval, and pupal development.

Mechanical handling and processing of diet can also reduce costs, e.g. in the former pink bollworm *Pectinophora gossypiella* (Saunders) rearing facility in Phoenix, Arizona, USA, diet was processed and sterilized in a twin-screw extruder (Edwards et al. 1996; Miller et al. 1996). The application of agricultural-engineering

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and food-processing techniques promises to produce significant further improvements (Cohen 2015).

### *8.2. Diet Quality*

It is important to ensure the quality of diet ingredients before purchase or use, e.g. by monitoring the physical and chemical variability of the ingredients, bacterial load, and any insecticide residues, and using bioassays to assess its impact on insect development, production, and quality parameters. Poor quality control of diet ingredients is a major problem in mass-rearing, and therefore requires attention, including specifying chemical and biological assays that the ingredients must pass. In the end, a cheaper ingredient may in fact be more expensive due to frequent "crashes" in production caused by low-quality ingredients.

In AW-IPM programmes that integrate the release of sterilized insects, the availability of an irradiator means that diet components can easily be decontaminated. In rearing tsetse flies, the bacterial load of the blood diet (FAO/IAEA 2019a) is critical, and can be reduced through irradiation (Feldmann 1994). Bacterial contamination of wheat bran used in an uncooked diet for rearing the carob moth *Ectomyelois ceratoniae* (Zeller) can also be reduced by irradiation.

### *8.3. Additives to Diets*

Additives to larval or adult diets can have a profound effect on the performance of insects (Augustinos et al., this volume; Dowell et al., this volume; Pereira et al., this volume). Supplementing boll weevil *Anthonomus grandis grandis* Boheman diet with beta-carotene increased dispersal and trap response (Reinecke 1991). In *An. arabiensis* mosquitoes, a vitamin mix (used as an additive) significantly decreased larval development time (Damiens et al. 2012).

Feeding methyl eugenol to adults of the oriental fruit fly *Bactrocera dorsalis* (Hendel) reduces the time to sexual maturity and the catch of sterile males in methyl-eugenol-baited traps (Shelly and Dewire 1994). Other minor components, that can be added to the adult diet or applied directly to modify the behaviour of released insects, are being identified (Papadopoulos et al. 2001; McInnis et al. 2002; Shelly et al. 2002; Lance and McInnis, this volume).

### *8.4. Micro-Organisms in the Diet*

Control of disease in the colony, and of micro-organism contamination of the diet, are intimately linked (Abd-Alla et al., this volume). The identification and control of microbiological contaminants have been reviewed by Sikorowski (1983), Goodwin (1984), Sikorowski and Goodwin (1985), Sikorowski and Lawrence (1994), and Inglis and Sikorowski (2009a, b). Apart from immediate mortality, microbial contamination can lead to changes in development, body composition, and susceptibility to insecticides (Sikorowski and Goodwin 1985). Control begins with preventing infection by appropriate handling techniques and sterilization of

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ingredients, as well as surface sterilization of eggs or pupae (Leppla et al. 1974). A wide range of antimicrobial compounds for insect diets has been tested (Gifawesen et al. 1975; Ludemann et al. 1979; Hartley et al. 1982; Funke 1983; Bathon 1977), but many are unsuitable, and none is suitable for all insect species. Alverson and Cohen (2002) found that some of the most common ones have a significant negative impact on rearing *Lygus hesperus* Knight. Other means of microbial control include irradiation and various forms of heat treatment.

Conversely, it is now recognized that many micro-organisms are important for many aspects of the biology of their hosts. It has been shown in fruit flies that probiotic application of *Enterobacter* sp. resulted in improvement of both pupal and adult productivity, as well as reduced rearing duration, particularly for males, without affecting pupal weight, sex ratio, male mating competitiveness, flight ability, and longevity under starvation (Augustinos et al. 2015; Yao et al. 2017; Cáceres et al. 2019; Stathopoulou et al. 2021). Kyritsis et al. (2017) described two bacterial isolates, an *Enterobacter* sp. (strain AA26) and a *Klebsiella oxytoca* strain, that were used as probiotics in larval and adult diets. These strains have been shown to be beneficial, affecting several aspects related to the rearing efficiency and biological quality of the Mediterranean fruit fly VIENNA $8^{D53+}$ genetic sexing strain, but other work did not find consistent benefits from probiotic diets (Niyazi 2004; Sacchetti et al. 2014), indicating that controlling the specific species and strains of micro-organisms in the diet and their concentrations will be important (Augustinos et al., this volume).

## 9. SEX SEPARATION

In the SIT, released sterile males mating with wild females produce the only sterilizing effect. If sterile females are also released, they can have (depending on the species) an additional positive effect by distracting wild fertile males and acting as a “sperm sink”. However, usually the simultaneous release of both sexes is less economical, and also less effective, than the release of only males, because there may be a tendency towards assortative mating (Robinson et al. 1999). In haematophagous disease vectors, the females are usually the vectors, and must be removed prior to release (Lees et al., this volume).

In most insects, the sexes can be separated on the basis of external morphology, but this may be possible only in the pupal or adult stages, and may be difficult to automate. For example, in programmes that release sterile male tsetse flies, teneral adult flies are usually hand-sorted in a chiller to separate the sexes, a very slow and laborious procedure. Research showed that a computer-based optical recognition system was too slow and too prone to error to be useful, but recent work on separating pupae according to sex by near-infrared imaging shows promise (Moran and Parker 2016). In many insects, e.g. some mosquitoes (Gerberg et al. 1994; Zacarés et al. 2018) and Lepidoptera, sexual dimorphism in size (apparent in the pupal stage) can be used to separate the sexes, but overlap in size (in some species) may render the sorting inefficient. In some insect groups, pupae show genitalia characters that identify the sex, but these are difficult to observe, require hand-sorting, and have not been automated. Spiking blood meals with ivermectin has

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shown potential as a viable treatment to eliminate female *An. arabiensis* from laboratory colonies, but its practical use in a mass-rearing facility still needs to be tested (Yamada et al. 2013).

The sexes often show a variation in developmental rate, and this can sometimes be used to separate them. Female tsetse flies emerge first and, by manipulating temperature conditions during pupal development, sex separation based on the timing of adult emergence is possible (Opiyo et al. 1999, 2000); in some species the efficiency was more than 99%, and eliminated laborious hand-sorting work in a chiller. Protandry in mosquitoes results in males pupating/emerging before females, and larvae, male and female pupae can be separated using mechanical sexing tools (Focks 1980) or by using sieves of different mesh size. Automated systems using the difference in size between male and female pupae are currently under development to sort *Aedes* mosquitoes (Zacarés et al. 2018; Lees et al., this volume). Recent coordinated research (Bourtzis and Tu 2018) explored methods for sex separation in mosquitoes (Bellini et al. 2018; Kittayapong et al. 2018; Mashatola et al. 2018; Papathanos et al. 2018).

Various methods to separate the sexes, based on Mendelian genetics or engineered sex-linked mutations, have been developed (Robinson and Franz 1999; Robinson et al. 1999; Marec et al. 2005; Zepeda-Cisneros et al. 2014; Dandalo et al. 2018; Häcker and Schetelig 2018; Lebon et al. 2018). These systems, together with other genetic developments, are discussed by Franz et al. (this volume), Häcker et al. (this volume), and Lees et al. (this volume).

## 10. MARKING

To mark or not to mark sterile insects for release, that is the question. It is usually assumed that marking is essential for field monitoring of sterile to wild ratios so as to follow programme progress, but the hugely successful New World screwworm eradication programme (Klassen et al., this volume; Vargas-Terán et al., this volume) has never used any form of marking. Progress is monitored by measuring egg-hatch rates, and such a method may work for some of the other target insects as well (Vreysen, this volume). No one method of marking is universally applicable, and all may have negative effects on the insects (Dowell et al., this volume; Hendrichs and Robinson, this volume; Vreysen, this volume).

Methods of marking were reviewed by Hagler and Jackson (2001); the various individual marking systems, including numbering and coding systems, are clearly not appropriate for production levels of hundreds of millions per week. Methods appropriate to mass-rearing include dye marking (that can be observed directly or with a simple UV system), chemical marking that requires some form of instrumentation for detection, and genetic markers.

For any marking technique, quality control of the marking — detecting and rapidly correcting any failure of marking — is important (Enkerlin et al. 1996; Kohama et al. 2003). The misidentification of a released sterile insect as a fertile insect can have huge financial consequences for an AW-IPM eradication programme, justifying both the commitment of significant resources to, and great care in, the marking.

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### *10.1. Dye Marking*

Dye marking falls into two categories, internal dyes fed through the larval or adult diet, and external dyes. Internal dyes are usually oil-soluble; considerable testing of dyes has been done (Vail et al. 1966; Hendricks and Graham 1970; Graham and Mangum 1971; Hendricks 1971). It was found that a dye suitable for one species is not necessarily appropriate for even a closely related species. The ease of administering the dye in the diet makes this system very attractive, but the disadvantages are that the dyes can be toxic or cause behavioural or other changes (Schroeder et al. 1974) and may not persist in later stages; also, it is relatively difficult to identify a series of different markers. Nevertheless, the dye Calco Red has been used extensively for marking Lepidoptera, e.g. pink bollworm and codling moth. Rhodamine B has been shown to be a less expensive and a viable body-marking technique for *Aedes aegypti* (L.) without the negative side effects of traditional marking methods (Johnson et al. 2017).

Almost as extensively, external dye marking has frequently been used in AW-IPM programmes that release sterile insects. Usually this involves a fluorescent dye that is dusted or sprayed onto the insects (Chang 1946; Taft and Agee 1962; Stern and Mueller 1968; Schroeder and Mitchell 1981; Reinecke 1990; Enkerlin et al. 1996; Verhulst et al. 2013), but oil-soluble dyes have also been used for external marking (Steiner 1965; Schroeder and Mitchell 1981). The procedure is simple to apply; several different colours and dyes can be used. The impact of externally applied dyes on the behaviour or longevity of insects is variable (Nakata 2008; Reid and Reid 2008; Dickens and Brant 2014); it has been reported that excessive dye may reduce the response of codling moth males to calling females (Logan and Proverbs 1975). There is evidence that reducing the quantity of dye used can significantly increase survival and quality in fruit flies. The disadvantages of using external dyes are that an additional processing step is involved, the dyes may affect insect quality, the marker may not be as reliable as an internal one, and the dye dusts can pose a health hazard to staff.

### *10.2. Elemental Markers*

An alternative to dye marking is using elemental markers (reviewed by Akey 1991 and Akey et al. 1991). Three forms of detecting elemental marking utilize radioisotopes, neutron activation, or one of several forms of atomic spectroscopy (Akey and Burns 1991) or isotope ratio mass spectrometry (Heiling et al. 2006). Elemental fingerprinting may also be included here — detecting the ratios of unsupplemented elements that will vary between field populations and mass-reared insects (Hood-Nowotny et al. 2009). Due to environmental concerns, radioactive-isotope marking has fallen out of favour. Neutron activation of rare elements is a very sensitive technique; in principle it is able to detect picogram quantities (Curtis et al. 1973; Hamann and Iwannek 1979), but the need for a fast-neutron source makes the technique extremely expensive and impractical.

Atomic absorption spectroscopy of stable isotopes offers a much more accessible technique (Akey 1991; Stimmann 1991; Van Steenwyk 1991; Fernandes et al. 1997;

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Maciel-de-Freitas et al. 2004; Wilkins et al. 2007). Different forms of spectroscopy can be used to identify the marker with varying levels of sensitivity (Akey and Burns 1991). For a programme that releases sterile insects, the cost of false positives is potentially so high that the additional equipment and procedure may well be justified, particularly in the later stages of eradication, and elemental marking could provide a valuable second means of identification to back-up dye marking.

Hood-Nowotny et al. (2006) suggested that it was possible to use an isotopic label in adult *An. arabiensis,* and to detect it at an appropriate concentration up to 21 days post-emergence; however, the optimum labelling treatment would cost about USD 250 per million mosquitoes. Enrichment of semi-natural mosquito larval habitats with stable isotopes of nitrogen and carbon resulted in effective marking of *Anopheles* and *Aedes* mosquitoes colonizing these habitats (Opiyo et al. 2016).

### *10.3. Other Markers*

An interesting development in chemical marking is the use of non-insect protein markers (Hagler and Cohen 1990; Hagler et al. 1992; Hagler and Miller 2002; Hagler 2004). The technique has been tested using rabbit IgG, fed in the diet or applied externally, and detected by sandwich enzyme-linked immunosorbent assay. The technique looks promising, requires only simple laboratory equipment, and rapid application should be possible. A big advantage is that the mark is completely invisible, and should have no biological effect on insects. Protein marking can be combined with dye marking to increase the marking efficacy (Irvin et al. 2012).

To distinguish released insects from wild ones, naturally occurring phenotypic mutations can be used in a similar manner to dyes, as long as the mutation is sufficiently rare in the wild population (Bartlett 1982). However, almost certainly, most mutations have some deleterious effect. For the SIT, the disadvantage is often not so great that their use is prevented, and a few such mutations have been investigated or used (Fay and Craig 1969; Schroeder and Mitchell 1981; Niyazi et al. 2005). The main problem with mutations is that, since they cannot be transferred between species by conventional genetics, they must be identified for each species to be marked.

Most mass-reared insect colonies have a limited genetic basis, resulting in limited genetic diversity. This can be exploited to distinguish colony insects from the target population, by identifying natural DNA markers specific to the colony or target insects. Mitochondrial DNA markers were used in South Africa to distinguish wild from released sterile insects (Barnes et al. 2004), but for identification this technique requires the use of polymerase chain reaction (PCR) amplification.

Once again, genetic engineering offers the prospect of new marking techniques (Häcker et al., this volume; Hendrichs and Robinson, this volume). In principle, any transgenic insect carries an identifiable marker (the transgenic construct), but for identification this also may require PCR amplification. It would be easier to use a gene that encodes a visible product. At present, the most popular genes are green fluorescent protein (GFP) and its derivatives (Peloquin et al. 2000), and DsRed (Alphey 2002). Fluorescent markers can be made sperm-specific to enable the sperm to be identified in mated field females (Scolari et al. 2008). Such transformations, if

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carefully constructed, should avoid most of the disadvantages of natural mutation markers.

## 11. STORAGE

Successful field operations using the SIT require a timely and predictable supply of sterile insects, and this can present logistical problems. There is usually a premium on the release of sterile insects at the beginning of the target population build-up, before the population level becomes too large, but colony rearing may be constrained by diapause or hibernal quiescence. Also, for a seasonal pest, to save on costs, the rearing must be reduced to a maintenance level during the winter, but this is an inefficient use of the facility. In an ideal situation, the rearing would be continuous, with some means to stockpile the insects over winter in preparation for release in the spring, e.g. onion maggot *Delia antiqua* (Meigen) (Loosjes 2000). For these reasons, the ability to store insects, and to manipulate quiescence or diapause, becomes very valuable (FAO/IAEA 2016). For shorter timescales, storage for a few days may be useful to synchronize insects for periodic release, and storage in a quiescent state for a period of hours may be desirable for delivery to the release site and for the release process itself. Leopold (1998, 2000) reviewed cold storage and cryopreservation of insects for each of these timescales.

Insects with an obligate or facultative diapause may be of better quality than non-diapause individuals (Bloem et al. 1997, 1998, 2000) and, assuming diapause termination can be controlled, form a convenient way of storing insects. Denlinger (2002) reviewed the regulation of diapause. Obligatory diapause in a one-generation-per-year species, e.g. western cherry fruit fly *Rhagoletis indifferens* Curran (Vankirk and Aliniazee 1982), may make continuous rearing impossible. The main aim is to find a means to induce and then break diapause early to permit the production of sterile insects in advance of the field population. The duration of induction, diapause, and breaking of diapause may last many months, and present a formidable obstacle to successful incorporation into the rearing system. The obligate duration of some of the cues may be circumvented by the application of hormones, and certain volatile organic solvents can influence the termination of diapause (FAO/IAEA 2016).

The exposure of insects to low temperature often results in damage. Denlinger and Lee (1998) as well as Sømme (1999) reviewed the physiology of cold damage. Below 0˚C the main threat to an insect is ice formation, but a range of hysteresis proteins, cryoprotectants, and stress proteins, and the elimination or masking of nucleating proteins and bacteria in freeze-susceptible species, lower the super-cooling temperature below that likely to be experienced. Without freezing, osmotic stress is minimized. In freeze-tolerant species, nucleating proteins induce rapid freezing at higher temperatures, and osmotic stress is countered mainly by cryoprotectants.

Diapausing insects often exhibit inherent cold tolerance. Also, in many non-diapause insects, it is possible to induce cold tolerance by exposing the insects to heat or less extreme cold before storage, producing so-called “rapid cold hardening” in a matter of minutes or hours (Lee et al. 1987; Denlinger et al. 1992; Rinehart et al.

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2000; Chidawanyika and Terblanche 2011; Nyamukondiwa et al. 2013). Cold or heat shock induces the production of specific stress proteins, commonly called heat-shock proteins (HSPs), which among other effects are thought to prevent protein denaturation (Parsell and Lindquist 1993). The rapid production of HSPs makes them useful for short-term storage, shipment, and release, but their effect can be longer term, and can be re-induced by brief warming to higher storage temperatures (Chen and Denlinger 1992). In the future, it may be possible to genetically engineer desirable traits to enhance the storage potential of a mass-reared strain (Robinson and Franz 1999).

Finally, during colonization and rearing, insects are subject to selection pressure of varying intensity that can result in rapid genetic drift and loss of heterozygosity. Cryopreservation provides a method for long-term storage of genetic material with specific desirable traits, and the storage of strains where diapause or conventional cold storage is not possible (Leopold 1998, 2000; Rajamohan et al. 2003, 2014; Rajamohan and Leopold 2007). Where it is possible, cryopreservation may reduce the labour and cost of maintaining continuously many strains.

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# CHAPTER 3.3.

# MANAGING PATHOGENS IN INSECT MASS-REARING FOR THE STERILE INSECT TECHNIQUE, WITH THE TSETSE FLY SALIVARY GLAND HYPERTROPHY VIRUS AS AN EXAMPLE

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## SUMMARY

Effective management of insect pathogens in insect mass-rearing is fundamental to the successful implementation of area-wide integrated pest management programmes (AW-IPM) with a sterile insect technique (SIT) component. Insect pathogens, notably viral pathogens, affect the sustainable production of high-quality males for the SIT, either by compromising colony establishment and stability or by reducing insect performance, including mating and flight ability. Many pathogenic bacteria, fungi, microsporidia, spiroplasma/mycoplasma and viruses can infect insects. Due to the broad subject of insect pathology, only the pathogens affecting insect mass-rearing facilities producing sterile insects are discussed here, with a special focus on tsetse flies, mosquitoes and codling moth viral pathogens. Special emphasis is given to the successful management of the tsetse salivary gland hypertrophy virus (SGHV), the current management of the codling moth granulovirus (CpGV), and the risk of infections of mass-reared mosquitoes with pathogenic viruses and other vector-borne diseases. Also discussed are the potential risks posed by these pathogens to staff working in mass-production facilities.

## 1. INTRODUCTION

For centuries, the main goal of organized insect mass-production was to provide reliable and cost-effective sources of high-quality insects for numerous important purposes (Parker, Vreysen et al., this volume). Recent purposes of insect mass-rearing include: (1) release of sexually sterilized males as part of sterile insect technique (SIT) applications (Parker, Mamai et al., this volume), or of genetically modified insects, for the suppression/eradication of target insect pest populations, (2) augmentative releases of parasitoids, predators or pollinators to control target pests or pollinate target crops, (3) application of insects as hosts for the production or dissemination of microbial control agents, (4) use of insects as food and feed (Van Huis 2013; Van Huis et al. 2013; Vantomme et al. 2014; Dossey et al. 2016), and (5) production of economic products such as honey, silk (Needham et al. 1937) and cochineal dyes (Greenfield 2005). Insects are also mass-reared for research and industrial purposes, e.g. the production of chemical components used in cosmetics, medicine for human therapy, and the production of biofuels (Dickerson and Leppla 1992). Additionally, insects are mass-reared for use in celebrations, as pets or objects of interest, and in conservation programmes for endangered or threatened species (Resh and Cardé 2003).

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To control insect pests of agricultural or medical importance, various methods including the SIT are used (El-Wakeil and Abdallah 2012). When applied within the concept of area-wide integrated pest management (AW-IPM), the SIT is a proven, effective and environment-friendly tool. This method has been successfully applied to control several insect pests around the world (Goodenough et al. 1983; Klassen et al., this volume). Efficient and effective mass-rearing of the target insect is a fundamental prerequisite when applying the SIT (Dyck 2010). For some purposes, e.g. as hosts for biological control agents or as food and feed sources, mass-rearing of large quantities of the insect is the main objective. However, when producing insects for release into the field where they have to perform a specific task, obtaining high-quality insects is paramount; in the case of the SIT the released sterile males must outcompete wild males in mating with wild females (Parker, Vreysen et al., this volume; Vreysen, this volume). Therefore, high standards must be implemented when producing insects for the SIT. However, microbial agents often compromise insect rearing, representing a major risk and challenge for managers of mass-rearing facilities. Consequently, an essential component to achieve high insect quality is investing sufficiently in expertise and infrastructure to manage effectively and control all potential insect pathogens.

The biological control of insect pests using microbial control agents applied in an augmentative way is a sustainable and environment-friendly tool (Hoffmann and Frodsham 1993; Lacey et al. 2001). Currently, approximately 50 agents, including entomopathogenic viruses, bacteria, fungi and nematodes, are commercially produced; this represents ~1–2% of the global pesticide market (Gallai et al. 2009; Lacey et al. 2015). One of the limitations of using biological control is the mandatory *in vivo* production in the insect hosts of all viruses and some of the other microbial agents. This *in vivo* production system must be highly efficient and economical for the large-scale production of sufficient numbers of the insect hosts, and plays a role in determining the success and the market competence of the biocontrol methods.

In addition to the important role of insect mass-rearing in pest control, in the agricultural sector insects are reared also for other economic reasons. The honeybee *Apis mellifera* L. is important not only for honey production, but also for other products like beeswax (Calderone 2012). More importantly, as the primary plant pollinators, honeybees enhance landscape diversity and are fundamental to the pollination of many agricultural crops, with a worldwide annual value of USD 153 thousand million; in the United States alone the annual value is estimated at USD 17 thousand million (Cohen 2004, 2015; Roeder et al. 2010).

## 2. MICROBIAL DISEASES AND INSECT MASS-REARING

The microbial contamination of artificial insect diets, particularly by moulds, fungi and bacteria, is a common problem in insect mass-rearing. The rearing conditions for insects are also conducive for microbial growth, especially in the absence of antimicrobial products. For example, Roeder et al. (2010) reported that, in the absence of antifungal agents, within three weeks 40% of the artificial diets used to rear *Heliothis virescens* (F.) larvae were contaminated with microbes. The control of

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microbial agents is essential to reduce their negative impact on reared insects and to maintain healthy insect colonies (Alverson and Cohen 2002). Generally, antibacterial and antifungal compounds are ingredients of insect diets to control the microbial contamination. However, many of these compounds can be quite toxic to insects, even at low concentrations (Roeder et al. 2010; Cohen 2015). For example, as the concentration of methylparaben (a common antifungal agent) increases in the diet, it significantly reduces the insect colony's performance (Shikata et al. 1998). Finding the right combination and doses of compounds to fight microbial growth, while at the same time reducing or eliminating any negative effects that these antimicrobial compounds have on the reared insects, can be quite challenging (Cohen 2015).

### *2.1. Bacterial Diseases*

Many types of bacteria can be associated with insects, including saprotrophic facultative parasites and obligate parasites. In insect mass-rearing, many pathogenic bacteria have been reported, but here the focus is on the bacterial diseases found in mass-rearing insects that are used for SIT projects. These insects include various species in the orders Diptera, Lepidoptera, Hymenoptera and Coleoptera.

Bacteria encountered in insectaries are usually innocuous. However, for insects exposed to stressors such as a change from natural to artificial diets, and exposure to elevated temperature and humidity, high density (overcrowding) and toxins, bacteria can multiply extensively within the bodies of insects with a weakened immune system, causing diseases that may be severe or even fatal (Sikorowski and Lawrence 1994). For example, some genotypes of *Bacillus sphaericus* produce insecticidal toxins that are active against mosquito larvae (Boucias and Pendland 1998).

In *Helicoverpa zea* (Boddie), contamination with certain bacterial species, e.g. *Pseudomonas maltophilia* and *Bacillus subtilis*, significantly affects larval mortality, pupal weight, larval and pupal deformation, egg production, and egg hatch. In the absence of antibiotics, *Serratia marcescens,* a highly contagious bacterium, can rapidly bring a moth rearing programme to a virtual standstill. Furthermore, these bacteria can cause sub-lethal infections, significantly compromising insect vigour (Sikorowski and Lawrence 1991).

In fruit flies, some bacteria could cause significant problems. For example, the gram-negative bacteria *S. marcescens* and *P. aeruginosa* can overwhelm Caribbean fruit fly *Anastrepha suspensa* (Loew) larvae and pupae when they are stressed, e.g. exposed to thermal stress by rearing at a temperature >30°C (Greany et al. 1977).

In rearing tsetse flies, bacterial contamination is crucial, and can cause a high abortion rate and high mortalities; this underscores the importance of precautionary measures taken during blood collection and storage, and during the feeding process, including irradiating and freezing blood, and conducting bioassays before exposing colonies to new batches of blood (Feldmann 1994). Although certain species of tsetse flies are more sensitive to bacterial contamination than others, according to Wetzel and Thiemann (1979) *Glossina m. morsitans* Westwood adult flies were more sensitive to *P. aeruginosa* bacterial contamination than adults of *G. p. palpalis*

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Robineau-Desvoidy; adults of the latter eliminated a bacterial infection at nine days post-ingestion, but *G. m. morsitans* adults required 14 days to clear the infection.

The best way to avoid bacterial contamination in mass-rearing is to maintain sanitary conditions (Inglis and Sikorowski 2009a, b). When using the membrane feeding technique in tsetse fly mass-rearing, this means applying prophylactic measures, i.e. working in aseptic conditions with sterile feeding membranes, aluminium trays and blood. Using sanitation protocols when rearing the Caribbean fruit fly *A. suspensa* avoided the negative impact of bacterial contamination with *S. marcescens* and *P. aeruginosa* (Greany et al. 1977). As a precaution, mass-rearing facilities often include either antibiotics, e.g. chlortetracycline, antifungals, e.g. nipagin and benzoic acid, or formalin as a general antimicrobial in adult and larval diets (Cohen 2015).

When sanitation is practised and antibiotics are available, bacterial contamination in insect mass-rearing is rare. However, it is important to note that prolonged application of antibiotics may lead to resistant bacterial strains. The wise use of antibiotics is not a substitute for, but a complement to, good sanitation for disease control (Sikorowski 1984).

### *2.2. Fungal Diseases*

There are many species of fungi that infect insects, e.g. species in the genera *Aspergillus, Beauveria, Entomophaga, Entomophthora, Hirsutella, Metarhizium, Nomuraea, Paecilomyces* and *Penicilium*. Species of *Entomophthora* have a worldwide distribution, and play effective roles in controlling many insects of economic importance in the orders Hemiptera, Homoptera, Diptera, Lepidoptera, Coleoptera, Orthoptera and Hymenoptera (MacLeod 1963; MacLeod and Muller-Kogler 1973). Fungi are unique among insect pathogens in that they infect their hosts primarily through the cuticle, although a few taxa may invade through the host's alimentary canal. By simple contact, dissemination of the infection among the individuals of a colony occurs rapidly. Due to the spread of fungal spores by wind and rain, fungal diseases can occur in field-collected insects. This scenario was reported for grasshoppers, where *B. bassiana* caused 90% mortality (Inglis and Sikorowski 2009b).

In general, fungus infection is humidity-dependent, a condition which is usually found in large-scale insect mass-production facilities where artificial diets are used. Furthermore, holding large numbers of insects in a limited space (for economic or convenience reasons) facilitates the spread of fungal diseases. In addition, many saprophytic species such as *Aspergillus* and *Penicilium* spp. growing on the artificial diet might be facultative pathogens, requiring the use of an antifungal compound to avoid infection. The use of fungicides to control fungal infections is a prerequisite for mass-rearing some insect species.

Some fungi such as *Aspergillus flavus* produce aflatoxins, among the most potent carcinogenic compounds; this can be a major health issue for workers in mass-rearing facilities. Also, fungi are health risks -- breathing hazards and possible contamination via the skin, eyes, and nasal passages (Burgner et al. 1998; Revankar et al. 1999; Butt et al. 2001).

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### *2.3. Protozoan, Microsporidia and Mycoplasma/Spiroplasma Diseases*

Most of the entomopathogenic protozoa and microsporidia (thought to have evolved from a fungus (Keeling et al. 2000)) produce chronic, non-lethal infections that reduce the host reproduction (Hurd 1993). These pathogens also cause irregular growth, loss of appetite and malformed larvae, pupae or adults with reduced vigour, fecundity and host lifespan. Although microsporidia have been reported to infect all insect orders, the majority of genera occur in dipteran hosts, which represent an important challenge when applying the SIT to insect disease vectors such as tsetse flies and mosquitoes, and to fruit flies. Microsporidia infection can occur in three different ways: (1) *per os*, through feeding on spore-contaminated food or on dead or moribund insects, (2) transovarially from a female to her offspring on or in eggs (which includes newly hatched larvae feeding on the egg chorion), and (3) directly through the insect cuticle. In certain cases, e.g. *Helicoverpa zea*, all offspring of microsporidia-infected females are infected. In addition, in a lepidopteran insectary, microsporidia spores may be disseminated via moth scales. Once ingested by an insect host, microsporidia spores germinate in the midgut and infect midgut tissue and hemocytes. The severity of the infection is dependent on the number of ingested spores, temperature and larval age. Ingestion of a large number of spores usually causes mechanical damage to the peritrophic membrane and midgut epithelium, thus allowing the ingress of bacteria present in the gut lumen. In such cases, the infected insects soon die. Although the ingestion of small numbers of spores does not kill the host insect, this causes a chronic infection (Inglis and Sikorowski 2009b).

### *2.4. Entomopathogenic Nematodes*

Infection with entomopathogenic nematodes in mass-rearing environments is a rare event. However, new insect colonies established using field-collected insects might be infected by nematodes. Although there are about thirty families of nematodes associated with insects, the majority of these are animal and plant parasites and use insects only as vectors. Nematodes that infect insects as primary hosts occur in seven families. Nematode infection causes sterility, reduced fecundity, development delay, aberrant behaviour, colour changes and host death. Most of the time the entomopathogenic nematodes do not reach adulthood in the hemocoel of the infected host. Rather, such nematodes emerge from the host as post-parasitic juveniles, moult to the adult stage, mate, and produce offspring (Kaya and Stock 1997). Infective juveniles directly penetrate the host's exterior integument using their stylets. Nematodes from the families *Steinernematidae* and *Heterorhabditidae* are obligate parasites of insects and carry mutualistic bacteria of the genus *Xenorhabdus* within their guts; once the nematodes gain access to the hemocoel, the bacteria are released into the hemocoel and kill the host within 24 hours (Kaya and Stock 1997).

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### *2.5. Viral Diseases in Insect Mass-Rearing*

Viruses are among the agents that have a severe pathogenic impact on insect populations, both in the field and the laboratory. Viruses have been found to be responsible for epizootics in mass-reared useful insects, e.g. silkworm (Watanabe 1986), crickets (Szelei et al. 2011; Weissman et al. 2012) and honeybee (Chen and Siede 2007; Wilfert et al. 2016). Insects can be infected with DNA or RNA viruses. Of the approximately 100 families of viruses infecting eukaryotic organisms (ICTV 2017), 16 families of RNA viruses (Ryabov 2017) and 12 families of DNA viruses (Tijssen et al. 2016; Williams et al. 2017) infect several important orders of insects, e.g. Diptera, Lepidoptera, Hymenoptera, Coleoptera and Orthoptera. Some of these viruses are specific to arthropods (largely insects), e.g. families Dicistroviridae and Iflaviridae encompassing small RNA viruses, or families Baculoviridae, Nudiviridae and Ascoviridae encompassing large DNA viruses. Other RNA viruses (Nodaviridae and Reoviridae) or DNA viruses (Iridoviridae, Parvoviridae and Poxviridae) consist of genera, e.g. *Alphanodavirus, Cypovirus* and *Chloriridovirus*, or subfamilies, e.g. *Densovirinae* and *Entomopoxvirinae*, specifically pathogenic to insects, whereas other genera or subfamilies of these families infect vertebrates or humans but not insects. The honeybee is infected by at least 12 viruses belonging to five different virus families. In addition, some arthropod-borne viruses replicate both in their insect hosts and their vertebrate hosts. Examples include members of the virus families Bunyaviridae and Flaviviridae (Ryabov 2017).

Viral infections in insect populations can be symptomatic (with overt disease symptoms) or asymptomatic (hidden symptoms). While the former can result in the loss of fitness or death of the infected insects, the latter seems usually not to have fitness costs to the host. Cryptic latent virus infections are frequently observed in wild insect populations as well as in small- and large-scale laboratory colonies. Under certain unfavourable abiotic conditions, e.g. temperature and humidity, or biotic stress, e.g. increase in population density, shortage or change of food or accidental contamination of food, these hidden viral infections can be activated and soon result in outbreaks of epizootic infections (Tanada 1963; Weissman et al. 2012). However, the most frequent origin of viral outbreaks is the accidental introduction into laboratory colonies of individuals from other colonies (or collected from the field) that carry asymptomatic viral infections.

Until the discovery of several small RNA viruses in *Drosophila* species, several laboratories that use *Drosophila* as a model for genetic research have been working for years with colonies carrying hidden virus infections (Jousset et al. 1972; Teninges and Plus 1972; Plus et al. 1975). Further, the development of deep sequencing and metagenomic analysis tools has led to the rapid discovery of novel virus strains in asymptomatically infected insects. Examples include the honeybee filamentous virus with a genome of circular dsDNA of ca. 500 kbp in size (Gauthier et al. 2015), the small circular ssDNA of ca. 2 kb viruses (family Circoviridae) isolated from crickets, dragonflies and mosquitoes (Rosario et al. 2012; Pham et al. 2013; Garigliany et al. 2015), rhabdoviruses (Longdon et al. 2015) and endogenous retroviruses (Akkouche et al. 2012; Pelisson et al. 2002) of *Drosophilidae*. Hidden latent viral infections represent a major challenge for insect mass-rearing for the SIT because rearing for many generations under a high density might represent a stress

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factor compromising the insect immune system and leading to the emergence of latent viral infections.

### *2.5.1. Viral Diseases in Lepidoptera*

Several lepidopteran species of economic importance are the targets in SIT projects (e.g. codling moth, false codling moth *Thaumatotibia leucotreta* (Meyrick), pink bollworm *Pectinophora gossypiella* (Saunders) and cactus moth *Cactoblastis cactorum* (Berg)) or the basis of industrial silk production (silkworm *Bombyx mori* (L)). Insects belonging to the order Lepidoptera are the hosts of many insect viruses, notably in the larval stages. Outbreaks of epizootics caused by viral agents of the family Baculoviridae were frequently reported to control natural dense populations of lepidopteran pests (Cory and Bishop 1997; Cory et al. 1997; Moscardi 1999; Fuxa 2004). Owing to their safety for humans and specificity in host range, usually limited to a single or a limited number of insects of the same species, these viruses are used for the biological control of several crop and fruit pests. On the other hand, baculoviruses represent a major problem for the mass-rearing of Lepidoptera used in SIT projects, e.g. the codling moth (Tanada 1964) or the silkworm (Summers 2006). More than 160 years ago the first insect viral infection, (i.e. baculovirus in the silkworm), was reported: in 1856 Cornalia and Maestri were able to associate the refractile occlusion bodies (now known as polyhedral) with the disease in silkworm (Summers 2006). The granulovirus CpGV in the codling moth was first reported in Mexico in 1964 (Tanada 1964). Currently, CpGV is widely used as a biological control agent to control this pest. On the other hand, strong measures to control CpGV are required to avoid a severe negative impact on the stability of the codling moth colony used for the SIT in the Okanagan Valley of British Columbia, Canada (section 3.2.). In addition to these two viruses, similar baculovirus infections have been reported in many other lepidopteran insects.

Densoviruses represent another major threat for the mass-rearing of Lepidoptera. These viruses are highly pathogenic to their hosts; they are responsible for epizootics in natural populations of some major lepidopteran pests, e.g. oil palm defoliators and sugar cane and corn borers, and in mass-reared insects used as pet food, e.g. crickets and the greater wax moth *Galleria mellonell*a (L.) (Fédière 2000; Bergoin and Tijssen 2010). The *Bombyx mori* densovirus (BmDV-1) is the causative agent of the infectious flacherie of the silkworm (Shimizu 1975), one of the most deleterious diseases in sericultural farms. As a result of a demonstration that the susceptibility of the silkworm to BmDV-1 varied from one strain to another, it was possible to select resistant strains (Eguchi et al. 1991).

### *2.5.2. Viral Diseases in Tsetse Flies*

In 1987 the first collapse of the *Glossina pallidipes* Austen colony, established at the FAO/IAEA Insect Pest Control Laboratory (IPCL) in Seibersdorf, Austria, occurred; it was caused by the salivary gland hypertrophy virus (SGHV) (Fig. 1). This colony was initiated from various batches (528 pupae) collected from the Lambwe Valley, Kenya, in November 1983. The colony was maintained and increased to a thousand productive females, but thereafter the colony stagnated at that level, having a low fly

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emergence rate, high daily mortality (1.8% in flies older than 50 days), low fecundity (<1.1 pupae per female per month) and a low insemination rate (<85%). Microscopic examination of dissected flies revealed that up to 85% of males and 70% of females had hyperplastic salivary glands (Fig. 1). Males with hyperplastic salivary glands showed testicular degeneration and aspermia. These problems led to discontinuation of the colony in 1988 (FAO/IAEA 1987, 1988). Thereafter, a new *G. pallidipes* colony was established with insects originating in Tororo, Uganda, and donated by the Tsetse Research Laboratory, Bristol, England. However, the new colony was established and successfully maintained without an investigation of the cause of the collapse of the previous colony (FAO/IAEA 2006).

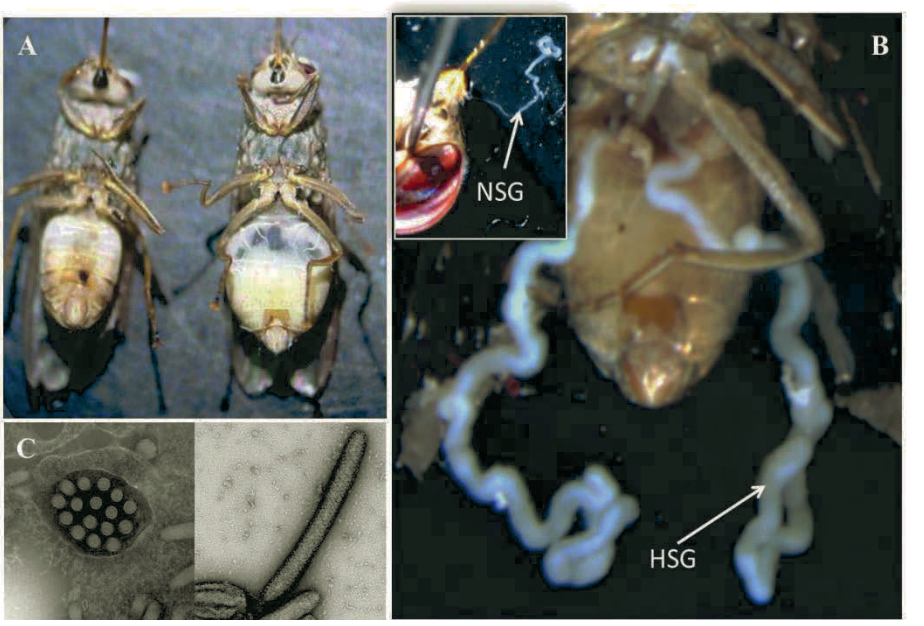



*Figure 1. Symptoms of* Glossina pallidipes *salivary gland hypertrophy virus (GpSGHV) in tsetse fly* G. pallidipes *males. (A) Males with asymptomatic (left) and symptomatic (right) infection (reproduced from Abd-Alla et al. 2009a, with permission from Elsevier); (B) Tsetse males with normal salivary glands (NSG) and hypertrophied salivary glands (HSG) (reproduced from Abd-Alla et al. 2013a, with permission from Elsevier); (C) Transmission electron microscopy (TEM) micrograph of GpSGHV virus particles (right), cross section (left). The TEM images were made by J. van Lent and H. M. Kariithi (Wageningen University, reproduced with permission).*

Following pest suppression and the release of sterile males, in 1997 the tsetse fly *G. austeni* Newstead was eradicated on the island of Unguja, United Republic of Tanzania (Vreysen et al. 2000; Hendrichs et al. 2007). This success prompted other African countries to consider applying the SIT to eradicate tsetse fly populations. A programme to create a zone free of *Glossina pallidipes* in the Southern Rift Valley of Ethiopia was initiated in 1996 (Vreysen et al. 2000; Feldmann et al., this volume).

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This project stimulated the IPCL to conduct research on the problematic *G. pallidipes* colony. Since the available colony was initiated from insects collected in Uganda, it was decided that a *G. pallidipes* colony should be started using flies from Ethiopia. Therefore, a new *G. pallidipes* colony was initiated in 1999 with pupae collected in Arba Minch, Ethiopia. This colony became well established, and in 2001 it reached about 15 000 productive females.

However, similar to the 1987 colony, the new colony declined steadily over a 2-year period, becoming extinct in 2002. Similarly, dissections revealed that up to 85% of both male and female flies had salivary gland hypertrophy (SGH), a syndrome first described in wild populations of *G. pallidipes* (Table 1) (Abd-Alla et al. 2011). To overcome the problems of managing a *G. pallidipes* colony, research was conducted for more than ten years, resulting in effective management packages (section 3.1.).

Symptoms of the SGH were initially reported in 1932 (Table 1). During dissection of *Glossina pallidipes* specimens to observe trypanosomes in Zululand, South Africa, swollen salivary glands were observed (Whitnall 1932; Whitnall 1934). Later, the SGH symptoms were reported in *G. morsitans* (Burtt 1945). In the 1970s, the SGH syndrome was associated with a virus found in cytoplasmic vacuoles of the salivary glands and midgut epithelial cells of *G. fuscipes* Newstead and *G. morsitans* (Jenni 1973; Jenni and Steiger 1974a, b; Jenni and Böhringer 1976). At that time the virus was described as "virus-like particles" (VLPs), and erroneously thought to be an arbovirus.

The first link between the SGH and the salivary gland hypertrophy virus was reported by Jaenson (1978) who examined the hypertrophied salivary gland in *G. pallidipes* from Kenya using electron microscopy; it was found that rod-shaped virus particles were present only in salivary glands that showed this syndrome. The particles were absent in non-hypertrophied salivary glands. Based on the assumption that the new virus might be a potential biological control agent against tsetse flies, many researchers made a concerted effort to explore this idea. Subsequently, SGH syndromes were reported in many tsetse species, e.g. *Glossina austeni, G. m. morsitans, G. nigrofusca nigrofusca* Newstead and *G. pallicera pallicera* Bigot (Burtt 1945; Ellis and Maudlin 1987; Gouteux 1987). Although containing a double-stranded DNA genome, the virion structure did not suggest that the tsetse virus should be assigned to any of the existing insect virus groups (Burtt 1945; Odindo et al. 1986; Ellis and Maudlin 1987; Gouteux 1987). Pathological effects noted in various countries included sex distortion and a reduction in insemination rates, fecundity and lifespan (Jaenson 1986).

The main research question was to explain why the Ethiopian-derived colony collapsed, unlike the Ugandan-derived colony from Tororo which was, and still is, sustainable. Notably, the prevalence of the SGH syndrome was about 10% in the Tororo colony compared with 85% in the Arba Minch colony. This significant difference in the prevalence of the SGH syndrome raised the question whether these colonies were infected by the same or different virus strains. Therefore, the complete genome of the virus isolated from the Tororo colony was sequenced and annotated. The sequencing data identified the genome of the virus as a circular double-stranded DNA molecule over 300 kb in size; this confirmed the previous observation of Odindo (1986) that this virus is unique and could not be assigned to any existing

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insect virus family. Therefore, a new family of insect viruses called Hytrosaviridae was created, encompassing the tsetse fly virus and a similar virus from the house fly *M. domestica* as two members of this family. The name Hytrosaviridae was derived from Hytrosa, sigla from the Greek '*H*ypertrophia' for '*hy*per*t*rophy' and '*s*ialo*a*denitis' for 'salivary gland inflammation' (Jaenson 1986; Abd-Alla et al. 2008, 2009b; Garcia-Maruniak et al. 2009; Lietze et al. 2011). The recent sequencing and annotation of the genome of the Ethiopian GpSGHV (GpSHGV-Eth) strain revealed deletions/insertions in 37 ORFs and 17 ORF deletions and 24 novel ORFs compared with the Ugandan GpSGHV, which might explain the differential pathogenesis between the two colonies (Abd-Alla et al. 2016).

In addition, it was demonstrated that the GpSGHV infects other tsetse species, and causes a negative impact on the flies' performance without developing salivary hypertrophy (Demirbas-Uzel et al. 2018a, b). Also, it was reported that the virus has different strains/genotypes circulating in the wild tsetse population, and the virus infection affects the flies' immune system (Kariithi et al. 2018a, b; Meki et al. 2018a, b, c).

The sequence data of the SGHV isolated from the Tororo-derived (Uganda) *G. pallidipes* represents the reference virus genome; the virus is named as the GpSGHV-Uga. The availability of the GpSGHV-Uga genomic data enabled the development of virus detection tools such as a conventional, non-destructive PCR and a quantitative PCR (qPCR) which were used to diagnose and quantify the viral infections in tsetse colonies. The PCR detection method revealed that, although only about 10% of the Tororo colony exhibited SGH symptoms as an overt infection, almost all flies harboured the virus infection as an asymptomatic infection.

Quantitative PCR provided further evidence that the virus titre unequivocally correlated with the SGH symptoms, thus providing additional proof that the GpSGHV causes SGH. Sequence analysis of the SGHV-Uga genome revealed that it shared several gene similarities with other large DNA viruses such as baculoviruses, nudiviruses or entomopoxviruses. Of particular interest was the similarity of the SGHV DNA polymerase (a gene responsible for replicating the DNA) with that of the human herpes virus. This similarity led to the use of the antiviral drugs commercially available against herpes virus infection to control the SGHV infection (section 3.1.1.) (Abd-Alla et al. 2008, 2009b, 2014).

The average low rate (0.5–1%) of virus infection with overt SGH symptoms or covert latent infection prevalence in tsetse wild populations significantly differed from the laboratory colonies; in the laboratory a covert infection occurred in almost all flies (Odindo 1982; Odindo and Amutalla 1986; Abd-Alla et al. 2007, 2009a). To explain this difference our attention was drawn to the *in vitro* feeding system which was developed at the IPCL in Seibersdorf, and is currently used in all tsetse mass-rearing facilities, i.e. flies (in holding cages) are fed in several successive rounds on the same membranes containing defibrinated blood. The detection of infectious SGHV in the blood remaining on the membranes after feeding flies infected with the SGHV clearly demonstrated that the successive feeding rounds significantly favoured virus transmission within the tsetse colony (Fig. 2) ( Feldmann 1994; Abd-Alla et al. 2010, 2011, 2013b). This important finding was the basis for modifying the feeding regime to manage the SGHV infection (section 3.1.2.).

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*Table 1. Chronological history of the discovery and distribution of SGHVs*[1]

| Major contribution | Reference |
|---|---|
| • First published record of SGH in *Glossina* spp. | Whitnall 1932, 1934 |
| • Suggestion that SGH is sex-linked | Burtt 1945 |
| • Description of virus particles in *G. morsitans* and *G. fuscipes fuscipes*; suggestion of Golgi-ER viral assembly | Jenni 1973; Jenni and Steiger 1974a, b; Jenni and Böhringer 1976 |
| • First published record of SGH in *Merodon equestris* F. | Lyon and Sabatier 1973 |
| • First clear association of viral particles with SGH | Jaenson 1978 |
| • SGH in *M. equestris* | Amargier et al. 1979 |
| • Demonstration that viral particles are infectious *per os*; first report that *Glossina* virus has dsDNA genome | Odindo 1982, 1988; Odindo and Amutalla 1986; Odindo et al. 1986 |
| • First report on reduced insemination rates, fecundity and lifespan in laboratory colonies of *G. pallidipes* | Jaenson 1986 |
| • SGH in wild tsetse from Zimbabwe and Côte d'Ivoire | Ellis and Maudlin 1987; Gouteux 1987 |
| • Poor productivity of *G. pallidipes* colonies at FAO/IAEA, Austria | FAO/IAEA 1987, 1988 |
| • Proposal of *Glossina* virus as a biocontrol agent | Odindo 1988 |
| • Demonstrated transmission of *Glossina* virus after artificial infection | Jura et al. 1988, 1989, 1993; Jura and Davies-Cole 1992 |
| • Cytopathology of virus particles in tsetse salivary glands | Kokwaro et al. 1990, 1991 |
| • SGH in *G. m. swynnertoni* Austen and *G. brevipalpis* Newstead | Shaw and Moloo 1993 |
| • First published record of SGH in *Musca domestica* L. | Coler et al. 1993 |
| • SGHV in tsetse milk glands, midgut and male accessory reproductive glands | Sang et al. 1996, 1997, 1998, 1999) |
| • Collapse of an Ethiopian-derived *G. pallidipes* colony at IPCL, Seibersdorf, Austria | FAO/IAEA 2002 |
| • Virus particles in male accessory reproductive glands of *G. m. morsitans* | Kokwaro 2006 |
| • *G. pallidipes* SGHVs genome sequenced | Abd-Alla et al. 2008 |
| • *M. domestica* SGHVs genome sequenced | Garcia-Maruniak et al. 2008 |
| • Establishment of Hytrosaviridae family | Abd-Alla et al. 2009b |
| • Transcription analysis of *M. domestica* SGHV | Salem et al. 2009 |
| • Description of proteome, ultrastructure and morphogenesis of *Glossina* virus | Kariithi et al. 2010, 2013a |
| • World-wide distribution of SGHV in *M. domestica* | Prompiboon et al. 2010 |
| • SGHV-like virus described in accessory gland filaments of the parasitic wasp *D. longicuadata* (Ashmead) | Luo and Zeng 2010 |
| • Negative impact of antiviral drug (valacyclovir) on SGHV replication in tsetse | Abd-Alla et al. 2012 |
| • Role of endosymbionts on transgenerational transmission of SGHV in *G. pallidipes* | Boucias et al. 2013 |
| • Successful management of *Glossina* hytrosavirus in *G. pallidipes* colonies using modified feeding system | Abd-Alla et al. 2013b |
| • Successful management of *Glossina* hytrosavirus and eradication of SGH in *G. pallidipes* colonies using combination of antiviral drugs and modified feeding system | Abd-Alla et al. 2014 |
| • Description of molecular pathways modulated by GpSGHV in *G. pallidipes* and *G. m. morsitans* | Kariithi et al. 2016 |
| • Genome of *G. pallidipes* SGHV (Ethiopia) sequenced | Abd-Alla et al. 2016 |

[1]Table adapted from Kariithi 2013, used with permission

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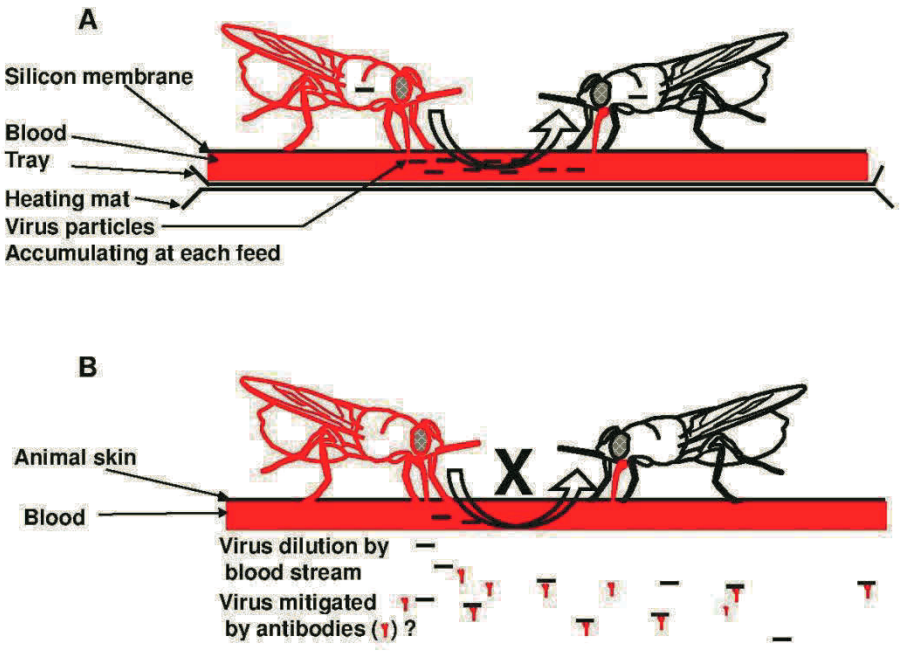



*Figure 2. Horizontal transmission of SGHV in* G. pallidipes*. (A)* In vitro *membrane feeding used in tsetse mass-rearing; (B) Simulation of the* in vivo *feeding on live animals. Red, flies with SGH; black, uninfected flies (Abd-Alla et al. 2011).*

### *2.5.3. Viral Diseases in Mosquitoes*

*Viruses Pathogenic to Mosquitoes.* There are several types of viral pathogens that cause diseases in mosquitoes, most of which belong to four major groups: (1) the baculoviruses (NPVs) (Baculoviridae: Nucleopolyhedrovirus) (the most common mosquito viruses), (2) cytoplasmic polyhedrosis viruses (CPVs) (Reoviridae: Cypovirus), (3) densoviruses (DVs) (Parvoviridae: Brevidensovirus) and (4) the iridoviruses (MIVs) (Iridoviridae: Chloriridovirus). Baculoviruses, densoviruses and iridoviruses are DNA viruses, while cypoviruses are the main RNA viruses in mosquitoes (Becnel 2006; Becnel and White 2007; Kariithi 2013; Tijssen et al. 2016).

The first reported mosquito baculovirus (AesoNPV) was isolated from *Aedes sollicitans* (Walker) in Louisiana in 1969, and over the next two decades, NPVs were isolated from 10 additional mosquito species in the genera *Aedes, Anopheles, Culex, Psorophora, Uranotenia* and *Wyeomyia* (Afonso et al. 2001). Baculoviruses are highly pathogenic, and are responsible for epizootics in field populations of mosquito larvae, e.g. the *Culex nigripalpus* Theobald nucleopolyhedrosis virus CuniNPV (Afonso et al. 2001). Moreover, a baculovirus infection can be destructive to mass-reared mosquito colonies.

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The highly pathogenic mosquito densoviruses (MDVs) are non-enveloped and relatively stable in the field or under laboratory conditions, making it very difficult to inactivate them. The MDVs are highly specific to mosquitoes in aquatic larval habitats. Larvae that survived an MDV infection and developed to the adult stage exhibit a dose-dependent shortening of their adult lifespan. Infected females vertically transmit the virus to their offspring at the new oviposition site. These characteristics make the MDVs suitable biocontrol agents against mosquito populations. In contrast, when densoviral diseases spread in laboratory colonies, these same characteristics usually cause control efforts to be unsuccessful. In most cases the colony has to be destroyed, and equipment and the facility decontaminated (Carlson et al. 2006). MDV infections can be latent, e.g. the *Aedes albopictus* densovirus chronically infecting an apparently healthy subclone of an *Aedes albopictus* C6/36 cell line; this was fortuitously discovered to be highly pathogenic by feeding *Aedes aegypti* (L.) larvae in C6/36 cell flasks (Jousset et al. 1993).

In addition to MDVs, mosquito infection with several other viruses has been reported. For example, recent deep sequencing of samples derived from three colonies of *Anopheles* spp. maintained at the Institute Pasteur (Carissimo et al. 2016) led to the identification of two "novel" RNA viruses, a cytoplasmic polyhedrosis virus (multisegmented dsRNA) and a cripavirus (bisegmented ssRNA) that chronically infected these colonies. A new group of ssRNA viruses with a large genome (20 kb), classified in the family Mesoniviridae, has been identified recently in different species of mosquitoes in the genera *Culex* and *Anopheles* in Côte d'Ivoire (Zirkel et al. 2011, 2013) and in *Culex vishnui* Theobald and *C. taeniorhynchus* Wiedemann in Vietnam (Nga et al. 2011).

*Vector-Borne Diseases and Risk of Infection to Staff.* The mosquito species currently reared in laboratory colonies, including for the SIT, especially in large-scale factories, belong to three genera of culicids: *Aedes, Anopheles* and *Culex.* These species encompass the vectors of the major human parasitic diseases, e.g. malaria or flavivirus arboviral pathogens such as yellow fever, West Nile encephalitis, dengue, Chikungunya and Zika viruses. The vertical transmission of several of these viruses in their natural mosquito vectors has been demonstrated experimentally under laboratory conditions and detected in wild populations of mosquitoes (Bolling et al. 2015; Tesh et al. 2016; Thangamani et al. 2016).

Recent investigations on the mosquito microbiome revealed that, in addition to these classical arboviral pathogens, mosquitoes house diverse varieties of endosymbiotic RNA viruses, many of which are phylogenetically related to human pathogenic arboviruses in the families Flaviviridae, Bunyaviridae and Togaviridae (Cook et al. 2013; Marklewitz et al. 2013; Huhtamo et al. 2014; Bolling et al. 2015; Li et al. 2015). In contrast to classical arboviruses, which replicate in their invertebrate and vertebrate hosts, these viruses are incapable of replicating in vertebrate cells, and are designated as insect-specific viruses (ISVs). Several of these ISVs are vertically transmitted (Saiyasombat et al. 2011; Bolling et al. 2012; Haddow et al. 2013), strongly suggesting their long-term association with their insect host. Experimental data have provided evidence that ISVs can alter the mosquito's susceptibility to certain pathogenic arboviruses (Kent et al. 2010;

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Bolling et al. 2012; Hobson-Peters et al. 2013; Kenney and Mertz 2014). These data have opened up new perspectives for the development of mosquito strains refractory to the transmission of vertebrate arboviruses.

Taken together, these data imply a risk to staff in mass-rearing mosquitoes – they might become infected with mosquito-borne diseases. Therefore, strict measures must be followed to avoid the introduction of infected mosquitoes into colonies, or exposure of infected humans to the mosquito colonies. In this respect, field-collected mosquito samples should first be quarantined and diagnosed for virus infections using sensitive diagnostic tools, e.g. by qPCR, prior to the introduction of such mosquitoes into the existing fly colonies or in the establishment of new colonies. To protect staff from infections with mosquito-vectored viruses, protective measures must be undertaken, e.g. minimizing and eliminating escapees, wearing protective clothing and adhering to control measures (ASTMH 2001). Personal protection is especially important in the event that mosquitoes escape from rearing cages. Moreover, strict hygiene in the environment around mass-rearing facilities is important to prevent escaped mosquitoes from finding breeding places; this also minimizes the chance of staff becoming infected with a virus and the reintroduction of a virus into the colonies. An additional risk in respect to mass-reared mosquito colonies is the emergence of a latent virus infection, especially under stressful rearing conditions, e.g. high densities, which might compromise the mosquito immune system. Therefore, to ensure colony safety and sustainability, it is recommended that mosquito colonies be surveyed routinely for both pathogenic and mosquito-borne viruses.

### *2.5.4. Viral Diseases in Reared Fruit Flies and Wild Populations*

As in mosquitoes (section 2.5.3.), several viruses have been identified in fruit flies (Webster et al. 2015). In addition to the covert infections of laboratory or wild strains of Drosophilidae (section 2.5.), several RNA viruses have been isolated from species of Tephritidae belonging to two genera of major economic interest - *Ceratitis* and *Bactrocera* (*Dacus*). Plus et al. (1981) isolated a reovirus and a picorna-like virus from the J. C. R. Ispra strain of *Ceratitis capitata* (Wiedemann). Bashiruddin et al. (1988) found a picornavirus in various life stages of the Queensland fruit fly *Bactrocera tryoni* (Froggatt) in a laboratory colony; the virus induced premature mortality and decreased fecundity in infected flies.

Pathogenicity tests using various insect viruses demonstrated the sensitivity of the olive fruit fly *Bactrocera oleae* (Rossi) to *per os* infection with the picornavirus Cricket Paralysis Virus (CrPV). Feeding the flies on a solution containing the virus resulted in 50% mortality in 5 days and more than 80% mortality within 12 days. The virus was found to be horizontally transmitted from infected to healthy flies by fecal contamination. The flies could also be infected with the type 21 iridovirus from *Helicoverpa armiger*a (Hübner) (Manousis and Moore 1987). A virus exhibiting the salient structural and genomic features of reovirus virions was isolated from wild *B. oleae* flies from Greece. This virus actively replicated in midgut cells where it aggregated in cytoplasmic paracrystaline arrays, and was abundantly released to the gut lumen through the microvilli (Anagnou-Veroniki et al. 1997).

The Mediterranean fruit fly *C. capitata* was reported to be infected with the

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*Drosophila melanogaster* Meigen C picornavirus (DCV); the infection resulted in increased virus titre and fly mortality (Jousset 1976). Recently, three novel picornaviruses infecting *C. capitata* were discovered and named as *C. capitata* iflavirus 1 and 2 (CcaIV1 and CcaIV2) and *C. capitata* noravirus (CcaNV). The CcaIV2 and CcaNV infections were abundant in most of the fly strains used in the various SIT projects around the world, as well as in field captures in the east of Spain. Although there is no direct proof that the virus infection affects the fly's performance, high viral titres of CcaNV were associated with a reduction in the lifespan of males released into the field for the control of this pest. Taken together, these data strongly suggest the possibility that CcaNV impairs the fitness of the sterile flies (Llopis-Gimenez et al. 2017).

In addition to tephritid fruit flies, several laboratory or wild strains of Drosophilidae have been reported to be infected covertly with many RNA viruses, e.g. the DCV (Arnold et al. 2015), the Sigma virus (Rittschof et al. 2013), the Nora virus (Cordes et al. 2013) and the Drosophila P virus (Plus and Duthoit 1969; Teninges and Plus 1972). Further, the spotted-wing drosophila *Drosophila suzukii* (Matsumura) was reported to be infected with Drosophila C virus and Flock House virus (Cattel et al. 2016).

### *2.5.5. Viral Diseases in Honeybees, Silkworms and Crickets*

Historically, honeybees and silkworms are the most important insects used in the production of economic products. Many diseases, including viral diseases, were first recorded in these insects (Trager 1935; Glaser and Stanley 1943). However, the viral diseases in honeybees and silkworms are not discussed here; they have been well documented by others (Aruga 1971; Cox-Foster et al. 2007; vanEngelsdorp et al. 2009; Francis et al. 2013; Jiang and Xia 2014).

Recently, the intensive mass-production of field crickets as pet food resulted in the presence of viral diseases, causing enormous losses and requiring urgent intervention (Weissman et al. 2012).The first epizootic of the European house cricket *Acheta domesticus* (L.), with a densovirus (AdDV) as the causative agent, was detected in a Swiss commercial factory (Meynadier et al. 1977). Several other outbreaks have been observed in Europe, but it was only in 1991 that an AdDV epizootic was reported in a cricket farm in south-eastern USA (Styer and Hamm 1991). In 2009/2010 severe AdDV outbreaks in cricket farms decimated the multimillion dollar cricket pet food industry all over North America, from Quebec to Alberta and Florida to California (Weissman et al. 2012). Sequence analyses of the viral genome revealed a 99% common identity between the 1977 European isolate and different 2009 North American isolates. This finding demonstrated the very high genetic stability of the virus, and provided evidence that these epizootics were not due to the emergence of novel virulent strains of the virus (Szelei et al. 2011).

## 3. STRATEGIES TO MANAGE PATHOGENS IN INSECT MASS-REARING

Some of the viruses, bacteria, protozoa and fungi found in insect mass-rearing are known disease-causing pathogens, but others have no known host-fitness costs. Pathogens may be introduced into mass-rearing facilities from the field-collected

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insects used to establish colonies, or could be latent and erupt as a result of intense and continuous rearing under stressful rearing conditions in the facilities. Some of the conventional pathogen management techniques in insect mass-rearing include isolation and rearing of healthy individuals from colonies that manifest mixed infections, changing colony environmental conditions and the application of antibiotics. Although these techniques are useful in the management of disease outbreaks, their effectiveness is mostly short-lived. Effective pathogen management largely depends on the type, transmission mode, and persistence of the pathogens as well as the manifestations of the disease.

The next five sections highlight the effective management of salivary gland hypertrophy syndrome (SGH) in mass-rearing tsetse flies. Also discussed are different approaches and techniques that are used in the management of viral and non-viral diseases in mass-rearing codling moths, honeybees, and silkworms.

### *3.1. Combined Approaches to Manage SGHV in Tsetse Mass-Rearing*

Outbreaks of the SGH syndrome in tsetse mass-rearing facilities are a rare occurrence in view that SGHV presence is mostly asymptomatic. However, when the SGH occurs, the fecundity of some tsetse species, especially *G. pallidipes*, is compromised. Except in *G. pallidipes* colonies, SGHV infections are mostly asymptomatic, but unknown factors, such as environmental stressors and perhaps host physiology, can promote SGH outbreaks, resulting in the loss of colony productivity and the decline of an entire tsetse colony over several generations (section 2.5.2.). The mechanisms by which SGHV infections persist in tsetse colonies are known partially. However, it is clear from various experiments and observations that the virus is vertically transmitted and persists in subsequent fly generations (Boucias et al. 2013). Horizontal SGHV transmission is well documented (Abd-Alla et al. 2010), and is the principal route through which viral infections spread in tsetse mass-rearing (Fig. 2). Therefore, it is important to minimize the risk of SGH outbreaks, which can be done through several approaches, as described below.

#### *3.1.1. Antiviral Drugs*

For viruses that are intermittently reactivated from latency to symptomatic disease manifestations, antiviral drugs can be administered to keep the virus infection in check. Studies in human herpesviruses (HHVs, family Herpesviridae) have shown that the administration of antiviral drugs such as acyclovir and valacyclovir reduced the prevalence, titre and shedding of the virus from infected host cells (De Clercq 2007). In principle, these antiviral drugs are used at concentrations that are not detrimental to the DNA synthesis of the host (Bras et al. 2001; De Clercq 2003; Miller et al. 2005), and are converted to active metabolites by virally encoded thymidylate synthase enzyme. In addition, evidence shows that the SGHV shares a high homology with HHVs, including DNA polymerase and thymidylate synthase enzymes (Kariithi et al. 2013b), which are the antiviral target genes, inhibiting viral DNA replication. Thus, these drugs are ideal chemotherapeutic agents against viral infections in mass-reared insects. Overall, the administration of antivirals results in

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reduced viral titre. Reduction of viral shedding results in reduced horizontal transmission of the virus into blood meals during *in vitro* membrane feeding, thereby keeping the virus undetected in the colonies. Success was achieved in controlling the SGHV in the tsetse mass-production facility in Ethiopia and in the Seibersdorf Laboratory; oral administration of valacyclovir (at a dose of 300 µg/mL blood) for over 18 months significantly reduced SGHV loads and suppressed SGH outbreaks to undetectable levels (Abd-Alla et al. 2008) (Fig. 3). Importantly, such long-term administration of valacyclovir resulted in acceptable levels of fly productivity and mortality. It is important to note that valacyclovir reduced viral DNA replication. It will be more effective when it is administered to flies with a low virus titre, but not to flies with a symptomatic infection.

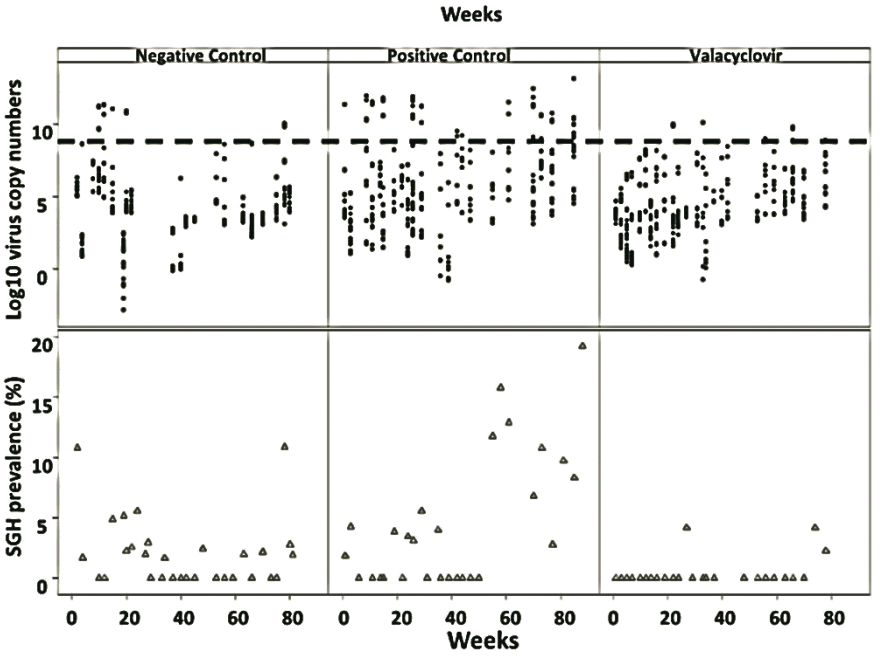



*Figure 3. Effects of long-term treatment with the antiviral drug valacyclovir on* G. pallidipes *biology and virus prevalence. SGHV virus loads and SGH prevalence determined by qPCR and dissection, at 60 days after feeding. (---): threshold virus load correlated with SGH symptoms. Negative control: flies fed exclusively on clean blood meals; positive control: flies fed on virus-contaminated blood meals; valacyclovir: flies fed on virus-contaminated blood supplemented with 300 µg/mL valacyclovir. Data points for virus load are for individual flies (Abd-Alla et al. 2012).*

### *3.1.2. Clean Feeding System*

In tsetse mass-rearing facilities, the *in vitro* membrane feeding system, which involves feeding of up to 10 sets of fly feeding cages on the same membrane in succession, favours horizontal transmission of the virus (Feldmann 1994; Abd-Alla et al. 2012). A single SGHV-infected individual is able to shed up to $10^7$ infectious

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viral particles into the blood meal during a 10–15 min feeding event (Abd-Alla et al. 2010) (Fig. 2), thereby infecting most of the non-infected flies in the cages that feed afterwards. This results in the spread and long-term maintenance of the virus in the colonies. Consequently, it was thought that, in principle, the horizontal SGHV transmission can be efficiently interrupted by modifying the membrane feeding regime. This was the origin of the clean feeding system (CFS), which is now one of the most cost-effective anti-SGHV management strategies in tsetse mass-rearing facilities (Abd-Alla et al. 2010, 2013b).

Implementation of the CFS involves providing each fly cage with fresh blood at each meal to prevent flies from picking up the virus shed during the feeding of flies in earlier cages without increasing the cost. The CFS consists of three basic steps: (1) teneral flies and their subsequent progenies are always fed first, (2) after feeding the maximum possible numbers of cages on this first round (determined by the available resources, i.e. number of feeding trays), the rest of the fly cages are fed on the same membranes in a second round, and (3) after the first and second feeding rounds, the remaining colony flies, i.e. the oldest, are fed last on the same membranes (Abd-Alla et al. 2013b). In the CFS setting (Fig. 4), one of the most important issues is to keep separate all the fly records and pupae collection and incubations from these three colony feeding groups. Within two years of its implementation, the CFS was hailed as a big success; virus prevalence was significantly reduced and the SGH symptoms were completely eliminated from the colonies (Abd-Alla et al. 2013b). As a result, no SGH outbreaks have been reported in the colony in the two years since the CFS was put in place at the Seibersdorf Laboratory. It is worth noting that the CFS was implemented using existing resources without adding extra cost.

### *3.1.3. Combined Approaches*

To manage SGHV infections effectively in tsetse mass-rearing, a multi-tactical approach is the best option. Based on experience in dealing with SGHV infections gained over the last decade, some of the extremely important tactics to manage viral infections include: (1) strict sanitation of facilities, equipment and adult cages, (2) regular monitoring of SGH symptoms (fly dissection and PCR diagnosis), and (3) quarantine, e.g. by keeping a virus-free stock colony separate from the main colony. In addition to existing colonies, many new tsetse mass-rearing colonies are being established from field-collected flies in different African regions, with little or no awareness of SGHV prevalence. Since the SGHV is present in wild tsetse populations, there is a risk of introducing the virus to the new colonies.

The implementation of the CFS or administration of antiviral drugs alone in the management of SGHV infections requires a long time to significantly bring down the SGH prevalence to acceptable levels (below 10%). Importantly, the trials described by Abd-Alla et al. (2013b) were based on small fly groups with relatively low SGH prevalence (compared with the usual high prevalence in normal colonies). The CFS or valacyclovir alone reduced the prevalence of symptomatic infections (SGH) to an average of 1.9% and 0.63% within 28 and 21 months, respectively (Abd-Alla et al. 2012). As administration of the antiviral drugs in mass-rearing facilities is very feasible given that these drugs are cheap and readily available

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commercially, a combination of the CFS (consisting of sanitation, modified blood feeding, SGH monitoring and quarantine) and the administration of valacyclovir was attempted on a large scale (Abd-Alla et al. 2014). Combined, the CFS and the supplementation of blood meals with valacyclovir resulted in complete elimination of the disease symptoms (from an initial 24% SGH prevalence) within six months of implementation. Taken together, an integrated approach can be used by combining these anti-SGHV strategies.

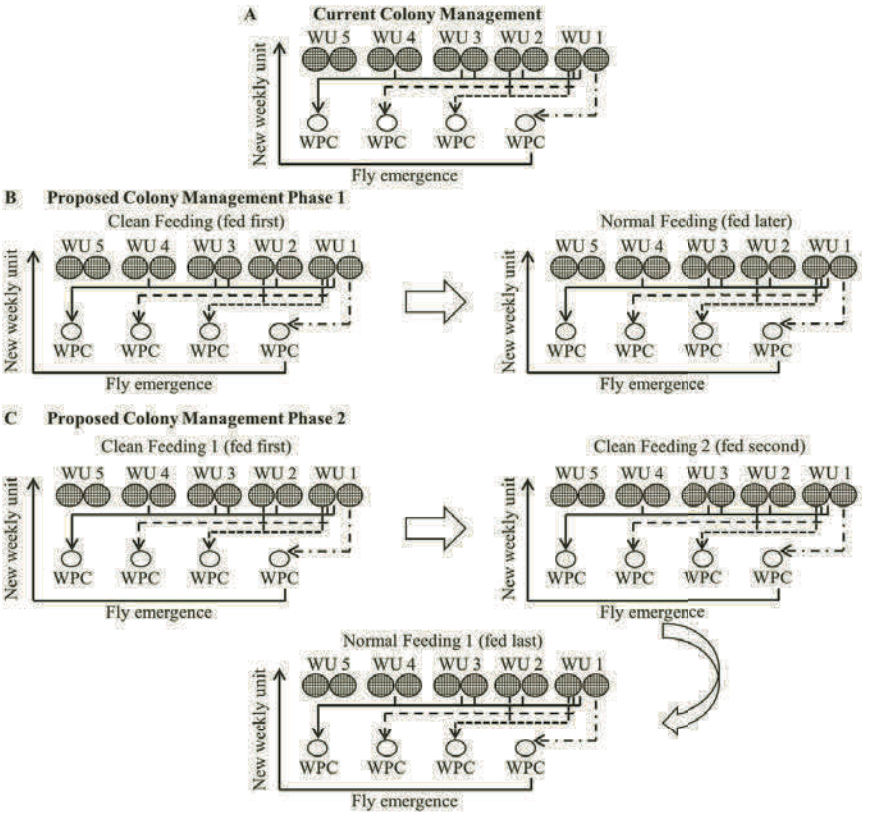



*Figure 4. Handling, feeding and management of a tsetse colony. (A) Regular* in vitro *membrane feeding system as routinely practised in tsetse mass-production factories, (B) intermediate phase, and (C) final phase of the clean feeding system. WU = weekly unit, WPC = weekly pupal collection (Abd-Alla et al. 2013b).*

### *3.1.4. Impact of Implementing the Combined Approach on Colony Performance*

The long-term application (more than 3 years) of antiviral drugs to manage SGHV infections in the mass-rearing of *G. pallidipes* colonies has not resulted in any observable negative effect on fly productivity and survival. The single-most important aspect of the combined approach, in the case of an SGH outbreak, is the ability to restore colony productivity rapidly (within six months) without any

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additional cost in specialized equipment or reagents, except that minimal training of staff on colony handling protocols is required. Importantly, in case supplementing blood meals with a drug results in an undesirable side effect, or there is a potential risk of resistance development, the drug can be withdrawn once the SGH outbreak has been successfully managed; thereafter, the CFS protocols alone should reduce an SGHV infection to an acceptable level. Ideally, if frequent and fluctuating SGH outbreaks occur in a mass-rearing facility, the administration of antiviral drugs would reduce SGH prevalence significantly; then the sustained use of the CFS should follow. For rearing facilities with low SGH or non-observable SGH outbreaks, the CFS should be maintained so that that disease symptoms are not manifested.

*3.1.5. Novel Prospective Approaches to Manage SGHV in Tsetse Mass-Rearing*

Paratransgenesis, i.e. the expression of antiviral molecules by transformed endosymbionts, is a viable option to manage viral infections in tsetse mass-rearing. Paratransgenesis was first achieved in *Rhodnius prolixus* Stål, vector of Chagas' disease, via transformation of its symbiont *Rhodococcus rhodii* (Abd-Alla et al. 2014). This approach has been hailed as a promising strategy in the creation of pathogen-refractory mosquitoes (Beard et al. 1992). *Wolbachia* is a potential endosymbiont for creating SGHV-refractory tsetse strains; it induces antiviral activity in insects (Wilke and Marrelli 2015). Another symbiont that can be targeted for paratransgenesis in tsetse flies is *Wigglesworthia*, which upregulates the host's cellular defence system ( Saridaki and Bourtzis 2010; Rances et al. 2012; Weiss et al. 2012). Recently, Boucias et al. (2013) showed that removal of *Wigglesworthia* from *G. pallidipes* significantly reduced SGHV replication in adult flies and suppressed virus vertical transmission to fly progeny. The symbionts can be artificially cultured, genetically engineered and stably fixed into the tsetse genome to express molecules that interrupt SGHV replication and transmission. Such virus-refractory tsetse lines are stable because the endosymbionts are maternally transmitted to the next fly generation. Attempts have been made to identify potential candidate transgenes that can be exploited in creating paratransgenic tsetse lines (Boucias et al. 2013).

Another potential approach to managing virus infections is applying RNA interference (RNAi), which can also be mediated via bacterial endosymbionts (Abd-Alla et al. 2016; Kariithi et al. 2016). The feasibility of this approach is underscored by the presence in the tsetse genome of the key components of the RNAi machinery, including several copies of Dicer (*dcr*), Argonaute (*ago-1* and *2*) and some components of the dsRNA-binding genes (Whitten et al. 2016). On the other hand, the genome of the SGHV contains some of the key genes involved in viral DNA replication, including late effector factors (*lef*), DNA polymerase (*dnapol*), DNA helicase (*dnahel*) and the *per os* infectivity factor (*pif*) genes (IGGI 2014). The identification of these key genes implies that the RNAi system is not only functional in tsetse flies but may also be actively involved in keeping the SGHV under control; apparently only a few of the viral genes are expressed during asymptomatic infections to avoid detection by the host's immune system. Preliminary results

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indicate that RNAi-mediated silencing of SGHV genes is feasible (I. K. Meki, personal communication).

#### *3.1.6. Technology Transfer from the Laboratory to Large-Scale Mass-Production Facilities*

It is a challenge to transfer the technology of a newly developed pathogen management strategy from small-scale laboratory trials to a wider operational application. This is particularly so because, unlike controlled laboratory settings, external uncontrolled environmental factors could limit the technology's effectiveness under mass rearing operations. Even before the technology is transferred to operational applications (in mass-rearing, and in the field where applicable), there is the challenge of sustaining the technology in the laboratory. For example, in the case of the application of transgenic and RNAi approaches, the stability of the transgenes requires finding ways to maintain the transgene constructs (preferably as embryos) and to make rigorous evaluations under mass-rearing conditions. These embryonic transgenes can be used as stock for the operational applications. If there is no effective means of ensuring the stability of these technologies under mass-rearing conditions, the advantage of having a fall-back plan is jeopardized because the transgenes will be lost. So far, most laboratory trials have not been successfully transferred to the operational level. However, there is a noteworthy exception -- the IAPV-RNAi laboratory trial was field-tested in beehives in the USA (Abd-Alla et al. 2016; Kariithi et al. 2016). Also, transgenesis in silkworm mass-rearing has made significant progress in technology transfer from the laboratory to contained trials at operational level in multiple locations (Hunter et al. 2010).

The case of the combined anti-SGHV management strategy developed at the IPCL tsetse production facility in Seibersdorf is an example of how technology can be successfully transferred from laboratory trials to an operational level with widespread field application (FAO/IAEA 2015a). The technology was transferred to a tsetse mass-rearing facility in Kality, Ethiopia. This was done primarily by developing standard operational procedures (SOPs), training staff in the mass-rearing facility on the correct manner of implementing the new technology and establishing ways to evaluate it. The implementation of the management package resulted in the complete elimination of SGH symptoms and, moreover, according to PCR, eliminating the GpSGHV infection (Fig. 5).

### *3.2. Management of CpGV in Mass-Rearing the Codling Moth*

In addition to infection with pathogenic fungi, microsporidia, nematodes and bacteria, mass-reared colonies of the codling moth are contaminated with *C. pomonella* granulovirus (CpGV) (Subbaiah et al. 2013). CpGV infections are mainly observed in larvae, but the infections are largely asymptomatic. CpGV is an extremely specific and highly virulent viral pathogen of the codling moth. Once ingested by the larvae, the virus infects the digestive tract causing the disease which kills within 3–7 days. The subsequent rupturing of the larval skin allows the CpGV virion particles to infect other larvae and to spread in the colony. The routes of entry

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of CpGV into mass-rearing facilities are through the air filtration systems, walls of the rearing rooms and spent diet (Zimmermann et al. 2013). Since handling is the main contributor to accidental contamination of the codling moth with CpGV (Cossentine et al. 2005), the most common practices to control CpGV infection include adding formaldehyde to the larval diet (Dyck 2010; Zimmermann et al. 2013) and general hygiene, e.g. washing egg sheets and disposing of spent diet and dead larvae.

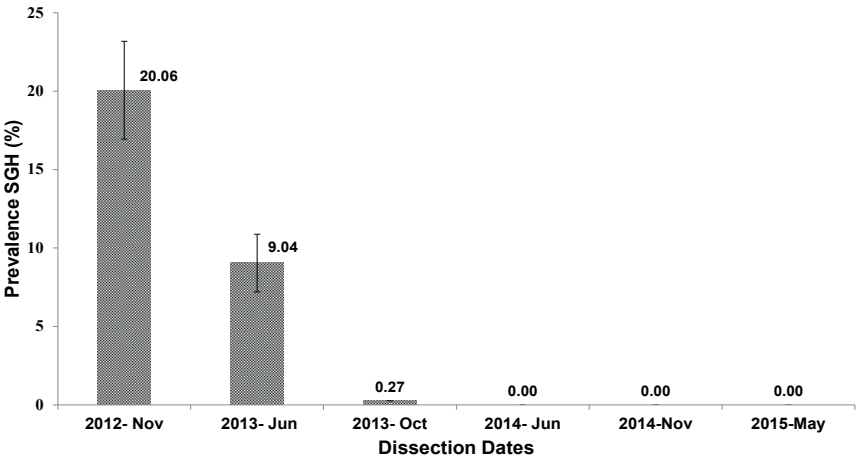



*Figure 5. Impact of implementing a combined virus management strategy on SGH prevalence in* G. pallidipes *in the tsetse mass-rearing facility in Kality, Ethiopia.*

An alternative strategy to control CpGV infections is to establish virus-free laboratory colonies. This can be achieved by using codling moth strains that are known to be CpGV-resistant. CpGV-resistant strains have previously been reported in various commercial orchards in Europe routinely subjected to CpGV treatments (Schmitt et al. 2013). The fact that the CpGV is vertically transmitted (transovum) in codling moth colonies ( Brinton et al. 1969) can be exploited in the establishment of virus-free colonies. For example, surface decontamination of the eggs sheets can successfully remove low levels of CpGV infection. This is a cheap procedure -- a 20-min egg sheet swabbing in simple solutions such as 0.5% sodium hypochlorite/1% Tween 20, or a 6-h UV-B exposure, followed by rinsing with distilled water to maintain egg viability (Etzel and Falcon 1976; Dyck 2010). Once such a virus-free colony is established, the possibility that a low-level virus infection still remains can be monitored easily by PCR. Primer and PCR amplification protocols for the detection of CpGV in codling moth populations have been described (Kundu et al. 2003).

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### *3.3. RNAi Approach to Manage Pathogens of the Honeybee*

In the absence of effective antiviral chemotherapeutics in hives of the European honeybee (*Apis mellifera* L.), symptomatic viral infections are minimized by reducing stress, e.g. maintaining healthy colonies, and chemical control of *Varroa*, disinfection and selection for disease-resistant honeybee races (Chen 2011; Martin et al. 2012). Modern antiviral strategies include RNA interference (RNAi), which can significantly reduce viral titres up to 100% and mortality up to 90%. Notable success stories include the reduction of honeybee mortality caused by the Israeli acute paralysis virus (Maori et al. 2009), significant reduction of infection (27–60%) of the Asian honeybee *Apis cerana* F. with the Chinese sacbrood virus (Liu et al. 2010) and protection of European honeybees against infection with the deformed wing virus (Desai et al. 2012). Additionally, the threat caused by *Varroa* mites (viral reservoirs, incubators, activators and competent viral vectors) can be significantly reduced, up to 97%, by RNAi (Campbell et al. 2010; Garbian et al. 2012).

### *3.4. Transgenic Approaches to Manage Pathogens of the Silkworm*

There are no curative measures against viral diseases in silkworms. Conventional management measures include preventing virus transmissions (destroying the virus transmission cycle and breeding for resistant races), enhancing silkworm vigour (rearing hardy races and eliminating unfavourable conditions), diagnosing early viral disease (culling out sick worms and therapeutics), and taking prophylactic measures (disinfecting silkworms and rearing sheets). These methods are inexpensive and quite effective, but are not sufficient. Modern (transgenic) antiviral methods have been successfully applied in the silkworm industry without compromising the cocoon crop yields, resulting in enhanced resistance to baculovirus (*Bombyx mori* nucleopolyhedrovirus BmNPV) in silkworms (Watanabe 2002; Isobe et al. 2004; Kanginakudru et al. 2007; Jiang et al. 2012a, b; Subbaiah et al. 2013; Jin et al. 2014). The effectiveness of the transgenic silkworms could be further optimized by combinations of various molecular technologies. The best approach would be pre-selecting silkworm parents with a substantial level of natural virus-resistance, creating hybrids and enhancing their refractoriness to virus infection.

### *3.5. Management of Non-Viral Diseases in Insect Mass-Rearing*

Among the non-viral disease-causing agents in mass-reared colonies are: bacteria (Bacillaciae, Pseudomonadaceae and Enterobacteriaceae), rickettsia (Rickettsiaceae), protozoa (Amebiases, Gregarine and Coccidian), fungi (Entomophthorales and Muscardine), microsporidia and spiroplasma (Jiang et al. 2013a, b). Most of these contaminating microbes are acquired through the diets, and can alter the nutritional value of insect diets. Deliberate measures must be put in place to monitor microbes, e.g. using bioassays, and then prevent microbial contaminations because they are a major problem affecting the mass-rearing of insects (Sikorowski and Lawrence 1994).

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Most of the bacterial diseases affect the larval stages of the insects (Gouli et al. 2011). Although the majority of these entomopathogenic bacteria are transmitted orally, they can also be vectored by nematodes and parasitoids. On the other hand, fungal diseases are transmitted (via infectious asexual spores) through penetration of the insect cuticle, but a few are orally transmitted. Upon infection, the fungus grows in the hemolymph and produces lethal toxins. New spores can be produced after the death of the host, thereby spreading the fungal infection in the colony. Amongst the bacterial control measures are disinfection and pasteurization, e.g. combinations of heating and antimicrobials to inhibit microbial growth. This approach should be practiced in combination with decontamination of the diet and rearing containers. For example, in tsetse mass-rearing, the feeding trays and membranes are frequently autoclaved, while the blood diet batches (which are usually decontaminated by irradiation prior to delivery to the mass-rearing facilities) are routinely monitored with petri dishes for contamination. In addition, other control measures include strict observance of personal hygiene of the workers, maintenance of a clean and sanitary environment, and sterilization of insectary equipment. Inflow and accumulation of air-borne microbial contaminants can be effectively prevented by air filtration (Feldmann 1994; Sikorowski and Lawrence 1994; FAO/IAEA 2006, 2015b, 2019).

Microsporidia are close relatives of fungi, and are transmitted either orally (via spores) or vertically (via eggs) (Lietze et al. 2010). Upon infection, these pathogens develop within the cytoplasm, eventually causing death, either directly (due to cellular damages) or through septicaemia (via secondary bacterial infections of the damaged cells). The highly species-specific microsporidia often infect insect populations chronically without disease epidemics, but they can be fatal in some mass-rearing facilities (Eilenberg et al. 2015). Microsporidia can be controlled by biennial (autumn and spring) applications of antibiotics, e.g. Fumagillin in honeybee colonies, but some species have shown signs of resistance to antibiotics (Huang et al. 2013). Applying antibiotics is usually combined with replacing old, dark brood combs.

Entomopathogenic rickettsia and spiroplasma are efficiently transmitted vertically and horizontally; a high prevalence of these pathogens results in mortality and severe losses of fecundity and general colony fitness. Due to the nature of their transmission in mass-production settings, i.e. mainly through contact, various infrastructural improvements can be implemented to significantly manage their infections and to improve rearing quality. These include circulating water and air filtration systems, continuous monitoring and waste removal, continuous larval/pupal separation and sterilization (routine hygiene), appropriate measures to reduce and prevent the development of pathogens, and a pathogen-free optimal diet.

Artificial mass-rearing conditions eventually lead to a decrease in the immune fitness of the insects (Yates and Antia 2006; Hawley and Altizer 2011; Ugelvig and Cremer 2012). Therefore, to replenish colonies with individuals having a high immune capacity, it is necessary to replace periodically or outbreed the colonies with fresh pathogen-free field-collected samples/specimens. For example, the colonies could be outbred with locally caught conspecifics (which should be PCR-screened for pathogen infection before introducing them into existing colonies). It is strongly recommended that, in the case of mass-rearing tsetse flies, newly

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introduced conspecifics should be maintained under the above-mentioned combined approach to prevent an SGH outbreak.

## 4. CONCLUSIONS

Pathogens affect the quality of mass-reared insects. Therefore, it is very important to manage microbial and in particular virus infections in insect colonies during the mass-rearing process. Although there is still much to be learned about insect pathogens, in general microbial and fungal pathogens are currently managed using antibiotic and anti-fungal compounds, in addition to implementing the required sanitary procedures, but viral disease management when mass-rearing moths requires the use of formalin. However, for working staff, formalin is a hazardous chemical, and also it cannot be used to manage viral diseases when mass-rearing tsetse flies or mosquitoes. Therefore, alternative virus management strategies are needed, and thus this chapter focused on the management of viral pathogens. Inglis and Sikorowski (2009a, b) reviewed in detail the management of other insect pathogens.

Applications of transgenics and RNAi-gene silencing drugs, and the oral administration of antiviral drugs, have proven to be potent measures in managing viral infections in tsetse mass-rearing, apiculture and sericulture. However, it is clear that no single method can be used alone to manage effectively viral infections in insect mass-rearing; instead, an integrated approach is required. Molecular-based methods are a good complement to conventional virus management practices. The application of these measures should be accompanied by accurate and routine diagnosis of infections, and proper documentation of disease manifestations. To avoid the risk of developing drug-resistance, the application of antiviral drugs, especially in tsetse mass-rearing, should be restricted to colonies with comparatively high SGH outbreaks; the unnecessary administration of antiviral drugs to healthy colonies should be avoided. For colonies with a low SGH prevalence, a best management practices (BMP) approach entails applying preventive measures such as CFS and an avoidance of stressors as the main pillars, while not using antiviral drugs. To develop sustainable virus management strategies for mass-rearing insects, the link between covert and overt infections needs further investigation.

There are several important aspects to note in managing pathogens: (1) it is important to monitor the occurrence of disease symptoms and perform regular diagnostics, (2) a hygienic and optimal environment in the colonies must be maintained; all insects showing disease symptoms must be removed immediately and appropriately destroyed, (3) insect colonies should be kept in different stocks – keeping them separate from the mass-rearing is easier then managing an outbreak that infects the whole facility, (4) it is advisable to outbreed colonies at intervals (to replenish genetic diversity, if possible with strains that are pathogen-resistant), and (5) hybrid lines should be reared separately; these can be used to establish highly productive colonies in the event that the productivity of the parent colony declines.

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# CHAPTER 3.4.

# STERILIZING INSECTS WITH IONIZING RADIATION

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## SUMMARY

Exposure to ionizing radiation is currently the method of choice for rendering insects reproductively sterile for area-wide integrated pest management (AW-IPM) programmes that integrate the sterile insect technique (SIT). Gamma radiation from isotopic sources (mainly cobalt-60 or decreasingly caesium-137) is commonly used, but high-energy electrons and X-rays are other practical options. Recently, the development of self-contained low-energy X-ray irradiators for insect sterilization is being encouraged because they only require access to electricity and cooling water, and avoid the complex and costly shipment of radioactive sources across international borders. Insect irradiation is safe and reliable when established safety and quality-assurance guidelines are followed. The key processing parameter is absorbed dose, which must be tightly controlled to ensure that treated insects are sufficiently sterile in their reproductive cells and yet able to compete for mates with wild insects. To that end, accurate dosimetry (measurement of absorbed dose) is critical. Data from more than 5300 studies on irradiation since the 1950s, covering more than 360 arthropod species (IDIDAS database), indicate that the dose needed for sterilization of arthropods varies from less than 5 Gy for the acridid *Schistocerca gregaria* (Forskål) to 400 Gy or more for some lyonetiid, noctuid, and pyralid moths. Factors such as oxygen level, insect age and stage during irradiation, and many others, influence both the absorbed dose required for sterilization and the viability of irradiated insects. Consideration of these factors in the design of irradiation protocols can help to find a balance between the sterility and competitiveness of insects produced for programmes that release sterile insects. Some programmes prefer to apply “precautionary” radiation doses to increase the security margin of sterilization, but this overdosing often lowers competitiveness to the point where the overall induced sterility in the wild population is reduced significantly.

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## 1. INTRODUCTION

The potential of ionizing radiation to interact with materials has numerous applications in industry, medicine, and agriculture (Arthur et al. 2015; IAEA 2018). Ionizing radiation breaks down molecules, causing various effects in irradiated material. Radiation can cause polymerization of plastics, and can kill pathogens and other micro-organisms, leading to applications in food processing and the sterilization of health-care products. In organisms, which are composed of differentiated and undifferentiated cells, mitotically active cells, such as stem and germ cells, are the most radiation-sensitive cells. In the case of the sterile insect technique (SIT) and related biocontrol applications, as well as post-harvest quarantine treatments (Heather and Hallman 2008; Bloem et al. 2009), radiation can make an insect reproductively sterile by damaging the chromosomes of gonial cells. Specifically it causes germ-cell chromosome fragmentation (dominant lethal mutations, translocations, and other chromosomal aberrations), leading to the production of imbalanced gametes and subsequently the inhibition of mitosis and death of fertilized eggs or embryos (Klassen and Vreysen, this volume; Robinson, this volume). In adult insects, midgut stem cells, which undergo continuing mitotic divisions (such as in the cotton boll weevil *Anthonomus grandis grandis* Boheman), are particularly sensitive to ionizing irradiation, and the irradiation of certain species may cause a significant reduction in lifespan and increased mortality (Riemann and Flint 1967). Nevertheless, the successful sterilization of other radiation-sensitive insect species, without a reduction in their lifespan, may indicate that the effect on cell replacement in the midgut could be mitigated (Sakurai et al. 2000). Differentiated somatic cells are generally less sensitive to radiation than are stem cells. Thus a lethal effect requires a higher radiation dose than a reproductive sterilization effect. The impact of radiation on somatic cells is expressed as the development of abnormalities, a reduction in lifespan, flight ability, mating propensity, and nutrition, and finally insect death.

Radiation sterilization of insects is a relatively straightforward process, with reliable quality control procedures. The key parameter is the radiation absorbed dose which is expressed in Système International d'Unités (SI) units as gray (Gy) (1 Gy = 100 rad), where 1 Gy is equivalent to 1 joule (J) of absorbed energy in 1 kg of a specified material (1 Gy = 1 J/kg). As long as the correct dose is delivered at the appropriate stage of development, efficacy of the irradiation process is guaranteed.

Other advantages of using radiation to sterilize insects include: (1) temperature rise during the process is insignificant, (2) sterile insects can be released immediately after processing, (3) irradiation, unlike chemosterilants, does not add residues that could be harmful to human health or the environment, and (4) radiation can pass through packaging material, allowing insects (or commodities in the case of post-harvest treatments) to be irradiated after having been packaged.

In the 1950s and 1960s, numerous mutagenic chemicals were tested as alternatives to radiation to induce sterility in insects (Knipling 1979; Klassen and Vreysen, this volume; Lance and McInnis, this volume). Chemosterilants were added to rearing diets, applied topically to insects, or even deployed in attractant-baited devices in the field. The efficacies of irradiating and chemosterilizing insects for population control were, in general, similar (Guerra et al. 1972; Flint et al. 1975; Moursy et al. 1988). However, today, chemosterilants are not used for sterilizing mass-reared insects. Most

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chemosterilants that were investigated in early studies are carcinogenic, mutagenic, and/or teratogenic, leading to environmental and human-health issues such as the integrity of ecological food chains, waste disposal, e.g. spent insect diet, and worker safety (Hayes 1968; Bracken and Dondale 1972; Bartlett and Staten 1996). More recently, a group of less broadly toxic compounds (insect growth regulators) has been studied as chemosterilants, but primarily as alternative insecticides, or in attractive autodissemination devices, rather than for use in SIT projects, e.g. Casaña-Giner et al. 1999; Moya et al. 2010; Bouyer and Lefrancois 2014). Insect resistance to chemosterilants is an additional concern (Klassen and Matsumura 1966).

Sterile insects are also being produced that are transgenic and contain conditionally expressed dominant lethals (Thomas et al. 2000; Harris et al. 2011; Häcker et al., this volume). However, at this time, exposure to ionizing radiation is still the principal method of inducing sterility for area-wide integrated pest management (AW-IPM) programmes that release sterile insects.

## 2. RADIATION SOURCES

The suitability of a radiation type for the SIT depends on properties, such as relative biological effectiveness (RBE), penetrability, availability, safety, and cost. The RBE of radiation is defined as the ratio dose X/dose R, i.e. the dose of 200–250 kV X-rays required to produce a specific biological effect to the dose of radiation (R) required to produce the same effect. The RBE of radiation for the induction of chromosome aberrations depends on its linear energy transfer (LET — the energy imparted to a medium by a charged particle of a specified energy, per unit distance). Radiation with a higher LET is more effective in inducing sterility, and most likely would yield insects that are more competitive (North 1975). However, a higher LET also means that penetration is limited. For example, alpha particles have a high value of LET, but can penetrate only a fraction of a millimetre into a container of insects, which makes them unsuitable to sterilize insects for release in AW-IPM programmes. Neutrons are more effective than gamma radiation or X-rays in sterilizing insects (Hooper 1971; North 1975; Offori and Czock 1975). However, neutrons can induce radioactivity in irradiated materials, which, along with the immobility of nuclear reactors (the usual source of neutrons), makes their use impractical for most programmes.

Considering this, the types of radiation that can be used practically in programmes that release sterile insects include gamma radiation, high-energy electrons, and X-rays (Bushland and Hopkins 1951, 1953; Baumhover et al. 1955; Lindquist 1955). All have similar effects on materials, and in particular on insects (since they have a similar RBE). For certain insect life stages and radiation doses, several studies found no significant difference between electrons and gamma radiation in their lethal effects (Hooper 1971; Adem et al. 1978; Watters 1979; Dohino et al. 1994).

To maintain the fitness of irradiated insects, and for the safety of workers, the induction of radioactivity in irradiated materials, such as canisters and insects, must be avoided. This is achieved by ensuring that the particle energy used for the SIT is less than 5 million electron volts (MeV) for photons (gamma radiation or X-rays), and below 10 MeV for electrons (Elias and Cohen 1977; Codex Alimentarius 1983; FAO/IAEA/WHO 1999; IAEA 2002a). Thus, gamma radiation from cobalt-60 (Co-

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60) (photon energies are 1.17 and 1.33 MeV) and caesium-137 (Cs-137) (0.66 MeV), electrons generated by accelerators with energy less than 10 MeV, and X-rays generated from electron beams with energy below 5 MeV, are acceptable for sterilizing insects.

### *2.1. Radioisotopes*

Currently, the most commonly used radiation for the SIT is gamma radiation from the radioisotopes Co-60 and Cs-137. These isotopes have long half-lives, and the energy of their gamma radiation is relatively high (Table 1). To provide the same throughput, caesium sources, because of the lower photon energy, require about four times more activity than cobalt sources. Cobalt-60 is produced by placing small cylinders of natural cobalt (which is 100% Co-59) into a nuclear reactor, where the Co-59 atoms absorb neutrons and are converted into Co-60. These cylinders are removed from the reactor after 1 or 2 years, and are further encapsulated in corrosion-resistant stainless steel cylinders to produce source "pencils". Caesium-137 is produced from the fission of uranium and plutonium, and must be chemically separated from other fission products and actinides present in used nuclear fuel. The use of caesium for radiation purposes is declining because this process is very elaborate, and also in view of the danger its water solubility represents in terms of potential use for terrorism.

*Table 1. Comparison of properties of Co-60 and Cs-137*

| Property | Co-60 | Cs-137 |
|---|---|---|
| Production mode | Neutron absorption in nuclear reactors | Chemical separation from spent nuclear fuel, e.g. uranium |
| Half-life | 5.271 years | 30.07 years |
| Photon energy | 1.17 and 1.33 MeV (in equal proportions) | 0.66 MeV |
| 50% dose-decrease (depth in water) | 23 cm | 21 cm |

### *2.2. Electron Beam*

Even though high-energy (5–10 MeV) electrons can be used to sterilize insects, because of the cost and size of an electron beam facility it is normally not a suitable option. The exception would be if there is such a facility in the vicinity that is being used for other purposes. In such cases, the facility could be leased for a limited time period and at an affordable cost. An example is the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) facility in Valencia, Spain. Such electrons are generated by an electron accelerator, which does not involve any radioactive materials. Electrons are

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introduced into an accelerating structure from an injector, where they are accelerated to the designed high energy that can be derived from a variety of sources depending on the type of accelerator. An electron accelerator yields a narrow and intense electron beam, and thus the dose rate can be up to 1000 times greater than from a gamma irradiator. However, gamma radiation from Co-60 or Cs-137 penetrates irradiated materials more deeply than electrons. For example, for Co-60 gamma rays, dose decreases only to half at a depth of about 23 cm in water, but for 10-MeV electrons the useful depth is only about 4 cm.

### *2.3. X-Rays*

When a beam of electrons strikes material with a high atomic number, e.g. tungsten, X-rays are generated. X-rays, like gamma radiation, are electromagnetic radiation. Radiation generated in this manner (by the rapid deceleration of a charged particle) is also known as "Bremsstrahlung". While gamma radiation from radioisotopes has discrete energies, Bremsstrahlung has a broad energy spectrum with a maximum equal to the energy of the incident electrons. The penetration of 5-MeV X-rays is similar to that for Co-60 gamma radiation and therefore higher than that for electrons.

## 3. RADIATION TECHNOLOGY AND STERILIZATION PROCESS

### *3.1. Irradiation Facilities*

The design of an irradiation facility affects the dose distribution and the attainable dose range. It may be designed either for a specific application (or product) or for multiple applications, depending on local considerations and user requirements. The basic components of an irradiation facility include:

- Radiation source (radioisotopic gamma source, X-ray tube or electron accelerator) and the associated control systems,
- System for transporting the product, e.g. insects, or in some cases the source, to and from the position at which irradiation occurs,
- Shielding to protect workers and the surrounding environment from radiation.

The irradiation facility should also include a dosimetry laboratory, and a product-handling system with areas designated for receiving and for segregated pre- and post-irradiation storage.

#### *3.1.1. Gamma Irradiators*

The radiation source consists typically of several source pencils of either cobalt or caesium. The dose rate is determined by the current activity (strength) of the source, and the operator controls the absorbed dose delivered to the insects by adjusting the time that they are exposed to radiation (an exception — in some large-scale irradiators, several dose rates can be obtained by raising different subsets of the source pencils into the irradiation room). The only variation in the source output is the known reduction in activity caused by radioactive decay, which can have a significant impact on the programme (financial as well as scheduling irradiation operations) if

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not taken into account. The activity of a cobalt source, for example, decreases by about 12% annually. The irradiator operator compensates for this loss of activity by incrementally increasing irradiation time (approximately 1% each month) to maintain the same dose given to the insects. Since irradiation times eventually become impractically long, sources need to be replenished at regular intervals, depending on the initial activity of the source and the operational requirements.

Typically there are two types of gamma irradiators used in programmes that release sterile insects — self-contained dry-storage irradiators, and large-scale panoramic irradiators.

*Self-Contained Dry-Storage Irradiators.* At present, most sterilization of insects is accomplished using gamma radiation from self-contained irradiators (Fig. 1). These devices house the radiation source within a protective shield of lead, or other appropriate high-atomic number material, and they usually have a mechanism to rotate or lower the canister of insects from the loading position to the irradiation position. These canisters, which are reusable and generally made of steel, aluminium, or plastic, hold packaging containers with insects during irradiation. To irradiate, a canister is placed in the irradiation chamber while it is in the loading (shielded) position and, depending on the current dose rate of the irradiator, the timer is set to deliver the pre-selected dose. On the push of a button, the chamber is automatically moved to the irradiation position. In most self-contained irradiators, the irradiation position is in the centre of an annular (circular) array of long parallel pencils that contain the encapsulated radionuclide. With this design, the dose is relatively uniform within the irradiation chamber (section 3.3.2.). In an alternative configuration, the radionuclide is contained in a single rod. In this case, the canister with insects is rotated on a turntable within the irradiation chamber to achieve an acceptably uniform dose. The axis of rotation is parallel to the source rod, which is usually vertical. The canister stays in the irradiation position for the set time interval, and then automatically moves to the unloading (shielded) position at the end of the treatment. Self-contained dry-storage irradiators provide a high dose rate but a small irradiation volume (1 to 4 litres), and are suitable for research as well as small-scale programmes that apply the SIT.

*Large-Scale Panoramic Irradiators.* For large-volume irradiation, panoramic irradiators are more suitable. The source consists of either several Co-60 rods (pencils) arranged in a plane or a single rod that can be raised/lowered into a large irradiation room. When retracted from this room, the source is shielded either by water (wet storage), or by lead or other appropriate high-atomic-number material (dry storage). Since isotopic sources emit gamma radiation isotropically (in all directions), they may be surrounded by canisters of insects to increase the energy utilization efficiency, and several canisters can be irradiated simultaneously.

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*Figure 1. In preparation for irradiation, a canister with insects is placed in the irradiation chamber (while it is in the shielded position) of a self-contained gamma irradiator. Depending on the dose rate of the day, the timer on the control panel (lower right) is set to give the desired dose.*

Many large-scale irradiators run in a continuous-operation mode, in which canisters of insects are carried on a conveyor around a central source. The canisters may pass by the source several times to increase dose uniformity in the canisters as well as energy utilization. The speed of the conveyor is selected so that the insects receive the intended dose. The source is moved to the storage position only when the irradiator is not in use. An alternate method is batch operation, where several canisters of insects are placed in the irradiation room while the source is in its storage position. The source is then moved into the irradiation room for the length of time required to achieve the desired absorbed dose. To improve dose uniformity, each canister may be rotated on its own axis during irradiation using turntables.

### *3.1.2. Self-Contained Low-Energy X-Ray Irradiators*

Recently, self-contained low-energy X-ray irradiators (Fig. 2) suitable for SIT applications have become commercially available (Wagner et al. 2009; Mastrangelo et al. 2010; Mehta and Parker 2011). The maximum X-ray energy for these irradiators is about 160 keV, and thus shielding requirements are less than for a typical self-contained gamma irradiator. These irradiators utilize X-ray tubes that consist of an electron source (generally a heated wire, a filament which emits electrons), an

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electrostatic field to accelerate these electrons, and a converter to generate X-rays. In the currently available irradiators, the converter is present throughout the curved surface of the tube, and hence the X-rays are emitted in all directions. One method for irradiating insects is to operate the irradiator in a batch mode where several canisters of insects are placed around and parallel to the X-ray tube, and revolve around the tube during irradiation while maintaining their orientation (much like chairs on a Ferris wheel), achieving acceptable dose uniformity. An alternate method is to continuously pass trays with insects between two X-ray tubes, providing irradiation from two sides.



*Figure 2. A typical X-ray irradiator (self-shielded X-ray irradiator) consisting of two units: the exposure chamber (in the foreground) houses the X-ray tube and the canisters with insects, and the second unit (in the background) houses the water tank and cooling fans. (Photo from A. G. Parker, reproduced with permission.)*

### *3.1.3. Electron Accelerators*

Accelerator-generated radiation has two modes, electrons and X-rays produced from these electrons (Fig. 3). The two principal electron-beam characteristics are beam (electron) energy in MeV, and the average current in milliamperes (mA). These accelerators generally produce electrons of energy up to 10 MeV, and hence the irradiators need to be housed in heavily shielded rooms (unlike self-shielded irradiators). The beam energy determines the penetration of electrons in a material (thus dictating the useful size of the canister for irradiation), and the average beam current affects absorbed-dose rate (thus determining throughput, e.g. the number of canisters treated per hour). Unlike gamma radiation, radiation beams for such

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irradiators are rather focused (for both modes), and typically conveyors are used to move canisters of insects continuously through the beam. Also, the electron beam (and also X-ray beam) is scanned to cover the length and width of the insect container, generally a tray. Since X-rays penetrate deeper than the electrons, from which they are generated, larger canisters of insects can be used when using the X-ray mode. To improve dose uniformity, each canister may be rotated on its own axis during irradiation using turntables.

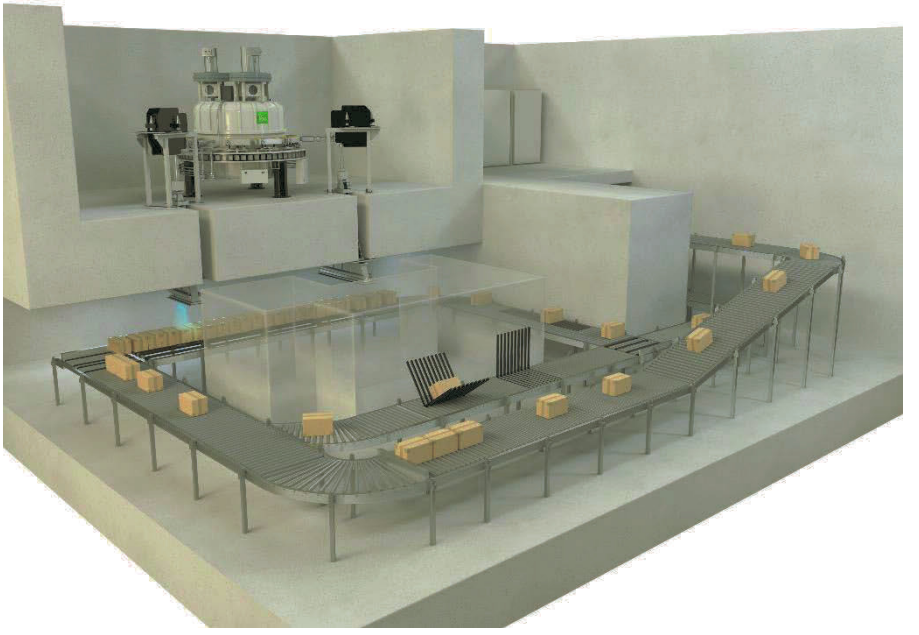


*Figure 3. Electron beam irradiation facility equipped with an electron accelerator and electron-to-X radiation converter. (Photo from Ion Beam Applications (iba), reproduced with permission.)*

### *3.1.4. Selection of Irradiator*

Since gamma radiation, X-rays and electrons have similar sterilizing effects, the choice of source for SIT irradiation is based on other considerations, such as penetration, cost, product throughput (Mehta 2009; DIR-SIT 2018), as well as expertise available at the site, and environmental, safety and security factors. The shallow penetration of electrons restricts the size of the canister used for irradiation. In addition, gamma irradiators, as well as the new self-contained X-ray irradiators, are usually simpler to operate and less expensive than large electron accelerators, at least within the range of the power required for SIT applications. However, X-rays and electron accelerators may have more public acceptance because they produce no radiation when switched off, and there are no transportation or radioactive waste issues (Cavalloro and Delrio 1974; Piedade-Guerreiro and Gomes da Silva 1983; Cleland and Pageau 1985; Smittle 1993; EBFRF 2004; FDACS 2018).

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The power emitted by a gamma-ray source containing 100 kCi of Co-60 is roughly equivalent to that of a 1.5 kW electron accelerator. The power capacity of currently available commercial accelerators with 5–10 MeV electrons is usually much greater than this, making them unsuitable for dedicated SIT use. X-ray irradiators have the advantages of both gamma irradiators (high penetrability) and accelerators (no radiation when switched off). Based on all of these factors, almost all current insect sterilization programmes have traditionally used gamma irradiators (Table 2).

*Table 2. Examples of insect mass-rearing facilities, and the types of irradiators used for reproductive sterilization (more extensive list found in DIR-SIT 2018)*

| Location of facility | Insect reared | Dose (Gy)[1] | Initial activity (kCi) | Irradiator model (MANUFACTURER) | Source |
|---|---|---|---|---|---|
| Argentina | *Ceratitis capitata*[4] | 110 | 20 | IMCO-20[2] | Co-60 |
| Brazil | *Ceratitis capitata* | 115 | | RS2400 (RadSource Technologies) | X-ray |
| | *Aedes aegypti*[5] | 65 | | 125 keV, 18 mA | |
| Burkina Faso | *Glossina palpalis gambiensis*[6] | 110 | 12 | GAAA (Groupement Atomique Alsacienne Atlantique) | Cs-137 |
| Canada | *Cydia pomonella*[7] | 150 | 24 | Gammacell® 220[2] (NORDION) | Co-60 |
| Chile | *Ceratitis capitata* | 120 | 16 | Gammacell® 220[2] (NORDION) | Co-60 |
| China | *Aedes albopictus*[8] | 50 | | Wolbaki (Tongda Dandong, China) 160 keV, 16 mA | X-ray |
| Costa Rica | *Ceratitis capitata* | 150 | | RS2400 (RadSource Technologies) 150 keV, 45 mA | X-ray |
| France (Reunion) | *Aedes albopictus* | 35 | | BloodXrad (Cegelec Actemium NDT-PES) 160 keV | X-ray |
| Guatemala | *Ceratitis capitata* | 100–145 | 11 | Gammacell® 220 E[2] (2 units) (NORDION) | Co-60 |
| | *Anastrepha ludens*[9] | 80 | 12 | Gammacell® 220 R[2] (J. L. SHEPHERD) | Co-60 |
| | | | 80 | Process Irradiator 484C-P[2] (J. L. SHEPHERD) | Co-60 |
| | | | 42 | Husman 521A[2] (ISOMEDIX) | Cs-137 |
| | | | 46 | Husman 521[2] (ISOMEDIX) | Cs-137 |

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*Table 2. Continued*

| Location of facility | Insect reared | Dose (Gy)[1] | Initial activity (kCi) | Irradiator model (MANUFACTURER) | Source |
|---|---|---|---|---|---|
| Israel | *Ceratitis capitata* | 100 | 12 | Gammacell® 220 E[2] | Co-60 |
| | *Bactrocera oleae*[10] | 100 | | | |
| Japan | *Cylas formicarius*[11] | 200 | 97.8 | C-188[3] (NORDION) | Co-60 |
| | *Euscepes postfasciatus*[12] | 150 | | | |
| Mexico | *Anastrepha ludens* | 80 | 59.9 | GB-127[3] | Co-60 |
| | *Anastrepha obliqua*[13] | 80 | 59.9 | GB-127[3] | Co-60 |
| | *Ceratitis capitata* | 100 | 27.9 | JS-7400[3] (2 units) (NORDION) | Co-60 |
| Panama | *Cochliomyia hominivorax*[14] | 55 | 19.5 | Model 7810-150[2] (J. L. SHEPHERD & ASSOCIATES) | Co-60 |
| Philippines | *Bactrocera dorsalis*[15] | 64–104 | 30 | GB 651 PT[3] (NORDION) | Co-60 |
| South Africa | *Ceratitis capitata* | 90 | 10 | (LOCAL MANUFACTURER)[3] | Co-60 |
| | *Thaumatotibia leucotreta*[16] | 150 | 20 | Point Source Panoramic[3] | Co-60 |
| Spain | *Ceratitis capitata* | 95 | | Rhodotron TT200 (Ion Beam Applications) 10 MeV, 8 mA | E-Beam |
| Thailand | *Bactrocera dorsalis* | 90 | 24 | Gammacell® 220[2] (NORDION) | Co-60 |
| | *Bactrocera correcta*[17] | 80 | 24 | Gammacell® 220[2] (NORDION) | Co-60 |
| USA (Hawaii) | *Ceratitis capitata* | 140 | 47 | Husman 521[2] (2 units) (ISOMEDIX) | Cs-137 |
| USA (Texas) | *Anastrepha ludens* | 70 | 38 | Husman 521[2] (ISOMEDIX) | Cs-137 |

[1]Sterility-inducing dose in hypoxia (except *Cydia pomonella*)
[2]Self-contained dry-storage irradiator
[3]Panoramic irradiator
[4]*C. capitata* (Wiedemann)
[5]*Ae. aegypti* (L.)
[6]*G. palpalis gambiensis* Vanderplank
[7]*C. pomonella* (L.)
[8]*Ae. albopictus* (Skuse)
[9]*A. ludens* (Loew)
[10]*B. oleae* (Rossi)
[11]*C. formicarius* (F.)
[12]*E. postfasciatus* (Fairmaire)
[13]*A. obliqua* (Macquart)
[14]*C. hominivorax* (Coquerel)
[15]*B. dorsalis* (Hendel)
[16]*T. leucotreta* (Meyrick)
[17]*B. correcta* (Bezzi)

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In recent years, because of the potential use of radionuclides in terrorist attacks, governments and the International Atomic Energy Agency (IAEA 2012, 2017) have encouraged the development of alternatives to gamma irradiators. Governmental and international controls on radioactive materials have increased the difficulty and expense of purchasing, transporting, or reloading gamma irradiators. In addition, in many jurisdictions, facilities with gamma sources are required to have comprehensive and expensive security systems that include such features as 24-h video monitoring, motion and tamper sensors, and access systems that incorporate multi-factor recognition of users. Users with unescorted access to the facilities are now often required to pass extensive background checks.

On the other hand, X -ray irradiators have the advantage that they can easily be shipped internationally, and only need access to electricity and the replacement of X-ray tubes after every few thousand hours of use. Nevertheless, their use can be energy-intensive, and experience thus far indicates that they require more maintenance, and are less reliable for continuous operation, than gamma irradiators (IAEA 2012).

### *3.2. Radiation Safety*

It is essential that written descriptions of specific safety procedures, for all activities at an irradiation facility, are prepared. Before using a radiation source, workers must be given detailed training on relevant national legislation and regulations, and on safety procedures for the installation and use of a radiation source (IAEA 2003, 2014).

Irradiators are designed to keep the radiation exposure and dose to workers "as low as reasonably achievable" (ALARA), and within pre-set limits. These dose limits are based on the recommendations of several agencies of the United Nations (UN), including the IAEA, Food and Agriculture Organization of the United Nations (FAO), and World Health Organization (WHO) (IAEA 2014). Appropriate safety methods and procedures have been developed for each type of irradiator, and when operated correctly with the appropriate safeguards, they are safe and easy to use. Irradiators are usually licensed by national atomic energy authorities which set certain requirements, such as restricting access (entry for authorized persons only), a periodic survey of the radiation field in the vicinity where workers could be present, the use of personal radiation dosimeters, and the availability of radiation survey meters. These requirements are specifically aimed at protecting all workers from radiation. In addition, irradiators incorporate interlocks that prevent unintentional access to areas with high radiation fields. Cases of accidental exposure to Co-60 gamma rays are usually reported by the IAEA (IAEA 1996; Gonzalez 1999), and data from such historic cases are useful for probabilistic risk assessment. When the useful life of a gamma source is over, the irradiator or the source pencils are usually returned to the supplier for storage, reuse, recycling, or disposal. This is now becoming a mandatory and elaborate procedure.

During handling of insects, especially adult Lepidoptera, irradiator operators may be exposed to insect allergens, and additional safety measures may be required to minimize the risk of allergy and health hazards (Parker, Mamai et al., this volume).

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### *3.3. Measurement and Distribution of Absorbed Dose*

#### *3.3.1. Radiation Dosimetry*

For the success of a programme using the SIT, the absorbed dose delivered to the insects needs to be accurately measured and controlled. Also, if contractual arrangements or national regulations prescribe specific doses, the programme will require adequate means to demonstrate compliance. Therefore, the programmes need to have an established system to accurately measure absorbed dose and estimate the associated confidence interval, a process known as dosimetry (ISO/ASTM 2013a). Dosimetry is performed using dosimeters — devices that, when irradiated, exhibit a quantifiable change in some property, e.g. colour, that can be related to the absorbed dose. A dosimetry system includes dosimeters (that are placed in different positions within the canister), measuring instruments (to read the change in the dosimeters) along with their associated reference standards, and procedures for using them (ISO/ASTM 2013b).

Dosimeters are commonly used in sterile insect production facilities for tasks such as absorbed-dose mapping (section 3.3.2.), qualification of the irradiator (section 3.5.2.), and process control (section 3.5.3.). Several dosimeters are suitable for these routine dosimetry procedures at SIT facilities (ISO/ASTM 2013a). Many facilities use radiochromic film systems because they are relatively affordable and are simple to use (avoiding extensive training) (IAEA 2004). Procedures for calibrating routine dosimetry systems, and for determining radiation fields in irradiators used for insect sterilization, are described in various ISO/ASTM standards (section 3.5.1.) (ISO/ASTM 2009; 2013a, b, c), which are updated periodically, and in an IAEA technical report (IAEA 2002b). Reference-standard dosimeters are used to calibrate the routine dosimetry system, and to determine the dose rate at a reference position in a self-contained irradiator. Sterile insect production facilities use reference-standard dosimeters for both of these purposes. Accredited dosimetry laboratories typically provide these dosimeters and make the readings, resulting in measurements that are “traceable” to national or international standards.

#### *3.3.2. Absorbed-Dose Mapping*

Ideally, it would be desirable to irradiate all insects in a container (or a canister) at the same dose. In practice, because of the characteristic of radiation interaction with matter, there is a systematic pattern of dose variation within the canister, and therefore not all insects receive the same dose. Dose distribution within the canister is determined by “dose mapping”, which typically is conducted by placing several dosimeters at known locations throughout the canister. Dose mapping provides operators of SIT irradiators with information on the absorbed dose within the canister, including areas of maximum and minimum dose, the dose uniformity ratio (maximum dose/minimum dose), and areas where the dose rate is relatively uniform (Fig. 4). Techniques for dose mapping are described in detail in Walker et al. (1997) and ISO/ASTM (2013a).

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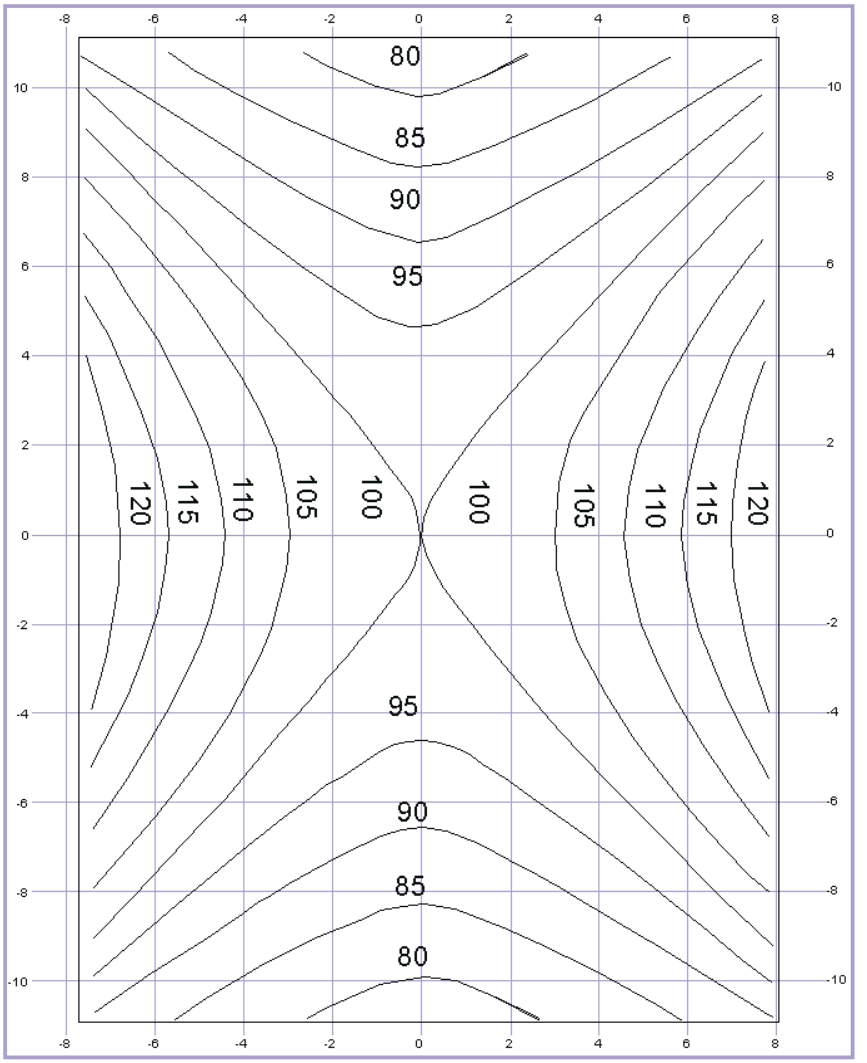



*Figure 4. Example of isodose curves in the irradiation chamber of a Gammacell® 220. Values are normalized to 100 in the centre of the gamma field. The field is most uniform in the centre. In this case the dose uniformity ratio (DUR) is 1.5 [120/80]. Grid is at 2-cm intervals from the centre of the chamber. (Figure from MDS Nordion, reproduced with permission.)*

### *3.4. Radiation Sterilization of Insects*

#### *3.4.1. Selecting Sterilizing Dose*

The dose that is used to induce sterility is of prime importance to programmes that release sterile insects. As the absorbed dose increases, sterility increases, but insect

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quality and competitiveness will decrease (Parker, Vreysen et al., this volume; Lance and McInnis, this volume). Insects that receive too low a dose are not sufficiently sterile, and those that receive too high a dose will be non-competitive, reducing the effectiveness of the programme. Quite often, full (100%) sterility is not the most favourable condition for a programme, and thus process optimization is necessary to balance sterility level and competitiveness, taking into consideration factors that could affect the radiation sensitivity of insects (section 4.) and programme requirements (Parker and Mehta 2007). If quarantine security is a consideration, 100% sterility may be required for any released females. Males, however, tend to be less radiosensitive, and, in many species, eliminating a residual egg hatch of 1% (or less) from fertile females mated to irradiated males (even though many of these eggs do not survive other stages) requires doses that substantially reduce the ability of males to compete with, and thus induce sterility into, wild populations (Fisher 1997; Toledo et al. 2004).

Therefore, given the unavoidable dose variability within a canister (as mentioned above), sterile insect production facilities define an acceptable range of doses that can be given to the insects. Most often, programmes or regulatory officials specify a minimum dose that all insects must receive to ensure sufficient sterility. Due to dose variability, most insects actually receive a dose that is somewhat higher than that minimum. An alternate approach is to specify an optimum (or central target) dose, and set this as the average or median dose within the irradiated volume of insects. In either case, the dose uniformity ratio (DUR) should be small, preferably below 1.3. The goal is to sterilize all insects sufficiently without treating large proportions with doses that are too high, substantially reducing competitiveness. Irradiator operators can often adjust process parameters to achieve a more uniform dose distribution (section 3.5.2.).

Induced mutations may exert lethality at any stage of insect development. Quite often, for reasons of simplicity and convenience, the induction of detrimental lethal mutations is measured solely on the basis of egg hatchability. However, because lethal mutations can affect any developmental stage, researchers should measure dose effects all along this developmental continuum, or the actual survivorship from egg to adult, to give a true picture of induced sterility. As a result, 99 or 100% sterility in the egg stage is not essential nor desirable if it drastically reduces the competitiveness and vigour of the sterile insect. In some moths, the $F_1$ generation is more sterile than the substerilized parent generation. These are unique genetic phenomena in more radiation-tolerant species that are responsible for inherited sterility (IS) in Lepidoptera and some other arthropods (Marec et al., this volume).

An informed decision on treatment dose requires accurate data on how factors such as dose, insect stage and age, and various process parameters affect levels of sterility and insect quality. For programmes that apply the SIT, the accuracy and value of such data depend on the use of standardized dosimetry systems, procedures, and reporting methods (ISO/ASTM 2013a; FAO/IAEA/USDA 2019). Published data on the radiation biology of the same or similar species can provide guidance, but, in many cases, are of limited value because dosimetric procedures, dose-measurement traceability, dose distribution, and other pertinent information are often not reported. In addition, the details of insect-handling procedures, and, perhaps, strain-related differences, can influence radiation sensitivity (section 4.).

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### *3.4.2. Preparing Insects for Irradiation*

*Stage/Age of Insects.* The selection of the insect development stage and age at which the insects will be irradiated is based on knowledge of the timing of maturity of insect reproductive organs (section 4.2.), handling suitability during irradiation and subsequent shipping, and sensitivity to somatic damage. For many holometabolous species (having complete metamorphosis), a good time for irradiation is late in the pupal stage, or early in the adult stage, when germ tissues have formed (Anwar et al. 1971; Ohinata et al. 1971, 1977, 1978). For example, tephritid flies are usually irradiated 1 or 2 days prior to adult emergence (pupae kept at about 25°C). Flies that are irradiated earlier in the pupal stage will tend to be of lower quality (in terms of mating propensity, flight, and sex pheromone production), an indication that somatic tissues were adversely affected (Fletcher and Giannakakis 1973). However, when tephritid pupae are irradiated too close to adult emergence, a proportion of females can already have some developed oocytes that, in spite of having been irradiated, can become viable eggs (Williamson et al. 1985). Ideally, the development and maturity stage should show an external physical indicator that acts as a quick and reliable identification tool, such as pupal eye-pigment colour in the Mediterranean fruit fly (Ruhm and Calkins 1981; Seo et al. 1987; Resilva and Pereira 2014).

In the pentatomid bug *Nezara viridula* (L.), sexual maturity and mating occur 5–17 days after adult emergence. Fourth- and fifth-instar nymphs are most frequently selected for irradiation because they are more radiosensitive than adults, and male and female reproductive systems are already well developed at this stage (Kiritani 1963; Mitchell and Mau 1969).

*Packaging for Irradiation.* Insects are usually irradiated in the primary packaging containers that are subsequently transferred, unopened, to an emergence and release facility where the adult insects are prepared for release (FAO/IAEA 2017). These containers provide protection to the sterile insects, and guard against their escape. They also prevent tampering. A variety of packaging containers has been used, e.g. 2- and 4-litre polyethylene bags, unwaxed paper cups (with lids), paper boxes, and plastic bottles of up to 15-litre capacity. SIT irradiation protocols may incorporate reusable canisters (typically steel, aluminium or plastic) that hold the primary packaging containers during irradiation. The size and shape of these canisters are usually a function of the size and shape of the irradiation chamber, especially in the case of self-contained irradiators (section 3.5.2.).

If insects are irradiated in a reduced-oxygen atmosphere, as a means of reducing the formation of free radicals (section 4.1.), the packaging container must be airtight. For example, tephritid pupae are sealed, with as little air space as possible, in plastic bags or bottles and then held at cool temperatures (12–20°C) for at least 1 hour before irradiation. During this period the insects exhaust most of the oxygen remaining within the container. Hypoxia (a deficiency of oxygen reaching the tissues of the body) can also be achieved by saturating the atmosphere within the container with helium or, more commonly, nitrogen, prior to and during irradiation (Ashraf et al. 1975; Ohinata et al. 1977, 1978; Hooper 1989).

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### *3.5. Quality Assurance*

#### *3.5.1. Quality Assurance Programmes*

Quality assurance (QA) is an important part of any successful AW-IPM programme using the SIT. A QA programme provides various benefits with respect to irradiation procedures, including:

- Success of the process — adequately sterilized insects of good quality can be produced consistently,
- Compliance with regulations — a QA programme makes it convenient to audit the process against established standards,
- Harmonization — as international trade of sterile insects is growing, it has become more important to ensure dependable uniformity across geographical and political regions,
- Public acceptance — when the public realizes that SIT facilities strictly follow set procedures and document the process, it has more confidence in the programme.

An effective QA programme includes standard operating procedures (SOPs) for all activities related to packaging, irradiation, and dosimetry. Also, processing equipment that controls key operating parameters (those affecting dose) is periodically tested and/or calibrated to verify that the irradiator is operating properly. This is then documented as part of the record for the QA programme.

For many processes related to insect sterilization using radiation, standards and guidelines are available that can be incorporated into a facility's QA programme. These include dosimetry standards developed by the International Organization of Standardization and the American Society for Testing and Materials (ISO/ASTM 2009, 2013a, b, c, d), an SOP for using the Gafchromic® film dosimetry system for the SIT (IAEA 2004), and a comprehensive quality-control manual for applying the SIT against fruit flies (FAO/IAEA/USDA 2019).

#### *3.5.2. Irradiator Operation and Configuration*

When an irradiator is installed, it is evaluated to ensure that it is working according to the manufacturer's specifications, and baseline data on its performance are developed. These two activities are known as *installation qualification* and *operational qualification*, respectively (ISO/ASTM 2013a). Operational qualification includes, among other things, initial dose mapping (section 3.3.2.), and measurement of the dose rate at a reference position, e.g. at the centre of a fully filled canister (section 3.3.1.). The reference dose rate is then used to establish the basic relationship between key operating parameters, such as timer setting or conveyor speed, and absorbed dose. Dosimetry standards recommend repeating periodically the reference dose-rate measurement, e.g. every 3 years for gamma irradiators (ISO/ASTM 2013a). A caesium-137 (Cs-137) source, in particular, may contain impurities (Cs-134) that affect the decay rate, and thus, over time, the dose rate. For the low-energy X-ray irradiators, it is recommended that the reference dose rate be measured annually. Reference-standard dosimetry and dose mapping are repeated as appropriate following any changes in the irradiator, such as source renewal in gamma irradiators or a new X-ray tube, which could affect the dose rate or dose distribution.

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Before insects are sterilized, key process parameters are established as part of performance qualification. For most insect irradiators, the absorbed dose delivered to the insects is controlled by adjusting a single parameter, such as timer setting (irradiation time) or conveyor speed. For X-ray irradiators, the absorbed dose also depends on the tube voltage and tube current. Values of these parameters depend on the dose specifications (section 3.4.1.) and the reference dose rate on the day of irradiation. Dose mapping is again performed to ensure that all insects within a given canister will receive an appropriate dose. If necessary, process parameters can often be adjusted to improve dose uniformity; common alterations include optimizing the size or shape of the canister, rotating the canister on a turntable during irradiation, using dose attenuators, and using plugs of simulated product, e.g. styrofoam, in the canister or irradiation chamber to exclude insects from areas with unacceptably low or high dose rates. This procedure establishes a canister design and a loading configuration of insects that result in an acceptably uniform dose distribution, and if possible reduce the DUR to 1.3 or less. If any of the changes listed above are made to improve dose uniformity, dose mapping should be repeated. The results of this mapping may also be used to establish a reference location for performing routine dosimetry as part of process control (section 3.5.3.).

### *3.5.3. Process Control*

The accidental release of insects that are not irradiated properly could potentially be disastrous (Knipling 1982; FAO/IAEA 2017), especially in programmes like those in California and Florida, USA, where the SIT is used to eradicate extremely small pest populations and/or as a prophylactic measure to prevent the establishment of newly introduced pests. To avoid this problem, programmes that release sterile insects implement various process-control elements to help ensure that all insects are irradiated according to specifications (FAO/IAEA/USDA 2019). In addition to the elements listed below, programmes applying the SIT often monitor relevant process parameters such as those related to the preparation and packaging of insects, setting of the irradiator timer, conveyor speed, canister specifications, and position and loading of the canisters. The results of process monitoring are routinely documented as part of the record of the QA programme.

*Sterility Testing.* Most AW-IPM programmes that integrate the SIT test samples of irradiated insects on a regularly scheduled basis to confirm that specified levels of sterility are being achieved. The quality-control manual for using the SIT against fruit flies suggests that this could be done for every shipment (FAO/IAEA/USDA 2019), comparing the egg hatch from pairings of irradiated and non-irradiated insects with that from crosses of non-irradiated insects. Besides making regularly scheduled sterility tests, unscheduled tests should also be conducted before any insects are shipped whenever changes are made to any equipment or procedures. However, it takes time to obtain the results of a sterility test, and the results may be known too late to prevent the release of incorrectly treated insects. Therefore, sterility testing must be supplemented with other methods, such as routine dosimetry.

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In addition, the competitiveness of the sterile insects needs to be checked with insects of the target population to ensure the efficacy of the programme. Such testing helps to ensure that all procedures are being followed correctly, including rearing, pre-irradiation preparation (e.g. age-based selection of insects), packaging for hypoxia or nitrogen (if used), temperature control, irradiation-dose control, and post-irradiation handling (FAO/IAEA 2017).

*Routine Dosimetry.* Regular dosimetry can help to confirm that insects are being irradiated according to programme specifications, and may be required for every shipment (FAO/IAEA/USDA 2019). This is usually done by placing dosimeters on packaging containers or canisters at a specific location (which may be the reference location identified through dose mapping performed during performance qualification (section 3.5.2.)), where the dose rate has a known and predictable relationship to the minimum and maximum dose rate within those canisters. Unlike sterility testing, routine dosimetry can identify problems in the irradiation process quickly enough so that improperly sterilized batches of insects can be intercepted prior to release in the field. Although routine dosimetry is faster than sterility testing, a third control described below provides an immediate visual confirmation check that a given container has gone through the irradiation process.

*Radiation-Sensitive Indicators.* A radiation-sensitive indicator is a material, such as a coated or impregnated adhesive-backed (or adhesive-fronted) substrate, ink, or coating that undergoes a qualitative visual change when exposed to a specified dose of radiation (ISO/ASTM 2013d). The dose at which the indicator changes should ideally be below, but near, the minimum dose required for the sterilization process. Since the degree of colour change is not proportional to the dose, these indicators cannot substitute for dosimeters.

Indicators are used as aids in tracking whether or not specific containers have been irradiated (Fig. 5). The relevant manuals (FAO/IAEA 2017; FAO/IAEA/USDA 2019) suggest that an indicator should be attached to each packaging container of sterile insects to help ensure (along with product segregation protocols and other procedural methods) that non-irradiated insects are not unintentionally released in the field. Also, indicators could potentially be used to assist in tracking multiple passes of containers through an irradiator when the sterilizing dose is fractionated into several smaller doses (section 4.1.).

Indicators that are exposed to excessive humidity, high temperature or UV radiation, e.g. sunlight, before or after irradiation may give erroneous readings; hence they are useful only within an irradiation facility where these conditions are controlled.

Recommended dosimetric procedures, including routine dosimetry and the use of indicators for programmes releasing sterile insects, are described in published standards and guides, e.g. ISO/ASTM 2013a, c, d; FAO/IAEA/USDA 2019.

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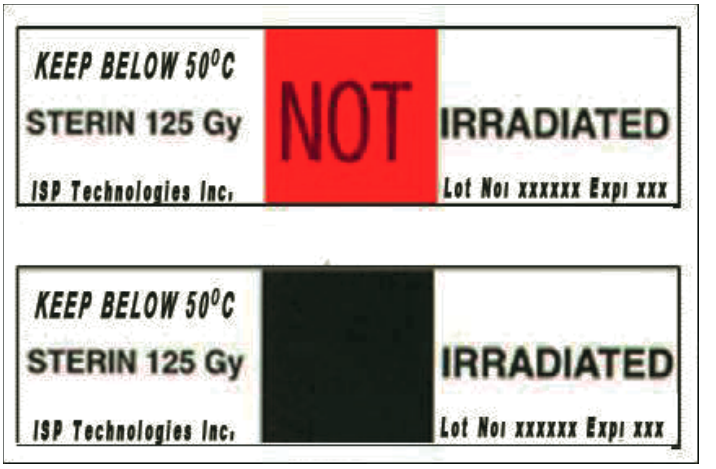



*Figure 5. Radiation-sensitive indicators before (upper) and after (lower) exposure at dose >125Gy (© ISP 2002). (Figure from FAO/IAEA/USDA 2019.)*

## 4. FACTORS MODIFYING INSECT RADIATION SENSITIVITY

The sensitivity of arthropods to radiation depends on many parameters. Radiation sensitivity varies widely among species (section 6.), but environmental conditions, and the biological state of the organism at the time of irradiation, also can have significant influences. These latter factors should be optimized so that sterilized insects are of the highest possible quality.

### *4.1. Environmental and Physical Factors*

#### *4.1.1. Ambient Atmosphere*

Oxygen levels affect the sensitivity of insects to radiation (Baldwin and Chant 1971; Economopoulos 1977; Ohinata et al. 1977; Rananavare et al. 1991; Fisher 1997). Damage induced by radiation is typically lower in an oxygen-reduced environment (hypoxia) than in air, so usually higher doses are needed to produce comparable reproductive sterility. However, because the magnitude of this protective effect tends to be greater for somatic damage than sterility, the use of oxygen-reduced atmospheres is a common strategy to improve sterile insect competitiveness without sacrificing sterility (Parker, Vreysen et al., this volume; Lance and McInnis, this volume). Methods for inducing hypoxia are described in section 3.4.2.

The increased radiation damage in a high-oxygen environment is a general phenomenon in radiobiology. Ionizing radiation initiates a chain of oxidative reactions along the radiation path in the tissues and the formation of simple free radicals ($H^0$ or $OH^0$) with very short lifetimes on the order of $10^{-10}$ sec, which in the absence of dissolved oxygen might be neutralized by combining with hydrogen radicals resulting in no net damage. In the presence of dissolved oxygen, damaging peroxy-radicals ($HO_2^0$ or $RO_2^0$), free radical species with greater stability and

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lifetimes, may be formed that irreversibly alter the organic molecules, including the germ cell chromosomes (Tubiana et al. 1990). For the protective effect of low oxygen to be seen, the tissues must be under anoxic or hypoxic conditioning prior to and during irradiation. Anoxic conditioning of adults prior to emergence led to an increase in antioxidant capacity driven by mitochondrial superoxide dismutase and glutathione peroxidase. When exposed to gamma irradiation, a strong oxidative stressor, males that received anoxic conditioning had lower lipid and protein oxidative damage at sexual maturity (López-Martínez and Hahn 2012). It must be noted that high-LET radiation (e.g. alphas, neutrons) is less affected by the presence or absence of oxygen than low-LET radiation (X-rays and gamma radiation). This may be because high-LET radiation causes several ionizations within one macromolecule, damaging it beyond repair (Pizzarello and Witcofski 1967).

#### *4.1.2. Dose Rate*

The adverse effects of radiation appear, in general, to be lessened by reducing the rate at which the sterilizing dose is delivered to the insects. This can be done by using a lower dose rate, and longer irradiation time, for a single irradiation (Yanders 1959; Nair and Subramanyam 1963; Hooper 1975; Mayas 1975; Ilao 1977).

An alternate approach to conserve insect quality is dose fractionation, where the sterilizing dose is delivered over time in a series of smaller irradiations (North 1975; LaChance and Graham 1984; Haynes 1993; Tamhankar and Shantharam 2001). However, because of its impracticality, current AW-IPM programmes applying the SIT usually do not follow this procedure (although see Kumano et al. 2012).

#### *4.1.3. Temperature*

There are some data to suggest that irradiation at reduced temperatures tends to reduce the sensitivity of arthropods to radiation (Rananavare et al. 1991). Low temperatures, to a certain limit, and hypoxia, also reduce the metabolic rate, and therefore the development rate of insects during irradiation. However, temperature does not affect the actual dose delivered to the insect.

### *4.2. Biological Factors*

#### *4.2.1. Cell Stage and Characteristics*

The most radiosensitive cells are those (1) with a high mitotic rate, (2) with a long mitotic future (i.e. under normal circumstances, they will undergo many divisions), and (3) which are of a primitive type. These generalizations, with some exceptions, have become known as the Law of Bergonie and Tribondeau (Casarett 1968). In this regard, germ cells are the most radiosensitive, and show different killing and sterilization susceptibility according to their development stage.

It is generally accepted that chromosomal damage (structural and numerical anomalies) is the cause of dominant lethal mutations. Dominant lethal mutations occurring in a germ cell do not cause dysfunction of the gamete, but are lethal to the fertilized egg or developing embryo (Robinson, this volume). The earlier stages of

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spermatogenesis (spermatocytes and spermatogonia) are generally more radiosensitive than later stages (spermatids and spermatozoa) (Proverbs 1969). Dey and Manna (1983) found that chromosomes in spermatogonial metaphase and anaphase I were more sensitive to X-rays than those in other stages. Germ-cell sensitivity in female insects is, however, complicated by the presence of nurse cells that are most susceptible to injury during mitosis (LaChance and Leverich 1962).

The dose required to inhibit mitosis is reported to be inversely proportional to the number of chromosomes, and correlates with the average interphase chromosome volume. The larger the nuclear volume, apparently the greater is the sensitivity. Similar relationships were determined in animals and plants, and used to predict their sensitivity to chronic irradiation (Sparrow et al. 1963, 1967; Casarett 1968; Whicker and Schultz 1982; Jacquet and Leonard 1983). Furthermore, radiosensitivity appears to be influenced by additional parameters including cell repopulation capacity, tissue and organ regeneration ability, and biological repair (Harrison and Anderson 1996).

Chromosome organization can also affect the response to radiation. Several insect orders (Hemiptera, Lepidoptera, Trichoptera, Odonata, and Dermaptera) have holokinetic chromosomes, i.e. properties of the centromere are distributed over the entire chromosome (Kuznetsova and Chubareva 1979; Marec et al., this volume). LaChance and Riemann (1973) suggested that, in these taxa, most dominant lethal mutations cause death after blastoderm formation. In other orders, dominant lethal mutations are expressed during the early cleavage divisions.

### *4.2.2. Developmental Stage and Age*

Age and developmental stage are important parameters to be taken into consideration when deciding on radiation process parameters for the SIT. In general, adults are less radiosensitive than pupae, which in turn are less sensitive than larvae. Similarly, older pupae tend to be less sensitive to radiation than younger pupae (Ismail et al. 1987; Ahmed et al. 1990; Hamed and Khattak 1991; Dongre et al. 1997). Also, there is a negative relationship between the age of eggs and their sensitivity to radiation (Chand and Sehgal 1978).

### *4.2.3. Gender*

Regarding sterilization or disinfestation, female arthropods are, in general, more radiosensitive than males (Cogburn et al. 1973; Hooper 1989; Hallman 1998), but there are numerous exceptions. For example, males were found to be more radiosensitive than females in the hemipteran families Pyrrhocoidae, Piesmidae, and Pentatomidae (Mau et al. 1967), the American cockroach *Periplaneta americana* (L.) (Wharton and Wharton 1959), certain Coleoptera (section 6.2.), and ixodid ticks (Purnell et al. 1972).

The wide variation reported among species in relative radiosensitivity of males versus females likely results in part from differences in the maturity of oocytes present when females are irradiated. For example, if Mediterranean fruit fly female pupae are irradiated one or two days before adult emergence, egg production is completely stopped by doses well below those needed to sterilize males. However, on the day before emergence and at later times, females contain increasing numbers of

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oocytes that mature into viable eggs even if irradiated at doses sufficient to sterilize males (Williamson et al. 1985).

*4.2.4. Size and Weight*

Early studies (Wharton and Wharton 1957; Willard and Cherry 1975) suggested that species with large adults tend to be more radiosensitive than those with small adults. Experiments have shown that the American cockroach *Periplaneta americana* (L.) is killed or sterilized by radiation doses to which smaller insects such as *Drosophila*, *Habrobracon*, and *Tribolium* are much less sensitive (Lanouette et al. 2017; Krüger et al. 2018). Similarly, other studies (Cole et al. 1959) found that the LD-50 varied inversely with the size of the insect species tested (body louse *Pediculus humanus humanus* L., house fly *Musca domestica* L., *Periplaneta americana*, German cockroach *Blattella germanica* (L.), firebrat *Thermobia domestica* (Packard), bed bug *Cimex lectularius* L., and Pharaoh ant *Monomorium pharaonis* (L.)). However, the correlation between size, weight, and radiosensitivity has not proved to be strong.

*4.2.5. Diapause*

The effects of diapause on insect sensitivity to radiation appear to vary. Mansour (2003) found that radiation-related reductions in adult emergence were greater following treatment of diapausing than that of non-diapausing larvae of the codling moth *Cydia pomonella* (L.), but other authors reported that diapausing and non-diapausing larvae of other species were equally sensitive to radiation (Ignatowicz 1997; Hallman 2000). Carpenter and Gross (1989) reported no interaction between inherited sterility (IS) and diapause with regard to several traits, although crosses involving moths that emerged from diapaused $F_1$ pupae produced significantly fewer eggs. In contrast, diapausing twospotted spider mites *Tetranychus urticae* Koch appeared more tolerant to radiation than non-diapausing mites (Lester and Petry 1995).

*4.2.6. Nutritional State*

Pre- or post-irradiation starvation, or the nutritional state, may influence radiosensitivity (Wharton and Wharton 1959; Stahler and Terzian 1963; Drummond et al. 1966). For example, to achieve 100% sterility, male and female lone star ticks *Amblyomma americanum* (L.) required about 10 Gy before engorgement and 24 Gy after engorgement (Drummond et al. 1966). The data suggested an attenuation of radiation-induced lethality in a blood-fed organism, but the mechanism remains unknown. Beuthner (1975) did not find such differences in *Amblyomma variegatum* (F.), *Hyalomma anatolicum excavatum* Koch or *Rhipicephalus appendiculatus* Neumann.

*4.2.7. Additional Factors*

An insect's state of hydration, or moisture content, could potentially influence the effects of radiation, but probably this is applicable mostly to commodity disinfestation. Diurnal rhythms apparently can influence the induction of sterility by

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radiation. Rananavare et al. (1991) found that potato tuberworms *Phthorimaea operculella* (Zeller) irradiated in scotophase were more sensitive than those treated in photophase. Finally, genetic differences related to geographical diversity within a species can potentially affect insect radiosensitivity (Fisher 1997; Hallman 2003; Azizyan and Ter-Hovhannesyan 2010).

*Enterobacter* spp. and other bacterial strains as probiotics can be useful where irradiation affects insect fitness. Probiotics in diets greatly improved various fitness parameters in irradiated insects, including pupal weight, longevity, adult size, flight ability, and adult emergence. Also, in terms of mating competitiveness, irradiated males reared on probiotics achieved more matings and transferred larger quantities of sperm to females (Stathopoulou et al. 2021; Augustinos et al., this volume).

## 5. ARTHROPOD SPECIES SUBJECTED TO RADIOSTERILIZATION

The International Database on Insect Disinfestation and Sterilization (IDIDAS) (Bakri et al. 2005; IDIDAS 2018) was developed to collect and share information about radiation doses for disinfestation and reproductive sterilization of arthropods, and to perform a comparative analysis and quality-assurance check on existing data. IDIDAS was based on a literature review and analysis of more than 5330 references that were published during the past six decades. Due to space limitations, these references are not included here but are available on the IDIDAS website (Fig. 6).

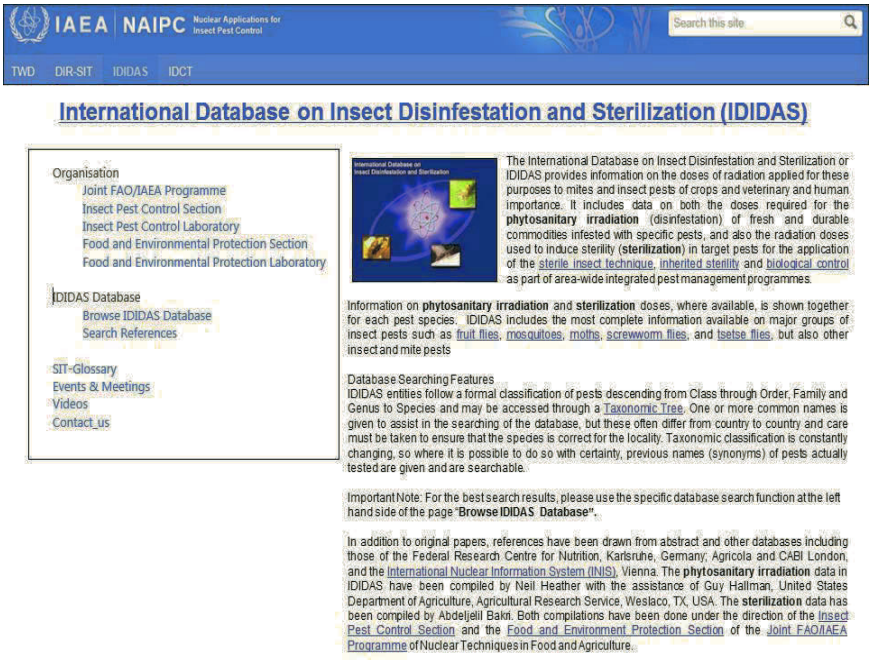



*Figure 6. Home page of the International Database on Insect Disinfestation and Sterilization that provides doses required for the phytosanitary irradiation of infested commodities, as well as for the induction of sterility, in more than 360 arthropod species (IDIDAS 2018).*

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In the past six decades, at least 360 species of arthropods of economic importance, belonging to 216 genera, 83 families, 8 insect orders and 2 arachnid orders, have been subjected to irradiation studies for the purposes of research, biological control, or pest management programmes (Table 3). Of these, 29% are Diptera, 24% Coleoptera, 24% Lepidoptera, 9% Hemiptera, 7% Acari, 3% Thysanoptera, 1.5% Hymenoptera, 1% Blattodea, 1% Araneae, and less than 1% Orthoptera and Phthiraptera. Out of 101 entries on Diptera from 17 families and 32 genera, 41 species belong to the Tephritidae; this group is important in pest management and international trade. The Curculionidae (Coleoptera), Tortricidae and Pyralidae (Lepidoptera), and Culicidae (Diptera) follow Tephritidae in the number of species radiosterilized.

Potential sources of error in any compilation of records, such as this database, are numerous. One of the main difficulties derives from taxonomy, an evolving science; during the past 60 years the names of many pest species, families and orders have been revised. In some taxa, e.g. Blaberidae, the mean radiation dose with a large confidence limit range raises questions about the source of the error.

Organisms for irradiation drawn from a cultured population should, therefore, be defined for posterity by lodging voucher specimens in an appropriately secure and curated collection. This is particularly important for groups subject to frequent taxonomic changes, such as the Tephritidae.

## 6. RADIATION DOSES FOR ARTHROPOD STERILIZATION

Arthropods are less radiosensitive than humans and other higher vertebrates (Table 4), but more sensitive than viruses, protozoa and bacteria (Ravera 1967; Rice and Baptist 1974; Whicker and Schultz 1982; Blaylock et al. 1996; Harrison and Anderson 1996). One of the main reasons for the lower radiosensitivity is that arthropods have a discontinuous growth during immature stages, and cells become active only during the moulting and metamorphosis processes. This is encoded in Dyar's Rule, i.e. insects double their weight at each moult and thus their cells need to divide only once per moulting cycle (Hutchinson et al. 1997; Behera et al. 1999). The low sensitivity of most adult insects to radiation is attributed to the fact that they are composed of differentiated cells, which do not undergo replacement (Sullivan and Grosch 1953). Such cells are much more resistant to death or damage induced by irradiation than are dividing or undifferentiated cells.

Radiation doses for sterilization (Table 3), as reported in the literature (IDIDAS 2018), were summarized using similar criteria. In general, the developmental stage irradiated was the pupa, but for some groups data are available only for eggs, larvae or adults. Other experimental parameters such as temperature, radiation source, dose rate, etc., may have differed. Even compiling the data was difficult because of the absence of uniform experimental procedures and dosimetry, and the influence of various parameters. Dose values reported below may also differ from doses that are routinely used to sterilize members of the reported taxa for the SIT, especially in cases where programmes irradiate insects in oxygen-reduced atmospheres. Therefore, the dose ranges presented should be considered only as guidelines for further investigation and to provide general introductory information (Bakri and Hendrichs 2004; Bakri et al. 2005).

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*Table 3. Calculated mean and 95% confidence limits (upper $L_2$, lower $L_1$) (Sokal and Rohlf 1995) for radiosterilization doses for insects and other arthropods. Data are for in-air irradiation of males treated mostly as pupae (or eggs, nymphs, adults as indicated). Other factors, e.g. radiation source, temperature, dose rate, and level of sterility achieved, are not necessarily consistent. (References for data from IDIDAS 2018)*

| Order | Family | Number of genera | Number of species | Sterilization dose (Gy) | | |
|---|---|---|---|---|---|---|
| | | | | $L_2$ | Mean | $L_1$ |
| Acari | Acaridae | 4 | 5 | 516 | 375 | 233 |
| | Argasidae | 2 | 2 | 20 | 20 | 20 |
| | Dermanyssidae (Nymph) | 1 | 1 | 7.5 | 7.5 | 7.5 |
| | Eriophyidae (Adult) | 4 | 4 | 350 | 350 | 350 |
| | Ixodidae | 4 | 7 | 23 | 20 | 16 |
| | Oligonychidae (Adult) | 1 | 1 | 200 | 200 | 200 |
| | Tenuipalpidae | 1 | 3 | 300 | 300 | 300 |
| | Tetranychidae | 2 | 4 | 300 | 300 | 300 |
| Araneae | Araneidae | 1 | 1 | 40 | 40 | 40 |
| | Eresidae (Adult) | 1 | 1 | 150 | 150 | 150 |
| | Pholcidae (Adult) | 1 | 1 | 20 | 20 | 20 |
| Blattodea | Blaberidae (Adult)* | 2 | 2 | 205 | 72 | -60 |
| | Ectobiidae | 1 | 1 | 32 | 32 | 32 |
| Coleoptera | Anobiidae (Adult) | 3 | 3 | 70 | 43 | 17 |
| | Bostrichidae (Adult) | 2 | 2 | 165 | 155 | 145 |
| | Bruchidae | 3 | 6 | 77 | 55 | 33 |
| | Cerambycidae (Adult) | 1 | 1 | 90 | 80 | 70 |
| | Chrysomelidae (Adult) | 3 | 3 | 28 | 28 | 28 |
| | Coccinellidae | 1 | 1 | 35 | 35 | 35 |
| | Curculionidae (Adult) | 21 | 29 | 71 | 54 | 38 |
| | Dermestidae (Adult) | 3 | 8 | 227 | 170 | 113 |
| | Elateridae (Adult) | 1 | 1 | 100 | 80 | 60 |
| | Laemophloeidae (Adult) | 1 | 3 | 200 | 200 | 200 |
| | Lyctidae (Adult) | 1 | 1 | 69 | 69 | 69 |
| | Nitidulidae | 1 | 1 | 75 | 75 | 75 |
| | Ptinidae | 1 | 1 | 300 | 300 | 300 |
| | Scarabaeidae | 5 | 5 | 111 | 72 | 33 |
| | Scolytidae | 1 | 2 | 69 | 43 | 18 |
| | Silvanidae | 2 | 3 | 117 | 117 | 117 |
| | Tenebrionidae | 8 | 15 | 186 | 140 | 94 |
| Diptera | Agromyzidae | 1 | 2 | 162 | 158 | 152 |
| | Anthomyiidae | 1 | 2 | 45 | 35 | 38 |
| | Calliphoridae | 3 | 5 | 32 | 30 | 18 |
| | Ceratopogonidae** | 1 | 1 | 300 | 300 | 300 |

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*Table 3. Continued*

| Order | Family | Number of genera | Number of species | Sterilization dose (Gy) | | |
|---|---|---|---|---|---|---|
| | | | | $L_2$ | Mean | $L_1$ |
| Diptera | Chironomidae (Egg) | 1 | 1 | 1000 | 1000 | 1000 |
| | Chloropidae | 1 | 1 | 38 | 38 | 38 |
| | Culicidae | 3 | 17 | 175 | 80 | 118 |
| | Drosophilidae | 1 | 6 | 179 | 140 | 100 |
| | Glossinidae (Adult) | 1 | 11 | 117 | 85 | 53 |
| | Muscidae | 4 | 6 | 31 | 25 | 19 |
| | Oestridae | 2 | 3 | 52 | 45 | 38 |
| | Piophilidae | 1 | 1 | 100 | 100 | 100 |
| | Psilidae | 1 | 1 | 43 | 43 | 43 |
| | Sarcophagidae | 1 | 1 | 52 | 36 | 19 |
| | Sciaridae (Adult) | 1 | 1 | 40 | 40 | 40 |
| | Tachinidae | 1 | 1 | 20 | 20 | 20 |
| | Tephritidae | 8 | 41 | 108 | 85 | 83 |
| Hemiptera | Aleyrodidae (Nymph) | 3 | 4 | 72 | 70 | 58 |
| | Aphididae (Adult) | 3 | 3 | 283 | 140 | 198 |
| | Cicadellidae (Adult) | 1 | 1 | 200 | 180 | 160 |
| | Coccidae (Nymph) | 1 | 1 | 250 | 250 | 250 |
| | Coreidae (Adult) | 2 | 2 | 80 | 80 | 80 |
| | Delphacidae | 2 | 2 | 118 | 81 | 44 |
| | Diaspididae (Adult f.) | 4 | 4 | 234 | 185 | 136 |
| | Lygaeidae (Adult) | 1 | 1 | 100 | 100 | 100 |
| | Miridae (Adult) | 1 | 2 | 200 | 200 | 200 |
| | Pentatomidae (Adult) | 3 | 4 | 62 | 55 | 48 |
| | Pseudococcidae | 4 | 5 | 183 | 125 | 148 |
| | Pyrrhocoridae | 1 | 1 | 70 | 70 | 70 |
| | Reduviidae | 3 | 3 | 90 | 70 | 50 |
| Hymenoptera | Apidae | 1 | 1 | 109 | 90 | 70 |
| | Formicidae | 4 | 5 | 92 | 80 | 67 |
| Lepidoptera | Arctiidae (Adult) | 2 | 2 | 430 | 350 | 270 |
| | Bombycidae (Adult) | 1 | 2 | 250 | 250 | 250 |
| | Elachistidae (Adult) | 1 | 1 | 200 | 200 | 200 |
| | Crambidae | 6 | 10 | 385 | 275 | 165 |
| | Gelechiidae (Adult) | 5 | 5 | 303 | 213 | 123 |
| | Gracillariidae | 1 | 1 | 450 | 450 | 450 |
| | Lymantriidae | 2 | 2 | 257 | 230 | 158 |
| | Lyonetiidae | 1 | 1 | 900 | 900 | 900 |
| | Noctuidae | 6 | 14 | 611 | 400 | 188 |
| | Pieridae | 1 | 1 | 350 | 350 | 350 |

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*Table 3. Continued*

| Order | Family | Number of genera | Number of species | Sterilization dose (Gy) | | |
|---|---|---|---|---|---|---|
| | | | | $L_2$ | Mean | $L_1$ |
| Lepidoptera | Plutellidae | 1 | 1 | 200 | 200 | 200 |
| | Pyralidae | 9 | 18 | 522 | 363 | 203 |
| | Sphingidae | 1 | 1 | 100 | 100 | 100 |
| | Thaumetopoeidae | 1 | 1 | 40 | 40 | 40 |
| | Tineidae | 2 | 2 | 120 | 120 | 120 |
| | Tortricidae | 19 | 22 | 452 | 375 | 298 |
| Orthoptera | Acrididae | 1 | 1 | 4 | 4 | 4 |
| Phthiraptera | Pediculidae | 1 | 1 | 750 | 750 | 750 |
| Thysanoptera | Phlaeothripidae | 1 | 1 | 200 | 200 | 200 |
| | Thripidae | 5 | 9 | 200 | 200 | 200 |

*Blaberidae: Questionable in view of very large confidence limit range
**Fertility recovery after first mating
(Adult f.) = Adult female

*Table 4. Ranges of $LD_{50}$ for acute irradiation of organisms from different taxonomic groups (length of time for survival is usually set at 30 days for mammals, but longer times may be needed for other organisms) (Table from Bakri et al. 2005, reproduced with permission)*

| Group | LD50 (Gy) | Reference |
|---|---|---|
| Bacteria, protozoa, viruses | 100–10 000 | Harrison and Anderson 1996 |
| Insects | 30–1500 | Whicker and Schultz 1982 |
| Molluscs | 50–500 | Ravera 1967 |
| Higher plants | 1.5–>130 | Harrison and Anderson 1996 |
| Fish | 4–100 | Harrison and Anderson 1996 |
| Amphibians | 7–22 | Harrison and Anderson 1996 |
| Reptiles | 3–40 | Harrison and Anderson 1996 |
| Birds | 5–20 | Harrison and Anderson 1996 |
| Humans | 3 | Rice and Baptist 1974 |

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### *6.1. Arachnidae*

#### *6.1.1. Acari*

The mean dose to sterilize Acari species ranged from 7.5 to 375 Gy (Table 3). The Chilean false red mite *Brevipalpus chilensis* Baker (Tenuipalpidae), the grain mite *Acarus siro* L. (Acaridae), the brownlegged grain mite *Aleuroglyphus ovatus* (Troupeau) (Acaridae), and the citrus red mite *Panonychus citri* (McGregor) (Tetranychidae) are among the least sensitive species. Hard ticks (Ixodidae), such as *Amblyomma* spp. and *Boophilus* spp., tend to be more sensitive than soft ticks (Argasidae). However, the radiation sensitivity of some tick species appears to change depending on whether the tick is engorged with blood or not (section 4.2.).

#### *6.1.2. Araneae*

A recent study (Magris et al. 2015) used the orb web spider *Argiope keyserlingi* (Karsch) (Araneidae) as a model to assess the effectiveness of male sterilization via 40 Gy irradiation and its consequences on male courtship behaviour. The results validated the thread assay and sterilization as legitimate tools for the study of male courtship behaviour and fertilization success. The other known cases of irradiation of spiders for sterilization were conducted to determine the pattern of sperm precedence (Lance and McInnis, this volume) in multiple-mated females. Kaster and Jakob (1997) used a 20-Gy dose to sterilize males of *Holocnemus pluchei* (Scopoli) (Pholcidae), whereas Schneider and Lubin (1996) applied a 150-Gy dose to *Stegodyphus lineatus* (Latreille) (Eresidae). These species showed last-male precedence and complete sperm mixing, respectively.

### *6.2. Insecta*

#### *6.2.1. Coleoptera*

The mean sterilization dose for Coleoptera ranged from 43 to over 200 Gy (Table 3). The leaf beetles (chrysomelids) are the most sensitive, followed by coccinellids, scolytids, anobiids, curculionids, and bruchids. Species requiring more than 100 Gy as sterilizing dose are by increasing order: silvanids, tenebrionids, bostrichids, dermestids, laemophloeids, and ptinids. Some data in this order suggested a differential response of males and females towards sterilizing doses of radiation. Males may be more sensitive than females, as in the case of the Japanese beetle *Popillia japonica* Newman (Ladd et al. 1973) and the beetle *Tribolium madens* (Charpentier) (Brower and Tilton 1973), or less sensitive as in the case of the khapra beetle *Trogoderma granarium* Everts (Carney 1959; Nair and Rahalkar 1963).

The effects of gamma radiation on the boll weevil were thoroughly studied with a view to applying the SIT (Earle et al. 1979; Villavaso et al. 1989; Haynes 1993). Males were sterilized by about 80 Gy, but their longevity was poor. The egg-laying capacity of females mated to these males was reduced at doses of 50 Gy or more, but they continued to produce some fertile eggs until doses approached 200 Gy, a dose which rendered the weevils non-competitive (McKibben et al. 2001). Studies were conducted on reducing the negative effects of radiation using improved mass-reared

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strains, oxygen-reduced atmospheres, and fractionated radiation doses (Earle et al. 1979; Haynes 1993; McKibben et al. 2001). Even though these effects could be mitigated, the large-scale application of the SIT was not practical and cost-effective, and boll weevil eradication succeeded without the SIT by area-wide integration of pheromone traps, and cultural and chemical controls (McKibben et al. 2001; El-Lissy and Grefenstette 2007).

The detrimental effects of radiation have also been one of the main obstacles in applying the SIT to the sweetpotato weevil *Cylas formicarius* (F.), and in particular the West Indian sweetpotato weevil *Euscepes postfasciatus* (Fairmaire). Digestive obstruction following the collapse of the epithelial tissue of the midgut was suggested as the cause of the short lifespan of gamma-irradiated adults (Sakurai et al. 2000). Nevertheless, mitigation measures, including partial sterility and fractionated-dose irradiation at low temperature, were successful in overcoming these effects (Kumano et al. 2010, 2012), and an AW-IPM programme in Kume Island, Japan, to eradicate these two weevils using the SIT is making good progress (Shimoji and Miyatake 2002; Shimoji and Yamagishi 2004); *C. formicarius* was declared eradicated in 2013 (Haraguchi et al. 2014).

### *6.2.2. Blattodea*

Several Blattodea species have been used in radiobiological studies exploring biochemical, physiological, and genetic properties (Shivaji and Rastogi 1974). In spite of the pest status of many cockroach species, there have been relatively few investigations related to pest suppression using sterile insects, due largely to potential problems with releasing large numbers of males into natural populations (Ross and Cochran 1963; Berndt 1978; Menon 1978; Ross et al. 1981; Gecheva and Apostolova 1986). In terms of sterility and mortality, cockroaches are among the most radiation-sensitive insects, with less than 5 Gy required in some cases (*Blaberus craniifer* Burmeister) to induce sterility (Wharton and Wharton 1959; Ross and Cochran 1963). Sexual differentiation in radiosensitivity was observed in *B. craniifer*, where males were more sensitive than females (Gecheva and Apostolova 1986). In Blattodea, it is the adult stage that is most frequently used for sterilization with ionizing radiation.

### *6.2.3. Diptera*

Radiation sterilization of dipterans generally requires doses from 20 to 160 Gy (Table 3). Drosophilidae and Agromyzidae are among the least radiation-sensitive families of Diptera tested, whereas tachinids are the most sensitive. The late pupal (often pharate adult) stage is preferred for the radiation of most fly species because it is practical to handle and ship pupae, and an acceptable balance between competitiveness and sterility is achieved. In the Culicidae, the late pupal in water, or the early adult stage, is irradiated (Helinski et al. 2006, 2009). Semi-field and field experiments have demonstrated that a radiation dose can be selected that gives sufficient sterility without significantly impacting the competitiveness of male mosquitoes. Furthermore, the ability of radiation-sterilized males to locate and participate in naturally occurring swarms, or to start new swarms, was confirmed in *Anopheles arabiensis* Patton (Ageep et al. 2014). A strategy combining a low radiation dose to ensure female

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sterility (SIT), and *Wolbachia*-based IIT (Incompatible Insect Technique), is being developed for *Ae. aegypti* and *Ae. albopictus* to ensure that any unintentionally released *Wolbachia*-infected females would not be able to transmit disease, and also would be sterile, thus avoiding population replacement (Lees et al. 2015).

Tephritidae, the major family in this order that is the target of the SIT, require on average about 85 Gy for sterilization. Tephritids are relatively homogeneous with respect to radiation sensitivity — less than 100 Gy are needed to achieve complete sterility in the five major pest genera (*Anastrepha, Bactrocera, Ceratitis, Zeugodacus, Rhagoletis*), and this confirms the generic recommendation of a dose in the range of 100–150 Gy to disinfest agricultural commodities for international trade (Hallman 2000). Many AW-IPM programmes applying the SIT against major pest tephritids have used 100–150 Gy for sterilization, well over the family "average" of 85 Gy. In some early programmes (LaChance et al. 1967), this was a "precaution" to increase the security margin for sterilization, but the overdose often has lowered competitiveness to the point where it reduced the overall ability of irradiated flies to induce sterility into the wild population (Toledo et al. 2004). In recent programmes, these higher doses are usually associated with the use of hypoxia to enhance sterile male competitiveness (section 4.2.) (Fisher 1997).

The Indian tropical midge *Chironomus ramosus* Chaudhuri (Diptera: Chironomidae) tolerated a higher dose of gamma radiation as compared with other known dipterans. The radiation doses required to cause 100% mortality immediately after radiation exposure of egg, larva, pupa and adult stages were 1000 Gy, 3000 Gy, 3200 Gy, and 3500 Gy, respectively (Datkhile et al. 2009). This is one of the least radiation-sensitive species of insects.

### *6.2.4. Hemiptera*

The mean sterilizing dose in the Hemiptera ranged from 55 to 250 Gy (Table 3), with *Circulifer tenellus* (Baker) (Cicadellidae) females being the least sensitive species tested thus far (Ameresekere and Georghiou 1971), and *Halyomorpha halys* (Stål) (Pentatomidae) adults being the most sensitive. In general, adult females required a gamma radiation dose of 50–60 Gy to achieve a high level of sterility. However, higher doses of up to 200 Gy (electrons in this case) were needed to achieve complete sterility in female *Myzus persicae* (Sulzer) (Aphididae) and *Pseudococcus comstocki* (Kuwana) (Pseudococcidae) (Dohino et al. 1997). Adult males typically required a dose between 70 and 200 Gy. For $4^{th}$- and $5^{th}$-instar nymphs, a lower dose was needed; 75 to 100% sterility was achieved with doses between 5 and 100 Gy. Patterns of relative radiosensitivity between females and males differ among species of Hemiptera (IDIDAS 2018). Considering the male adult stage, the most sensitive bugs are the pentatomids followed by pyrrhocorids, reduviids, and coreids. The least sensitive are cicadellids and mirids.

Only 19 species belonging to 10 out of 53 families of Hemiptera have been subjected to radiation for sterilization. For several species, the feasibility of releasing sterile males for pest suppression was investigated (Shipp et al. 1966; Baldwin and Chant 1971; Tadic 1972; Dyby and Sailer 1999; Calvitti et al. 2000). Some hemipterans are facultatively parthenogenetic, but Steffan and Kloft (1973) argued that, with proper timing and climate, effective genetic control might still be possible.

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*6.2.5. Hymenoptera*

The Hymenoptera include a number of serious pests, such as Africanized honey bees, and various Formicidae (ants) and sawflies. Since bees and ants are social insects with complex life histories, SIT studies have been limited to a few laboratory experiments (Sakamoto and Takahashi 1981). Only data of Apidae and Formicidae species are shown in Table 3. Most experimental irradiations of other hymenopterans (Braconidae, Eulophidae, Pteromalidae), e.g. the parasitic wasp *Habrobracon hebetor* (Say) (Braconidae), have been conducted in conjunction with relatively basic radiobiological, behavioural and physiological investigations (Hoch et al. 2009).

For male honey bees *Apis mellifera* L. (Apidae), the sterilizing dose is 80–100 Gy (Lee 1958). For queens of ants (Formicidae), such as the invasive fire ant *Solenopsis invicta* Buren, the African big-headed ant *Pheidole megacephala* (F.), the little fire ant *Wasmannia auropunctata* (Roger), and the Argentine ant *Linepithema humile* (Mayr), a radiation dose of 150 Gy has been proposed as a phytosanitary treatment (Follett et al. 2016). However, this proposed dose is higher than the dose required to prevent reproduction in the most tolerant ant species tested to date.

*6.2.6. Lepidoptera*

Lepidopterans as a group are relatively not sensitive to radiation; mean doses for sterilization range from 40 to 500 Gy, with *Thaumetopoea pityocampa* (Denis and Schiffermüller) (Thaumetopoeidae) requiring the lowest documented average dose of 40 Gy (Baccetti and Zocchi 1962), while three pyralid moths *Plodia interpunctella* (Hübner), *Ephestia eleutella* (Hübner), and *Ephestia calidella* Guenée, the noctuid *Xestia c-nigrum* (L.), the gelechiid *Phthorimaea operculella* (Zeller), the tortricid *Grapholita molesta* (Busck), the crambid *Crocidolomia binotalis* Zeller, and the arctiid *Diacrisia obliqua* Walker (Syn. *Spilosoma obliqua* Walker) required the highest recorded doses (> 400 Gy) for complete sterility of pupae and adults (IDIDAS 2018).

For some moth species, e.g. the coffee leaf miner *Leucoptera coffeella* Guérin-Méneville (Katiyar and Ferrer 1968), even when treated with doses as high as 600 Gy and more, the few male survivors retained some fertility. In contrast to other insect orders, the $F_1$ progeny of irradiated male lepidopterans are typically more sterile than their parents (inherited sterility). Moreover, the sex ratio in the $F_1$ generation is biased towards males. Thus, substerilized males can sire completely sterile offspring, and this phenomenon has been exploited in a number of inherited sterility programmes (Marec et al., this volume; Simmons et al., this volume).

*6.2.7. Orthoptera*

Acrididae (Orthoptera), along with Blaberidae (Blattodea) cited above, are among the most radiosensitive insects known (less than 5 Gy needed for sterilization). This is in agreement with Willard and Cherry (1975) who suggested that large long-lived adults are more radiosensitive than small short-lived adults. However, due to the voracious feeding by nymphs and adults, the release of sterile acridids is not applicable for the control of species such as *S. gregaria.*

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*6.2.8. Thysanoptera*

No species of Thysanoptera has been investigated for pest suppression directly using the SIT. However, in Japan, radiation sterilization has been investigated as a quarantine treatment to disinfest cut flowers of thysanopteran pests. Doses up to 400 Gy (electron beam) and 100 Gy (gamma rays) were given to suppress the pests *Frankliniella occidentalis* (Pergande) (EPPO 1994) and *Thrips* spp. (Dohino et al. 1996; Hayasi et al. 1999; Bansiddhi 2000), respectively.

## 7. CONCLUSIONS

Although radiation is a key component of the SIT, it is generally not given the attention it deserves - in terms of procedures for irradiation, dosimetry, and the choice of an appropriate dose to maximize the induction of sterility in wild females. The development of accurate dose-response curves for the target insect, using precise dosimetry, is a prerequisite of any programme releasing sterile insects. It is important to note here that the focus needs to be on two types of responses: 1) the sterility in the irradiated insect, and 2) the competitiveness for mating. These are opposing responses, and both need to be considered. There are much data for the first kind, but not enough for the second (Rull et al. 2012; Parker, Vreysen et al., this volume). The survey of the available literature presented here shows the wide variation in the response of the different insect species to radiation, and also highlights the need for accurate dosimetry.

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# CHAPTER 3.5.

# STERILE INSECT QUALITY CONTROL/ASSURANCE

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## SUMMARY

The sterile insect technique (SIT) depends greatly on the production of good-quality sterile male insects that are released into target wild populations. Quality is controlled through a system of bioassays of quality parameters that reflect the insect's ability to survive natural enemies, interact with its environment, forage for food and water, and locate, mate and fertilize females of the target population. The system was developed by compartmentalizing the essential survival and mating behaviours of the species involved, and then developing a series of tests to confirm that these behavioural traits are present in the mass-reared insects. The system also has a feedback loop to correct problems in the production portion of the system before they become evident. Nevertheless, regular implementation of field or field-cage tests under semi-natural conditions, where sterile males have to compete with wild males for wild females, is required to provide the ultimate confirmation that the sterile insects have the ability to fulfil their mission after release.

## 1. INTRODUCTION

A major concern of entomologists, and managers of area-wide integrated pest management (AW-IPM) programmes that integrate the sterile insect technique (SIT), is that released sterile males are adequate to fulfil the mission for which they were produced, i.e. that they compete well with wild males, and successfully mate and inseminate the targeted wild females. The components that are most important in such programmes include dispersal, survival under field conditions, location of mating arenas, courtship, mating, and sperm transfer (Koyama et al. 2004; Vreysen, this volume).

The ability of entomologists to evaluate and quantify the effectiveness of mass-reared sterilized insects in interactions with wild insects did not exist in the first decade of applying the SIT (Boller 2002). Initially, the effectiveness of released sterile insects was evaluated according to whether or not the SIT worked in the field. If it failed, managers were able to speculate only on what went wrong, or where and when the problem occurred.

In large suppression and eradication programmes using the SIT (Hendrichs, Vreysen et al., this volume), large numbers of insects must be reared for sterilization and release. Often the emphasis is on the numbers, and insect quality is overlooked or marginalized (Simmons et al. 2010; Itô et al., this volume). For many years, the SIT was considered as a "numbers game", i.e. if a programme began to fail, the remedy was to increase the sterile insect release rate. Only when a programme failed completely might the quality of the sterile insects be doubted, e.g. early in the New World screwworm *Cochliomyia hominivorax* (Coquerel) programme in the south-western USA (Klassen et al., this volume; Vargas-Terán et al., this volume). The controversy surrounding the possible causes of this failure (Bush et al. 1976; LaChance et al. 1982) led to the adoption of regular strain renewal in the mass-rearing facility, and the development of a quality-control system to continuously

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monitor the insect colony using a series of behavioural and physiological tests to detect colony changes. This fostered the development of "quality control of mass-reared insects" as a recognized entomological discipline (Boller 1972).

This chapter discusses the history of the development of quality-control technology, the principles and philosophy of assessing insect quality, and the relative importance of the various parameters used to assess insect quality in the context of mass-rearing for the SIT. Quality control is most developed for various fruit fly species (FAO/IAEA/USDA 2019) and for tsetse flies (Mutika et al. 2001, 2002), but some advances have been made recently with Lepidoptera (Simmons et al. 2010; Vreysen et al. 2016) and mosquitoes (Balestrino et al. 2017). Quality control in rearing biological control agents is beyond the scope of this chapter.

## 2. HISTORY AND PHILOSOPHY OF QUALITY CONTROL/ASSURANCE

Scientists have long been aware that insects produced for laboratory purposes should be of a quality appropriate to the experimental system being tested. However, with the advent of mass-rearing and sterilization for the SIT, the quality of mass-produced insects became even more important since appropriate behaviour in the field was now critical to the success of field programmes (Simmons et al. 2010).

Boller (2002) reviewed the history of the quality control of mass-reared insects. He divided this history into four distinct periods: (1) before 1969 — little concern about behavioural quality in rearing programmes, (2) 1969–1975 — a growing awareness of the ideas and concepts of quality control, (3) 1976–1979 — international collaboration began, and prototypes of quality-control systems were initiated, and (4) 1980 to the present — finally the concept, and practical applications in most mass-rearing facilities, were generally accepted (Bernon and Leppla 1994; Bigler 1994; Leppla 2009).

The publication of an "idea book" on fruit fly quality control by Boller and Chambers (1977) is probably responsible for developing the subject of quality control into what is now recognized as a discipline of economic entomology. These authors were able to bring together the diverging schools of thought on quality control, and were able to combine the groups into a global Arthropod Mass Rearing and Quality Control Working Group (later renamed Mass Rearing and Quality Assurance (MRQA)) under the International Organization for Biological Control (IOBC). It had its first workshop in 1982, and subsequently a workshop has been held every three or four years (Leppla and De Clercq 2019). The proceedings of several of the workshops are available from MRQA/IOBC (2019) or in Leppla et al. (2002).

The terms "quality control" and "quality assurance" are closely related and often confused. The objective of quality control is to identify (and correct) defects in the finished product, while that of quality assurance is to prevent defects with a focus on the process used to make the product (Singh 2018). Therefore, quality control is reactive but quality assurance is proactive. The two terms are largely confused in the insect-rearing literature; few authors make a clear distinction, and most work to date is on quality control. Only a few rearing facilities have adopted quality assurance and achieved certification through the ISO 9000 standards (ISO 2019). Therefore,

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we will use the expression "quality control" throughout this chapter unless referring to specific work on quality assurance, and our use of "quality control" should be understood to include "quality assurance" unless otherwise indicated.

The fundamentals of quality control are basic to any manufacturing process. Rearing insects to fulfil a specific function is analogous to manufacturing a product. The principles and philosophy of quality control in the realm of manufacturing were established many years ago (Charbonneau and Webster 1978), and these same principles are adaptable today to the quality of mass-reared insects. These principles are described in an excellent book by Feigenbaum (2004), who defined total quality control as:

> an effective system for integrating the quality-development, quality-maintenance and quality-improvement efforts of the various groups in an organization so as to enable production and service at the most economical levels which allow for full consumer satisfaction.

Quality control is divided into three categories:

(1) *Production quality control*, where the inputs to rearing are addressed, including diet ingredients, equipment, etc.,

(2) *Process quality control*, measuring how things are done, such as diet preparation, environmental conditions, infestation rate, larval separation, pupal holding, irradiation dose, etc., and

(3) *Product quality control*, where the insects produced are evaluated for effectiveness in completing the purpose for which they are required.

This chapter concentrates on product quality control and some aspects of process control, while production and process control are covered in Parker, Mamai et al. (this volume).

The "control" in quality control is a management tool consisting of: (1) setting quality standards, (2) appraising conformance to these standards, (3) acting when the standards are breached, and (4) planning for improvements in the standards (Barnes et al. 2015). The difference between mass-producing insects and rearing small colonies for laboratory experiments is in the product rather than the process. In other words, the "bottom line" of a mass-rearing programme is the performance of the released insects in the field (FAO/IAEA 1992).

While the factors affecting product quality are both technological and human, the human factors, involving operators, section leaders, and other personnel, are by far the most important, and the problems most likely to occur are often related to the human factors. The purpose of a quality-control programme is to identify and address the factors leading to low quality during the rearing process, instead of focusing on enhancing low-quality insects after they have been reared (Pereira et al., this volume). Such a programme results in improvements in product quality and employee morale, and reductions in production costs and production "bottlenecks". It also provides improved inspection methods, definite schedules for preventive maintenance, and a factual basis for standards during the rearing process.

The development of a functioning quality-control programme depends on a continuous chain of responsibility for quality through all the workers. To achieve this, responsibilities for quality should be assigned to all key personnel in the rearing facility. Certain workers should be assigned to evaluate insect quality using

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scheduled bioassays; their only responsibility should be product quality, and they must be answerable only to the programme manager. These people serve as the "eyes and ears" of the programme manager; they must never be answerable to the rearing manager. "Control" in quality control is achieved when there is constructive feedback, from the quality-control workers through the programme manager, on activities and factors that may be responsible for the lack of quality.

Quality-control procedures both incur costs and provide benefits. The costs are in the appraisal costs of evaluating inputs, processes and product quality, while benefits accrue from savings associated with avoiding: (1) defects during the rearing process, (2) internal failure costs caused by defective equipment, materials, or substandard rearing ingredients, and (3) external failure costs caused by allowing defective products to reach a customer, e. g. insects that do not survive under natural conditions, are not competitive with wild males, are incompatible with the target population, or were inappropriately irradiated.

## 3. STRAIN DOMESTICATION, MAINTENANCE, AND REPLACEMENT

Over time, under artificial rearing conditions, important behavioural and physiological traits undergo change characterized by Ochieng'-Odero (1994) as acclimatization, selection, and domestication. Examples of such affected traits are: fecundity, preoviposition period, courtship song, flight, oviposition, rate of development, production of pheromone, response to pheromone, eye morphology, visual sensitivity, metabolic rate, and resistance to stress (Mangan 1992). The potentially negative impact of changes resulting from this domestication process was discussed by Boller (1972), Rössler (1975a, b), Huettel (1976), Mackauer (1976), Van Keymeulen et al. (1981), Shimoji and Miyatake (2002), and Caprio (2009).

When insects are brought into the laboratory to initiate mass-rearing, the conditions to which they are subjected are very different from those to which the species is adapted in the field. These conditions exert different selection pressures on the individuals brought from the field to initiate a colony, selecting a small subset of the population with a reproductive advantage under the new conditions, potentially creating a genetic "bottleneck". The drive to reproduce may overcome some of the inappropriate environmental aspects, but the accumulated changes in courtship and other behaviours resulting from the selection process may be detrimental once the insect is released back into the field, reducing survival and mating competitiveness with wild insects.

The constraints of mass-production of insects can lead to selection for rapid larval development, short pupal period, early sexual maturity, reduced pheromone production, abbreviated courtship behaviour, and early fecundity (Miyatake 1993, 1998a, b; Miyatake and Yamagishi 1999; Cayol 2000). Changes in mating behaviour will reduce the competitiveness of the reared male insects (Miyatake and Shimizu 1999), but other changes caused by mass-rearing may be linked to other detrimental changes, such as reduced predator avoidance during male courtship (Hendrichs et al. 1994; Hendrichs and Hendrichs 1998). In the melon fly *Zeugodacus cucurbitae* (Coquillett), changes in the circadian rhythm and time of mating can be linked to selection for early mating (Miyatake 2002; Miyatake et al.

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2002) and rapid development (Miyatake 1996, 1997a), changes in longevity to early fecundity (Miyatake 1997b), and mating success to rearing density (Miyatake and Haraguchi 1996). However, Liedo et al. (2007) showed that quite small changes in adult colony rearing conditions may improve quality.

To produce large numbers of insects, an appropriate artificial diet is usually required (Lees et al., this volume; Parker, Mamai et al., this volume); often this diet has already been developed and proven adequate for the development of immature stages. However, when a large number of eggs is placed on a limited amount of diet, the situation may change. Waste products from larvae can create an intolerable situation for the insects and/or workers. Metabolic heat could raise the temperature, resulting in too-rapid development. The nutritional value of the diet may change. In any case, selection occurs for individuals that can tolerate these conditions. Pupal handling and storage also create conditions for selection. Each of these parameters can cause a genetic "bottleneck" that severely limits the number of individuals that survive the first few generations. As each subsequent generation becomes better adapted to the rearing conditions, a larger proportion of insects survives.

Severe "bottlenecking" does not always result in a permanently limited gene pool, but the gene frequency may change. When the population is increased substantially, the number of mutations also increases. Nei et al. (1975) speculated that, when populations expand, genetic diversity increases through mutations. The reduction in average heterozygosity per locus depends on the size of the bottleneck, and on the rate of population growth. If, after going through even an extremely small "bottleneck", the population size increases rapidly, there may be significant recovery of heterozygosity. That does not mean that the population returns to the identical diversity of the original gene pool. The western spruce budworm *Choristoneura occidentalis* Freeman, reared for 88 generations in a laboratory, lost 15 alleles, but the quality of the insects (in terms of size, fecundity, vigour, longevity, and disease resistance) was adequate for research purposes (Stock and Robertson 1982); the researchers made no mention of interaction with wild populations. Vale et al. (1976) reported no changes in activity or behaviour of the tsetse flies *Glossina morsitans* Westwood and *Glossina pallidipes* Austen due to laboratory rearing or artificial *in vitro* feeding.

In an AW-IPM programme that applies the SIT, the parameters necessary for the reared and sterilized males to be effective are: successful emergence and good field survival to the age of sexual maturity, sufficient mobility to find food, shelter, and wild females in a mating arena, swarming sites or other sites, mating competitiveness with the wild male for the wild female, mating compatibility with a wild female, successful transfer of sperm and accessory gland fluids, and adequate mobility and survival of the sperm.

To ensure that a rearing procedure is producing insects with these necessary traits, it is essential to have a system of quality-control tests that measures these parameters, as well as overall compatibility and competitiveness with the wild target population. The tests must be conducted on a regular basis so that the rearing process maintains the product quality in the long term (Parker, Mamai et al., this volume). If detrimental changes in insects begin to appear, a feedback loop to the rearing system is necessary so that the shortcoming can be identified, the cause determined, and the problem corrected (Calkins et al. 1988; FAO/IAEA/USDA

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2019). A filter rearing system is usually used to maintain the stability of genetic sexing strains, from which the normal mass-rearing colony is derived (Franz et al., this volume; Parker, Mamai et al., this volume). However, the same concept can be used to provide a means to control the selection pressure on the "mother" stock used for the colony while avoiding many of the worst traits from the pressure of mass-rearing (Fisher and Caceres 2000). A further step in this direction is the application of a "pre-filter" – a small adult colony held under semi-natural situations at low densities, e.g. a greenhouse within the mass-rearing facility, where it is exposed to variable conditions, natural-light cycles, hosts and predators to maintain as long as possible the viability of a newly introduced mother colony – instead of regularly bringing in new colonies that rapidly lose, in a few generations, their natural characteristics (Hendrichs and Robinson, this volume).

Strain replacement becomes essential when the properties of the reared strain become too different from the target population, and corrective measures are no longer effective. Replacing a strain can be a major task, takes considerable time, and also involves substantial cost. Newly collected field material may take several generations to adapt to colony rearing, with the colony stabilizing after about five generations (Bartlett 1984). If this involves an obligatory diapause, the time investment is clearly large. In the case of tsetse flies, the colonization of *G. pallidipes* in Ethiopia required more than 60 000 wild females over a period of 14 months to establish a viable colony, representing 4 or 5 generations of adaptation (Alemu et al. 2007).

Another potential hazard is the contamination of the new strain by insect pathogens such as densovirus (Carlson et al. 2006), hytrosavirus (Abd-Alla et al. 2013) or even human pathogens including arboviruses such as dengue that can be transmitted vertically in the case of a colony of mosquitoes (Buckner et al. 2013; Abd-Alla et al., this volume).

## 4. PARAMETERS OF QUALITY CONTROL

Product quality control covers the biological parameters of the reared insects. While the emphasis in this chapter will be on product control, some aspects of process control are also considered. Measurements for different groups of insects may involve different procedures. Information relating to tephritid fruit flies for most of the following sections can be found in FAO/IAEA/USDA (2019), most of which can be adapted to other insect groups, e.g. FAO/IAEA (2006).

### *4.1. Egg Hatch*

Changes in egg hatch or eclosion may indicate problems with mating in the colony, insufficient sperm quality or quantity in the males, inadequate sperm transfer to the females, insufficient sperm motility or infection with a pathogen. Samples of eggs should be monitored regularly for hatch rate, and also to ensure correct larval density in the diet.

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### *4.2. Larval Development Time*

As observed previously, changes in development time can be related to inadequate temperatures in the larval diet resulting from an incorrect egg seeding density, diet composition or rearing environment, as well as to changes in courtship behaviour. Development period should be monitored, and selection for rapid development avoided.

### *4.3. Pupal Size*

Pupal size is a good indicator of larval diet quality, rearing density, and any contamination or infestation problems. It is also an indirect measurement of reserves accumulated, affecting the longevity parameter under the stress test (section 4.9.). Pupal size is measured either by minimum diameter or weight. In some insects adult size, e.g. wing length in mosquitoes, is also used.

### *4.4. Percentage Adult Emergence*

The percentage of insects that emerges successfully, and may be affected by larval nutrition (pupal energy reserve), excessive temperature during the rearing and pupal-holding periods, and inappropriate relative humidity. Mishandling of pupae, such as excessive jarring, tumbling at early pupal stages, and excessive radiation dose may also influence percentage emergence and flight capacity.

### *4.5. Sex Ratio and Timing of Emergence*

Sex ratio may be affected by poor pupal-holding conditions, or in the case of a sexing strain that is based on a *temperature-sensitive lethal* (*tsl*) mutation, inappropriate temperature at any stage in the rearing. Timing of emergence indicates the uniformity of pupal age (pupal collection and handling control), and may warn of selection for inappropriate accelerated development. Uniformity of pupal age at irradiation is also a critical determinant of the extent to which exposure to radiation procedures affects the quality of flies (FAO/IAEA/USDA 2019).

### *4.6. Flight Ability*

Released sterile insects need to be sufficiently mobile and have adequate dispersal capabilities to reach, in a timely fashion, all the ecological niches that are occupied by the wild insects (Vreysen et al. 2016). Therefore, the ability of sterile insects to fly, after having been released in the field, is an essential attribute. Those insects that cannot fly to shelter or to food, or cannot reach the pheromone-calling or mating sites, are lost to the programme.

"Percent flight" is the percentage of insects that can fly, based on the number of pupae put into an emergence/flight tube. This may also be corrected for percentage emergence, a parameter called "absolute fliers", and which determines the number of adults that can be released (FAO/IAEA/USDA 2019).

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Reductions in flight, dispersal, and survivability due to rearing and/or irradiation have been noted by a large number of researchers, e.g. Dame et al. (1969), Rajagopalan et al. (1973), Sharp (1976), Nelson and Milby (1980), Nakamori and Soemori (1981), and Smith et al. (1981). Little and Cunningham (1978), Sharp et al. (1980), Little et al. (1981), and Sharp and Little (1982) found that sifting of fruit fly pupae at a critical stage in their development was the reason that indirect flight muscles did not insert properly into the cuticle of the exoskeleton during pupal development. This created the "*droopy-wing syndrome*" in which various degrees of droopy wings resulted in non-flying flies (Little and Cunningham 1978).

Knowledge about the mobility and dispersal characteristics of released insects is essential for developing and designing appropriate release strategies (Vreysen, this volume). A simple quality-control test suitable for routine testing, the flight-ability test, measures the ability of the insects to fly out of a restricted space (Carpenter et al. 2012; FAO/IAEA/USDA 2019; Seck et al. 2015; Balestrino et al. 2017; Culbert et al. 2018; Lees et al., this volume). In the last decade, several more elaborate methods have been developed to assess flight ability of mass-reared Lepidoptera, e.g. flight mills (Zhang et al. 2016), wind tunnels (Stringer et al. 2013), and flight assessment cages (Saour 2016), that would be appropriate for periodic testing.

Recent developments in machine vision, to record and analyse insect behaviour, would offer the opportunity to quantify important quality factors such as flight ability, mating propensity, and competitiveness. The flight tracks of irradiated male light brown apple moths *Epiphyas postvittana* (Walker) in a wind tunnel responding towards a calling female were different from those of untreated moths. This approach is likely to be amenable to automation, and could potentially be used in routine quality control (Suckling et al. 2011).

### *4.7. Pheromone Production and Response*

For certain insect groups such as fruit flies and Lepidoptera, pheromone emission and response are the earliest interactions that sterile males have with wild females. For fruit flies, the response of wild females to pheromone-calling wild or sterile males can be compared using the pheromone compatibility test carried out in field cages (FAO/IAEA/USDA 2019). For Lepidoptera, the response of released males to synthetic pheromone can be measured in a wind tunnel (Stringer et al. 2013) and to calling females in mating cages (Saour 2016) or on mating tables (Snow et al. 1976; Flint and Merkle 1984; McBrien and Judd 1996). A commercial insect-locomotion-activity meter was used to assess responses of wild and irradiated *E. postvittana* towards repeatable pheromone pulses. Non-irradiated males responded towards the pheromones significantly better than irradiated males. A high-temperature shock did not change the response of the non-irradiated moths, but it slightly diminished the response of irradiated moths. The system showed potential to assess routinely the quality of mass-reared moths, and might be suitable for factory-scale quality control (Brown et al. 2016).

Continuous laboratory rearing may create subtle changes in components of a pheromone emitted by a sterile male (or female), rendering it unattractive to a wild female (or male) (Minks 1971), but this does not always happen (Richmond and

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Berisford 1980; Sower et al. 1973); in the case of field-caged males of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), more wild females were attracted to sterile males than wild males. Close examination of the behaviour of the field-caged males revealed that sterile males "pheromone-called" more frequently, and for longer periods of time, than wild males (C. O. Calkins and T. R. Ashley, unpublished data); this may appear to be an advantage, but actually exposes sterile males to more predation attacks (section 4.10.) (Burk 1982; Hendrichs and Hendrichs 1998).

Protein feeding by adult male Mediterranean fruit flies enhances pheromone production and hence competitiveness (Kaspi and Yuval 2000; Shelly et al. 2002; Yuval et al. 2002, 2007). Protein feeding by males, together with size, also enhances post-copulatory sexual selection (Taylor and Yuval 1999). Therefore, providing sterile males with pre-release access to protein can enhance their field performance (Pereira et al., this volume).

Some insects require specific nutrients and semiochemicals to be able to synthesize their pheromone; supplying this to adults during their maturation can improve field performance, and reduce time spent by the released insects searching for this component in the field (Ji et al. 2013; Obra and Resilva 2013; Tan and Tan 2013; Haq et al. 2014, 2015, 2016; Pereira et al., this volume).

### *4.8. Vision*

Agee and Park (1975) developed the use of an electroretinogram to measure the quality of the vision of fruit flies; irradiation debilitated both the sensitivity and spectral accommodation. Rössler (1980) discovered that the sexual competitiveness of Mediterranean fruit fly males, possessing an apricot eye-colour mutant, was reduced. Using the apricot-eye strain for mating and acoustical tests, it was observed that, during courtship, a mutant male could not follow closely the wing waving of a normal female, resulting in the female rejecting the male's advances.

Visual impairment was also observed in mass-reared New World screwworms (Bush et al. 1976), necessitating strain replacement (Klassen et al., this volume).

### *4.9. Longevity under Stress*

In many insect species, the nutrient reserves acquired during the larval stage and carried through the pupal stage affect the longevity of the adult stage. The longevity/stress test, often related to pupal size, measures the percentage of adults that survives for a set time period, depending on species, without food or water (Orozco-Dávila et al. 1983; Ashley 1987; Mutika et al. 2002). Since this test produces stress, it is indicative of the amount of nutritional reserves present when adults emerge. It has recently been adopted as a quality-control parameter in tsetse flies (Seck et al. 2015).

While protein feeding improves pheromone production in Mediterranean fruit flies, it also reduces survival when they face starvation (Taylor and Yuval 1999). However, Maor et al. (2004) demonstrated that Mediterranean fruit flies fed on protein are successful at exploiting both protein and carbohydrate resources in the

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field, and their inability to overcome starvation is not a concern. Yuval et al. (2007) reached the same conclusion, stating that it may be better to release short-lived flies that are highly competitive instead of long-lived sexually ineffective ones.

### *4.10. Startle Activity*

A common problem in laboratory-reared insects is the loss of irritability. Mass-rearing conditions apparently select inadvertently for insects that, to maximize their fitness, can afford under the protected but very dense colony environment to ignore any movements in their immediate surroundings. As a result, they also disregard any potential dangers after being released in the field, and thus are much more susceptible than wild insects to many of the predators they will encounter (Hendrichs and Hendrichs 1998). The "startle test", developed by Boller et al. (1981), measures levels of irritability, and this or a similar test that measures capacity for predator evasion (Hendrichs et al. 2007; Dor et al. 2014; González-López et al. 2016; Dor and Liedo 2018; Rathnayake et al. 2019) could be used to select for increased irritability. The level of irritability appears to be inherited genetically (C. O. Calkins, unpublished data), and hence a proper management of the mother colony could probably maintain some degree of irritability.

### *4.11. Mating Propensity, Compatibility, and Competitiveness*

#### *4.11.1. Mating Propensity*

Mating speed or mating virility is one indicator of fitness (Pendlebury and Kidwell 1974; Lance and McInnis, this volume). This test measures the propensity or willingness of mass-reared sterilized males to mate with virgin females. This is appropriate only for male-choice mating systems; it provides no information for female-choice systems (Lance and McInnis, this volume).

Mating propensity presumably measures, by the use of the mating index, how "eager" the sterile males are to mate. However, as this test is usually conducted in the laboratory with reared males and females, results are often not representative of laboratory male performance when exposed to wild females in the field. This is confirmed by the fact that laboratory-reared males usually have a higher mating index than wild males.

Rapid matings tend to be controlled by the male genotype, while the female genotype may assume importance in slower matings (Parsons 1974). Therefore, for female-choice mating systems, this test is often misleading because mating speed largely reflects the fact that males, selected under extremely high-density adult-colony conditions (where most females become receptive at the same time, and thus a 1:1 operational sex ratio to courting males prevails), obtain rapid matings without going through the proper courtship sequence. These males are often rejected by wild females while attempting to court them in leks under natural conditions (Briceño and Eberhard 1998; Hendrichs et al. 2002).

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*4.11.2. Mating Compatibility/Assortative Mating*

The ability of reared sterilized males to compete successfully in mating with the males of the target population is crucial for the SIT. Reduced competitiveness can arise from problems in rearing, irradiation or handling, but also from inherent incompatibility between different strains. For example, the Mediterranean fruit fly arrived in southern Europe from West Africa via the Cape Verde Islands in the 1700s, while it reached Hawaii from East Africa via Australia in the late 1800s, and the populations in South America probably arrived from West Africa. The resulting isolation of these populations might allow the accumulation of changes, which will eventually lead to reproductive isolation and incipient speciation. Some evidence of reproductive isolation in island situations has been found (Hibino and Iwahashi 1991; McInnis et al. 1996; Miyatake 1998a), but an extensive comparison of Mediterranean fruit fly strains from around the world with the main mass-rearing strains showed no significant mating barriers (Cayol et al. 1999, 2002), indicating that in this species any mass-reared strain can be used against any wild population.

Similar results, i.e. no evidence of mating barriers, were obtained with codling moth populations from around the world (Taret et al. 2010), and with olive fruit fly *Bactrocera oleae* (Rossi) populations from the Mediterranean basin (Ahmad et al. 2018). The same result was observed between strains of *G. palpalis gambiensis* originating from Burkina Faso, Mali, and Senegal (Mutika et al. 2013), even though the strain from Senegal, for example, displayed substantial ecological and genetic divergence from the other strains (Bouyer et al. 2010; De Meeûs et al. 2015).

Reisen et al. (1980) detected assortative mating in releases of *Culex tritaeniorhynchus* Giles, as did Raulston et al. (1976) with the tobacco budworm *Heliothis virescens* (F.). The latter was caused by a change in the temporal mating period of the laboratory-reared population which initiated courtship 2 h earlier than the wild population. Zervas and Economopoulos (1982) observed that laboratory-reared olive fruit flies also began to mate 2 h before wild flies, although this was not observed in field-cage studies using newly established colonies (Ahmad et al. 2018). Wong et al. (1982) noted assortative mating between laboratory-reared and wild oriental fruit flies *Bactrocera dorsalis* (Hendel). On the other hand, Spates and Hightower (1967) found that New World screwworm males became sexually more aggressive when reared in the laboratory. However, assortative mating in *Anastrepha fraterculus* (Wiedemann) led to the realization that this widespread South and Central American species is actually a complex of sibling species (Cáceres et al. 2009; Segura et al. 2011; Hernández-Ortiz et al. 2012; Devescovi et al. 2014).

Differential mating preferences of wild and released sterile insects will reduce the efficacy of the SIT component in AW-IPM programmes (Hibino and Iwahashi 1991), but these negative impacts have often been underestimated (Vreysen et al. 2006). It is known that selective mating occurs between colonized New World screwworm strains of different age, with males from strains that have been cultured for many generations being non-selective in mating with old-line or new-line females, but new-line males copulated more readily with females from newly colonized strains than with females from older strains (Spates and Hightower 1970). This asymmetric mating isolation between the behaviours of old and new screwworm strains has been associated with reduced female contact-pheromone

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activity (Hammack 1987), but its relevance and effects on the efficiency of SIT projects have never been researched properly in the field. Vreysen et al. (2006) present models that examine this effect, and it was demonstrated clearly that, for older screwworm strains, a doubling in the number of sterile male insects released per surface area is required to obtain a suppression effect similar to that when no asymmetric mating isolation is present. Consequently, the discriminatory behaviour of wild screwworm males against sterile females has significant economic implications.

#### *4.11.3. Mating Competitiveness*

In-depth knowledge about the mating behaviour of the target insect, and the factors that could impair this behaviour, is indispensable for improving the performance of the sterile insects – to increase the overall efficiency of the SIT, and hence to reduce its cost (Hendrichs et al. 2002). The size of males can also be an indirect indicator of mating competitiveness. It affects mating success in the Caribbean fruit fly *Anastrepha suspensa* (Loew) (Burk and Webb 1983) and the Mediterranean fruit fly (Churchill-Stanland et al. 1986, 1987; Bloem et al. 1993a, b, c; Economopoulos et al. 1993; Orozco and Lopez 1993). A larger male is more competitive against rival males, and females tend to select larger males over smaller ones, but Hunt et al. (2002) found an effect of size on pheromone calling but not on overall mating success. In field releases, larger males disperse farther and live longer than smaller males (Bloem et al. 1994) (section 4.9.). Pupal weight is a good indicator of adult size, and Mediterranean fruit fly programmes (to comply with quality standards) require that late-stage pupae should weigh about 7 mg. In New World screwworm programmes, late-pupal weight should not fall below 44 mg.

However, in *Aedes aegypti* (L.) mosquitoes, it was demonstrated that large females showed no evidence of a mating preference, whereas small males were relatively more successful than large males when mating with small females, exhibiting an advantage of around 20–25% (Callahan et al. 2018). This result shows that size might matter, and should be monitored in mosquito SIT programmes, particularly when considering that laboratory-reared mosquitoes can often be larger than their wild counterparts (which experience more competition for food in their larval habitats, especially in the case of *Aedes* species that exploit micro-habitats).

Laboratory bioassays often do not indicate accurately the field performance of reared insects (Katsoyannos et al. 1999). Quality-control tests (that usually are more appropriately carried out under semi-natural conditions in field cages containing vegetation) are: pheromone attractiveness, mating compatibility, and mating competitiveness (Zapien et al. 1983; Cayol et al. 1999, 2002; FAO/IAEA/USDA 2019). It has been recommended that, at least once a year, AW-IPM programmes releasing sterile insects obtain insects from the field population being targeted, and compare them with mass-reared insects in field cages that permit as large a range of natural behaviours as possible, including female choice (FAO/IAEA/USDA 2019; Lance and McInnis, this volume; Parker, Mamai et al., this volume). Field-cage and field tests are discussed below (section 8.).

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### *4.12. Remating*

A further important component of mating behaviour for the SIT is remating by wild females (Lance and McInnis, this volume; Whitten and Mahon, this volume). Female monogamy is not required for the SIT, but if there is a differential rate of remating by females first mated to a sterile or a wild male, or there is sperm selection following multiple matings, there will be an effect on the SIT (Bonizzoni et al. 2007; Pérez-Staples et al. 2013).

Remating may be controlled by factors transferred by the male during copulation; if the mass-reared males do not transfer the necessary factors, the incidence of remating may rise differentially (Jang 2002; Vera et al. 2002). This may be measured in an extension to the field-cage compatibility/competitiveness tests listed above. The females from pairs collected in the compatibility/competitiveness test are marked according to the male with which they first mated, and the field-cage test repeated on subsequent days. Remating pairs are then scored, and the results examined for evidence of differential remating (McInnis et al. 2002).

Multiple mating of female insects is common in Lepidoptera; this was recently confirmed with *Eldana saccharina* Walker under controlled laboratory conditions. The multiple-mating potential of both males and females might have consequences for obtaining adequate sterile to wild male overflooding ratios (Walton and Conlong 2016a; Barclay, this volume). Multiple mating is also common for females of the cluster caterpillar *Spodoptera litura* (F.); a study on sperm-use patterns revealed that females preferentially use the sperm of the last male to mate. In addition, female mating success, remating propensity, and fertility were significantly influenced by mating sequences that included irradiated males (Seth et al. 2016). Multiple mating is not uncommon in insects, e.g. fruit flies, tsetse flies, and mosquitoes. Barclay (this volume) discussed modelling the impact of multiple mating on the SIT.

## 5. EFFECTS OF IRRADIATION ON QUALITY

An essential step in the SIT is the induction of sterility. In many insect groups, irradiation results in a reduction in competitiveness (Bakri et al., this volume), and much recent work has been aimed at reducing this negative effect.

The timing of irradiation can be adjusted to reduce the effects without creating logistical and handling problems. Normally, the least amount of damage to adult males is caused when irradiation is carried out shortly after emergence, but the problem of irradiating large numbers of adult insects at one time is often challenging; therefore, usually pupae are irradiated as pharate adults shortly before emergence (Bakri et al., this volume). For various fruit flies, pupal development is monitored by eye colour to determine the optimal timing for irradiation (Ruhm and Calkins 1981; Resilva and Pereira 2014; Resilva et al. 2019).

Irradiation in air creates free radicals that are detrimental to the quality of insects. When oxygen is excluded by flooding the containers with nitrogen, this problem is reduced in fruit flies and codling moths (Ashraf et al. 1975; Robinson 1975). Later, it was discovered that when containers of fruit fly pupae were sealed, pupal metabolism quickly exhausted the oxygen and produced carbon dioxide, resulting in

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similar protection (Ohinata et al. 1977; Bakri et al., this volume). The effects of hypoxia have also been investigated in several other insects (Vreysen and Van Der Vloedt 1995; Hallman 2005; López-Martínez et al. 2014, 2016; Yamada et al. 2019).

In many insect species, females become 100% sterile at lower doses than males. Attempts to attain 100% sterility in males usually reduce quality; often it will be better to reduce the dose so as to obtain a better induction of sterility in the field females through more competitive males (Hooper 1972; Toledo et al. 2004; Parker and Mehta 2007).

In Lepidoptera, the higher radiation sensitivity of females has been exploited to adopt an $F_1$ sterility or inherited sterility (IS) strategy; very high radiation doses are required to induce dominant lethal mutations in Lepidoptera (Proverbs and Newton 1962; Blomefield et al. 2010; Soopaya et al. 2011; Cagnotti et al. 2012, 2016; Jang et al. 2012; Saour 2014; Fu et al. 2016; López-Martínez et al. 2016; Walton and Conlong 2016b; Chakroun et al. 2017; Marec et al., this volume). In these insects, a lower dose that partially sterilizes the males but completely sterilizes the females can be employed to induce sterility in the subsequent generation. A higher percentage of male moths exposed to substerilizing doses respond to virgin females than those treated with higher doses (Bloem et al. 2001; Simmons et al., this volume).

Irradiation can affect various quality parameters to varying degrees, including survival (Vreysen and Van Der Vloedt 1995; Opiyo 2001), mating competitiveness and seminal-fluid transfer (Mutika et al. 2002), flight ability (Stringer et al. 2013; Saour 2016; Zhang et al. 2016), and production of and response to pheromones (McGovern et al. 1975; White and Hutt 1975). Both dose fractionation (Vreysen and Van Der Vloedt 1995; Kumano et al. 2011a, b) and hypoxia (Ohinata et al. 1977; Vreysen and Van Der Vloedt 1995; Hallman 2005; López-Martínez et al. 2014, 2016; Yamada et al. 2019; Bakri et al., this volume) may reduce these negative impacts.

## 6. EFFECTS OF CHILLING ON QUALITY

Before irradiation and prior to shipping, pupae are often chilled to lower their metabolic rate. Therefore, understanding tolerance of pest insects to thermal extremes is essential to ensure optimal insect quality after shipment; tolerance traits can provide insights into constraints on their activity and survival. Long-distance shipments of irradiated Mediterranean fruit fly pupae (either chilled or not chilled) were compared by Brazzel et al. (1986). Pupae shipped for 18 h in hypoxia averaged 77% emergence and 70% fliers, but those shipped in hypoxia in chilled boxes averaged 82% emergence and 76% fliers. Pupae shipped for 40 h in bottles in hypoxia averaged 48% emergence and 34% fliers, but those in hypoxia and chilled boxes averaged 73% emergence and 62% fliers.

Prolonged chilling (exceeding 26 h) can be detrimental to fly emergence at the release point, but not as detrimental as hypoxia with no chilling (FAO/IAEA 2017a). Serghiou (1977) discovered that the competitiveness of irradiated sterile Mediterranean fruit flies decreased as their exposure to chilling increased, but chilling did not have an adverse effect on survival.

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In view of the need to synchronize releases of sterile male tsetse flies (*G. pallidipes*), Mutika et al. (2002) stored pupae at various low temperatures, and found that no significant differences in emergence, survival without a blood meal, or insemination capacity occurred. In fact, male adults emerged from pupae that had been stored at 15ºC started mating more quickly, and formed more mating pairs, than the controls (stored at room temperature). However, if adult males were chilled (and then removed from the low-temperature container for testing), insemination capacity was reduced, and mortality was 10–32%. Later, it was observed that storing the pupae at 4°C actually reduced the emergence rate significantly, down to 76.1 ± 13.2% when they were chilled for 1 d in the source insectary before transport to Senegal, and to 72.2 ± 14.3% when they were chilled for 2 d (Pagabeleguem et al. 2015).

In *Anopheles arabiensis* Patton, chilling the males at 4–10°C for 24 h did not have any significant negative effect on their survival (Culbert et al. 2017). Also, in *Ae. aegypti,* chilling males for 2 h at 4–10°C did not reduce the survival rate significantly, but chilling them at 0°C for the same duration reduced their survival significantly. Moreover, the full insemination rate of females by the treated males, as well as their flight ability, declined from exposure to temperatures of 8°C and lower (Culbert et al. 2018).

Insect thermal limits may vary with microclimatic fluctuations, and may be influenced by biotic or abiotic factors. Chill-coma temperature (CTmin) and critical thermal maximum (CTmax) were significantly different for *E. saccharina* moths collected from two host plant species, i.e. sugar cane and papyrus. However, the lower lethal temperature (LLT) of these moths, and the freezing temperature of the pupae, did not vary with the host plant species. Therefore, host plants may strongly mediate the lower critical thermal limits, but not necessarily the LLT or freezing temperatures (Kleynhans et al. 2014).

Male and female laboratory-reared *E. saccharina* were more heat tolerant than wild moths, but the latter were more cold tolerant than their laboratory-reared counterparts. Irradiation had a deleterious effect on the CTmax and the CTmin. Moths irradiated with a lower dose were more heat and cold tolerant than those irradiated with a higher dose, highlighting again the importance of treating lepidopterans with a lower dose rather than with the full sterilizing dose (Mudavanhu et al. 2014).

False codling moths *Thaumatotibia leucotreta* (Meyrick) exposed to -4.5°C for 2 h or to -0.5°C for 10 h had a 50% probability of survival, and their low-temperature tolerance was not affected by gender or adult age. Only limited evidence for cold hardening was found; survival did not increase after exposure to non-lethal, low- and high-temperature pre-treatments (Stotter and Terblanche 2009). The colony was found to be adequate under most temperature conditions, but not during the coldest months (Boersma and Carpenter 2016); it is proposed to develop a second, cold-adapted strain for release during the winter months.

Recapture rates of codling moths (that had been acclimated to low temperatures) were significantly greater under cooler conditions in the wild than either warm-acclimated or control moths. However, improvements in low-temperature performance of cold-acclimated moths came at a cost to performance under warmer conditions. Conversely, moths acclimated to higher temperatures performed better

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under warmer ambient conditions in the field when compared with cold-acclimated moths or control moths. Laboratory data matched field results, indicating that assessment of thermal activity of codling moths in the laboratory may also apply to their field performance (Chidawanyika and Terblanche 2011).

Rearing codling moths through diapause improved their competitiveness in orchards (Dyck 2010), but their radio-sensitivity was similar to that of moths reared under non-diapause conditions (Carpenter et al. 2010).

## 7. POST-SHIPMENT QUALITY CONTROL AT EMERGENCE AND RELEASE FACILITIES

In operational programmes covering large areas (or when shipping sterile insects to transnational customers), it may be necessary for efficient operation to have one or more emergence and release facilities separate from the rearing facility that are close to the release sites (Dowell et al., this volume). Pupae (or chilled adults) can be shipped in bulk from the rearing facility to the emergence and release facility (FAO/IAEA 2017a) where they can be held for emergence and maturation, and be supplied with appropriate environmental conditions, diet components and other treatments to condition them for release (Chidawanyika and Terblanche 2011; Ji et al. 2013; Obra and Resilva 2013; Tan and Tan 2013; Haq et al. 2014, 2015, 2016; Dowell et al., this volume; Pereira et al. this volume).

The FAO/IAEA/USDA (2019) manual (sections 6 and 7) provides a number of tests for process and product quality control for emergence and release facilities receiving shipments of sterile insects. This international standardization of post-shipment quality-control tests allows the supplier and receiver to compare the quality of commercial and transboundary shipments of sterile insects.

## 8. FIELD-CAGE AND FIELD TESTS

### *8.1. Field-Cage Tests*

As noted above, laboratory tests of competitiveness or compatibility generally do not reliably indicate the performance in the field (Katsoyannos et al. 1999; Itô et al., this volume; Vreysen, this volume). A much better measure can be obtained by using a walk-in field cage (White et al. 1977; Calkins and Webb 1983; Chambers et al. 1983). Standard procedures for field-cage operation have been defined for fruit flies (Cayol et al. 1999, 2002; FAO/IAEA/USDA 2019); increasingly, these have been adapted for other species such as tsetse flies (Mutika et al. 2001), mosquitoes (Oliva et al. 2012; Damiens et al. 2016), and Lepidoptera (Taret et al. 2010; Mudavanhu et al. 2016).

A walk-in field cage consists of a mesh cage, at least 2.3-m high and 3-m wide, erected over a host plant or other suitable vegetation (Calkins and Webb 1983). Equal low numbers of wild males and females of the target population, as well as sterile mass-reared insects of both sexes, are introduced into the cage, and mating pairs are observed. When a genetic sexing strain is being tested, tests with only sterile males may also be conducted. The four possible outcomes (SS, SW, WS, and

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WW) are then examined for trends in the mating (Box 1). The low-density and semi-natural conditions permit near-normal courtship and mating behaviour, such that any changes in the behaviour of the mass-reared insects should be apparent. The results of field-cage experiments are affected by the conditions under which the mass-reared insects have been reared; therefore, it is essential that conditions be tightly controlled. Each test should be replicated a minimum of 8–10 times, but data discarded when low proportions of both males and females from any strain participate in mating activities (FAO/IAEA/USDA 2019).

It is recommended that field-cage tests be performed against wild-collected insects about once per year (FAO/IAEA/USDA 2019; Lance and McInnis, this volume). Field-collected larvae must be handled properly. For example, if coffee berries infested with Mediterranean fruit flies are placed on screens and the larvae collected in pans below, the berries tend to dry out causing the larvae to exit the fruit prematurely. Then the emerged flies are smaller than normal, and this can invalidate bioassays comparing wild fly and laboratory-reared fly behaviours.

Mating competitiveness and compatibility of non-irradiated and irradiated *E. saccharina* assessed under semi-field conditions showed that wild moths did not discriminate against irradiated or laboratory-reared moths (Mudavanhu et al. 2016). Irradiated males mated significantly more times than their wild counterparts, regardless of the type of female mate, indicating that colony males were still as competitive as their wild counterparts. When male *C. sinensis* moths irradiated with 250 Gy competed with untreated wild males for untreated female mates in walk-in field cages, the Fried Competitiveness Index (Haisch 1970; Fried 1971) C value of 0.48 obtained indicated that a substerilizing dose of 250 Gy would be adequate for programmes that include an IS component (Fu et al. 2016).

Field-cage assessments are also useful for more detailed assessments of behaviour, e.g. time of mating, mating duration, remating, and the sequence and timing of specific behavioural components of courtship and pheromone calling (FAO/IAEA/USDA 2019). Standard walk-in field-cage tests were validated for use with *E. postvittana*. Mating frequency of *E. postvittana,* and the production of sex pheromone by the females, are significantly reduced with increasing radiation dose (Stringer et al. 2013). Modified test procedures were developed in which eggs are collected individually from the moths, after they had sufficient opportunity to mate in field cages, and then the females are dissected to determine the presence of one or more spermatophores (Woods et al. 2016).

In *Ae. albopictus*, field-cage experiments conducted in La Réunion island demonstrated that the Fried Competitiveness Index of sterile males was only 0.14 when they were released at 1-day-old, but improved to 0.53 when the release occurred after a 5-day holding period in laboratory conditions, indicating the appropriate age for release (Oliva et al. 2012). Furthermore, it was demonstrated that increasing the release ratio of sterile males led to a significant reduction in the competitiveness index, with 0.51, 0.20, and 0.18 recorded for the ratio of 100:100:100, 500:100:100, and 1000:100:100, respectively (Damiens et al. 2016).

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*Box 1. Indices of Compatibility and Competitiveness*

A standard field-cage experiment results in four figures, the numbers of each of the four possible pairings formed during the test period, SS, SW, WS and WW, where S represents sterile, W wild (fertile), and males are indicated first. The categories WS and SS will be missing in the case of genetic sexing strains where sterile females are not produced. These four figures have been combined in various ways to produce indices of competitiveness and compatibility, each emphasizing a different aspect, and each with advantages and disadvantages. (For more details, see section 3.1. in FAO/IAEA/USDA (2019)).

The *relative isolation index* (RII) gives an indication of mating compatibility between two strains. It is calculated as:

$$RII = \frac{SS \times WW}{SW \times WS}$$

A value of 1 indicates random mating, with larger values indicating assortative mating. This index is sensitive to changes in a single type of mating (e.g. a drop in the important category SW), is not affected by the overall level of participation of the different insect types, and does not depend on the ratio of sterile to wild insects in the test. The disadvantages are that the index is undefined if either SW or WS is zero, and it changes rapidly for a difference of only one if a category is small. The value of RII can be interpreted as the number of sterile males that have to be employed to be equivalent to one wild male. Values normally vary between 1.5 and 5, and values consistently above 3 are a cause for concern.

The *isolation index* (ISI) is defined as:

$$ISI = \frac{(SS + WW) - (SW + WS)}{SS + WW + SW + WS}$$

It ranges from -1 (complete negative assortative mating) through 0 (random mating) to +1 (complete positive assortative mating). The main advantages of the ISI over the RII are that it is easier to interpret values from -1 to +1 than from 0 to infinity, it is not as sensitive to a change in a small category and is defined even when one category is zero. Values normally lie between 0.1 and 0.4, and values over 0.5 are a cause for concern.

The ISI is normally combined with the *male relative performance index* (MRPI) and *female relative performance index* (FRPI) defined as:

$$MRPI = \frac{(SS + SW) - (WS + WW)}{SS + SW + WS + WW} \qquad FRPI = \frac{(SS + WS) - (SW + WW)}{SS + SW + WS + WW}$$

which show the proportion of sterile males and sterile females taking part in matings, and should be used to interpret values of ISI over 0.5.

These index values can be displayed graphically (then easier to interpret), as shown below.

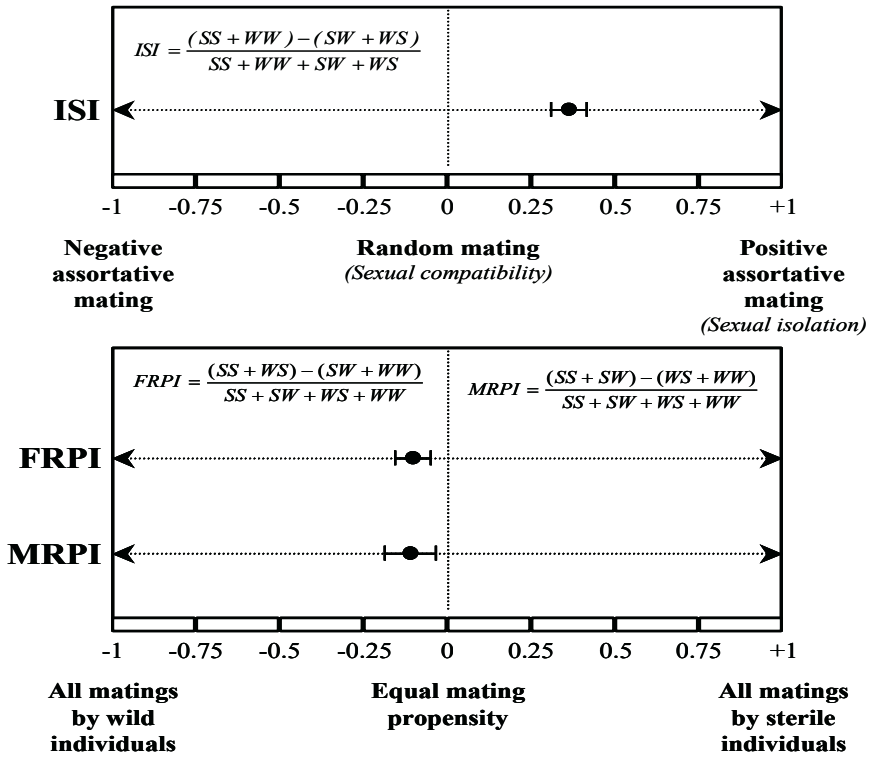
In contrast to the above, the Fried Competitiveness Index (Haisch 1970; Fried 1971) is based on the fertility of a particular cross combination rather than on the number participating in the cross. It is defined as:

$$C = \frac{N}{S} \times \frac{H_n - H_e}{H_e - H_s}$$

where C is competitiveness, $H_n$ and $H_s$ are defined as the fertility of crosses between normal and irradiated males with normal females (in laboratory tests), and $H_e$ is the observed fertility in an experimental set-up with N normal males and S irradiated males. A C value of 1 indicates equal competitiveness; observed values usually fall between about 0.3 and 1. It was originally devised for field tests where $H_e$ was measured by collecting egg masses in the field. The variance of C is given in Hooper and Horton (1981), and is a minimum when $H_e$ lies halfway between $H_n$ and $H_s$. (See also Pagendam et al. (2018) for an improved estimate of the Fried Competitiveness Index.)

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*Box 1. Continued*




*Graphic representation of isolation index (ISI) and female and male relative performance indices (FRPI, MRPI) (FAO/IAEA/USDA 2019).*

### *8.2. Field Tests*

The capacity of sterile insects to survive in the field, and to disperse to feeding, mating, and resting sites, is also critical (Hendrichs et al. 1991). Survival and dispersal rates of different rearing strains can be compared using various release/recapture methods (Itô et al., this volume; Lance and McInnis, this volume; Vreysen, this volume). Such rates are often not correlated with a flight-ability test of the same lot of sterile insects in the laboratory, or even survival in field cages, because they also measure the ability of the sterile insects to find food, to respond to attractants, and to evade predators (Hendrichs et al. 1993). C. O. Calkins and T. R. Ashley (unpublished data), using mass-reared Mediterranean fruit flies irradiated at the usual dose, and at twice this dose, monitored dispersal in the field. Flies that had been exposed to twice the usual radiation dose had very low rates of dispersal and survival. Other dispersal studies by Bloem et al. (1994) compared large flies (from pupae 8–8.5 mg in weight) with small flies (from pupae 5–5.5 mg); a higher

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percentage of large than small flies was captured initially, and over a longer period of time.

Ultimately, the competitiveness of the insects should also be tested in the field, e.g. White and Mantey (1977) and Villavaso et al. (1980). It is usually not possible to detect mating pairs directly in the field, but the competitiveness of the released sterile males can be estimated by comparing the egg hatch obtained from egg masses collected in the field or from captured wild females (some with sterile, others with fertile matings) (Haisch 1970; Fried 1971; Hooper and Horton 1981; Iwahashi et al. 1983; Parker and Welch 1991). In the case of tsetse flies, the proportion of females having mated with a sterile or a wild fertile male can be estimated from dissecting and examining the reproductive status of trapped wild females (Vreysen, this volume). An open-field study, using codling moths from Canada and South Africa, indicated that moths of both origins were equally attracted to calling females from Canada and South Africa; therefore, the codling moths from Canada were compatible with codling moths established in South Africa (Bloem et al. 2010). Such an estimate takes into account all the factors that affect the induction of sterility in the wild population, including survival, dispersal, mating competitiveness, sperm transfer, remating, and sperm competition (Vreysen, this volume). The same test can also be conducted in a field cage (FAO/IAEA/USDA 2019).

In *G. palpalis gambiensis*, it was demonstrated that a strain colonized for more than 40 years was still competitive in the field in its country of origin (Burkina Faso) (Sow et al. 2012), and more surprisingly that it was more competitive than an autochthonous and recently colonized strain in Senegal (Bassène et al. 2017). In *Glossina austeni* Newstead, it was also demonstrated that sterile males were able to aggregate exactly in the same sites as the wild males, leading to a homogeneous sterile to wild ratio during the successful eradication campaign in Zanzibar (Vreysen et al. 2011).

## 9. CONTROL CHARTS AND DASHBOARDS

Data generated by quality-control assays are not very useful until they can be graphed in a sequential manner to show trends. In 1924, W. Shewhart of Bell Telephone Laboratories first developed control charts (Feigenbaum 2004). A chart consists of sequential plots of a specific quality criterion on a graph that has a central line, and upper and lower control limits (Box 2). The control limits may either be defined a priori, or more usually are derived from long-term data. For this, many samples are taken over an extended period, and the mean or median computed for the central line; the upper and lower control limits are usually three standard errors on either side of the central line (Charbonneau and Webster 1978). Each new quality-control value is plotted on the chart, and both its position and the trend over the last seven points are assessed for conformity with a set of standard rules. A single value out of bounds is less a concern than a continuing trend away from the central line. Control charts have been proposed for the Mediterranean fruit fly (Calkins et al. 1982), Lepidoptera (Fisher 1983), and tsetse flies (Timischl 1980).

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A tool is now available specifically for monitoring the key parameters in insect mass-rearing in the manner of a Dashboard (FAO/IAEA 2018). This tool links production parameters with product quality ones, and presents the data in an easily understandable graphical format to facility the management of large or small colonies by both management and technical staff.

## 10. IMPACT OF LOW-QUALITY INSECTS ON ERADICATION PROGRAMMES USING THE SIT

Calkins and Ashley (1989) attempted to put an actual monetary value on the impact of producing low-quality insects. Using Knipling's (1979) model, Calkins et al. (1996) inserted varying values of the quality of Mediterranean fruit flies established during a pilot test for quality control. Using the New World screwworm as an example, with a growth rate of 5 and an overflooding ratio of 9:1, it would take five generations to achieve eradication. Since the Mediterranean fruit fly has a much greater biotic potential, and based on its life-history parameters, a growth rate of 12 was established; an overflooding ratio of 9:1 resulted in an increase in the population each generation. The "rule of thumb" for Mediterranean fruit flies, selected during the numerous fly invasions into California, USA, is a ratio of 100:1. If all flies were 100% effective, theoretically this ratio achieves eradication in three generations.

*Box 2. Shewhart Control Charts*

Control charts are a well-established tool for stabilizing industrial processes. They are formed by plotting sequentially the successive values of a control parameter. These are then compared with a central line (CL), upper control limit (UCL), and lower control limit (LCL), using certain criteria to determine if the process is in control.

The graphed parameter will usually be the mean of a sample taken from the process. In industrial processes, this may be compared with a priori limits, e.g. the design diameter of a shaft and the specified tolerance limits. In insect rearing, we do not usually have such a priori limits, and base the assessment of the graphed value by comparing it with long-term historical values. The long-term mean is taken as the central line, and the upper and lower control limits are defined as three standard errors above and below the mean. By standard statistical theory, if the parameter plotted is normally distributed, random fluctuation will cause about 0.1% of the values to lie above the UCL or below the LCL. If more than one value lies outside the control limits, this indicates a systematic influence, and the process is said to be out of statistical control.

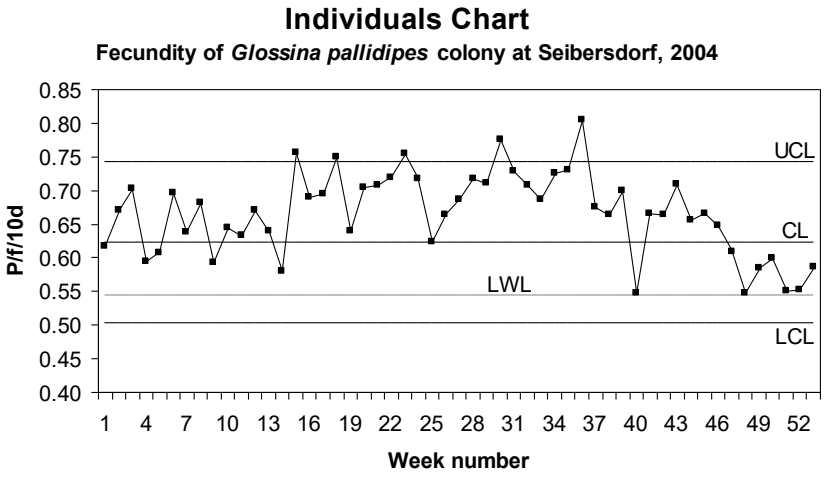
Two examples of control charts are given below — monitoring the fecundity of the *G. pallidipes* colony at the FAO/IAEA Seibersdorf laboratories. The parameter plotted is weekly fecundity, expressed as pupae per live female per ovarian cycle (10 days) (P/F/10d). The central line is the average value over a 105-week period (2003–2004). Since only a single measurement is taken each week, variability is estimated by using the difference between successive pairs of values, yielding a moving range, from which the standard deviation is derived.

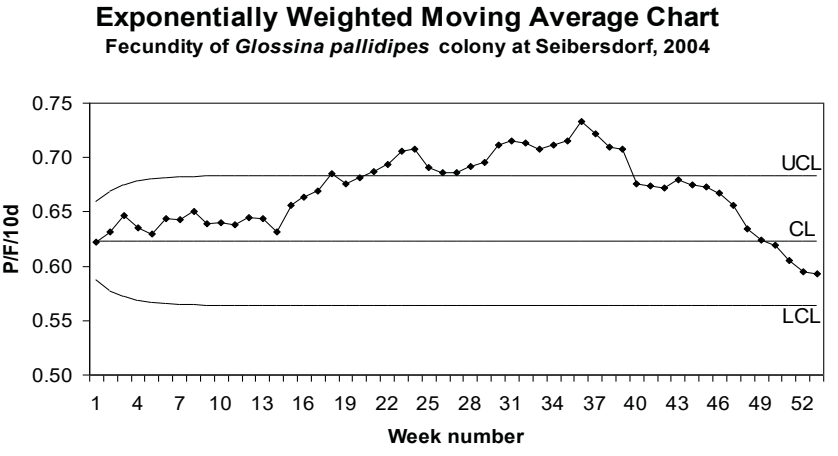
The first chart shows the individual values plotted for 2004, and the UCL, CL, and LCL. Since increased fertility is not a problem, only the LCL is considered further. Another line has been added, the lower warning line (LWL), at two standard errors below the CL; this draws attention to values approaching the limit.

The second chart uses an exponential weighted moving average (includes all historical data points but weights the most recent more highly). This is less sensitive to individual extreme values, but more clearly shows longer-term trends. In this case, the control limits are not straight lines, but are asymptotic to the control limits in the previous example. Both charts show that, in 2004, fecundity was usually better than average, but fell towards the end of the year.

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*Box 2. Continued*




However, when the fly quality equals the minimum acceptable level of quality, the effective release ratio becomes 23:1, and it takes five generations to achieve eradication (Calkins and Ashley 1989).

When the actual level of fly quality was inserted into the model, giving an effective ratio of 54:1, only four generations were needed for eradication. However, if the flies were only half as effective, the cost per hectare (to produce and utilize sterile flies that are of only moderate quality) becomes almost twice as much. Furthermore, increasing the frequency of releases or the number of released sterile insects can only compensate for low quality up to a point – beyond which increased

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numbers cannot make up for such deficiencies (Itô et al., this volume). Therefore, it is economically beneficial to rear high-quality rather than low-quality insects (Calkins and Ashley 1989).

A population model developed for *E. postvittana* indicated that releasing 300 Gy-irradiated moths would result in a 95% probability of extinction (if the ratio of released to wild moths exceeded 6.4:1). The model indicated an optimal release interval of 1 wk, but higher overflooding ratios would achieve eradication more rapidly. The model also predicted little advantage to releasing only male moths as compared with both sexes. Using a dose of 200 Gy, as part of an inherited sterility programme, would reduce (due to the resulting $F_1$ sterility) by ⅓ the required number of factory moths as compared with releasing lower quality moths irradiated with 300 Gy (Kean et al. 2011; Rull et al. 2012).

For the year 2001, a cost of USD 216 per million sterile male Mediterranean fruit flies was calculated when mass-reared at the El Pino facility, Guatemala (using a *tsl* genetic sexing strain) (see Table 1 in Hendrichs et al. 2002; Enkerlin 2003). At these large-scale production levels, these are probably the cheapest sterile insects in the world, although the cost increases significantly at lower production levels. The cost per million *tsl* males is more or less equivalent to the cost per million males of the usual bisexual strain. However, the big difference (in terms of cost savings) is in the transport and release operations, where sterile *tsl* males cost half as much as males of the bisexual strain (Caceres et al. 2004). In addition, male-only releases introduce three or four times more sterility into the target population than do bisexual releases (Robinson et al. 1999; Rendón et al. 2000, 2004).

There were several technical and managerial reasons why the ill-fated New World screwworm eradication project on the island of Jamaica was not successful (Vreysen et al. 2007; Dyck, Reyes Flores et al., this volume). However, the reduction in the quality of the sterile pupae received from the screwworm mass-rearing facility in Mexico (as evidenced by a gradual reduction in the average weight of the pupae) was certainly one of the critical technical issues that contributed to the failure of the programme (see Fig. 3 in Vreysen et al. 2007).

## 11. CHANGES IN INSECT BEHAVIOUR CAUSED BY LABORATORY REARING

Selection during colonization and mass-rearing usually changes the biology and behaviour of the reared insects (Iwahashi et al. 1983; Calkins 1989; Miyatake 1993, 1998a, b, 2002; Miyatake and Haraguchi 1996; Miyatake and Yamagishi 1999; Cayol 2000; Shimoji and Miyatake 2002; Maor et al. 2004; Caprio 2009; Meza et al. 2014; Aceituno-Medina et al. 2017). The oviposition medium is often completely different from that in natural conditions, e.g. fabric, paper, a membrane, a plastic bottle with small holes. Adult-emergence cages serve as both mating and oviposition cages, and males often harass ovipositing females. In these colony-holding cages, insects are maintained at abnormally high densities, and there is no space to develop mating aggregations or swarms, or to attract females through producing a pheromone. All insects are of the same age, and frequently more females are placed in a production cage than males, reducing the need for courtship or competition

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between males. Many matings consist of brief courtships or, in some cases, even forced matings. Adult insects are discarded as soon as egg production begins to wane, thereby selecting for early maturing insects. In this situation, it is advantageous for females to mate quickly and maximize the number of progeny. It is disadvantageous for males to retain elaborate courtships, or for females to discriminate in mate selection. If abbreviated courtship becomes fixed in the population, this courtship repertoire may not be acceptable to wild females (Briceño and Eberhard 1998).

The mating competitiveness of laboratory-reared and wild Mediterranean fruit flies was examined in natural mating arenas (leks) (Zapien et al. 1983; Shelly 1995). Although the laboratory-reared males readily joined leks, and displayed calling behaviour similar to that of wild flies, relatively few laboratory-reared males were able to mate with wild females. The sterile males either failed to attract wild females to their positions in the lek, or the courtship repertoire was not acceptable to attracted wild females. In addition, on cloudy days, sterile males refrained from participating in sexual activities, and thus could not compete for wild females during such low-light-intensity conditions (Zapien et al. 1983).

In a mass-rearing environment (unlike the field), light, temperature, and relative humidity are often constant. Exposure to constant and optimal conditions of light, temperature, and relative humidity can select for individuals that are better adapted to these conditions, but lack the ability to adjust to fluctuating environments (Cayol 2000). If insects are reared for generations in constant light, changes in the temporal mating periods may result. In field-cage tests in Florida, laboratory-reared Caribbean fruit flies mated in the afternoon and completed mating activities long before wild flies began to mate near dusk (C. O. Calkins, unpublished data).

## 12. REMEDIAL ACTIONS TO RESTORE EFFECTIVENESS OF REARED INSECTS

When a colony of insects, used for the SIT, begins to deteriorate in effectiveness, there are several ways to restore it, including a change in colony management procedures. However, if genetic selection is involved, the problem may be more difficult to solve, and the establishment of a new colony from the target population may be required. Changed management procedures may reverse the selection, or at least slow down colony deterioration (Fisher and Caceres 2000; Liedo et al. 2007; Parker, Mamai et al., this volume).

If the causes are identified, specific problems detected by various tests can be addressed to reverse selection (Cáceres et al. 2007), e.g. adult cages, designed to increase the internal surfaces, significantly improved the mating competitiveness of mass-reared males (Liedo et al. 2007). Alternatively, adult cages designed to require insects to fly before having the opportunity to eat and drink automatically selects against non-flyers. In the cases of the New World screwworm (Wyss 2002) and codling moth (Dyck et al. 1993), adult insects must be able to fly to be collected in a cold room; they are then taken to the field for release.

Another problem that often develops in a reared colony is flies lacking irritability (section 4.10.). As a result, released sterile insects become easy prey to many of the

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predators that they encounter in the field. If an automatic technique to select against such adults in the adult cage could be developed, this problem would be reduced.

Mating compatibility appears to involve heritable traits that, to a certain extent, can be manipulated. Changes in temporal mating periods may be corrected, at least partially, by implementing egg collection and other regimes for the colony production that maintain the normal distribution of relevant characters of the colonized population (Miyatake 1995), and holding adult colony flies under natural light/dark cycles. Also, fluctuating temperatures in the adult colony room would increase the tolerance for changing field conditions.

The mating performance of sterile fruit flies may decrease under extreme or marginal habitats, such as high altitudes where cooler temperatures prevail. While developing a RAPID quality-control system for early warning of poor fly performance, Boller et al. (1981) reared Mediterranean fruit flies in a laboratory at cool temperatures to determine if they would still mate and perform other tasks. They found that there was enough genetic variability in a colony, normally reared at optimal constant temperature, to perform at cool temperatures after being reared at these temperatures for only a few generations.

Haynes and Smith (1989) discovered that, when sterile boll weevils were fed on squares (cotton blossom buds) instead of plugs of artificial diet before release, locomotor activity increased, mortality decreased, and mating attractiveness increased by 16% when compared with that of control weevils fed on diet plugs.

There are several possible reasons why a reared insect becomes incompatible with a wild one, such as genetic changes caused by the rearing process, genetic drift, or a change in a wild population that has been exposed to released sterile insects for "too many" generations. Hibino and Iwahashi (1991) reported that wild female melon flies of an island population, exposed to sterile males late in an AW-IPM programme that integrated the SIT, became unreceptive to sterile males (Lance and McInnis, this volume; Whitten and Mahon, this volume). Such changes may appear as temporal changes in the mating period, subtle changes in the courtship behaviour or in the chemical make-up of a pheromone, changes in acoustical signals, etc. (Sivinski et al. 1989; Briceño and Eberhard 1998). In these cases, there may be no alternative except to replace the mass-reared colony with a new strain from the target population.

However, in situations where the rearing process itself is responsible for the change, changing the strain without correcting the process will not solve the problem. As described above, the changes that occur while domesticating an insect population are major ones, and do not happen in one or two generations. One solution is to continually develop new strains, just in case the current one deteriorates. In any case, this may be necessary to prevent possible incompatibilities as the SIT activities expand into new geographic areas that have potentially different target populations. However, strain replacement is extremely expensive (Mangan 1992), and a successful strain should not be discarded unless it is found to be inadequate. It is costly to expand a colony, conduct quality-control tests, determine rearing characteristics, and evaluate field performance. Nevertheless, regular strain replacement in the New World screwworm colony in Mexico became a standard procedure (Hofmann 1985; Parker, Mamai et al., this volume).

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A less drastic cure is to incorporate wild genes into the colony genome. Wild corn earworm *Helicoverpa zea* (Boddie) moths from St. Croix, US Virgin Islands, were incorporated into a colony in Tifton, GA, USA; as a result, released moths mated much more frequently with wild moths (Young et al. 1975). However, this approach is not always successful. The wild individuals being introduced must compete in the artificial conditions to which moths in the domestic colony have already adapted. In the case of fruit flies, one solution is to replace only the males in the colony with wild males. For wild females, the most problematic aspect of adaptation is the oviposition device (Ahmad et al. 2014); by excluding wild females from the new introduction, this part of the selection process is avoided.

In the case of tsetse flies, trying to incorporate wild genes from the wild target population of *G. palpalis gambiensis* from Senegal into an old domestic colony from Burkina Faso (through back-crossing on males) failed. Even though it was maintained for many generations, the cross-bred line did not develop a satisfactory fecundity, whereas the new colonized strain rapidly improved during the same time to reach the same fecundity as the domestic one (Pagabeleguem et al. 2016).

Sometimes an improvement in the general vigour of a colony can be achieved by reducing the stress of the mass-rearing regime of the mother colony (Orozco-Dávila et al. 2014). Boller and Calkins (1984) used a relaxed low-density rearing method for a strain of Mediterranean fruit flies, and within two generations the vigour and size of the males increased. A system for limiting stress on a mother colony, called filter rearing (Fisher and Caceres 2000), also prevents the accumulation of deleterious traits from the high-density rearing stages back to the mother colony (Parker, Mamai et al., this volume). In this system, the mother colony is held under low-density low-stress conditions. Surplus insects from this colony are continuously fed into a sequence of high-density amplification steps to the final release colony, but the mother colony is kept separate and never receives material back from the high-density steps. The use of such a filter system has several advantages. Since the mother colony is small, it is possible to keep the insects under low-density conditions, and apply sexual competition or directed selection to them to restore or maintain desirable traits (Tejeda et al. 2017; Quintero-Fong et al. 2018). If necessary, by establishing a new mother colony, the whole production can be easily switched to a new strain.

If a colony is replaced because the rearing process is responsible for developing an inferior strain of insects, and the particular feature of the rearing method responsible for the change in insect behaviour is not adjusted, the new colony will rapidly develop the same inferior behaviour. This domestication takes place in just a few generations during the "bottleneck" that occurs in the initial phase of colonization (Zygouridis et al. 2014). Leppla et al. (1980) reported the complete adaptation of a noctuid moth to a laboratory environment in five to seven generations. The adaptation of the Caribbean fruit fly to a rearing regime, where performances in the laboratory bioassays were the same as those for the reared colony, took three or four generations (C. O. Calkins, unpublished data). Pashley and Proverbs (1981) noted a gradual change over time in the allozymes of reared codling moths, and predicted that this change could affect mating interactions with a wild population.

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## 13. QUALITY-CONTROL MANUALS

The first system of quality control was developed in 1978 by E. Boller for the Mediterranean fruit fly (Boller et al. 1981). It consisted of behavioural tests that included a pupal calibration sorting process to determine the array of pupal sizes, a startle test to measure irritability, an olfactometer to determine the age and time of day when males began to produce pheromone and females began to respond, a mating propensity test, a new ratio test, and an isolation index.

An operational quality-control manual for Mediterranean fruit flies, used at the Moscamed factories in Mexico and Guatemala and in other fruit fly programmes in Latin America, was developed by Orozco-Dávila et al. (1983), and subsequently modified or updated by Brazzel et al. (1986), Ashley (1987), and Calkins et al. (1996). To harmonize tests in these manuals, standardize quality-control tests, and allow comparisons for commercial and transboundary shipments of sterile insects, an international quality-control manual was produced in 2003 and updated in 2019 (FAO/IAEA/USDA 2019); it is being used in most fruit fly programmes.

Specific quality-control manuals have been produced for some other insects, including the New World screwworm (Lopez et al. 1995), tsetse flies (Feldmann 1994; Gooding et al. 1997; IAEA 2012), and Lepidoptera (Fisher 1983). This latter one included process-control charts that were sensitive tools for identifying changes in insects during colonization, and a feedback loop back to the production. Manuals for mass-rearing specific insects often include sections on quality control, including those for some other fruit fly species (Spencer and Fujita 1997), codling moth (Dyck 2010), tsetse flies (FAO/IAEA 2006), and *Anopheles* mosquitoes (FAO/IAEA 2017b).

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# CHAPTER 3.6.

# SUPPLY, EMERGENCE, AND RELEASE OF STERILE INSECTS

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## SUMMARY

The mass-production and sterilization of insects, as well as subsequent shipment, adult emergence, holding, feeding, collection, and field-release activities based at production or emergence and release facilities (ERFs), are vital components of applying the sterile insect technique (SIT). Mass-rearing, and these post-factory SIT activities, have evolved into a well-established modular/industrial process that has adapted and adopted manufacturing principles such as the development of standard operational procedures (SOPs) and quality control (QC) standards, allowing a common understanding/agreement between producers and users of sterile insects at satellite ERFs. Shipment practices for short- and long-distance transport are well documented, and it is likely that they will continue to be developed and improved for current and new insect species according to their biological needs. Procedures for insect emergence, feeding, and holding for sexual maturation of sterile insects (including the designs of new holding containers) have gradually evolved because of a better understanding of their biological requirements. ERFs specialize in insect emergence, holding, providing food and water to the adults, maturation, and collection for release. For some target pests, such as moths, adults can be collected after emergence for direct transport and release, without previous holding and feeding of adults. Release of sterile insects varies depending on the species and the nature of the area-wide integrated pest management programme (AW-IPM). This chapter also reviews the methods of release – static, ground, and aerial (using aircraft with fixed-wing or rotary-wing, and unmanned aerial vehicles (UAVs)). Recently, developed technology has allowed prototyping of release equipment designed for UAVs. New release boxes and machines for a wide range of insect species have been developed, aiming to optimize equipment that enables variation in release densities, and takes into account other operational considerations according to the needs of action programmes. New ground- and aerial-release equipment has been tested and put into operation -- increasing the quality of the released insects. Through the use of combined GPS/GIS, dedicated software, and devices plugged into computers and the internet, additional progress has been made in implementing electronic/navigating equipment that enables knowing the precise location of insect releases and recording the conditions of their aerial broadcast. These developments enable the delivery, monitoring, and supervision of releasing sterile insects, with the results available in real time. The private sector has actively participated and demonstrated continued interest in the provision and processing of sterile insects, as well as aerial GPS/GIS guided release.

## 1. INTRODUCTION

During the past decades the mass-rearing of insects has been developed further, with major improvements in the industrialization of the production process for the application of the sterile insect technique (SIT). By documenting their procedures

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and practices, some mass-rearing facilities (MRFs) have established/received ISO 9001–2015 certifications for insect rearing, thereby bringing about greater consistency in production numbers and insect quality (ISO 2019; E. M. Ramírez-Santos, personal communication). All these mass-rearing activities are the focus of the chapter by Parker, Mamai et al. (this volume). In addition to these improvements in the production of sterile insects, there have been significant advances in the quality-control processes (covered in detail in the chapter by Parker, Vreysen et al., this volume) that have increased the availability and supply of sterile insects for their use in area-wide integrated pest management programmes (AW-IPM) worldwide.

This chapter focuses on the receiver/end-user of SIT implementation, i.e. the post-factory activities after sterile-insect production, marking and sterilization activities at MRFs. They take place mostly at emergence and release facilities (ERFs) located either at or near production sites, or near distant target release sites to which the sterile insects are usually shipped in bulk as pupae. Activities carried out at ERFs and during preparation for release are discussed, including the adoption of improved processes related to the chilled adult release technique (Tween and Rendón 2007), through which sterile insects are emerged, maintained, and fed for several days, and then chilled and released as robust and competitive sterile insects via novel technologies.

After leaving a MRF, nutritional, semiochemical, and other treatments applied to adult sterile insects (to improve their field performance) are the focus of the chapter by Pereira et al. (this volume).

## 2. SUPPLY OF STERILE INSECTS

### *2.1. Availability from Established Mass-Rearing Facilities*

The most rapid and cost-effective way (at least in the short-term) to obtain a continuous supply of sterile insects is by their purchase/importation from an established MRF that rears the required target species (Dowell et al. 2000; Blomefield et al. 2011; Bjeliš et al. 2016; Horner et al. 2016; section 4.6.). In the case of long-distance transport, of course cost-effectiveness depends on the shipment; for long distances the shipping cost can be considerable, and the shipping duration can affect sterile-insect quality. However, especially when containment or eradication programme activities are urgent, outsourcing sterile insects enables operational programmes to avoid spending time and economic resources on the training of personnel, planning, and construction required to establish an MRF. Establishing and maintaining an operational MRF is a demanding economic endeavor that is time-consuming and requires major investment. Instead, by procuring sterile insects, emergency programmes can rapidly integrate the SIT, e.g. eradicating a pest outbreak such as the 2015–2017 eradication of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) in the Dominican Republic (Zavala-López et al. 2021). The tsetse eradication programme in Senegal decided not to build an MRF but to outsource the sterile flies from Burkina Faso (Vreysen et al. 2021) -- a wise decision for programmes that will no longer require the MRF after achieving eradication.

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Also, there are examples of programmes that routinely purchase sterile flies because the target areas are relatively small, e.g. the *C. capitata* fruit fly programmes in Croatia (Bjeliš et al. 2016) or Patagonia (De Longo et al. 2000), or where no MRF can be established for regulatory reasons in pest-free areas, such as the preventive release programme (PRP) in California (Dowell et al. 2000). Other programmes begin by buying sterile flies (for some years) so as to establish and gain experience with SIT operations, while planning and eventually establishing their own mass-rearing facilities -- examples are the *C. capitata* programmes in Israel, Morocco, and Spain (Primo 2003; Cayol et al. 2004; Qadda 2017).

There is a limited number of insect species for which MRFs are well established: Mediterranean fruit fly, Mexican fruit fly *Anastrepha ludens* (Loew), West Indian fruit fly *Anastrepha obliqua* (Macquart), melon fly *Zeugodacus cucurbitae* (Coquillett), Queensland fruit fly *Bactrocera tryoni* (Froggatt), codling moth *Cydia pomonella* (L.), false codling moth *Thaumatotibia leucotreta* (Meyrick), and pink bollworm *Pectinophora gossypiella* (Saunders). Additionally, mass-rearing facilities for some veterinary and medical pests have been successful: New World screwworm *Cochliomyia hominivorax* (Coquerel), tsetse flies *Glossina austeni* Newstead, *Glossina fuscipes fuscipes* Newstead, *Glossina palpalis gambiensis* Vanderplank, and *Glossina pallidipes* Austen, and Asian tiger mosquito *Aedes albopictus* (Skuse).

In general, MRFs are built to produce sterile insects for their own programme requirements, and therefore only a few of them have the capacity or interest to produce sterile insects for customers in addition to those needed for their own use. Despite the perceived shortage of sterile-insect supply, there has been much collaboration between programmes to supplement sterile insects when the need arises. The Joint FAO/IAEA Programme maintains a database of rearing facilities, capacities, and contact information (IDIDAS 2019; Bakri et al., this volume).

Due to their role in national plant protection or animal/human health, most of the sterile insect release programmes have some form of government involvement (Dyck, Reyes Flores et al., this volume). They operate with limited budgets, and are driven by an urgent need to manage serious pests to limit pest damage, confine the pest to the smallest area possible to reduce operational costs, and maintain or open markets for locally-produced commodities. Therefore, as a complement to other control activities that the programmes may implement, a reliable source of good-quality sterile insects at an affordable cost is always in demand. The provision of insects by government-operated institutions is usually conducted through bilateral agreements and on a full cost-recovery basis, including the depreciation of facilities and equipment. Often the "excess" capacity of an MRF, where output exceeds direct programme needs, may not always be available as it is dedicated to others through contractual agreements to prioritize such partners in case of emergency needs.

The production cost per million insects (eggs, pupae, adults) can be greatly reduced with economies-of-scale (Parker, Mamai et al., this volume). As a rule, due to their cost of construction, most MRFs were built undersized rather than basing the production output on a per-insect unit cost. In small rearing facilities, the fixed costs are relatively high, therefore increasing the production cost per million sterile insects.

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One major improvement in the rearing and release of some insect species is the feasibility of deploying only sterile males, through the use of genetic sexing strains (GSSs) (Franz et al., this volume) or other approaches (Papathanos et al. 2018). In general, this option reduces by half the costs of insect dyeing/marking, irradiation, shipping, and aerial release, while increasing SIT effectiveness when compared with standard/both sex releases (Hendrichs et al. 1995; Rendón et al. 2004). The introduction of GSSs that remove females before their shipment, coupled with rearing efficiencies and economies-of-scale, greatly reduce product and shipping costs in addition to reducing the cost of processing insects at ERFs.

### *2.2. Processing Insects for Release*

There are three common approaches to managing sterile insect production and release:

- Operate a centralized facility that combines an MRF with an ERF that prepares sterile insects for release (Schwarz et al. 1985; Ortiz et al. 1987; Nakamori et al. 1992; Liedo et al. 1993; Msangi et al. 1999; Bloem and Bloem 2000; Koyama et al. 2004).
- Operate an MRF for rearing and sterilization, and one or more satellite ERFs to which sterile insects are shipped and where they are processed for the releases (IAEA 2008).
- Receive eggs from another MRF for local rearing, sterilization, and release (section 4.6.2.).

#### *2.2.1. Centralized Facilities Combining Production with Emergence and Release*

The decision to establish a combined production-release facility versus separate ERFs is normally linked to convenience (directly at the MRF or located nearby close to or at the local airport used for aerial releases), as well as the size and distance from the target release areas. When these can be serviced from the local MRF/ERF without excessive ferry times, there is no need to establish satellite ERFs.

One of the advantages of locating an ERF next or close to an MRF is that preparing insects for release at the place where they are reared eliminates the extra operations of packing the sterilized insects for shipment and sending them to a satellite ERF; it also has the added advantage that, due to the lower hypoxia time during pupal shipment, the quality and quantity of sterile insects available for releases increases (Arredondo et al. 2018). Due to the progress in control activities within the Moscamed action programme in Guatemala, an ERF has been established directly at the El Pino mass-rearing facility; this unit is experiencing an increase of insect quality due to reduced hypoxia and handling (E. M. Ramírez-Santos, personal communication).

For the eradication of *Glossina austeni* in Zanzibar, sterilized tsetse fly adults, produced and emerged in Tanga, Tanzania, were released aerially in degradable cardboard boxes over Unguja Island, resulting in the elimination of the trypanosomosis disease in cattle (Msangi et al. 2000; Vreysen et al. 2000). In the pink bollworm programme in the USA, moths were reared, sterilized, and emerged

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in Arizona, but then flown to and directly released in California (USDA 1995; Walters et al. 2000; Staten and Walters 2021). A single centralized facility produces and releases sterile codling moths in British Columbia, Canada (Bloem and Bloem 2000; Nelson et al. 2021), and also ships them successfully to South Africa and New Zealand (Blomefield et al. 2011; Horner et al. 2016; Dyck, Reyes Flores et al., this volume). In the 2016–2017 eradication of the New World screwworm outbreak in Florida, sterilized pupae from the MRF in Panama were shipped to the USA, where the pupae were directly placed into ground-release chambers in the field from which adults emerged (USDA/APHIS 2017; Skoda et al. 2018).

Regardless of whether there is a single centralized production-release facility, or additional satellite ERFs, it is an advantage to have a single management structure for a programme, allowing efficient decision-making and the rapid exchange of information among the components of the mass-rearing and release processes (FFEPO 1999; Dyck, Reyes Flores et al., this volume).

#### *2.2.2. Satellite Facilities for Emergence and Release Only*

Flying emerged sterile adults to distant target areas for release can result in long and costly ferry times that may be detrimental to insect quality; the establishment of ERFs strategically located near the release sites to minimize ferry times is the more viable alternative in these cases.

The operation of satellite ERFs has several additional advantages: (1) sterile insect programmes can work in areas that, for regulatory reasons, cannot have MRFs, (2) one large MRF, operating at increased economies-of-scale, can provide insects to several satellite facilities, often in different countries, and (3) several insect species can be simultaneously prepared for release.

The shipment of sterile insects to satellite ERFs enables them to be placed in areas generally free of the target insect without the risk of fertile pest insects escaping from a local MRF (Dogan et al. 2016). This is important if the goal of the programme is the eradication of isolated invasions of the target pest, or its exclusion from an area free of it through preventive releases. Thus, sterile pupae, for example of the Mediterranean fruit fly, Mexican fruit fly, and New World screwworm, can be shipped from MRFs located in areas where these pests occur to ERFs in areas free of permanent infestations (USDA 1995; CDFA 2002).

The use of satellite ERFs gives maximum flexibility to the release of sterile insects. One large MRF can provide sterile pupae to several satellite facilities scattered throughout a region. For example, producing sterile fruit flies in large MRFs in Guatemala and southern Mexico, and shipping them as pupae to a chain of ERFs in northern Mexico, as well as California and Florida, has been more cost-effective from an operational standpoint (USDA/APHIS 2009; Enkerlin, this volume).

Satellite ERFs are less complex in their design than centralized MRFs, and often do not require permanent construction. As programme needs change, satellite ERFs can be relocated. The satellite ERFs, used to prepare sterile New World screwworms for release, moved steadily southwards from Mexico to Panama, as the screwworm eradication programme progressed through Central America, but the MRF continued to operate in Mexico (Wyss 2000) until a new MRF became operational at Pacora,

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Panama (Scott et al. 2017; Vargas-Terán et al., this volume). This approach should be followed when several countries/regions have a common pest problem, avoiding duplication of efforts in the construction and operation of rearing facilities, while reducing costs due to economies-of-scale and servicing several countries with the large production of sterile insects.

Several species of sterile insects from different MRFs can be handled in a single ERF facility. In California, the same staff processes Mediterranean and Mexican fruit flies at the same satellite ERF located at Los Alamitos. Recently, the states of Florida (Fig. 1) and California produced floor plans to renew their existing ERFs after the successful establishment of the concept of preventive release programmes (PRPs) (which have greatly reduced the cost of eradicating invasive populations while avoiding the establishment of Mediterranean and Mexican fruit flies) (Hendrichs, Enkerlin et al., this volume). These new ERFs already included additional space to allow for the emergence and release of other pest species.



*Figure 1. United States Department of Agriculture (USDA) sterile insect emergence and release facility, Sarasota, FL, USA (USDA 2019). (Photo from Halfacre Construction Co., Aerial Innovations of Florida, and J. Renshaw, 2018, reproduced with permission.)*

The different goals and physical separation of MRFs and ERFs can result in more attention and focus on field operations, and in the case of independent customers provide a more objective assessment of sterile insect quality. Conducting product quality control tests in accordance with agreed procedures, and exchanging these results between MRFs and ERFs, provide a better way to monitor quality and address end user/client concerns.

There are rearing issues where real cause/effect needs to be documented to avoid supply or quality reductions. In some instances, the problems observed in the rearing or transportation processes are only brought to light after the satellite ERFs note a decrease in the quality of the resulting insects being prepared for release, while sometimes the problem might also be the result of mishandling at the EFRs. Currently, with the improved availability of electronic means of communication and electronic data storage, shared boxes or drives (with access by sterile insect producers and receivers/users) can be dedicated to report results on quality by independent testing at MRFs and ERFs for feedback and comparisons. Statistical analysis of the data has also enabled the preparation of quality-control charts that

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gradually raise, or at a minimum monitor and maintain, insect quality over time (FAO/IAEA 2018a).

### *2.3. Commercial Production and Release of Sterile Insects*

There are advantages and disadvantages to both governmental and private suppliers of sterile insects (Dyck, Reyes Flores et al., this volume). Government suppliers, unlike private suppliers, may be willing to develop insect MRFs for which there is a potential need, but not necessarily a continuous demand, as in the case of eradication programmes (Quinlan and Enkerlin 2003). They fund programmes to develop the SIT against new pests such as the sweetpotato weevil *Cylas formicarius* (F.) (Kohama et al. 2003; Kumano et al. 2011) or the cactus moth *Cactoblastis cactorum* (Berg), or programmes that demonstrate the feasibility of funding and implementing the SIT in new ways, such as taxing the local community for codling moth suppression, or applying public-private partnerships for false codling moth AW-IPM programmes. Governments can also establish programmes that cross several political entities, such as the pink bollworm programme on both sides of the Mexico-USA border (Simmons et al., this volume), the Mexican fruit fly preventive release programme along the California-Mexico border (Enkerlin, this volume), or the release of New World screwworms along the Panama-Colombia border (Skoda et al. 2018; Vargas-Terán et al., this volume). Governments are more likely to invest in research designed to develop potential new technologies for use in mass-rearing and release programmes if the pest is considered to be a potential animal/human health or phytosanitary threat.

However, government-funded facilities have several drawbacks, one being the tendency to change policies and/or funding priorities, e.g. after elections. Thus, a programme that had the strong support of one administration may find its budget reduced or eliminated by another (Dyck, Reyes Flores et al., this volume). This can make programme managers feel insecure, postpone needed changes in programme procedures or facility upgrades, and make growers, ranchers, and other stakeholders reticent to support the programme. Another major drawback of government funding is the tendency to ask the programme to do more and more with a static budget. This leads to decisions that tend to lower the quality of sterile insects produced while keeping the numbers of insects produced and released high (Liedo et al. 1993). This can have a negative impact on programme success, which consequently will often lead to further funding cuts that continue the cycle of problems and often cause reinfestations to occur, making the suppression or eradication efforts appear not to be technically feasible because of the budgetary inconsistencies.

The affected agricultural industries can play a major role in creating awareness of the importance of sterile insect programmes among their members, government leaders, and the public (Dyck, Regidor Fernández et al., this volume). They can help develop confidence in the use of sterile insects for eradication or pest suppression. This will generate a demand for the sterile insects that, in turn, will attract private companies to invest in production plants necessary to meet this demand.

In the Mediterranean region, bait sprays have traditionally been used to suppress major pests such as the Mediterranean fruit fly and the olive fruit fly *Bactrocera*

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*oleae* (Rossi). Encroachment on traditional production areas by "urban sprawl", and the expansion of tourist resorts, makes the future of aerial-bait applications increasingly untenable. In 2009 the European Union prohibited aerial spraying of pesticides (EU 2009), thereby effectively ending most aerial application in all member states and overseas territories. Nowadays, aerial spraying in the EU requires special authorization by local governments (but only when justified by emergency conditions).

As a consequence, the integrated use of sterile insects, with the objective of routine area-wide pest suppression, has great potential for commercialization (Hendrichs et al. 1995; Hendrichs, Vreysen et al., this volume). The agricultural industry itself is searching for, and promoting the development of, more environmentally acceptable measures to substitute less acceptable technologies developed more than 50 years ago. Private companies are increasingly participating in the production and provision of sterile insects, e.g. de Groene Vlieg in the Netherlands (De Groene Vlieg 2019), BioBee in Israel (Bassi et al. 2007; BioBee 2019), and in South Africa FruitFly Africa Ltd. (Venter et al. 2021) and Xsit (Boersma 2021), although most of these private-sector services are providing sterile insects to customers in their own countries.

Most programmes contract with private firms to provide the aerial services for sterile insect release. Some also contract for part or the whole of sterile insect emergence and release processes. For example, the Mexican National Fruit Fly Campaign has outsourced the operation of five Mexican fruit fly and West Indian fruit fly ERFs in Mexico (Leal Mubarqui 2005; Bassi et al. 2007). Private companies are paid per flight hour, from take-off to landing, or in some instances for actual engine-time utilized (based on the Hobbs meter); both are valid practices. An exception is the Mexican fruit fly programme in Texas, where the aircraft is owned by the United States Department of Agriculture (USDA), and the pilot is an employee of the US government. The Animal and Plant Health Inspection Service (APHIS) of the USDA uses this aircraft to design and develop new technologies for releasing sterile insects.

Experience in the USA and elsewhere indicates that usually it is cheaper to contract with a private company (to provide aircraft and pilots) than to purchase aircraft and pay for maintenance, full-time pilots, and mechanics. However, this may not be true in all countries (due to limitations in the offer of such services), and a detailed economic analysis needs to be conducted for each programme. Due to the continuous search for operational efficiencies, action programmes are actively looking for alternatives to conduct aerial releases through the use of unmanned aerial vehicles (UAVs); this initiative has already developed some alternatives for some pest species, for example mosquitoes, where releases are often concentrated over villages or towns (section 5.3.3.).

## 3. SHIPPING STERILE INSECTS

There is a major advantage to producing, marking, and sterilizing insects in an MRF, and then shipping them to an ERF that is dedicated to emerging, holding, and releasing the sterile insects. Shipping procedures are well known and tested. Sterile

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insects, e.g. codling moth, Mediterranean fruit fly, New World screwworm, and tsetse flies are shipped routinely to near and distant places (Enkerlin and Quinlan 2004; Blomefield et al. 2011; Rull et al. 2012; FAO/IAEA 2014, 2016b, 2017a, b, 2018b; Horner et al. 2016; Dyck, Reyes Flores et al., this volume).

### *3.1. Shipping Time, Distance, and Insect Quality*

At present there is still a limit on how far sterile insects can be shipped. Adult emergence and flight ability decrease with increasing shipment time, even when shipping pupae in a low-oxygen atmosphere (hypoxia) – an effect that seems to be reduced in some species such as *Bactrocera tryoni,* where a decline in adult emergence of 0.1%/h (inside sealed plastic bags for periods up to 192 h at 17°C) has been reported (Dominiak et al. 2011a). For example, sterile Mediterranean fruit fly pupae have been shipped successfully from Guatemala and Mexico to Argentina, Austria, Chile, the Dominican Republic, Israel, South Africa, and the continental USA (Dyck, Reyes Flores et al., this volume). They have also been shipped from Argentina to Spain, from Israel and Spain to Croatia, and from Portugal and Spain to Morocco, etc. Since 2010, sterile Mexican fruit flies have also been shipped from Guatemala to South Texas, and Reynosa and Tijuana in northern Mexico, with high-quality parameters reported after shipment. In general, the hours of pupal hypoxia have been in the range of 43–45 h (departure to arrival) (M. López, personal communication).

Enkerlin and Quinlan (2004) developed an international standard to facilitate the transboundary shipment of sterile insects. A summary of worldwide transboundary sterile insect shipments during the last 50 years can be found under FAO/IAEA (2018b).

Shipments of sterile insects are often conducted by renting space in the cargo compartment of commercial airlines (fitting in the containers/boxes that will deliver an agreed number of sterile insects). Usually a broker company is contracted to arrange documentation for customs and the airlines. Optimized flight routing is mandatory to reduce time in transit, the number of airports used, and detours made. Delays in shipments do occur -- some of them are due to security measures, mistakes in paperwork, changes in routing or the airlines shifting their priorities to baggage (cargo is then not loaded, generating delays in the arrival time at the final destination). If pupae are kept under hypoxia for longer than the documented/expected periods of time, the quality of the resulting adult insects decreases (FAO/IAEA 2017a, b; Arredondo et al. 2018).

When shipping to exceedingly distant destinations, the packing configuration is modified so as to contain coolers and ice packs in sufficient number to ensure that temperature and relative humidity conditions are maintained (Pagabeleguem et al. 2015). Although not under hypoxia, the quality of tsetse adults suffers if the pupae are kept chilled too long (Mutika et al. 2002, 2014). Before starting routine shipments, trial shipments between interested rearing and release facilities should be sent so as to document shipping routes, costs, and final product quality before assuming viability or non-viability of the supply.

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### *3.2. Shipping Eggs*

An additional form of insect supply is to ship the early development stages of insects, which will then be reared at the recipient MRF. Mediterranean fruit fly thermally-treated eggs (to kill female eggs for the production of only males), and the black-pupae strain (BPS) of the Mexican fruit fly, are routinely shipped with little or no loss in yield or quality of the resulting sterile adults (IAEA 2002; H. Conway, personal communication). This procedure avoids the problems associated with maintaining pupae under hypoxia for long periods of time. As the volume and weight of eggs are far less than those of pupae, shipping costs are substantially reduced. For example, one litre of eggs is equivalent to almost 5 million pupae. Of course, egg shipment still requires MRFs for producing males and sterilizing them at the receiver end. Currently, 9 litres of Mediterranean fruit fly eggs of a *tsl*-genetic sexing strain are shipped daily from the El Pino production facility in Guatemala to the production facility in Metapa, Mexico. Purchasing eggs is a very reliable process, enabling the receiving programme to avoid maintaining a viable colony of fertile adult insects for egg production (since escapes are always a security concern in areas declared or about to be declared free of the pest). One additional advantage is that it also avoids the costs and the intricacies of the maintenance of filter-reared colonies of GSSs (Fisher and Caceres 2000; Franz et al., this volume; Parker, Mamai et al., this volume).

### *3.3. Shipping Adult Lepidoptera*

In general, lepidopteran species are shipped as chilled sterile adults. One reason is that larval or pupal separation from diets is complicated; another is that sterilizing adults instead of pupae results in more competitive insects (Stewart 1984; Dyck 2010; Boersma 2021; Bakri et al., this volume). Lepidopteran species (such as codling moths) shipped as adults can tolerate more than 60-h shipments (Blomefield et al. 2011). Shipments of codling moth pupae and adults from Canada to New Zealand and South Africa provided support to the suggestion that the SIT for codling moth (in pome-fruit production areas) might be implemented by the importation of irradiated chilled moths from rearing facilities in a different country or hemisphere (Blomefield et al. 2011; Horner et al. 2016). The success in these shipments points out the need to explore new methods or conditions for transporting pupae and/or emerged sterile adults from the MRF to where they are to be released (Dyck, Reyes Flores et al., this volume).

### *3.4. Shipping Costs*

One important consideration related to the establishment of satellite ERFs is the cost of shipping sterilized insects (Wyss 2000). For example, the shipping cost alone to send New World screwworm pupae to Libya (packed in cardboard boxes for adult emergence and direct release) was four times the purchase price, and four times the dispersal cost. Shipping cost is reduced by 50% if using GSSs (where females are removed, enabling the shipment of only males).

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The shipping cost of sterile Mediterranean fruit flies from El Pino in Guatemala to California and Florida PRPs is about 16–18% of the total cost of one million pupae plus shipment (E. M. Ramírez-Santos, personal communication), while for the Mexican fruit flies shipping from Guatemala to the continental United States is about 11% (M. López, personal communication); both facilities produce and ship males-only of their respective rearing species. As in the case of mass-rearing costs, the unit shipping costs depend largely on the volume of pupae that are shipped. Shipments of low sterile fly numbers (less than 5 million per shipment) to pilot projects usually result in high unit costs due to the fixed costs of the customs broker company.

In addition to issues related to insect quality, increasing shipping distance results in increasing shipping costs and delays that are also a concern for the supply of sterile insects to EFRs and action programmes. The New World screwworm production facility in Tuxtla Gutierrez, Mexico, provided the sterile flies for the Central American eradication programme. However, with increasing shipping distance, both the shipping costs, and the potential for problems, including delays in the shipping process, increased (FAO 1992; Wyss 2000). By 2006, a new mass-production plant for the New World screwworm was inaugurated in Panama, and now supplies the sterile fly barrier in the Darien region along the border between Panama and Colombia (Hendrichs, Vreysen et al., this volume; Klassen et al., this volume; Vargas-Terán et al., this volume).

## 4. EMERGENCE AND RELEASE FACILITIES

### *4.1. Location, Design, Building Materials, and Construction*

Strategic placement of the ERFs, and therefore their sterile insect release activity, is relevant to operational efficiency and costs. ERFs that are located close to the areas where control activities are conducted by the action programme will see their operating costs reduced. Having ERFs equidistant from all the areas where releases are conducted reduces costs in expensive ferry times to conduct releases and return to airport landing strips. For the selection of sites to establish an ERF, action programme decision-makers need also to consider all the adverse conditions that will prevent aircraft from conducting their insect release activity and avoid/minimize them while selecting the location. Among the various factors to consider are rain patterns, prevailing winds, and the presence of commercial airports in close proximity that could limit the operation, etc. When choosing a site for the establishment of an ERF, there are other requirements (which facilitate the stability of the operations) that need to be considered, e.g. having an adequate site not too far from all the facilities of a medium-sized metropolitan city (for the convenience of the workers and obtaining equipment-repair services) but still a distance from it so as to avoid disruption of daily operations (FAO/IAEA 2004, 2012; Parker, Mamai et al., this volume).

It is relevant that ERFs have a dependable supply of electricity; nevertheless, the facility should also budget for the provision of an emergency plant generator. The provision of potable water is essential for the ERF; however, the provision of

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municipal water and/or the drilling of its own well and water purification system will avoid any shortages in the amounts of water that are required for this type of operation. A water-treatment plant may also be needed because the sewage in ERFs is often contaminated with fluorescent powder arising from the washing of holding boxes or trays and racks; this can be a big problem unless properly addressed.

An ERF also needs to be close to a good ground-transportation system. This reduces the cost of transporting sterile pupae or adults, and also reduces the time that pupae or adults are in transit, thereby increasing the quality of the resulting adult insects. When eradication programmes are progressing in their control activity, it may be necessary to change the ERF's location to increase operational efficiencies and insect quality, as well as to reduce operational costs. Therefore, in long-term eradication campaigns, the ERFs may be designed to be movable or permanent, and their layout will be influenced by these needs.

Some emergency programmes use trailers or refrigerated trailers and basic insect containers (such as buckets or Plastic Adult Rearing Containers (PARC) used for the PARC system) and paper bags (in which to emerge and release adults either aerially or by ground) (Fig. 2), thereby rapidly solving the problems that appear in an emergency programme in which the goal is to eradicate the pest as soon as possible with minimal investment in equipment and infrastructure. On the other hand, in other pest control situations, such as the PRPs or long-term exclusion/eradication programmes, in which continuity of operations is foreseen, the design and selection of building materials for the establishment of ERFs shifts to more permanent constructions, choosing in this case materials such as metal structures combined with block and concrete.

In general, ERFs are designed with a modular layout. Each of the modules/rooms is implemented to maintain sterile insects for several days with adequate environmental conditions (temperature and relative humidity) that will enable insects to emerge and mature sexually – for subsequent release using the release method of choice. The size of rooms is designed to emerge and hold the number of insects that are released each operational day; this usually coincides with the number of sterile insects that is received daily from one or sometimes two MRFs. Some ERFs process and release sterile insects every day, others only during weekdays; the capacity of the ERF is based on the operating and release requirements.

### *4.2. Environmental Control Equipment*

The environmental conditions under which sterile insects are maintained prior to their release play an important role in their adult emergence time, sexual maturity, feeding status and survival in the field. ERFs need to consider the relationship time/temperature accumulation, e.g. degree days, to set adult emergence room temperature to ensure adequate time (in hours) for emergence and associated age/sexual maturity reached at the time of insect release (Parker, Mamai et al., this volume).

Also, relative humidity plays an important role. If it is too low, it may be difficult for an adult to emerge, and thus it uses up critical energy reserves prior to release. Regarding the feeding status of the insects, low humidity dehydrates sterile

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insects, as can some food sources, reducing vitality and numbers. On the other hand, in a high-humidity environment, insects stick to one another or to the screen surfaces and food; excess humidity also promotes the presence of unwanted fungal development on top of the food provided. Therefore, adequate humidity levels, along with appropriate regular cleaning of emergence rooms and equipment, are relevant to avoid contamination of surfaces (Abd-Alla et al., this volume).

Since the ERFs operate 24 h/day year-round, even if releases are only conducted in some instances during weekdays, the environmental conditions need to be monitored constantly because deviations from the required conditions can have great impact on the release numbers and insect quality.




*Figure 2. Mediterranean fruit fly emergence and release facility in Retalhuleu, Guatemala, showing clockwise: A: Worker placing sterilized dyed pupae into a PARC emergence box; B: Stapling paper bags in the centre to permit flies to leave the bag; C: Placing one modified agar-block diet; D: Stacks of six PARC boxes inside an adult emergence room. At the Neretva Valley, Croatia, Mediterranean fruit fly programme: E: Machine for loading pupae into paper bags; F: Adult emergence room containing bags for fly emergence. Water is provided/sprayed through the surface of the bag.*

### *4.3. Facility Operation*

In general, the ERFs that receive shipments of sterile pupae operate with a building layout as follows:

- *Reception area* – workers assigned to this area are responsible for receiving and preparing the pupae when they arrive from the MRF. In this area, the conditions

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of the biological material are documented on arrival, e.g. temperature, the irradiation dosimeters are visually verified, and the weights of the biological material received are checked to calculate the total number obtained in the shipment. Additionally, an observation of the external dust-dye is conducted to determine if there are problems with water condensation, lack of fluorescent-dust marking, or others (nowadays MRFs document the percentage marked pupae prior to shipment departure) (section 4.3.). The pupae are then moved to the next step in the process.

- *Pupae distribution* – this area is used to place, manually or by automated systems (Fig. 2E), the biological material inside containers (PARC boxes or adult emergence towers) that will enable adult emergence and feeding for a predetermined number of days. In parallel to this process, the adult food is applied or placed prior to insect emergence (food is usually prepared ahead of time in diet-cooking rooms) (section 4.4.).
- *Diet-cooking room* – a kitchen that usually has several boilers or kettles to prepare agar-based food. As alternatives to this type of diet, there are currently two additional options, a liquid diet and a diet that is a combination of both in which solid and liquid diets are combined inside the Mubarqui tower (Fig. 4).
- *Adult emergence and holding rooms* – usually as many as the number of days per week of programmed releases.
- *Aromatherapy room* – for the species that have this option to enhance mating activity (Pereira et al., this volume), the size of this room allows for at least one-day-release capacity; alternatively, this type of "sexual pre-conditioning" treatment can be conducted within the last adult emergence room a day prior to insect release using existing adult emergence rooms.
- *Chilling room* – for insect species where chilled adults are transported and released in aerial or ground releases (Tanahara et al. 1994); some programmes supplement aerial releases with ground releases of chilled adults.
- *Washing area* – for cleaning and drying materials and equipment used in the adult emergence and release process; in this area industrial washing machines are employed to clean PARC boxes or tower trays.
- *Quality control (assurance) area* – the biological characteristics of the sterile insects received, as well as those that are released in the field, are measured and documented (Parker, Vreysen et al., this volume). Early efforts at judging the quality of sterile insects focused mainly on the adult insect (Calkins 1989; Calkins et al. 1996; Dominiak et al. 2014; Fanson et al. 2014; FAO/IAEA/USDA 2019), and only pre-/post-irradiation on arrival at an ERF. These insects are subjected to measurement of various parameters: emergence, survival, flight, and mating competitiveness (Ortiz et al. 1987; FFEPO 1999; Koyama et al. 2004). The current approach is also to include in the measurements all those parameters that will describe the quality characteristics of sterile insects before and after their field delivery (FAO/IAEA/USDA 2019).
- *Administration* – area to provide administrative support to all technical activities of the ERF operations, processing bills, personnel technical assistance, and other logistic needs.
- *Methods development* – area designed to evaluate and validate rearing and

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quality-control practices prior to or during their implementation at the ERF operations.

- *Maintenance* – for personnel dedicated to resolve operational and maintenance problems with equipment and buildings, as well as prototyping equipment and other needs.
- *Warehouse* – space must be provided for storing high-quality diet materials. Delivery times, rates of consumption and others (local vs. imported diet materials) should be calculated to allow sufficient areas for process/human safe storage of products. For the operation of ERFs, the number of ingredients to prepare adult diets is reduced when compared with those in an MRF. For most insects, this means access to locally produced products (IAEA 2008). It is very important that these diet materials are free of insecticide residues.

## 5. EMERGENCE AND COLLECTION OF STERILE INSECTS

### *5.1. Holding, Marking, Feeding, and Collecting Adults*

In some target species, such as fruit flies, tsetse flies, and mosquitoes, adults need to be held after emergence for various periods in different types of containers for feeding and maturation; then they are collected for release by moving the holding containers to cold rooms (where the low temperature makes them quiescent).

#### *5.1.1. PARC System*

In many AW-IPM programmes that integrate the SIT, sterile pupae are shipped to ERFs where they are placed into containers in which adults emerge. Systems for the emergence, holding and feeding of fruit flies prior to their field release have changed over time. An early system was the "bucket method" used for roving ground release (FAO/IAEA 2017a, b). This was followed by the paper bag system for aerial release (Nadel et al. 1967). Later, the PARC system was developed for the aerial release of chilled flies (shown in Fig. 2, where preparations are being made for Mediterranean fruit fly emergence using PARC boxes). This system was developed by the USDA (Mabry 1986) to replace the earlier paperboard Tanaka box, developed by the California Department of Food and Agriculture (CDFA). The PARC system, an improvement over the Tanaka box, permitted the containers to be sanitized after each use, and reduced the number of escaped flies within an ERF.

The use of these systems requires that the sterile pupae be dispensed into paper bags at a predefined amount; six bags are placed into each PARC box. Once filled with pupae, the bags are then loosely stapled at the top to allow emerging sterile flies to escape and live and feed inside the container until they are ready for field release. Screen panels on the sides of the container provide ventilation, and a screen panel in the lid permits the flies to feed on a gelatinous slab consisting of agar, sucrose, water, and a food preservative -- usually sodium benzoate or methylparaben. This stackable PARC system (Fig. 2D) requires much controlled-environment space to hold a large number of these bulky containers during the emergence and fly maturation period. It is also labour-intensive, although recently

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automated systems have been designed for both loading the pupae (Figs. 2, 3) and cleaning the boxes using industrial washing machines. Paper bags are costly, both in direct and waste-disposal/littering cost. Also, some flies are lost when the bags are destroyed. This PARC system is still used today in some release programmes (although there is a gradual trend to implement newer technologies) or in emergency situations e.g. the 2015–2017 eradication of *C. capitata* in the Dominican Republic (Fig. 2).

*5.1.2. Tower System*

In a gradual progress towards an ideal system, a sterile insect "emergence or Worley tower" was developed by the USDA in Mission, TX (Salvato et al. 2004) to improve upon the PARC boxes. This new "tower technology" also facilitates the concept of chilling insects and loading them into release containers for aerial dispersal using the chilled adult release technique (Tween and Rendón 2007). This tower system is currently used for emerging, feeding, and chilling/immobilizing Mediterranean fruit flies in Florida and California, and Mexican fruit flies in Texas (Fig. 3).

Each tower consists of interlocking screen-panelled aluminium frames (trays) stacked on a portable/movable base. Pupae are placed into a channel around the inside perimeter of each aluminium tray. One or two slabs of gelatinous food are put on each screen panel. Up to 80 trays can be stacked in one tower, producing a solid tridimensional aluminium block. To provide air circulation, a small direct-current axial exhaust fan is placed on the top of each tower (stack of trays) to provide forced upward ventilation within the tower. When flies emerge they move from the perimeter channel to the screen of a tray, while the empty puparia are left in the channel. The flies remain on the screen and feed for 4–6 days, the period required for maximum adult emergence. On the day of field release, the towers are moved into a cold room where higher-volume exhaust fans suck cold air (~4ºC) into each tower to accelerate fly immobilization. After a period of exposure to the cold, the puparia are vacuumed from the channels, and the screened trays are manually turned upside down over a hopper (and pan beneath) to collect the chilled flies (Fig. 3). After the collected chilled flies are weighed, they are transferred to the aerial release box and transported to, and loaded into, a release aircraft (Figs. 10, 11).

Two additional tower systems (Hernández et al. 2010) are currently being used at the Moscamed programme in Mexico (Fig. 4) and Guatemala (Fig. 5). The Mubarqui tower system in Mexico has a larger surface area and volume for the flies to expand their wings, feed, and mature. The system uses a predesigned "Mubarqui diet" for feeding adults of Mediterranean and Mexican fruit flies (Fig. 4).

The two systems shown in Figs. 4 and 5 use the environmental room conditions (temperature and relative humidity) without the need for exhaust fans on top of the towers; this was the reason for not using the previously described "Worley tower system". Electricity fluctuations generated too many inconsistencies in the operation of the many fans, thus their use was discontinued in favour of a new tower design.

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*Figure 3. Tower system for Mediterranean fruit fly -- stages in the emergence and collection of fruit flies using the tower system (Worley Tower). Upper left: Screen tray loader, showing an upper rotating-revolver mechanism (transparent containers) to load each tray with pupae. Upper right: Aluminium frame/tray, showing a slab of agar-based food diet and dyed pupae in a channel around the edge. Lower left: Stacks of trays forming towers, each connected (yellow cables) to a power outlet to operate a fan that draws air upwards. Lower right: Tower system for Mexican fruit fly -- screen trays in cold room with puparia removed from channels by suction, and chilled flies on screen being collected in a hopper. USDA/APHIS, Sarasota, FL, USA.*

The other difference of the tower system used in Guatemala compared with the previous system is that it uses a "liquid diet", replacing the expensive and cumbersome preparation, handling, and discarding of agar diets, and enabling the liquid diet to be replenished externally. This permits flies to be kept with no refill until they are released, or with a refill for longer periods when overcast climatic conditions prevent aerial release on the scheduled date (or for some different insect species that have longer maturation periods). These two additional tower systems use the same chilling process as the one described above for the "Worley tower". Automated washing of the screens is also available for the liquid-diet tower.

Compared with the PARC system, the tower system improves considerably the quality of the flies to be released. Also, it reduces fly escapes as well as the required controlled-environment space, with a consequent reduction of fixed costs, although

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there are obvious variations in the space requirements of the different systems described above. However, the optimal holding density of sterile insects in the tower system must be carefully determined because higher densities result in decreased sterile-insect quality (Hernández et al. 2010).

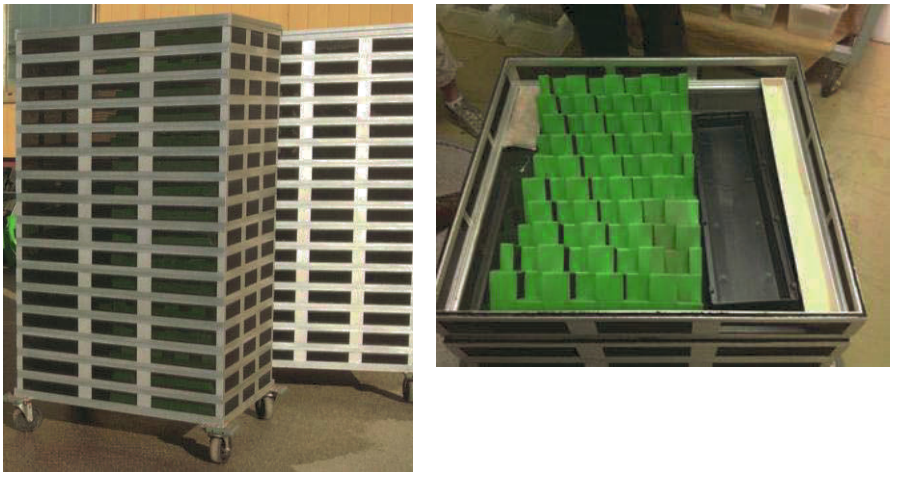


*Figure 4. Left photo: Self-stackable 16-tray Mubarqui tower system for Mediterranean and Mexican fruit flies. Right photo: Inner components of an emergence tray -- left upper corner, agar slab for water provision; centre, green panels for increased fly-resting space; right, black tray for pupae placement and white tray for placing the "Mubarqui diet". Medfly Programme, Neretva Valley, Croatia, and Mexican Fruit Fly Programme, Mexico.*



*Figure 5: Left: Fly emergence and maturation room inside the new ERF at El Pino, Guatemala, using liquid-diet towers, with air-conditioning units for room conditioning. Right: Liquid-diet tray showing (through the screen) two white plastic trays holding pupae (pupae marked with orange-coloured dye). A cotton cloth (white) and a sponge (blue) draw the liquid diet by capillary action from the PVC-pipe system placed on the side of the trays (as shown in the left photo).*

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The only waste materials produced in the tower system are puparia, food residues, and water from the mechanical tray washer. Overall, labour requirements are also reduced, primarily because of the automated pupae loading, puparia separation and disposal, and tray-washing processes. On the other hand, since there are fewer flies in each tower tray than in a PARC box, collecting chilled flies from tower trays is slower; thus, during the critical time in which chilled flies must be collected from tower trays, more manual labour (than from PARC boxes) is required. Hence, on balance, even though the tower system requires more hand-labour in the final step of the process, i.e. collecting chilled flies, its overall cost is lower than that of the PARC system because of the optimization of space and savings in hand-labour costs in the other steps of the process (as well as saving the cost of buying and disposing of paper bags, and the agar-diet preparation process which includes the cost of agar, electricity, and hand-labour), while adding the main advantage that tower-reared flies are better fed and less "stressed" than those reared using the PARC system.

### *5.1.3. Marking Emerging Adults*

To enable identification after capture in the field, many species are normally marked at the MRF prior to shipment, emergence, and release (Hendrichs and Robinson, this volume; Parker, Mamai et al., this volume; Vreysen, this volume). Calco and tinopal dyes have been used successfully to mark lepidopteran larvae (Stewart 1984; Dyck 2010; Boersma 2021). Fluorescent dyes, used on fly pupae (Fig. 6), are transferred to the ptilinum of the adult as it emerges at an ERF (Steiner 1965; Schroeder et al. 1972; Vreysen et al. 1999; Dominiak et al. 2010; FAO/IAEA 2016b). The transferred dye becomes incorporated into the head capsule as the ptilinum is withdrawn into the insect's head (Fig. 7). Upon capture, as adults are viewed under a long-wave ultraviolet (blacklight) lamp; the dye fluoresces, enabling wild unmarked and reared marked flies to be distinguished from each other. Currently, there are several insect species for which there is technology available to supersede the described marking process (Niyazi et al. 2005). This new technology is based on the insertion of genes that allow fluorescence of body parts and sometimes other insect tissues, e.g. testis – sperm. Some of these species/strains have already been tested for their rearing characteristics (Ramírez-Santos et al. 2017), but at this time this technology is not approved for use in the field by most government regulations.

### *5.1.4. Provision of Food and Water Prior to Release*

For most adult insects, it is important that they are provided with a source of food and water shortly after emergence. Most SIT target species are anautogenous, and the food reserve accumulated during larval development is only sufficient to sustain the adult for 1 or 2 days under ideal environmental conditions. Intake of protein (Papanastasiou et al. 2019) and other essential resources is required by emerged sterile males and females to permit reaching sexual maturation after a time-delay following feeding (Hendrichs and Prokopy 1994). Studies have shown that providing food and water to newly emerged adults results in much higher recapture rates following release (FAO 1992; Hernández et al. 2010; Bellini et al. 2014; Maïga

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et al. 2014; FAO/IAEA 2016b, 2017a, b; Parker, Mamai et al., this volume) (sections 3.3., 4.1., 4.2.; Figs. 2, 3, 4, 5). Increasing the food surface available for emerging sterile insects is important; all adults need access to the food within the crowded conditions of PARC boxes or tower trays (USDA/APHIS 2009).

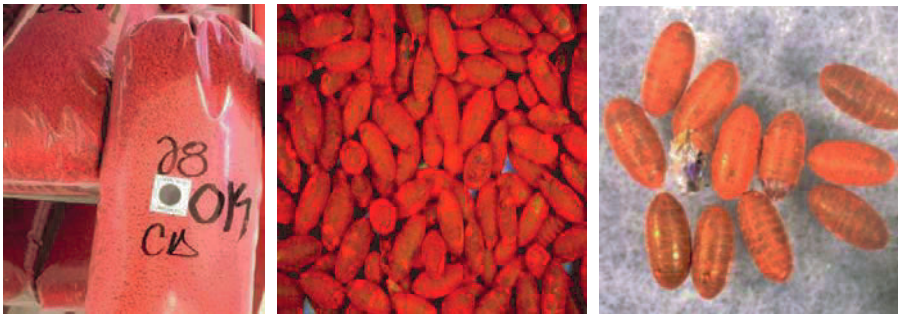


*Figure 6. Left: Bags containing DayGlo™-dusted pupae -- showing also an irradiation dosimeter that has become black (Bakri et al., this volume), proving that the dose administered reached the required level. Centre: Pupae showing DayGlo dye on the surface of the puparia (a high percentage of marked adult flies is desirable for field traceability). Right: Mediterranean fruit fly males emerging from pupae, marking their body parts during the process. (Photos from E. M. Ramírez-Santos, reproduced with permission.)*



*Figure 7. Upper photos: Unmarked flies. Lower photos: Marked flies showing sterile flies that have emerged from DayGlo™-dyed pupae (from left to right: ptilinum mark, dorsal body marks including wings, and ventral position marks mainly on the legs). Observations conducted using an epifluorescent microscope, El Pino MRF. (Photos from E. M. Ramírez-Santos, reproduced with permission.)*

The addition of specific food supplements to the adult diet, including proteins, for emerging male fruit flies to enhance their post-factory field performance and competitiveness, are discussed in detail by Pereira et al. (2013) and Pereira et al.

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(this volume); these papers also discuss the interaction between nutritional and hormonal treatments to advance sterile male sexual maturation, as well as semiochemical treatments to improve sterile male competitiveness.

Sterile insects are often released soon after emergence to minimize space requirements in an ERF, or a few days later, but well before full sexual maturation is reached (avoiding mating between sterile males and sterile females in the case of bisexual strains). However, the use of GSSs (Franz et al., this volume), emerging only sterile males at ERFs (and processing half the volume of a bisexual strain), enables holding them closer to sexual maturation when they are better able to compete for wild females (this also reduces the often high losses that sterile males suffer in the field, due to predation and other causes, between the time of their release and reaching maturity).

McInnis et al. (2013) found (for the Mediterranean fruit fly in large field cages) that holding sterile males longer at the ERFs and releasing them closer to maturity (and also providing food and water during the maturation period) increased adult survival and thus the number of sexually mature males active in the field.

### *5.2. Collecting Emerged Adults by Positive Phototaxis for Direct Release*

In some insect species, adults are directly collected after emergence, without previous holding and feeding of adults. Often, they are positively phototropic, and thus show a strong positive phototaxis to a light source. This behavior has been used to attract newly emerged adults into cold rooms at ERFs directly for release, e.g. New World screwworm flies (Wyss 2002). Also, adults of pink bollworm moths, codling moths, and false codling moths (Fig. 8) are collected after emergence, often directly at MRFs, using fluorescent, ultraviolet or incandescent lights (Hutt et al. 1972; Stewart 1984; Dyck et al. 1993; Wyss 2002; Dyck 2010; Boersma 2021). Cold temperatures are used to temporarily immobilize the adults following collection (Dyck 2010; Hofmeyr and Pretorius 2010; FAO/IAEA 2016b; Boersma 2021), as well as while they are being transported to an aircraft or ground-release vehicle, and then during release operations.



*Figure 8. Cabinets for emergence and collection of false codling moths (Xsit, Citrusdal, South Africa). (Photo from Boersma 2021, reproduced with permission.)*

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The importance of having adequate control of relative humidity during chilling, holding, and release of adult insects cannot be overemphasized (Tween and Rendón 2007). If the relative humidity is too high, it can affect insect quality. This is particularly problematic when chilling adults (covered externally with a dust-dye) at high humidities and loading them into metal release boxes. Condensation on the inside of the release box can lead to excessive moisture that completely saturates the insects, causing them to become tangled in a large ball at the bottom of the box, resulting in fly damage during release. Commercial dehumidification systems can be used to reduce the humidity during chilling, transporting, and releasing insects.

## 6. RELEASE OF STERILE INSECTS

There are three approaches of releasing sterile insects -- static ground-based receptacles (static release), mobile ground-based vehicles (ground release), and releases using aircraft (aerial release). Each approach has advantages and disadvantages. Usually field action programmes use a combination of them for their operations, according to the conditions, needs, and strategic objectives.

The aforementioned chilled adult release technique is complementary to the release methods. It is used just before releases and aims to produce easy-to-handle quiescent sterile insects that can then be loaded into different-sized release containers, usually metal boxes or bags. The chilling process takes place inside a wall-insulated cold room, where emergence/feeding containers are rolled in. Cold rooms are pre-chilled at the appropriate temperature, usually at ca. 4ºC. The actual chilling process requires 40–60 min. Chilled insects are then collected and loaded into an additional container for field release.

### *6.1. Static Release*

Typically static release receptacles are containers deployed in the field into which sterile pupae or adult insects are regularly placed (Liu and Yeh 1982; Williamson et al. 1983; Cuisance et al. 1984; Oladunmade et al. 1990; FFEPO 1999; Msangi et al. 2000; Yamagishi and Kakinohana 2000; Koyama et al. 2004; Sutantawong et al. 2004; Dominiak et al. 2011b). When pupae are placed into them, adults are able to emerge in synchrony with local diurnal and temperature cycles, leave the receptacle, and disperse from the site during the day as the temperature rises. Static releases require a uniform distribution of release sites throughout the target area, and the pinpointing of areas to receive increased numbers of sterile insects. This approach is possible only for relatively small-sized areas that are accessible.

This method was used in the 2016–2017 outbreak of the New World screwworm in Florida, where ground release sites covering several islands were used. These static sterile-insect releases, combined with other quarantine, treatment, and inspection procedures, achieved eradication of the pest a few months after its detection (USDA/APHIS 2017; Skoda et al. 2018; Vargas-Terán et al., this volume).

Placing pupae in release receptacles eliminates the need to establish ERFs, reducing labour and infrastructure costs. Pupae are easily handled; they can be poured into the release receptacle or included in a pre-prepared insert placed into the

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release receptacle. Simple volumetric measurements provide a way of estimating the number of pupae in each receptacle.

Static releases have major disadvantages: deployment is limited by ground access to release sites, distribution of sterile insects is clumped (with foci separated by areas of low insect density), provisioning release receptacles is labour-intensive, and emerged insects are very susceptible to bad weather and predators that learn to visit the release sites. However, for some species and conditions, this release method is still considered (Dominiak et al. 2011b; Reynolds et al. 2012). Most programmes that have used static releases have changed to either ground or aerial releases (Liu and Yeh 1982; Koyama et al. 2004).

### *6.2. Ground Release*

In a mobile ground-release system, adult sterile insects are dispensed from slow-moving trucks (CMFFEPP 2019), all-terrain vehicles (ATVs) (OKSIR 2019; Boersma 2021; Nelson et al. 2021), or mountain bicycles (Horner et al. 2016). The insects are released by hand or machine, directly into the environment, or in open bags from which they escape after release (Holler and Harris 1993; Fisher 1996; FFEPO 1999; Bloem and Bloem 2000; Covacha et al. 2000; Loosjes 2000; Koyama et al. 2004; Bjeliš et al. 2013, 2016). To maximize their dispersal away from the release vehicle or bag, the insects may be kept at ambient temperatures during the release procedure. The Carnarvon Western Australia eradication project uses a "Medfly blower" consisting of a refrigerated cooler that keeps the flies immobile at about 5ºC, and incorporates an auger, heater, and blower fan. The unit is mounted on the back of a truck (Fig. 9) which, combined with a remote dispersal release interface, enables the insect releases (B. Woods, personal communication).

Ground releases can treat a larger area more quickly and uniformly than static releases. The vehicle routes can easily be changed to meet changing demands of the programme, and additional release trips can be made in areas that need higher numbers of sterile insects. Fewer workers are needed to make ground than static releases. The negative impact of predators on the sterile insects is minimized when the adults are released directly into the environment (Fig. 9). Since pupae are kept indoors at relatively constant temperatures, the adults emerge at a predictable rate, making long-range scheduling possible. Adult insects can be held at lower temperatures during periods of inclement weather, and then released when acceptable weather conditions return. Ground releases are used regularly in fruit fly programmes to supplement aerial releases in areas with difficult access. In the *C. capitata* eradication programme in the Dominican Republic (Fig. 9) (Zavala-López et al. 2021), ground bag releases were conducted to supplement aerial releases along the coastal areas, which were affected by wind drift. Ground releases may not be as uniform as aerial releases, and greater sterile insect densities will occur close to areas bordering the path of the release vehicles. There are also safety issues associated with having slow-moving vehicles travelling on normal roads, and inaccessible areas of dense vegetation pose problems for ground-release systems. In the case of bag releases, bird and vespid predators will learn to exploit the insects contained in them, and bags themselves may constitute a litter problem.

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ERFs are still needed to prepare/feed insects prior to their release. Since adults will emerge even during periods of inclement weather, when releases are not possible or advisable, the accumulation of insects may necessitate their destruction. However, in the Lower Rio Grande Valley at the Mexican fruit fly programme in Texas, when aerial releases cannot occur due to weather conditions, ground releases are a practical solution (Fig. 9). They are also used to increase sterile insect densities in pest hotspot areas where it is required, as well as sensitive areas such as bird sanctuaries and butterfly gardens where other means of control cannot be used (H. Conway, personal communication). If long periods of overcast conditions, e.g. rainy season, are expected, one might need to choose an alternative emergence and release system to prevent/reduce foreseen losses.



*Figure 9. Upper photos (left and centre): Ground release of sterile insects along coastal areas during the recent eradication of the Mediterranean fruit fly in the Dominican Republic. Flies emerged in paper bags housed in PARC boxes, and were taken to field locations where the bags were opened (ripped), enabling the males to fly. (Photos from R. Nina, reproduced with permission.) Upper right: Sterile male Mediterranean fruit flies being ejected from a ground-release machine in Croatia. (Photo from FAO/IAEA, L. Popović and Z. Marinović, Neretva SIT Programme Croatia, Center for Plant Protection, HAPIH, reproduced with permission.) Lower left: Testing of ground-release equipment in Texas for the Mexican fruit fly. The equipment uses vehicle speed and a potentiometer to deliver a known number of flies/ha. (Photo from H. Conway, reproduced with permission.) Lower right: "Medfly blower" releasing flies in Carnarvon, Western Australia. (Photo from B. Woods, reproduced with permission.)*

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### *6.3. Aerial Release*

Aerial releases from aircraft directly discharge chilled sterile adults (chilled adult release technique) or eject bags or boxes containing the adults (Howell et al. 1975; Nakamori and Kuba 1990; FAO 1992; USDA 1995; Vreysen et al. 1999; Msangi et al. 2000; Pereira et al. 2000; Villaseñor et al. 2000; Vreysen et al. 2000; Walters et al. 2000; Wyss 2000; Yamagishi and Kakinohana 2000; Kohama et al. 2003; Rendón et al. 2004; Staten and Walters 2021).

The direct release of sterile adults requires that they be taken from adult emergence containers or rooms, chilled (or first chilled in the containers), and put into large refrigerated boxes that are then loaded onto a specially equipped aircraft (FFEPO 1999; Dowell et al. 2000; Villaseñor et al. 2000; Walters et al. 2000; Wyss 2002; Koyama et al. 2004; Staten and Walters 2021) (Figs. 10, 11, 12). The insects are dispensed from the aircraft using a motor-driven conveyor or screw auger that delivers the insects from the release box to the release tube. The release rate is controlled by varying the speed of the auger and the aircraft. Since screw conveyors cause some damage to flies, an improved release machine design uses instead flat vibrating conveyor belts that reduce sterile-insect damage prior to their release (Leal Mubarqui et al. 2014).

In some programmes, the aerial release of sterile insects has been done with paper bags or boxes (Hentze and Mata 1987; FAO 1992; Vreysen et al. 1999; Covacha et al. 2000; De Longo et al. 2000; Msangi et al. 2000; Opiyo et al. 2000; Villaseñor et al. 2000; FAO/IAEA 2006a). Usually pupae are placed in bags/boxes, held in the release containers until adult emergence, and then the release bags or boxes are loaded into larger holding containers and placed into the aircraft. The release bags or boxes are held at ambient temperatures and dispensed from the aircraft by hand or conveyor belt. Releasing sterile insects in their emergence bags or boxes reduces preparation time, compared with the chilled-insect procedure, but there can be some damage due to bag compaction if too many are transported to the aircraft or too many placed within it, and the sterile-insect dispersal achieved is more limited compared with the chilled adult release technique.

Aerial releases enable the covering of large areas quickly, regardless of the difficulty of the terrain, lack of roads, or high density of vegetation. They distribute the sterile insects over the treated area as required, especially if the aircraft flight paths are controlled by a Global Positioning System (GPS) guidance programme (Villaseñor et al. 2000; USDA 2019) (section 6.4.). The mechanized release system can be linked to a computer to provide flexibility in the release rate during a flight. This allows more sterile insects to be delivered to those areas needing them, without having to make multiple flights over any given area. If boxes or bags are used, the release rate is increased by ejecting more of them in a given time period (section 6.4.).

On the other hand, aerial releases are more weather-sensitive than ground releases, and an ERF is required. The aerial release of sterile insects in bags or boxes is problematic if litter is a concern (but a biodegradable container mitigates this problem). Bags and boxes are expensive, and for some insects (such as tsetse flies that are extremely sensitive to insecticides) the cardboard boxes must be free of any chemical residue (this increases the price of the boxes). They also take up

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considerable space in an aircraft that could instead be devoted to holding many more chilled insects. Aerial releases of the Mediterranean fruit fly in Argentina, using bagged adults, averaged only 1.2–1.3 million flies per flight (De Longo et al. 2000), whereas aerial releases by directly releasing chilled flies can release 10 million per flight (in Spain with a Cessna 206) and up to 40 million flies per flight (in Mexico with a Cessna Caravan). The number of sterile insects that can be released by an aircraft is also a function of the type of insect, the release rate, and the aircraft size. Flight duration is usually determined by the amount of time that sterile adult insects can tolerate chilling without a reduction in quality (usually about 4 h), but this depends on the temperature and size of the insects. Pereira et al. (2000) found that aerial releases are less expensive than ground releases.

Both fixed-wing (Figs. 10, 11) and rotary-wing (Fig. 12) aircraft can be used to deliver sterile insects aerially to the target area. The choice of aircraft type will depend on programme needs and local environmental conditions, as well as the availability of aerial services or the cost of the aircraft. Releasing with rotary-wing aircraft is usually more expensive, although gyrocopters are less expensive than fixed-wing aircraft. Rotary-wing aircraft are used usually for the spot-treatment of restricted host areas, or canyons, where fixed-wing aircraft cannot operate easily.

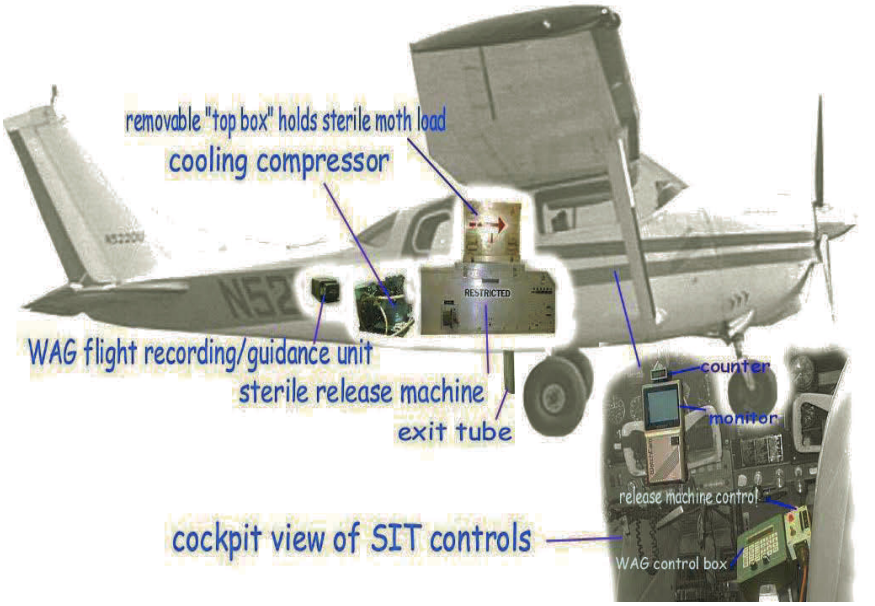



*Figure 10. Fixed-wing aircraft used to release sterile pink bollworm moths in 2007. Placement of the release machine into the aircraft is shown. Locations of the moth-holding and moth-release equipment, flight recording/guidance unit (Wag-Aero (Brand name) = GPS navigation unit that tracks the flight path), and SIT controls are labelled. (Photo from the California Department of Food and Agriculture, reproduced with permission.)*

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*Figure 11. Releases from fixed-wing aircraft. Upper photos: Preparing aerial bag releases in Mendoza, Argentina. Lower photos: Loading refrigerated boxes with chilled sterile Mediterranean fruit flies in Retalhuleu, Guatemala. RT: Refrigerated Truck; RB: Refrigerated Box; SA: Screw Auger; RS: Release Shut. (Photos from G. Iriarte, reproduced with permission.)*




*Figure 12. Aerial release of sterile male tsetse flies in the Niayes region of Senegal using a gyrocopter (Dixit 2015). (Photo from J. Bouyer, reproduced with permission.)*

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Due to recent technological developments in aerial technology and guidance systems, programme managers look for improved and alternate ways of releasing sterile insects, so that the process is more efficient in its coverage and more cost-effective. There are different release devices being considered for this exploratory new wave of technology; the initial step is to acknowledge that there are very dissimilar requirements in terms of the release rates/unit area needed to achieve pest control. For fruit flies and mosquitoes, usually thousands of insects per ha are required, in lepidopteran species hundreds of insects per ha, in screwworms tens of insects per ha, and in tsetse only individual flies per ha (Table 5 in Hendrichs, Vreysen et al., this volume). This variation in release rate changes the aircraft requirements. For pest species that require an extensive coverage at a very low release rate, such as tsetse flies, fuel-tank capacity in fixed-wing aircraft is a more limiting factor than the capacity to hold sterile insects. On the other hand, for fruit flies, in view of the relatively large volumes to be released per area, larger fixed-wing aircraft are required to maximize the release per flight. However, because of the difference in millions of insects to be released in the diverse programmes, there is also a gradient in fixed-wing aircraft size/capacity. For lepidopteran species, fixed-wing aircraft have been used for pink bollworm moths (Simmons et al., this volume) (Fig. 10); in other operations such as releases of the false codling moth in South Africa, ATV squads and gyrocopters have been used. However, both practices were discontinued due to worker issues in the former and security concerns in the latter (Boersma 2021; S. Groenewald, personal communication). In the Niayes region of Senegal, the programme to eradicate tsetse flies (which required very low rates of sterile males per ha) used a gyrocopter for the aerial releases (Dixit 2015) (Fig. 12).

New ways of releasing sterile insects from the air are being explored and adopted – type of aircraft as well as new technologies for release. For the capacity of aerial release machines, there is now an adaptable or "universal" delivery system; it was developed so as to meet the needs of very different operations, sizes and release rates, i.e. size of the aircraft needed, as well as the number of sterile insects being released in the field. This "smart delivery system" is capable of releasing at varying densities, ranging from 10 flies per $km^2$ for tsetse flies at one extreme up to more than half a million per $km^2$ for fruit flies on the other (Leal Mubarqui et al. 2014) (sections 6.3.3., 6.4.).

### *6.3.1. Fixed-Wing Aircraft*

In the early years of sterile insect release, degradable bags or boxes were released from aircraft, e.g. New World screwworm flies, Mediterranean fruit flies (Fig. 11), and tsetse flies. For a long time, fixed-wing aircraft were the standard vehicle for releasing sterile insects, e.g. New World screwworm flies, pink bollworm moths (Fig. 10), Mediterranean fruit flies (Fig. 11), Mexican fruit flies, and tsetse flies. Today, in many cases, chilled insects are released from special machines with augers or conveyor belts that eject the insects automatically according to a predetermined rate, enabling the planned density of sterile insects to be achieved on the ground, e.g. false codling moths in South Africa (Xsit 2019a; Boersma 2021).

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### *6.3.2. Rotary-Wing Aircraft*

Compared with fixed-wing aircraft, rotary-wing aircraft are appropriate for making release flights over mountainous terrain or small target areas (Vargas et al. 1995). Melon flies were released with helicopters in Okinawa, Japan (Nakamori and Kuba 1990; FAO/IAEA 2016c; Enkerlin, this volume), and a gyrocopter was used to release sterile tsetse flies in Senegal (Fig. 12) (Dixit 2015; Vreysen et al. 2021; Feldmann et al., this volume). For a period of time, false codling moths were released in South Africa with gyrocopters, but currently these moths are being released with small helicopters (Boersma 2021).

### *6.3.3. Unmanned Aerial Vehicle (UAV) (Drone)*

Unmanned aerial vehicles (UAVs), remotely piloted aircraft systems (RPASs) or drones are the latest development in aerial release technology; their main components are shown in Fig. 13. Using these systems is attractive to operational programmes because of the potential cost reduction in some sterile-insect release activities (Tan and Tan 2013). Also, the adoption of this technology can avoid the loss of human lives that sometimes results from aircraft accidents (Dyck, Reyes Flores et al., this volume).

The advantages of the RPAS technology (accuracy, increased safety, and cost-efficiency for small- and medium-scale operations) are counterbalanced by limitations at the technical level (reduced payload and flight duration) as well as at the regulatory level (mandatory special operational permits from regulatory agencies for operations beyond the visual line of sight) (Benavente-Sánchez et al. 2021). Compared with ground releases, sterile insect dispersal and survival should be improved when using UAVs.

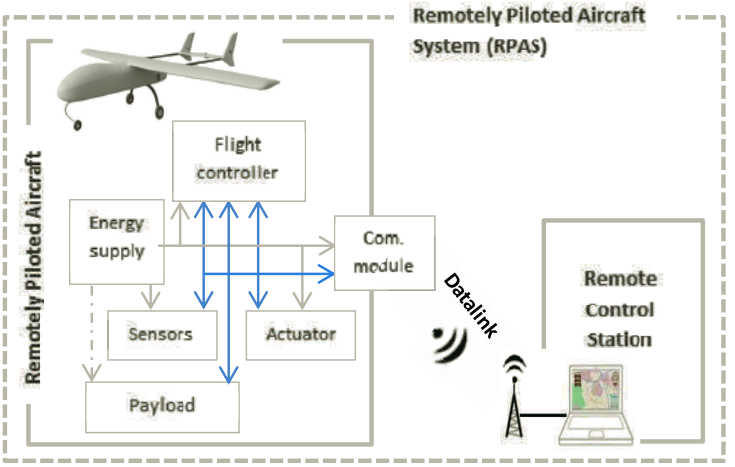



*Figure 13. Main components of a Remotely Piloted Aircraft System (RPAS). Modified after Maxorazon, Wikipedia CC BY-SA 4.0. (Wikipedia Commons 2018). (Drawing from Benavente-Sánchez et al. 2021, reproduced with permission.)*

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Following the development of commercially available UAVs, they are increasingly being tested for releasing insects in several SIT projects (Table 1): *Aedes* mosquitoes (Fig. 14), chilled tsetse flies (Benavente-Sánchez et al. 2021) (Fig. 15), codling moths (Figs. 14, 16) (Horner et al. 2016; M3 2018; PFR 2018a, b; Proskiw 2018; Seymour 2018; FGN 2019), false codling moths (Xsit 2019b; Boersma 2021), and pink bollworm moths (USDA/APHIS 2018).

*Table 1. Technical parameters of Remotely Piloted Aircraft System (RPAS) applications releasing sterile insects (data adapted from Benavente-Sánchez et al. 2021, reproduced with permission)*

| Parameter | | Release of sterile insects | | |
|---|---|---|---|---|
| | | Male *Aedes* mosquitoes[1] | Male *Glossina* tsetse flies[2] | Codling moths (♂+♀)[3] |
| Adult weight (mg) | Min | 0.9 | 14 | 23 |
| | Max | 1.1 | 18 | 25 |
| Release density (nr/ha) | Min | 1000 | 0.5 | 200 |
| | Max | 3000 | 1.0 | 1000 |
| Insect biomass ($g/km^2$) | Min | 90 | 0.7 | 690 |
| | Max | 330 | 1.8 | 2500 |
| Area covered per flight (ha) | Min | 40 | 4000 | 40 |
| | Max | 60 | 8400 | 100 |
| Swath width (m) | Min | 75 | 500 | 120 |
| | Max | 100 | 700 | 180 |
| Flight distance (without ferry) (km) | Min | 5.3 | 80 | 3.3 |
| | Max | 6.0 | 120 | 5.6 |
| Flight time (without ferry) (min) | Min | 11 | 67 | 6 |
| | Max | 13 | 100 | 9 |
| Speed during operation (m/s) | | 8 | 20 | 10 |
| Payload (g) | | 300 | 800 | 7000 |
| Type of aircraft | | Rotary; micro/small | Fixed-wing, rotary; medium | Fixed-wing, rotary; medium |
| Type of operation | | VLOS[4]; urban area | BVLOS[5]; rural area | BVLOS[5]; rural area |
| Special flight authorization | | Mandatory | Mandatory | Exempted |

[1] Trial release of *Aedes aegypti* in Juazeiro, Brazil, 2018 (Benavente-Sánchez et al. 2021; J. Bouyer, personal communication)
[2] Planned release of *Glossina* spp. in Chad and Ethiopia (Benavente-Sánchez et al. 2021)
[3] Routine release of *Cydia pomonella* in a pilot area of 391 ha in Central Hawke's Bay, New Zealand (PFR 2018a, b; Benavente-Sánchez et al. 2021; R. M. Horner, personal communication)
[4] VLOS = Visual Line Of Sight
[5] BVLOS = Beyond Visual Line of Sight

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UAVs are particularly appropriate for situations requiring the release of relatively small sterile-insect quantities, and on small areas (including residential areas), e.g. releasing mosquitoes over urban areas (an emerging need due to the recent explosion of mosquito-borne diseases) (Lees et al., this volume). UAVs have been tested to release sterile *Aedes aegypti* (L.) in Brazil (Fig. 14) and Mexico. A prototype (Remotely Operated Mosquito Emission Operation -- ROMEO) has been developed (HEIGHT TECH 2019) that can carry half a million mosquitoes per load, and has flight independence up to 30 min, enough to cover an area of 1 $km^2$ (FAO/IAEA 2016a; Garnier et al. 2018; UN 2018; Bouyer et al. 2020) (Fig. 14).

Benavente-Sánchez et al. (2021) reviewed the progress and prospects for using RPASs in SIT projects. They present a table with data on tests releasing sterile insects (mosquitoes, tsetse flies, and codling moths) – comparing the weight of adults, payload, release density, area covered per flight, and flight distance, speed, and time, etc. (Table 1) (see Table 5 in Hendrichs, Vreysen et al., this volume, for another cross-species comparison). Details of appropriate equipment and procedures are provided. They also reviewed the progress made in different countries in regulating and harmonizing internationally the use of different-sized RPASs for various agricultural applications. Legislation, regulation, certification, and authorization are essential for any routine use of RPASs in support of SIT projects.



*Figure 14. Left: Drone ROMEO (Remotely Operated Mosquito Emission Operation) (From FAO/IAEA 2016a; UN 2018; Bouyer et al. 2020). Centre: RPAS and mechanism to release sterile male mosquitoes* Aedes aegypti *in Brazil in 2018 (From J. Bouyer, reproduced with permission). Right: Drone releasing sterile codling moths in Canada (From M3 Consulting Group -- hd-mediaitemid51688-3698 (M3 2018); Proskiw 2018; Seymour 2018).*



*Figure 15. Prototype Unmanned Aerial Vehicle (UAV) (drone) for releasing sterile tsetse flies. The pods under the wings carry the chilled adult males in biodegradable boxes that are dropped according to a pre-planned flight programme. (Photo from Benavente-Sánchez et al. 2021, reproduced with permission.)*

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*Figure 16. Remotely piloted fixed-wing drone, operated in visual line-of-sight, releasing sterile codling moths in New Zealand (PFR 2018a, b). (Photo from R. M. Horner and Plant and Food Research Institute, New Zealand (PFR 2018a), reproduced with permission.)*

### *6.4. Global Positioning System (GPS) for Guiding and Monitoring Insect Release*

Guidance of aircraft used to release sterile insects has evolved from a system based on compass headings, landmarks, and flight times to one that now uses the Global Positioning System (GPS) (Slagell 2000; Bouyer et al., this volume) (Figs. 10, 17, 18). Using the GPS, programme managers can lay out the exact flight paths for the aircraft. This technology allows the distribution of sterile insects precisely where desired. It has also reduced flight times and pilot error, eliminating duplication of flight paths and the unintentional omission of some of them. Today, most programmes making aerial releases use the GPS to guide the aircraft. The GPS is especially useful in areas that lack the visual landmarks that pilots may use to determine where they are and where they need to fly.

A computer with its software enables recording of pre-programmed flight lanes, release elevation, and airplane speed, information that can be classified and displayed using Geographic Information Systems (GIS) (Bouyer et al., this volume). Programme managers greatly benefit from the combination of these tools to supervise the work and guide the release and other control activities (Figs. 17, 18). GPS and GIS location of the sterile and fertile insect captures is also used to map out presence and distribution of the flies as well as the sterile to wild ratios relevant for the success of the SIT (FAO/IAEA 2006b).

In the Mediterranean fruit fly programme in Valencia, Spain, differential release rates of sterile males are applied according to pest density as the aircraft flies over release blocks with non-regular shapes, so curvilinear release paths are adapted to them. The flow of sterile males is adjusted automatically by the release machine during the flight to fit the target release map that was previously created using the most recent trapping information on apparent densities of wild flies in the field (Fig. 18) (section 6.3.).

The MACX system is a sterile-insect release monitoring option that was designed to track the quality of chilled-adult releases. The data are recognized, analysed, translated, and re-transmitted to a website where it is available to supervisors and programme managers; the system can follow the releases in real time (Leal Mubarqui 2005; Leal Mubarqui et al. 2014).

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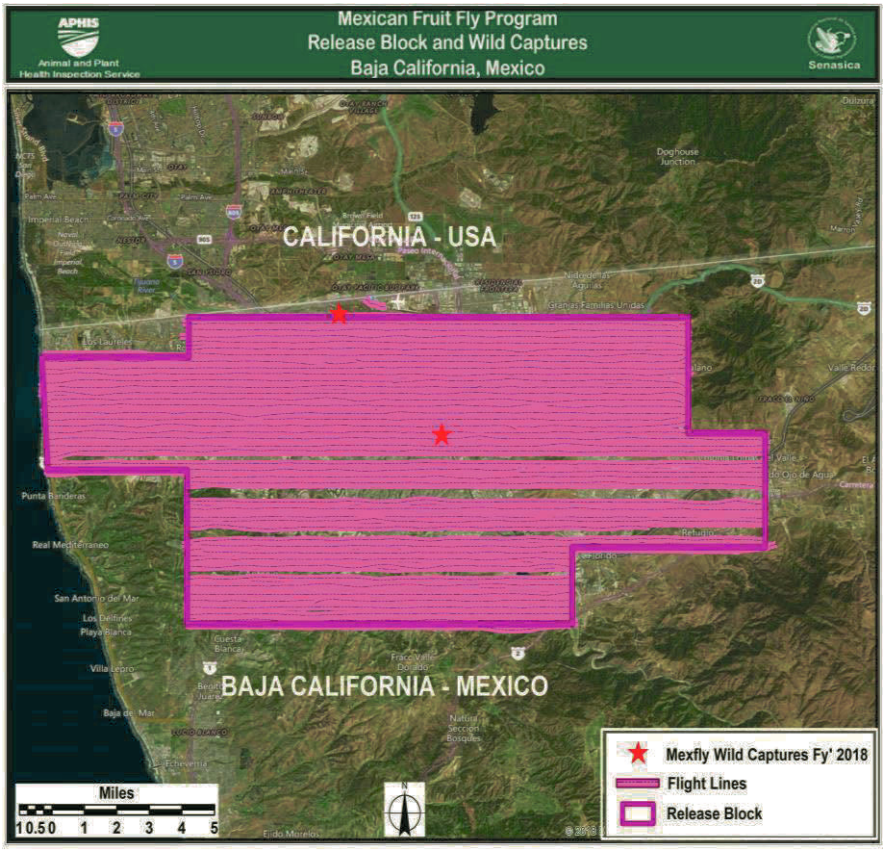



*Figure 17. Aircraft flight lanes (thin red lines) for sterile insect releases in the Mexican Fruit Fly Programme in Baja California, Mexico. (Illustration from L. Charles, USDA/APHIS/IS Mexico, and E. Lira, USDA/APHIS/IS Guatemala, reproduced with permission.)*

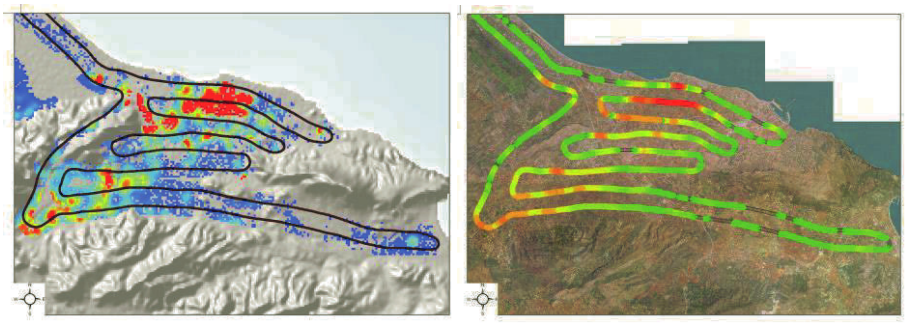


*Figure 18. Left: Release block with non-regular shape and curvilinear flight path adapted to it, showing the target release intensities (red colour means high densities of sterile males are needed). Right: Map showing the differential release actually conducted (the red colour of the release flight line confirms higher release flows of sterile males).*

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To release boxed tsetse flies, an electronic counter is used; this device has an optic sensor and a GPS antenna. It counts the biodegradable boxes as they are released from a fixed-wing UAV (Fig. 15), and saves a digital file recording the position at which each box has been dropped. The file can be imported into GIS software for later analysis of the quality of the flight (FAO/IAEA 2016b; Benavente-Sánchez et al. 2021).

## 7. CONCLUSIONS

Most of the large operational programmes that integrate the SIT have established emergence and release facilities (ERFs) in the vicinity of the release areas, usually separated from the mass-rearing facilities (MRFs) that supply them with the sterile insects, commonly in the form of pupae. This operational scheme results in lower costs for the programme and better quality insects in the field, provided the duration of shipments and temperature of the shipped insects are kept at acceptable levels. However, in some insect species, adults can be collected after emergence (Fig. 8) (without the need for holding and feeding) for direct transport and release.

MRFs can also supply sterile insects to independent field programmes, thereby avoiding large initial investments in the mass-production of sterile insects. Shipping sterile insects across national borders has become a major activity, with improved shipping procedures resulting in higher-quality sterile insects.

More efficient methods for emerging, holding, and feeding high-quality insects, such as the tower system for fruit flies, have been developed, optimizing the space needed in the ERFs and reducing the workload for the daily operations. However, earlier methods are still useful depending on the particular action programme, e.g. emergency response vs. permanent activity.

The incorporation of GPS technologies into surveillance systems has enabled the determination of the precise location of pests in the field and at which sterile-insect releases should be directed. Navigation technologies, using GPS, GIS, and electronic communication systems have greatly improved the accuracy, reliability, efficiency, and recording of sterile-insect releases.

Similarly, insect ground- and aerial-release methodologies have evolved, using available industrial technologies. Although still in the development and validation phase, in some situations unmanned aerial vehicles (UAVs) or drones appear to be a realistic and cost-effective possibility for releasing sterile insects within their technical limitations of payload and endurance. However, the regulation and international harmonization for using different-sized remotely piloted aircraft systems (RPASs) are still in progress.

Quality-control manuals have been updated (FAO/IAEA/USDA 2019; FAO/IAEA 2017a, b) to include assessments of sterile insects prior to, and even after, their field release, ensuring their viability for the expected introduction of sterility into feral populations. A spreadsheet on sterile-insect release density calculations and its manual, recently published (FAO/IAEA 2019), has enabled release densities (as well as the corresponding ratios of sterile to wild insects based on actual field trapping data) to be determined; its programmatic use has already contributed to achieving an improved use of the SIT. All of these improvements add

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up to a more cost-effective implementation of the SIT as part of an area-wide integrated pest management approach to pest control.

## 8. ACKNOWLEDGEMENT

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# CHAPTER 3.7.

# MONITORING STERILE AND WILD INSECTS IN AREA-WIDE INTEGRATED PEST MANAGEMENT PROGRAMMES

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## SUMMARY

Insect pest control programmes, which integrate the release of sterile insects, can be efficient only if the released insects have an optimal biological quality. Frequent monitoring of the quality of reared insects after being released in the field is an important but often neglected component of area-wide integrated pest management (AW-IPM) programmes that integrate the sterile insect technique (SIT). Parameters of sterile insects, which should be monitored regularly, are sexual competitiveness of the released insects, and related components, e.g. survival, mobility, dispersal characteristics, and spatial occupation of the habitat. A well-balanced monitoring programme will, at any given time, provide essential feedback on the target and sterile populations in the field, and therefore on whether progress is being made. This information is prerequisite to efficient implementation of the release and cost-efficient use of sterile insects. The type of monitoring to be done will be determined largely by the particular biology of the target insect species. The rate of sterility induced in the wild insect pest population is the most important parameter in relation to the release of sterile insects; an increased rate of induced sterility in the native female population can unequivocally be linked to a decrease in the density of the target insect population.

## 1. INTRODUCTION

Successful area-wide integrated pest management (AW-IPM) programmes using the sterile insect technique (SIT) against insect pests require reliable data on the biology of the target insect, especially its sexual behaviour, population dynamics, and temporal and spatial fluctuations in population density and distribution (Itô et al., this volume). This information is essential to accurately interpret data accumulated during the monitoring of a programme. Monitoring sterile and wild insects is a critical aspect of any SIT operation and includes: (1) the performance of sterile insects after release, and (2) the impact of sterile males and other control tactics on the wild target population.

The efficient implementation and success of any control programme using the SIT will depend on factors related directly to the quality of the released insects (Parker, Vreysen et al., this volume). It is imperative that the released sterile insects intermingle rapidly with the wild population after being released, and mate at the same rate as the wild insects. The production of insects in a rearing facility, that have a "biological quality" or "competitiveness" comparable with that of wild insects, is much more complicated than might be anticipated. This quality can easily be impaired due to: (1) aspects inherent to the colonization and mass-rearing procedures (artificial environment, holding density, stress) (Parker, Mamai et al., this volume), (2) the sterilization treatment with ionizing radiation (Bakri et al., this volume), (3) the physical handling and marking procedures at the production or release centres, and (4) the transport of the insects to the release site (Dowell et al., this volume; Parker, Mamai et al., this volume; Parker, Vreysen et al., this volume). In addition, the behaviour of the reared and released insects could be altered drastically due to changes induced in the genetic traits of the mother stock kept for numerous generations under artificial conditions. Continuous rearing may select for traits that favour mass-

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production (early maturation, high fecundity, mating at high densities), but which could negatively affect field performance (courtship behaviour, release of pheromones, territorial behaviour) and even prevent released insects from mating with wild insects (Lance and McInnis, this volume).

Ideally, to assure optimal quality of reared insects, rigorous quality control procedures must be implemented at all times and in all phases of production, not only on an ad hoc basis or after the emergence of a problem (Spradbery 1994). However, regardless of how meticulously quality control procedures are implemented, the laboratory criteria of fitness may have little bearing on the ability of released males to survive and mate with wild females (Krafsur and Hightower 1979). Admittedly, measurement of the "biological quality" of sterile insects in a laboratory or field cage is a convenient way to assess the effect of several factors related to sexual competitiveness (FAO/IAEA/USDA 2019; Parker, Vreysen et al., this volume), but it does not assess other parameters that are also of relevance to the insect's performance in the open field. Therefore, the frequent monitoring of the competitiveness of the released insects and related parameters, such as survival, dispersal rate, and spatial occupation in the natural habitat, is an indispensable component of AW-IPM programmes that integrate the release of sterile insects.

Efficient programme implementation and technical management of an AW-IPM programme are only possible through the regular and frequent analysis of accurately collected field data. In practice, not all programmes using the SIT grant the same importance to monitoring and data analysis, and the emphasis given depends on factors such as: (1) level of experience of the managers, and their confidence in the programme, (2) economics and availability of sterile insects (is it cheaper to release more insects than develop an extensive monitoring programme?), and (3) efficiency and economics of the available monitoring tools. Scientifically sound monitoring activities require significant funding for personnel, equipment, logistics, and recurrent expenses, and as a consequence, insufficient importance is often given to this component. However, when progress does not match expectations, accurate field data are a prerequisite to detecting the causes of the problem and applying corrective measures. Otherwise, programme managers are doomed to "guess work" and making decisions based on assumptions, frequently resulting in technical and financial failure.

History teaches us that any AW-IPM programme will face external criticism, especially when the goal of the programme is to eradicate the target insect population in a circumscribed area (Klassen and Vreysen, this volume). Unfortunately, it is usually the uninformed "outsider", lacking appropriate scientific background or insight into the activities and challenges being faced by the programme, who is criticizing it. A scientifically sound monitoring programme is an essential element in successfully refuting such criticism.

## 2. MONITORING INSECT QUALITY IN THE FIELD

Surprisingly little attention has been given to monitoring the competitiveness of sterile insects in the field. In part, this is due to the technical difficulties involved in making these types of observations. In recent years, however, significant progress has been made by using larger walk-in field cages to study a set of parameters (e.g. preferred

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host location, courtship and mating behaviour, mating compatibility, spatial distribution related to female location, mating success, etc.) that are relevant to the sexual competitiveness of the reared sterile insects in a semi-controlled natural environment (FAO/IAEA/USDA 2019; Parker, Vreysen et al., this volume). Even with this important development, the successful interaction of mass-reared sterile males with wild females in the field is still the key to the success of the SIT, and every effort should be made to assess the extent of this interaction. A Field Quality Control group, that designs a set of tests tailored to the needs of a target species, must be an essential part of all field programmes. This group should be composed of full-time employees of the programme who have the required expertise in conducting field evaluations, and extensive knowledge about the ecology and behaviour of the target insect (FAO/IAEA/USDA 2019).

### *2.1. Field Monitoring of Sexual Competitiveness*

The competitiveness of an organism is defined as its ability to compete with conspecific organisms for a limited environmental resource (FAO/IAEA/USDA 2019). Fitness in wild insects involves their genetic contribution to the next generation's gene pool relative to the average for the population. Sterile insects do not reproduce, and therefore do not contribute to the next generation's gene pool. Their competitiveness is largely a matter of survival, dispersion (spatial occupation of the habitat), adequate behavioural responses, habitat finding, and successful mating, i.e. it ends with mating or insemination (LaChance 1979). Therefore, the general sexual competitiveness of a sterile insect is largely defined and influenced by components such as survival, mating propensity, mating compatibility, post-mating factors, etc. (FAO/IAEA/USDA 2019; Lance and McInnis, this volume). In view of the drastic changes, due to continued mass-rearing, that can be induced in the genetic traits of reared insects, the frequent measurement of sexual competitiveness and its related components under field conditions is indispensable for success (Haisch 1970; Itô and Koyama 1982).

The sexual competitiveness '*c*' of sterile insects was defined by Fried (1971), and its value will normally fluctuate between 0 and 1 (Itô and Koyama 1982; Iwahashi et al. 1983; Itô et al., this volume). The importance of regularly monitoring the sexual competitiveness in the field was demonstrated in the programme against the melon fly *Zeugodacus cucurbitae* (Coquillett) on Kume Island, Japan. In the beginning of the release programme, mating competitiveness measured in the field was high ($c = 0.8$), and comparable with that obtained in laboratory cage tests. After 18 generations of continuous mass-rearing, the field value of *c* dropped to 0.2, whereas the laboratory value remained high. This difference was attributed to the inferior mating performance of wild males in small laboratory cages, and a real decline in the field competitiveness of sterile males due to rearing-induced genetic changes, i.e. domestication, and the development of SIT resistance in the wild population (Iwahashi et al. 1983; Lance and McInnis, this volume; Whitten and Mahon, this volume). As a result of monitoring the field *c* value, programme managers were alerted, took corrective action in a timely manner, and the melon fly was eradicated in Kume Island.

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An interesting trend in the field competitiveness of released insects, in relation to the operational size of an AW-IPM programme, is provided by the New World screwworm *Cochliomyia hominivorax* (Coquerel) eradication programme in the USA and Central America. In the first decade of the programme in the USA (Curaçao, Florida, Texas 1954–1962) (Klassen et al., this volume), less than 50 million sterile insects per week were released at low densities, and competitiveness was 0.29–0.43. However, when production increased to 500 million sterile insects per week, the competitiveness dropped to below 0.1 (Mayer et al. 1998), indicating that increased production levels have a negative impact on field competitiveness. Measures such as regularly renewing the strains when the programme moved from the arid south USA to tropical Central America mitigated the problem.

Fried's model indicates that the sexual competitiveness of released insects is inherently linked to the sterile to wild insect ratio obtained in the field. Calculations of critical sterile to wild ratios for any target insect (Barclay, this volume) are usually based on experience or the results of mathematical models, and may vary considerably among insect species, and between theory (models) and practice (operational programmes). Critical sterile to wild ratios varied between 7:1 for the tsetse fly *Glossina palpalis gambiensis* Vanderplank (Politzar and Cuisance 1984), 25:1 for the olive fruit fly *Bactrocera oleae* (Gmelin) (Tzanakakis 1974), 40:1 for the codling moth *Cydia pomonella* (L.) (Dyck et al. 1993), 60:1 for the pink bollworm *Pectinophora gossypiella* (Saunders) (Staten et al. 1993), 80:1 for the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Villaseñor et al. 2000), and 25–100:1 for the New World screwworm (J. W. Snow, personal communication). Operational sterile to wild ratios can even be different for species and subspecies within a genus, e.g. a sterile to wild male ratio of 7–10:1, greater than 10:1, and greater than 30:1, was applied against *G. palpalis gambiensis* in Burkina Faso, *Glossina palpalis palpalis* Robineau-Desvoidy in Nigeria, and *Glossina austeni* Newstead in Unguja Island, Zanzibar, respectively (Politzar and Cuisance 1984; Oladunmade et al. 1990; Vreysen et al. 2000). Most likely these differences are related to distinct ecological affinities of the target insect, their mobility, spatial occupation of the habitat, population regulation, sterile male quality, etc.

Even though the critical ratio of sterile to wild males for a target insect can be influenced by factors such as the reproductive potential of the females, climatic conditions, biological quality of the insects before leaving the rearing facility, funds available to disperse sterile insects, and time available to achieve suppression or eradication, it is the true density and distribution of the wild insect population that is the most determining factor. The number of wild insects per unit of habitat surface determines the release rate of sterile insects that is required to achieve the desired sterile to wild ratio in the target area. In turn, the production capacity of the rearing facility must be adequate for the chosen release rate. Underestimating the actual population density can result in a shortage of available sterile insects in the production facility, whereas overestimating it will lead to overproduction and unnecessarily increased costs (Bloem and Bloem 2000). The density of insect populations is usually strongly correlated with habitat and vegetation cover, e.g. melon fly density in Okinawa, Japan, varied from less than 10 flies per hectare in the mountains to greater than 600 flies per hectare in crop fields and bushy areas (Yamagishi et al. 1993).

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Therefore, the density in all vegetation types in the target area should be estimated so as to adjust accordingly the number of sterile males released. It is important that the critical sterile to wild ratio be obtained in all trap sites, indicating that sterile insects have been appropriately placed in the target area (Krafsur et al. 1980). Several simple mathematical models are available to assess absolute insect population densities, e.g. Lincoln Index (Southwood 1978), and Jackson's positive and negative method (Jackson 1939), but these methods have rarely been routinely used in operational programmes integrating the SIT (Itô et al., this volume).

The patchy distribution of most insect species (Itô et al., this volume; Lance and McInnis, this volume), and the strong correlation between insect density and vegetation type, has implications for interpreting "overflooding ratios" when releasing sterile insects, i.e. one should take into account not only the overall sterile to wild male ratio obtained in all traps in a given area over a period of time, but also the ratios achieved in each type of habitat and vegetation cover must be adequate. Therefore, a suitable network of traps, strategically deployed in all habitats, is needed (Vreysen et al. 2000).

### *2.1.1. Marking Insects*

To calculate sterile to wild ratios, sterile insects are usually marked before release (FAO/OIEA 2018; Dowell et al., this volume; Parker, Mamai et al., this volume). Marking is traditionally done using physical markers (e.g. fluorescent dye on the entire body, acrylic paint on the thorax (for small pilot trials) or Calco Red in the diet of some Lepidoptera), but some of these markers have issues of cost, human health, sustainability or negative effects on the insects' competitiveness (Hagler and Jackson 2001; Parker, Mamai et al., this volume). More stable morphological markers have become available, such as the VIENNA 8-*Sergeant* ($Sr^2$) strain of the Mediterranean fruit fly, that in addition to the *temperature sensitive lethal* (*tsl*) and *white pupae* (*wp*) mutations, carries the dominant *Sergeant 2* ($Sr^2$) mutation that is expressed in males as three abdominal stripes, whereas wild-type males have only two stripes (Nyazi et al. 2005). The transgenic strain VIENNA 8–1260 presents another stable marker for the Mediterranean fruit fly. The strain expresses the Ccb2t promotor driver tGFP in the testes as green fluorescence and DsRed in the body as red fluorescence (Scolari et al. 2008) (Fig. 1).

Irrespective of which marker is being used, it needs to be ascertained whether there are negative effects on production in the mass-rearing facility or on their sexual competitiveness, survival, dispersal, etc. In that respect, Rempoulakis et al. (2016) assessed production parameters and competitiveness of the above-mentioned Mediterranean fruit fly strains in walk-in field cages. VIENNA 8–1260 females produced fewer eggs than the other two strains, but the egg hatch of all strains was similar. Differences in male mating competitiveness of the three strains against wild-type males were gradually reduced with successive generations under semi mass-rearing conditions. However, VIENNA 8 males adapted faster to laboratory conditions as compared with VIENNA 8–$Sr^2$ and VIENNA 8–1260 males with respect to mating competitiveness. VIENNA 8 males of the $F_{10}$ generation were equally competitive with wild-type males, whereas the mating competitiveness of VIENNA 8-$Sr^2$ and

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VIENNA 8–1260 males was similar but lower as compared with wild-type males. Males from all three strains copulated earlier than wild-type males.



*Figure 1. Mediterranean fruit fly strain VIENNA 8-1260 expressing green fluorescence in the testes and red fluorescence in the body. (Photo from G. Franz, reproduced with permission.)*

Recently, the biomarker dye rhodamine B has shown promise as a marker for Lepidoptera and mosquitoes. Feeding male tobacco budworm *Heliothis virescens* (F.) on 0.1% rhodamine dissolved in 10% sucrose solution did not only stain the body of the males but could also be detected in the spermatophores of female moths that had mated with the males. The intake of the dye had no effect on life span and mating performance of the males and production of eggs (Blanco et al. 2006). Sugar-feeding with rhodamine was used to stain the seminal fluid and bodies of male *Aedes aegypti* (L.) with no significant impact on their survival or mating competitiveness (Johnson et al. 2017).

In programmes releasing sterile New World screwworms, the flies are not marked, and estimations of the ratio of sterile to wild insects are based on catches of female insects. Females are dissected, and the atrophied ovaries of sterile females distinguish them from wild females. However, caution is required in interpreting these data. Sterile to wild ratios are derived from trapped insects, and if the distribution and response to traps is different for sterile and wild male insects, the ratios are prone to error (Meats 1996). One must also be cautious in interpreting female screwworm ratios; they might not reflect actual male screwworm ratios in the field due to sex-related differences in longevity, response to the trapping device, and dispersal characteristics.

Insects may be trapped in areas where their presence is not expected or anticipated, or where it is paramount to know their origin, e.g. in pest free areas, in recently cleared areas, in traps that are deployed in the vicinity of mass-rearing facilities, or an unmarked insect trapped at the end of an eradication campaign that potentially has lost

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its physical marker. In all these cases there is a need to identify the origin of the insect, or whether the insect is a sterile released or a wild relic fly. For some insect species, such as the Mediterranean fruit fly, molecular markers have become available that allow an assessment of the origin of the trapped flies (Meixner et al. 2002; Silva et al. 2003). The use of release strains that differ in their mtDNA background from that of the target population facilitates discriminating between unmarked flies and new incursions into a free area (San Andres et al. 2007; Franz et al., this volume).

Similarly, a molecular technique has been developed, based on the determination of cytochrome oxidase haplotypes to discriminate between wild and sterile male tsetse flies *Glossina palpalis gambiensis* with a high level of accuracy. The DNA was isolated from the heads of flies and a portion of the 5' end of the mitochondrial gene cytochrome oxidase I was amplified for sequencing. All sterile male flies from a Burkina Faso strain had the same haplotype but differed from that of wild male flies trapped in Senegal and in Burkina Faso. The method allows 100% discrimination between sterile and wild male *G. p. gambiensis* and can be used in release programmes in case there is doubt regarding the origin of a trapped fly (Pagabeleguem et al. 2016).

Stable isotopes have been used successfully to determine the natal origin of insects, their feeding strategies and mating behaviour (IAEA 2009; Vreysen et al. 2016). Enriching the water in which larvae of the mosquito *Culex pipiens* L. (Diptera: Culicidae) developed with $^{15}$N-labeled potassium nitrate and $^{13}$C-labelled glucose allowed the differentiation of marked from unmarked adult mosquitoes throughout their entire lifespans, e.g. 55 days. The labelling had no effect on immature mosquito survival or adult body size, and the technique was also applied to mark naturally breeding mosquitoes in standing water bodies in the field (Hamer et al. 2012). Hood-Nowotny et al. (2016a) labelled seven species of lepidopterans: cactus moth *Cactoblastis cactorum* (Berg), *Eldana saccharina* Walker, *Spodoptera litura* (F.), *Epiphyas postvittana* (Walker), diamondback moth *Plutella xylostella* (L.), *Lobesia botrana* (Denis and Schiffermüller), and pink bollworm *Pectinophora gossypiella* (Saunders), which made them distinguishable from wild conspecific moths based primarily on the difference in the ratio of the stable isotopes $^{13}$C:$^{12}$C. Depending on whether the wild host plants had a C3 or C4 metabolism, the diets were prepared with sugar derived either from sugarcane or sugar beet, so that the moths reared on the meridic diet had a ratio different from wild moths. The technique also worked with the tsetse fly *Glossina pallidipes* (Austen); laboratory-reared flies remained distinguishable from wild flies for more than 80 days (Hood-Nowotny et al. 2011). Mass-reared Mediterranean fruit flies fed on a larval diet containing C4 sugar could be distinguished with >95% confidence from wild flies that had developed mostly within the fruits of C3 plants. The C4 marker was detectable up to 12 days after release (Hood-Nowotny et al. 2009).

Elemental analysis-isotope ratio mass spectrometry (EA-IRMS) is the standard tool to measure isotope signatures, but this technology is expensive. A simpler technology called combustion module-cavity ring-down spectroscopy (CM-CRDS) is now available on the market. The technology is cheaper and more convenient than EA-IRMS, and data obtained were in good agreement with those obtained using EA-IRMS in terms of accuracy and precision (Hood-Nowotny et al. 2016b).

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## *2.2. Field Monitoring of Parameters Related to Sexual Competitiveness*

### *2.2.1. Apparent Density and Survival*

In routine monitoring, the most immediately available parameter is the proportion of sterile insects recaptured within a certain time frame, i.e. the recapture rate, and a sudden decrease in this rate could reflect a change in the quality of the released insect or in distribution methods (Hutt 1979; Yamagishi et al. 1993).

To increase their chances of encountering a receptive virgin female, sterile males need to survive and have a sexually active life in the field for as long as possible (Curtis and Langley 1982). It is important to estimate this parameter and investigate methods to increase the survival (longevity) of released sterile males (Lance and McInnis, this volume; Parker, Vreysen et al., this volume). After leaving a rearing facility, released sterile insects must find a food source or a host to replenish their limited energy reserves. In the absence of a host, their life expectancy is determined by the available initial energy reserves and the post-factory treatments before release (Pereira et al., this volume). Therefore, it is a critical parameter, and can be assessed in the laboratory (FAO 1992) or in field cages under natural conditions (Vreysen 1995).

An exponential decline in the recapture rate of marked released insects gives an indication of the daily survival (or mortality) rate in the area (Fig. 2). The survival rates will determine the frequency of sterile insect releases, ensuring appropriate sterile male densities over the entire target area at all times (Dowell et al., this volume). Sterile insect survival is influenced by factors such as host availability, climatic conditions, predator avoidance capacity, and vegetation cover, and therefore the survival of released insects should be assessed in all seasons and in all representative areas of the target zone.

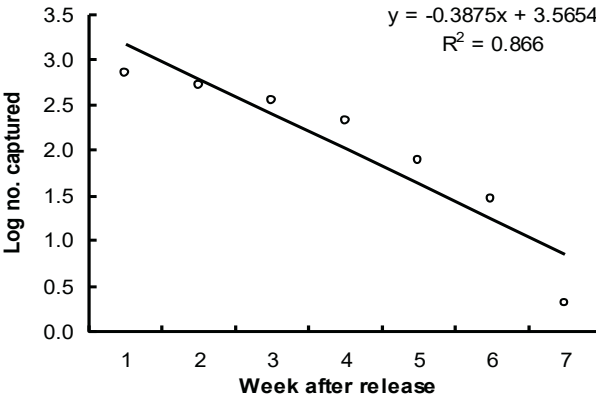



*Figure 2. Survival of sterile male* Glossina austeni *aerially released in Unguja Island, Zanzibar, Tanzania.*

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*2.2.2. Mobility, Dispersal and Distance between Isolated Populations*

Released sterile insects need to be sufficiently mobile, and have adequate dispersal capabilities, to reach (in a timely fashion) all the ecological niches occupied by the wild insect population (Itô et al., this volume; Lance and McInnis, this volume). Mobility and the potential of released insects to disperse are often underestimated. The recorded maximum dispersal distance for released New World screwworms is 290 km, 107 km for melon flies, 48 km for oriental fruit flies *Bactrocera dorsalis* (Hendel), and 18.5 km for the tsetse fly *G. p. gambiensis* (Hightower et al. 1965; Proverbs 1974; Van der Vloedt et al. 1980). Lance and McInnis (this volume) discuss the implications of this high mobility for programmes using the SIT.

Knowledge about the mobility and dispersal characteristics of released insects is essential for developing and designing appropriate release strategies (Itô et al., this volume). Admittedly, this is complicated; there are many variables that influence the dispersal of insects, e.g. wind direction (Peterson et al. 1980), wind velocity (Parman 1945), vegetation density (Krafsur and Hightower 1979), relative humidity (Brenner 1984), host availability (Spradbery 1994), radiation dose (Cuisance and Itard 1973; Wong et al. 1982), and the release of only males or both sexes (Proverbs et al. 1973; Hendrichs et al. 1995). A release strategy should aim at deploying sterile insects in close proximity to wild virgin females. Release lanes for aerial distribution, or release points for ground release, should be separated by a distance not exceeding the average maximum dispersal distance of the released insect (Dowell et al., this volume). Evidence from New World screwworm programmes suggests that the efficiency of released males, as measured by their mating frequency, is strongly influenced by operational considerations of sterile fly distribution. Distance between parallel flight lanes, and fly density in release containers, are especially critical variables (Krafsur et al. 1980). The sterility of wild populations increased significantly if flies were distributed in small boxes of 400 flies in release swaths of 2 km instead of in larger boxes of 2000 flies in swaths of 8 km (Krafsur and Garcia 1978). Obviously a denser network of aerial release lanes will require more aircraft time and is more expensive, but the programme will be more efficient, and releases of sterile insects would possibly be needed for a shorter time period.

The mobility and dispersal capacity of released sterile insects should be monitored frequently and compared with those of wild insects. This will require the capture, marking and release-recapture of large numbers of wild insects, and unfortunately these are not always available. Although in the past several complex mathematical models have been developed that describe the movement and mobility of insect species (Williams et al. 1992), a simple but useful index is the "mean distance of dispersal" from a release site to a trap (Itô and Koyama 1982; FAO/IAEA/USDA 2019):

$$x = \frac{\sum_{j=1}^{n} x_j N_j}{\sum_{j=1}^{n} N_j} \qquad (1)$$

where $n$ = number of traps, $x_j$ is the distance between a release point and the $j$-th trap, and $N_j$ is the number of flies recaptured with the $j$-th trap.

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The mean distance of dispersal can easily be measured by deploying traps along a regular grid of at least 6 x 6 traps, the dimensions of the grid depending on the insect species. Grids are usually easier to set up than a series of concentric circles, but circles are better for measuring dispersal. Caution is required in selecting the sampling device, and especially in using traps in combination with powerful attractants (e.g. pheromone traps for Lepidoptera), that could mask the natural dispersal characteristics of the insect. For lepidopteran species, passive, non-attractant interception traps are an alternative to male pheromone traps; they have been used to determine natural flight paths and flight patterns of insects in search of mates or hosts. The disadvantage of this technique is that catches are usually very small, making statistical analysis difficult or impossible (Knight 2000).

Caution is likewise required in generalizing results from mark-release-recapture studies (Itô et al., this volume), as these are, in the case of mosquitoes, influenced by the ecological characteristics of the study sites, and these studies should always be made in the area where the mosquito project is being conducted (Petrić et al. 2014; FAO/IAEA 2020b).

Dispersal is an important factor to consider when making strategic decisions on the selection of target areas for AW-IPM approaches, be it suppression or eradication (Hendrichs, Vreysen et al., this volume). For example, in the past, estimates of the dispersal capacity of the tsetse fly *G. p. gambiensis* at the microscale were obtained using direct methods (mark–release–recapture) as well as indirect ones (genetic isolation by distance). In this species, genetic isolation was strongly correlated with distance; however, populations separated by 15 km of rice plantations were more isolated than those separated by 100 km of gallery forest, indicating that for this species the friction of riparian vegetation to dispersal was significantly lower than that of rice plantations (Bouyer et al. 2009; De Meeûs et al. 2012). As no useful friction maps were available for tsetse flies at any scale, a friction map was developed to identify natural barriers that isolate populations of *G. p. gambiensis* in West Africa. A statistical model was used to assess the genetic distance between 37 tsetse populations sampled in West Africa, using a set of remotely sensed environmental data as predictors. The least-cost path between these populations was then estimated using the predicted friction map. The method avoids the subjectivity inherent in expert-based weighting of environmental parameters. As a result, potentially isolated clusters of *G. p. gambiensis* habitat were identified based on a species distribution model and ranked according to their predicted genetic distance to the main tsetse population (Bouyer et al. 2015).

Dispersal of female mosquitoes to seek a host is epidemiologically important because it influences the capacity of the females to acquire and disseminate pathogens. Dispersal to find suitable oviposition sites is also important because it increases the spreading of potentially infected progeny (Petrić et al. 2014). The dispersal of mosquitoes is influenced by the density and distribution of blood sources, availability of oviposition sites, weather, terrain features, vegetation, housing characteristics in urban and rural settings, etc. There appears to be a correlation between mosquito dispersal into new areas and proximity to major transportation routes, commercial movement of tyres, ornamental-plant trade, and individual, public, and commercial transportation out of infested areas (Petrić et al. 2014).

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*2.2.3. Spatial Distribution within Habitat*

Released sterile insects must also disperse into the ecological niches occupied by wild insects, and detailed data on temporal changes in spatial distribution of sterile and wild insects are needed (Lance and McInnis, this volume). This is very challenging; most insects are not uniformly or randomly distributed, but have patchy distributions in both space and time. Spatial heterogeneity is related mostly to host availability and vegetation, making it difficult but not impossible to determine the optimal release rate (number of flies released per $km^2$) (Krafsur et al. 1979). An adequate trapping network, covering all types of vegetation, is needed to provide frequent (weekly) detailed information on the density and spatial distribution of wild and released insects. Geographic information systems (GIS) and remote sensing (RS) tools can greatly facilitate the selection of these trapping sites, ensuring adequate coverage but also avoiding the deployment of too many sampling devices (Bouyer et al., this volume). Data on temporal and spatial changes in occupation of the habitat, by both sterile and wild insects, can be used to regularly adapt the scheme for distributing sterile insects.

In the tsetse project in Senegal, selection of trap sites was initially guided using remote sensing techniques and vegetation classifications obtained from Landsat 7 Enhanced Thematic Mapper Plus (ETM+) images that enabled the identification of suitable habitats to harbour *G. p. gambiensis* with high sensitivity, but low specificity (Bouyer et al., this volume). These areas were denominated "wet areas" and the strategic deployment of Vavoua traps (Laveissière and Grébaut 1990) in these habitats allowed the delimitation of the tsetse-infested area. In the area of zero catches adjacent to the infested area, a mathematical model was used to assess the risk that flies were present despite a sequence of zero catches (Bouyer et al. 2010; Barclay et al., this volume). The analysis showed a risk of >0.05 in 19% of the area which was considered likewise as infested and included in the control operations.

Species distribution models have been used more and more to optimize insect pest control activities in general and to obtain more accurate information on the spatial distribution of the insect pest in particular (Barclay, this volume). Whereas previous distribution models were critical for a better understanding of the distribution of the pest insects, they suffered from inadequate spatial resolution. High-resolution satellite images and recent advances in species distribution modelling methods can be used to improve the accuracy of prediction. For example, in the tsetse eradication campaign in Senegal, the use of a Maxent Model enabled the relocation of 22 of the 97 monitoring traps to more suitable sites according to habitat suitability (Dicko et al. 2014).

On a finer spatial scale, the dispersion of insects such as tsetse flies, e.g. *G. palpalis palpalis*, is influenced by sex, age (young virgin females occupy different parts of the habitat than older flies), and the state of gravidity in females (Gouteux 1987). These microspatial differences in an apparently similar habitat should be monitored carefully in important target areas, such as "hot spots" (areas with unusually high insect densities).

An example is the spatial occupation of wild and sterile *G. austeni* in an apparently uniform primary forest ecosystem (Fig. 3). A total of 422 wild female, 679 wild male and 3318 sterile male fly catches from 12 trapping sites over a period of 10 months was used to study the spatial occupation of the flies in a primary forest. The aerial release of the sterile male flies assured their random distribution over the very homogeneous habitat. Despite these uniform aerial release rates, the sterile males redistributed

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themselves and occupied microhabitat niches similar to those occupied by wild insects. This ability of sterile males to aggregate (and therefore locate to) those areas preferred by wild males is of primary importance to ensure adequate sterile-to-wild male overflooding ratios everywhere (Vreysen et al. 2011).

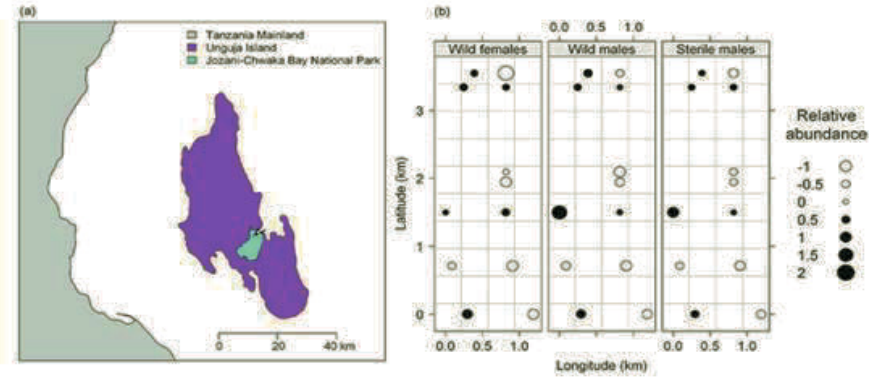



*Figure 3. (a) Unguja Island and the Jozani forest (now called the Jozani-Chwaka Bay National Park), (b) The spatial and standardized abundance of wild and sterile* Glossina austeni *as sampled with 12 sticky panel traps in the Jozani forest. (Figure from Vreysen et al. 2011.)*

## 3. MONITORING PROGRAMME PROGRESS

### *3.1. Monitoring and Insect Biology*

#### *3.1.1. Monitoring and Insect Behaviour*

The feasibility of developing and deploying efficient insect monitoring tools will be determined and influenced by the behaviour and the biology of the insect. Species with adults that respond to a trapping device may be sampled directly in sufficient numbers to accurately assess fluctuations in population density and structure. The biological mechanisms involved in attracting insects from a distance, and luring them into a trapping device, are usually related to their host-, food-, or mate-seeking behaviour, which is often regulated by volatile components (semiochemicals) emitted by the host/food/mate, and at close range influenced by visual characteristics (Colvin and Gibson 1993; Tan 1993; Green 1994; Hall and Wall 1995; Lühken et al. 2014). Sometimes direct trapping of insects is difficult (e.g. in inaccessible areas), inefficient (e.g. a good attractant and trap are not available), or uneconomic, and therefore alternative indirect methods of monitoring have been developed, usually involving assessments of damage inflicted by the pest on its hosts (Iwahashi 1977; FAO 1992; Bloem and Bloem 2000; Dyck et al. 2000).

Surveillance programmes for mosquitoes, such as the Asian tiger mosquito *Aedes albopictus* (Skuse), rely on the use of ovitraps, rather than adult trapping systems.

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Ovitraps are simple devices that consist of plastic cups that have a shiny black surface, are filled 2/3 with water, and contain a Masonite strip on the inner wall that is suitable for oviposition (Carrieri et al. 2009). The maximum time interval allowed for checking the ovitraps is 1 week; longer periods would turn the traps into mosquito reproductive breeding sites (Ritchie 1984). The egg-density data obtained can provide a good estimate of the biting-female density (Carrieri et al. 2011). Monitoring with ovitraps has the advantage of being low cost, but the catch data might be influenced by factors such as siting, micro-environment, presence or absence of other breeding sites, and local adulticide treatments (Carrieri et al. 2017).

### *3.1.2. Direct Sampling of Adult Insects*

In AW-IPM programmes, it is important to be able to monitor both high and low population densities. The densities of pests such as screwworms tend to be low but highly aggregated (Krafsur et al. 1979; Spradbery 1994), and the most efficient and appropriate sampling device should be used (Katsoyannos 1994) for the relevant geographical area (Baylis and Nambiro 1993). However, most trapping systems are biased, and samples are rarely representative of the insect population (Vale and Phelps 1978; Vreysen and Saleh 2001). Therefore, it is always a sound practice to estimate population densities using more than one method (Bloem et al. 2005; Petrić et al. 2014; Lees et al., this volume). To interpret trapping data correctly, it is imperative to understand these "trap biases", and the factors that affect the size and structure of trap samples over time and space. Some of the most significant factors that affect insect population samples are: (1) activity of the insects, which depends on the insect's physiological state and on climate (Rogers 1978; Turner 1987; Williams et al. 1990b), (2) efficiency of the trap, which is influenced by the elements of attractant, trap construction, habitat, and climate (Hargrove and Vale 1980; Dransfield et al. 1982; van de Straat et al. 2019; FAO/IAEA 2020a), and (3) intrinsic trap biases (Williams et al. 1990a).

In addition, when selecting a trapping device for a monitoring programme, the following aspects have to be taken into account: performance of the sampling device in relation to economics (number of traps needed is inversely correlated with trap efficiency), servicing, time required to deploy a trap, unit cost and its components, durability, "user friendliness" (i.e. time required to remove trapped insects), efficiency of the bait (very good baits may overestimate the local population density), and species specificity (Flores et al. 2017; FAO/IAEA 2018). Trapping large numbers of non-target organisms is inefficient; e.g. biting flies (Stomoxyinae) caught in tsetse fly traps (more than 1000 individuals per trap per day) (Saleh et al. 1999), species of *Chrysomya*, morphologically similar to New World screwworms, caught in traps at a ratio of 2600:1 (Spradbery 1994), and a wide range of tephritids and non-tephritids in fruit fly traps baited with food lures (Katsoyannos 1994; Miranda et al. 2001).

Smart traps are now available on the market and are becoming more common for monitoring purposes in action programmes. These sampling devices, which automatically record the target insects sampled and provide feedback using appropriate software systems, have several advantages, e.g. traps need to be checked only when relevant catches have been recorded, preventing unnecessary visits to empty traps and making the entire monitoring process much more cost-effective. In

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addition, new systems are being developed that allow the automatic identification of the insects, reducing or even eliminating the need for trap visits (Vreysen et al. 2016). One of these trapping systems consists of a multi-funnel trap equipped with a camera connected to an internal modem for General Packet Radio Service. Images taken are stored on a memory card and are adequate for identifying larger insects such as longhorn beetles (Chinellato et al. 2013).

The potential use of these systems in sterile insect release programmes would also lead to more cost-effective and efficient field surveillance, as real-time feedback on released insects could lead to more efficient decision-making (Simmons et al. 2010). For example, in Australia, a system called RapidAIM (2018) was developed that combined the knowledge of fruit fly behaviour with proprietary hardware and software used in a grid of instrumented, low-powered smart traps. The traps detect the presence of a fruit fly, send the data to the cloud for analytics, and generate an alert. This system visualizes the location and occurrence of fruit fly presence and/or outbreaks which can result in a rapid and efficient response.

### *3.1.3. Indirect Sampling*

Monitoring a population through direct sampling of adult insects can, in many instances, be supplemented by indirect sampling procedures, both to obtain additional information on the progress of the programme and to verify the data obtained by direct sampling procedures. In addition to direct sampling, indirect sampling is used routinely in programmes against veterinary pests (screwworm and tsetse), crop pests (Lepidoptera and fruit flies), and human disease vectors (e.g. mosquitoes).

*Monitoring Host Organisms.* In many holometabolous insects, immature stages represent a large percentage of the population (up to 97% for the Mediterranean fruit fly (Carey 1982; Liedo and Carey 1996)), and in view of this demography these stages should also be sampled (FAO/IAEA 2019).

Since it is easy to detect maggots in animal wounds, indirect sampling through the surveillance of myiasis cases in livestock, game animals, and humans has become the standard method of monitoring progress in New World screwworm eradication programmes. Depending on resources, surveillance can be done either passively (livestock owners check their animals and report positive myiasis cases (Robinson et al. 2000)) or actively (programme staff physically inspect at regular intervals all host animals in the target zone (FAO 1992; Vreysen et al. 2007)). Even though passive surveillance is obviously less expensive, the absence of standardization and a "reference-sampling unit" make temporal comparison of such field data very difficult or even impossible. Also, passive surveillance is influenced by: (1) the accessibility that farmers have to their livestock, (2) the willingness of farmers to inspect their animals on a regular basis (and remove screwworm larvae and send them to the responsible authority in the country), (3) the vastness of the grazing area, and (4) the efficiency of veterinary services. Therefore, a decrease in "reported cases" does not necessarily reflect a lower population density of screwworms (i.e. progress in the programme), but is probably correlated more with the reporting efficiency and level of farmer cooperation (Vreysen et al. 2007). The highly successful New World

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screwworm eradication programme in Libya (1990–1992) provides a good example of an efficiently executed active surveillance programme; 94 field teams inspected 16.2 and 30.5 million animals in 1990 and 1991, respectively, in an area of 40 000 km$^2$ (FAO 1992). Accurate reporting of the number of animals inspected, wounds detected, and wounds infested, provided excellent feedback to programme managers to evaluate the progress and guide eradication activities (Lindquist et al. 1992).

*Monitoring Disease Transmission.* In the case of vectors of diseases such as tsetse flies (Glossinidae) that transmit *Trypanosoma* spp., and mosquitoes that transmit malaria, dengue, chikungunya, Zika, etc., insect-trap data can be supplemented with data on the transmission, prevalence, and incidence of the disease in livestock and humans (Barclay et al., this volume). These data become especially valuable if and when the density of the insect population drops below the detectable limit of the trapping device used. In the case of tsetse, the careful screening of sentinel animals, i.e. not infected with trypanosomes, introduced into the target area can significantly increase confidence that the tsetse fly has been eradicated (Dyck et al. 2000). However, interpreting these veterinary surveillance data is complex, and preferably should always be correlated with entomological monitoring data because:

- The density of a tsetse population and the incidence of the disease are not necessarily positively correlated, e.g. an insecticide spraying campaign in Kenya reduced the tsetse population by 98%, whereas 6 months after completion of the campaign the disease prevalence was reduced only from 5 to 2% (Otieno et al. 1990).
- The time required for a fly to develop a mature trypanosome infection is between 5 and 25 days, depending on the species and the temperature (Molyneux et al. 1982). Consequently, the potential for a tsetse population to transmit the disease is increased proportionally to its average age (Harley 1965), and the removal of the younger section of the fly population as the result of control actions does not significantly reduce its transmission capability.
- A parasitological survey will not show transmission if a tsetse population is thriving on livestock free of trypanosomes.

A series of indices has been used traditionally to monitor indirectly densities of *Aedes* spp. and the efficiency of control programmes. These include the:

- house index (HI), i.e. percentage of houses with a least one active breeding site,
- container index (CI), i.e. percentage of containers with larvae,
- Breteau index (BI), i.e. number of active breeding sites per 100 premises, and
- ovitrap index (OI), i.e. average proportion of ovitraps with mosquitoes.

Although widely used, these indices have some disadvantages when used in epidemiological studies, e.g. the CI considers only the percentage of positive containers, and not absolute numbers, and the HI is limited as it does not take into account the number of positive containers. The BI is the only index that combines positive containers with the density per premise (Petrić et al. 2014).

Other indices that have proven to be very useful are the number of pupae per premise (PPI), and the number of pupae per hectare (PPH), because they exploit the

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strong correlation between the number of adults in a defined area and the number of pupae (in view of the low pupal mortality) (Carrieri et al. 2011).

*Monitoring Crop Damage.* The damage that insects inflict to crops can be assessed, providing indirect information on the density and distribution of the pest insect population. Crop damage can be measured at regular intervals, e.g. 80–100 cotton bolls per field were collected each week in the pink bollworm programme in California, USA (Staten et al. 1993). Also crop damage can be assessed at harvest, e.g. in the codling moth programme in Canada, a random examination of fruit is made in about one-third of the treated orchards (n = 600) at harvest (Bloem and Bloem 2000). Caution is required in interpreting the data, because damage to a specific crop is not always caused by the target insect, e.g. all insect damage in fruit orchards in Washington State, USA, was attributed to the codling moth, but careful study revealed that fruit injury from codling moth larvae was only 0.3% while that from leaf rollers averaged 1.1% (Calkins et al. 2000). In some instances, the results of direct monitoring (insect trapping) do not correlate with data from indirect sampling (crop damage), e.g. there was no spatial correlation between damage, i.e. defoliation, from gypsy moths *Lymantria dispar* (L.) and the counts of adult male moths in traps (Liebhold et al. 1995).

### *3.2. Monitoring Impact of Sterile Insect Releases on a Wild Population*

Three important indicators provide essential information on the impact of released insects on the target population: (1) proportion of the female target population that mated with sterile males (level of induced sterility), (2) changes in the age structure of the target population caused by variations in the recruitment rate of young insects, and (3) decline in the density of the target population. The characteristics of the reproductive biology of the species, and the trap-orientation behaviour of females, will determine which of these parameters can be used. For example, all three parameters can be monitored in tsetse fly programmes integrating the SIT, but in Lepidoptera, pheromone traps attract only male moths, and thus the information obtained is restricted to the apparent densities of wild and sterile males.

Analysing and interpreting temporal and spatial monitoring data can be accurate only if standardized sampling procedures are used. Especially in the case of direct sampling, uniformity of procedures is required for the entire duration of the monitoring programme, particularly with respect to: (1) trapping device (type, colour, material used for trap construction, shape, etc.), (2) sampling sites (number and location), (3) trap mounting and deployment, (4) lures or odour attractants (chemical composition, pH, volatile release rates, etc.), and (5) sampling interval. In addition, baseline data (collected before control actions are initiated) on spatial and temporal fluctuations in the density and structure of the target population (Itô et al., this volume), collected over a period of at least 1 year, are essential for making correct interpretations of the field data collected during the suppression and sterile insect release phases (Vreysen and Khamis 1999; Vreysen et al. 1999a; De Longo et al. 2000) (Box 1). An alternative is to collect comparable data from an untreated area

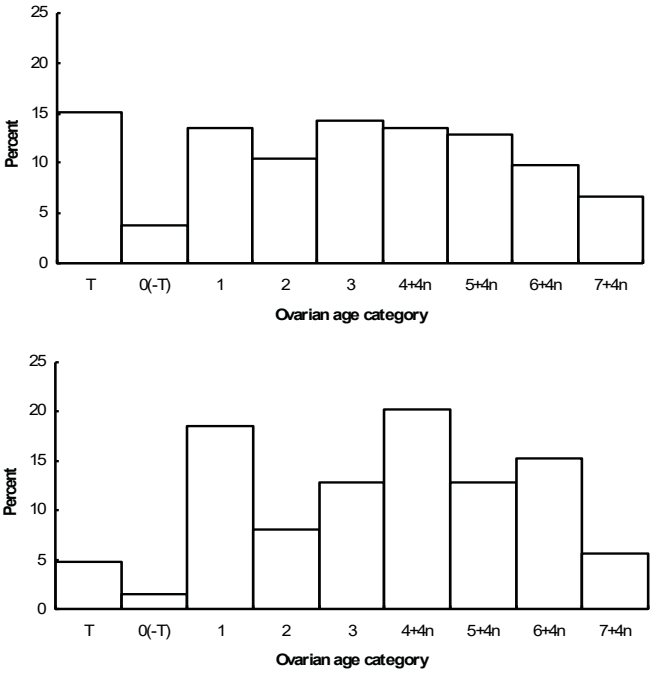
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during the phase when control actions are applied, but it is often difficult to select ecologically comparable areas (Waterhouse et al. 1976).

*Box 1. Pre-Control Entomological Baseline Data Collection: Prerequisite for Correct Interpretation of Trap Catch Data during Control Activities*

The application of a control measure against an insect population will change the size of that population (section 3.2.4.) and its age structure (section 3.2.3.). Even when not subjected to a control measure, insect populations (in terms of both size and structure) are not stable in time and space. Consequently pre-control data on the structure of the target population must be collected to interpret correctly sampling data obtained during the control activities.

Physiological age grading is feasible for tsetse flies due to the uniqueness of the female reproductive system (section 3.2.1.). The example below demonstrates the spatial differences in the age structure of the tsetse fly *Glossina pallidipes* Austen sampled in riparian forest vegetation along the Kulfo River and in the Chamo bush thickets of the Nechisar National Park in Ethiopia. The flies were sampled in both habitats during the same period of the same day. The data show that the female fly population along the Kulfo River was significantly younger (18.8% young females, tenerals and nulliparous) than the female fly population in the Chamo thickets (6.4% young females) ($\chi^2 = 10.80$, df = 4, $P < 0.03$ — females in the older categories 4 and >4 were pooled in this analysis). A change in fly population structure due to applied control measures must be analysed in relation to these natural spatial (and also temporal) differences.




*Population structure of* G. pallidipes *sampled in (upper graph) riparian forest along the Kulfo River (n = 133) and (lower graph) bush thickets (n = 124) of the Nechisar Park in Ethiopia (T = teneral, 0 = nulliparous, 1–7 = number of ovulations; note that categories i = 4–7 contain flies that have ovulated i + 4n times; more details in Challier 1965).*

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Quantifying a reduction in the reproductive potential of a target population constitutes the most powerful and straightforward tool to assess progress in a programme using the SIT (Waterhouse et al. 1976; Vreysen 2001). An accurate knowledge of the level and spatial distribution of sterility induced in wild females will permit a more strategic use of the sterile insects, resulting in increased programme efficacy and reduced programme costs. Assessing the rate of induced sterility in a wild target population is only possible when the female portion of the population can be sampled, and when morphological indicators or "visual markers" are present in the female reproductive system to differentiate between matings with wild or sterile males. Alternatively, female insects can be trapped live and then maintained in controlled conditions for a certain time period to monitor the production of viable offspring (result of a fertile mating) or non-viable eggs (result of a sterile mating). In some insects, egg masses can be collected in the field and the level of sterility determined (section 3.2.2.).

The rate of induced sterility is not only the most essential, but also the most reliable, parameter to assess progress in programmes that release sterile insects. Although a reduction in the number of insects caught in traps can be an important indicator, the number trapped is strongly affected by numerous (often unknown) factors (section 3.1.2.), making the interpretation of trap catches complex (section 3.2.4.). A progressive increase in the sterility of the target population, combined with declining numbers of insects trapped, will provide unequivocal evidence that the eventual collapse of a target insect population is due solely to the loss of fertility, without interference of other factors (Vreysen et al. 2000).

### *3.2.1. Monitoring Reproductive Capacity of a Wild Population — Tsetse Flies*

Tsetse flies have a unique reproductive system, making them very suited to the assessment of sterility levels in a population subjected to sterile insect releases (Van der Vloedt et al. 1978; Vreysen et al. 1996). Tsetse flies reproduce by adenotrophic viviparity (Hagan 1951). The four polytrophic ovarioles in the reproductive system of females develop sequentially (Saunders 1960) (Fig. 4A), with only one oocyte maturing per pregnancy cycle lasting 9 or 10 days (Tobe and Langley 1978). Consequently, the maturation stage of the developing oocyte (the next to ovulate) in fertile females is always in sequence with a particular development stage *in utero*, i.e. embryogenesis or one of the three larval stages (Challier 1965). Mating a virgin female tsetse with a sterile male will result in fertilization of the egg *in utero* by the sperm, carrying dominant lethal mutations that will result in the death of the embryo (embryonic arrest) (LaChance et al. 1967), which is later aborted (Van der Vloedt et al. 1978). Consequently, aberrations between the size of the maturing follicle and the development stage *in utero* (dead embryo or uterus empty due to expulsion of the embryo) will become apparent (Vreysen et al. 1996) (Fig. 4B).

Dissection of a reasonably sized sample of wild female flies from a population subjected to sterile male releases will show the proportion of females in the sample that have aberrations in their reproductive system — a direct indication of the rate of induced sterility in the target population (Vreysen et al. 2000). The only weakness in the methodology is the 1- or 2-day time lag between fertilization of the egg with the irradiated sperm and embryonic arrest becoming visible with a microscope (Van der

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Vloedt and Barnor 1984). This could result in underestimating the level of induced sterility.

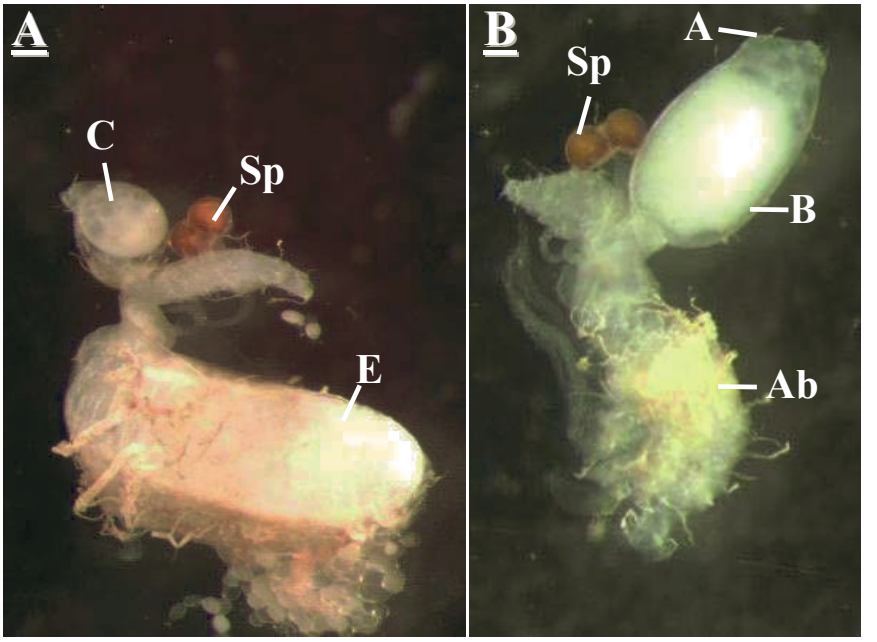



*Figure 4. A. Reproductive system of female tsetse fly mated with fertile male, showing one ovulation (C = follicle next in ovulation sequence (FNOS)) and viable egg (E)* in utero. *B. Reproductive system of female mated with sterile male, evidenced by imbalance between size of FNOS (B) and uterus content (Ab = abortion, Sp = spermathecae).*

Pre-control baseline data from female tsetse flies on Unguja Island, Zanzibar, showed that 50 and 46.1% of females had a viable egg or larva *in utero,* respectively, and only 3.5% of females had an empty uterus showing the loss of an egg or larva (natural abortion rate) (Vreysen and Khamis 1999) (Fig. 5). During the initial 8 months of the SIT activities (mid 1994–early 1995), an insufficient number of sterile males was released, and consequently the ratio of sterile to wild males remained below 10:1. In spite of this low ratio, during this period 19.9% of sampled females had mated with sterile males (Vreysen et al. 2000). In 1995, the number of sterile males released constantly increased, and more than 50 sterile males for each wild male were trapped after week 34. Simultaneously, the frequency distribution of the uterus content of the sampled females became progressively more distorted compared with the pre-control distribution, i.e. $\chi^2 = 70.3$ in early 1995 and $\chi^2 = 196.6$ in late 1995 (df = 4; $P < 0.0001$), due to a gradual increase in the proportion of females that had aborted dead embryos or displayed eggs *in utero* in embryonic arrest (Vreysen et al. 2000; Vreysen 2001).

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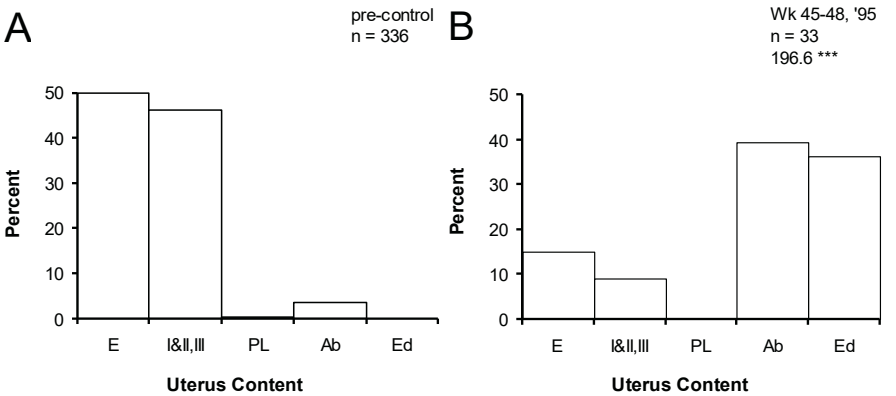



*Figure 5. Frequency distribution of uterus content of* Glossina austeni *females sampled during (A) pre-control phase and (B) late in sterile male release phase (E = egg, I, II, III = first-, second-, and third-instar larva, PL = post larviposition, Ab = abortion, Ed = degenerating egg). Number is chi-square value of comparison of frequency distribution of uterus content with that of pre-control sample (*** P < 0.001). (Figure adapted from Vreysen 2001, reproduced with permission.)*

A similar trend can be observed in Fig. 6, which shows data on the reproductive status of young parous females (1 or 2 ovulations) that had mated with a sterilized male 2 or 3 weeks previously. It is very evident that the sterility level in the young female population gradually increased as the sterile to wild male ratio increased, i.e. from 26% in the last quarter of 1994 to 32, 48, and 72% in the 2nd, 3rd, and 4th quarters of 1995, respectively. Concurrently, as sterility in the young female fly population increased, the proportion of young female flies with a viable larva *in utero* (females mated with a wild fertile male) decreased.

### *3.2.2. Monitoring Reproductive Capacity of a Wild Population – Screwworms, Lepidoptera, and Fruit Flies*

Assessing the rate of induced sterility in screwworms, Lepidoptera, and fruit flies is more challenging. No differentiation between sterile and fertile matings can be made by direct examination of the reproductive system of sampled wild females. Any quantification of sterility levels in wild females requires the collection and maintenance of eggs, and an assessment of the ratio of hatched (fertile) to non-viable (sterile) eggs (Thomas and Mangan 1989). For screwworm, fruit fly and lepidopteran species, special egg collecting methods have been developed (Davis et al. 1968; Snow et al. 1976; Parker and Welch 1991; Katsoyannos et al. 1999), but most are labour-intensive and expensive to implement in large operational programmes. The usefulness and applicability of these methods vary for each insect group (see below).

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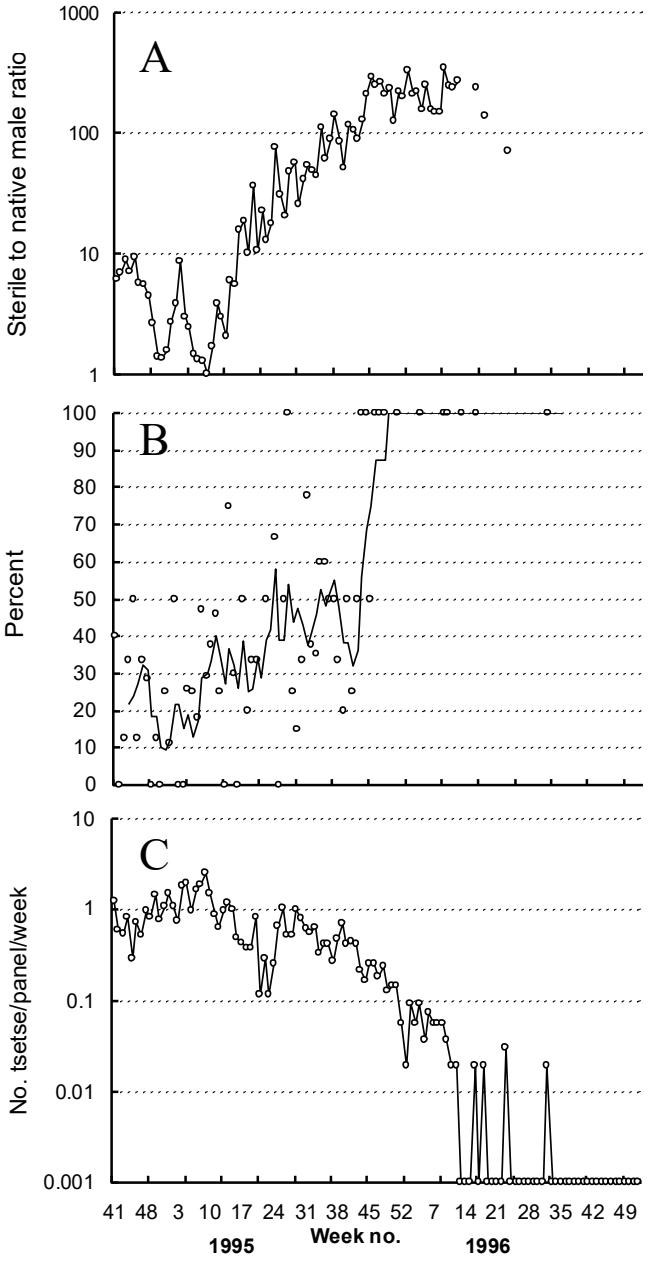



*Figure 6. A: Sterile to wild male ratio. B: Rate of induced sterility as proportion of young parous females (1 or 2 ovulations). C: Apparent density (+ 0.001) of wild males and females.* Glossina austeni, *Unguja Island, Zanzibar. (Figure adapted from Vreysen et al. 2000, reproduced with permission.)*

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*Induced Sterility and Egg Collection.* In female screwworm flies, with 100–150 ovarioles per ovary, egg development is synchronous, and an average of 200 eggs are deposited (Thomas 1993), regardless if eggs are fertilized by fertile or sterile sperm (LaChance and Bruns 1963). Egg masses can be collected in several ways: (1) from artificially inflicted wounds on sentinel animals, maintained in fixed capture stations (Davis et al. 1968; Parker and Welch 1991; FAO 1992), (2) from wounds found by field inspectors during routine surveillance activities (Robinson et al. 2000), and (3) from gravid females that were attracted to, and caught in, liver-bait stations and "egged" in glass tubes (Parker and Welch 1991). Maintaining sentinel pens is cumbersome, difficult to implement in some countries in view of animal right issues, and rather problematic in tropical remote environments because of the paucity of suitable animals, water, shade, and animal food (Krafsur and Highthower 1979; Parker and Welch 1991). (It would be convenient if some kind of artificial wound were available to collect eggs from wild screwworm females; such a methodology needs to be developed.)

Training and expertise are needed to detect and identify screwworm fly eggs. Also they are short-lived, and inappropriate handling often causes increased mortality, resulting in overestimates of the rate of induced sterility. In addition, one egg mass is not necessarily the output of one female, as was originally assumed (Krafsur et al. 1979; Brenner 1984), but possibly the product of more than one female (eggs laid beside eggs already deposited by another fly) (Thomas and Mangan 1989), making it difficult to interpret sterility data. Nevertheless, the most manageable option at present is the systematic collection of egg masses from animal wounds during routine surveillance along a predetermined grid network, and adequate geo-referencing of the sampling sites for incorporation into GIS (Bouyer et al., this volume), providing accurate information on the spatial and temporal distribution of induced sterility in the wild population.

The development of female lures has recently brought about significant progress in monitoring the Mediterranean fruit fly (IAEA 1999) (Box 2). However, as with screwworms and Lepidoptera, dissection of female fruit flies does not reveal any difference between fertile and sterile matings. Therefore, live-trapped female Mediterranean fruit flies can be transferred to a cage containing a natural or artificial oviposition substrate, egg masses collected, and egg hatchability assessed (IAEA 1999; Katsoyannos et al. 1999). Except for experimental trials, methods such as maintaining fruit fly eggs dissected from collected fruits to assess hatchability (Wong et al. 1986; McInnis et al. 1994), or measuring the size of the head of spermatozoids collected from spermathecae of female flies to differentiate between sterile and fertile sperm (McInnis 1993), have never been applied because they are cumbersome. Nevertheless, eggs from wild Mediterranean fruit flies were collected from coffee berries in Guatemala and induced sterility calculated (Rendón et al. 2004). Also, an artificial oviposition device, to collect eggs from female melon flies netted in the field, was developed and used successfully to assess egg hatchability during the programme on Kume Island, Japan (Iwahashi et al. 1976; Iwahashi 1977).

A significant development is the availability of a reaction-restriction fragment length polymorphism-based method (PCR-RFLP) to identify the origin of sperm in captured wild female Mediterranean fruit flies. The method identifies Y chromosomes

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in DNA extracts from the spermathecae, enabling the identification of the genetic origin of the sperm, i.e. wild, sterile or both, as an indicator of the degree of induced sterility in the target population (Juan-Blasco et al. 2013; Krafsur and Ouma, this volume). Another method is to use rhodamine dye to mark the sperm of male insects that can then be detected in the spermathecae or spermatophores of their female mates (Johnson et al. 2017). The potential of using stable isotopes for labelling sperm was studied under laboratory conditions for the mosquito *Anopheles arabiensis* Patton. Spermathecae filled with labelled sperm could be successfully distinguished from spermathecae with unlabelled sperm (Helinski et al. 2007). All these methods open new avenues to assess mating frequencies (and hence programme progress) in programmes that have an SIT component.

*Box 2. Monitoring Mediterranean Fruit Fly AW-IPM Programmes that Integrate the SIT*

Sampling the male portion of the population provides important feedback on sterile to wild male ratios, and on dispersal characteristics of released sterile males. Sampling females is equally vital; they constitute the "reproductive component" of the wild population, are the target of the SIT activities, and provide crucial information on the rate of sterility induced in the population (section 3.2.1.).

In earlier Mediterranean fruit fly programmes applying the SIT, both sexes were released, and population monitoring was done mainly with trimedlure-baited Jackson traps, which attract mostly male flies. This resulted in a reduction in efficiency of the programmes due to the trapping-out of a significant portion of the sterile males and the large effort to sort trapped sterile from wild flies. For example, in the Mediterranean fruit fly programme in the Los Angeles Basin, California, USA, about 0.5 million males were captured each week, resulting in a very costly and inexact process attempting to find any wild males among all the recaptured sterile males (J. Hendrichs, personal communication). The female portion of the wild population was sampled with traps containing generic liquid food lures, which unfortunately also catch many non-target species, making it time-consuming and laborious to sort out the female Mediterranean fruit flies.

The development of Mediterranean fruit fly genetic sexing strains (Franz et al., this volume) allowing male-only releases in operational programmes (Rendón et al. 2004), and the development of a lure for female Mediterranean fruit flies (IAEA 1999), have given a new dimension to monitoring programmes that have an SIT component. Female attractant-baited traps focus mainly on the detection of wild females and their offspring (no sterile females are released), thereby significantly reducing sterile male recapture and the laborious trapping and the sorting of hundreds of thousands of males (Hendrichs et al. 1995). In addition, these traps collect enough males to monitor the ratio of sterile to wild males and the distribution of sterile male releases (Midgarden et al. 2004). The monitoring component of Mediterranean fruit fly programmes can account for up to one-third the total cost during the fly-free stage of AW-IPM programmes (Enkerlin 2001; Hendrichs, Vreysen et al., this volume). The release of male-only strains, combined with the availability of female attractant traps, increased the efficiency of sterile males, and significantly reduced monitoring costs.

*Problems Associated with Trapping Female Lepidoptera.* Monitoring progress in lepidopteran programmes relies mainly on crop damage assessments and on traps using potent female sex pheromones as lures (Riedl et al. 1986; Bloem and Bloem 2000; Walters et al. 2000). The pheromone traps attract only male moths, and sterile moths can be distinguished from wild moths if appropriate marking techniques are used (Dyck et al. 1993; Parker, Mamai et al., this volume) (section 2.1.). Therefore, trap samples will indicate the apparent densities and ratio of wild and sterile moths, but the absence of females in the samples precludes any data on sterility levels induced in the wild population. In addition, the deployment of pheromone traps has to

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be well balanced to prevent the trapping of too many sterile male moths, which reduces the efficiency of the SIT component of the programme.

Several methods have been developed to assess sterility in moth populations using tethered (Alford and Silk 1983) or clipped-wing sentinel/decoy virgin females placed on mating tables (Snow et al. 1976; Shaver and Brown 1993), in virgin-female traps (Snow et al. 1969), or in mating houses (Mastro and Schwalbe 1980). These female moths will attract male moths, and it is assumed that the virgin females mate with sterile and wild male moths at the same periodicity and frequency as occurs in nature. Mated females are then transferred to controlled conditions, where deposited eggs are screened for sterility. The "tethering" or "clipped-wing" method is, however, hampered by the small size of many lepidopteran species, and by numerous escapes, although Teflon®-walled mating tables may prevent escapes (McBrien and Judd 1996). Also, if the design of mating houses interferes with the entrance response of males, such cages would not be suitable.

A pear-derived volatile (ethyl (2E,4Z)-2,4-decadienoate), which acts as a kairomone (chemical emitted by one species and attracting another) for codling moth males and virgin and mated females, has been available for the last two decades (Light et al. 2001). It enables the trapping of live female codling moths; this greatly facilitates the collection of egg masses for screening sterility but also permits the sampling of females in an economic and systematic way over large geographical areas in operational programmes that use the SIT. This discovery has promoted the search for kairomones that are efficient for other lepidopteran pest species, such as the diamond back moth.

In that respect, a recent study on the morphology and distribution of antennal sensilla of the diamondback moth showed that each sensillum contained three co-compartmentalized olfactory receptor neurons (ORNs). Each ORN class showed a narrow response spectrum, with some ORNs specialized for green-leaf volatiles and (±)-linalool that are present in brassicaceous hosts, while several other ORNs responded to two non-host volatile sesquiterpenes, (E)-β-farnesene and germacrene D, as well as (E)-β-caryophyllene, a host-related sesquiterpene volatile (Wee et al. 2016). In addition, other studies showed that female moths were significantly more attracted to conspecific larvae-infested cabbage plants, had significantly shorter flights than in fields with intact uninfested cabbage hosts, and oviposited significantly more eggs on larvae-infested cabbage than on intact uninfested cabbage (Wee 2016). These data indicate the potential of developing a brassica host-derived kairomone attractant for female diamondback moths that could be used for sampling purposes.

*Distinguishing $F_1$ Males from Wild Males in Inherited Sterility Programmes of Lepidoptera.* Inherited sterility in lepidopteran programmes has a distinct advantage over full sterility in that the released semi-sterile males have a better mating competitiveness than fully sterile males, in addition to the resulting multiplier effect from viable but sterile progeny from every mating. The deleterious effects induced by irradiation are inherited by the $F_1$ generation, but it is very difficult to measure progress in population suppression because the $F_1$ offspring are not marked and therefore cannot be distinguished from wild males. Using light microscopy, the incidence of chromosomal aberrations in $F_1$ male larvae can be used to reveal the

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proportion of moths that mated with released substerile males. Fragmentation, and non-reciprocal, reciprocal, and multiple translocations, are the most common types of aberrations encountered (Marec et al., this volume).

A forensic biosecurity method, based on a cytological assessment of sperm bundles of wild and $F_1$ males, has been developed and shows great promise. The technique can successfully distinguish the homogeneous nuclei clusters of eupyrene bundles of the normal fertile males from the heterogeneously stained nuclei clusters of the $F_1$ progeny. This technique has been demonstrated in six lepidopteran species: codling moth, cactus moth, diamondback moth, painted apple moth *Teia anartoides* Walker, corn earworm *Helicoverpa zea* (Boddie), and fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Carpenter et al. 2009; Wee et al. 2011). However, the technique only yielded good results when good specimens for cytological diagnosis were obtained. In addition, the percentage of positive staining results was strongly correlated with survival. Moths that had spent 24 h on a sticky base in a monitoring trap were equivalent to freshly killed specimens, but the efficacy of the technique decreased after that (Wee et al. 2011).

### *3.2.3. Monitoring Variations in Age Structure of a Wild Population*

A change imposed on an insect population through variations in mortality, emigration, and invasion, will be reflected in the age structure of that population (Van der Vloedt et al. 1980; Rogers and Randolph 1986; Vreysen et al. 1999a). A method to determine the age of individual insects (or a group of insects) would enable variations in population structure resulting from the control measures to be assessed, and would be another powerful tool to monitor progress in programmes. The effects of season, habitat, and other factors on the age structure of a population must be separated out from those of applied control measures.

There are several methods that have routinely been used in operational programmes to estimate the age of tsetse fly populations (Van der Vloedt et al. 1980; Vreysen et al. 2000) — the development stage of ovarioles in females (Saunders 1960; Challier 1965), and the wing-fray analysis (rate of wear of the wings) (Jackson 1946). Wing-fray measurements are a convenient way to give a reasonable, albeit crude, indication of the mean age of a population, but since fraying is influenced by the activity pattern of flies, the rate of wing fraying varies between species and the sexes (Ryan et al. 1980). Determining the physiological age structure in tsetse, using ovarian development (section 3.2.1.), is labour-intensive but very accurate. However, it is not suitable for determining the chronological age of tsetse populations, in view of the influences of temperature and nutritional state on the development rate of each gonadotrophic cycle (Saunders 1972), and inter- and intra-species differences (Wall 1990).

The measurement of fluorescent pigments (pteridines), which accumulate linearly with age in the heads of tsetse flies (Lehane and Mail 1985; Langley et al. 1988) and New World screwworms (Thomas and Chen 1989), and curvilinearly in the Old World screwworm *Chrysomya bezziana* (Villeneuve) (Wall et al. 1990), may provide a cheap, convenient, and rapid indicator of the mean age of these insect populations. However, the level of pteridine accumulation is highly dependent on temperature and fly size. Also, the precise age of individual insects cannot be determined because the

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levels of residual variation in pteridine fluorescence remain unexplained in all cases studied, and appreciable confidence limits must be placed around pteridine-derived age estimates (Wall et al. 1990). According to field studies, this method was not suitable for accurate age determination in Mexican fruit flies *Anastrepha ludens* (Loew) (Tomic-Carruthers et al. 2002).

Near-infrared spectroscopy (NIRS) has been used to estimate the chronological age of stable flies *Stomoxys calcitrans* (L.), house flies *Musca domestica* L., and face flies *Musca autumnalis* De Geer. NIRS has several advantages over the pteridine fluorescence technique for age-grading field-collected insects (e.g. speed and portability of instruments). The technique is independent of the sex and size of the insects being studied, and of the temperature to which adult insects were exposed (Mendoza et al. 2002).

Releasing competitive sterile males will gradually increase the proportion of wild females that do not produce viable offspring. Consequently, fewer young insects will be recruited into the population, and the age structure will gradually become skewed towards older age groups, e.g. data from the tsetse programme in Zanzibar (Fig. 7). During the early stages of the release programme, the monthly averages of the proportion of teneral and nulliparous (i.e. young) females in the samples (determined by ovarian ageing) fluctuated between 16 and 19%, whereas the monthly averages of the proportion of old females (with 4 or more ovulations) fluctuated between 18 and 28%. Thereafter, the proportion of young flies decreased progressively, whereas the proportion of old females (≥ 4 ovulations) gradually increased (Vreysen et al. 2000).

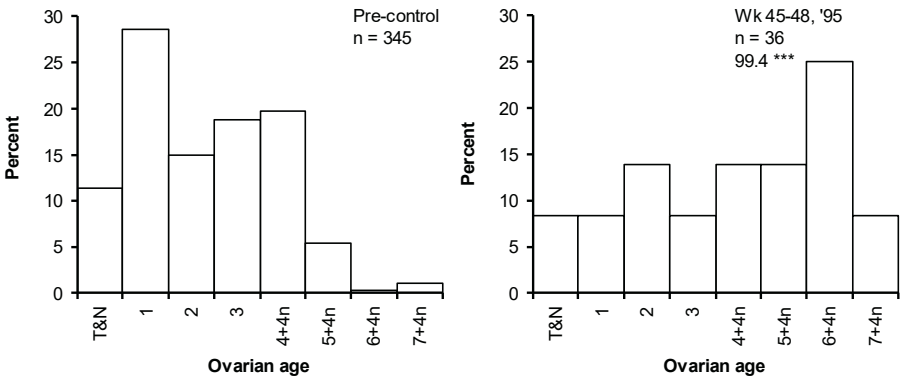



*Figure 7. Frequency distribution of ovarian age categories of* G. austeni *females sampled during (left) pre-control phase and (right) late release phase (T = teneral, N = nulliparous, 1–7 = number of ovulations; more details in Challier 1965). Number indicates chi-square value of comparison of frequency distribution of ovarian age with that of pre-control sample (*** $p < 0.001$). (Figure adapted from Vreysen 2001, reproduced with permission.)*

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*3.2.4. Monitoring Relative Abundance of a Wild Population*

Decline in the apparent density of a wild population, as shown by the number of insects trapped in a sampling device, is a commonly used parameter to assess the progress of AW-IPM programmes for fruit flies (Iwahashi 1977), Lepidoptera (Bloem and Bloem 2000; Walters et al. 2000) and tsetse flies (Vreysen et al. 2000) (Fig. 6). In the case of insects with a very long lifespan, such as tsetse flies (Vreysen et al. 1996), monitoring this parameter has an inherent weakness; no insects are actually killed by the SIT technology, and thus there is an inevitable delay in the decline in the number of wild insects available to be trapped.

When sampling insects to obtain an indicator of programme progress, the main difficulty is related to interpreting catch data. For example, is a temporal decline in the number of insects trapped, even when using a standardized monitoring programme, always an indication of progress in a control programme? Numerous factors influence the size of trap samples (section 3.1.2.), and the importance of these factors for the interpretation of monitoring data is illustrated below.

The density of a natural insect population rarely remains stable, but fluctuates in both space and time. Knowledge of these fluctuations is prerequisite to correctly interpreting monitoring data. In the absence of any control measure, monthly trapping data for the tsetse fly *Glossina swynnertoni* Austen, over a period of 23 years in Tanzania (Fig. 8), show high seasonal variations in the apparent density of the population, with the average highest apparent density being 18 times that of the lowest average density (Glasgow and Welch 1962).

Differences in population density from one place to another can be great. Locations with unusually high densities are called “hot spots” and require special attention in AW-IPM programmes (sections 2.1. and 2.2.3.) (Box 3).

In addition to the need for baseline data on the temporal and spatial fluctuations in the density of a wild insect population, the factors that influence changes in the behavioural responses of insects towards trapping devices, in both space and time, need to be understood to correctly interpret monitoring data (Vreysen and Saleh 2001). An analysis of weekly trap catches of sterile *Glossina austeni* released on Unguja Island, Zanzibar, over a period of more than 2 years, showed that, in each 12-month period, the size of catches fluctuated by a factor of more than 10, independent of the actual sterile fly population density (which was estimated from the number of sterile males released) (M. J. B. Vreysen, unpublished data). These data led to some important lessons. Even dramatic increases in trap catches, especially during control operations, could wrongly be attributed to sudden explosions of the pest population, migration from adjacent areas, decrease in mortality, or failure of the applied control method. This study indicates that the probability of trapping insects during a post-control monitoring phase would be increased significantly by deploying traps during strategic periods, e.g. when the behavioural response of insects to the trapping device is at a high point (Saleh et al. 1999).

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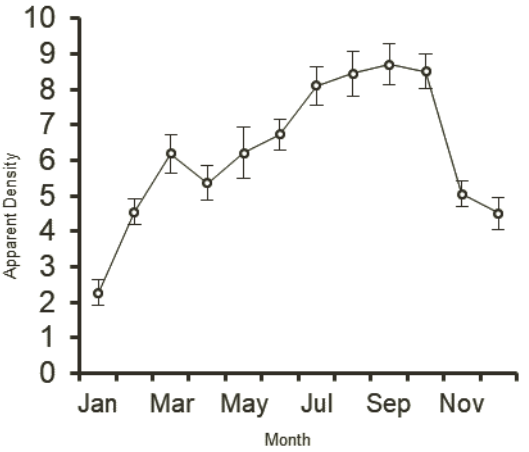



*Figure 8. Fluctuations in average monthly apparent density (as percentage of total catches) of* Glossina swynnertoni *population sampled over period of 23 years in Tanzania. (Figure adapted from Glasgow and Welch 1962, reproduced with permission.)*

*Box 3. Monitoring "Hot Spots"*

Localized infestations or "hot spots" are of particular interest in AW-IPM programmes (Shiga 1991). Strategies that allow quick action to manage these situations are required. To detect hot spots quickly, programme managers must always be alert throughout the duration of the programme. The early detection of relic populations, or reinfestations in localized areas, is a determining factor in reducing programme cost, in increasing programme effectiveness, and in the ultimate successful completion of the programme (Itô and Kakinohana 1995). Although baseline surveys might reveal that the pest infestation is widespread, hot-spot areas are often concealed, and are revealed only after the programme has advanced.

The reasons for hot spots are numerous — localized ideal climatic conditions, abundant hosts, and in some cases also presence of difficult topography that hampers the effectiveness of pre-release suppression activities (Nakamori and Shiga 1993).

When hot spots are discovered or persist, surveillance in the vicinity should immediately be increased. The size of the area under surveillance will depend on the mobility of the insect, and should be increased systematically until no more wild insects are trapped. The traps deployed must be serviced/emptied on a very frequent (daily) basis, or the frequency and intensity of indirect sampling procedures (fruit inspection, disease monitoring, etc.) (section 3.1.3.) increased, to amplify confidence in the data. For some insect species such as the Mediterranean fruit fly, DNA analysis can indicate whether the infestation is new, or originates from a relic population or from insects that accidentally escaped from a rearing facility (McInnis et al. 2017). If the infestation is new, it is essential that live wild insects are collected in the new infestation zone, and their mating compatibility with factory-reared and sterilized insects assessed. In addition, the overall monitoring efficiency needs to be increased by immediately installing a high-density delimitation trapping grid around each incursion (FAO/IAEA 2018), and improving communication and/or feedback mechanisms with crop farmers or livestock keepers to reduce the time between an infestation being discovered and it being reported to programme management or relevant authorities.

After assessing and compiling the necessary information, using GIS (which can greatly facilitate the analysis and management of localized infestations (Bouyer et al., this volume)), corrective measures must be taken immediately, e.g. apply an insecticide, increase the number of sterile insects released, create a buffer zone or quarantine programme, and limit the movement of livestock, crops, etc.

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## 4. ESTABLISHING THE ABSENCE OF INSECTS

After the "last" wild insect has been caught, a difficult decision must be made. When should the release of sterile insects be terminated? Stopping the release too soon could jeopardize the success of the programme, but if releases are continued after eradication has been achieved, useful resources are wasted (Proverbs 1974). The time period of continued releases but zero captures will be influenced by the life cycle of the insect, the efficiency of the sampling system used, and the financial resources available.

To increase the confidence of detecting wild insects during and after the final stages of an eradication programme, the monitoring strategy could be adjusted by: (1) increasing the density and frequency of the direct and indirect monitoring activities (Yamagishi et al. 1993), (2) increasing the proportion of sampling devices biased for the female segment of the population (to obtain more information on fertility or induced sterility) (Vreysen et al. 2000), and (3) releasing sterile females as sentinels (Vreysen and Van der Vloedt 1992). The decision to stop releasing sterile insects is frequently made on an ad hoc basis, and is highly influenced by financial and political circumstances. In the eradication programmes in Central America and in Libya, after the "last" case had been detected, the dispersal of sterile New World screwworms continued for 6–18 months (FAO 1992; Wyss 2000). In fruit flies, it is standard procedure that delimitation trapping continues for at least three fly generations (using degree-day models) after the "last" fly has been detected (FAO 2016).

A problem related to the issue of when to stop dispersing sterile insects is how long to continue post-release monitoring to obtain sufficient confidence that a pest has been eradicated (Barclay and Hargrove 2005; Hargrove 2005; Barclay et al., this volume). A sample can only confirm that individual insects are present in a given area; sampling can never prove a negative (McInnis et al. 2017). However, samples can demonstrate that the number of individuals is within a specified range, with a known degree of confidence (Venette et al. 2002). The probability of detecting rare individuals is directly related to the number of sampling units and the density of the population (McArdle 1990; Lance and Gates 1994). Therefore, verification sampling should be implemented in high-risk areas and previous hot spots after termination of releases so as to maximize the probability of detecting relic insects in the field (McDermotte 2000; FAO/IAEA 2018). If too few samples are taken, an error could be made in concluding that a pest is absent from a habitat (Venette et al. 2002).

As the declaration of the absence of an insect in a target area cannot be guaranteed, it must always be qualified by probability or confidence levels (Barclay et al., this volume). Unfortunately, standardized probability-based entomological criteria to confirm the status of eradication have rarely been applied in insect eradication programmes.

An option is to follow the approaches used by ecologists to assess species extinction (McDermotte 2000). In one approach, after extinction is assumed to have occurred, the number of negative sightings required to confirm extinction at a given probability level is assessed; this requires an accurate knowledge of the sensitivity of the sampling method (Reed 1996). A second approach takes into account data from pre-eradication sampling, assuming a declining population. In this method the probability of extinction is estimated as a function of the frequency of pre-eradication

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sightings, and the proportion of the total time (pre- and post-eradication) during which no sightings have been made (Solow 1993).

## 5. DATA MANAGEMENT

It is essential that information from the monitoring activities is reliable, comprehensive, and clear, and is delivered in a synthesized and timely manner to decision-makers (Reyes et al. 1988). The amount and diversity of data that have to be handled and analysed can be staggering, especially in large-scale operational programmes. Thus each programme requires a properly developed data-flow structure, e.g. from field teams via field sub-offices to programme headquarters, and an efficient data-analysis unit. A programme website is very useful; it permits all concerned to have access anytime to the raw and analysed data.

Comprehensive field data recording sheets are indispensable, and must be adapted to the biological characteristics of each target species and to the needs of each programme. Data sheets should include all information relevant to a proper data analysis, e.g. details on animals screened, details on the composition of trap samples, geo-referenced trap deployment sites, baits, types of traps, etc. Using electronic data collectors, and transmitting data to computers at programme headquarters via e-mail, facsimile, or HF radio, even if rather sophisticated compared with traditional paper methods, permit rapid and efficient data collection and compilation (Bouyer et al., this volume). In addition, field-monitoring data should be complemented with climatic data from remote automated weather stations in the target area.

Using identical sampling periods, standardized sampling procedures, and uniform compilation methods ensure homogeneous data sets; they greatly facilitate the analysis and interpretation of the data (Box 4). The Gregorian year with 365 days can be divided into equal periods for data collection and compilation, e.g. in the tsetse programme in Zanzibar, traps were checked 1–5 times per week, depending on the importance of the area, but all of the data were compiled on a weekly basis (Vreysen et al. 2000). Data can be compiled using EXCEL spreadsheets or an ACCESS-based database; the latter is more appropriate for large amounts of data, and allows easy incorporation into most GIS. Particularly useful are databases which have been developed to manage the field data of specific pest control programmes, and which can promote the standardization of data reporting and analysis on a regional scale, e.g. the Disease and Vector Integrated Database (DAVID) (Robinson 2001).

The frequency of analysing the data is related to the regularity of sampling and compilation of the data, but is usually done every 1–3 months; however, if the insect has a high reproductive rate, it will be done much more frequently. In the analysis of trap catches, indices, such as daily or weekly catch per trap, and the proportion of positive traps, can be used. However, Clift and Meats (1998) showed that the proportion of positive traps is not a good indicator in the early stages of a programme, since the proportion of positive traps is only slightly reduced when the catch per trap declines from 10 to 1. Standard statistical methods such as analysis of variance in a randomized block design (with fixed time units as blocks) can, after proper transformation of the data, be used for the temporal and spatial comparison of data (Sokal and Rolf 1995).

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## 6. CONCLUSIONS

The importance of reliable monitoring data before, during, and after the release of sterile insects cannot be overstated. In spite of the availability of efficient "direct" and "indirect" surveillance methods suitable for a variety of target species, the monitoring component in operational programmes is too often neglected. Consequently, programme management and decision-making are based more on established protocols, availability of financial resources, and political inspiration rather than on sound scientific principles.

Programmes that release sterile insects are inherently complex, with many critical components in the production process (aimed at delivering high-quality insects), methods of handling and transport, and dispersal procedures. The multifaceted nature of these programmes also implies that the probability of problems occurring is higher than in conventional pest control programmes.

*Box 4. Concept of Reference Sites for Monitoring or Surveys*

Notwithstanding the importance of sound and robust monitoring activities in an AW-IPM programme, funds are needed to deploy each sampling device. Monitoring activities should be planned as a compromise between cost-efficiency and providing adequate and sufficient data. AW-IPM programmes tend to be implemented over large geographical areas, and monitoring (or surveying) in detail the entire area is not feasible, practical or cost-effective. Selecting reference (or fixed) monitoring sites, representative of a certain area, is a useful approach to efficiently monitor or survey large geographical areas (Leak et al. 2009).

Depending on the size of the target area, this is divided into Universal Transverse Mercator (UTM) squares, e.g. 5 x 5 km or 10 x 10 km, and each UTM grid square is characterized by parameters that are important to the distribution of the pest, i.e. vegetation, land use, land cover, hydrology, soil type, altitude, etc. Pending the availability of these specific data layers, GIS can facilitate the characterization of the grid squares (Bouyer et al., this volume). Therefore each grid square has a certain number of classes, which are of relevance to the abundance and distribution of the pest. After considering accessibility, logistics, personnel, etc., a reference monitoring site can be selected for each class of each grid, and a certain number of sampling devices deployed in each reference monitoring site, which will then be representative for that class in that specific grid square.

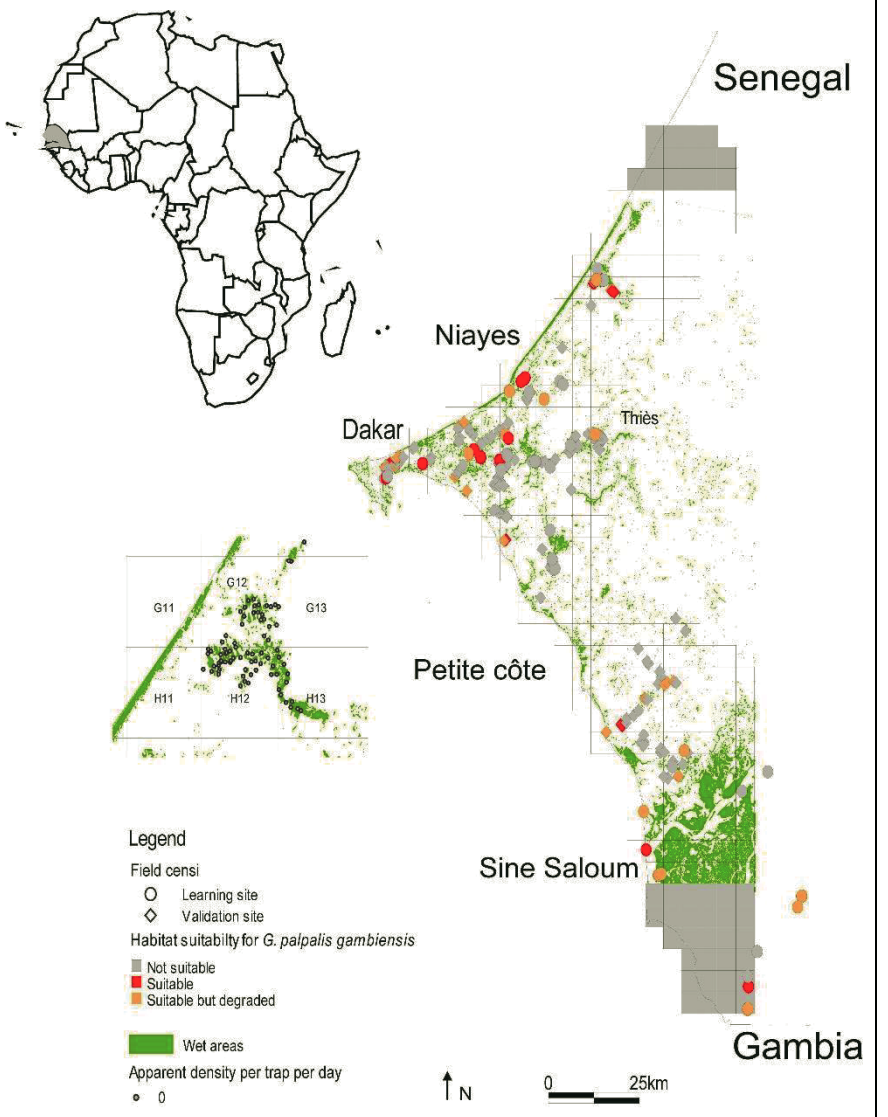
This approach was applied to develop and implement an efficient sampling strategy for the collection of entomological baseline data in the tsetse project in the Southern Rift Valley of Ethiopia (Vreysen 2000) and in the Niayes of Senegal (Bouyer et al. 2010).

In the case of Ethiopia, about 15–18 trapping sites were selected in each UTM square (total of 105 squares, each 10 x 10 km) to sample the wild fly populations during four surveys within 1 year. Accurate data on the spatial and temporal differences of the tsetse populations were collected with limited resources over a large geographical area (more than 10 000 $km^2$) using carefully selected trapping sites in representative areas (Vreysen et al. 1999b; Vreysen 2000).

In the case of Senegal, a 5 x 5 km grid overlaying the entire target area (286 cells) was developed to facilitate the field sampling procedures. During preliminary surveys, a phytosociological census achieved a supervised classification of the vegetation in addition to some entomological data collected in the various habitats. This enabled an assessment of the suitability of the habitat to harbour the tsetse fly *G. p. gambiensis* in the area, denoted as wet areas since the suitability was correlated with the presence of ground water at the end of the dry season. All these wet areas were identified in each of the grids and georeferenced for the deployment of tsetse traps (Bouyer et al. 2010).

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*Box 4. Continued*




*The target area in Senegal with the overlaying grid structure and the identified wet areas as obtained by the supervised classification (right) and the selection of trapping sites in the identified wet areas in grids G12, G13, H12, and H13 (insert). (Figure modified from Bouyer et al. 2010.)*

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Only the availability of reliable field data can: (1) provide clear evidence that observed programme progress is due to released sterile insects and other measures applied to suppress the pest population, (2) identify the causes of problems and suggest possible solutions, and (3) increase the efficiency of the programmes by more strategic deployment of sterile insects. It is acknowledged that monitoring methods are often time-consuming, labour-intensive, and even laborious, but in many instances the financial losses, resulting from inefficient management decisions made without the benefit of reliable field data, outweigh the costs of monitoring activities.

Several shortcomings in implementing the monitoring activities of operational programmes have been pointed out. The need for more standardization, research, and development to improve several aspects of monitoring the field component of these programmes has been highlighted. Examples of these aspects are: (1) development of guidelines to standardize sampling procedures (trap types for specific species, lures and attractants, trap deployment, trap densities, etc.) (FAO/IAEA 2018, 2020a, b), (2) refinement and development of more efficient lure and trapping systems, (3) better understanding of insect ecology, in relation to insect densities, aggregation patterns, and dispersal characteristics, (4) research on visual markers in the sperm of sterile males, (5) better methods of measuring induced sterility, and (6) simple statistical probability methods, which can easily be applied by field entomologists, to assess the absence of a target species.

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# CHAPTER 4.1.

# ROLE OF POPULATION GENETICS IN THE STERILE INSECT TECHNIQUE

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## SUMMARY

The detection and analysis of genetic variation in natural and laboratory populations of pest insects are reviewed. The application of population genetic methods and theory can help to plan and evaluate the implementation of area-wide integrated pest management (AW-IPM) programmes that use the sterile insect technique (SIT). Population genetic studies can play an important role in estimating dispersal rates and thus the degree of isolation or gene flow among target populations, determining if sibling species exist, establishing the origin of outbreaks or reintroductions, and supporting the quality control of mass-reared colonies. The target's population history may be examined in terms of "bottlenecks", range

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fragmentations, and expansions. Genetic methods can be helpful in distinguishing wild insects from released sterile or semi-sterile ones, and in ascertaining, together with mating compatibility studies, the compatibility of mass-reared colonies with target wild insects.

## 1. INTRODUCTION

Population genetics, in the broad sense, is the study of gene frequencies in and among subdivided populations. It encompasses estimations of variation in terms of allelic and genotypic frequencies. Given appropriate data, the application of population genetic theory allows estimates of rates of gene flow within and among populations, estimates of effective, i.e. reproductive, population sizes, tests for past "bottlenecks" and rapid expansions of population size, and pairwise genetic distances. Since all evolutionary change is accompanied by change in gene frequencies, population and evolutionary genetics overlap.

Methods of population genetics can be used to support the application of the sterile insect technique (SIT) for the integrated control of target pest populations in nature. Briefly, the degree of genetic isolation of target populations from each other, and from untargeted populations, can be estimated. The existence of sibling species may be investigated, in combination with behavioural, cytogenetic and other approaches, by careful sampling and genetic analysis of pest populations. Immigration rates from unchallenged populations may be estimated, in terms of reproducing females per generation. The origin of pest outbreaks in treated areas may thereby be investigated. The genetic relatedness of mass-reared strains and target populations may be estimated in terms of allelic composition and gene frequencies. The question of laboratory "degradation" of strains destined for release can be examined objectively in terms of inbreeding and genetic distances from founding and target populations.

Given what may be done by using modern techniques and adequate sampling of natural populations, it should nevertheless be noted that the New World screwworm *Cochliomyia hominivorax* (Coquerel), tsetse fly *Glossina austeni* Newstead and Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) area-wide integrated pest management (AW-IPM) programmes were executed successfully without any substantial knowledge of the population genetics (section 8) (Enkerlin, this volume; Feldmann et al., this volume; Vargas-Terán et al., this volume).

Here we outline the kinds of genetic variation that may be examined efficiently, and briefly review tests of hypothesis and analytical methods that may be applied to genetic data. In addition, the causes of genetic differentiation are discussed.

Population genetics is built on well-developed and sophisticated mathematical and statistical theory (but not reviewed here). Crow and Kimura (1970) and Wright (1969a, b; 1978a, b) covered the theoretical basis of population genetics. The analysis of population genetic data was examined by Nei (1987), Weir (1996), Rousset and Raymond (1997), and Nei and Kumar (2000). Luikart and England (1999) reviewed the methods of data analysis, and tabulated the necessary software. Wright (1969a, b; 1978a, b), Avise (2004), Gillespie (2004), Hartl and Clark (2007), Hedrick (2011), and Nielsen and Slatkin (2013) provided general treatments of population and evolutionary genetics. We recommend Hartl and Clark as the

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"friendliest" treatment. Roderick (1996) and Black et al. (2001) have written particularly useful reviews of insect population genetics.

## 2. WHY POPULATION GENETICS?

- Sibling or cryptic species are reproductively isolated forms that are morphologically undifferentiated. If pre-mating isolating mechanisms exist, the application of sterile males of one member of a species complex may be totally ineffective against other members. Sibling species are common, e.g. among tsetse flies *Glossina morsitans* Westwood, *G. fusca* Walker, *G. palpalis* Robineau-Desvoidy and *G. fuscipes* Newstead (Gooding and Krafsur 2005), the *Drosophila willistoni* Sturtevant species complex, the *Anopheles maculipennis* Meigen complex (Bates 1949), the *Anopheles gambiae* Giles complex (Coluzzi et al. 2002; Dorn et al. 2011), the *Aedes scutellaris* (Walker) complex (Kambhampati et al. 1992), the *Simulium damnosum* Theobald complex (Vajime and Gregory 1990), the sand fly *Lutzomyia longipalpis* (Lutz and Neiva) (Lanzaro et al. 1993) and the ladybird beetle *Coleomegilla maculata* (De Geer) complex (Krafsur and Obrycki 2000). Most complexes uncovered to date are medically important Diptera or genetically interesting *Drosophila* species, but this is probably sampling bias. Complexes important to agriculture include the tephritid flies *Anastrepha fraterculus* (Wiedemann), where the existence of at least seven morphotypes has been confirmed recently, while for *Bactrocera dorsalis* (Hendel) the putative complex was shown to represent intra-specific variation, resulting in the synonymization of four pest species (Hendrichs et al. 2015). Colonies of medically important Diptera are routinely established to study insecticide resistance, vector-pathogen transmission, etc., and intercrosses among strains may produce sterile progeny. Examination of polytene chromosomes, well developed in many Diptera, may reveal reproductively isolated forms, as demonstrated in some *Drosophila* species (Dobzhansky 1970). Many insect pest species are not easily reared or studied genetically, particularly herbivorous beetles and moths, hence the question of cryptic speciation may not arise and cannot be investigated easily.
- Natural populations tend to be discontinuously distributed. Gene flow varies among them as per many factors, including the historical, geographical, and environmental. There may be local selection regimes that cause one or more populations to differ biologically in ways that could make the SIT less effective due to mating barriers. Such barriers might be ecological, temporal, or behavioural, and their strengths, in principle, could vary greatly. It should be noted, however, that no such mating barriers were detected in the New World screwworm (Krafsur 1985, 1994) and Mediterranean fruit fly programmes (Cayol et al. 2002).
- Estimating rates of gene flow among populations can provide an estimate of the rate of invasion or reinvasion of eradicated areas from unchallenged populations. The rates can be described in terms of the average numbers of reproducing flies per generation. Moreover, the likely source(s) of pests in an outbreak can be examined and, in many instances, specified. For easily transported pests such as

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the Mediterranean fruit fly, the geographic origins of new infestations can, in principle, be specified. Determining the source of new infestations is an important undertaking to determine and close pathways, up to establishing embargos on produce originating from areas suspected of having shipped infested commodities (Aguirre-Ramirez et al. 2017).

- Construction of laboratory strains for sterilization and release can be improved. If adequate numbers of insects are to be sterilized and released, it is necessary to establish thriving colonies. How much of the existing natural genetic diversity should be incorporated into a release strain? The geographic range of many pest species is very large, and locally adapted populations may exist. How then should the genetic make-up of release strains be formulated?
- An analysis of genetic variation may also reveal genetic markers in laboratory colonies that can be used to differentiate released insects or the offspring of semi-sterile insects from their wild counterparts during the monitoring of programmes that release sterile insects.

## 3. CHIEF KINDS OF GENETIC VARIATION

Chromosome morphologies and structural rearrangements may vary within species and provide markers that distinguish populations (Robinson, this volume). Examples include paracentric inversions, easily detected in stained squashes of polytene chromosomes found in *Drosophila*, *Anopheles* mosquitoes, and the supernumerary chromosomes of some *Glossina morsitans* group tsetse flies (Gooding and Krafsur 2005). These kinds of variation rarely provide the high degree of resolution afforded by molecular variation in DNA or its transcription and translation products such as allozymes. Moreover, chromosome rearrangements are not selectively neutral as has been demonstrated in *Drosophila* species and *Anopheles gambiae* (Coluzzi et al. 2002). They may nevertheless provide important information about reproductive isolation and the operation of natural selection.

Allozymes are allelic forms of an enzyme. Isozymes are enzymes that act on a common substrate. Alleles are codominant. They are readily demonstrated by electrophoresis of tissue homogenates on starch, cellulose acetate, or polyacrylamide followed by histochemical staining that involves a dye or UV-fluorescence (Richardson et al. 1986; Murphey et al. 1996). Allozyme banding patterns correspond to genotypes, i.e. one band for a homozygote, two bands for a heterozygote at a monomeric locus, and three bands for a heterozygote at a dimeric locus. Most allozyme loci occur on the nuclear genome, but some loci, e.g. nicotinamide adenine dinucleotide phosphate (NADP)-dependent malate dehydrogenase, are mitochondrial. Allozyme analysis requires that tissue samples be fresh or frozen to preserve enzyme activities. This restriction does not apply to analysis of DNA.

Mitochondrial DNA (mtDNA) is single-copy, hence haploid, self-replicating, and is maternally transmitted as a circular molecule of about 16 kilobases. It encodes 22 transfer RNA genes and 13 proteins, including the cytochrome oxidases, NADH, RNAase, and ribosomal RNAs. There are many highly conserved regions from which oligonucleotide primers have been designed, e.g. Simon et al. (1994), which

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are commercially available in kits. These primers allow the amplification of regions of the mitochondrial chromosome by the polymerase chain reaction (PCR). Mitochondrial variation can be assessed by several methods. Nucleotide sequencing of amplified regions can be followed by the comparison of sequences among individuals. Sequencing is recommended now that its costs have greatly decreased.

Microsatellites are simple sequence repeats (SSRs) of nucleotide motifs that are repeated *n* times: $(CA)_n$, $(GA)_n$, $(TA)_n$, $(CAG)_n$, etc. (where C is cytosine, A is adenine, G is guanine, T is thymine) that may occur throughout the nuclear genome. Each locus may vary among individuals in the number of repeats. Alleles are codominant. Conserved flanking sequences allow the design of oligonucleotide primers with which to amplify via PCR the microsatellite region. Comparatively sophisticated procedures are necessary to find microsatellites in the genome, and to develop oligonucleotide primers to amplify them. A substantial number of protocols are available to do so, e.g. Abdelkrim et al. (2009), Malausa et al. (2011). Some protocols involve enrichment of DNA for repeat sequences to maximize the yield of desired repeats (Hamilton et al. 1999). Alternatively, one may choose to hire a commercial organization to isolate and characterize microsatellite loci, and for some laboratories this may be the least expensive and most efficient way to proceed.

Single Nucleotide Polymorphisms (SNPs) are sites in the genome where DNA sequences differ by a single base when two or more individuals are compared. Sequence differences occur due to single nucleotide substitutions or insertions/deletions. SNPs occur in coding and non-coding regions of nuclear DNA, and constitute the most abundant markers in eukaryotic genomes. They are widely distributed and most loci are biallelic. Improved sequencing technology and available genomic sequence data have made it possible to analyse directly genetic variation in DNA. Signatures of adaptive genetic variation in wild populations can be detected without prior identification of candidate genes (Elmer and Meyer 2011). This is done by conducting genome scans of SNPs by using "next generation" DNA sequencing (section 4). Gloria-Soria et al. (2016) developed a panel of ~73,000 SNPs distributed across the genome of *G. f. fuscipes* (*Gff*) that was used to estimate genome-wide genetic diversity, differentiation and linkage disequilibrium in wild *Gff* populations in Uganda.

## 4. RELATIVE ADVANTAGES AND DISADVANTAGES OF PRINCIPAL METHODS

### *4.1. Obsolete Methods*

Several methods have become obsolete because much more efficient techniques have become available. Falling into disuse are studies of allozymes, used to assess enzymatic protein variation. Also, obsolete are the SSCP (single strand conformational polymorphism) technique to examine mitochondrial variation, RFLPs (restriction fragment length polymorphisms), RAPDs (randomly amplified polymorphic DNA), and AFLPs (amplified fragment length polymorphisms), all of which can be used to examine elements of genomic DNA.

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### *4.2. Mitochondrial Variations*

Regions of the mitochondrial genome are highly variable, and some may be useful as population-specific markers. The mitochondrion is inherited as a single locus, and the effective population size (Box 1) is about a quarter of the size estimated by nuclear loci. This makes mitochondrial variation more sensitive to the effects of historical reductions in population size ("bottlenecks") and colonizing episodes. The reason is that the mitochondrial genome is single copy (haploid) and maternally inherited.

*Box 1. Basic Formulae Used in Population Genetics*

Gene heterozygosity (diversity) at a locus is one minus the frequency of homozygotes expected by Hardy-Weinberg criteria, corrected for sampling bias (Nei 1987):

$$h_e = 2n(1 - \Sigma x_i^2)/(2n - 1)$$

where $x$ is the frequency of allele $i$, and $n$ is the sample size. For measuring diversity at haplotypes, use $n/(n-1)$ as the correction factor. The average heterozygosity $He$ over $s$ loci is,

$$H_e = \Sigma h_e/s$$

with variance

$$\text{Var} = \Sigma(H_e - h_e)^2/[s(s - 1)]$$

Under Hardy-Weinberg assumptions, the relationship between diversity $H_e$, mutation rate $\nu$, and effective population size $N_e$ is:

$$H_e = 4N_e\nu/(4N_e\nu + 1)$$

Effective population size is, roughly, the number of successfully reproducing individuals. Note that $4N_e$ is likely to be a very large number and the mutation rate a very small number, of the order $10^{-6}$ for allozyme loci; both are difficult to measure.

The index $F$ describes departures from random mating by taking the difference between expected heterozygosity $h_e$ and the observed $h_O$ as a fraction of the heterozygosity expected on Hardy-Weinberg criteria:

$$F = (h_e - h_O)/h_e, \text{ or } F = 1 - (h_e/h_O)$$

It gives the equilibrium value of the proportion of homozygous genotypes. $F$ is a sensitive index of breeding structure, and may be expressed for hierarchical levels in subdivided populations. (It is described further in Box 3.)

### *4.3. Chromosomal Polymorphisms*

Chromosomal polymorphisms occur in many species. Cytological examination of pest taxa can provide important evidence of population subdivisions, variation in sex-determining mechanisms, and indicators of natural selection. There are a number of common structural variations in chromosome morphology that can be observed by microscopic examination of appropriate tissues. Such tissues include brain, testis, ovarian and trichogen cells, and salivary glands. Giant multi-banded

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"polytene" chromosomes can be found in tsetse trichogen and mosquito salivary glands and ovarian nurse cells; polytenes arise from a large number of chromosome reduplications without cell separation -- thus giant cells with greatly enlarged chromosomes. They are particularly well-developed in nematocerous Diptera, and can also be found in higher Diptera, e.g. *Glossina* spp.

Sex chromosomes ("heterosomes") in many taxa indicate the phenotypic sex, e.g. in most Diptera, homogametic XX for females and heterogametic XY for males, but male Lepidoptera are homogametic and the females heterogametic. High frequencies of sex chromosome polymorphisms were detected in *G. p. palpalis* (Maudlin 1979) indicating that further cytological work on the *G. palpalis* complex is warranted.

Among the most common chromosomal polymorphisms are paracentric inversions, where two breaks occur in a chromosome arm followed by a 180-degree rotation of the interstitial piece and a rejoin of the three pieces. Most, if not all, seem to respond to selective forces, and therefore do not provide unbiased estimators of gene flow. Nevertheless, they may provide striking indicators of genetic differentiation of populations. Pericentric inversions include the centromere within the interstitial central part of the chromosome; such an inversion can greatly change the appearance of a chromosome.

Cytological analysis can be time-consuming and requires much skill, and therefore adequate and representative sampling of natural populations can be a problem (Augustinos et al. 2014).

### *4.4. Microsatellites and Single Nucleotide Polymorphisms*

SSRs can be well distributed throughout the genome and highly polymorphic, which has both advantages and disadvantages. Microsatellite alleles can prove useful in pinpointing the source or sources of immigrant insects in a treated area. Automated scanning and scoring of acrylamide gels allow high throughputs [by using Genemarker (SoftGenetics, USA) or Genescan®, Genotyper® and GeneMapper® software (Applied Biosystems, ThermoFisher Scientific, USA)]. Null alleles, usually caused by mutations in primer annealing sites, lead to underestimates of heterozygotes. Some taxa have few SSRs, including some culicine mosquitoes and ticks (Fagerberg et al. 2001). High mutation rates occur at some loci, violating a key Hardy-Weinberg assumption, and leading to homoplasy and biased estimates of gene flow and genetic differentiation of populations. Homoplasy, the convergent development of genotypically different but phenotypically similar repeats, is common in microsatellites. Highly variable loci with many alleles provide downwardly biased estimates of gene flow and genetic differentiation (because as diversities $h_o/h_e \rightarrow 1$, $F \rightarrow 0$). Since many microsatellite loci do not conform well to the infinite allele model (the stepwise mutation model would be more appropriate) (Box 2), some analytical procedures, e.g. *F* statistics estimation, may be inappropriate, and it then becomes necessary to use other methods. Some problems related to SSRs are avoided by using SNPs. Nevertheless, microsatellite loci and SNPs have become the principal tools with which to examine the genetics of

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populations, leading to an explosion of analytical methods to examine the plethora of data now available (Luikart and England 1999; Excoffier and Heckel 2006).

*Box 2. Stepwise Mutation Model (SMM)*

Change in microsatellite repeat number is thought to occur principally in a stepwise fashion by unequal crossing over and replication slippage. Two or more independently arisen alleles can have the same molecular weight, and therefore appear as one on gels (homoplasy). Clearly, there are upper and lower limits to the number of repeats a polymorphic locus can have, so the infinite alleles model of mutation is inappropriate. Thus, in theory, the stepwise mutation model (SMM) is most appropriate when dealing with microsatellite loci. Slatkin (1995) developed a model based on the SMM analogous to Wright's *F* statistics. He termed the index of gene flow $R_{ST}$. The basic difference is that *F* statistics are based on variance in allele frequencies, and $R_{ST}$ is based on variance in repeat number. However, simulations have shown that, when sample sizes are less than 50 and the number of loci scored are less than about 20, *Fst* provides less biased estimates of gene flow than $R_{ST}$ (Gaggiotti et al. 1999).

### *4.5. DNA Sequencing*

Next generation (high throughput) sequencing describes several advanced, rapid and efficient sequencing methods now available (Mardis 2008; Schuster 2008; Seeb et al. 2011; EMBL-EBI 2017). Refer to Danecek et al. (2011) for formatting high throughput DNA sequencing for further use in population and phylogenetic work. Also, refer to Frías-López et al. (2016) for appropriate software for the same purpose. High throughput sequencing now enables a more comprehensive view of how genomes vary spatially and temporally than do the older methods.

## 5. BASIC PRINCIPLES OF POPULATION GENETICS

As cited earlier, several academic texts offer comprehensive treatments of population and ecological genetics. Here we offer only an elementary treatment of the subject.

The Hardy-Weinberg theorem, or rule, is the fundamental template on which genetic data are tested. The rule specifies that in diploid, sexually reproducing organisms, genotypic frequencies remain unchanged from generation to generation if the locus under consideration is selectively neutral, mating is random, generations are discrete, the mutation rate negligible, and population size infinite, so that no sampling errors occur in the transmission of gametes from one generation to the next. The foregoing assumptions, however, apply to few, if any, natural populations. Violations of Hardy-Weinberg assumptions may cause departures from random mating within and among populations. The principle cause is genetic drift — the random change in small gene pools due to sampling errors that occurs when the gametes of a parental generation unite to form the individuals of the next generation.

For diploid genomes, the probability of fixation of a neutral allele, i.e. attains a frequency of 1, is $1/2N_e$, where $N_e$ is the "effective" population size (Box 1). For uniparentally inherited loci, the probability is $1/N_e$. Thus, fixation, i.e. loss of diversity, is inversely proportional to the effective population size $N_e$. It should be

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noted that the effective population size may be much less than the census number because not all individuals reproduce, and some generate more progeny than others.

Most species are distributed discontinuously in space and time, so individuals in a population will have a greater opportunity to interbreed with receptive individuals in their own population than with individuals in other populations. Thus, matings will not ordinarily be random among populations separated by distance, altitude, or age structure. The degree of genetic differentiation among populations provides a measure of gene flow.

Selection acting on a locus can also cause departures from Hardy-Weinberg expectations. Most genetic markers used in population genetics research are assumed to be selectively neutral. However, many allozyme loci are not selectively neutral, but respond to balancing selection. An interesting example of balancing selection is that of the tsetse fly *Glossina pallidipes* Austen. Populations in Zambia and Zimbabwe show about the same level of allozyme heterozygosity as populations in Kenya, but with much reduced levels of microsatellite and mitochondrial variation (Krafsur 2002).

## 6. PROCESSING AND INTERPRETING POPULATION GENETIC DATA

Having scored haplotype or genotypic frequencies, it is necessary to test hypotheses about their distributions within individuals (if dealing with nuclear variation) and at hierarchical levels of population structure. Much software is available for these tasks. (A website maintained by Joseph Felsenstein at the University of Washington lists, with annotations, 392 free software packages and 54 free web servers.) Excoffier and Heckel (2006) offer a helpful list of programmes, while Lischer and Excoffier (2012) provide an automated data-conversion tool for connecting genetic data with analytic programmes.

The first step is to examine gene diversities for each population, and record the observed heterozygosity $h_o$ at each locus. Next, calculate the expected single locus heterozygosities $h_e$, together with the expected heterozygosity averaged over loci $H_E$ (Box 1). Allele or haplotype frequencies can be tested for homogeneity over sampling units by using the Chi-square contingency tests (Rice 1989) (these are incorporated into most software packages). If gene frequencies at all or most loci are homogenous among populations, then there is little likelihood of genetic differentiation among populations, assuming one is dealing with selectively neutral variation. It is then likely that the sampled populations behave as a single randomly breeding unit, and the SIT would likely have to contend with high immigration rates. Such a result does not necessarily argue against the successful application of the SIT, as is discussed below. However, it is most likely that allele frequencies among populations will differ, a consequence of population structure and genetic drift. Over time, drift and genetic differentiation can lead to the development of reproductive isolation and, eventually, to speciation (Dobzhansky 1970; Futuyma 1998; Avise 2004).

The magnitude of genetic drift is inversely proportional to the effective population size (Box 1), i.e. the geometric mean number of individuals that contributes progeny to a subsequent generation, and more formally, the size of an

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ideal population experiencing the same rates of random genetic change as the real population being considered. Genetic drift is most expeditiously quantified by estimating $F_{ST}$ or its analogues (Box 3).

*Box 3. F Statistics*

Wright's $F$ statistics and analogues are used to describe the breeding structure of subdivided populations. The original statistical theory was formulated in terms of two alleles segregating at a single locus. $F$ can be defined in terms of inbreeding, as departures from random mating, and as correlations between uniting gametes.

$F_{IS}$ describes departures from random mating within populations; it is the correlation of random alleles within individuals averaged over populations, relative to the correlation of two random alleles from the population as a whole. Thus, the heterozygote deficiency (increase of homozygosity or inbreeding) in individuals is (Hartl and Clark 2007): $F_{IS} = (H_S - H_I)/H_S = 1-(H_I/H_S)$. Here $H_I$ is estimated as the average observed heterozygosity among populations, and $H_S$ is the expected heterozygosity.

$F_{ST}$ indicates departures from random mating among populations; it is the correlation of two random alleles in a population relative to the correlation of two random alleles chosen from the entire population ($F_{ST} = 1-[H_S/H_T]$), where $H_T$ is the heterozygosity averaged over all populations. It was termed by Wright as the standardized measure of genetic variance among populations, and also as the "fixation index" that measures reduction of heterozygosity, or heterozygote deficit compared with Hardy-Weinberg.

$F_{IT}$ describes departures from random mating accruing from all causes ("inbreeding"); it is the correlation of alleles in individuals relative to that in the entire population ($F_{IT} = 1- [H_I/H_T]$). Important extensions of $F$-statistics include $G_{ST}$ (Nei 1987) and Weir and Cockerham's (1984) theta ($\theta$).

$F_{ST}$, $G_{ST}$, and $\theta$ underestimate differentiation of subdivided populations progressively as gene heterozygosity $h_e$ increases because as diversity $h \rightarrow 1$ in Hardy-Weinberg demes, $F_{ST}$, $G_{ST}$, and $\theta$ will approach zero. Indeed, they cannot take a value equal to or greater than the mean level of homozygosity (Hedrick 1999). Slatkin (1985), Barton and Slatkin (1986) and Slatkin and Barton (1989) offered a way around the problem of high diversities. Slatkin observed a nearly linear relationship between the mean frequency of private alleles $p_I$ and the mean number of reproducing migrants per generation $N_em$: $\log p_I = a \log_{10} Nm + b$. Constants $a$ and $b$ vary with mean sample size. The method was found to be robust.

$F_{ST}$, $G_{ST}$, and $\theta$ are variance statistics. $\theta$, for example, may be used in an analysis of variance for both diploid and haploid loci (Weir 1996). Thus, one can obtain variance statistics and estimates of $\theta$ for each level of hierarchy (Rice 1989).

The inverse of $F_{ST}$ is proportional to the level of migration that satisfies the equation, $F_{ST} = (4N_em + 1)^{-1}$, where the term $N_em$ is, very roughly, the harmonic mean number of reproducing migrants per generation (Box 4). Fig. 1 shows the relationship between $N_em$ and $F_{ST}$. Wright (1978a, b) suggested that a critical level of migrants is about one reproducing organism per generation. A lower sustained migration rate among equilibrium populations would lead to further genetic differentiation by drift.

For discontinuously distributed, i.e. subdivided, populations, it is helpful to make estimates of $F_{ST}$ between all possible pairs of populations. In this way, one can test hypotheses that $F_{ST}$ increases with geographic distance or other physical measure of interest. Highly differentiated populations can easily be identified in this way. Such local populations could, in principle, have a measure of pre-mating reproductive isolation that might work against the SIT. If deemed to be of interest, colonization,

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and laboratory and field-cage testing for pre-mating isolation, could then be carried out (Cayol et al. 2002).

*Box 4. Gene Flow*

According to Wright's island model of gene flow, $F_{ST}$ can be defined in relation to the effective population size $N_e$, mutation rate $\nu$, and migration rate $m$. $F_{ST} = [4N_em + \nu + 1]^{-1}$ for populations at mutation-drift-migration equilibrium. This equation allows the estimation of a migration rate in terms of the mean number of reproducing organisms per generation $N_em$. Thus, where the mutation rate $\nu$ is negligible (i.e. $\rightarrow 0$), $N_em \approx (1 - F_{ST})/4F_{ST}$ for diploid loci. For mitochondrial haplotypes, $N_em \approx (1 - F_{ST})/2F_{ST}$ (where the sex ratio approaches unity).

$F_{ST}$ is non-linear because it is a reciprocal function of $N_em$ (Fig. 1). Therefore, mean values of $N_em$ can be quite meaningless, and caution is required in their interpretation (Whitlock and McCauley 1999). Pairwise estimates of $F_{ST}$ and its linear transformations are useful, however, and can be obtained by using "Arlequin" software (Excoffier and Lischer 2010).

Some assumptions of the island model of gene flow include populations of equal size in which drift does not occur, all of which exchange alleles with equal probability.

Other models have been formulated, including the stepping-stone model, in which only adjacent populations exchange alleles.

The island model, however, has proved sufficiently robust for many purposes, and it remains the most generally used. Theoretical developments have led to new applications, for example, the cladistic nested analysis of phylogeographical data (Templeton 1998).

An additional caution is necessary when using microsatellite loci to estimate $F_{ST}$. Mutation rates can be substantial at some microsatellite loci, leading to downwardly biased estimates of $F_{ST}$.

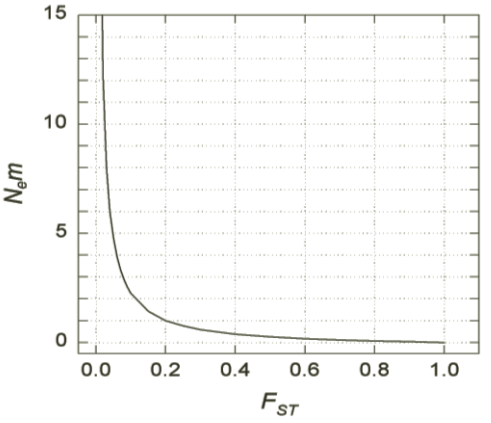



*Figure 1. Mean numbers of reproducing migrants per generation exchanged among populations ($N_em$) as a function of the mean departures from random mating ($F_{ST}$) according to the island model of gene flow. $N_em = (1 - F_{ST})/4F_{ST}$.*

Do high rates of dispersal argue definitively against the SIT? History indicates that they do not. Epizootiological and experimental studies have shown that the New World screwworm has a great capacity for dispersal, as might be expected in such a colonizing species. High dispersal rates of the screwworm flies did not fatally

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compromise the effective application of the SIT to this species wherever it was applied.

## 7. POPULATION GENETICS AND CONSTRUCTION OF RELEASE STRAINS

How may the rules of population genetics be used to construct strains for eventual release? Thriving mass-reared colonies of the target insect are required, the progeny of which, when radiosterilized, must be competitive with wild males in seeking to mate with wild females (Lance and McInnis, this volume; Parker, Mamai et al., this volume; Parker, Vreysen et al., this volume).

It is often assumed that the "degradation" of laboratory stocks occurs via adaptation through selection and inbreeding, with a correlating loss of field competitiveness. Work on *Drosophila* showed a great loss of gene diversity (Briscoe et al. 1992) and fitness that was proportional to the duration of laboratory colonization (Jungen and Hartl 1979). Also, Cioci et al. (2014) detected a significant loss of microsatellite diversity arising from a genetic "bottleneck" in a ca. 38-year-old *Glossina pallidipes* colony. On the other hand, no substantial loss of mitochondrial variation was detected in *Glossina pallidipes* (Krafsur et al. 2016) or *Glossina morsitans* Westwood (Wohlford et al. 1999). Thus, each case must be taken on its own merits.

During laboratory adaptation, predation pressure is absent in colonies. Equally, sexual selection that operates in nature may be relaxed because of the proximity of mates in cages. For example, contact cuticular hydrocarbon pheromones in higher Diptera (Carlson et al. 1993; Brown et al. 1998), courtship behaviour in tephritid fruit flies, and swarming behaviour in nematocerous Diptera, could, in principle, inadvertently become modified during laboratory adaptation (Eberhard 2000; Briceño and Eberhard 2002; Briceño et al. 2002). The genetics and evolution of pheromone signalling and response behaviour have been studied in moths (Löfstedt 1993; Phelan 1997). Inbreeding causes a loss of genetic variation that could, in principle, reduce competitiveness. Are these realistic concerns? Is competitiveness in the field inversely proportional to laboratory adaptation? Do laboratory strains/colonies "deteriorate" over time?

The theory of inbreeding is well known; a simple account of elementary inbreeding theory is given in Box 5. A substantial loss of heterozygosity may occur if a release strain is formed from too few founding insects, followed by a prolonged "bottleneck" in colony size. However, it is by no means certain that even a great loss in diversity will have pronounced effects on laboratory fitness or mating competitiveness in the field. Many colonizing species undergo periodic genetic bottlenecks in nature, with little adverse effect. The question of laboratory adaptation and competitiveness must be addressed on a case-by-case basis. In the New World screwworm, for example, estimates of sterile mating rates among geographically diverse target populations were found to be unrelated to the strain of sterile flies released (Krafsur 1985, 1994). Experience with *Glossina morsitans* suggested that colonized and irradiated flies dispersed and mated with their wild cousins at approximately the expected frequencies (Vale et al. 1976; Dame 1979; Vreysen et al. 2011).

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*Box 5. Inbreeding*

Some consequences of inbreeding and drift include high frequencies of homozygosis and fixation of mildly deleterious alleles. Correlations between uniting gametes in "inbred" populations are greater than the correlations drawn from reference populations higher in the hierarchy. Loss of genetic diversity through drift is to be expected in closed populations. The inbreeding coefficient $F$ (probability of identity by descent) in generation $t$ is related to the effective population size $N_e$ as follows:

$$F_t = 1 - (1 - 1/2N_e)^t \quad \text{As } t \text{ increases, } F \to 1, \text{ hence diversity} \to 0$$

Manipulating this equation shows that about half the diversity at selectively neutral loci is lost in $1.4N_e$ generations. For a breeding colony established from 2, 5, 10, 25, and 100 pairs the initial loss of diversity in a single generation is 22, 10, 5, 2, and 0.5%, respectively. These are trivial decreases. In a constantly expanding insect culture there would be little additional loss of variation. After 10 generations of "inbreeding" at the foregoing population sizes, however, heterozygosity losses become far greater, at 92, 63, 39, 12, and 5 %, respectively. Thus, the duration of a "bottleneck" has profound effects on genetic variation. It is well to remember that these formulae apply to selectively neutral loci, but insects in culture are subject to various forms of selection, some of which may be for traits not advantageous in the wild. It should be noted, however, that weak selection intensity in very small populations can be overridden by drift (Black et al. 2001).

In some cases, genetic phenomena have been invoked to explain pest outbreaks during sterile insect release programmes (section 8), but few genetic data were available to support the contentions. Nowadays, however, it is easy to obtain genetic data for target populations, and for laboratory colonies and their source populations. Inbreeding coefficients ($F$) and genetic distances can (and should) be estimated continuously as part of quality control programmes. A progressive loss of diversity in laboratory colonies, coupled with a progressive decay in physiological quality control indices, could provide prima facie evidence of degradation, and possibly predict a decline in field competitiveness. If no data are collected, little of substance can later be said about genetic causes of, or correlations with, the failure of a particular programme.

The rationale for constructing strains for propagation and release should include the number of founding organisms and their geographical origins. If there is good reason to believe that a target pest population may constitute a species complex, then it is necessary to ensure that only the pest species is cultured. Isofemale strains can be constructed for insects that reproduce readily in colony (an isofemale strain is based upon the progeny of a single mated pair). Many such lines can be constructed, and each evaluated for laboratory fitness and field competitiveness. If it can be shown that geographically adapted populations exist (Vera et al. 2006), then it would be prudent to conduct mating preference tests under semi-natural conditions (Cayol et al. 2002). Such tests have strong environmental correlations, and thus experimental designs must be adequate to detect unambiguously a true genetic component. Where strong, genetically determined mating preferences exist, of course it would be advisable to rear, sterilize, and release the most compatible strains.

The question of loss of diversity and fixation of deleterious alleles because of small effective population size can, in principle, become acute when constructing

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strains by classical or modern molecular tools; the progenies are descended from a unique genome that may or may not be subject to recombination with wild-type alleles (Franz et al., this volume).

Finally, it is most important to put in perspective the issue of "strain degradation". It is difficult and expensive to conduct field experiments that estimate the genetic and environmental components of competitiveness. In projects that release sterile insects, however, sterile mating rates in target pest populations should be monitored routinely (Parker, Vreysen et al., this volume; Vreysen, this volume). Experience has shown that rearing, handling, irradiation, and release procedures are much more important for competitiveness than the laboratory "degradation" of release strains. A good example is the North and Central American New World screwworm eradication programme (see below).

There are new, more efficient, and faster computer programmes suitable for the analysis of population genetic data. Principal component analysis (PCA) has become a standard analytical tool in population genetics. Many applications employ Bayesian methods that allow complex and subtle inferences of such data. Although beyond the scope of this chapter, Box 6 provides a brief introduction to PCA and Bayesian applications.

*Box 6. Principal Components and Bayes Theorem*

Principal component analysis is convenient and easy to use. Graphic presentations can be colorful and informative. In PCA, eigenvectors and eigenvalues are calculated from allele frequency covariance matrices of all pairwise comparisons. Covariance is the mean value of the product of deviations of two variates from their respective means. Patterson et al. (2006) offer further information and cite convenient computer programmes.

The application of Bayes theorem or rule offers a useful tool to estimate probabilities of events based on prior knowledge related to the event of interest and to compare gene flow models. Bayesian methods can be used to infer phylogenies (Felsenstein 2004), i.e. evolutionary relationships among taxa or geographically diverse populations.

At its mathematically simplest, Bayes theorem is given by $P(A \mid B) = [P (B \mid A \text{ X } P(A)] / P(B)$ where P(A) is the probability of A and P(B) is the probability of B; $P(A \mid B)$ is the probability of A given B, and $P(B \mid A)$ is the probability of B given A. Here, P(A) is the "prior" probability, and $P(A \mid B)$ is the "posterior" probability.

Approximate Bayesian Computation (ABC) bypasses some difficult mathematical procedures (Beaumont 2010), and provides a flexible platform to carry out complex models. ABC is useful in investigating demographic histories and evolutionary questions. ABC is incorporated into a growing list of statistical procedures and genetical software packages, e.g. DIYABC v2.1.0 ("Do It Yourself ABC") (Cornuet et al. 2014) and MrBayes 3 (Ronquist and Huelsenbeck 2003). DIYABC is software for comprehensive analysis of population history using approximate Bayesian computation (ABC) on DNA polymorphism data. MrBayes is a programme for Bayesian inference and model choice across a wide range of phylogenetic and evolutionary models. MrBayes uses a Markov chain Monte Carlo simulation method to estimate the expectation of a statistic in a complex model. Another software is ABCtoolbox (Wegmann et al. 2010).

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## 8. APPLICATIONS AND EXAMPLES

Experience suggests that studies of gene flow throughout a species' range are not necessary to implement and successfully carry out programmes that release sterile insects. Mediterranean fruit fly and New World screwworm programmes were conducted successfully for years without any substantial population genetic data available. However, the spectre of reproductively isolated screwworm populations was raised repeatedly, particularly when progress was slow or releases deemed ineffective (Krafsur et al. 1987). Some investigators were so certain of the existence of assortative matings between wild and released flies, and of reproductively isolated forms, that numerous claims were made in anticipation of their certain discovery. Indeed, a few geneticists played a controversial and unhelpful role (in the New World screwworm eradication programme in the USA and Mexico) by claiming evidence of assortative matings (Bush et al. 1976), or later (Richardson 1978; Richardson et al. 1982), by claiming evidence that screwworms constituted a hitherto unrecognized complex of reproductively isolated forms.

This situation, precipitated by screwworm epizootics in 1972–1976 in the south-western USA, was exacerbated by the practice of presenting and evaluating eradication programme results solely on screwworm case incidence. Programme officials invoked screwworm migration across the border from Mexico and rainfall to explain the outbreaks, but no gene flow or meteorological data were ever provided. The possibility of genetic changes in target populations was also acknowledged. In the absence of published meaningful data to support programme claims, interested commentators offered their own explanations, attracting much notice in the scientific community, and leading to attempts to stop the development of the Mexico screwworm eradication programme until critics could solve the very problems they claimed to have identified.

There was very little evidence that the screwworm outbreaks could be explained by powerful genetic phenomena or the existence of cryptic species. There was much evidence to falsify the claims, had it been recognized and invoked by programme officials (LaChance et al. 1982; McInnis 1983; Krafsur et al. 1987; Krafsur 1998). One of the lessons to be learned from this episode is that periodic estimates of gene frequencies from wild and mass-reared insects of a targeted species, together with predictive quality control measures, can be used to track changes that could, in principle, point to a decline in strain effectiveness for SIT implementation. The availability of such data, particularly when published in peer-reviewed scientific journals, can do much to defuse potentially damaging and confounding criticism from the scientific community. It would also be necessary, in those species where it is practicable, to maintain estimates of sterile mating frequencies -- to show a relationship between sterility and sterile insect dose rates and target population densities (Krafsur and Hightower 1979; Krafsur 1994; Vreysen, this volume).

Pest outbreaks during a sterile insect release programme can reasonably be expected, and it will be necessary to specify the most likely causes. It must be anticipated that natural events, such as weather or the evolution of assortative mating, can, in principle, occur, but experience has shown that it is much more likely that failures in insect production, the distribution of sterile insects or the

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implementation of complementary suppression measures will have caused this breakdown in control.

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# CHAPTER 4.2.

# POPULATION SUPPRESSION IN SUPPORT OF THE STERILE INSECT TECHNIQUE

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## SUMMARY

Suppression or eradication of insect pest populations by the release of sterile insects is often dependent on supplementary methods of pest reduction to levels where the target pest population can be overflooded with sterile insects. Population suppression activities often take place in advance of, or coincide with, the production and release of sterile insects. Supplementary methods to remove breeding opportunities, or management methods that prevent access of pests or vectors to the hosts, may reduce the population or prevent damage or disease transmission. Insecticides have been used widely in direct applications or applied as baits, in traps, or on specific sites where the pest makes contact or reproduces, although they are increasingly being replaced by biopesticides. As sterile insect release does not kill the pest, adult biting pests or fertile mated females of the pests will continue to attack hosts after the release of sterile insects. Thus supplementary pest suppression programmes and quarantine measures are essential to prevent damage or the spread of disease. Eradication or effective pest management requires that the entire population of the pest be treated, or that the programme apply immigration barriers. It also requires taking into account interactions among control methods; they can be additive, synergistic or antagonistic. When supplementary pest control activities directly benefit the human population in areas being treated, such as in mosquito or screwworm control programmes, these area-wide suppression activities are usually acceptable to the public, but when the public receives no direct benefit from supplementary control activities such as in crop pest programmes, social resistance may develop unless public information is managed properly.


## 1. INTRODUCTION

The sterile insect technique (SIT) is highly species-specific and non-polluting; the target is the reproductive system of sexually reproducing pests. Supplemental systems to reduce pest populations are often required, prior to the release of sterile insects, to reduce the target pest population to the degree that the sterile insects have an advantageous numerical ratio to induce sterility. Most of the successful programmes releasing sterile insects were applied when field populations were at low densities, either after a natural population decrease (such as winters in subtropical or temperate climates) or after the application of area-wide suppression activities.

The decision to use suppression before the release of sterile insects may also be for economic reasons. Quarantine decisions, on market access of commodities attacked by pest outbreaks, are frequently based on adult trapping data. Reducing the adult population close to the detection level with adulticide sprays and/or other means adds the benefit of meeting quarantine protocols and reopening markets sooner than when the sterile insects have eliminated the population.

In other cases, the action of released sterile insects on the pest population is indirectly associated with reduction in pest damage. Mosquitoes and tsetse flies can continue to bite and spread disease after they are mated to sterile flies. Fertile-mated screwworms can, for the rest of their lives, continue to destroy livestock. These activities are not reduced by the release of sterile flies. Decisions to use pesticides or other methods to protect hosts will, in these cases, for example in the case of an epidemic, be largely independent of the success of the sterile insects.

In eradication programmes, multiple suppression methods may be combined, but interactions among them must be considered, particularly when they interact with the dynamics of the pests' Allee threshold (Suckling et al. 2012). Combinations of methods can thus be considered to have synergistic (greater efficiency from the combination in achieving extinction), additive (no improvement over single methods alone), or antagonistic (reduced efficiency from the combination) effects on Allee

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dynamics. When planning the integration of methods it is crucial to take these effects into account.

In this chapter the various pest control techniques that are used to suppress pest populations, in conjunction with the application of the SIT, are reviewed. Suppression activities applied as precursors, or in tandem with sterile insect release, will be emphasized. Quarantine treatments are not considered.

## 2. OVERVIEW OF PEST CONTROL TECHNIQUES

Major benefits of the SIT are species specificity and the possibility of eradication. Knipling (1979) outlined the techniques for reducing insect pest populations with respect to the species specificity (Table 1), and to their effectiveness with respect to pest density levels (Table 2). Table 2 is of particular relevance to the SIT as its effectiveness relates to the density of the pest population. He followed this overview with a discussion on integrating these techniques with the SIT.

*Table 1. Classification of degrees of selectivity of various methods of insect control (adapted from Knipling 1979)*

| Highly selective | Moderately selective | Non-selective |
|---|---|---|
| Insect attractants (specific) | Attractants (baits) | Conventional insecticides |
| Insect pathogens (specific) | Biopesticides (specific) | Biopesticides (general) |
| Insect parasitoids (specific) | Parasitoids (general) | Mechanical control |
| Insect predators (specific) | Predators (general) | Cultural measures |
| Insect-resistant plant varieties | Insect entomopathogenic fungi (specific) | Insect entomopathogenic fungi (general) |
| Genetic techniques | Autoinoculation devices | |
| Mating disruption | Light traps | |

Knipling (1979) also discussed a series of exceptions and modifications to this classification, and recent research has greatly extended the number of available suppression methods. The method of application was also recognized as being of crucial importance in selectivity. For example, aerial application of broad-spectrum insecticides will have a greater impact on non-target organisms than application of the same insecticide as a bait or to a specific location on a plant or animal that is to be protected from a pest.

Natural biological control is inevitably a part of any area-wide integrated pest management (AW-IPM) programme, but specific releases and manipulation of parasitoids and predators are being used as part of AW-IPM systems that include the SIT (Knipling 1992, 1998, 1999; Wong et al. 1992; Bloem et al. 1998; Vargas et al. 2004; Montoya et al. 2007). Marec et al. (this volume) describe the synergism between inherited sterility (IS) and natural enemies (Bloem et al., 1998; Carpenter et al. 2004). In New Zealand, irradiated male painted apple moths *Teia anartoides* Walker were released, and *Bacillus thuringiensis* Berliner variety *kurstaki* (*Btk*) was

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simultaneously applied to suppress the pest population (O'Callagahan et al. 2003; Suckling 2003; Simmons et al., this volume).

Knipling (1979) followed this table with an introduction to the concept of IPM, including descriptions of the purposes of pest management: to slow population growth, suppress and maintain populations below a certain level, or eliminate populations. Since then, there has been a number of recorded eradication successes using different IPM strategies combining various control methods, including insecticides, mating disruption, SIT, host removal, *Bt* plants, lure and kill, etc. (Table 3) (Suckling et al. 2014; Klassen and Vreysen, this volume).

*Table 2. Classification of insect control techniques by efficacy at various pest densities (adapted from Knipling 1979)*

| Methods equally effective at all densities | Methods most effective at lowest densities | Methods most effective at highest densities |
|---|---|---|
| Conventional chemical insecticides[1] | Sterile insect and other genetic techniques | Host-specific pathogenic organisms |
| Chemical sterilants applied to natural populations | Sex- or aggregating-attractant traps | Host-specific parasitoids |
| Cultural and mechanical control methods | Sex-attractant diversion sources or mating disruption | Host-specific predators |
| Insect-resistant crop varieties | Sex-attractant vapours | |
| Genetically modified crops[2] | | |
| Light traps | | |
| Attractant baits | | |
| Trap crops | | |
| Synthetic non-pheromone attractants | | |
| Sex pheromones that block responses | | |

[1]Some factors, e.g. spatial aggregation and partial protection by vegetation cover, can make their efficiency density-dependent

[2]Although leaving sufficient refugia with non-resistant plants to avoid rapid selection for insect resistance

## 3. CULTURAL, ENVIRONMENTAL, AND MECHANICAL CONTROL

Activities in agricultural production, property management or lifestyles can all influence insect pest densities, and a major advantage of cultural control is that it is pest density-independent. The disadvantage is that many cultural control activities reduce the population but do not protect the crops or animals being attacked. In cases where very low pest populations can have high economic or health impacts, cultural control through habitat manipulations alone probably will not provide the desired level of suppression.

Development of the SIT technique for control of the New World screwworm *Cochliomyia hominivorax* (Coquerel) relied heavily on activities that prevented infestation of livestock. The pest was subjected to considerable cultural control because of the value of livestock and the critical damage from infestations to animal

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and humans. Programmes for treating wounds, and preventing reinfestations of individual animals, were the primary actions taken to control screwworm damage (FAO 1992; Vargas-Terán et al., this volume). Dove (1938) proposed a combination of livestock management measures such as special care with wound protection, protection of females and offspring following pregnancy, curing tick bites, castrating and branding animals only during winter, protecting castrated and branded animals, and other management activities. Protecting animals from infestation, and wound treatment, are still the key suppression activities in screwworm programmes where this pest has not been eradicated.

*Table 3. Examples where multiple suppression methods have been combined during successful eradication programmes (modified from Suckling et al. 2012, 2014)*

| Number of suppression methods | Combinations and their assumed density-dependence[1] | Insect scientific name | Insect common name | References |
|---|---|---|---|---|
| 3 | Mating disruption (DD)<br>+*Bt*-cotton (DI)<br>+SIT (DD) | *Pectinophora gossypiella* (Saunders) | Pink bollworm | Tabashnik et al. 2010; Simmons et al., this volume |
| 5 | Ground insecticides (DI)<br>+Aerial *Btk* sprays (DI)<br>+Mass-trapping (DD)<br>+Host-plant removal (DI)<br>+SIT (DD) | *Teia anartoides* | Painted apple moth | Suckling et al. 2007; Simmons et al., this volume |
| 3 | Pour-on (DD)<br>+ Insecticide targets (DD)<br>+ SIT (DD) | *Glossina austeni* Newstead | Tsetse fly | Vreysen at al. 2000; Feldmann et al., this volume |
| 3 | Pour-on (DD)<br>+ Insecticide targets (DD)<br>+ SIT (DD) | *Glossina palpalis gambiensis* Vanderplank | Tsetse fly | Dicko et al. 2014; Feldmann et al., this volume |
| 3 | Wound treatment (DD)<br>+ Cultural controls[2] (DD)<br>+ SIT (DD) | *Cochliomyia hominivorax* | New World screwworm | FAO 1992; Vargas-Terán et al., this volume |

[1]Density-independent (DI) indicates a control method that removes a proportion of the population, independent of density (e.g. broad-spectrum insecticides). Density-dependent (DD) methods work better on bigger populations (e.g. pour-on applied on cattle will work better at high tsetse densities if they mainly feed on cattle but will not kill the small part of the population feeding on alternative hosts) or on the contrary on smaller populations, accelerating the extirpation towards the end (e.g. SIT works better because the overflooding ratio of sterile to wild individuals favours sterile individuals).
[2]Example of cultural control: dehorning, branding or castration during cold months.

Cultural control methods for crop pests can include area-wide destruction of crop residues, general orchard sanitation and removal of infested fruit, and enforcement of planting and harvesting dates.

Cultural control of the pink bollworm (Nobel 1969) in the USA was developed in the early 1950s as part of an area-wide approach as the pest spread across Texas, New Mexico, and Arizona. Activities included evaluation of stalk-shredding machines for

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killing the potential overwintering insects and incorporating the use of shredders. Devices were developed that killed bollworm larvae in cotton gin trash. In contrast to the screwworm programme, the impact of these activities was to decrease the pink bollworm population, rather than directly protecting the crop.

Early reviews of fruit fly pest control focused on environmental modifications to reduce reproduction and the survival of immature stages, and chemical control to kill adults. Back and Pemberton (1918) described covering of immature fruit with a bag or cloth material to prevent infestation. They also described a system used in Australia of bagging the canopy of trees with mosquito netting, but considered the method too expensive for large-scale use. Individual bagging of fruit was successful, but the bag had to be impermeable to oviposition, and problems with scale insects on the protected fruit developed. More recently, this concept has been revived to protect cabbage crops in Africa (Martin et al. 2013).

Another cultural control approach to fruit fly pests was described as "clean culture" (Back and Pemberton 1918). This method is based on removing all hosts from the infested area. Crawford (1927) reviewed clean-culture methods used in Mexico and determined that the approach was effective for the Mexican fruit fly *Anastrepha ludens* (Loew), but extreme measures such as the destruction of trees were not practical or effective (although see Kovaleski and Mumford (2007) for successful *Cydia pomonella* (L.) eradication in Brazil). He compared ranches that cleaned up fallen oranges once a week, and found the approach ineffective for complete control but more effective when coordinated with the application of poison bait. Crawford also recognized the value of trap crops in controlling fruit fly damage; he suggested that a few grapefruit trees (a preferred citrus species) could be used for this. He also recognized that the trap-crop trees could be sprayed.

The application of "clean culture" to fruit fly management programmes has been widely practiced in AW-IPM programmes integrating the SIT in the USA and the joint programmes in Mexico and Guatemala. Sanitation and the destruction of host material is widely carried out in the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) eradication programme (Moscamed) in Mexico and Guatemala. During a series of outbreaks in the Palenque district of northern Chiapas, from 8332 hectares treated (e.g. bait sprays), 33 978 kg of host material were collected and destroyed (Moscamed data sheets for 2002). In Guatemala, from 411 739 hectares treated, 345 633 kg of host fruit were destroyed (USDA/APHIS/IS 2002). As an immediate response in 2003 to a large infestation (106 hectares) of Mexican fruit flies in Valley Center, California, the California Department of Food and Agriculture removed and buried host fruit. A total of 2 941 070 kg of fruit (mostly citrus) was collected from the ground and stripped from trees at sites ranging from 0.26 to 36 hectares. The removal and destruction of host material is a logical complementary suppression activity because it destroys immature stages of the pest that cannot be controlled by other methods. However, there is a possibility that the removal of host material induces adult females to migrate in search of hosts, and actually causes an outbreak to spread.

Where the primary purpose of the SIT is to eliminate or prevent diseases through eliminating or suppressing the vectors, cultural and mechanical controls have been applied widely. In addition to pesticide application as a supplementary treatment incorporated into programmes that also release sterile insects for mosquito control,

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Musil (2002) outlined improvements in integrated activities that reduce the vector populations, and in health care supplemental activities:

- Habitat management that interferes with the mosquito life cycle, including physical barriers, use of beneficial organisms, and removal of breeding sites,
- Community education and participation to improve public understanding of the mode of disease transmission,
- Public health strategies that integrate detection, diagnosis, and prompt treatment of the disease to prevent spread.

In the case of mosquito control, active source reduction, together with larviciding, adulticiding, and public education through door-to-door campaigns (source reduction through education) could achieve up to 75% reduction of adult densities in the case of *Aedes albopictus* (Skuse) (Fonseca et al. 2013). Such integrated strategies will be crucial when integrating the SIT, thus facilitating the overflooding of wild populations and inducing more than 50% sterility in females to reduce adult populations further (Bellini et al. 2013).

## 4. CHEMICAL CONTROL

### *4.1. Direct Insecticide Application for Population Suppression*

Broadcasting insecticides has often been done in conjunction with AW-IPM programmes that integrate the SIT. However, public acceptance of insecticide application in this context has usually been limited to situations where the treated properties are owned by persons who receive some benefit. Eradication experiments, and programmes with medically important pests such as mosquitoes and tsetse flies, have frequently used applications of persistent insecticides. Programmes for pests such as the pink bollworm, with a distribution largely restricted to certain hosts, were also implemented with insecticides but with little publicity, although more recent supplementary suppression activities were based on attractants (Walters et al. 2000a). Suppression of the codling moth in Canada required initial insecticide treatments to reduce the pest population (Bloem and Bloem 2000). If an insecticide is applied at the same time that sterile insects are being released, the chemical may kill some of the sterile insects; however, as long as the ratio of sterile:wild insects remains the same, this would not impair the efficacy of the SIT.

In *C. capitata*, models demonstrated that it is crucial to determine optimal spraying schedules to ensure an efficient suppression of target populations in preparation of SIT application (Barclay et al. 2019). The most important biotic parameters to be considered were the relative length of the larval period, the fertility rate, and the age to first oviposition. The stage targeted by sprays, and the percent mortality caused by each spray, were also found to be important in determining the required number of sprays.

Mangan (2014) reviewed adulticidal insecticide bait sprays for fruit flies, and summarized the development of organic baits that replaced organophosphate baits.

In the 1960s when the first attempts to use the SIT, or other genetic modifications of released mosquitoes, were made, broadcasting insecticides to suppress mosquitoes was widespread. Patterson et al. (1980) described a field trial to control the stable fly

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*Stomoxys calcitrans* (L.) with the SIT as an adjunct to insecticidal and physical methods. Insecticides were also applied for the control of tsetse flies in Africa; applying insecticides against these species was a component of the programmes as they developed.

According to Douthwaite (1992), the first area-wide attempts to control tsetse flies with insecticide sprays began in 1945 in South Africa using organochlorine insecticides. This programme resulted in the successful elimination of *Glossina pallidipes* Austen from 11 000 $km^2$ through the aerial application of DDT or lindane, and supported by game destruction, habitat clearing and massive trapping operations (Du Toit 1954). Although the negative effect of these treatments on beneficial insects was recognized, and an impact on bird populations was reported by Graham (1964), persistence and bioaccumulation of residues was not understood at the time.

The first tsetse programme using sterile mass-reared insects was carried out against *Glossina morsitans morsitans* Westwood in Tanzania (Dame et al. 1980; Williamson et al. 1983). The strategy of this test was to suppress the tsetse population with two aerial applications of endosulfan (28-day interval), and then control the population with sequential releases of sterile males. A 195-$km^2$ area was surveyed for 14 months using various trapping methods, and the reproductive status and density of the population were assessed. A 105-$km^2$ area was selected for treatments. A 1-km barrier was cleared and treated with manual backpack spray applications of DDT in a 300-m-wide swath to prevent the migration of flies into the treated area. Fly surveys showed that, after the first endosulfan application, there was nearly 100% reduction in *G. m. morsitans* and 91.5% reduction in *G. pallidipes*. Following the first spray, sterile *G. m. morsitans* males were released twice per week at a rate of 135 males per $km^2$, resulting in an average male sterile:wild ratio of 1.12:1. A comparison of the female reproductive status between control and treated areas showed that, following the second spray, the sterile males were highly effective. Over the 15-month sterile-treatment period, Williamson et al. (1983) reported an 81% reduction in *G. m. morsitans*. The population of *G. pallidipes*, which received only the insecticide treatment, recovered to pre-spray levels within 5 months.

The sequential aerosol technique (SAT) involves spraying ultra-low-volume formulations of insecticides from the ground (fogging) or air (fixed-wing aircraft or helicopter) with limited environmental impact. The goal is to kill adult tsetse flies in the first spraying cycle by direct contact, and kill emerging flies in subsequent cycles. Recently, the SAT has been used against *Glossina palpalis gambiensis* and *Glossina tachinoides* Westwood, and achieved reduction levels higher than 98% (Adam et al. 2013). Against savannah species in an open environment, it is even more efficient, and achieved complete elimination of *Glossina morsitans centralis* Machado (Kgori et al. 2006). The SAT might represent a rapid solution to reduce tsetse populations before releasing sterile males, provided that habitat specificities and individual insecticides sensitivities are taken into account (De Deken and Bouyer 2018).

The need for innovative tools in managing mosquitoes was recently pointed out by the Global Vector Control Response 2017–2030 (WHO 2017). This includes suppression tools in the case of *Aedes aegypti* (L.) and *Ae. Albopictus*; at present there are only limited possibilities because of their disseminated larval microhabitats. For species living in large water habitats, spraying *Bacillus thuringiensis* var. *israelensis* (*Bti*) is considered to be the most efficient larvicide strategy to control mosquito

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populations with negligible environmental impact. For example, it is used widely in French Atlantic and Mediterranean coastal wetlands (Lagadic et al. 2014).

Pal and LaChance (1974) reviewed early trials of genetic control during the late 1960s and 1970s. They cite the need for supplemental suppression activities to reduce the number of released sterile insects that are required. They suggested that, if insecticide-based control methods are to be applied concurrently with the release programme, the best chemical control would be a larvicidal programme that killed target insects without affecting released insects. If a pre-release suppression programme is used, then an adulticide treatment would be preferable.

Weidhaas et al. (1962), Morlan et al. (1962), and Patterson et al. (1970) performed field trials with sterilized mosquitoes in Florida, USA. In these tests DDT applications were made in perimeter areas to prevent the immigration of pests or as treatments to reduce populations. Patterson et al. (1970) concluded that:

> Obviously, other population suppressants such as insecticides, reduction in breeding sources, and biological control will have to be used to decrease a total population in a large area to a level commensurate with the mass-rearing capabilities.

Trials of genetic control of *Culex pipiens fatigans* Wiedemann in villages near Delhi, India, in a programme developed with the World Health Organization (WHO), were among the first to apply insecticides as part of an integrated supplemental population suppression experimental approach. After several attempts to introduce sterility into the populations (reported from 1971 and 1972 tests with negative results), tests were designed to distinguish the effects of rearing, chemosterilization and strain genetics from the effects of strain contamination with females and immigration (Pal 1974; Pal and LaChance 1974). Yasuno et al. (1976) reported the results of a 5-month sterile insect release (February to July 1973) of chemosterilized males into an area with mosquito fish *Gambusia affinis* (Baird and Girard) or larvicide-treated (temephos) breeding sites in buffer zones. A second treatment of an adulticide, pyrethrum, was applied to one set of villages. Although the Delhi programmes, summarized by Pal (1974), Yasuno et al. (1976) and Curtis (1977), were not permitted to continue to the stage of measuring population suppression, the programmes proposed in these tests included population monitoring and integration of adult and larval chemical control. Curtis and Andreasen (2000) cited the importance of insecticide-based barriers to females immigrating into areas treated for mosquito control. Immigrant females not only serve to increase the target population and impede eradication, but may also reintroduce the disease and set back the ultimate goal of eradication.

SIT treatments for *Anopheles* spp. will need to consider the increased resistance to pesticides (Mattingly 1957; Pal and LaChance 1974; Asman et al. 1981; Whalen 2002; Ranson et al. 2011). Alternatives (to the general spray programmes applied in previous mosquito SIT trials to suppress adult populations) are required in any new control programme -- preferably classic alternate methods of population reduction (Ross 1902) such as habitat modification, and more recent alternative methods both to reduce mosquito populations and protect people from bites and disease.

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### *4.2. Stationary Devices (Traps and Insecticide-Impregnated Targets)*

The goal of stationary attractive devices such as traps and insecticide-impregnated targets is to impose a modest daily mortality on tsetse females by attracting them to a device that either kills the flies by contact with an insecticide or retains them in a non-return cage. Pyrethroid compounds were identified as the principal insecticides used but sterilizing compounds, and compounds that inhibit reproduction such as triflumuron, may also be effective (Oloo et al. 2000). A review of using insecticides in traps and targets against tsetse is found in Bouyer and Vreysen (2018).

The deployment of insecticide-impregnated targets, and the release of sterile males, successfully eliminated *G. palpalis gambiensis*, *G. tachinoides*, and *Glossina morsitans submorsitans* Newstead from 3500 km$^2$ of agropastoral land in Burkina Faso (Politzar and Cuisance 1984). Prior suppression of the native fly populations was achieved by placing insecticide-impregnated screens along 650 km of gallery forest at a density of 10 screens per linear km for 4 months during the dry season. Subsequent releases of sterile males, at the rate of 20–35 per linear km, were sufficient to obtain sterile:wild ratios of 10:1 and to eliminate the target populations.

A trial against *Glossina palpalis palpalis* Robineau-Desvoidy was carried out in central Nigeria. The population was first reduced by deploying insecticide-impregnated screens and by removal-trapping with traps (Oladunmade et al. 1985; Takken et al. 1986) that reduced the native fly population by 90–99% over a 6–12-week period. Extending the period of control, using traps and targets, did not achieve eradication. A further major concern was the loss of the screens due to theft, flooding, and fire (Takken et al. 1986). Nevertheless, the target population was eventually eliminated over the entire 1500-km$^2$ area by weekly releases of sterile male flies from the ground (Oladunmade et al. 1990).

Recently, in Burkina Faso, insecticide-impregnated targets, in combination with pour-on treatments of livestock and ULV spraying of the gallery forests, enabled a reduction of 83% for *G. palpalis gambiensis* and a 92% reduction for *G. tachinoides* (Percoma et al. 2018). Also, recently in Senegal, the use of insecticide-impregnated targets set in suitable landscapes at a density of 1–3.4 per km$^2$ reduced populations of *G. p. gambiensis* by more than 95%; this was followed by releasing sterile males to reach population elimination (Dicko et al. 2014).

For fruit flies, because broadcast insecticides were not acceptable, Mangan and Moreno (2007) tested various baits in stations to suppress *Anastrepha ludens* populations. Besides a toxicant, baits contained attractants, feeding stimulants and other materials to help preserve the effectiveness of the bait over time.

Regarding mass-trapping for pest suppression, to be effective trap densities must be very high. The deployment and maintenance of large numbers of trapping devices is costly and logistically complex, and thus mass-trapping is generally not practicable or economically viable over large areas, and may be applicable only in special areas where other suppression approaches are not possible (Navarro-Llopis and Vacas 2014). Furthermore, two issues must be addressed when deploying traps at high densities for population suppression: (1) direct effects on non-target animals, and (2) indirect environmental effects related to trap placement and servicing (Nagel and Peveling, this volume).

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### *4.3. Pour-Ons — Insecticide Applied to Moving Baits*

Live-bait technology (pour-ons) is an efficient technology for tsetse flies, stable flies, and other nuisance pests or disease-transmitting vectors in infested areas with a high density of cattle, but the disadvantages are the high frequency of treatment, the high cost of insecticides, and the impact on the dung fauna (Vale et al. 2004).

A programme to control tsetse flies or trypanosomosis by treating livestock with insecticides can be effective by killing the flies as they attack animals. Leak (1999) noted that three conditions are required to achieve optimum control of tsetse populations through pour-on treatments: (1) a large proportion of feedings are taken from domestic rather than wild animals, (2) a large proportion of the livestock are treated, and (3) the level of fly reinvasion is relatively low. Leak also reviewed the use of artificial odours, colours, or targets attached to workers. Targets on workers were a component of the eradication of tsetse in Principe in 1910 (Hendrichs, Enkerlin et al., this volume; Klassen et al., this volume), and a tsetse-control technique using odour attractants and traps was proposed by Balfour (Balfour 1913).

Initially, pour-on insecticides consisted of DDT mixed with resins (Leak 1999). Other tests were done feeding lindane to cattle. Although applying these pesticides to cattle was terminated because of environmental concerns, the development of synthetic pyrethroids revived this treatment method (Bauer et al. 1992). These insecticides have the advantages of low human toxicity, high insect toxicity (especially to tsetse flies), and rapid movement through the epidermis. Ivermectin compounds were also discussed, but the effective dose is very close to the limit for toxic effects on the hosts, and cost is prohibitive (Pooda et al. 2013).

The eradication programme against *G. austeni* on Unguja Island (Zanzibar) was specifically planned to meet environmental concerns for supplementary population control (Vreysen et al. 2000). It was found that the pour-on treatment alone was not sufficient to eradicate the tsetse population in the island. Maybe this was due to flies feeding on hosts other than cattle, such as bush pigs, which enabled some flies to be unaffected by the cattle treatment. The programme relied on the use of live-bait technology (in areas of high cattle density), and the deployment of insecticide-impregnated screens (in the forested areas), to reduce the native tsetse population before releasing sterile males (Vreysen et al. 2000). The fly densities in the primary forest habitats were reduced 80–98% by using insecticide-impregnated screens, deployed at densities of 40–70 per $km^2$ for a period of 18 months (Vreysen et al. 1999). The same strategy was applied recently in Senegal (Vreysen et al. 2013).

In Burkina Faso, the application of deltamethrin to cattle failed to eradicate *G. tachinoides* because the preferred hosts, monitor lizards, were available for feeding by tsetse (Bauer et al. 1999). Apparently pour-on insecticides can reduce tsetse fly populations drastically, but untreated wild animals may serve as alternate food sources for the sustenance of the population (Bouyer et al. 2013).

### *4.4. House, Bednet, and Other Treatments*

The use of insecticide-impregnated bednets has proven to be a successful treatment to reduce malaria morbidity. The development of pyrethroid insecticides, that are safe

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for human contact, has provided a substitute (for DDT, organophosphate and carbamate insecticide treatments) that gives more direct protection than outdoor sprays to control populations. According to Curtis (2002), treated nets can irritate, drive away or kill biting mosquitoes. Numerous tests have shown that this treatment greatly reduces both populations of mosquitoes and rates of disease. The use of insecticide-treated bednets, as well as treatment of curtains, wall hangings and clothing, have also been tested, but bednets, which act as a trap baited with the sleeping person, have proven more effective.

More than 20 tests of bednets have demonstrated a reduction of 20–63% in malaria disease rates. Tests carried out in The Gambia, Kenya, and Ghana showed a significant (25–39%) reduction in mortality of children. Mathenge et al. (2001) found that bednets reduced the rate that some mosquitoes (but not others) entered houses, and the action of bednets against mosquitoes was also species-specific. Slight shifts in feeding times were also noted for one species (but not the other). The success of bednets is reduced for mosquito species, such as *Aedes* spp., that bite earlier in the day. Treatment of other things in a household, and indoor spraying, may help control disease transmission by these species. However, it must be noted that the efficiency of these strategies tends to decrease with the spread of resistance in malaria vectors (Ranson et al. 2011). They are also challenged by the development of behavioural resistance, like outdoor feeding (Russell et al. 2011), hence the necessity of developing alternative methods (WHO 2017).

### *4.5. Chemical Treatments to Protect Hosts from Biting Adults or Immature Stages*

Wound treatments have been an important and consistent part of the New World screwworm programme in North America (Graham 1979). In addition to reducing overall screwworm populations and protecting livestock from larval damage, the research programme to develop wound dressings had direct effects on the programme. The early development of a treatment called “Smear 62” (Knipling 1939) led to research methods that included rearing larvae on artificial diets. Not only are wound treatments an essential supplementary component of this programme that releases sterile insects, but research in developing these treatments also led to the implementation of the programme.

The use of small packets of coumaphos, chlorfenvinphos or similar insecticides, applied either as a spray on cattle or as an individual treatment to infested wounds, was an essential part of the screwworm eradication programme in Florida, southern Texas and Latin America. Unlike attempts to trap out or reduce adult screwworm populations by applying insecticides area-wide, larvicidal applications of insecticide directly saved livestock, and producers could easily observe the application’s benefit. During the breakdown of the programme in 1972, larvicide treatments were the main mechanism that saved livestock and slowed the spread of cases. During the late 1970s, when the programme was stalled in northern Mexico (Coppedge et al. 1980a), the lack of progress was attributed to both ineffective sterile flies and the lack of animal protection by livestock producers.

The efforts of the Mexican-American programme to use the coumaphos packets in conjunction with releasing sterile insects were very successful. Through grower

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education and publicity, producers were informed that these packets were provided by the inspectors of the programme. The ability to provide producers with an effective and free treatment for infested cattle was surely a major factor in gaining access to ranches in Mexico and Central America. Historically and culturally, ranches in these regions were not open to outsiders.

### *4.6. Insecticide Baits*

An area-wide insecticide-bait treatment to control screwworm adults was developed in the 1970s, in conjunction with activities improving baits for monitoring populations. Mackley and Brown (1985) reviewed the development of swormlure, an attractant, and SWASS (Screwworm Adult Suppression System) pellets. In Texas, screwworms were believed to migrate hundreds of kilometres, so extremely large plots were needed to avoid the confounding effects of flies migrating into the treated areas.

SWASS pellets were tested, through "before and after treatment" observations, in Curaçao, Texas (Coppedge et al. 1980b), Colima (Tannahill et al. 1982), and Veracruz (Spencer and Garcia 1983). The bait swormlure was used to attract and sample adult screwworm flies. Wounded animals were used to collect eggs, and thus sample the reproductive capacity of the fly population. The general pattern in the experiments was a reduction in populations trapped and in reproduction. It was concluded that swormlure and SWASS were attractive and toxic, respectively. However, since the experiments were not replicated, no statistical conclusions about the effects of the SWASS treatments can be drawn. Although the baits reduced the total population, they may not have reduced the reproductive potential of localized populations. Another consideration is that, in wet areas such as Veracruz, the formulation of the SWASS pellet was such that it dissolved when wet (Mackley and Brown 1987), and the pellets may not have persisted long enough to reduce the populations.

Moreno and Mangan (1995, 2002) and Piñero et al. (2014) reviewed the development of improved insecticide baits for fruit flies. At the beginning of the 20th century entomologists discovered that fruit flies would feed on toxic chemicals contained in baits composed of various sugars. In search of an insecticide programme for the control of the recently established Mediterranean fruit fly in Hawaii, Back and Pemberton (1918) reviewed the research status of edible baits for use with arsenic poisons. The principal baits were carbohydrates and fermenting substances such as sugars, molasses, syrups, and fruit juices. McPhail (1937) found that sugar-yeast solutions attracted several species of *Anastrepha* and, in 1939, found that protein lures were attractive to these species. In 1952 Steiner demonstrated the use of hydrolysed proteins and partially hydrolysed yeast in combination with organophosphate insecticides to control fruit flies, leading to the attracticides currently used. The first protein-hydrolysate baits contained protein hydrolysate, sugar, and parathion (Steiner 1952). The early fruit fly eradication programmes in the USA relied on attracticide baits using DDT or organophospate pesticides. Flies responding to the attracticide needed only fume exposure, or to contact, taste, or ingest the mixture, whereupon in a short time they died.

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Bait formulations that meet both the attraction and gustatory requirements of the pest permit the use of a wide range of contact and stomach insecticides (Moreno and Mangan 1995, 2002). The concentration of the active ingredient in bait can be reduced more than 90%. To be active, the formulations require consumption, either because the toxicant cannot penetrate, or the concentration is not sufficient to penetrate, the insect cuticle. Other important components include conditioners such as oils, humectants, and adjuvants. These components protect the spray drops from evaporation and running off vegetation, help keep the drops wet for fly ingestion, and enhance the toxicity of the insecticide. Mangan and Moreno (2001) showed that a series of commercial adjuvants varied widely in their interaction with dyes and fruit fly mortality, and under field conditions adjuvants could significantly increase bait effectiveness by about 30%.

A series of insecticides was tested in the laboratory with SolBait formulated for tropical fruit fly control (Moreno and Mangan 2002). In that study, 16 insecticides, with mammalian toxicity values at least 40x lower than malathion, were identified. As part of this study, Moreno and Mangan developed, and adopted for commercial use, spinosad for fruit fly control. This spinosad-based toxic bait, currently marketed as GF120, was formulated with proprietary modifications by Dow Agrosciences to optimize attraction, edibility, and stability. In addition, since spinosad is derived from naturally occurring soil bacteria, *Saccharopolyspora spinosa* Mertz and Yao, after being combined with a selected series of bait components, the product was eligible for organic registration, and is now used widely.

Benavente-Sánchez et al. (2021) indicated that drones could be used to apply bait sprays against fruit flies in "hot spots" and larvicides against mosquito breeding sites.

### *4.7. Autodissemination Techniques*

A founder trial by Devine et al. (2009) against *Ae. aegypti* in an Amazon city (Iquitos, Peru) showed that adult mosquitoes might be used, as vehicles for insecticide transfer by harnessing their fundamental behaviours, to disseminate a juvenile hormone analogue (JHA) between resting and oviposition sites. Setting up JHA dissemination stations, in 3–5% of the available resting area, resulted in increased larval mortality in 95–100% of the larval cohorts developing at those sites. During these trials, overall reductions in adult emergence of 42–98% were achieved. The method has since been validated against various *Aedes* species, but to reach a good level of suppression, it is quite expensive; due to low attractiveness, a high density of dissemination stations is needed.

It has been suggested that released sterile male mosquitoes could be used as vehicles of JHA or other biocides (Bouyer and Lefrançois 2014); this was tested successfully on a small scale in Kentucky (Mains et al. 2015).

The autodissemination of entomopathogenic fungi in fruit flies is under study (section 7); this might represent an effective suppression technique compatible with the SIT (Dimbi et al. 2009; Flores et al. 2013; Toledo et al. 2017).

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## 5. BEHAVIOURAL MODIFICATION WITH CHEMICALS

The use of pheromones for the detection and management of lepidopterous pests has become a standard procedure. Tamaki (1985) summarized the chemistry and application of pheromone technology for pest management. The chemical structures of pheromone compounds are known for 160 lepidopterous species in 20 families. Pheromone technology could be applied in three ways (Tamaki 1985):

- Monitoring and surveying for early detection of introduced exotic insects, forecasting pest outbreaks, and estimating population density,
- Mass-trapping for population suppression and detailed monitoring,
- Communication disruption to inhibit mate-finding and suppress the population.

Cardé and Minks (1995) reviewed the use of pheromones for mating disruption as a pest control strategy. Success with this strategy has been restricted to moths that have a mating behaviour that involves males following a pheromone plume as the principal means to locate females. Cardé and Minks described a series of modes of action that results in mating disruption, including effects on the sensory mechanisms of the target males, and control of behaviour and orientation. They described programmes for 9 pest moth species that have successfully used mating disruption, and 14 additional species that, at that time, had formulations available. An example of combining mating disruption with the SIT is the use of gossyplure in the pink bollworm control programme in the south-western United States (Walters et al. 2000a; Staten and Walters 2021; Simmons et al., this volume).

Gossyplure is a mixture of two isomers of 7,11-hexadecadienyl acetate (Hummel et al. 1973). This mixture was shown to be the effective attractant, with more than 56 times the attraction to males than hexalure (cis-7-hexadecenyl acetate) or than the less effective propylure mixtures reported to be sex pheromones (Jones et al. 1966; Jones and Jacobson 1968; Keller et al. 1969). Flint et al. (1974) used gossyplure for monitoring early season populations. The delta trap, previously used to control the gypsy moth *Lymantria dispar* (L.), was shown by Foster et al. (1977) to be a superior *P. gossypiella* monitoring tool. The first registered use of a pheromone to control the pink bollworm through mating disruption was developed by Gaston et al. (1977) in tests carried out in Arizona and California, USA.

Jenkins (2002) discussed current commercial formulations of gossyplure and modes of action of disrupting pink bollworm mating. Formulations exist as three types: (1) reservoir type such as the PB-ROPE L, which is containerized into a plastic tube or band, has a long field life (60–90 days), and is applied at 250–1000 units per hectare, (2) a low-rate, female equivalent, sprayable product has a field life of 7–21 days, contains an insecticide additive, and is contained in a paste, flake, or hollow fibre at 750–32 000 units per hectare, and (3) a low-rate, microdispersible system that can be applied as a fog or in a capsulated form, and has a field life of 7–28 days.

In addition to the application of insecticides against the codling moth, one of the pest suppression methods used in the Okanagan-Kootenay Sterile Insect Release (OKSIR) Program in British Columbia, Canada, was mating disruption (Judd et al. 1992; Dyck et al. 1993; Bloem et al. 2001; Nelson et al. 2021; Simmons et al., this volume). The integration of mating disruption and the SIT has also been successfully implemented against the codling moth in Washington State, USA, and south of the Canadian/USA border (Calkins et al. 2000). In organic orchards in British Columbia,

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Judd and Gardiner (2005) showed that within several years these two measures, coupled with removing overwintering larvae using cardboard tree bands (mechanical control), suppressed the codling moth population to non-detectable levels. These findings, as well as the integration of mating disruption as part of the successful pink bollworm eradication, point to a favourable interaction of mating disruption with the SIT, but precisely how these two approaches complement one another remains to be determined (Suckling 2011; Cardé 2021).

Parapheromones or synthetic lures, such as trimedlure, ceralure, cuelure, and in particular methyl eugenol, are effective attractants for fruit fly males (Cunningham 1989), and can be deployed in traps, panels or blocks from the ground by placing on host trees, or nailing to posts. Alternatively, insecticide-treated baited blocks (wood chips) or wicks can be released from aircraft for area-wide population suppression (Vargas et al. 2014). Such male annihilation technique (MAT) campaigns can be applied alone, prior to the release of sterile males, or more effectively simultaneous with the release of the males (Barclay et al. 2014).

More recently, it was proposed that sterile male fruit flies, e.g. Mediterranean fruit flies, could be used as pheromone sources to implement mobile mating disruption against moths, e.g. light brown apple moths (Suckling et al. 2011), a strategy called "ménage-à-trois" (Suckling et al. 2007b).

## 6. RESISTANT PLANTS

The development of cotton cultivars resistant to the pink bollworm has been a long-term component of the pest management programme. Nobel (1969) reviewed the characteristics of the components of resistance used in breeding programmes initiated in the 1950s. The cultivars screened, *Gossypium thurberi* Todaro and *G. thurberi* x *Gossypium hirsutum* L., were recognized as the least attractive for oviposition. Pink bollworm larvae attacking these varieties experienced reduced larval survival and lengthened development time, apparently due to a protective response by the seeds.

Characters of varieties that induced oviposition away from the bolls exposed larvae to increased contact with pesticides and increased predation or parasitism. Boll, stem, and leaf morphologies that reduced oviposition were developed, and also nectariless cultivars to reduce the supply of food for adults. Although these characters appeared to reduce survival or oviposition, field trials did not show an increase in protection from attack. Increases in gossypol in the plant, though reducing pink bollworm survival, rendered the seed unusable for animal feed and the seed oil unusable for human food. Wilson et al. (1992) reported a 36% reduction in seed damage in a breeding line with a combination of nectariless, okra leaf, and early maturity; nevertheless, it still required insecticide treatment to control the pink bollworm.

Transgenic cotton varieties were developed by Monsanto, and released in the early 1990s. Tests in Arizona by Wilson et al. (1992) established that the pink bollworm adult populations were greatly reduced in transgenic cotton plots. The number of pests per 100 bolls was 87.5 for control varieties, and only 0.2 for transgenic varieties. Seed damage was similarly reduced in transgenic plots (0.14%) compared with 4.83% damage in control varieties. In summary, Wilson et al. reported that, in transgenic

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compared with susceptible lines, there was a reduction of 95–99% in rosetted blooms, pink bollworm populations in the bolls, and seed damage.

In China, the widespread adoption of *Bt*-cotton resulted in reduced insecticide sprays in this crop, leading to a marked increase in abundance of three types of generalist arthropod predators (ladybirds, lacewings, and spiders), and a decreased abundance of aphid pests in cotton fields and also in neighbouring crops (maize, peanut, and soybean) (Lu et al. 2012).

In the areas applying the SIT against the pink bollworm, the use of transgenic cotton has positively affected programme execution (Walters et al. 2000a, b). Already in 1997, 81% of the plantings in the Imperial Valley were genetically engineered cotton. As a result, Walters et al. (2000a) evaluated the possibility of moving from the containment to an eradication programme by integrating engineered cotton with the SIT and other control methods. They divided the components of eradication into five treatments — sterile insect release, genetically engineered cotton, high-rate pheromone release, mid-season pheromone application, and monitoring. Eradication was only possible by including all adjacent cotton growing areas in California, Arizona, and New Mexico in the USA, and in Baja California Norte, Chihuahua, and Sonora in Mexico (NAPPO 2004; Staten and Walters 2021).

By targeting the larval stages of the pest, this strategy is highly compatible with the SIT. A good example of using genetically modified crop plants that express a toxin is the area-wide programme against the pink bollworm in south-western USA and north-western Mexico. It demonstrated that planting *Bt*-cotton could effectively suppress the native moth population to a level where SIT application became very cost-effective, and enabled progress towards an eradication programme (Tabashnik et al. 2010). At the same time, releasing sterile pink bollworm moths was a viable alternative to the official refuge strategy for preventing the development of resistance to *Bt*-cotton; the sterile moths mated with the rare resistant insects, leaving no offspring, thereby delaying the evolution of *Bt* resistance (Wu 2010). As a result, susceptibility to *Bt*-cotton did not decrease in the target pink bollworm population, insecticide sprays against this pest were completely eliminated, and the density declined dramatically, leading eventually to regional pink bollworm eradication (Staten and Walters 2021).

## 7. BIOLOGICAL CONTROL

If natural enemies targeting the immature stages of a pest are released, the efficacy of sterile insects is enhanced because the number of pest insects reaching the adult stage is reduced; this increases the sterile:fertile overflooding ratio. Therefore, associating the SIT with biological control can result in synergistic associations (Suckling et al. 2012).

The integration of augmentative parasitoid releases and inherited sterility is especially synergistic (Carpenter et al. 2004; Carpenter 2013). When parasitoids develop normally on $F_1$ eggs, larvae, and pupae that result from the release of substerile moths (Marec et al., this volume), the increased number of hosts available will permit an increase in the parasitoid population. As most of the $F_1$ generation will not reach the adult stage, any parasitoids completing their development in these hosts

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will increase the efficacy of an area-wide programme. Most importantly, $F_1$ hosts can enable natural enemies to survive during critical times of low pest population, thereby increasing natural enemy populations prior to the time when the target pest reaches its economic threshold (Carpenter 2013).

Laboratory and field evaluations of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Hypocreales) have shown that these entomopathogenic fungi have the potential to suppress fruit fly populations (Ekesi et al. 2002; FAO/IAEA 2019; Abd-Alla et al., this volume). In recent large field trials on the Mediterranean fruit fly, sterile males, inoculated with *B. bassiana* as vectors to spread the fungus into the target pest population, were released (Toledo et al. 2017). By the end of the release period, the wild population had been reduced by more than 90% when compared with non-treated areas. If infected sterile males live long enough, even if sexual encounters do not result in matings, there might be horizontal transmission at leks (male aggregations visited by receptive females) that will result in the death of wild males and females; the fungus will also prevent infected wild females from remating and reproducing with wild males. Since *B. bassiana* is a generalist fungus, potentially infecting a wide range of arthropods, the environmental impact of such releases will need to be assessed further. Nevertheless, Flores et al. (2013) showed that the release of sterile flies as vectors is highly specific, and did not cause infection in bees or coffee berry borers *Hypothenemus hampei* (Ferrari); only trace quantities of conidia are required to inoculate sterile flies (0.0001 g per fly). The combined methods would be particularly useful for fruit fly population suppression during the rainy season in the tropics, when ground and aerial insecticidal sprays become largely ineffective (Toledo et al. 2017).

A similar strategy, coating sterile male *Aedes* mosquitoes with specific Densovirus, is presently under study (Bouyer et al. 2016). Also, *Bt* can be applied during the release of sterile insects to suppress a target population of a moth (Suckling et al. 2007a).

## 8. SUPPLEMENTARY TREATMENTS IN STERILE INSECT RELEASE PROJECTS

Releasing sterile insects in an area-wide pest control programme requires that the target population be isolated from adjoining populations, and that the target population is sufficiently reduced so that a high enough ratio of sterile to fertile matings inhibits reproduction. Current programmes achieve isolation by relying on combinations of quarantine barriers, geographic barriers, or treatment of buffers or barriers at the target population's margins (Hendrichs, Vreysen et al., this volume). To achieve the needed overflooding ratio, suppression/eradication programmes usually also require sufficient sterile insect production in conjunction with pest population suppression.

In addition to a lack of immigration and sufficiently high sterile:fertile ratios, other factors are required to achieve success in suppression or eradication programmes using the SIT. A key component of successfully applying pest management techniques is the effectiveness of the application in preventing pest damage. Of course, reducing pest populations by reducing their reproductive potential eventually

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reduces damage. However, sterile matings with the pests reviewed above do not kill the pest. Therefore, economic losses continue -- arising from reproduction by wild females mated before sterile insect release, and the continued biting and disease-transmission by infected blood-feeding females, independent of their sterile or fertile mating status.

The major supplementary treatments reviewed above provide direct and immediate reduction of crop damage by the pest or disease transmission by the vector, and are particularly needed before and during the first generation of sterile insect releases. Protection from damage by pests is practiced whether the programme releases sterile insects or not, so these treatments are usually widely applied components of pest suppression programmes. However, when these treatments are practiced in areas where the recipients (environmental and human) receive no direct benefits, as frequently must be done in AW-IPM programmes that integrate the SIT, the programmes can face social and political opposition, unless public opinion is preventively managed (Dyck, Regidor Fernández et al., this volume).

Target population suppression activities supplemental to sterile insect releases tend to be methods that were developed to control the pest populations or prevent damage from the pests, independent of sterile insect releases. Public acceptance of pest management programmes is best related to the benefits derived directly and indirectly from the activities, but the success of suppression or eradication efforts is dependent on pest population reduction (Klassen and Vreysen, this volume).

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# CHAPTER 4.3.

# PRACTICAL AND OPERATIONAL GENETIC SEXING SYSTEMS BASED ON CLASSICAL GENETIC APPROACHES IN FRUIT FLIES, AN EXAMPLE FOR OTHER SPECIES AMENABLE TO LARGE-SCALE REARING FOR THE STERILE INSECT TECHNIQUE

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## SUMMARY

Through genetic and molecular manipulations, strains can be developed that are more suitable for the sterile insect technique (SIT). In this chapter the development of genetic sexing strains (GSSs) using classical genetic approaches is described, while the development of GSSs using molecular approaches is discussed by Häcker et al. (this volume). GSSs increase the effectiveness of area-wide integrated pest management (AW-IPM) programmes that use the SIT by enabling the large-scale production and release of only sterile males, and generally increasing sterile-male effectiveness when competing for wild females in the absence of sterile females. For species that transmit disease, the removal of females is mandatory. GSSs have been developed for several species, including Tephritidae and mosquitoes. For the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and the Mexican fruit fly *Anastrepha ludens* (Loew), genetic sexing systems have been developed, and they have been shown to be stable enough at large-scale mass-rearing levels to be used in operational programmes for extended periods of time. In this chapter, the basic principle of translocation-based sexing strains is described, and Mediterranean fruit fly and Mexican fruit fly strains are used as examples to indicate the problems encountered in developing and using such strains. Furthermore, the strategies used to solve these problems are described.

## 1. INTRODUCTION

The sterile insect technique (SIT) is an increasingly important component of area-wide integrated pest management (AW-IPM) programmes for certain key insect pest species (Table 1). The application of the SIT in operational programmes, and its expansion to additional pest species, continues to reveal areas where technology can improve the SIT. An example of using improved technology is the transfer of genetic sexing technology to Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Rendón et al. 2000, 2004), and more recently to the Mexican fruit fly *Anastrepha ludens* (Loew) (Orozco et al. 2013; Zepeda-Cisneros et al. 2014) programmes. In future, in view of their important benefits, it is expected that such strains will be required for many other insect species of agricultural, veterinary or human health importance. Beyond application for the SIT, genetic sexing technology has been applied to natural enemies and to the silkworm *Bombyx mori* (L.). In the latter case, males produce more silk. W-autosome translocation sexing strains, based on a cocoon marker, were developed (Nagaraju 2002) and, in India, are reared at a male-only production level of 125 million per week (J. Nagaraju, personal communication).

Based on the reproductive biology of most insects, and on the basic principle underlying the SIT, it is clear that generally only released sterile males are important for this technique to be effective. Wild females determine the population size of the next generation, but wild males, or more precisely the sperm they produce, are present in such great excess that it would be necessary to remove about 99% of all wild sperm before obtaining a significant reduction in the size of the next generation (Koyama et al. 1984; Barclay and Hendrichs 2014). Sterility is introduced into a wild population only through sterile males, even when they are released together with sterile females. Recognizing this principle, the SIT was initially called the “sterile-male method” (Knipling 1959). However, at that time, the only AW-IPM programme integrating the SIT was against the New World screwworm *Cochliomyia hominivorax* (Coquerel),

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and both sexes were released. The name was meant to highlight the fact that only the sterile males are the active agent in the SIT. As demonstrated very clearly for the Mediterranean fruit fly by McInnis et al. (1994) and Rendón et al. (2000, 2004), bisexual releases are far less effective than male-only releases in introducing sterility into a wild population, and this will probably apply to many pest species. The obvious reason is that the released sterile males and females tend to mate with each other. As a result, the proportion of matings between sterile males and wild females is reduced, and less sterility is introduced into the wild population.

*Table 1. Approximate worldwide maximum mass-rearing capacities for SIT application achieved at one time for different species (some data from FAO/IAEA DIR-SIT 2020)*

| Species | Maximum production reached in millions per week | Genetic trait that defines the sexing strategy |
|---|---|---|
| **Screwworm flies** | | |
| New World screwworm *Cochliomyia hominivorax* (Coquerel) | 500 | No |
| Old World screwworm *Chrysomya bezziana* (Villeneuve) | 6 | No |
| **Fruit flies** | | |
| Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) | 3500 (only males) | Y-autosome translocation, using a *temperature-sensitive lethal* (*tsl*) |
| Mexican fruit fly *Anastrepha ludens* (Loew) | 250 (only males) | Y-autosome translocation, pupal colour separation |
| Melon fly *Zeugodacus cucurbitae* (Coquillett) | 200 | No (Y-autosome translocation, pupal colour separation[1]) |
| West Indian fruit fly *Anastrepha obliqua* (Macquart) | 60 | No |
| Oriental fruit fly *Bactrocera dorsalis* (Hendel) | 50 | No (Y-autosome translocation, pupal colour separation[2]) |
| Queensland fruit fly *Bactrocera tryoni* (Froggatt) | 15 | No[3] |
| *Bactrocera carambolae* Drew and Hancock | | (See Häcker et al., this volume) |
| Sapote fruit fly *Anastrepha serpentina* (Wiedemann) | 5 | No |
| South American fruit fly *Anastrepha fraterculus*(Wiedemann) (species 1) | 2 | No (Y-autosome translocation, pupal colour separation[4]) |
| Olive fruit fly *Bactrocera oleae* (Rossi) | <1 | No |
| Malaysian fruit fly *Bactrocera latifrons* (Hendel) | <1 | No |

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*Table 1. Continued*

| Species | Maximum production reached in millions per week | Genetic trait that defines the sexing strategy |
|---|---|---|
| **Onion maggot** | | |
| Onion maggot *Delia antiqua* (Meigen) | 7.5 | No |
| **Moths** | | |
| Pink bollworm *Pectinophora gossypiella* (Saunders) | 150 | No |
| False codling moth *Thaumatotibia leucotreta* (Meyrick) | 30 | No |
| Codling moth *Cydia pomonella* (L.) | 14 | No |
| Gypsy moth *Lymantria dispar* (L.) | 1 | No[5] |
| Carob moth *Ectomyelois ceratoniae* (Zeller) | <1 | No |
| **Tsetse flies** | | |
| *Glossina pallidipes* Austen | <1 | No (Manual, sex-specific, time of emergence[6]) |
| *Glossina palpalis gambiensis* Vanderplank | <1 | No (Manual, sex-specific, time of emergence[6]) |
| *Glossina fuscipes fuscipes* Newstead | <1 | No (Manual, sex-specific, time of emergence[6]) |
| **Mosquitoes** | | |
| *Anopheles albimanus* Wiedemann | <1 | Pupal size, Y-autosome translocation, propoxur resistance |
| *Aedes aegypti* (L.) | <1 | No (Sex separation by pupal size) |
| *Aedes albopictus* (Skuse) | <10 | |
| *Anopheles arabiensis* Patton | <1 | Y-autosome translocation, dieldrin resistance |

[1]A sexing strain is available, but is not reared on a large scale (McInnis et al. 2004).

[2]A sexing strain is available, but is not reared on a large scale (McCombs and Saul 1995).

[3]A strain was developed where the females carry a wing mutation (bent wing), and are thereby disabled, so effectively only males are active in the field (Meats et al. 2002). This strain has not yet been used in large-scale programmes.

[4]A sexing strain is available, but is not reared on a large scale (Meza et al. 2019).

[5]IAEA 2008.

[6]Infrared sex separation of pupae for some species; it has not yet been used in large-scale programmes (Dowell et al. 2005).

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To address this problem, it was suggested that strains producing only males should be developed (Whitten 1969; Hendrichs et al. 1995). Besides improved effectiveness in the field, it was expected that mass-rearing costs would be reduced significantly, but this is not always the case (Caceres 2002; Caceres et al. 2004). Nevertheless, significant cost reductions can be achieved in the post-production processes. Only half the volume of pupae is handled for marking, irradiation, transport, emergence, feeding to maturation, and release activities, and the cost of monitoring is reduced significantly, especially in combination with female-specific traps (Epsky et al. 1999; Vreysen, this volume).

Another very important consideration is that, in some cases, sterile females simply cannot be released. Fruit fly females may cause damage from "sterile stings" in certain fruit types, females of biting flies reduce meat production in livestock, and females of bloodsucking species may transmit disease (Lance and McInnis, this volume; Lees et al., this volume).

For a few species, natural characters can be used for large-scale separation of the sexes, e.g. sex-specific pupal-size differences (Dame et al. 1974; Papathanos et al. 2018; Zacarés et al. 2018), and sex-specific differences in the timing of adult emergence, e.g. tsetse fly *Glossina austeni* Newstead (Opiyo et al. 2000; Parker, Mamai et al., this volume). For species where only females suck blood, it is possible to feed them with a toxic substance, and then release the males (Yamada et al. 2013). However, the biology of most species does not permit sex separation on the scale required for the SIT and, therefore, specific sex separation strains have to be developed. To date, this has been achieved using Mendelian genetics, specific mutations, and chromosome rearrangements, as well as, more recently, using molecular-based approaches (Häcker et al., this volume).

Genetic sexing strains (GSSs) have been developed for several species, including the Australian sheep blow fly *Lucilia cuprina* (Wiedemann) (Whitten 1969), and recently the South American fruit flies (Meza et al. 2019), using the same basic principle of sex-specific linkage of a selectable marker. However, only for *Anopheles albimanus* Wiedemann (Seawright et al. 1978; Bailey et al. 1980), *C. capitata* (Franz et al. 1994), and *A. ludens* (Zepeda-Cisneros et al. 2014) were GSSs developed to the point where they could be mass-reared at levels required for AW-IPM programmes integrating the SIT (Table 1), and only in *C. capitata* and *A. ludens* was the sexing system improved enough for large-scale application over extended periods of time.

As described below, constructing a basic sexing strain is rather trivial. However, such "prototype" strains do not normally show the characteristics that are essential for successful long-term integration of the SIT as part of area-wide programmes. These characteristics include aspects of feasibility and economy, i.e. using the appropriate type of sex-separation mechanism, as well as productivity and stability, i.e. the overall genetic structure of the strain. To transfer a sexing system from an experimental laboratory to a mass-rearing facility requires very intensive research into the genetic behaviour of the sexing system. This approach is usually supplemented by incorporating appropriate modifications that provide sustainability for extended periods under large mass-rearing conditions. This requires basic genetic knowledge of the species involved, and until now this has been achieved for only very few pest species.

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Even though GSSs for operational AW-IPM programmes releasing sterile insects are available for the Mediterranean and Mexican fruit flies, nevertheless only the Mediterranean fruit fly strains have been analysed sufficiently to permit general conclusions; this species is referred to extensively in this chapter. It illustrates the problems encountered and modifications introduced to obtain improved sexing strains that are advanced enough to allow a current worldwide production of about 3500 million male flies per week (Table 1).

The lessons learned from this species can very likely be extrapolated to all translocation-based strategies for other species and, to some extent, to new methods using molecular biology. The enormous numbers of insects that must be reared under relatively stressful conditions dictate that even extremely rare genetic or molecular phenomena can threaten the integrity of the sexing system.

## 2. PRINCIPAL STRUCTURE OF SEXING STRAINS

All GSSs developed to date are based on the same principle, and require two separate components (Fig. 1) (Whitten 1969; Franz and Kerremans 1994):

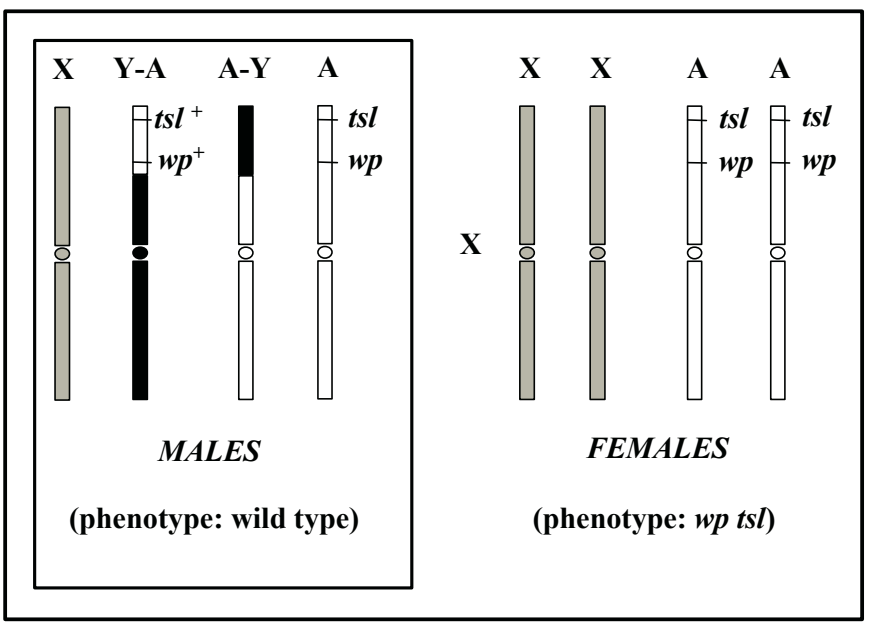



*Figure 1. Basic structure of GSS carrying Y-autosome translocation linking normal "wild-type" alleles of the selectable markers* white pupae (wp) *and* temperature-sensitive lethal (tsl) *to the male sex. Y-A: translocation fragment carrying Y chromosomal centromere, A-Y: reciprocal translocation fragment carrying autosomal centromere, X: X chromosome, A: autosome.*

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- Mutation that can be used as a selectable marker for sex separation (a list of potential selectable markers is given in Table 2 in Robinson 2002),
- Y-autosome translocation to link the inheritance of this mutation to sex.

In the Mediterranean fruit fly, and probably also in many other pest Diptera, the Y chromosome carries a dominant *Maleness* factor (Willhoeft and Franz 1996). To construct a GSS, the wild-type allele of the selectable marker is physically linked to this chromosome via a Y-autosome translocation (Fig. 1). In the resulting strain, the males are heterozygous (normal "wild-type" phenotype), and the females homozygous for the selectable marker and thus express the mutation, and can be separated from males. Y-autosome translocations are induced by irradiation, followed by a genetic screen that is based either on the inherited sterility associated with translocations or on a mutation to detect male-linked inheritance. The former method can be used in all species, even where no, or only very limited, genetic information is available. Not all translocations are equally appropriate for use in a GSS, e.g. translocations involving multiple autosomes have a high level of sterility. As outlined below, the stability and productivity of a strain depend crucially on the structure of the translocation.

### *2.1. Mutations for Large-Scale Separation of Sexes*

The choice of a suitable selectable marker involves decisions regarding the feasibility and economics of the resulting sexing mechanism. In this context, several criteria must be considered.

#### *2.1.1. Sex Separation versus Female Killing*

Killing females is the best option, except for special cases where females are needed to maintain the colony (species with a low reproductive capacity, such as tsetse flies).

#### *2.1.2. Timing*

To minimize rearing costs, females should be killed as early as possible during development. Timing also determines the stage, and corresponding amount of biomass, during which the females must be killed, and this has an effect on the practicality, cost, and accuracy of the treatment, e.g. for fruit flies large numbers of eggs can be treated more easily than large numbers of larvae or pupae.

#### *2.1.3. Physical or Chemical Treatment to Kill Females*

The egg stage is the most practical stage, but in most cases chemicals cannot penetrate the eggshell, and a selectable marker that responds to physical treatment (e.g. temperature) is required. If only selectable markers that are sensitive to chemicals are available, the larval stage is the earliest possible stage for sexing. The use of chemicals raises additional points: cost of the chemical, human toxicity (to workers), accessibility to the chemical by feeding larvae (solubility, distribution, and stability in the diet), effect on symbionts or bacteria in the diet, and disposal of spent diet containing the chemical.

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*2.1.4. General Considerations*

It is important also to know the accuracy of the sexing mechanism, the additional costs for equipment, chemicals, etc., and the reduced productivity (in comparison with a standard strain). The accuracy of eliminating females should be close to 100%, but the selectable marker should not negatively affect the viability and productivity of mutant females in the colony.

Choosing the right selectable marker is a vital decision. Several different markers for the Mediterranean fruit fly are available (Robinson 2002). However, most of them are not ideal for large-scale application. Current GSSs for this species carry two mutations, *temperature-sensitive lethal* (*tsl*) (Franz et al. 1994) that is used to eliminate females, and the closely linked mutation *white pupae (wp)* (Rössler 1979) used as a visible marker. The *tsl* mutation has certain biological properties that impact on its usefulness and also on the procedures required for mass-rearing or sexing.

*2.1.5. Temperature-Sensitive Period in Individuals Homozygous for the* tsl *Mutation*

In a GSS, females are homozygous for the *tsl* mutation. The standard protocol to test temperature sensitivity is a 24-hour treatment at temperatures from 31 to 35˚C, compared with the control at 25˚C. To determine the sensitive period of the *tsl* mutation, eggs are collected for 1 hour, and temperature tests done at different stages of development. As shown in Fig. 2A, homozygous *tsl* individuals are very sensitive to temperature during the embryonic stage, and to a lesser extent during moulting from first- to second-instar, and from second- to third-instar, larvae. Fig. 2B shows the results of temperature tests with pupae of known age. The pupae are also sensitive to temperature, especially during the first 3 days.

Additional tests were done with adults, maintaining the cages at 25, 28, 31, and 34˚C, and daily mortality was measured. In the *tsl* homozygous strain, significant mortality was observed already at 28°C (Fig. 3).

In conclusion, temperature sensitivity is strongest during the egg stage, and elimination of homozygous *tsl* individuals is achieved by incubating eggs at 34˚C for 24 hours. Secondly, later stages are also temperature sensitive. Therefore, the temperature during mass-rearing must be carefully controlled to prevent damage to the *tsl* homozygous females. Thirdly, even though the sensitivity of the adults is a complication for mass-rearing, it also has a benefit in that any homozygous *tsl* individuals that escape will not be viable at elevated temperatures. This applies to any flies that may escape from a production facility, and to the few remaining females among the released sterile males.

*2.1.6. Maternal Effect in Individuals Heterozygous for the* tsl *Mutation*

In a GSS, the males are heterozygous for the *tsl* mutation, but does the presence of a wild-type allele completely suppress the sensitivity to temperature? Originally the temperature treatment to eliminate females was done relatively early during embryogenesis, i.e. eggs were collected for 24 hours, followed immediately by the 24-hour temperature treatment. However, this resulted in a reduced recovery of males.

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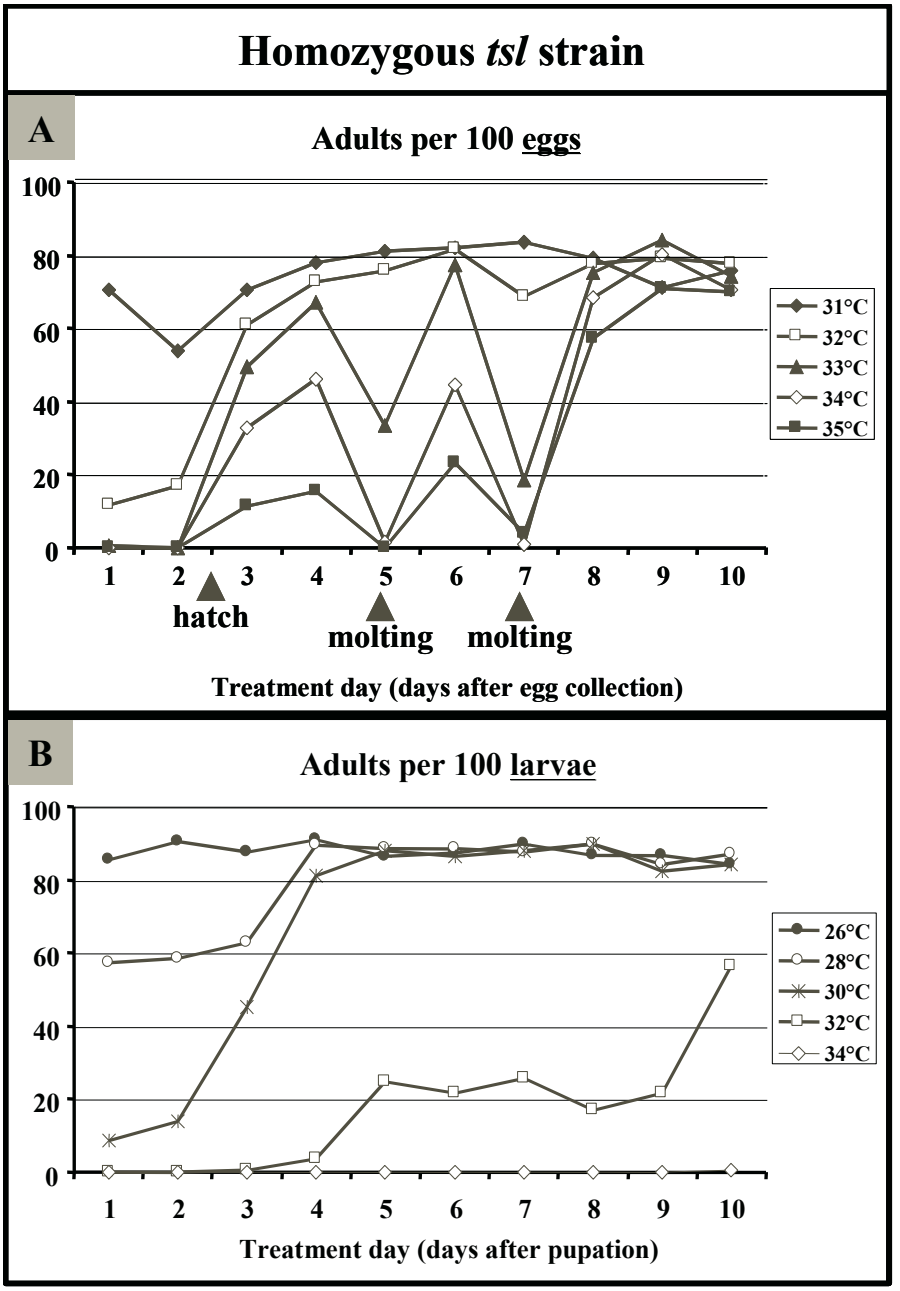



*Figure 2. Temperature pulse experiments with homozygous* tsl *strain. Eggs (A) or pupae (B) were collected for 1 hour followed by temperature treatment during different days of development with temperatures indicated.*

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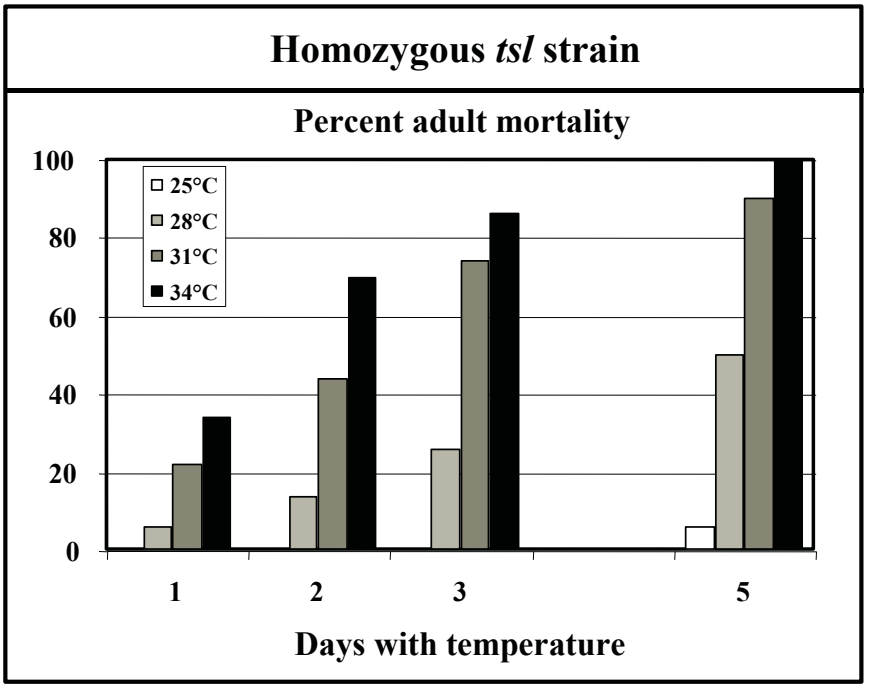



*Figure 3. Temperature treatment of adults homozygous for* tsl *mutation. After emergence, adults were maintained constantly at temperatures indicated.*

Tests of $F_1$ individuals from reciprocal crosses, between a wild-type strain and a homozygous *tsl* strain, demonstrated that the temperature sensitivity of the $F_1$ differed significantly, although in both cases the genetic constitution of the $F_1$ is identical (*tsl*/*tsl*$^+$). Fig. 4B shows that, in the first cross (wild type *tsl*$^+$ females and *tsl* males), the *tsl*$^+$ allele is completely dominant over the mutant allele, i.e. the same level of lethality is observed in $F_1$ offspring as in a wild-type strain (Fig. 4A). However, in the reciprocal cross where homozygous *tsl* females are used, a significant temperature sensitivity of the $F_1$ offspring is observed (Fig. 4C).

This difference is due to a maternal effect. During the early stages of development, the embryo's own genome is inactive, and apparently it utilizes a *tsl*-gene product that it receives in the egg from the mother. Consequently, during these early stages of development, the embryo is dependent on the genotype of the mother, and in a GSS the mother is homozygous for the *tsl*. Only after activating their own genetic material are the heterozygous *tsl*/*tsl*$^+$ males protected against elevated temperatures. Based on these findings, the temperature-treatment regime was modified, i.e. after collection, and before the treatment is applied, eggs are maintained at 25˚C for at least 24 hours. This led to a significantly improved recovery of males (Fisher 1998).

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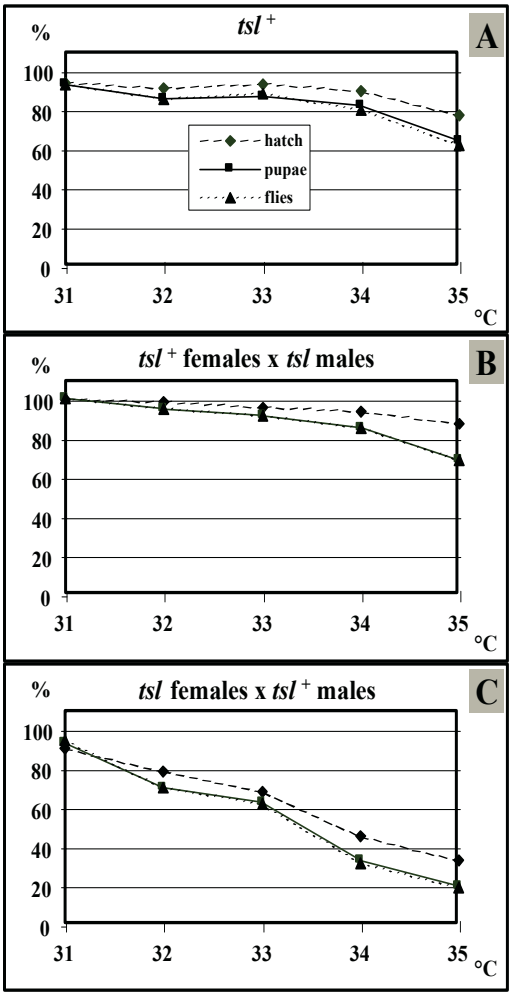



*Figure 4. Difference in temperature sensitivity of* tsl$^{+}$/tsl *genotypes depending on direction of parental cross (maternal effect). Values shown for hatch, pupae, and adults are given as percentage of values obtained at 25°C. Homozygous* tsl *strain shows no hatch starting at 34°C.*

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*2.1.7. Delayed Development*

Individuals homozygous for the *tsl* mutation develop more slowly than those either heterozygous or homozygous for a wild-type allele. Already at 25˚C, homozygous *tsl* individuals pupate 1 day later than the wild type. If eggs and larvae are subjected to an extended incubation at elevated but sub-lethal temperatures, e.g. 29˚C, the speed of development is affected. As expected, the development of wild-type individuals is accelerated, i.e. pupation occurs 2 days earlier than at 25˚C. However, in the homozygous *tsl* strain, pupation occurs only 1 day earlier than at 25˚C, i.e. 2 days later than the $tsl^+$ individuals.

Differences in pupation time are a good indicator of the temperature increase during larval rearing. For example, poor rearing conditions, like excess heat in the larval diet, become apparent as increased differences in the pupation time of $tsl^+$ males and homozygous *tsl* females.

In principle, the delayed development of the homozygous *tsl* females could be used to separate the sexes at the larval or pupal stages, e.g. for mass-rearing of larval or pupal parasitoids. In mass-rearing a *ts*l-based GSS, the first day of pupal collection yields virtually only male pupae, but the collection on the 5$^{th}$ day contains mostly females (Fig. 5 in Caceres 2002). This permits loading production cages with an excess of females (to increase egg production) without using a mechanical pupal separator. The surplus of males can be added to the insects to be irradiated and released.

*2.1.8. Unwanted Recombinants and Filter Rearing System*

An additional improvement to maintain GSS stability or purity is the filter rearing system (FRS) (section 4.3.), i.e. the maintenance of a mother colony as a pure GSS where any exceptional flies are removed according to their pupal colour phenotype (Cáceres et al. 2000; Parker, Mamai et al., this volume). As described below, GSSs can generate unwanted recombinant individuals, some of which cannot be removed by inspection of the pupal colour (Table 2).

*Table 2. Recombinant types produced in T(Y;5)* $wp^+tsl^+$/wp tsl *males*

| Recombination type | $F_1$ genotype | $F_1$ phenotype | Accumulation in mass-rearing | Detection in filter rearing system |
|---|---|---|---|---|
| Type-1a | T(Y;5)*wp tsl*/*wp tsl* males | white pupae, *tsl*, 50% sterile | No | Yes |
| Type-1a | $wp^+$ $tsl^+$/*wp tsl* females | brown pupae, $tsl^+$ | Yes | Yes |
| Type-1b | T(Y;5)$wp^+$ *tsl*/*wp tsl* males | brown pupae, *tsl*, 50% sterile | No | No |
| Type-1b | *wp* $tsl^+$/*wp tsl* females | white pupae $tsl^+$ | Yes | No |
| Type-2 | Y/*wp tsl*/*wp tsl* males | white pupae, *tsl*, 100% fertile | Yes | Yes |

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One type of recombinant female, *wp tsl*$^{+}$*/wp tsl,* emerges from white pupae, and therefore is not removed by the FRS, and will accumulate in the colony, where it becomes evident when an increasing number of white-pupae females survive the temperature treatment. However, such females can be distinguished from the non-recombinant females since the presence of the *tsl*$^{+}$ allele causes them to pupate early, together with the males. Consequently, it is recommended for the FRS that new adult cages be set up with only brown-pupae males that have pupated early, and with white-pupae females that have pupated late. It has been shown that this selection scheme significantly improves the accuracy of the FRS.

### *2.1.9. Large-Scale Temperature Treatment of Embryos*

In mass-rearing, eliminating female embryos requires only a simple and cheap water bath. The temperature treatment is applied during the "egg bubbling stage" when incubating eggs are oxygenated and maintained at a high density in water. The accuracy of the treatment is almost 100%, even in very large facilities where hundreds of millions of eggs are treated every day (Caceres 2002). Male quality and quantity is not affected significantly by the treatment. In rearing individuals for the colony, temperature must be carefully controlled; if not, female production is reduced, and, as described below, the stability of the sexing system is negatively affected (section 4).

## *2.2. Sex Linkage of Selectable Marker*

Selectable markers are generally not sex-specific. Therefore, the respective wild-type allele must be linked to the male-determining Y chromosome. In Mediterranean fruit fly, this linkage is achieved by irradiating wild-type pupae with 40–50 Gy, and backcrossing individual $F_1$ males with mutant females carrying the selectable markers. The $F_2$ is screened for lines where the mutation used in the screen is inherited in a sex-specific manner, i.e. males are wild type and the females mutant. The choice of the mutation determines which autosome is involved in the translocation.

Several independent experiments to induce Y-autosome translocations in the Mediterranean fruit fly have been performed (Robinson and Van Heemert 1982; Kerremans et al. 1992; Franz et al. 1994; Kerremans and Franz 1995; Delprat et al. 2002). On average, about 7% of $F_1$ crosses involve male-linked translocations. This frequency depends on the genome size and the size of the chromosomes involved. In total, more than 30 Y-autosome translocations involving chromosome 5 were generated. Chromosome 5 carries both the *wp* and *tsl* mutants. This diverse availability of translocations is a very important prerequisite for the successful development of sexing strains — it allows a choice of the most appropriate translocation (with reference to stability and productivity).

The productivity of a sexing strain is correlated primarily with the segregation behaviour of a Y-autosome translocation during male meiosis. There are two ways in which a Y-autosome translocation can segregate — alternate and adjacent-1 (Fig. 5).

In nearly all cases, these two types occur at equal frequencies (Franz 2000; G. Franz, unpublished data). Only alternate segregation leads to genetically balanced offspring, but adjacent-1 segregation results in severe deletions and triplications.

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Deletions usually cause lethality already during embryogenesis, but triplications can survive, depending on the size and resulting sex, until the pupal or even adult stages. As a consequence of this segregation pattern, only 50% of the offspring produced by males carrying a simple translocation, involving only one autosome, are genetically balanced, i.e. males are 50% sterile. In males with a more complex translocation, where more than one autosome is involved, sperm sterility is increased accordingly (Franz 2000).

The structure of the translocation, in particular the structure of the Y chromosome and the location of the break point on the Y chromosome, affects the segregation behaviour and the viability of the resulting adjacent-1 offspring (Willhoeft and Franz 1996). If the break point is located between the Y-chromosomal centromere and the *Maleness* factor, the triplication-type adjacent-1 individuals will be female, e.g. strains VIENNA 7 and 8 (sections 4.1. and 4.2.; Robinson et al. 1999). It has been shown that females are about 100-fold more sensitive to triplications in most regions of chromosome 5 than males with triplications of similar size (G. Franz, unpublished data), and consequently they do not survive to the pupal stage. Additional rearrangements of the Y chromosome can lead to an increase in the alternate segregation frequency and, thereby, to an increase in the productivity of the strain.

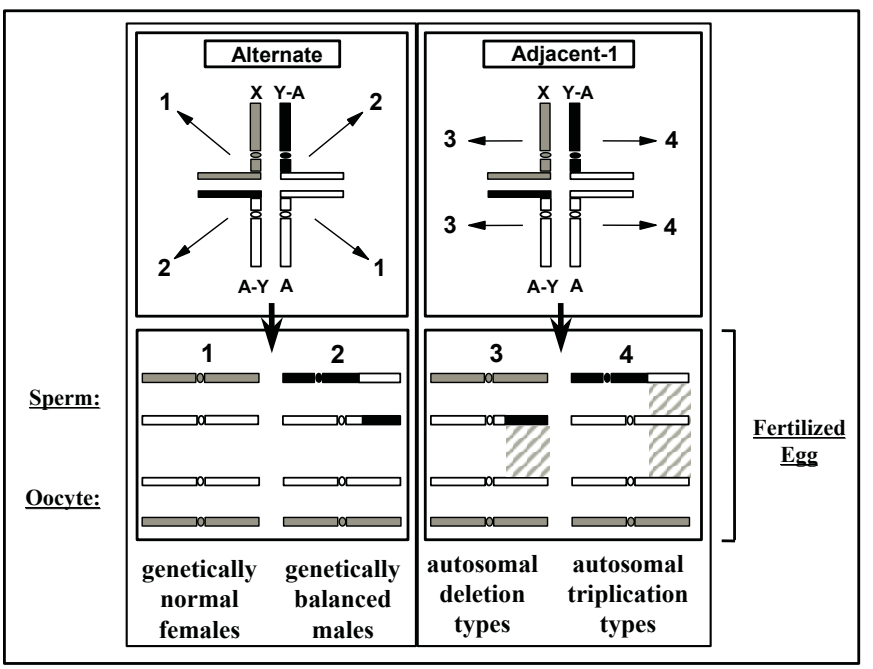



*Figure 5. Two different types of segregation of Y-autosome translocations during male meiosis. Y-A: translocation fragment carrying Y chromosomal centromere, A-Y: reciprocal translocation fragment carrying autosomal centromere, X: X chromosome, A: autosome. Adjacent-2 segregation represents a non-disjunction of homologous centromeres, and therefore is not considered here.*

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## 3. INSTABILITY IN SEXING SYSTEM

The first Mediterranean fruit fly sexing strain was based on the *wp* mutation and the translocation T(Y;5)101 (Robinson and Van Heemert 1982). In laboratory-scale rearing, no obvious signs of instability were detected. In 1985, this strain was tested in mass-rearing. Although the scale of production was rather limited (about 1 million males per week), within a relatively short period the sexing system started to degrade (Hooper et al. 1987). The following actions were taken to remedy the situation:

- *Improving cytology, and developing chromosome maps.* During the development of polytene chromosome maps, it was discovered that there are two classes of tissues that contain polytene chromosomes with completely different banding patterns (Bedo 1986; Zacharopoulou 1987, 1990, 1991), and the Y chromosome is visible only in polytene chromosomes isolated from trichogen cells in male sub-orbital frontal bristles.
- *Induction, analysis, and mapping of new mutations.* Many new mutations were isolated and genetic maps constructed (Rössler et al. 1994). To date, seven phenotypic mutations are known for chromosome 5, several of which were mapped on polytene chromosomes by deletion mapping or *in situ* hybridization (Fig. 6).

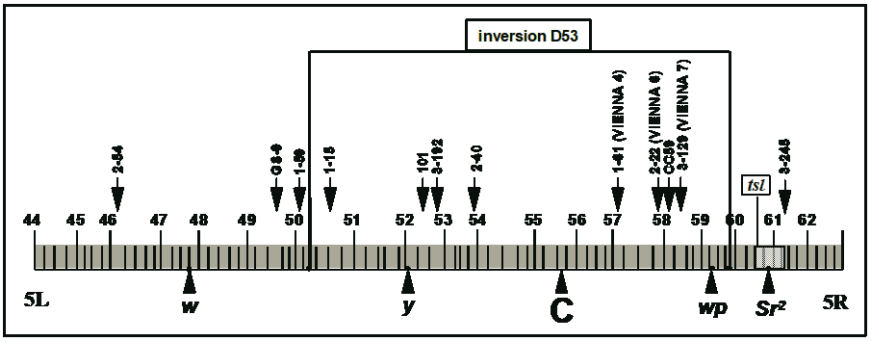



*Figure 6. Schematic polytene Mediterranean fruit fly chromosome map of autosome 5 from trichogen cells: map positions of mutations* white (w), yellow body (y) *and* Sergeant$^2$ (Sr$^2$) *are shown in addition to location of selectable markers* wp *and* tsl. *Arrows indicate break points of several Y-autosome translocations. Extent of chromosomal region inverted in D53 is shown. C: centromere. (VIENNA 8 includes translocation 101 combined with inversion D53.)*

### *3.1. Type-1 Male Recombination*

The most frequent cause of strain instability is autosomal recombination in males heterozygous for the selectable marker(s), i.e. recombination between the translocated wild-type chromosome and the free autosome carrying the mutant alleles (type-1 recombination, Franz 2002). In the GSSs being used in operational programmes that apply the SIT, where *wp* and *tsl* are used as selectable markers, genetic recombination

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in two chromosomal regions is of relevance, i.e. in the region between the translocation break point and *wp* (type-1a), and the region between *wp* and *tsl* (type-1b, Fig. 7). Each sub-type produces two reciprocal recombinants; type-1a replaces the wild-type alleles of both markers on the translocation with the mutant alleles from the free autosome, while type-1b exchanges only the wild-type allele of the *tsl*. This results in the generation of several new combinations of these markers and sex (Table 2).

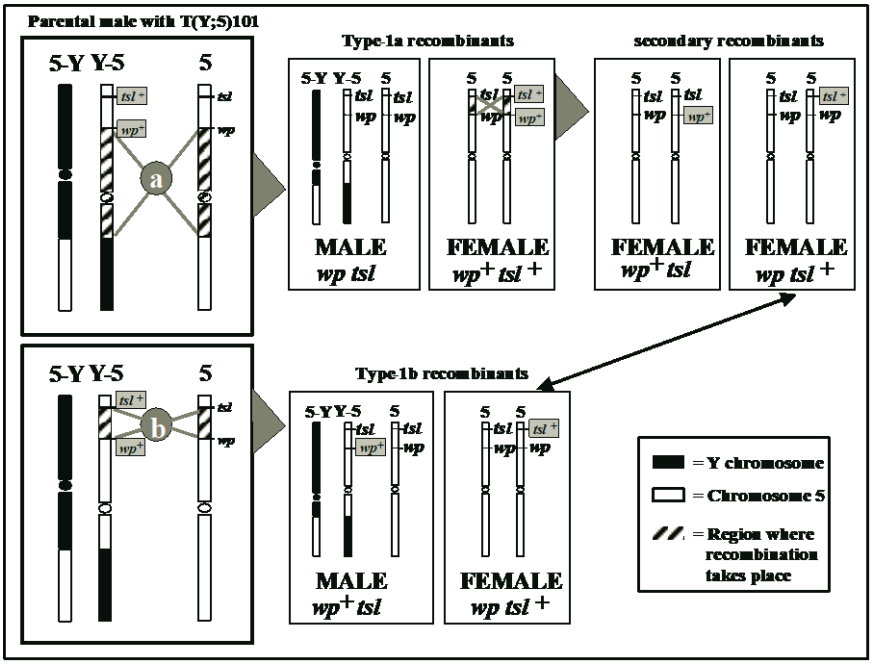



*Figure 7. Consequences of type-1 recombination in Y-autosome 5 translocation T(Y;5)101, either in chromosomal region between translocation break point and* wp *(type-1a) or between* wp *and* tsl *(type-1b). Also, the genotype resulting from secondary recombination in type-1a recombinant females is shown. 5-Y: translocation fragment carrying chromosome 5 centromere, Y-5: reciprocal translocation fragment carrying Y chromosomal centromere.*

In Mediterranean fruit fly males, the recombination frequency is very low, especially compared with recombination in females. For example, the recombination frequency between the relatively distant mutations *white* (*w*), on the left arm of chromosome 5, and *wp* on the right arm, is 0.176% in males (Franz 2002) but 48.4% in females (Rossler and Rosenthal 1992), i.e. these two markers behave as if they were unlinked in females. In spite of its rare occurrence, male recombination is nevertheless a threat to the integrity of the sexing system since some of the resulting recombinants have a selective advantage as compared with normal non-recombinant flies. In a continuous mass-rearing system, where exceptional individuals cannot be removed,

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they will gradually replace the non-recombinant genotypes. To illustrate this, a GSS, based on the translocation T(Y5)101 (Robinson and Van Heemert 1982) and the markers *wp* and *tsl*, was reared in standard small-scale conditions for more than 3 years. In each generation, 34 ml of pupae were taken to initiate the next generation without removing any recombinants; in parallel, flies emerging from 40 ml of pupae were screened for recombinants (Fig. 8). A typical pattern of strain degradation due to type-1 recombination emerges from generation 20 onwards. During male meiosis the two reciprocal types of recombinants, $wp^+$ females and *wp* males, occur at equal frequencies. However, from generation 20 onwards, the $wp^+$ female recombinants accumulate rapidly, while the number of *wp* males remains insignificant. The accumulation of $wp^+$ females is even faster than expected based on a purely additive build-up of the recombinants occurring in each generation. This effect is caused by the selective pressure in favour of these wild-type females during the mass-rearing process.

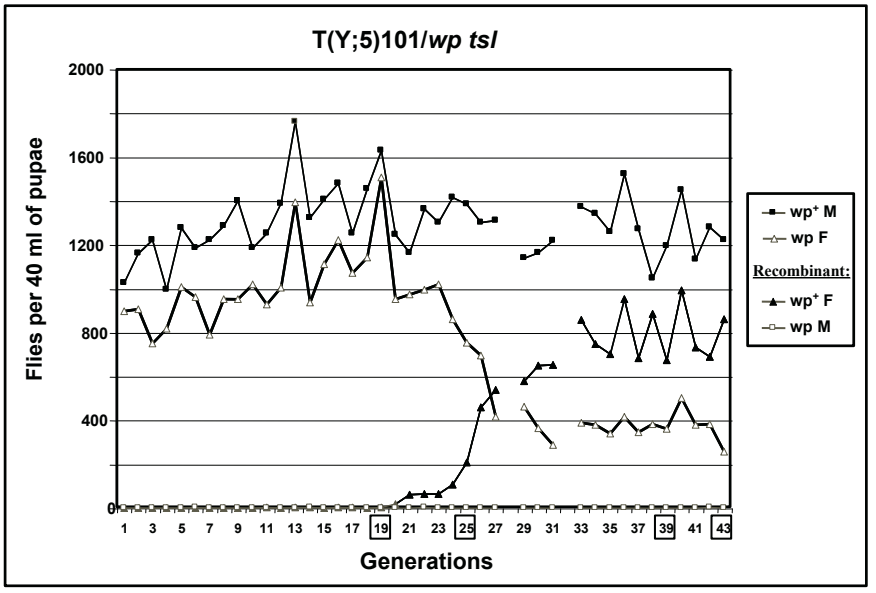



*Figure 8. Breakdown pattern of genetic sexing strain T(Y;5)101/*wp tsl*. In each generation, 34 ml of pupae were taken to initiate the next generation, without removing any recombinants, and a parallel sample of 40 ml of pupae was analysed. Temperature tests were conducted with females from highlighted generations (Fig. 9). F: females, M: males.*

The reverse argument applies to *wp* males; they have a selective disadvantage compared with normal non-recombinant males, and therefore do not accumulate. Furthermore, the accumulation of $wp^+$ females continues until, in about generation 33, a certain equilibrium is reached, where these wild-type females are about twice as abundant as the non-recombinant mutant females.

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Temperature tests were needed to assess the exact nature of the recombinants, with respect to the *tsl* mutation. In generations 19, 25, 39, and 43, eggs from the colony were treated for 24 hours with temperatures between 31 and 35˚C, and the phenotype of the resulting adults was scored. Fig. 9A shows that the $wp^+$ females are also wild type for the *tsl* mutation (no reduction in numbers with increasing temperature), confirming that they are type-1a recombinants that resulted from recombination between the translocation break point and *wp*. As expected from the results shown in Fig. 8, such recombinants are not present in generation 19. However, in generation 25, $wp^+$ $tsl^+$ females are detected, and their frequency reaches a maximum in generations 39 and 43. Fig. 9B shows the survival of *wp* females following temperature treatment. In generation 19, all *wp* females are temperature sensitive, i.e. their genotype is therefore *wp tsl*. However, already in generation 25, about half of the *wp* females have lost the *tsl* (genotype *wp* $tsl^+$) (they survive even at 35˚C).

In generations 39 and 43, all *wp* females are $tsl^+$. As indicated in Fig. 7, there are two ways to explain the occurrence of these *wp* $tsl^+$ females, i.e. either by type-1b recombination in the males, or by a secondary recombination in the recombinant *wp tsl*/$wp^+$ $tsl^+$ females. The latter is the most likely explanation since recombination in females is much higher than in males. Furthermore, results with other strains show that type-1b recombination appears to be very rare (see below).

In conclusion, type-1 recombination, in combination with a secondary recombination in females, leads to a strain where the normal *wp tsl* females are completely replaced by a 2:1 ratio of $wp^+$ $tsl^+$ and *wp* $tsl^+$ females. The rate at which these recombinant females increase in number cannot be explained by simply adding the recombinants that occur in each generation. A very strong selection is acting on the colony, leading to the rapid accumulation of recombinants that display an advantageous phenotype. The primary increase in selective advantage is caused by the loss of temperature sensitivity and, consequently, no other types of recombinants accumulate. The presence of the *wp* mutation also causes a selective disadvantage (also noted in other combinations) (Fig. 7 in Franz 2002). The speed of accumulation will depend greatly on the rearing conditions, i.e. if they are sub-optimal, this effect will be accelerated.

An additional exceptional phenomenon can be observed only in large mass-rearing facilities. In the colony production line, pupal trays resulting from the first larval collection day, which normally consist of $wp^+$ $tsl^+$ male pupae, may also contain very few *wp* pupae. It is known that homozygous *tsl* individuals do not accelerate their development in response to elevated temperatures ("slow development") as a wild type strain (or a heterozygous $tsl^+$/*tsl* individual) would. So, the earlier pupal collections in a GSS are mostly $wp^+$ $tsl^+$ males, while later pupal collections primarily consist of *wp tsl* females. So, white pupae females detected in early pupal collections are expected to be due to recombination events between the *wp* and *tsl* loci. There has also been preliminary evidence that the "slow development" phenotype may not be directly associated with the *tsl* locus itself, suggesting that this is due to a different locus, namely slow larvae (*sl*), which should be close to the *tsl* locus on chromosome 5.

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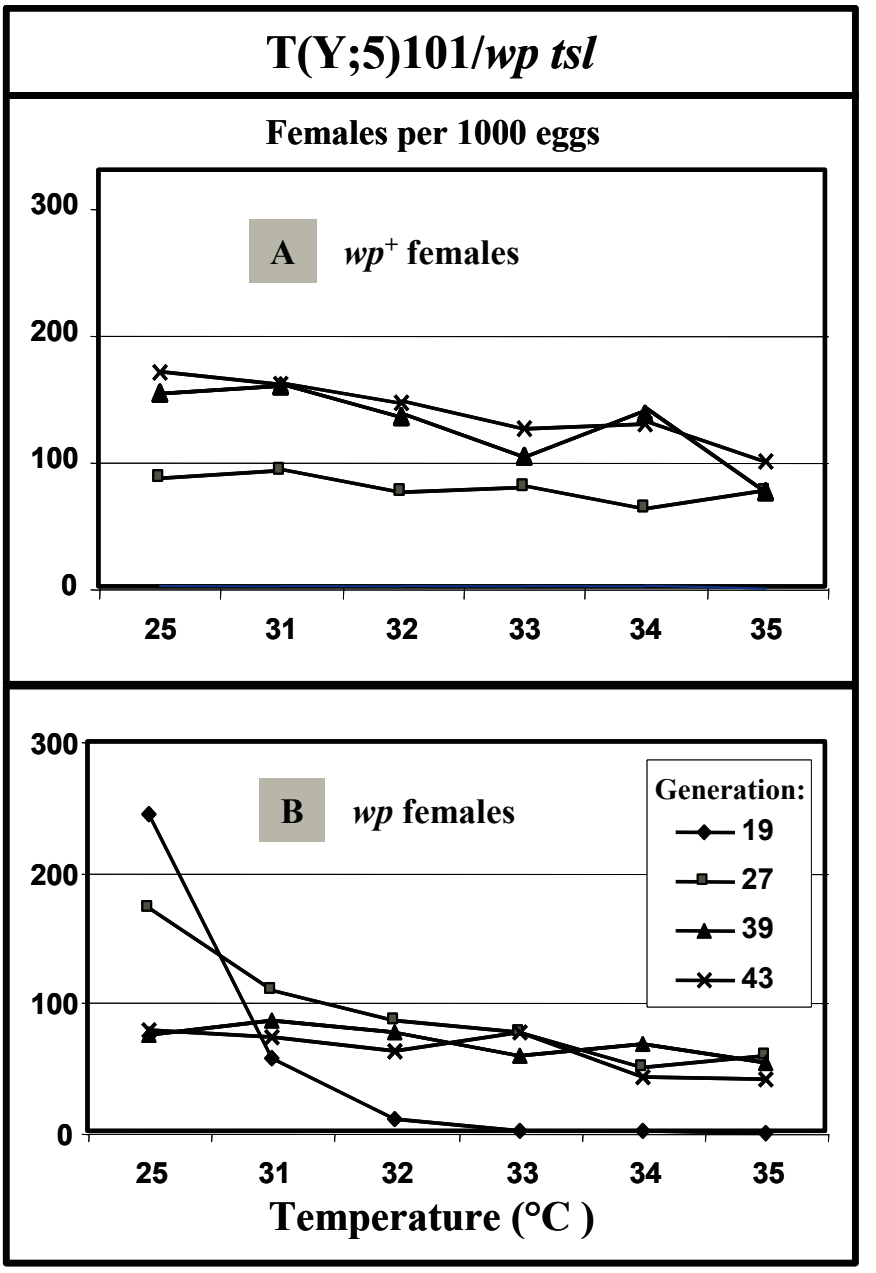



*Figure 9. Temperature tests with females of sexing strain T(Y;5)101/*wp tsl*. Temperature tests were done in generations 19, 27, 39, and 43.*

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In an effort to confirm this hypothesis, early *wp* females were crossed with males from the balancer line 68b (Gourzi et al. 2000), the $F_1$ $wp^+$ progeny was inbred, and the $F_2$ *wp* individuals were screened for lines homozygous for *wp tsl* exhibiting an accelerated development. Temperature response tests confirmed that a few lines, which were homozygous for *wp* and *tsl*, exhibited faster development during the larval stage, confirming that this phenotype is due to its close association with *wp* and *tsl* loci in region 61 of the polytene chromosome map (Fig. 6). After the establishment of $sl^+$/$sl^+$ lines, crosses were performed as described in section 2.2., which resulted in lines with *wp tsl* female larvae that develop faster, almost like the $wp^+$ $tsl^+$ male larvae (M. Porras, personal communication). Rare recombination events, which can only be detected under mass-rearing conditions, may result in the loss of this phenotype (Type-1c male recombination).

The isolation of the *sl* locus is of major applied importance because a *wp tsl* line in which females develop almost as fast as males would significantly increase the rearing efficiency and reduce the overall costs. This is because males and females will have access to the same amount and quality of larval food, so facilities will reduce larval rearing time, and thus space and energy consumption required for colony maintenance. In any case, protocols still need to be developed and validated for this new strain at mass-rearing facilities prior to its incorporation in large-scale operational programmes (M. Porras, personal communication). Interestingly, a similar slow larval development phenotype was described recently in *A. ludens* (Meza et al. 2019).

### *3.2. Type-2 Male Recombination*

During mass-rearing, a second type of male recombination was detected (G. Franz, unpublished data). It is very rare (estimated frequency about $10^{-5}$ or less), and generates males with free untranslocated Y chromosomes that are homozygous for *wp* and *tsl* (Table 2). Such males accumulate rapidly in the mass-rearing colony (even though they are sensitive to temperature) because they are completely fertile. Their primary effect is on the productivity of the colony, and not on the accuracy of sexing, because together with females they are eliminated during the temperature treatment. The current hypothesis is that recombination occurs between the two translocated Y fragments. Choosing the appropriate Y-autosome translocation, e.g. where the Y-chromosomal break point is close to the centromere, can minimize this problem.

## 4. STRATEGIES TO IMPROVE STABILITY

Genetic tools are required to improve the stability of sexing strains, and two types of positional information are absolutely essential: (1) the position of the translocation break point, and (2) the position of the selectable marker(s). Also, at least one mutation per chromosome is needed to determine the structure (e.g. which autosomes are involved) and the genetic behaviour of the translocations.

There are two principal strategies to increase the stability of sexing strains. Firstly, by selecting translocations where the break point and the marker are close together, type-1 recombination can be reduced. Secondly, by incorporating inversions that cover

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the critical region between the translocation break point and the selectable marker into the strain to eliminate, or at least reduce, type-1 recombination.

### *4.1. Improved Y-Autosome Translocations*

The *wp* mutation is at position 59B on the trichogen polytene map, and the *tsl* is located in the interval 60B–61B (Fig. 6) (Kerremans and Franz 1994; G. Franz, unpublished data). More than 30 translocations were analysed to determine: (1) the position of the break point on chromosome 5 (Kerremans et al. 1990; Franz et al. 1994; Kerremans and Franz 1995), (2) the position of the break point on the Y chromosome (Willhoeft and Franz 1996), and (3) the genetic behaviour (e.g. sterility and segregation behaviour). Based on this information, three strains were selected, i.e. T(Y;5)1-61 (VIENNA 4), T(Y;5)2-22 (VIENNA 6), and T(Y;5)3-129 (VIENNA 7). They were subjected to extensive stability tests, following the same protocol as described above for strain T(Y;5)101 (Franz 2002).

After small-scale rearing for up to 95 generations, no recombinants accumulated in the strains. The overall frequency of type-1a recombination was between 0.017 and 0.021%, and that of strain T(Y;5)101 was 0.084% (if only the generations before recombinants started accumulating were considered). Furthermore, no obvious decrease in sensitivity was shown by the temperature tests, indicating that here also type-1b recombination did not occur at any significant level. Since the distance between the *wp* and *tsl* in the three new strains is the same as in T(Y;5)101, the absence of *wp* $tsl^+$ individuals support the hypothesis that the occurrence of such genotypes during the rearing of T(Y;5)101 was caused by a secondary recombination event in the *wp tsl*/$wp^+tsl^+$ recombinant females.

### *4.2. Inversions*

The second strategy is to use the known recombination-reducing properties of chromosomal inversions. Chromosomal inversions render recombinants non-viable if the recombination has occurred within the inversion. Therefore, the aim was to induce an inversion in the *wp tsl* chromosome. Ideally, it should cover the interval between the translocation break point and the *tsl* mutation, and it must be viable in a homozygous condition. The genotype of the resulting strain would be T(Y;5) $wp^+tsl^+$/Inv *wp tsl* males x Inv *wp tsl*/Inv *wp tsl* females.

In a large screen for inversions, using reduced recombination in the interval between the mutation *yellow body* (*y*) and *wp* as indicator, one homozygous viable inversion, D53 (*wp tsl*), was detected (A. Zacharopoulou, C. Cáceres, and G. Franz, unpublished data). Unfortunately, it does not cover the *tsl*, i.e. it would not reduce type-1b recombination. However, recombination tests with *wp* and the mutation *Sergeant*$^2$ (*Sr*$^2$), located very close to *tsl* (Fig. 6) (Niyazi et al. 2005), showed that inversion D53 also has a strong recombination-reducing effect in neighbouring areas (G. Franz, unpublished data).

Based on this finding, D53 was combined experimentally with the translocation T(Y;5)101, and a new strain VIENNA 8 was constructed. This strain was reared for 5 years (60 generations), under the same conditions as T(Y;5)101, without inversion.

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When the results in Fig. 10 are compared with those in Fig. 8, it is apparent that stability is improved significantly, i.e. no $wp^+$ females (type-1a) were detected. Temperature tests with *wp* females showed also that no *wp* $tsl^+$ female recombinants (type-1b) accumulated in the colony (Fig. 11), even though the inversion covers only a small proportion of the *wp-tsl* interval.

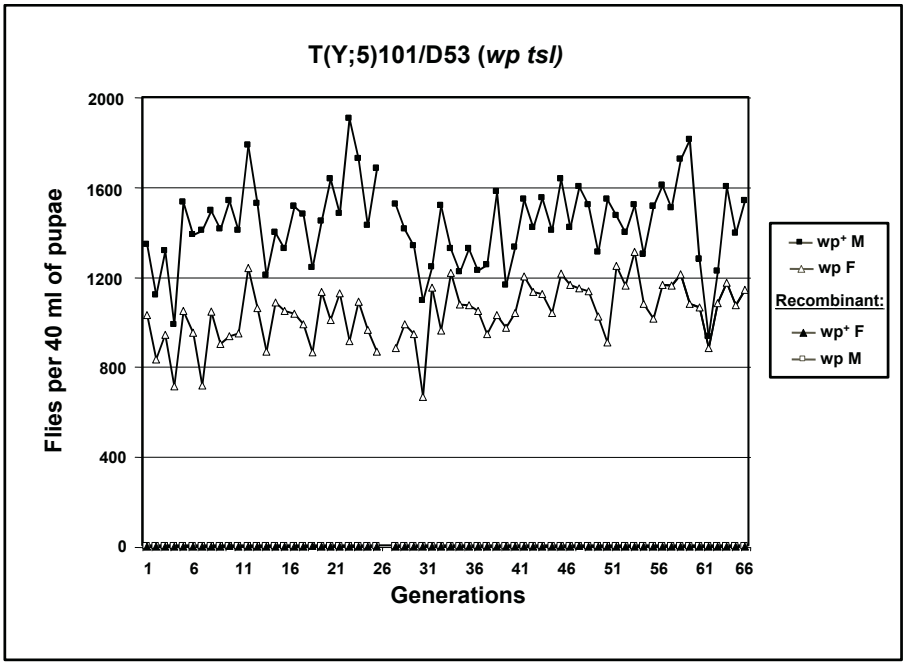



*Figure 10. Increased strain stability due to presence of inversion D53. Neither* wp$^+$ *females nor* wp *males are detected at significant levels. Rearing and analysis were identical to the experiment without inversion. In several generations,* wp *females were tested for temperature sensitivity (Fig. 11). F: females, M: males.*

Both genetic strategies to improve the stability of sexing strains have been successful. Using the appropriate translocation reduces the recombination frequency by 0.65, and if an inversion is added, the frequency is reduced by another 0.80. Inversions have the added potential that they permit translocations based on criteria other than stability to be used. For example, translocation T(Y;5)101 causes less sterility (10–30%, G. Franz, unpublished data) than other translocations, but is not very stable. However, in combination with inversion D53 in strain VIENNA 8, stability is very high. VIENNA 8 has now been provided to several operational AW-IPM programmes that integrate the SIT (Augustinos et al. 2017).

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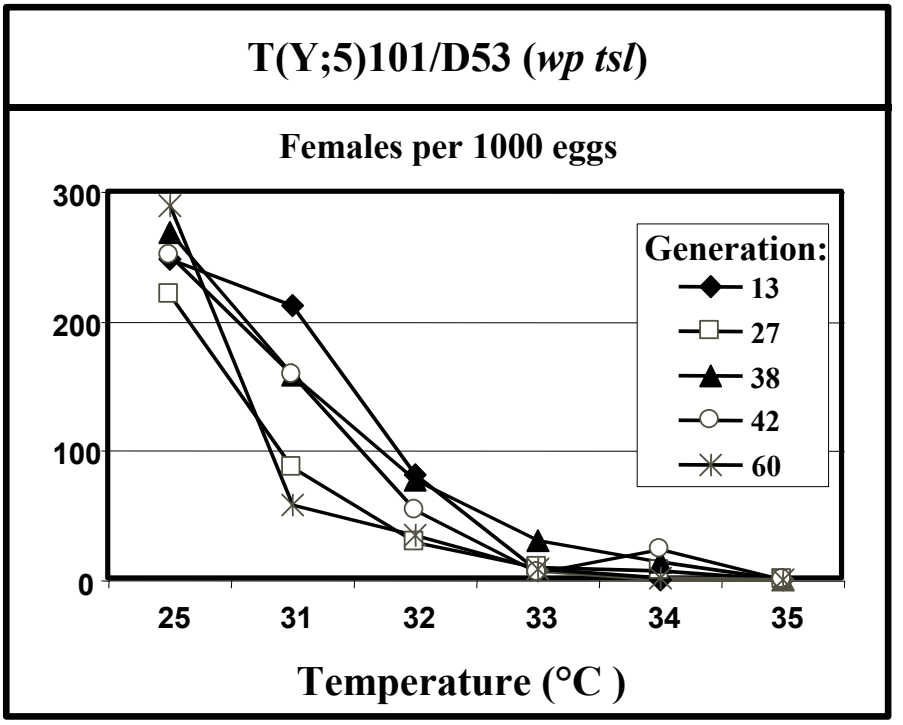



*Figure 11. Temperature tests with* wp *females from five different generations during rearing of sexing strain T(Y;5)101/D53.*

### *4.3. Filter Rearing System (FRS)*

Nevertheless, no GSS will be absolutely stable under conditions of large-scale mass-rearing. Experience with GSSs in different mass-rearing facilities has shown that rearing conditions and the scale of mass-rearing determine whether or not the stability of a strain can be maintained. Up to a certain level of mass-rearing, a facility with well-trained personnel, appropriate equipment, and adjusted operating procedures can maintain the stability. However, if the level of mass-rearing is very high, or any of the aforementioned requirements are not met, stability cannot be guaranteed, and additional measures are required (Caceres et al. 1993). It is for this purpose that the FRS was developed to help maintain the purity of the mother colony (Cáceres et al. 2000; Fisher and Caceres 2000; Parker, Mamai et al., this volume). In a systems approach, by avoiding the accumulation of recombinants, the FRS provides a second independent procedure to maintain strain integrity. Only with both strategies in place (genetically improved strains and improved mass-rearing procedures that also include a FRS) is it possible to produce more than 3500 million Mediterranean fruit fly males per week (Table 1). However, it must be emphasized that the FRS can be used only when a visible mutation is included in the GSS.

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## 5. ADDITIONAL FEATURES: MARKERS

GSSs should carry markers (internal as well as external) that make them distinguishable from wild flies or other strains. Markers make it possible to: (1) detect the deliberate or accidental contamination of a strain with wild flies or flies from a different strain, (2) determine if non-irradiated flies (especially females) were released, and (3) reduce the biological and economic costs of, and ambiguities in, the currently used procedure for marking released flies (adults emerge from pupae coated with powdered fluorescent dye) (Dowell et al., this volume; Parker, Mamai et al., this volume; Vreysen, this volume).

To achieve this goal in the Mediterranean fruit fly, several strategies have been pursued. First, a particular type of mitochondrial DNA (mtDNA) haplotype was introduced into the GSS. This mtDNA haplotype was detected in a wild-type strain from Egypt, and it can be used to differentiate the GSS from most field populations where AW-IPM programmes integrating the SIT are being carried out. The mitochondrial genome of the Mediterranean fruit fly has been fully sequenced (Spanos et al. 2000). It was used for the development of a molecular approach to assess the mating success of sterile Mediterranean fruit fly VIENNA 8 males in SIT programmes (San Andrés et al. 2007; Juan-Blasco et al. 2013). In addition, microsatellite markers have been developed which have been used for genotyping purposes (Bonizzoni et al. 2000, 2001, 2004; Stratikopoulos et al. 2008; Karsten et al. 2013; Todd et al. 2017). The genome of the Mediterranean fruit fly has recently been sequenced, thus providing a wealth of information which can be exploited for the development of novel, faster, and more accurate genotyping (Papanicolaou et al. 2016). An alternative approach was based on the introduction of the dominant mutation $Sr^2$ (Niyazi et al. 2005) into GSSs, with males distinguishable from wild males because they show three instead of two white stripes on the abdomen.

## 6. FUTURE PERSPECTIVES

Currently, the *wp tsl*-based Mediterranean fruit fly and the *bp*-based Mexican fruit fly GSSs are used worldwide. The capacity to produce sterile males of the Mediterranean and Mexican fruit flies using these GSSs is expected to increase steadily. In many existing facilities, production will increase, and several new facilities are being constructed or expanded, e.g. Mexico, Morocco, and USA (Mission, Texas). Several countries want to use the technology for pest suppression to decrease the reliance on insecticides. The Mediterranean and Latin-American regions may eventually represent a market of 4000 million sterile Mediterranean fruit fly males per week.

Beyond the Mediterranean and Mexican fruit flies, there is an increasing need to develop GSSs for other insect species of agricultural or public health importance, particularly in the case of mosquitoes where the females are the disease vectors, and thus the removal of the females prior to the release of sterile males is mandatory (Marec et al. 2005; Bourtzis et al. 2016; Hendrichs and Robinson, this volume). Since the genetics of many pest species is not well known, using Mendelian genetics to develop a GSS takes some time. Part of the reason for this long lead time is that Mendelian genetics involves several unknowns. For example:

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- Inducing desirable mutations, like the *tsl* mutation in the Mediterranean fruit fly, in other species is a very unpredictable process, with no guarantee that such selective markers will be identified. Genetic markers, potentially useful for sex separation at the pupal stage, are relatively easy to isolate. Therefore, they should be exploited until an efficient and robust embryonic female killing system is developed.
- Translocation induction is also a random process, and based on experiences with the Mediterranean fruit fly, there are at least two criteria that have to be used to select useful translocations: (1) only one autosome should be involved, and (2) the positions of the break points on the autosome and the Y chromosome have to be known.
- Inversions will be very important in any GSS based on classical genetics, but they can also be used to facilitate screening for mutations like the *tsl* and for outcrossing of GSSs. Screening for homozygous viable inversions is very time-consuming and labour-intensive, and requires appropriate mutations and a well-developed cytology.

In contrast to classical genetics, molecular strategies may offer the possibility of developing generic "sexing cassettes" in one species that can be transferred with only minor modifications to other closely related species (Robinson and Franz 2000; Robinson et al. 2004), using non-transgenic or transgenic approaches; this is discussed in more detail in another chapter (Häcker et al., this volume).

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# CHAPTER 4.4.

# INSECT SYMBIOSIS IN SUPPORT OF THE STERILE INSECT TECHNIQUE

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## SUMMARY

Recent estimations from various studies suggest that the total number of insect species ranges between 2.8–30 x $10^6$; an estimate of 5.5 x $10^6$ is probably closer to reality (Stork et al. 2015; Stork 2018). Insect species have established sophisticated symbiotic associations with a plethora of diverse microorganisms including bacterial species. These symbiotic associations can drastically influence the biology, ecology, and evolution of insect hosts, including insect species of agricultural, veterinary, and human-health importance (Margulis and Fester 1991; Zilber-Rosenberg and Rosenberg 2008). Despite the widespread presence of symbiosis, only in the last decades have technological advances enabled us to take a first glance at the complexity of such interactions, at least at the genomic level. Although studies increasingly describe and assess different possible effects of insects' symbionts on their hosts, including in mosquitoes, tsetse flies, and other pest and disease-vector species, this chapter summarizes the knowledge, gained mainly in the last two decades, on the structure and importance of gut symbiotic communities of tephritid fruit flies of economic importance, focusing mainly on their beneficial impact and prospects for improvement in the sterile insect technique (SIT).

## 1. INTRODUCTION

Defining symbiosis and its types has been quite challenging. It was first defined as "the living together of unlike organisms" (De Bary 1879). However, the complexity underlying this term is extensive, and additional terms have been proposed to further elaborate this widely diverse phenomenon. For practical purposes, it is important to follow generally accepted criteria to define symbiotic relationships. Although not fully determined and quite dynamic, such criteria are necessary to categorize and study the different aspects of symbiosis.

A symbiotic relationship (and more specifically, a symbiont) may fall into more than one category, and categorization depends also on the perspective or the aim of the researcher studying the symbiont or the symbiotic relationship. Some of the prevailing terms and classifications of symbiosis are summarized in Table 1. Although the clear majority of insect symbiotic relationships can still be considered as unknown, there are some well-studied examples among different insects of economic, veterinary or human health importance. Their elucidation has been achieved due to numerous studies which have revealed the existence of specific bacteria that clearly are important in certain insect species; some of them are summarized below.

- *Buchnera aphidicola* in aphids: This gammaproteobacterium is a primary symbiont of aphids that provides a clear nutritional benefit to its host. It is an Enterobacteriaceae-like bacterium, but a long-standing symbiotic relationship, estimated to have started 160–260 x $10^6$ years ago, has led to the gradual loss of important genes from the *B. aphidicola* genome and the development of an obligatory symbiotic relationship with aphids (van Ham et al. 2003; Andersson 2006; Pérez-Brocal et al. 2006; Douglas 2009, 2013; Akman Gündüz and Douglas 2012).
- *Wigglesworthia glossinidia*: Like *B. aphidicola,* this bacterium is a primary obligatory symbiont of tsetse flies, offering primarily a nutritional benefit to its host. It has also lost many genes, resulting in a very small genome and dependency on the tsetse host (Aksoy 1995; Chen et al. 1999; Akman et al. 2002; Pais et al. 2008; Weiss and Aksoy 2011; Bing et al. 2017).

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*Table 1. Different types and classifications of symbiosis*

| Term | Definition/examples |
|---|---|
| Mutualism *vs* commensalism *vs* parasitism | Mutualism is when both partners benefit from the relationship, commensalism when one benefits while the other partner is not affected, and parasitism is the relationship where one partner benefits at the expense of the other. |
| Obligatory *vs* facultative | In an obligatory symbiosis, partners cannot survive without each other. In a facultative symbiosis, the partners benefit from living with each other, but they can still live without each other. Alternatively, symbionts can be defined as primary (obligatory) or secondary (facultative). As an example, *Wigglesworthia glossinidia* is considered as a primary endosymbiont of tsetse flies. |
| Ectosymbiosis *vs* endosymbiosis | Depending on whether the symbiont lives on the outer surface or within the body of the host, symbiosis is defined as ecto- and endosymbiosis, respectively. In the case of endosymbiosis, if the symbiont lives either within the host's cells or outside them, it can be considered as intra- or extracellular symbiont, respectively. As examples, *Wolbachia* is considered as intracellular, while the different Enterobacteriaceae localized in the gut are primarily extracellular. |
| Tissue specificity | A symbiont may have a "universal" presence in the host or may be restricted to specific tissues. This may also indicate the specific role of the symbiont. In this respect, Enterobacteriaceae are considered mainly as "gut symbionts", while the alphaproteobacterium *Wolbachia* is considered as a "reproductive symbiont", although additional functions have been reported for it. |
| Type of benefit gained | Symbionts can be beneficial for their hosts in different ways. Among them, those delivering nutritional, reproductive, and immunity/defense advantages, are receiving much attention. |
| Autochthonous (or resident) *vs* allochthonous (or transient) symbiosis | One of the most difficult categories to define is whether we are dealing with "actual" symbiosis, which implies the establishment of long-standing symbiotic relationships, or the observed symbiotic communities are, at least partially, defined by chance (meaning by the random acquisition of symbionts from the environment that are not able to sustain themselves in the host without their continuous supply from the environment). Such relationships, although they may be beneficial for the host, will be eliminated after transfer to a different "ecological niche" (a laboratory can be considered as a different niche as well). In such observed changes, the question regarding symbionts that are no longer present is: are these microorganisms actual symbionts that were no longer beneficial in the new environment, or do they represent transient symbionts that disappeared since there was no longer an external continuous supply from the environment? |
| Type of partner | The usual assumption is that it is bacteria that have established relationships with their eukaryotic host. Although probably bacteria cover most of such symbiotic relationships, there are also symbiotic relationships involving archaea, viruses, and eukaryotic microorganisms (such as fungi and yeast). |

- *Candidatus* Erwinia dacicola: This bacterium is considered as a primary obligatory symbiont for the olive fruit fly *Bactrocera oleae* (Rossi). It is important for helping *B. oleae* larvae feed on a "challenging" environment, such as unripe olives. It is also a characteristic example of the confusing limits of obligatory and secondary symbionts, since it is no longer necessary for domesticated olive flies not reared on olives. Therefore, studying only laboratory populations would lead to the conclusion that this bacterium is not a symbiont of *B. oleae* (Capuzzo 2005; Estes et al. 2009, 2012; Kounatidis et al. 2009; Ben-Yosef et al. 2014) .

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- *Wolbachia pipientis*: This group of Alphaproteobacteria is widespread in insects. It is found mainly in reproductive tissues, and is usually referred to as a "reproductive parasite" due to its ability to manipulate reproductive functions, leading to diverse phenotypes including parthenogenesis, feminization, male-killing, and cytoplasmic incompatibility (CI). It is a facultative intracellular symbiont that has developed preferential relationships with many insect species. Although referred to mainly as a reproductive parasite, there are studies indicating that *Wolbachia* also has a role in nutrition, metabolism, and immunity (Werren et al. 2008; Saridaki and Bourtzis 2010; LePage and Bordenstein 2013). Current knowledge on the status of *Wolbachia* in tephritids, regarding both presence and possible effects, has been summarized recently by Mateos and her colleagues (Mateos et al. 2020). In addition, it has been suggested that the SIT and *Wolbachia* symbiosis can be combined (SIT/IIT, Incompatible Insect Technique) into a safe and biosecure approach for the population suppression of *Aedes* mosquito species transmitting major human pathogenic diseases such as dengue, chikungunya, Zika, and yellow fever (Zheng et al. 2019; Lees et al., this volume).

## 2. MAPPING SYMBIOTIC DIVERSITY

An important step towards utilizing insect symbionts is mapping their diversity. This rather descriptive step is crucial, since we need to know the identities of the symbionts so that later on we can decide what roles symbionts have and which ones are important. Characterizing symbionts and symbiotic communities has been laborious, but recently technological advances in sequencing technology have enabled their quick identification, at least at the DNA level. However, the complete characterization of a symbiont requires a combination of approaches, and is still a labour-consuming task (Table 2).

Prior to DNA-based characterization, the classification of bacteria was based only on phenotypic characteristics. These include morphological, biochemical, and cultivation characteristics, including the ability to grow in selective media, production of characteristic substances that can be identified easily, and resistance/sensitivity to a variety of antimicrobials. As a prerequisite for classification, the bacteria had to be cultivable.

Systematic genotyping started with molecular methods that also required a cultivation-dependent step, especially in the time before the introduction of the Polymerase Chain Reaction (PCR). Sequencing of the 16S *rRNA* gene (or others), and related methods (such as the Denaturing Gradient Gel Electrophoresis (DGGE), Restriction Fragment Length Polymorphism (RFLP), and PCR-RFLP) based methodologies (Krafsur and Ouma, this volume), were utilized and are still being used. Reduction in the cost of Sanger sequencing, and advances in bioinformatic analysis during previous decades, enabled the development of Multi Locus Sequence Typing (MLST) and Multi Locus Sequence Analysis (MLSA) approaches, based on a combination of selected genes, universal for a species or closely related species. However, these methods are still time- and labour-consuming, as well as costly, permitting only a limited number of samples to be analysed and a rather restricted look into the genetic diversity of bacteria. A brief history on the transition from the

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era of sole microscopy to the NGS-supported (Next Generation Sequencing) DNA genotyping is provided by Escobar-Zepeda et al. (2015).

*Table 2. Symbiont classification approaches*

| Criteria | | Approach | Outcome |
|---|---|---|---|
| Phenotypic | Morphological | Microscopic | Characterize the morphology of a selected bacterium |
| | | Macroscopic | Characterize the morphology of a selected bacterial colony |
| | Biochemical | Growth | Characterize the growth profile of a selected bacterium under different conditions (aerobic *vs* anaerobic *vs* microaerophilic, temperature, pH, selective growth media) |
| | | Antibiotic resistance | Characterize the resistance of a selected bacterium to different antimicrobials |
| | | Metabolic pathways | Characterize the presence/absence of specific enzymes through their effect on metabolism |
| Genotypic | Single gene (16S *rRNA* gene) | PCR-RFLP | Characterize genetic diversity based on differences in sequences that are recognized by restriction enzymes; mainly used for 16S *rRNA* gene |
| | | DGGE | Characterize genetic diversity based on differences in the DNA sequence of single genes that alter melting point and electrophoretic mobility |
| | | Sanger sequencing | Characterize genetic diversity of single gene; it can be effective without cultivation in the presence of species-specific primers and absence of multiple genetically close strains |
| | | NGS sequencing | Characterize the genetic diversity of mixed samples, based on a single gene; massive parallel sequencing that generates thousands or millions of short reads (amplicon sequencing) |
| | MLST | Sanger sequencing | Characterize genetic diversity of the DNA sequence of different genes; not suitable for mixed bacterial samples; it can be effective without cultivation in the presence of species-specific primers and absence of multiple genetically close strains |
| | Metagenomics | Genome sequencing | Characterize the genetic diversity based on the whole genome (complete or draft) in complex samples; more effective with cultivated material |
| | Single cell genomics | Genome sequencing | Characterize the genetic diversity based on the whole genome (complete or draft), derived from a single cell |

The recent advances in sequencing technologies, such as High Throughput Sequencing, have enabled quick and massive parallel sequencing at a reduced cost, thus allowing a better look into bacterial communities and not just isolated bacteria. These technologies have been widely used in the last decade for the simultaneous analysis of the 16S *rRNA* gene profile of environmental samples, producing thousands

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to millions of reads per sample, based on the set-up and the needs of the researcher. Still, the major limitation of this approach was the reading length, which is restricted to a few hundred base pairs, thus compromising the robustness of 16S *rRNA* gene-based taxonomy.

These sequencing advances, along with advances in molecular biology and bioinformatic analysis, now enable a faster analysis of bacteria or bacterial communities of interest at a reduced cost. This is extremely important because single gene or MLST approaches cannot fully resolve the genetic diversity among bacteria of interest, especially considering that even strains of the same species can have quite diverse properties. Based on these advances, metagenomic approaches that can analyse almost the complete genomic content of communities of organisms, and single cell genomics (that is the ability to have the complete genome of a single cell), are offering opportunities for the analysis of symbiotic communities that did not seem realistic one or two decades ago.

## 3. EXPLOITING INSECT GUT SYMBIONTS TO ENHANCE THE SIT

There are several studies describing many different possible effects of insects' symbionts on their hosts, as well as their potential role to enhance SIT application. There are studies in mosquito species (for recent reviews see Guégan et al. (2018), Strand (2018), and Scolari et al. (2019) and references therein) and tsetse species (less) (for reviews see Snyder and Rio (2013) and Wang et al. (2013) and references therein), but most of the data derive from experiments on fruit flies in the family Tephritidae (Niyazi et al. 2004; Behar et al. 2008; Ben Ami et al. 2010; Gavriel et al. 2011; Hamden et al. 2013; Sacchetti et al. 2014; Augustinos et al. 2015; Kyritsis et al. 2017; Cai et al. 2018; Khaeso et al. 2018; Ventura et al. 2018; Akami et al. 2019; Augustinos et al. 2019).

### *3.1. Mapping Symbiotic Diversity in Fruit Flies of Economic Importance*

Numerous studies have been performed on different tephritids. Among them, most studies are on the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), which is a model for SIT application, and *B. oleae*. Recently, other species, mainly of the *Anastrepha, Bactrocera,* and *Zeugodacus* genera, have also been targeted. Studies that address the structure of symbiotic communities in tephritids are summarized in Table 3. As already stated, the first steps are descriptive; many studies deal with identifying which bacterial species are found in which hosts. The next step is to describe the relative abundances, and how bacterial symbiotic communities change under different conditions. Subsequently comes the question of functional roles; the mere analysis of presence and/or abundance of certain symbionts cannot answer such questions.

#### *3.1.1. Gut Symbiotic Communities in the Mediterranean Fruit Fly*

Starting with the Mediterranean fruit fly, many studies have been performed on the characterization of its gut symbiotic communities (Table 3). In more recent years,

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beginning in 2005, gut symbiotic communities were described for this species under different conditions. *Klebsiella oxytoca*, an Enterobacteriaceae diazotroph common on plants and able to fix atmospheric nitrogen, was identified as a major symbiont of natural populations (Behar et al. 2005). Minor but persistent *Pseudomonas* communities were also identified using PCR-based assays (Behar et al. 2008).

The effect of irradiation on the structure of gut symbiotic communities was also documented (Ben Ami et al. 2010), and communities of reduced variability were identified in long-established laboratory populations and strains of SIT importance (Ben Ami et al. 2010; Augustinos et al. 2015). Although not strictly referring to gut communities, the reduced diversity of symbionts in a line of the Mediterranean fruit fly VIENNA 7 genetic sexing strain (GSS), reared in Australia (~99% *Enterobacter* sp.) (Morrow et al. 2015), is in accordance with these two studies. Utilization of NGS approaches enabled a more detailed look at the symbiotic communities of both wild and laboratory populations and at different developmental stages (Aharon et al. 2013; Malacrinò et al. 2018).

### *3.1.2. Gut Symbiotic Communities in the Olive Fruit Fly*

Among tephritids, the olive fruit fly is probably the species that most clearly demonstrates how advances in sequencing and bioinformatics have enlarged our ability to study and understand insect symbiotic communities (Table 3). More than a century ago, Petri described cultivable endosymbionts of *B. oleae* (Petri 1909). In 2005, *Candidatus* Erwinia dacicola was identified as the main symbiont of olive fruit fly's natural populations, while later other potential symbionts were identified in natural and laboratory populations of the species (Capuzzo 2005; Estes et al. 2009, 2012; Kounatidis et al. 2009; Ben-Yosef et al. 2014; Augustinos et al. 2019; Koskinioti et al. 2019).

Interestingly, it was also recognized that the primary symbiont of natural populations, *Candidatus* Erwinia dacicola, is almost absent from artificially reared populations ( Estes et al. 2009, 2012; Kounatidis et al. 2009). The presence of abundant and minor components of the communities could be better described, at least to the resolution of genus level (Ben-Yosef et al. 2014, 2015). Using single cell genomics and NGS sequencing, the draft genome of *Candidatus* Erwinia dacicola was obtained, starting from DNA of a single cell, which is important for bacterial species that cannot be cultivated (Blow et al. 2016).

### *3.1.3. Gut Symbiotic Communities in Some* Anastrepha, Bactrocera*, and* Zeugodacus *Species*

Except for the Mediterranean fruit fly and the olive fruit fly discussed above, there are only a few studies in other tephritids (Table 3). Using classical microbiological approaches, a small number of bacterial taxa has been characterized in *Anastrepha ludens* (Loew) (Kuzina et al. 2001), *Bactrocera dorsalis* (Hendel) (Pramanik et al. 2014; Gujjar et al. 2017; Khaeso et al. 2018; Khan et al. 2019), *Bactrocera zonata* (Saunders) (Naaz et al. 2016), *Zeugodacus cucurbitae* (Coquillett) (Hadapad et al. 2016; Gujjar et al. 2017), and *Zeugodacus tau* (Walker) (Prabhakar et al. 2013; Khan et al. 2014).

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*Table 3. Efforts for mapping gut associated bacteria species in tephritids of economic importance*

| Species | Approach | Material used | Main outcome | Reference |
|---|---|---|---|---|
| *Bactrocera oleae* | Direct PCR and sequencing of the 16S *rRNA* gene of extracts | Wild material collected as pupae | *Candidatus* Erwinia dacicola is the main symbiont of wild populations | Capuzzo 2005 |
| | Microscopy, culture dependent, and culture independent approaches | Wild material kept on olives in the lab | *Candidatus* Erwinia dacicola is the main symbiont *Enterobacter* sp., *Citrobacter* sp., and *Raoultella* sp. also present | Estes et al. 2009 |
| | 16S *rRNA* gene libraries and sequencing | Wild material collected as pupae and laboratory colonies | *Acetobacter tropicalis* is present in laboratory colonies and wild sample; *Enterococcus* sp. and *Paenibacillus* sp. also detected; *Candidatus* Erwinia dacicola detected only in natural populations | Kounatidis et al. 2009 |
| | 16S *rRNA* gene PCR and *Candidatus* Erwinia dacicola specific assay | Wild material collected as pupae and laboratory colonies | *Candidatus* Erwinia dacicola spread in all wild populations *Candidatus* Erwinia dacicola present in laboratory colonies reared on olives; *Candidatus* Erwinia dacicola is rare in laboratory colonies reared on artificial diet; Presence of different Enterobacteriaceae in laboratory populations | Estes et al. 2012 |
| | 16S *rRNA* gene libraries and sequencing | Wild material collected as pupae (only oesophageal bulbs) | Two haplotypes of *Candidatus* Erwinia dacicola | Savio et al. 2012 |
| | High throughput sequencing -- 16S *rRNA* gene | Wild material of different developmental stages | High prevalence of *Candidatus* Erwinia dacicola; Low presence of *Pectobacterium*, *Pseudomonas*, and *Kluyvera* | Ben-Yosef et al. 2014 |
| | High throughput sequencing – 16S *rRNA* gene | Wild material kept on olives in the laboratory | High prevalence of *Candidatus* Erwinia dacicola in wild flies but not in mass-reared flies; Low presence of *Pantoea* and *Burkholderia* both in wild flies and mass-reared flies; High prevalence of *Providencia* in mass-reared flies but not in wild flies | Ben-Yosef et al. 2015 |

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*Table 3. Continued*

| Species | Approach | Material used | Main outcome | Reference |
|---|---|---|---|---|
| *Bactrocera oleae* | Single cell genomics and metagenomics | Wild material | Draft genome of *Candidatus* Erwinia dacicola | Blow et al. 2016 |
| | High throughput sequencing – 16S *rRNA* gene | Wild material plus 1st generation in the laboratory | Detection of different OTUs including *Candidatus* Erwinia dacicola, *Providencia, Enterobacter,* and *Klebsiella,* Prevalence of *Candidatus* Erwinia dacicola | Koskinioti et al. 2019 |
| | 454 pyrosequencing | Laboratory population, different developmental stages, age, and sex | Mainly Enterobacteriaceae, *Morganella* prevailing, developmental stage and age influence structure of gut microbiota community | Augustinos et al. 2019 |
| *Bactrocera cacuminata* | 16S *rRNA* gene libraries and sequencing | Wild material | Dominant Gammaproteobacteria Mainly Enterobacteriaceae (*Enterobacter, Serratia, Klebsiella, Escherichia)* | Thaochan et al. 2015 |
| *Bactrocera carambolae* | Illumina sequencing | Wild material | Gammaproteobacteria dominant | Yong et al. 2017 |
| *Bactrocera dorsalis* | PCR of the 16S *rRNA* gene, DGGE, sequencing | Laboratory colonies and wild material | Dominant Gammaproteobacteria Diverse symbiotic community | Wang et al. 2011 |
| | Culture dependent, morphological and biochemical characterization | Laboratory colonies | 11 genera identified Dominant Enterobacteriaceae community (*Citrobacter, Enterobacter, Serratia, Klebsiella, Morganella*) | Pramanik et al. 2014 |
| | Pyrosequencing | Wild material | 172 OTUs identified Effect of developmental stage on symbiotic communities Firmicutes and Proteobacteria dominant | Andongma et al. 2015 |
| | Illumina sequencing | Wild material | Gammaproteobacteria dominant | Yong et al. 2017 |
| | Culture dependent, morphological, biochemical, 16S *rRNA* gene sequencing | Laboratory colonies | Mainly different Enterobacteriaceae genera (*Providencia, Klebsiella, Enterobacter, Citrobacter*) | Gujjar et al. 2017 |
| | Culture dependent, 16S *rRNA* gene sequencing | Labortory colonies | 13 bacterial species Firmicutes and Proteobacteria more abundant | Khaeso et al. 2018 |

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*Table 3. Continued*

| Species | Approach | Material used | Main outcome | Reference |
|---|---|---|---|---|
| *Bactrocera dorsalis* | Illumina sequencing plus culture dependent 16S *rRNA* gene sequencing | Laboratory colonies | Dominance of Enterobacteriaceae, presence of other minor communities that increase their relative abundance after irradiation | Cai et al. 2018 |
| | Culture dependent 16S *rRNA* gene sequencing, morphological characterization | Laboratory colonies | Dominance of Enterobacteriaceae, reduced diversity | Khan et al. 2019 |
| | Illumina sequencing | Laboratory colonies and wild material | Differences due to developmental stage, age, and origin (wild versus laboratory) | Hadapad et al. 2019 |
| *Bactrocera minax* | 454 pyrosequencing | Wild material | Proteobacteria dominant<br>Genera of Enterobacteriaceae the most represented (*Enterobacter*, *Klebsiella*, *Serratia*, *Citrobacter*) | Wang A. et al. 2014 |
| | 454 pyrosequencing | Wild material | Proteobacteria dominant<br>Genera of Enterobacteriaceae the most abundant | Andongma et al. 2019 |
| *Bactrocera tryoni* | 16S *rRNA* gene libraries and sequencing | Wild material | Proteobacteria dominant, mainly Gammaproteobacteria | Thaochan et al. 2015 |
| | 16s *rRNA* gene NGS amplicon sequencing | Laboratory colonies and wild material | Presence of Acetobacteraceae, Enterobacteriaceae, and Leuconostocaceae in wild samples<br>Differences between laboratory and wild populations<br>Presence of *Asaia* sp. in samples deriving from wild | Deutscher et al. 2018 |
| *Bactrocera zonata* | Culture dependent, morphological, biochemical, 16S *rRNA* gene sequencing | Wild material | Three dominant bacterial genera (*Klebsiella*, *Microbacterium*, *Rhodococcus*) | Naaz et al. 2016 |
| *Ceratitis capitata* | PCR-DGGE analysis and sequencing | Wild material | Identification of different Enterobacteriaceae (*Enterobacter*, *Citrobacter*, *Klebsiella*, *Pectobacterium*)<br>*Klebsiella* is the most dominant<br>Variability among developmental stages | Behar et al. 2005 |

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*Table 3. Continued*

| Species | Approach | Material used | Main outcome | Reference |
|---|---|---|---|---|
| *Ceratitis capitata* | Specific assays for targeting minor symbiotic communities (16S *rRNA* gene) | Wild material | Presence of a minor, previously undetected, *Pseudomonas* community | Behar et al. 2008 |
| | Culture dependent approach, 16S *rRNA* gene, DGGE, sequencing | Mass-reared Vienna 8 GSS | Effect of irradiation on gut symbiotic communities<br>Differences between laboratory VIENNA GSS strain and the wild population<br>Different Enterobacteriaceae present (*Salmonella*, *Citrobacter*, *Providencia*, *Morganella*, *Enterobacter*, *Pectobacterium*, *Klebsiella*)<br>Presence of a stable *Pseudomonas* community | Ben Ami et al. 2010 |
| | In situ, metabolic profiles, culture, pyrosequencing | Wild material | Change in the diversity, size and distribution of symbionts in different developmental stages<br>Communities of different Enterobacteriaceae | Aharon et al. 2013 |
| | Culture dependent approach, 16S *rRNA* gene sequencing | Mass-reared Vienna 8 GSS, different developmental stages and ages | Three genera identified (*Enterobacter*, *Providencia*, *Acinetobacter*)<br>Changes in the composition of the symbiotic communities with development | Augustinos et al. 2015 |
| | Illumina MiSeq sequencing, 16S *rRNA* gene | Wild material from different hosts | More than 3000 OTUs present, mainly Firmicutes and Proteobacteria<br>Significant differences among different developmental stages<br>Effect of the host fruit on the profile of the communities | Malacrinò et al. 2018 |
| *Anastrepha fraterculus* | 454 pyrosequencing | Laboratory populations of two morphospecies, different developmental stage, age, and sex | Mainly Enterobacteriaceae, *Morganella* prevailing, developmental stage and age influence structure of gut microbiota community | Augustinos et al. 2019 |
| *Anastrepha grandis* | 454 pyrosequencing | Laboratory population, different develop. stage, age, and sex | Mainly Enterobacteriaceae, *Morganella* prevailing, developmental stage and age influence structure of gut microbiota community | Augustinos et al. 2019 |

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*Table 3. Continued*

| Species | Approach | Material used | Main outcome | Reference |
|---|---|---|---|---|
| *Anastrepha ludens* | Culture dependent, metabolic profile | Laboratory colonies | 18 species in 13 genera Enterobacteriaceae prevailing community (*Enterobacter*, *Providencia*, *Serratia*) | Kuzina et al. 2001 |
| | 454 pyrosequencing | Wild material | Mainly Enterobacteriaceae | Ventura et al. 2018 |
| | 454 pyrosequencing | Laboratory population, different developmental stages, age, and sex | Mainly Enterobacteriaceae, *Morganella* prevailing, developmental stage and age influence structure of gut microbiota community | Augustinos et al. 2019 |
| *Anastrepha obliqua* | 454 pyrosequencing | Wild material | Mainly Enterobacteriaceae | Ventura et al. 2018 |
| *Anastrepha serpentina* | 454 pyrosequencing | Wild material | Mainly Enterobacteriaceae | Ventura et al. 2018 |
| *Anastrepha striata* | 454 pyrosequencing | Wild material | Mainly Enterobacteriaceae | Ventura et al. 2018 |
| *Zeugodacus cucurbitae* | Culture dependent, morphological, biochemical, 16S *rRNA* gene sequencing | Wild material | Nine genera identified, mainly Enterobacteriaceae (*Enterobacter*, *Klebsiella*, *Citrobacter, Providencia*) | Hadapad et al. 2016 |
| | Culture dependent, 16S *rRNA* gene sequencing | Laboratory population | *Bacillus* and *Morganella* | Gujjar et al. 2017 |
| | Illumina sequencing | Laboratory colonies and wild material | Differences due to developmental stage, age, and origin (wild versus laboratory) | Hadapad et al. 2019 |
| *Zeugodacus tau* | Culture dependent, morphological, biochemical, 16S *rRNA* gene sequencing | Laboratory populations | Three species (*Klebsiella oxytoca*, *Pantoea agglomelans*, *Staphylococcus* sp.) | Prabhakar et al. 2013 |
| | Culture dependent, morphological, biochemical, 16S *rRNA* gene sequencing | Laboratory population | Mainly Enterobacteriaceae identified (*Proteus*, *Klebsiella*, *Erwinia*) but also others | Khan et al. 2014 |

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Characterization based on construction of 16S rRNA gene libraries and/or Sanger sequencing has been performed in *Anastrepha fraterculus* (Wiedemann) (Juárez et al. 2019), *Bactrocera cacuminata* (Hering) (Thaochan et al. 2015), *B. dorsalis* (Wang et al. 2011; Khaeso et al. 2018), *Bactrocera minax* (Enderlein), (Andongma et al. 2019), and *Bactrocera tryoni* (Froggatt) (Thaochan et al. 2015).

Finally, using NGS approaches, the structure of gut symbiotic communities was resolved in higher resolution for some species, including both laboratory and natural populations. Among them are natural populations of *A. ludens* (Loew), *A. obliqua* (Macquart), *A. serpentina* (Wiedemann), *A. striata* Schiner (Ventura et al. 2018), *Bactrocera carambolae* Drew and Hancock (Yong et al. 2017), *B. dorsalis* (Andongma et al. 2015; Yong et al. 2017), *Bactrocera minax* (Enderlein) (Wang A. et al. 2014), *Z. cucurbitae* (Hadapad et al. 2019), as well as laboratory populations of two morphotypes of *A. fraterculus*, *A. grandis* (Macquart), and *A. ludens* (Augustinos et al. 2019), *B. dorsalis* (Hadapad et al. 2019), and *Z. cucurbitae* (Hadapad et al. 2019).

### *3.2. Gut Symbiotic Communities – Limitations*

Despite the advances in sequencing approaches that result in enormous data sets, we must keep in mind that such sequencing-based studies are descriptive in principle since a) the resolution provided does not easily go beyond genus level and, b) the "actual" bacteria are not isolated and cultivated, resulting in reduced usefulness regarding possible applications. Moreover, the differences in the methodological set-up (samples collection, DNA extraction, sequencing platform, number of sequences analysed, and algorithms applied for analysis of sequences) make comparisons among studies difficult.

Although the focus until now has been on bacteria, they are not the only symbiotic players in the insect microbiota. A few recent studies are investigating other symbionts that could be important. For example, there is a fungal survey of olive fruit fly wild populations, based on a metabarcoding analysis of ITS2 (Malacrinò et al. 2017), and the cultivation-based identification (coupled with sequencing of selected genes) of yeast in natural populations of *B. tryoni* (Deutscher et al. 2017).

## 4. GUT SYMBIONTS IN THE INSECT MICROBIOTA – ROLE ASSESMENT

It is rather difficult to document the role and importance of symbionts for their host, mainly because:

a. Symbionts may play a role in specific environments but may be redundant, unnecessary or outcompeted in others. *Candidatus* Erwinia dacicola plays a critical role in adult olive fruit flies by providing amino acids or converting inaccessible nitrogen. This symbiont is also important when olive fruit fly larvae feed on unripe olives. In such cases, poor sampling, or transfer of insects to a new environment for experiments, can compromise the results and interpretation.
b. Symbiotic communities probably function in a "redundant" way, meaning that some of the symbiotic bacteria may be important, but their absence can be compensated by others, based on availability and chance. Provided that we are

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dealing with "actual" and not transient symbionts, this may explain why different bacterial species can be found in the same host under different circumstances.

c. There may be changes in preferential relationships during ontogeny. This is also difficult to prove and monitor, but may explain differences observed among developmental stages. During development, communities comprising different bacterial operational taxonomic units (OTUs) during development, or presenting quantitative differences, are commonly found. This means that the same OTUs are present but with significantly different relative abundances. This is what makes interpretation of data from natural populations difficult, where only a limited view of the life cycle is available.
d. Bacteria with the highest representation are not necessarily the most important symbiont. Sometimes, underrepresented members of the community may provide something crucial. Based on the methodological approaches used, such symbionts may remain cryptic and their role underestimated.
e. It is difficult to measure directly the effect of a symbiont. This is because it has to be isolated to test possible properties and functions or it must be studied as a part of the whole symbiotic association (but then it is not clear if a certain effect can be attributed to this specific symbiont).

To provide answers that are as robust as possible, different approaches are followed, and even better, a combination of them. However, all these approaches make certain compromises which must be considered both prior to planning and during the interpretation of results. Some of the main approaches used to estimate the importance of gut-related bacteria to their host are summarized below:

- *Characterization of the symbiotic communities of a species across its range:* As in all experiments, proper sampling is crucial. In this case, proper sampling means collecting material that represents different geographic origins, different seasons, and different hosts. In addition, different developmental stages, sex, and age are parameters that may shape the profile of symbiotic communities. Approaches that take into consideration the above could determine the "core microbiome" of an insect host. Obviously, ideal sampling targeting the symbiotic profile of a species can be a very laborious task.
- *Providing an excess amount of a cultivated symbiont under artificial-diet rearing:* Providing a symbiont through feeding can demonstrate its beneficial effects. However, this does not necessarily mean that these effects are present under standard conditions. Moreover, the positive effect may not have a linear correlation with the quantity; in such cases the effect will not be demonstrated by artificial accumulation of the symbiont.
- *Creation and test of aposymbiotic (symbiont-free) organisms:* This approach is useful because it provides evidence based on the negative (or positive) effects identified after the removal of symbionts. It can be done through antibiotic feeding, which results in the suppression or elimination of the symbiotic community. However, there are certain limitations. It is difficult to attribute an observed effect to a specific symbiont, since the whole community is influenced. Also, antibiotic treatment can influence non-symbiont mediated metabolic pathways (such as those of mitochondria), which makes the interpretation of results questionable. In such experiments, giving time for some generations to

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develop without the effect of the antibiotic would reduce the possibility that the observed results are associated with anything but the removal of the symbionts.

- *Creation of aposymbiotic organisms and reintroduction of selected bacteria:* Although useful, this approach is partially compromised by the fact that an important effect may be identified, but this is in an artificial system and not in the framework of studying the host organism together with its symbionts as a single entity. That means that this effect could be substantially different in the presence of the other autochthonous symbionts.
- *Cultivation of symbionts and assessment of possible effects* in vitro*:* Cultivation in artificial growth media is necessary to find the metabolic pathway through which a symbiont affects its host. *In vitro* cultivation provides evidence about the potentially useful metabolites produced. However, it is not possible to assess *in vitro* whether the symbiont will produce the same effect under the complex *in vivo* interactions of its host and all associated symbionts.

## 5. SYMBIONTS AND THEIR POTENTIAL APPLICATION TO ENHANCE THE SIT

Symbionts can be useful in different ways to boost SIT application (Deutscher et al. 2019). The first application is what we refer to as "probiotic diets". A probiotic diet can be beneficial in different ways. Based on the experimental set-up and the aim of the researcher, a valid research goal is to determine how diets involving symbionts are beneficial. Symbionts can be provided as live material; they can be used as a source of nutrients or to establish their beneficial properties. Live or dead symbionts can also act as food sources for resident symbionts, thus offering an indirect positive effect. Another possible application is to explore the attractancy potential of symbionts, e.g. to make sterile males more attractive for mating with wild females or to include them as a lure in a "trap package" (Pereira et al., this volume). The focus of this chapter is mainly on probiotic applications of gut-associated bacteria.

### *5.1. Positive Effects of Symbiont-Enriched Diets*

Most of the studies have been performed on the Mediterranean fruit fly because it has been a model for the development of the SIT in tephritids (Table 4). This also means that there are universal protocols for rearing and handling, and laboratory rearing is quite easy and optimized. More importantly, the application of the SIT, involving the mass-production of the target species, makes these studies worthwhile. The potential positive effect of symbiont-enriched diets can be adopted easily in fly-rearing facilities, and may have a significantly favourable financial impact. However, the logistics aspect related to the large-scale production of probiotics still needs to be addressed.

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*Table 4. Summary of probiotic studies in Tephritidae and the main findings*

| Species | Bacteria | Provision | Effect | Reference |
|---|---|---|---|---|
| *Ceratitis capitata* | Enterobacteriaceae mix (*Enterobacter agglomerans, Klebsiella pneumoniae*) | Adult diet | Positive on male mating competitiveness of irradiated males (diet-dependent)<br>Positive on male longevity of irradiated males (diet and age-dependent)<br>Positive on sexual calling of irradiated males (diet-dependent) | Niyazi et al. 2004 |
| | Enterobacteriaceae mix isolated from Mediterranean fruit fly (*Enterobacter* spp., *Klebsiella oxytoca, Citrobacter freundii, Pectobacterium cypripedi*, *Pantoea* spp.) | Adult diet | Positive on adult longevity (nutritionally restricted flies) | Behar et al. 2008 |
| | *Klebsiella oxytoca* (Mediterranean fruit fly gut isolated) | Adult diet | Positive on mating latency time (irradiated, nutritionally restricted males) | Ben Ami et al. 2010 |
| | *Klebsiella oxytoca* (Mediterranean fruit fly gut isolated) | Adult diet | Positive on mating competitiveness of irradiated males<br>Positive on mating receptivity of nutritionally restricted females<br>Positive on longevity in starvation of nutritionally restricted females | Gavriel et al. 2011 |
| | Enterobacteriaceae mix of unknown isolation source: *Enterobacter* spp., *Klebsiella pneumonia, Citrobacter freundii* | Larval diet | Positive on pupal weight<br>Positive on flight ability (irradiated males)<br>Positive on adult size<br>Positive on mating competitiveness of irradiated males<br>Positive on sperm transfer | Hamden et al. 2013 |
| | *Enterobacter* sp. AA26 | Larval diet | Replacement or partial replacement of yeast with dead *Enterobacter* can:<br>positively affect pupal and adult recovery<br>positively affect immature development duration (decrease)<br>positively affect female pupal weight<br>positively affect longevity under stress | Kyritsis et al. 2019 |

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*Table 4. Continued*

| Species | Bacteria | Provision | Effect | Reference |
|---|---|---|---|---|
| *Ceratitis capitata* | *Enterobacter* sp. AA26 (Mediterranean fruit fly gut isolated) | Larval diet | Positive on immature developmental duration (decrease)<br>Positive on immature stages survival | Augustinos et al. 2015 |
| | *Enterobacter* sp. AA26, *Klebsiella oxytoca* | Larval and adult diet | *Klebsiella oxytoca* has a positive on immature developmental duration (decrease) as a larval diet probiotic<br>*Klebsiella oxytoca* has a positive on flight ability as a larval diet probiotic | Kyritsis et al. 2017 |
| *Bactrocera oleae* | *Pseudomonas putida* (olive fly gut isolated) | Adult diet | Positive on the fecundity of nutritionally restricted females | Sacchetti et al. 2014 |
| *Bactrocera dorsalis* | Various preparations of the identified bacteria | Larval diet | Effect on immature developmental duration (reduction or increase)<br>Positive effect on pupal weight | Khaeso et al. 2018 |
| | *Klebsiella oxytoca* and *Citrobacter coseri* separate live feeding | Larval diet | Feeding with live bacteria can restore ecological fitness of males that are sterilized through irradiation | Cai et al. 2018 |
| | *Proteus* sp. | Larval diet | Improved adult emergence, male ratio, and survival under stress | Khan et al. 2019 |
| *Bactrocera tryoni* | *Klebsiella* | Adult diet | No effect on fecundity | Meats et al. 2009 |
| | *Asaia* sp., *Enterobacter* sp., *Lactobacillus* sp., *Leuconostoc* sp. | Larval diet | *Enterobacter* sp. and *Asaia* sp. shortened immature development duration | Shuttleworth et al. 2019 |
| *Zeugodacus tau* | *Klebsiella* and *Proteus* | Adult diet | No effect on ovariole number or fecundity | Khan et al. 2014 |
| *Anastrepha obliqua* | Mix of endogenous gut symbionts | Adult diet | No effect on male longevity | Rull et al. 2015 |

### *5.1.1. Probiotic Supplements for the Adult Diet of the Mediterranean Fruit Fly*

Probiotics can be added to the larval or adult diets (or both). A positive effect of adult diets (enriched with an Enterobacteriaceae mix) on the mating competitiveness, longevity, and sexual calling of irradiated males was described by Niyazi et al. (2004). A different Enterobacteriaceae mix had positive effects on adult longevity

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(Behar et al. 2008). Later, a positive impact (of the addition of *K. oxytoca* to the adult diet of the VIENNA 8 GSS) on the mating latency time of irradiated males was found by Ben Ami et al. (2010). By enriching the adult diet with the same isolate, and for the same Mediterranean fruit fly strain, positive effects on the mating competitiveness of irradiated males, and on the mating receptivity and longevity of females under starvation, were described (Gavriel et al. 2011) (see also Bakri et al., this volume; Barclay, this volume; Hendrichs and Robinson, this volume; Itô et al., this volume; Lance and McInnis, this volume; Marec et al., this volume; Parker, Mamai et al., this volume; Robinson, this volume; Vreysen, this volume; Whitten and Mahon, this volume). However, in an independent study a few years later, using the same bacterial isolate and the VIENNA $8^{D53+}$ strain, a positive effect on the mating competitiveness of irradiated males was not detected (Kyritsis et al. 2017).

*5.1.2. Probiotic Supplements for the Larval Diets of the Mediterranean Fruit Fly*

Regarding probiotic enrichment of larval diets, the addition of three Enterobacteriaceae strains, isolated from sources other than the Mediterranean fruit fly, showed positive effects on pupal weight, adult size, flight ability, and the mating competitiveness and sperm transfer of irradiated males (Hamden et al. 2013). The addition of an indigenous bacterial symbiont *Enterobacter* sp. AA26 decreased the duration of immature development and increased the survival of immature stages, leading to increased productivity in the fly strain. However, no positive effects on the mating competitiveness of irradiated males were observed (Augustinos et al. 2015).

The use of *K. oxytoca* as an additive to the larval diet led to a decrease in the duration of immature development, and also had positive effects on flight ability (Kyritsis et al. 2017). Finally, although not strictly probiotic, the partial or full replacement of yeast (the costliest diet ingredient) with dead *Enterobacter* sp. AA26, in the form of dry biomass, gave promising results regarding the reduction or elimination of yeast from the larval diet, which could lead to a decreased cost. More specifically, replacement had a positive effect on the survival of immature stages, on pupal weight, and on adult survival under starvation. At the same time, no effect was observed regarding the sex ratio, fecundity, flight ability, and male mating competitiveness, showing that it is an approach worthy of further investigation (Kyritsis et al. 2019; Stathopoulou et al. 2021).

*5.1.3. Probiotic Supplements for the Larval and Adult Diets of Tephritids Other than the Mediterranean Fruit Fly*

Several recent studies have described the effects of adding a bacterial inoculant to the larval diet of a fruit fly other than the Mediterranean fruit fly. In *B. dorsalis* and *B. tryoni,* adding various bacterial cultures either reduced or increased the immature developmental duration, and had a positive or no effect on pupal weight, emergence, sex ratio, flight ability or survival under stress (Khaeso et al. 2018; Khan et al. 2019; Shuttleworth et al. 2019). Also, studies were done on using probiotic-enriched adult diets. Adding *Pseudomonas putida* to an olive fruit fly adult diet had positive effects on female fecundity (Sacchetti et al. 2014), but adding *Klebsiella* sp. in a *B. tryoni* adult diet, and K*lebsiella* sp. combined with *Proteus* sp. in a *Z. tau* diet, had no effect

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on fecundity (Meats et al. 2009; Khan et al. 2014). In a comprehensive analysis of virgin olive fruit fly females naturally infected with their symbionts, treated with antibiotics or treated with antibiotics and then provided with probiotic supplements of symbionts isolated from the olive fruit fly or Mediterranean fruit fly, it was shown that the first group with the natural microbiome (primarily consisting of *Candidatus* Erwinia dacicola) attempted oviposition significantly more times than the treated flies (Jose et al. 2019). Finally, adding a mix of endogenous symbionts in an *A. obliqua* diet had no effect on male longevity (Rull et al. 2015). In a recent report on *B. dorsalis* it was shown that a probiotic supplement of the gut-isolated symbiont *Klebsiella oxytoca* (BD177) can restore the biological quality of insects irradiated at the pupal stage (Cai et al. 2018).

### *5.2. Non-Probiotic Applications of Symbionts, and Alternative Documented Positive Effects*

Besides probiotic diets, there are other ways to exploit putative positive effects of symbionts (Table 5). Moreover, feeding in different stages is not the only starting point to assess a potentially beneficial effect. Comparisons between symbiotic and aposymbiotic olive fruit flies have shown that aposymbiotic flies have decreased fecundity, and their larvae cannot develop on unripe olives (Ben-Yosef et al. 2014, 2015). NGS characterization of symbiotic communities, along with the metabolic profile of *Citrobacter* sp., and accompanying insecticide resistance assays in *B. dorsalis*, demonstrated that this bacterium enhances its host insecticide resistance (Cheng et al. 2017). The use of aposymbiotic and reinfected *B. dorsalis* females revealed that symbionts influence mate selection (Damodaram et al. 2016), while aposymbiotic *B. dorsalis* flies exhibit a different foraging behaviour than symbiotic ones (Akami et al. 2019). Also, it is worth noting that commensal microbiota were shown recently to modulate larval foraging behaviour, development rate, and pupal production in *B. tryoni* (Morimoto et al. 2019). Finally, using bacterial cultures of different species in attractancy assays showed that *A. ludens*, *B. dorsalis*, *B. zonata*, *Rhagoletis mendax* Curran, and *Z. cucurbitae* adults can be attracted by various bacterial cultures (Maccollom et al. 1992; Reddy et al. 2014; Wang H. et al. 2014; Hadapad et al. 2016).

## 6. SYMBIONTS AS UNIVERSAL PROBIOTICS: REALITY OR ILLUSION?

Until now, results are encouraging but sometimes contradicted by other studies. Variations and inconsistencies among studies show that “universal probiotics” for the SIT-important species are feasible, at least for Tephritidae, but not yet applicable. The main origins of controversy are described below:

- *Obvious ones:* For several reasons it is not easy to compare directly the findings of different studies: the experiments are conducted with different insect species or strains, and use different bacterial species/isolates and feeding strategies (larval stages versus adults). Moreover, not all studies answer the same questions, e.g. they shed light on different parameters that are considered important in SIT application (such as rearing productivity or biological quality).

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*Table 5. Summary of non-probiotic effects of symbiotic communities on Tephritidae*

| Species | Material | Methodology | Main effect | Reference |
|---|---|---|---|---|
| *Ceratitis capitata* | Laboratory colony | Symbiotic vs aposymbiotic flies | Aposymbiotic females showed an accelerated oviposition rate under restricted diets<br>Symbionts affect mating latency time | Ben-Yosef et al. 2008b |
| | Laboratory colony | Symbiotic vs aposymbiotic flies | Aposymbiotic adults have decreased longevity under restricted diets | Ben-Yosef et al. 2008a |
| *Bactrocera oleae* | Wild material | Symbiotic vs aposymbiotic flies | Aposymbiotic females have decreased fecundity under various restricted diets | Ben-Yosef et al. 2010 |
| | Wild material | Symbiotic vs aposymbiotic flies | Aposymbiotic females have decreased fecundity under various restricted diets | Ben-Yosef et al. 2014 |
| | Wild material | Symbiotic vs aposymbiotic flies | Gut microbiota are important for larvae when developing in unripe olives, but not in ripe olives | Ben-Yosef et al. 2015 |
| *Bactrocera dorsalis* | Wild material | Bacterial cultures as baits | Adults are attracted to bacterial isolates (autoclaved) | Wang H. et al. 2014 |
| | Laboratory colony | Aposymbiotic and reinfected females | Symbionts influence mate selection | Damodaram et al. 2016 |
| | Laboratory colony | NGS characterization of symbiotic communities, culture and metabolic profile of *Citrobacter* | *Citrobacter* sp. enhances insecticide resistance | Cheng et al. 2017 |
| | Laboratory colony | Symbiotic vs aposymbiotic flies | Suppression of the microbiome results in changes of foraging behaviour of males and females | Akami et al. 2019 |
| *Bactrocera tryoni* | Laboratory colony | Assessment of the effect of vertically and horizontally acquired bacteria to development | The presence of both vertically and horizontally acquired bacteria improves larval foraging behaviour, development rate, and pupal production | Morimoto et al. 2019 |
| *Bactrocera zonata* | Laboratory colony | Attractancy assays for bacterial cultures | *Enterobacter* sp. and *Klebsiella* sp. are highly attractive for adults | Reddy et al. 2014 |
| *Zeugodacus cucurbitae* | Laboratory colony | Cultivation of bacteria and attractancy assays | Adults are attracted to bacterial isolates | Hadapad et al. 2016 |
| *Rhagoletis mendax* | Wild material | Traps with *P. agglomerans* | Adults are more attracted to traps baited with *P. agglomerans* | Maccollom et al. 1992 |

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Even in cases where the same questions are asked, the methodological set-up may be quite different, e.g. differences in rearing practices, and how male mating competitiveness is evaluated in laboratory, semi-field or field conditions. For all these reasons, results may vary or in some cases even be contradictory.

- *Differences in rearing practices:* Even in cases where the same insect strains (or strains sharing the same origin) and similar bacterial isolates have been used, results derived from different laboratories could be different. An important parameter influencing data interpretation is how each laboratory defines "standard (or control) rearing conditions". In the larval diet, different substrates that can influence the nutritional value of the control diet are being used. Diets such as wheat-bran, carrot-based or liquid diets are employed in various laboratories, and the percentage of yeast and other ingredients may vary as well. Diet has a strong effect on shaping the associated microbiota, as was shown recently in *B. tryoni, C capitata,* and *Z. cucurbitae* (Asimakis et al. 2019; Augustinos et al. 2019; Woruba et al. 2019). These aspects could strongly affect the results, and consequently the probiotic role assigned to the bacteria; some probiotic supplements could be beneficial only under specific conditions, e.g. in nutritionally poor diets.
- *Developmental stage specificity and dynamics:* Comparing the findings of related studies shows that the same (or similar) bacterial taxa do not consistently have the same effects. Bacterial taxa show great variations in the various developmental stages (Ben Ami et al. 2010; Aharon et al. 2013; Andongma et al. 2015; Augustinos et al. 2015). Over the last decade, many studies have tried to shed light on the characterization of gut symbiotic communities of tephritids, either conducted with long-time laboratory-adapted fly populations or with ones recently introduced into the laboratory. These studies suggest that gut symbiotic communities, although relatively simple in their structure, consisting of few bacterial taxa, show great variations in the different developmental stages at lower taxonomic levels (Ben Ami et al. 2010; Aharon et al. 2013; Andongma et al. 2015; Augustinos et al. 2015). These differences are usually evident at the taxonomic level of bacterial species or genus, may reflect the development of preferential relationships, and may explain why specific bacteria do not "retain" their beneficial effects when provided in different feeding stages (such as larval stages versus adults).

  From a different point of view, it is possible that different isolates belonging to the same bacterial species may not have the same effect. This can be related to either "real" differential properties of these strains, due to restricted but crucial genetic variability, or their "false" identification as being genetically close, due to the genotyping methodology used. Advances, that allow the complete metagenomic analysis at a relatively low cost, are expected to solve such potential misidentification issues.
- *Methodological and experimental set-up:* A major issue when trying to compare results regarding the effect of adult probiotic feeding on SIT-important parameters, such as male mating competitiveness and longevity under stress conditions, comes from experimental assumptions and limitations. The use of live instead of dead bacteria, the nutritional profile of the control diet, and the wild

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populations used for mating competitiveness studies are important parameters that may affect both the results and their interpretation.

## 7. CONCLUSIONS

Insects have established coevolved complex and sophisticated symbiotic associations with diverse microorganisms, including bacterial species. Recent advances in molecular biology and microbiology, sequencing methods, and bioinformatics have enabled the characterization of these symbiotic interactions, indicating the importance of the symbionts in many aspects of insect host biology, ecology, and evolution (including nutrition, mating behaviour, reproduction, and immunity). These advances in insect symbiosis enable us to exploit these symbiotic associations to support improving SIT applications against insect pests of agricultural, veterinary, and human health importance. In particular, there appears to be considerable potential in harnessing insect gut-associated microbiota to improve mass-rearing cost-effectiveness, as well as the biological quality (including male mating competitiveness) of the resulting sterile insects for SIT operational programmes.

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# CHAPTER 4.5.

# IMPROVING POST-FACTORY PERFORMANCE OF STERILE MALE FRUIT FLIES IN SUPPORT OF THE STERILE INSECT TECHNIQUE

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## SUMMARY

The sterile insect technique (SIT) is being applied against tephritid fruit fly pests in many areas of the world. Currently, fruit fly factories have the capacity to produce and sterilize several billion (thousand million) sterile insects per week, and to make them available for shipment to their final destinations. At sterile fly emergence and release facilities, the emerging flies are fed and held close to maturity, and then collected for area-wide release. While much research effort has been invested in improving mass-rearing and quality-control procedures at the fly-factory level, the post-factory handling of sterile flies has received much less attention. However, research (conducted mainly from 2000 onwards) has focussed on developing and validating ways of improving sterile male performance through better management during a critical period (starting with the arrival of pupae at the fly emergence and release facility and ending with the release of the sterile flies in the field). This chapter summarizes the progress made on this subject for fruit fly species (in the genera *Anastrepha, Bactrocera*, *Ceratitis* and *Zeugodacus*) against which the SIT is being applied. To increase the effectiveness of fruit fly SIT programmes, exposure of sterile males to nutritional, hormonal, and semiochemical treatments has been assessed for improvement in sterile male performance, and enhancement of post-factory handling and release methods. Incorporation of protein and juvenile hormone into pre-release *Anastrepha* spp. diets significantly accelerates sterile male maturation and improves sexual performance in several species. Improved or probiotic adult diets and semiochemical treatments using ginger root oil or citrus oils in *Ceratitis capitata*, and methyl eugenol and raspberry ketone in *Bactrocera* and *Zeugodacus* species, significantly increase sterile male mating competitiveness. Some of these treatments and improvements have been transferred to, and are being applied routinely in, operational programmes. However, these efforts need to be further strengthened to assess the interaction among different environmental and holding conditions, treatments and release systems, and to improve further the performance of mass-produced sterile males, a critical component of increasing the effectiveness of operational programmes.

## 1. INTRODUCTION

Application of the sterile insect technique (SIT), as part of an area-wide integrated pest management (AW-IPM) approach against fruit fly (Diptera: Tephritidae) pests, is gaining momentum, with active programmes targeting fruit fly pest species of economic importance in North, Central and South America, Europe, Middle East, Asia, Africa, and Australia (Enkerlin, this volume). There are now fruit fly factories with the capacity to mass-rear several billion (thousand million) sterile male insects per week (for detailed information, note the World-Wide Directory of SIT Facilities (DIR-SIT 2020)) (Parker, Mamai et al., this volume). After irradiation (Bakri et al., this volume), pupae are shipped to their destination, where they are processed in fly emergence and release facilities (FAO/IAEA 2017; Dowell et al., this volume). Several days after emergence, feeding, and sexual maturation, the sterile flies are released in the field, where the sterile males are expected to mate with, and transfer an effective ejaculate to, wild females. An industrial process, consisting of numerous complex steps, is required to achieve a biological goal (Pereira et al. 2013a).

Several quality-control and quality-assurance protocols have been developed to ensure that the released flies fulfil a series of minimum quality standards (FAO/IAEA/USDA 2019; Parker, Vreysen et al., this volume), but exigencies of the industrial process often affect the biological qualities of the final product.

The mass-rearing of fruit flies up to the pupal stage, including sterilization, takes place in specially designed insect factories (where insects are adapted to indoor conditions) (FAO/IAEA 2018). As late-stage pupae, the mass-reared insects are irradiated and shipped to fly emergence and release facilities, where they are placed in containers/release bags, boxes or towers for adult emergence, feeding and holding

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before they are released into target field areas (FAO/IAEA 2017; Dowell et al., this volume). During this critical period at these facilities, there is the potential to manipulate sterile males in a manner that will improve their mating success in the field (Pereira et al. 2013a). This includes optimizing the provision of nutritional supplements (Yuval et al. 2002), and hormonal (Teal et al. 2000) and semiochemical treatments (Shelly 2001), as well as the holding conditions and preparations for release (Tween and Rendón 2007; USDA/APHIS 2009; FAO/IAEA 2017).

An FAO/IAEA Coordinated Research Project (CRP) on "Improving Sterile Male Performance in Fruit Fly SIT Programmes" was conducted (2004 to 2009) with the objective of increasing the effectiveness of the SIT by improving the performance of sterile males after mass-rearing. The outcome of this research network, involving 31 research institutes in 17 countries, was summarized by Pereira et al. (2013a). Since then, the fields of applying nutritional, hormonal, and semiochemical treatments, as well as the work on optimizing pre-release conditions and operations that improve male quality, have continued to evolve. This chapter summarizes the current status in improving the performance of sterile males after mass-rearing.

## 2. NUTRITIONAL SUPPLEMENTS

In a majority of tephritid fruit flies, both males and females are anautogenous, emerging as adults with mostly undeveloped gonads. Both sexes rely on foraging during early adult life to ingest carbohydrates to fuel metabolic activities, as well as nitrogenous compounds for gonadal and accessory gland development and pheromone production (Epsky and Heath 1993; Hendrichs and Prokopy 1994). Thus, the survival and reproductive success of males in most tephritid species of economic importance is linked to the access to these nutrients. Nevertheless, several fly emergence and release facilities still do not include nitrogenous compounds in the pre-release diet of sterile males. The sterile males are generally offered a pre-release diet of only sugar or highly concentrated sucrose, presented in agar blocks, which are also a source of water (USDA/APHIS 2009; FAO/IAEA 2017).

The results on assessing nutritional supplements in the last 20 years are described thematically in more detail, together with relevant references, in Table 1. Studies on four genera of tephritids (*Anastrepha*, *Bactrocera*, *Ceratitis* and *Zeugodacus*) indicate that providing yeast hydrolysate to males, as part of the pre-release diet in the days following emergence, can enhance male sexual performance and reduce wild-female remating, although the optimal dosage and most practical form of presentation in an operational context still needs to be refined further (Kaspi and Yuval 2000; Aluja et al. 2001; Pérez-Staples et al. 2007; Haq et al. 2010a, 2014a). The implementation of such a strategy depends on a specific understanding of the nutritional needs of males (while feeding on nitrogenous sources in nature), trade-offs between sexual performance and survival, and operational costs and benefits. Another benefit of providing a protein-enriched pre-release diet is that sterile males will be much less attracted to nitrogenous baits in sprays or traps (such as Biolure or CeraTrap) that are increasingly being used for monitoring wild females when releasing only sterile males (Vargas et al. 2002; Maor et al. 2004; Vargas and Prokopy 2006; San Andrés et al. 2009).

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*Table 1. Summary of results, by thematic subject, of research conducted on the incorporation of nutritional supplements into pre-release diets and male performance of tephritids of the genera* Anastrepha, Bactrocera, Ceratitis, *and* Zeugodacus *(modified after Pereira et al. 2013a, reproduced with permission)*

| Subject addressed | Summary of results |
|---|---|
| Natural food | • Males feeding on orange (*Citrus sinensis* (L.) Osbeck) or mango (*Mangifera indica* L.) exhibit modified pheromone composition and improved sexual performance in *Anastrepha ludens* (Loew) and *Anastrepha obliqua* (Macquart) (Liedo et al. 2013; Utgés et al. 2013).<br>• Male pheromone production is quantitatively affected by natural food in *A. ludens* and *A. obliqua* (Liedo et al. 2013).<br>• Wild *Bactrocera tryoni* (Froggatt) males feeding on natural sources of food mature much slower than wild males with access to yeast hydrolysate; effects of diet are far less pronounced in mass-reared males (Weldon and Taylor 2011). |
| Dietary supplements | • Adding yeast hydrolysate (protein) to the pre-release diet significantly improves male sexual performance in *Anastrepha fraterculus* (Wiedemann), *A. ludens, A. obliqua, Anastrepha serpentina* (Wiedemann), *Anastrepha striata* Schiner, *Anastrepha suspensa* (Loew), *Bactrocera correcta* (Bezzi), *Bactrocera dorsalis* (Hendel), *B. tryoni, Bactrocera zonata* (Saunders), *Ceratitis capitata* (Wiedemann), *Ceratitis quilicii* De Meyer, Mwatawala, and Virgilio, and *Zeugodacus cucurbitae* (Coquillett) (Taylor and Yuval 1999; Aluja et al. 2001; Shelly et al. 2005; Pérez-Staples et al. 2007, 2008b, 2009; Yuval et al. 2007; Pereira et al. 2009, 2010a, b, 2011; Haq et al. 2010a, b, 2013; Haq and Hendrichs 2013; Liedo et al. 2013; Liendo et al. 2013; Orankanok et al. 2013; Quilici et al. 2013; Ndzana et al. 2016; Shelly 2017a).<br>• Adding protein to the pre-release diet improves male weight and body protein content in *A. suspensa, B. dorsalis,* and *Z. cucurbitae* (Haq et al. 2010c; Pereira et al. 2011; Reyes-Hernández et al. 2019). It also enhances male sexual organ development in *B. dorsalis* (Reyes-Hernández et al. 2019) and *B. tryoni* (Pérez-Staples et al. 2011).<br>• Some studies in *C. capitata* found no difference between sugar-only fed males and yeast hydrolysate-fed males in terms of mating success (Shelly and McInnis 2003; Shelly et al. 2003, 2006a), survival rate (Shelly and McInnis 2003), and dispersal (Shelly and Edu 2008a). |
| Ratio of protein to carbohydrates | • Protein in dry diets early in adult life contributes to male sexual performance, but the ratio of protein to carbohydrates affects survival in a dose-dependent manner in *A. fraterculus, A. ludens*, *A. obliqua,* and *B. tryoni* (Prabhu et al. 2008; Gómez et al. 2013; Liedo et al. 2013; Liendo et al. 2013; Utgés et al. 2013).<br>• As little as 4–10% of protein content in the pre-release diet is sufficient to significantly enhance male sexual performance in *A. fraterculus*, *A. ludens*, *A. obliqua,* and *B. tryoni* (Pérez-Staples et al. 2008a; Pereira et al. 2011; Gómez et al. 2013; Liedo et al. 2013; Liendo et al. 2013). |
| Optimal formulation/ delivery system | • A formulation based on soy whey protein and incorporating methoprene has been developed and tested for *A. suspensa* (Teal et al. 2013).<br>• Concurrently, a commercial product "Mubarqui" containing protein has been introduced in a dry formulation into release programmes for *A. ludens* and *A. obliqua* (Gómez and Teal 2010; Gómez et al. 2013). |
| Dietary effects on remating inhibition | • Feeding on a nitrogen-rich food significantly improves sterile male ability to inhibit female remating in *A. fraterculus* (Abraham et al. 2011, 2012, but see Abraham et al. 2013), *Bactrocera carambolae* Drew and Hancock, *B. tryoni, C. capitata,* and *Z. cucurbitae* (Pérez-Staples et al. 2008a; Gavriel et al. 2009; Haq et al. 2014a). |

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*Table 1. Continued*

| Subject addressed | Summary of results |
|---|---|
| Interaction between diet, dispersal, and survival | • Protein-rich diets are related to a lower resistance to starvation in *A. fraterculus, A. ludens*, *A. obliqua, C. capitata,* and *B. tryoni* (Levy et al. 2005; Utgés et al. 2013; Reynolds et al. 2014; Juárez et al. 2019).<br>• Adult diets that contain protein are related to lower recapture rates in protein-baited traps, and to reduced mobility and longevity in sterile *A. ludens*, *A. obliqua* (but not in *C. capitata*) males (Maor et al. 2004; San Andrés et al. 2009; Utgés et al. 2013).<br>• Protein-rich post-teneral diets do not adversely affect the survival and movement in the field of sterile *B. tryoni* and *C. capitata* males (Yuval et al. 2007; Gavriel et al. 2010; Taylor et al. 2013b).<br>• In the laboratory, protein-fed males live significantly longer than only sugar-fed males of *B. tryoni* and *Z. cucurbitae* (Haq et al. 2010b; Taylor et al. 2013b). The same was found for *A. ludens* and *A. obliqua* when the sugar:yeast ratio is 9:1 or 24:1, but not with a 3:1 ratio (Liedo et al. 2013).<br>• In the laboratory, *B. tryoni* fed a 3:1 ratio of sugar:yeast for two days following emergence are more vulnerable to starvation if food is unavailable in the following days than if the flies are fed only sugar (Taylor et al. 2013a). |
| Contribution of micro-organisms | • Supplementing post-teneral diets with symbiotic bacteria reduces mass-rearing costs and increases mating success in fruit fly pests in SIT application (Cáceres et al. 2019; Noman et al. 2020; Raza et al. 2020; Augustinos et al., this volume).<br>• The significant contribution of micro-organisms to the fitness of non-sterile *Bactrocera oleae* (Rossi) and *C. capitata* has been established (Behar et al. 2008a; Ben-Yosef et al. 2008a, b, 2010, 2014, 2015; Yuval et al. 2013; Stathopoulou et al. 2021). In *B. oleae,* the survival of males feeding on a probiotic diet is reduced compared with those fed on a sugar-only diet (Sacchetti et al. 2014).<br>• Presence of commensal bacteria affect mate-selection behaviour of *B. dorsalis* (Damodaram et al. 2016).<br>• Manipulating the microflora of sterile males by supplementing probiotic bacteria in adult diets can improve male sexual performance in *C. capitata* (Niyazi et al. 2004; Behar et al. 2005, 2008a, b, c; Ben-Ami et al. 2010; Gavriel et al. 2011; Hamden et al. 2013; Kyritsis et al. 2019), and in *A. obliqua*, *B. dorsalis,* and *Z. cucurbitae* (Rull et al. 2015; Yao et al. 2017; Cai et al. 2018; Stathopoulou et al. 2021). |
| Irradiation effects mitigated by dietary supplements | • Feeding with live bacteria can restore ecological fitness of males that were sterilized through irradiation in *B. dorsalis* (Cai et al. 2018).<br>• Diet and irradiation affect micro-organism diversity in *B. tryoni* (Woruba et al. 2019).<br>• Microflora diversity in laboratory-reared and irradiated males is low when compared with wild *C. capitata* males (Ben-Ami et al. 2010).<br>• Irradiation significantly diminishes the ability of *B. tryoni* flies to tolerate protein deprivation (Pérez-Staples et al. 2007; Taylor et al. 2013b). |

Studies on the nutritional requirements of males of several tephritid species and their effect on sexual success (involving various species targeted by the SIT) have reached different levels of understanding. For example, studies in nature on *C. capitata* provide evidence that wild males feed on sources of protein (Hendrichs and Hendrichs 1990; Hendrichs et al. 1991). However, while providing yeast hydrolysates or other sources of protein together with sugar to males generally improved mating success in *Anastrepha*, *Bactrocera,* and *Zeugodacus* (Table 1), in *Ceratitis* there is a variance in results reflecting the influence of other factors.

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Different effects of protein-enriched diets have been found, in some cases reducing male survival and dispersal; the adverse effect was reduced when the protein ratio in the diet was lowered, but there are no clear procedural guidelines available currently for this and other target species (Blay and Yuval 1997; Shelly and Kennelly 2003; Shelly and McInnis 2003; Prabhu et al. 2008; FAO/IAEA 2017).

Moreover, there is evidence that the integrity of micro-organisms in the male gut contribute to fly health, foraging behaviour, and sexual performance (Yuval et al. 2013; Akami et al. 2019; Deutscher et al. 2019; Juárez et al. 2019; Augustinos et al., this volume). Adult diets enriched with different probiotic bacteria strains showed in irradiated *C. capitata* males a positive effect on the mating competitiveness, mating latency time, sexual calling or longevity (Niyazi et al. 2004; Behar et al. 2008b; Ben Ami et al. 2010; Gavriel et al. 2011).

Nevertheless, despite the increasing scientific evidence of the benefits of nutritional supplements, currently, possibly out of convenience, sterile males are often offered only a pre-release diet of highly concentrated sucrose, normally presented in an agar block, but without any protein addition. The formulation and testing of optimal pre-release diets, containing sugar, protein and probiotic bacteria (and possibly other ingredients) in proportions that will result in enhanced sterile male performance in the field, is still not fully understood and accepted by many operational programme managers, and remains to be implemented. However, the Moscamed programme in Mexico uses the Mubarqui adult diet for *C. capitata*, which contains proteins from diverse plant seeds (Gómez et al. 2013), and the Moscafrut programme in Mexico releases sterile *A. ludens* and *A. obliqua* flies fed with a 24:1 sugar:yeast adult diet. Managers should evaluate this matter in relation to their fruit fly species and decide on the most appropriate pre-release adult diet and feeding regime for sterile males.

## 3. HORMONAL TREATMENTS

Age is a significant factor affecting sexual signalling and reproduction in numerous tephritid species (Liedo et al. 2002; Pereira et al. 2013a). Being anautogenous, tephritid fruit fly pests in nature require a considerable period (over a week in *Ceratitis* spp.; 2–3 weeks in *Anastrepha* spp*., Bactrocera* spp., and *Zeugodacus* spp.) under adequate nutritional conditions to reach sexual maturation (Teal et al. 2013). Even though mass-rearing inadvertently selects for accelerated sexual maturation, the discrepancy between the standard holding periods at fly emergence and release facilities, and the time to reach sexual maturation, poses a significant problem for SIT programmes. After release but before reaching full sexual maturation, sterile males suffer significant losses due to predation and other causes, resulting in far fewer males surviving to maturity and copulation (Hendrichs and Hendrichs 1998; Rao et al. 2013; González-López et al. 2016).

Holding sterile males for more days also increases the management costs, and additional investment is required to expand the infrastructure at fly emergence and release facilities (USDA/APHIS 2009). In addition, in those programmes where sterile males are released together with sterile females, longer holding times are not advisable because males may start mating before release, thereby transferring their

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limited sperm to sterile females. Clearly, the development of cost-effective methods to accelerate sexual maturity in released flies would have a significant positive impact on the efficacy of the SIT (Teal et al. 2000).

Research on several tropical *Anastrepha* species shows that juvenile hormone is a critical hormone, regulating sexual maturity and sexual signalling in these species (Table 2). Furthermore, application of juvenile hormone or the analogues, methoprene or fenoxycarb, can accelerate reproductive development and sexual signalling in sterile males of some species (Pereira et al. 2013a, b; Teal et al. 2013). Significant progress has been made in evaluating hormonal treatments using methoprene to accelerate reproductive development and, in some cases, improving further male sexual performance. It was found in many species (but not all) that sterile males become sexually mature significantly earlier when hormone exposure is included in pre-release holding protocols, but this optimum effect was only achieved when hormone treatment was coupled with a protein-enriched pre-release diet (Teal et al. 2013). Thus, irradiated males become sexually mature earlier and can be released earlier. This advantage is particularly important for SIT application against those species of *Anastrepha* spp*.,* *Bactrocera* spp*.,* and *Z. cucurbitae* that have long pre-copulatory periods. The improvement in male sexual performance by applying hormonal treatments was extensively studied in *Anastrepha* spp., *B. dorsalis*, *B. tryoni*, *Z. cucurbitae,* and *C. capitata*, although in these last two species no benefits were found. In addition, considerable progress was made in developing delivery systems to treat large numbers of flies with methoprene in operational programmes. The results on hormonal treatment assessments are described thematically in more detail, together with the relevant references, in Table 2.

*Table 2. Summary of results, by thematic subject, of research conducted on the effects of pre-release application of hormonal treatments on male performance of tephritids of the genera* Anastrepha, Bactrocera, Ceratitis, *and* Zeugodacus *(modified after Pereira et al. 2013a, reproduced with permission)*

| Subject addressed | Summary of results |
|---|---|
| Age of sexual maturation of males determined | • Age at which laboratory-reared and/or wild males of *A. fraterculus*, *A. ludens*, *A. obliqua*, *A. serpentina*, *A. striata*, *A. suspensa*, *B. correcta*, *B. dorsalis*, *B. tryoni*, *C. capitata, C. quilicii,* and *Z. cucurbitae* become sexually mature has been determined (Teal and Gómez-Simuta 2002; Aluja et al. 2009; Pereira et al. 2009; Gómez et al. 2013; Obra and Resilva 2013; Orankanok et al. 2013; Pereira et al. 2013b; Quilici et al. 2013; Segura et al. 2013; Sookar et al. 2013; Adnan 2019). |
| Improvement in reproductive maturation and sexual performance | • Hormonal treatment has positive effects on accelerating reproductive development and improving sexual performance in *A. fraterculus*, *A. ludens*, *A. obliqua*, *A. serpentina*, *A. striata*, *A. suspensa*, *B. tryoni,* and *Z. cucurbitae* (Aluja et al. 2009; Pereira et al. 2009; Segura et al. 2009; Haq et al. 2010a, 2013; Gómez et al. 2013; Collins et al. 2014; Gomez-Simuta et al. 2017; Bachmann et al. 2017; Adnan et al. 2020a).<br>• In *A. fraterculus,* the accessory glands of males treated with methoprene are less effective at inhibiting female remating than males not treated with methoprene (Abraham et al. 2012).<br>• There is no effect of hormonal treatment on either *B. dorsalis* or *C. capitata* (Faria et al. 2008; Shelly et al. 2009). |

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*Table 2. Continued*

| Subject addressed | Summary of results |
|---|---|
| Interaction of protein and hormone treatments on sexual performance | • Males treated with hormone and feeding on a protein-rich diet effectively compete with mature wild males, but at significantly earlier ages for *A. fraterculus*, *A. ludens,* and *A. suspensa* (Gómez and Teal 2010; Liendo et al. 2013; Pereira et al. 2013b; Gómez et al. 2013).<br>• Males of *Z. cucurbitae* treated with methoprene and with access to a protein-rich diet have an accelerated maturation and improved mating performance (Haq et al. 2010a). |
| Interaction of irradiation and hormone treatments on sexual maturity | • Interaction of irradiation and hormone treatments has no negative effects on male acceleration of reproductive maturity in *A. fraterculus*, *A. ludens*, *A. obliqua*, *A. suspensa,* and *B. tryoni* (Teal et al. 2007; Segura et al. 2013; Adnan et al. 2019).<br>• Females mated with 6-day-old, methoprene-treated *A. fraterculus* males remated more and sooner than females mated with naturally matured males, either sterile or wild (Abraham et al. 2013). |
| Optimal dose to accelerate maturation | • Optimal hormone treatment dose has been determined using topical application for *A. fraterculus*, *A. ludens*, *A. obliqua*, *A. suspensa,* and Z. *cucurbitae* (Teal et al. 2007; Pereira et al. 2009; Segura et al. 2013; Haq et al. 2010a). |
| Response in males and females to hormone treatments | • A differential response in males and females to methoprene exposure is found in *A. fraterculus* and *B. tryoni;* it can act as a physiological sexing system, minimizing matings between sterile males and sterile females (Segura et al. 2009; Liendo et al. 2013; Adnan et al. 2019).<br>• No differential response to methoprene exposure has been found in *A. ludens* males and females (Pereira et al. 2013b). |
| Development of delivery systems other than topical application | • Agar-based diet with 5–10% protein along with 0.05% methoprene, tested with *A. ludens* and *A. suspensa*, produces large amounts of waste and is not cost-effective (Pereira et al. 2013b; Teal et al. 2013).<br>• Pupal dipping in an acetone bath containing 0.05% methoprene, tested with *A. fraterculus*, *A. ludens,* and *A. suspensa*, does not impact emergence or survival and accelerates reproductive development, although it can cause humidity problems when handling large volumes of pupae (Liendo et al. 2013; Pereira et al. 2013b; Segura et al 2013).<br>• Pupal dipping is impractical due to health risks, safety, and disposal issues associated with the use of acetone (Pereira et al. 2013a).<br>• A dry sugar–protein pre-release diet containing 0.0015% methoprene for *A. ludens* and *A. obliqua* is practical and effective in accelerating development when applied in operations of fly emergence and release facilities (Gómez et al. 2013).<br>• Dietary treatment with mosquito larvicide containing methoprene in a dry 3:1 mix of sugar to yeast is effective in *B. tryoni* (Adnan 2019; Adnan et al. 2019, 2020a). |
| Efficient large-scale methods for incorporation of JH analogues | • The dry sugar–protein–methoprene diet method was assessed in a 3500-ha pilot area for *A. ludens* in Mexico (Gómez et al. 2013).<br>• The hormone and protein delivery system has tested positive in the "Moscafrut" action programme to control *A. ludens* fruit flies in Mexico (Gómez et al. 2013). |

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## 4. SEMIOCHEMICAL TREATMENTS

Males of most *Anastrepha* spp., *Bactrocera* spp., *Ceratitis* spp., and *Zeugodacus* spp. are attracted to natural compounds known as semiochemicals (Cunningham 1989; Kumaran et al. 2013; Segura et al. 2018). Some species are known to sequester these chemicals from plants for use in pheromone synthesis; for example, ingestion of methyl eugenol (ME) from natural sources by males of *B. dorsalis* and *B. carambolae* results in the storage of metabolites in the rectal gland and their subsequent release as part of the pheromone during fanning performed in courtship (Tan and Nishida 1996). Providing sterile *B. dorsalis* males with a source of ME to feed on before release could increase their mating competitiveness (Shelly et al. 2005). ME feeding may also confer a survival advantage because metabolites appear to act as a very potent allomone to deter vertebrate predators (Wee and Tan 2001).

In the case of *C. capitata*, exposure to ginger (*Zingiber officinale* Roscoe) root oil (GRO) or citrus oils (by contact or vapour) enhances considerably the mating competitiveness of wild or mass-reared males (Katsoyannos et al. 1997; Papadopoulos et al. 2001; Shelly 2001).

Exposure to guava (*Psidium guajava* L.) fruit volatiles increased the mating success of both wild and laboratory *A. fraterculus* males relative to non-exposed males (Vera et al. 2013). Also, citrus and grapefruit volatiles have potential to enhance the mating performance of male *A. ludens* and *A. fraterculus* (Bachmann et al. 2015; Morató et al. 2015). However, the attraction and mating enhancing effects of the semiochemicals so far found for *Anastrepha* effects are not as dramatic as seen with *Bactrocera*, *Ceratitis* and *Zeugodacus,* and suggest that similar compounds may exist in the *Anastrepha* ecological sphere and await discovery (Segura et al. 2018).

The research carried out in this area has helped to understand these phenomena, to extend them to other species, and to transfer and validate them under the large-scale conditions of action SIT programmes. Results obtained recently confirmed the compounds that have the potential to improve sterile male performance of target fruit flies in the field, and some additional ones have been identified. These chemicals are the following:

- Methyl eugenol (ME) is found in more than 450 plant species (Tan and Nishida 2012); exposure to holy basil (*Ocimum tenuiflorum* L.) and sweet basil (*Ocimum basilicum* L.) oils, as well as ripe fruits of tropical almond (*Terminalia catappa* L.), improve the mating competitiveness of several *Bactrocera* species, including *B. correcta, B. dorsalis*, and *B. zonata* males (Cunningham 1989; Tan and Nishida 1996; Quilici et al. 2004; Shelly et al. 2005; Shelly and Edu 2007; Obra and Resilva 2013; Orankanok et al. 2013).
- Cuelure (CUE) has potential to enhance the performance of *B. tryoni* and *Z. cucurbitae* males (Weldon et al. 2008; Shelly 2019).
- Raspberry ketone (RK) and/or zingerone (ZG) have potential to enhance attraction, maturation, and sexual performance of *B. tryoni* and *Z. cucurbitae* males (Khoo and Tan 2000; Akter et al. 2017b; Akter and Taylor 2018; Shelly 2019); however, Fezza and Shelly (2018) did not find that access to a RK-supplemented diet at an early age accelerated sexual maturation.

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- Ginger root oil (GRO) and α-copaene enhance male performance of *C. capitata* and *C. quilicii* (Shelly 2001; Shelly et al. 2007a; Quilici et al. 2013).
- Manuka oil (rich in α-copaene) from the manuka tree (*Leptospermum scoparium* Forst and Forst) of New Zealand improves *C. capitata* male mating (Shelly et al. 2008c).
- Orange oils (OO) and other citrus oils enhance male performance of *C. capitata* and *C. quilicii*, and potentially *A. fraterculus* (Shelly 2001; Shelly et al. 2007a; Quilici et al. 2013; Vera et al. 2013).
- Citrus, grapefruit, and guava fruit volatiles have potential to enhance the mating performance of male *A. ludens* and *A. fraterculus* (Vera et al. 2013; Bachmann et al. 2015; Morató et al. 2015).

The results of assessing semiochemical treatments, together with the relevant references, are described in more detail in Table 3.

Methodologies for exposing large numbers of *C. capitata* males through GRO or citrus-oil aromatherapy on a large scale in adult-holding rooms at fly emergence and release facilities have been developed (Shelly et al. 2007c, 2008a). They are now applied routinely in a very cost-effective manner in on-going SIT programmes in Australia, Croatia, Guatemala, Israel, Mexico, Spain, and the USA, resulting in a significant improvement in mating performance of the released sterile males.

It is envisaged that providing sterile *Bactrocera* spp. males with a source of ME to feed on before release will place them on at least an even "playing field" against wild males, thereby potentially reducing the number or frequency of sterile males released (Barclay et al. 2014; Vargas et al. 2014). However, the common methods for holding sterile males in fly emergence and release facilities do not enable the feeding of ME to millions of adult flies after their emergence. Additionally, exposure to ME must be brief in view of ME toxicity when access is unlimited. In response, Tan and Tan (2013) designed an automated ME machine prototype for briefly feeding ME to sterile males on a belt impregnated with ME after which they are brushed off and collected. However, this approach is not really suitable for industrial processing of millions of sterile males. Considering this scenario at fly emergence and release facilities, Haq et al. (2014b, 2015, 2018) demonstrated that ME application by aromatherapy also enhanced the mating success of males of *B. carambolae* and *B. dorsalis*, finding that ME aromatherapy produced a mating boost as early as one day after exposure. This alternative method appears to have merit for adoption but needs to be evaluated at larger scales in fly emergence and release facilities.

Another advantage of exposure to ME is that it reduces significantly the response of sterile males to ME in male annihilation treatments, thus potentially enabling the simultaneous application of the SIT and male annihilation (Barclay et al. 2014; Vargas et al. 2014). Similarly, pre-release exposure to RK has been found to diminish the subsequent response of *B. tryoni* to CUE sources (Akter et al. 2017a).

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*Table 3. Summary of results, by thematic subject, of research conducted on the effects of pre-release application of semiochemical treatments on male performance of tephritids of the genera* Anastrepha, Bactrocera, Ceratitis, *and* Zeugodacus. *Abbreviations explained in the text (modified after Pereira et al. 2013a, reproduced with permission)*

| Subject addressed | Summary of results |
|---|---|
| Search for semiochemical compounds that affect *Anastrepha* spp. male sexual performance | • Grapefruit volatiles increase male mating competitiveness in *A. ludens* (Morató et al. 2015).<br>• Male mating performance of *A. fraterculus* is positively affected by exposure to guava volatiles (Vera et al. 2013; Bachmann et al. 2015).<br>• Exposure to lemon fruit volatiles enhances male mating performance in *A. fraterculus,* but there are also some detrimental effects (Vera et al. 2013). GRO exposure also increases male mating success in *A. serpentina* (Flores et al. 2011). |
| Assessment of various semiochemicals in enhancing *Bactrocera* spp. male performance | • Exposure to basil oil (containing ME) has a positive effect on sterile male mating performance of *B. dorsalis* (Obra and Resilva 2013).<br>• Exposure to ripened tropical almond fruit (containing ME) enhances *B. dorsalis* male mating success (Shelly and Edu 2007).<br>• Exposure to ME significantly improves sterile male mating performance of *B. correcta* and *B. dorsalis* (Shelly et al. 1996, 2005, 2008b, 2009, 2010a; Hee and Tan 1998; Shelly and Edu 2008b; Ji et al. 2013; Orankanok et al. 2013).<br>• Some negative effects on the male ejaculate have been found in *B. dorsalis* (Reyes-Hernández et al. 2019).<br>• ME-mediated male mating enhancement also found in *Bactrocera cacuminata* (Hering) (Raghu and Clarke 2003), *B. carambolae* (Wee et al. 2007), and *Bactrocera umbrosa* (F.) (Wee et al. 2018).<br>• Exposure to commercial ME, and interaction with post-teneral adult diet, increases male mating performance of *B. correcta* and *B. dorsalis.* For both species, ME-treated sterile males are being released in operational SIT programmes in Thailand (Orankanok et al. 2013).<br>• Sterile males, feeding on commercial ME prior to release, induce higher egg sterility in wild populations than control sterile males denied ME (McInnis et al. 2011).<br>• Exposure to ME does not affect dispersal or survival of sterile flies of *B. correcta* and *B. dorsalis* (Orankanok et al. 2013). |
| Assessment of various semiochemicals in enhancing male performance of other *Bactrocera* spp. | • Feeding on CUE increases *Z. cucurbitae* mating success (Shelly and Nishimoto 2017). Feeding on RK enhances male mating performance of *Z. cucurbitae,* but only for one day post-feeding (Shelly 2000, 2019). However, exposure to zingerone has no effect on *Z. cucurbitae* male mating competitiveness (Shelly 2017b) or a positive effect only one day after exposure (Inskeep et al. 2019).<br>• Exposure to CUE and RK increases the mating competitiveness of *B. tryoni*, but young males fail to respond to CUE, OO, and GRO (Weldon et al. 2008; Kumaran et al. 2014a; Akter et al. 2017b).<br>• *B. tryoni* feeding on yeast hydrolysate enhances attraction to CUE (Weldon et al. 2008), and on CUE increases male signalling, female attraction, and male mating success (Kumaran et al. 2013, 2014b).<br>• *B. tryoni* feeding on RK (or caffeine supplements) promotes male early sexual maturation, greater mating success and increased multiple mating, without affecting remating receptivity in females (Akter et al. 2017b; Akter and Taylor 2018; Khan et al. 2019; Adnan et al. 2020b; GFV 2020).<br>• Exposing sexually mature adult *B. oleae* to the aroma of α-pinene significantly increases the mating performance over non-exposed males and females (Gerofotis et al. 2013; Kokkari et al. 2017). |

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*Table 3. Continued*

| Subject addressed | Summary of results |
|---|---|
| Optimal age and feeding dose for *Bactrocera* spp. male flies | • For *B. dorsalis,* Obra and Resilva (2013) determined the optimal age and duration of ME exposure, and the diurnal pattern of ME feeding.<br>• Optimal age of ME exposure, in combination with nutritional supplements, has been determined for sterile *B. correcta* and *B. dorsalis* males (Orankanok et al. 2013). |
| Effect of semiochemical treatments on *C. capitata* sexual signalling (pheromone calling) | • GRO-exposed males exhibit higher rates of sexual signalling compared with unexposed *C. capitata* males (Shelly 2001, 2007a, 2008, 2010b; Papadopoulos et al. 2006), although the effect found by Briceño et al. (2007) was lower.<br>• During sexual courtship GRO-exposed sterile males exhibit the same wing-beat duration as wild males, and this is shorter than for unexposed sterile males (Morelli et al. 2013).<br>• Exposure to OO and their components increases sexual signalling and mating success of *C. capitata* males (Shelly et al. 2004, 2006b; Kouloussis et al. 2013); this effect is more pronounced in protein-fed males, independent of age (Katsoyannos et al. 2004; Papadopoulos et al. 2006; Kouloussis et al. 2013, 2017).<br>• Exposure to volatiles of leaves of the tea tree increases mating competitiveness of sterile *C. capitata* males (Shelly and Epsky 2015). |
| Identification of active compounds other than GRO, and their effects on sexual performance of *C. capitata* | • Exposure to oranges and grapefruits (*Citrus paradisi* Macfad) improves mating success of male *C. capitata* (Papadopoulos et al. 2001; Shelly 2009).<br>• Exposure to fruits of five citrus species shows that sweet oranges confer the highest increase in male mating performance of *C. capitata*. Exposure to commercial citrus oils is similarly effective (Kouloussis et al. 2013).<br>• There is a positive effect of OO components (limonene, β-myrcene, and linalool) on protein/sugar-feeding males, but not on sugar-only-feeding males (Kouloussis et al. 2013).<br>• A mixture of limonene, β-myrcene, linalool, α-pinene, and geraniol (1:1:1:1:1) is very effective for both wild and sterile *C. capitata* male sexual performance (Kouloussis et al. 2013). |
| Effects of GRO and OO exposure to *C. quilicii* | • Exposure of sugar-only-fed *C. quilicii* males to GRO and OO increases their mating performance, but only GRO increases the mating performance of protein/sugar-fed males (Quilici et al. 2013). |
| Effect of semiochemical treatments on *C. capitata* female remating frequency | • The remating frequency of *C. capitata* females mated with sterile males treated with 0.1 ml/$m^3$ GRO is similar to that of females mated with wild males and lower than that of females mated with sterile unexposed males (Morelli et al. 2013). |
| Effect of semiochemical treatments on *C. capitata* dispersal and survival | • Field dispersal and survival rates of GRO-exposed sterile males are similar to those of unexposed *C. capitata* males (San Andrés et al. 2009; Paranhos et al. 2010; Juan-Blasco et al. 2013). |
| Determining the dose of semiochemical exposure for *C. capitata* | • OO compounds show positive effects in increasing *C. capitata* male performance (Kouloussis et al. 2013). The optimal dose of OO exposure was determined for both protein-sugar and sugar-only *C. capitata* adult diets. There are positive effects of OO for a protein-sugar diet, but no effects for a sugar-only diet (Kouloussis et al. 2013). |
| Optimal delivery system for large-scale semiochemical application in *Ceratitis* spp. SIT programmes | • The cost-effective GRO exposure to sterile males has been established, and is in operation in several *C. capitata* SIT programmes (Shelly et al. 2007b, c, 2008a; Paranhos et al. 2008, 2013; Juan-Blasco et al. 2013; Silva et al. 2013; Steiner et al. 2013). |

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## 5. CONCLUSIONS

Extensive laboratory and semi-field tests indicate that pre-release diets nutritionally enriched with some yeast hydrolysate improve sterile male performance; they can result in increased SIT efficiency for a majority of fruit fly species tested, and thus can be adopted by programme managers, although some confounding factors still need to be better understood for some target species, as well as the optimal protein to carbohydrate ratios and physical form of diet provision. Also, a better understanding of the effect of nutrition during the larval stage on the nutritional requirements of the adults is needed (Aluja et al. 2009).

In addition, hormone therapy coupled with feeding protein to adults generally accelerates male maturation and sexual performance among *Anastrepha* species and some *Bactrocera* species, with no negative side effects on survival. Furthermore, in some species, methoprene treatment has increased male mating and competitiveness. However, negative effects have been recorded on female remating in *A. fraterculus*, and on sperm storage and male ejaculate in *B. dorsalis*.

Accelerated male maturation and improved sexual performance can represent significant cost savings associated with infrastructure investment, reduced holding periods for sterile males prior to release, and fewer males dying in the field before reaching sexual maturity. Validation in a few fruit fly species has resulted in some of these major breakthroughs being incorporated into several action programmes, although others, for technical or practical reasons, have been reluctant to incorporate these innovations into their operations.

The development of cost-effective semiochemical treatments that improve sterile male sexual signalling and attractiveness to wild females, and thus overall mating performance, is highly desirable. Implementation of aromatherapy has significantly increased the effectiveness of on-going programmes integrating the SIT against C. *capitata*. On the other hand, while the incorporation of semiochemical treatments for *Bactrocera* species appears to be feasible, it still remains to be implemented operationally; major benefits result from the simultaneous field application of male annihilation to eliminate wild males. Thus, incorporating an ME pre-release treatment has considerable potential to significantly increase sterile to wild male overflooding ratios (Barclay et al. 2014; Vargas et al. 2014; Hendrichs and Robinson, this volume). For some *Anastrepha* species and other species such as *B. oleae* and *C. quilicii,* potentially similar semiochemicals and fruit volatiles probably exist, and more research funding should be allocated to find and investigate them (Segura et al. 2018).

Also critical for sterile male performance are the processes to which sterile males are subjected at fly emergence and release facilities (USDA/APHIS 2009; FAO/IAEA 2017; Dowell et al., this volume), the environmental conditions in which they are held, and their interaction with exposure to hormonal, nutritional and/or semiochemical treatments (Pereira et al. 2013a). In addition, holding densities (Díaz-Fleischer et al. 2009; Andress et al. 2015), the timing of providing treatments in relation to fly age and sexual maturation, and holding periods are also very relevant. For example, results indicate that in *C. capitata* sexing strains, the release of older males, closer to sexual maturity, is preferable; this results in more flies reaching mating age in the field (McInnis et al. 2013). Species with longer pre-copulatory

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maturation periods, such as *Anastrepha* spp. and *Bactrocera* spp., benefit more from combined nutritional and hormonal treatments to reduce long pre-release holding periods (Gómez et al. 2013; Pereira et al. 2013a).

Assessing the interaction of different environmental and holding conditions, combined with hormonal, nutritional, and/or semiochemical treatments, on sterile fly performance has resulted in the routine application of GRO under large-scale holding conditions in operational *C. capitata* SIT programmes (USDA/APHIS 2009; FAO/IAEA 2017). Also, a hormone-and-protein-delivery system has been developed for the SIT programmes suppressing *A. ludens* fruit fly populations in Mexico (Gómez et al. 2013). Furthermore, the effects on fly performance (in terms of dispersal and recapture) of different sterile fly shipping and release systems, and their interaction with hormonal, nutritional, and/or semiochemical treatments, have been assessed for some aerial- and ground-release systems for *C. capitata* (Paranhos et al. 2010, 2013; Silva et al. 2013). Finally, parameters of sterile-fly quality, treatments, space, labour, and economics were assessed and compared at eight fly emergence and release facilities in Guatemala, Mexico, and the USA (USDA/APHIS 2009); there is a good baseline on which to improve.

Knowledge gained so far, and the practical procedures developed, are transferable, at least in part, to other insect pest species with management programmes that include an SIT component. Nevertheless, much remains unknown about the effects of manipulating the environmental holding conditions, either separately or in combination with nutritional, hormonal, and semiochemical treatments, on subsequent male quality in the field. Release methods, while operationally convenient, are not always optimal in terms of sterile male performance (Dowell et al., this volume). For example, the effect of chilling (to immobilize) collected flies for aerial release can at least temporarily affect sterile male flight ability and mating competitiveness, including pheromone quantity or quality (Andress et al. 2012, 2013; Shelly et al. 2013; Arredondo et al. 2016). Therefore, the effects and interactions of the different processes, treatments, and systems need to be further assessed and refined, tailoring them to the biology of each target fruit fly species.

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# CHAPTER 4.6.

# APPLYING MODERN MOLECULAR TECHNOLOGIES IN SUPPORT OF THE STERILE INSECT TECHNIQUE

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## SUMMARY

The accelerating development of modern biotechnology, resulting continuously in new molecular techniques, has significantly increased the potential of understanding and modifying the genetic information in various organisms, from mammals to many insect species. Key molecular techniques developed over the past three decades include classical transformation technologies via transposable elements, and site-specific genome modification techniques using recombinases, both permitting the introduction of new genes or regulatory elements into the genome and their subsequent modification or (partial) removal. Tools like TALEN or CRISPR permit the precise editing of genomes at pre-defined positions, ranging from single-base changes to the knockout of complete genes, or the introduction of exogenous genetic material. On the other hand, RNA interference (RNAi) does not change genomic information but instead influences gene expression levels to various degrees, including complete suppression of expression. All of these tools can be used to develop or refine insect strains to improve the efficiency of existing pest control programmes, or to develop new SIT-based control systems. Modern biotechnology tools are being exploited to develop self-limiting as well as self-sustaining approaches. While the latter aim at population replacement strategies using gene drive or *Wolbachia*-based approaches and are mostly developed for vector and disease control, SIT-based control systems are self-limiting strategies that are supposed to leave no ecological footprint. Molecular technologies have already been applied to create sterility and sexing strains, and to introduce stable markers for monitoring in several insect species including the Mediterranean fruit fly, Mexican fruit fly, Australian sheep blow fly, New World screwworm, pink bollworm, and various mosquito species. Moreover, several of these strains have undergone an initial evaluation under mass-rearing scenarios or in open-field trials, although their wider adoption has so far been slow in view of low public acceptance of transgenic approaches, and the regulatory requirements and approvals that are required in most countries for their application.

## 1. INTRODUCTION

Successful implementation of the sterile insect technique (SIT) for insect pest control depends on several key factors, e.g. mass-rearing and irradiating the target pest insect. Both procedures can result in reduced biological quality and/or mating competitiveness of the produced sterile insects (Hooper 1972; Bloem et al. 2004; Andreasen and Curtis 2005; Parker and Mehta 2007; Guerfali et al. 2011). Besides sterilization by irradiation, it has been shown that sometimes other sterilization strategies, such as transgenic or symbiont-based approaches, also come at a cost to the mating competitiveness of the sterilized males (Catteruccia et al. 2003; Irvin et al. 2004; Bourtzis et al. 2014; Häcker et al. 2017; Kyritsis et al. 2019). This reduction in biological quality and mating competitiveness of the produced sterile insects influences the efficacy of SIT projects, and thereby their cost and feasibility. However, it should be noted that recent developments on insect symbiosis have shown that probiotic bacterial applications may significantly improve the biological quality (including male mating competitiveness) of the sterile insects (Ben Ami et al. 2010; Hamden et al. 2013; Ras et al. 2017; Augustinos et al., this volume).

Another important and challenging factor is the efficient and robust sex separation (sexing) for male-only releases. In the case of agricultural pests, this may affect the efficiency in action as well as the economy in production (Hendrichs et al. 1995; Cáceres et al. 2004). However, in the case of vector species such as mosquitoes, male-only releases are a prerequisite for SIT applications since females bite, blood-feed, and transmit major human pathogens. Therefore, efficient sexing on a large scale is needed (Gilles et al. 2014; Parker, Mamai et al., this volume).

For some insect pest species, morphological traits or markers have been developed in the last decades that help to discriminate between sterile and wild

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insects in the target area, and in particular to separate sexes. This is the case for the naturally occurring pupal size difference between males and females in some *Aedes* mosquitoes, which is being exploited for mechanical sexing (Bellini et al. 2018; Zacarés et al. 2018), although it is difficult to achieve a 100% pure separation and is very labour-intensive when applied at larger scales. Genetic sexing strains (GSS) (Franz et al., this volume) offer the possibility of efficient and large-scale sexing. This has been achieved for several species including *Anastrepha ludens* (Loew) (Orozco et al. 2013), *Bactrocera carambolae* Drew and Hancock (Isasawin et al. 2014), *Bactrocera dorsalis* (Hendel) (Isasawin et al. 2012), *Ceratitis capitata* (Wiedemann) (Franz 1995), *Anopheles albimanus* Wiedemann (Kaiser et al. 1978), *Anopheles arabiensis* Patton (Yamada et al. 2012), *Aedes albopictus* (Skuse) (Lebon et al. 2018), and *Zeugodacus cucurbitae* (Coquillett) (McInnis et al. 2004), and for some species is successfully being used for sexing under mass-rearing conditions in current SIT programmes, while being further developed for others.

These genetic GSS were created by mutagenesis and classical genetics, and are based on two components: a mutation that can be used as a selectable recessive marker, and the placement of the dominant wild-type allele of this marker on the Y-chromosome that links it to the male sex. Thus, the males are heterozygous for the recessive mutation and therefore are phenotypically wild type, while in homozygous females, the mutation can be used for sorting or killing the females. However, genetic sexing systems produced by mutagenesis and classical genetics in one species might not be transferable to another species, as the underlying mutation is often a random product. Molecular technologies offer new perspectives and strategies to create insect strains possessing the traits needed for an effective pest control programme (Häcker and Schetelig 2018). These modern biotechnologies involve transgenic as well as non-transgenic approaches, and can be applied to optimize existing, or develop new, strategies and strains, thereby complementing classical approaches. Molecular technologies can be used either as stand-alone techniques, e.g. sexing systems for population suppression (Häcker and Schetelig 2018), or could be combined with the SIT and/or the incompatible insect technique (IIT) (Bourtzis et al. 2016; Lees et al., this volume), to improve efficiency and to increase the range of pest insects targeted by pest control strategies that are environment-friendly. Moreover, once the mechanism of a classical GSS is uncovered, molecular technologies could help build similar non-transgenic sexing strains in other species.

In support of and as an alternative to classical genetic approaches, molecular technologies and IIT approaches are being exploited to create population suppression, sexing, or marking systems in several insect pest species. The current key molecular technologies that can be used to support the SIT, including site-specific recombination technologies, genome editing tools, and RNA interference (RNAi), are described below. Insect transformation via transposable elements is also included; it is still a major tool to produce transgenic insects in spite of the fast development and adaptation of new biotechnologies, which may help address the needs of population suppression programmes against major insect pest species effectively and in a relatively short period of time. Progress made in developing such molecular technologies for use in the SIT is described, including their

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preliminary applications in the first field trials, as well as the regulations and public acceptance issues that must be considered when transgenic insects are involved.

To assist in understanding the terminology used in this chapter, some definitions are given in Box 1.

*Box 1. Definition of Terms*

*Biotechnology*
Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use (Cartagena Protocol on Biosafety to the Convention on Biological Diversity - CBD 2000, 2007).

*Cytoplasmic Incompatibility (CI)*
In diplo-diploid organisms, a phenomenon that is expressed as embryonic lethality in matings between *Wolbachia*-infected males and females that are either uninfected or infected with a different *Wolbachia* strain. CI is induced by symbiotic bacteria such as *Wolbachia* (Bourtzis et al. 2003).

*Endogenous vs Exogenous*
Endogenous substances, processes or genes originate from within an organism; correspondingly, exogenous substances, processes or genes originate externally.

*Gene Drive*
A phenomenon in genetics, where the inheritance of a gene does not follow the Mendelian rules of being inherited to 50% of the offspring of heterozygous parents. Instead, inheritance of this gene is favourably biased, resulting in more than 50% of the offspring carrying the respective gene. Thus, the gene spreads into a population even if it has no benefit for the organism. Natural forms of gene drives exist and can function by copying themselves onto homologous chromosomes during meiosis. Thus, every gamete carries the gene instead of only 50%. Gene drives can be used to replace a wild population with a genetically altered one that has desired traits (like pathogen refractoriness in insect vectors). Gene drives could be designed in different ways making them self-limiting or self-sustaining.

*Genetically Modified (GM)*
Any organism whose genetic material has been altered in a way that cannot be the product of natural mutation or recombination, using molecular biology/genetic engineering techniques.

*Incompatible Insect Technique (IIT)*
A method to suppress insect pest populations analogous to the SIT; it is based on the mechanism of cytoplasmic incompatibility (CI) (see above).

*Living Modified Organism (LMO)*
Any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology (CBD 2000; FAO/IPPC 2017).

*Modern Biotechnology*
The application of: (a) *in vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles; or (b) fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection (CBD 2000; FAO/IPPC 2017).

*Non-GM*
An organism, in which the genetic material has been altered in a way that could occur also naturally by mating and/or by natural recombination (Article 2(2) of the Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC).

*Non-Transgenic Approaches*
Pest control strategies not involving transgenic insects.

*Population Replacement*
A target population of pest insects is replaced by another population of the same species that has been specifically altered to bear new traits or remove old ones. For example, a genetically modified mosquito species that is refractory to pathogen infections could be generated and used to replace a natural population. This could be achieved by using a gene drive mechanism (see above).

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*Box 1. Definition of Terms -- Continued*

*RNA Interference*
A conserved biological process to suppress gene expression or translation by neutralizing the corresponding messenger RNA (mRNA) molecules. Two types of small RNA molecules, microRNAs (miRNAs) and small interfering RNAs (siRNAs) in combination with several protein factors play a major role in identifying and cleaving the target mRNA. RNAi functions as an important defence mechanism against parasitic nucleotide sequences, but has also an important role in the regulation of gene expression. While transient RNAi is limited to a certain time-window in the development of one generation of an insect, systemic RNAi is inherited to the next generation(s).

*Self-Limiting vs Self-Sustaining*
In self-limiting control strategies like the SIT, the released insects do not persist in the environment. Therefore, repeated releases are necessary to control a population. In contrast, in a self-sustaining approach, the released insects are able to persist in the wild and spread into the wild populations. Therefore, a one-time release may be sufficient, depending on several factors including the fitness and spreading capacity of the released insects, so that the replacement of the target population happens in a reasonable amount of time.

*Sex Ratio Alteration*
Manipulation of the natural sex ratio of 50% male and 50% female offspring in favour of one of the sexes. This can be a means to produce male-only strains of a transgenic insect, and can be achieved by manipulating the sex determination pathways in embryonic development, for example by using RNAi or by influencing sex-specific splicing.

*Transgenic Insect*
Relating to an insect, whose genome has been altered by the transfer of an exogenous gene. Transgenesis can occur naturally or by genetic engineering techniques. A transgenic insect created by genetic engineering falls in the category of GM insects.

*Transgenic Population Suppression*
Molecular technologies, that use foreign DNA transferred into an organism, resulting in the death of its progeny, fall under the term transgenic population suppression. While the effects of the SIT and transgenic population suppression are similar, i.e. population size reduction, the modes of action are different. In the SIT, population reduction is achieved through sterile matings by the released males due to irradiation-induced damaged sperm, but in transgenic approaches the population reduction is often achieved by overexpression of lethal genes at certain stages of development in the offspring of the released males (Robinson, this volume).

## 2. KEY MOLECULAR TECHNOLOGIES FOR POPULATION CONTROL

### *2.1. Transposable Elements*

A frequently applied method to produce transgenic insects is using highly efficient class II transposable elements like *piggyBac* (Cary et al. 1989; Elick et al. 1996; Handler et al. 1998; Handler and Harrell 1999), *Minos* (Franz and Savakis 1991; Loukeris et al. 1995), *Mariner* (*Mos1*) (Jacobson et al. 1986; Lidholm et al. 1993) or *Hermes* (Warren et al. 1994; O'Brochta et al. 1996). These DNA transposons consist naturally of a transposase-encoding gene flanked by inverted repeat sequences. These elements integrate into short, defined, and randomly distributed recognition sequences in the insect genome (Handler 2002). For all transposons used commonly as molecular tools, the transposase gene has been separated from its flanking inverted repeats to create a bipartite system -- the transposase source in one vector (helper element) and a second transformation vector containing the inverted repeats flanking the transgene construct to permit its genomic integration (Handler and

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Harrell 1999, 2001a). To increase the germline transformation frequency, mutagenesis approaches have resulted in hyperactive transposases with the *piggyBac* variant hyPBase being highly active in diverse insects (Eckermann et al. 2018). Due to their broad applicability to many different insect species, bipartite transposable systems have become a key tool for insect transgenesis (Wimmer 2003); nevertheless, they have some drawbacks. Although the rapid loss of the non-integrated helper element separates the integrated transgenes from a source of transposase, the possibility of unwanted transgene remobilization in the presence of an endogenous transposase remains. In addition, the essentially random integration of the transposable elements into the genome can lead to insertional mutagenesis that can be deleterious or lead to reduced fitness of the transgenic strains (Woodruff 1992; Spradling et al. 1995; Catteruccia et al. 2003; Scolari et al. 2008; Häcker et al. 2017). Moreover, the inserted transgene is subject to genomic position effects, where the presence of nearby enhancers or silencers influences transgene expression levels (Schetelig et al. 2009b). Transgenes inserted close to heterochromatic regions may also be subject to silencing due to position effect variegation, i.e. the inactivation of a gene in some cells through its abnormal juxtaposition with heterochromatin. Some of these effects can be avoided by using chromatin insulator elements (Horn and Wimmer 2003; Sarkar et al. 2006; Scolari et al. 2008) or site-specific genome modification tools.

### *2.2. Site-Specific Recombination Systems*

Site-specific recombination (SSR) systems have become important and universal molecular tools for targeted genome modification (Bode et al. 2000; Kolb 2004; Coates et al. 2005). These systems depend on a recombinase enzyme that recognizes and binds short recombination sequences, induces double-strand breaks in the central crossover region of the recombination sequence, and catalyses strand exchange. The Cre recombinase was the first enzyme to be established as a molecular tool, and it paved the way for genomic modification and conditional gene targeting in mammalian cell lines and mouse models (Sauer and Henderson 1988; Orban et al. 1992; Tsien 2016). SSR systems commonly used in mammals and insects include Cre/*lox* from the *Escherichia coli* phage P1 (Siegal and Hartl 1996, 2000), Flp/*FRT* from the two-micron plasmid of *Saccharomyces cerevisiae* (Andrews et al. 1986), as well as the phiC31/*att* derived from the *Streptomyces* phage phiC31 (Thorpe and Smith 1998). Recombination sequences of the tyrosine recombinases Cre and Flp (*lox* and *FRT*, respectively) range from 34 to 48 nucleotides, and consist of two nearly identical motifs with an inverted-repeat symmetry flanking an eight base pair (bp) central crossover sequence where the recombination takes place (Fig. 1A). Site-specific genomic integration occurs by recombination between a single sequence integrated in the genome (acceptor site) and a second sequence (donor site) in a vector containing the transgene of interest. Recombinase protomers bind to each inverted-repeat motif at both recombination sites to catalyse the strand exchange, leading to donor vector integration. The donor and acceptor sequences are identical in case of the *lox* and *FRT* sites. Thus, they remain unchanged upon completion of the recombination and are available for

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successive reactions (Fig. 1B). Site preservation, however, also has a disadvantage: donor vector integration results in duplication of the recombination sites in the genome, flanking the transgene construct (Turan et al. 2013; Häcker et al. 2017). As the reaction between cis elements is thermodynamically and kinetically favoured over the reaction in trans, the excision reaction is much faster than the integration, leading to a very low frequency of stable integration events (Baer and Bode 2001; Wimmer 2005; Häcker et al. 2017). Adding the donor plasmid in excess can help to push the reaction towards the product (Turan and Bode 2011; Turan et al. 2013).

The recombination mechanism of serine recombinases, such as the phiC31 system, follows the same basic rules as Cre and Flp. However, it strongly differs on the sequence level and the architecture of the recombination sites. The recombination sequences recognized by the phiC31 integrase are termed *attP* and *attB*, and are much longer (about 200 bp) than the *lox* or *FRT* sites. The two arms of the *att* sites flank a 3-base-pair crossover region, and in contrast to *lox* and *FRT* sites they share only limited inverted repeat symmetry (Fig. 2A). Moreover, the *attP* and *attB* sites share no sequence identity besides the central crossover region. Therefore, recombination between *attP* and *attB* results in different and incompatible products, *attL* and *attR*, making it a one-time unidirectional reaction (Fig. 2B). In principle, however, the reverse reaction should be possible in combination with additional factors/enzymes that are naturally used by the phage to excise its DNA from the host genome.

Site-specific integration into insect genomes has been established with the integrase phiC31 for several species besides drosophilids, including two *Aedes* species (Nimmo et al. 2006; Labbé et al. 2010; Franz et al. 2011), *Anopheles gambiae* Giles (Meredith et al. 2011, 2013; Isaacs et al. 2012), and the Mediterranean fruit fly (Schetelig et al. 2009b). The drawback of site-specific integration via a single recombination site is the integration of the complete donor vector at the genomic target site, including bacterial resistance genes and regulatory elements within the vector backbone that may be unnecessary or even interfere with transgene expression.

The discovery that the mutation of certain bases in the central crossover region of *lox* or *FRT* sites does not abolish cleavage by the recombinase, but renders the sites incompatible with the wild-type sites (Hoess et al. 1986; Lee and Saito 1998; Missirlis et al. 2006), opened up new possibilities (Fig. 1A). These mutant sites recombine with an identical (homospecific) mutant site with equal efficiency as two wild-type sites, while the interaction between sites with non-identical core sequences (heterospecific sites) is usually prevented. This was the basis for a more sophisticated targeting strategy, the recombinase-mediated cassette exchange (RMCE). Cre- or Flp-RMCE is based on the double-recombination between two pairs of heterospecific recombination sites, flanking DNA cassettes in the donor vector and the genomic target site (Schlake and Bode 1994; Turan and Bode 2011), which leads to a cassette exchange instead of complete donor plasmid integration (Fig. 1C). Both, Flp/*FRT* and Cre/*lox*-based RMCE, were first demonstrated in *Drosophila melanogaster* Meigen (Horn and Handler 2005; Oberstein et al. 2005; Wimmer 2005).

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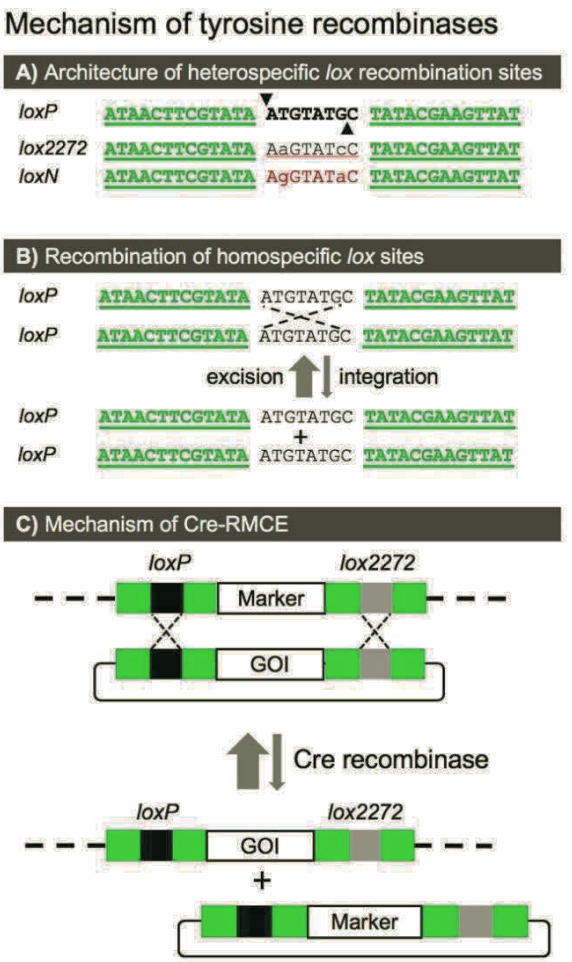



*Figure 1. Comparison of site-specific recombination mechanisms catalysed by tyrosine recombinases. A) General architecture of heterospecific recombination sequences recognized by the tyrosine recombinases shown exemplarily for the Cre/lox system. Note that the FRT sites of the Flp/FRT system show the same architecture and differ only in the recognition sequence. Inverted repeat sequences flanking the 8-bp core are underlined. The heterospecific lox sites differ from the wildtype loxP site in the core sequence (mutations shown in lowercase). Only two of the existing heterospecific lox sites are shown here, B) Due to the inverted repeat architecture of the lox sites, their sequence is preserved upon recombination between identical (homospecific) sites and the reaction is reversible by the Cre recombinase. Due to the thermodynamically and kinetically favoured reaction between recombination sites in cis, the excision reaction is much faster than the integration, resulting in a very low integration efficiency. C) The genomic landing site for Cre-RMCE consists of a marker cassette flanked by heterospecific lox sites. Recombination with the corresponding homospecific sites, that flank the gene of interest (GOI) cassette on the donor plasmid, leads to a cassette exchange. Recombination sites are preserved, the reaction is reversible.*

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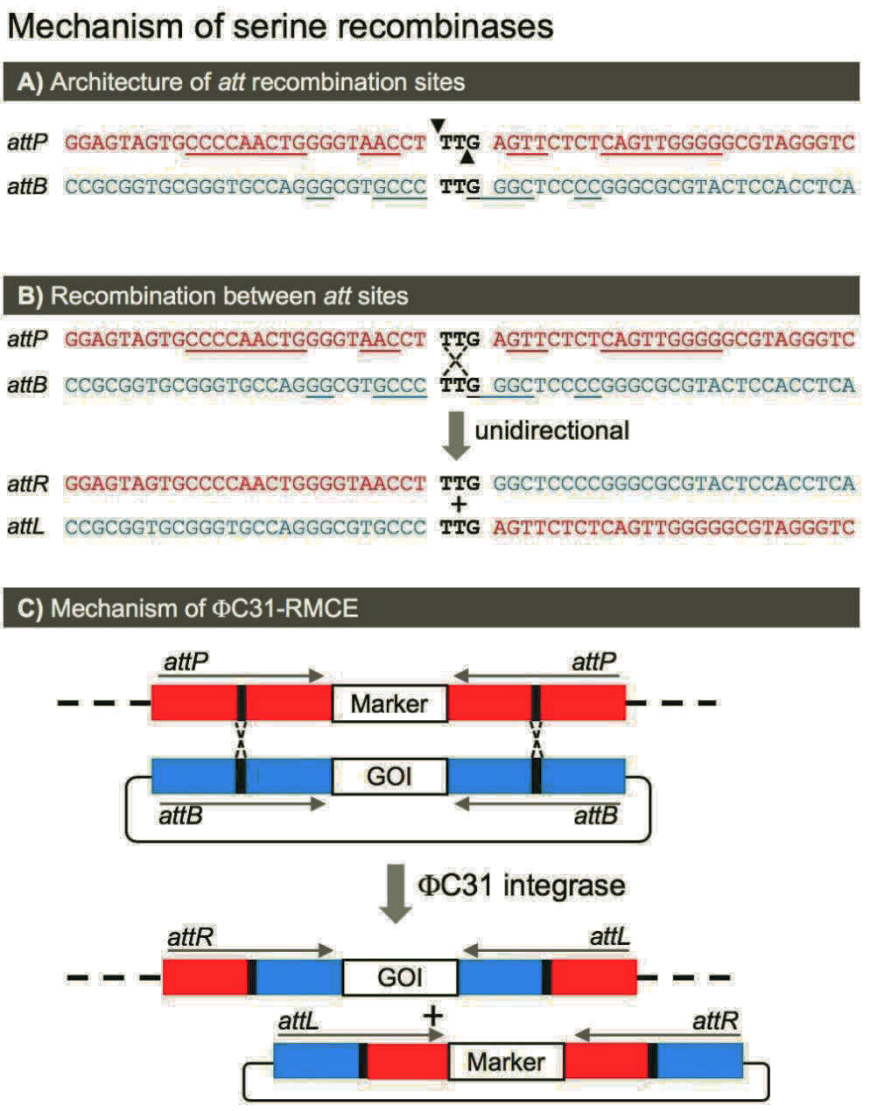



*Figure 2. Comparison of site-specific recombination mechanisms catalysed by serine recombinases. A) Serine recombinase recognition sequences show very limited repeat symmetry (underlined bases). Moreover, the attP and attB recombination sites recognized by the phiC31 recombinase share almost no sequence identity besides the 3 bp central crossover region (TTG). B) In contrast to lox sites (and tyrosine recombinases in general), the recombination between attP and attB results in incompatible attL and attR sites that can't recombine anymore. The absence of the reverse reaction enhances the integration efficiency via att sites in contrast to lox of FRT sites. C) For phiC31-RMCE, the genomic marker cassette is flanked by inverted attP sites, while the donor cassette is flanked by inverted attB sites. Recombination leads to cassette exchange but is irreversible due to the resulting incompatible attR and attL sites that are not recognized by the phiC31 integrase enzyme.*

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Until now, Cre/*lox*-RMCE was applied to only two non-drosophilid species, the tephritid *Anastrepha suspensa* (Loew) (Schetelig and Handler 2013a) and the mosquito *Aedes aegypti* (L.) (Häcker et al. 2017). Very recently, it was also achieved in the highly invasive pest *Drosophila suzukii* (Matsumura) (Schetelig et al. 2019). The functionality of Flp-RMCE was also demonstrated in the silkworm *Bombyx mori* (L.) (Long et al. 2012). In both systems, Cre- and Flp-RMCE, the heterospecific sites are restored after recombination. Therefore, both approaches allow for the repeated manipulation of the same target site including the reversion of the reaction.

The difference in the sequence of *attP* and *attB* sites, and the resulting directionality of the reaction, abolishes the need for heterospecific sites for RMCE using the phiC31 system. For phiC31-RMCE, the genomic acceptor cassette is flanked by *attP* sites in inverted orientation, while the donor cassette is flanked by inverted *attB* sites (Fig. 2C). This advantage, however, comes with certain shortcomings, such as the lack of control over the directionality of the cassette integration. Moreover, as for the donor plasmid integration via *att*-sites, the phiC31-RMCE is a one-time, unidirectional reaction due to the resulting incompatible *attR* and *attL* sites. PhiC31-RMCE has been successfully demonstrated for *D. melanogaster* (Bateman et al. 2006), *B. mori* (Long et al. 2013), and *Ae. aegypti* (Haghighat-Khah et al. 2015). A different approach combines phiC31 and Flp- or Cre-recombination to achieve cassette exchange in a two-step process via a donor plasmid integration intermediate (iRMCE) (Haghighat-Khah et al. 2015).

### *2.3. Genome Editing Methods*

Genome editing methods use engineered nucleases, "molecular scissors", to site-specifically modify the genome, making use of two naturally occurring and conserved DNA repair mechanisms in the cell. The nucleases create double-strand breaks (DSBs) at desired locations in the genome. These are then repaired, either by the cell's homology directed repair (HDR) or the non-homologous end-joining (NHEJ) pathway, which results in targeted mutations. Molecular biologists have engineered four families of nucleases as genome editing tools: meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector-based nucleases (TALEN), and the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system.

Meganucleases have long recognition sequences (>14 bp) which makes them very specific. By modifying their recognition sequence through protein engineering, their rather limited choice of target sequences can be expanded (Sussman et al. 2004; Arnould et al. 2006; Rosen et al. 2006). Protein engineering, however, can be time-consuming and costly.

The concept behind ZFNs and TALENs is based on a fusion protein consisting of a non-specific DNA cutting enzyme linked to peptides that recognize specific DNA sequences, such as zinc fingers and transcription activator-like effectors (TALEs). The DNA cleavage domain used for both systems is the restriction endonuclease *Fok*I (Kim et al. 1996; Li et al. 2011) that is active in yeast, plant and animal cells (Wood et al. 2011). The DNA binding domain (ZNF or TALE) can be

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engineered to bind nearly any desired DNA sequence. Both methods have been applied successfully to edit a variety of insect genomes (Bozas et al. 2009; Takasu et al. 2010, 2013; Liu et al. 2012; Aryan et al. 2013; Smidler et al. 2013), and they have been shown to work with satisfying efficiency and precision. However, as for meganucleases, the number of naturally occurring target sites is limited, and the engineering of the proteins to target new sites is cumbersome and costly, preventing the widespread use of these systems.

The CRISPR/Cas9 system has recently emerged as a very powerful tool for targeted genome editing in a variety of organisms. CRISPR/Cas is the prokaryotic equivalent of acquired immunity. CRISPR are repeat-spacer sequences found to date in approximately 40% and 90% of the sequenced bacterial and archaea genomes, respectively. The short spacer DNA originates from foreign DNA, e.g. virus or plasmid, and has been acquired by the bacteria and archaea as part of the immune response to a previous infection. These spacer DNAs are separated by short repeat sequences. Small clusters of Cas (CRISPR associated) genes are located next to the repeat-spacer array (for comprehensive explanations and illustrations, see Horvath and Barrangou 2010, Marraffini and Sontheimer 2010 or Bhaya et al. 2011). These repeat-spacer arrays are transcribed and processed into short CRISPR RNAs (crRNA) by nucleases of the Cas family of proteins. The crRNAs associate with other Cas proteins into ribonucleoprotein complexes that recognize foreign DNA by sequence complementarity with the crRNA. The foreign DNA is then cut by a Cas nuclease activity in a way similar to RNA interference in eukaryotic organisms (Marraffini and Sontheimer 2010), thus conferring immunity against infection with previously encountered infective agents.

Three different CRISPR/Cas systems are known so far, consisting of Cas proteins with different functions. The most used nuclease for genome editing is the multifunctional Cas9 protein from the type II system. Cas9 requires both the crRNA and a transactivating CRISPR RNA (tracrRNA) to function. For more convenient use, scientists engineered the crRNA and tracrRNA into one single-guide RNA (sgRNA) (Jinek et al. 2012). By adjusting the sequence of the sgRNA, the CRISPR/Cas9 system can be programmed easily to target any desired genomic location that is in the vicinity of a so-called PAM (protospacer adjacent motif) sequence (Jinek et al. 2012). The system can be used to knock out existing genes or add new DNA sequences, and has been adapted to different species. One drawback of CRISPR/Cas is the potential off-target effects, requiring very careful sgRNA design. However, the system is under constant adjustment and improvement, e.g. engineering the Cas protein (Kleinstiver et al. 2016), characterizing and adapting new Cas proteins, and engineering sgRNAs (Fu et al. 2014; Nowak et al. 2016). For insects, CRISPR/Cas was first developed in *D. melanogaster* in 2013 (Ren et al. 2013; Yu et al. 2013; Bassett et al. 2014), and by now has been adapted for several other species including *Ae. aegypti* (Kistler et al. 2015), *An. gambiae* (Hammond et al. 2016), *B. mori* (Wang et al. 2013; Ma et al. 2014), *Gryllus bimaculatus* De Geer (Awata et al. 2015), *Danaus plexippus* (L.) (Markert et al. 2016), and *Spodoptera litura* (F.) (Bi et al. 2016), and was recently transferred to the highly invasive agricultural pests *C. capitata* (Meccariello et al. 2017; Aumann et. al. 2018) and *D. suzukii* (Li and Scott 2016; Kalajdzic and Schetelig 2017; Li and Handler 2017).

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This rapid adaptation of CRISPR/Cas to a variety of insects within just 4 years demonstrates the versatility of this method and its potential for future applications.

### *2.4. Binary/Conditional Expression Systems*

Genetic sexing or sterility systems are useful only if the trait conferring sexing or sterility can be switched off for strain rearing. Therefore, binary or conditional expression systems are required. The GAL4-UAS system is a binary expression system first established in *D. melanogaster* (Brand and Perrimon 1993). It consists of a driver line containing the budding yeast GAL4 transcriptional activator under the control of a promoter of interest, and an effector line containing the upstream activating sequence (UAS) to which GAL4 binds to activate a gene of interest. The system becomes activated only when both parts of the system are present in an insect, i.e. by crossing the two lines. Additional control of the system is possible by the repressor GAL80 (Lee and Luo 1999) or by temperature-sensitive GAL4 versions (Mondal et al. 2007). The GAL4-UAS system has become a milestone tool for functional genomics studies in *D. melanogaster*, with thousands of driver and effector lines (Rorth 1998; Hayashi et al. 2002; Pfeiffer et al. 2008). The system has also been applied to several other insect species (*B. mori* (Imamura et al. 2003), *Tribolium castaneum* (Herbst) (Schinko et al. 2010), *Ae. aegypti* (Kokoza and Raikhel 2011), and *An. gambiae* (Lynd and Lycett 2012)), and could be exploited to create genetic sexing or sterility systems. However, the necessity of constantly keeping two strains to be crossed only for the release generation hinders its potential and practical application, because it increases mass-rearing complexity and costs.

The recently established bipartite Q system utilizes regulatory genes of the *qa* gene cluster from the fungus *Neurospora crassa* Shear and B. O. Dodge (Potter et al. 2010), and resembles the GAL4-UAS system in that it consists of an activator QF and an upstream activating sequence QUAS. The system shows low basal expression in the absence of QF, and high expression in the presence of QF. Expression can be efficiently suppressed by the repressor QS. The advantage of the Q system over GAL4-UAS is the additional level of control via quinic acid. Feeding quinic acid to flies temporarily relieves QS suppression, thus permitting the creation of a conditional expression system. The Q system, which was established in *D. melanogaster* and recently transferred to *An. gambiae* (Riabinina et al. 2016), might advance to become another conditional tool in insect genetics, biotechnology, and pest control. This would allow developing insect strains with an additional and completely independent lethality or sterility system to counter the possibility of resistance development against the first system (Eckermann et. al 2014).

Food supplement-controlled conditional expression systems like the “Tet-on” and “Tet-off” systems were developed by Bujard and Gossen (Gossen and Bujard 1992; Gossen et al. 1995) as mechanisms for inducible and reversible gene expression in eukaryotic cells. They are controlled by the antibiotic tetracycline (Tet) or one of its derivatives, e.g. doxycycline. The Tet-system is based on a naturally occurring bacterial defence system against antibiotics. It consists of the tetracycline-controlled transactivator protein (tTA) and the TetO operator sequence. tTA represents a fusion of the DNA-binding domain from the bacterial tetracycline

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repressor (TetR), and the transcriptional activation domain of the HSV1 protein VP16. For most Tet-on/Tet-off systems, several repeats of the TetO (mostly 7) are fused together with a minimal promoter, forming the Tet response element (TRE). tTA binds to the TRE and thereby triggers the expression of a downstream gene. In the Tet-off system, the binding of Tet to tTA inactivates tTA binding to TRE, therefore the downstream gene is not expressed, and the effect is "off". For Tet-on, binding of Tet to the reverse tetracycline-controlled transactivator protein (rtTA) enables binding to the TRE site and activates downstream gene expression – the system is "on". In contrast to other binary expression systems, like the GAL4-UAS system, the Tet-systems are controllable via the external addition of tetracycline. Moreover, the expression control is tighter, i.e. less leaky, than for example the estrogen receptor-based conditional expression systems (Sohal et al. 2001).

In insects, the Tet system was first tested in *D. melanogaster* (Bello et al. 1998), and has been applied to create conditional sterility or gender-specific lethality systems. Heinrich and Scott reported the first conditional female-specific lethality system in *D. melanogaster*, causing death in late pupal or early adult stages by expressing the pro-apoptotic gene *head involution defective* (*hid)* in the adult female fat body (Heinrich and Scott 2000). Such conditional lethality systems could, in general, also be constructed as a unisex lethality system that could be used in population control programmes instead of using radiation for sterilization. Since the released insects are not sterile in the strict sense, but carry a dominant lethal that would kill their offspring, Thomas et al. (2000) introduced the term RIDL – "Release of Insects carrying a Dominant Lethal" as an alternative to the SIT. Gong and colleagues created a one-component Tet-suppressible RIDL system in the Mediterranean fruit fly *C. capitata* (Gong et al. 2005). Instead of a specific promoter driving tTA, which then activates a lethal gene, they created an autoloop where tTA activates its own expression. This leads to the accumulation of toxic levels of tTA in the late larval or pupal stages in the absence of Tet. Fu et al. (2007) then combined this system with the sex-specifically spliced *Cctra*-intron (section 3.1.) to conditionally kill females, and named the system female-specific RIDL (fsRIDL). The fsRIDL system was subsequently transferred to a number of insect species using either exactly the same system as in *C. capitata* or by adjusting for species-specific regulatory elements (section 3.1.).

The first conditional transgenic embryonic sexing system (TESS) based on Tet-off was developed in *A. suspensa,* using a combination of an early promoter, acting at the egg stage, driving the tTA expression, and *hid* as a lethal effector gene to cause embryo-specific lethality in the progeny. Sex-specificity is achieved again by including the *Cctra*-intron into the *hid* gene (Schetelig and Handler 2012a). Like RIDL, also the early-acting Tet-off system can be designed as a unisex lethality system based on embryonic promoters and lethal effectors (Horn and Wimmer 2003; Schetelig et al. 2009a; Schetelig and Handler 2012a, b). The use of female-specific lethality systems for sexing is described in detail in section 3.1.; for the application of unisex lethality to create sterility systems, see section 3.3.

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### *2.5. RNAi*

RNA interference (RNAi) is a conserved eukaryotic mechanism of defence against viruses and foreign double-stranded RNA (dsRNA). Long dsRNA molecules are recognized and cleaved by the Dicer protein. The resulting small interfering RNA (siRNA) molecules are incorporated into the RNA induced silencing complex (RISC), and guide it to the complementary target messenger RNA (mRNA) that is cleaved by RISC and thereby silenced. RNAi has become an important tool to study gene functions, also in insects (Bucher et al. 2002). Many studies were performed by microinjection of dsRNA into eggs, larvae or adults, but this procedure is not applicable for mass-producing insects. Therefore, the finding that oral ingestion of dsRNA can trigger RNAi opened the door for its use in pest control. Oral delivery of dsRNA in insects was first described in the triatomine bug *Rhodnius prolixus* Stål and the light brown apple moth *Epiphyas postvittana* (Walker) (Araujo et al. 2006; Turner et al. 2006).

The first topical application of dsRNA that also induced systemic RNAi (when the signal spreads throughout the insect's body) was shown in the Asian corn borer *Ostrinia furnacalis* (Guenée) (Wang et al. 2011). Systemic RNAi by topical application of IAP1 dsRNA in *Ae. aegypti* could not be confirmed despite a first positive report (Pridgeon et al. 2008, 2016). In contrast, it was shown that topical application of the *Aae* IAP1 dsRNA did not to have a significant knock-down effect on the mRNA level, and even microinjection of IAP1 and other IAPs into adult *Ae. aegypti* females did not cause significant mortality (Puglise et al. 2016). Therefore, it cannot be determined if failure of the topical application in *Ae. aegypti* was due to the method or the target selection.

Both methods, oral ingestion and topical application, require *in vitro* RNA synthesis, which on a large scale can become costly. Another option is the *in vivo* delivery via bacteria (Tian et al. 2009; Zhu et al. 2011), host plants (Baum et al. 2007; Mao et al. 2007; Pitino et al. 2011; Zha et al. 2011), or host-plant viruses (Kumar et al. 2012) expressing the dsRNA. All three strategies have been shown to induce systemic RNAi in the investigated species: western corn rootworm *Diabrotica virgifera virgifera* LeConte (Baum et al. 2007), cotton bollworm *Helicoverpa armigera* (Hübner) (Mao et al. 2007), tobacco hornworm *Manduca sexta* (L.) (Kumar et al. 2012), brown planthopper *Nilaparvata lugens* Stål (Zha et al. 2011), and green peach aphid *Myzus persicae* (Sulzer) (Pitino et al. 2011). The bacteria-based system had a stronger effect than delivering the dsRNA via host plants. A possible reason for the reduced effect via host plant delivery might be the processing of the produced dsRNA by the plant's own RNAi machinery and Dicer enzymes into siRNA molecules, which might be less effective in the insect than the dsRNA (Mao et al. 2007; Kumar et al. 2012). This hypothesis is in line with an observation in the western corn rootworm where dsRNAs (but not siRNAs) are internalized into the midgut tissue (Ivashuta et al. 2015).

Given careful target design (which includes target selection and design of dsRNA taking into account polymorphisms and paralogous genes), RNAi can be a very specific approach. This has been demonstrated by Whyard et al. (2009) who showed that, by targeting the same enzyme in four different species, one can selectively kill only the species for which the dsRNA was designed. However,

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biological barriers, and not the proper target gene selection, are the most important limiting factors in insect RNAi. First, an efficient uptake by the midgut epithelial cells is required. Second, the alkaline environment in the insect midgut, as in lepidopterans (Dow 1992), could present a problem due to lysis of the dsRNA. Third, another significant barrier is the presence of nucleases in the midgut and salivary glands of insects. These nucleases might also be the reason for the lack of systemic RNAi in some insects. This is supported by a comparative study which showed that, in the RNAi-insensitive lepidopteran *M. sexta*, the dsRNA is rapidly degraded in the hemolymph, but in the hemolymph of the RNAi-sensitive cockroach *Blattella germanica* (L.) the dsRNA is stable (Garbutt et al. 2013). Along the same line, the desert locust *Schistocerca gregaria* (Forskal) shows a potent systemic RNAi response to injected dsRNA, but is insensitive to ingested dsRNA (Wynant et al. 2012). This phenomenon was explained when four different midgut RNAses were identified in *S. gregaria* (Wynant et al. 2014). Nevertheless, it might still be possible to target insects not susceptible to systemic RNAi by targeting midgut-specific genes, e.g. genes involved in detoxification pathways that are accessible for ingested dsRNA, provided that there are no midgut RNAses present.

In contrast to transgenic insects, the effect of RNAi is mostly transient (except for some cases of systemic or transgenerational RNAi). Therefore, the dsRNA must be supplied constantly during relevant developmental stages to guarantee the silencing effect. Depending on the production costs for the dsRNA and the required amounts (which depends on the production scale of the insects and the effectiveness of the dsRNA to silence the target gene), RNAi might be a costly method for pest control. An alternative to the synthetic production of dsRNA is the use of dsRNA-producing transgenic plants for agricultural pests or feeding the insects during mass-rearing with bacteria expressing the dsRNA. While this decreases the costs of dsRNA production, it involves using transgenic organisms, and with them come regulatory constraints in most countries, thus eliminating the advantage of RNAi (which by itself is a non-transgenic approach). Moreover, as the bacterial strain commonly used to express the dsRNA does not colonize the insect gut, this delivery method also has only transient effects. Recently, Whitten et al. (2016) succeeded in modifying symbiotic gut bacteria to express the dsRNA; these bacteria recolonized the gut of the target species and thereby induced a long-term silencing effect. Another option to circumvent the only transient effect of RNAi is by using transgenic insects expressing the dsRNA themselves. However, this would require again a conditional expression system (section 2.4.) to suppress dsRNA production for rearing the strains.

Several comprehensive reviews summarize the current insights and achievements in insect RNAi, and describe the challenges that are faced when developing this method as a tool for use in pest control (Baum and Roberts 2014; Joga et al. 2016; Darrington et al. 2017; Cooper et al. 2019).

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## 3. APPLYING MOLECULAR TECHNOLOGIES TO IMPROVE THE SIT

Modern molecular technologies can be used potentially to address and improve key aspects of programmes integrating the SIT such as sexing, sterilization, and marking of the insects destined for release. Combining available knowledge on insect biology, e.g. sex determination pathways, with specific and targeted genome modifications, could pave the way for new pest control systems. The following sections describe the current knowledge on sexing, sex determination and distortion, sterilization, monitoring, and transgene stabilization, as well as existing genetic (transgenic) systems with potential application for the SIT.

### *3.1. Sexing*

The release of only male insects can enhance the efficacy of SIT applications because the presence of sterilized females appears to prevent some sterilized males from competing with wild males for matings with wild females. Large-scale field studies comparing mixed sex versus male-only releases of the Mediterranean fruit fly in Guatemala showed a three- to five-fold increase in efficacy (Rendón et al. 2004). Moreover, male-only releases are a prerequisite in the case of vector control programmes because, for example, released female mosquitoes (even if sterile) would increase the number of biting and potentially disease-transmitting insects. For agricultural control programmes, it may also be beneficial not to release females, because the sterilized females could still cause damage by laying their eggs into fruits. Another important factor of male-only rearing is the reduction in costs, space, and logistics required at all levels of pest control programmes that apply the SIT, i.e. mass-rearing, quality control, distribution, and release as well as monitoring (Cáceres et al. 2004).

Significant savings can be gained if females are eliminated early in the rearing process (Franz et al., this volume). The role model for sexing is the Mediterranean fruit fly *temperature-sensitive lethal* (*tsl*) strain developed by classical genetic approaches, where the *tsl* mutation specifically and effectively kills all female embryos upon heat shock (Franz and McInnis 1995; Franz et al., this volume). Recently, another *tsl* mutation was created successfully by chemical mutagenesis in the mosquito *An. arabiensis* (Ndo et al. 2018). However, at the present time, the *tsl* mutations are not identified molecularly, and the creation of such a mutation using classical mutagenesis often takes a long time. Nevertheless, once the molecular basis of such a mutation is identified, modern molecular tools, as described above, could be used to build this highly efficient system in other species to create new conditional sexing strains based on heat shock, thus avoiding chemicals and antibiotics. These new strains might be classified as non-transgenic since no foreign DNA is transferred to the insect of interest, and potentially even as non-GM since the modification is based on a naturally occurring mutation. Thereby these sexing strains would evade the regulatory restrictions that apply to the release of GM organisms.

Other strategies could rely on the use of sex-determining factors to produce male-only strains. Sex-determination pathways in insects are very diverse, but all

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appear to be centred around a highly conserved sex-determining factor in Diptera, Lepidoptera, and Hymenoptera, *doublesex (dsx),* at the end of a sex-determination cascade (Funaguma et al. 2005; Verhulst et al. 2010; Geuverink and Beukeboom 2014). *Dsx* is sex-specifically spliced into a male and female isoform. In many tephritids this is controlled by *transformer* (*tra*) (Saccone et al. 2011) as a master epigenetic switch. A functional *tra* protein seems to be produced only in females (XX), while the male pre-mRNA is not spliced, and therefore contains an early stop codon leading to a non-functional protein (Verhulst et al. 2010). The female *tra* protein functions as splicing enhancer, leading to the female-specific splicing of *dsx*. In contrast, the absence of functional *tra* protein in males results in male-specific splicing of *dsx*. Female-specific *tra* expression is maintained through a positive feedback loop. When zygotic activation of this loop is prevented, male development follows. Although the function of *tra* appears to be conserved across many species, its sequence is not (Pane et al. 2005; Verhulst et al. 2010). This not only makes the identification of species homologues difficult, but also allows diversity of upstream-acting factors in different species. Therefore, deciphering the sex-determination pathways and other factors involved in different insects can be challenging.

Recently, breakthroughs were achieved in several insect species. Scientists identified the long-sought M-factor *Nix* in *Ae. aegypti* and *Ae. albopictus* (Hall et al. 2014, 2015; Liu et al. 2020), the maleness gene *Yob* in *An. gambiae* (Krzywinska et al. 2016), *GUY1* in *Anopheles stephensi* Liston (Criscione et al. 2013, 2016), *Mdmd* in *Musca domestica* L. (Sharma et al. 2017), and the *Maleness-on-the-Y* (*MoY*) gene in *C. capitata* (Meccariello et. al. 2019). *Nix, Yob, GUY1*, and *Mdmd* are located upstream of *dsx* in the cascade, and are needed for male development by directly or indirectly inducing male-specific *dsx* splicing. In the Mediterranean fruit fly, where *tra* is maternally deposited in the embryo to induce its auto-regulatory female-specific splicing, *MoY* is necessary and sufficient to suppress this female-specific *Cc-tra* splicing, resulting in male development. However, the mechanism of suppression has not yet been solved. Interestingly, *MoY* is functionally conserved in two other tephritids, *Bactrocera oleae* (Rossi) and *B. dorsalis*, providing a candidate gene for genetic-control strategies. Another mechanism of female-specific splicing of *dsx* was discovered in the WZ sex-determination system of the lepidopteran *B. mori*, where it is induced by a female-specific piRNA (piwi-interacting RNA) (Kiuchi et al. 2014).

The sex-determining factors could be promising tools to eliminate females or convert them to males for SIT projects (Schliekelman et al. 2005). The sex-specifically spliced *C. capitata tra*-intron is already successfully being used to robustly eliminate females. The female-specific splicing of the *tra*-intron leads to a functional protein, whereas the male isoforms result in truncated, non-functional versions. This has been exploited to develop a Mediterranean fruit fly sexing system using the *Cctra*-intron to female-specifically express a toxic gene in the fsRIDL system (Fu et al. 2007), which was also directly applied to the olive fruit fly *B. oleae* (Ant et al. 2012). Recently, the system was adapted to the Australian sheep blow fly *Lucilia cuprina* (Wiedemann) and the New World screwworm *Cochliomyia hominivorax* (Coquerel) using the sex-specific first intron of the screwworm *tra* gene for both species to female-specifically overexpress tTA to toxic levels (Li et al.

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2014; Concha et al. 2016). The successful characterization of the lepidopteran *doublesex* (*dsx*) gene was the basis for transferring the fsRIDL to the pink bollworm *Pectinophora gossypiella* (Saunders) and the diamondback moth *Plutella xylostella* (L.) using sex-specific splicing of *dsx* to accumulate tTA in females (Jin et al. 2013). fsRIDL kills females in the late larval or pupal stage (Fu et al. 2007). In contrast, in the TESS, the early embryonic and female-specific expression of the pro-apoptotic gene *hid* using the *sry-alpha* promoter kills females early in embryonic development in *A. suspensa, A. ludens,* and *C. capitata* (Schetelig and Handler 2012a; Ogaugwu et al. 2013; Schetelig et al. 2016), which in a large-scale production would reduce the cost of rearing. Similar systems have recently been developed for *Lucilia cuprina* (Yan and Scott 2015) and *C. hominivorax* (Concha et al. 2016; Scott et al. 2017). Following this approach, the *An. gambiae Yob* might be a promising new candidate gene to create a mosquito sexing strain, because overexpression results in female lethality, potentially due to a role in dosage compensation (Krzywinska et al. 2016; Krzywinska and Krzywinski 2018).

Instead of eliminating females, male-only populations could be created by reversing females into males. This would have the advantage of doubling the male output. Sex reversion via a transient RNAi effect has been successfully performed in several species by targeting *transformer* genes, whose function is required only for female development. In *A. suspensa*, injection of dsRNA against *Astra* and *Astra-2* into fertilized embryos led to at least 97% male progeny, of which half were XX males. These XX phenotypic males mate successfully but are infertile due to hypertrophic testes and reduced sperm motility (Schetelig et al. 2012). In contrast, transient RNAi against *tra* in *C. capitata*, *B. oleae*, and *L. cuprina* (Pane et al. 2002; Lagos et al. 2007; Concha and Scott 2009) resulted in fully transformed XX males, which were also fertile. Sex conversion could also be achieved by overexpressing suitable sex-determination genes or maleness factors, like *Nix* in *Ae. aegypti* (Hall et al. 2015; Aryan et al. 2019).

A different approach to sex distortion is the so-called X shredder. The endonuclease I-*Ppo*I specifically cuts X-chromosomal ribosomal rDNA sequences in *An. gambiae*. Tissue-specific expression of I-*Ppo*I in the testes using *beta2-tubulin (β2-tub)* 5' and 3' regulatory sequences leads to shredding of X chromosomes during gametogenesis, resulting in a strong bias for Y gametes, but also male sterility in crosses of heterozygous males with wild-type females. This is due to the long half-life of the I-*Ppo*I protein, which is transferred via sperm into the zygote where it shreds the X chromosome of the oocyte upon fertilization (Windbichler et al. 2008). The system was optimized to create a pure sex-distortion system without male sterility by reducing the half-life of the I-*Ppo*I protein through protein engineering (Galizi et al. 2014). Recently, the same group successfully rebuilt the X shredder in *An. gambiae* based on CRISPR/Cas. The guideRNAs were designed to recognize a conserved rDNA target sequence of the *An. gambiae* complex. By expressing the Cas9 protein specifically during spermatogenesis, the X chromosome in sperm is shredded. The selected guideRNA target sequence is conserved among *An. arabiensis*, *An. gambiae*, *An. bwambae* White, *An. melas* Theobald, and *An. merus* Dönitz. Therefore, this CRISPR/Cas-based X shredder should be applicable to the complete *An. gambiae* complex (Galizi et al. 2016).

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### *3.2. Marking/Monitoring/Sex Sorting*

To be able to monitor the effect of SIT releases, sterile insects have to be distinguished from wild insects in the field (Dowell et al., this volume; Franz et al., this volume; Parker, Mamai et al., this volume; Vreysen, this volume). Traditionally, colourful adhesive powders are used to mark the sterile insects before release (Schroeder and Mitchell 1981). However, the colourful powders are error-prone in some species because of a possible transfer of the marker during contact with wild insects, or removal of the powder by grooming. Moreover, some of the dusts can be harmful to workers handling the dye. An alternative would be replacement with genetic markers coding for fluorescent proteins stably integrated into the genome of insects (Hagler and Jackson 2001; Hendrichs and Robinson, this volume). Such genetic fluorescent markers are available in a variety of colours, and can be selectively expressed by using specific promoters. Transgenic strains carrying fluorescent markers have been developed in a wide variety of insect pests, including the mosquitoes *Ae. aegypti* and *An. gambiae* (Catteruccia et al. 2005; Smith et al. 2007) as well as fruit flies like *A. suspensa* and *C. capitata* (Handler and Harrell 2001a, b; Scolari et al. 2008; Zimowska et al. 2009). Important for the use of fluorescent markers in field monitoring is the stability of the fluorescent proteins over a long time under trapping conditions. This has been shown for the pink bollworm, where DsRed proteins were stable for at least 2 wk after trapping of specimens as confirmed by fluorescence microscopy and PCR (Simmons et al. 2011). A similar study with a transgenic strain of *A. suspensa* proved the stability of DsRed fluorescent marker protein over a period of 3 wk in field traps, monitored by visual inspection and PCR (Nirmala et al. 2011). Studies in *A. ludens* confirmed the marker stability under dry conditions over several months (Meza et al. 2011). As the fluorescent protein degrades over time, PCR assays have been developed to verify the DsRed marker molecularly (Zimowska et al. 2009).

Besides monitoring released insects in the field, fluorescent markers could serve multiple purposes. By using the *β2-tub* promoter, the marker is expressed in the male sperm (Catteruccia et al. 2005; Scolari et al. 2008). Thus, via marker expression in the gonads, such strains can be used to monitor the mating success of the released males by screening for the fluorescent sperm in the spermathecae of wild females (Juan-Blasco et al. 2013). Moreover, the male- (or sex-) specific expression of fluorescent markers allows (automated) sexing, as shown for *Aedes* and *Anopheles* species. A transgenic *Ae. aegypti* strain has been constructed in which DsRed expression is driven by the *β2-tub* promoter, allowing reliable sorting of DsRed males from non-marked females using the Complex Object Parametric Analyser and Sorter (COPAS) (Smith et al. 2007). In a transgenic strain of *An. stephensi*, *β2-tub* drives the expression of an enhanced green fluorescent protein (EGFP) permitting late larval automated sorting (Catteruccia et al. 2005). Marois and colleagues used the *dsx* promoter to drive EGFP in *An. gambiae*, which permits early larval separation by COPAS (due to the higher expression of EGFP in the midgut in male L1 larvae compared with females) (Marois et al. 2012). Importantly, the COPAS sorting does not significantly affect the viability and competitiveness of sorted males (Catteruccia et al. 2005; Smith et al. 2007).

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Instead of using suitable promoters to express the markers sex-specifically, transgenic lines with insertions of the marker on the Y chromosome can be used, as available for *A. suspensa*: four Y-linked *Dmel*-PUb-DsRed lines have been created, of which one expresses the marker already in early embryogenesis (Schetelig and Handler 2013b). Ideally, a Y-chromosome insertion should also contain a landing site, e.g. *attP*, *lox*, or *FRT,* for site-specific integration, such that any construct of interest can be inserted into the Y chromosome. Such lines are available for *An. gambiae* (Bernardini et al. 2014) and *A. suspensa* (Schetelig and Handler 2013b) having a Y-linked *attP* site.

### *3.3. Sterilization*

Genetic sterilization approaches can also contribute to the population control of insect pests and disease vectors. One of the first genetic sterilization systems causes reproductive sterility by the transfer of embryonically lethal transgenes. It was developed in *D. melanogaster* (Horn and Wimmer 2003), and was later successfully adapted and transferred to important pest insects like *C. capitata, Anastrepha ludens*, and *A. suspensa* (Schetelig et al. 2009a, 2016; Schetelig and Handler 2012b). It is based on the conditional expression of a lethal effector molecule, *hid*, controlled by an early embryonic promoter, to cause embryo-specific lethality in the progeny. During mass-rearing the conditional systems can be switched off by a food supplement, or the antibiotics tetracycline or doxycycline. Such strains, if released into the field where no antibiotics are present, will lead to biologically fertile matings, but their progeny will die due to the embryonically active lethal system. This results in the reduction of the wild-type population.

In contrast, RIDL was designed as a late-acting conditional lethality system that kills progeny in late larval or pupal stages by accumulation of toxic levels of tTA. RIDL systems that confer unisex lethality in the next generation, and can be used as sterility systems, were designed for *C. capitata* and *Ae. aegypti* (Gong et al. 2005; Phuc et al. 2007; Harris et al. 2011). More details about the design and function of the RIDL and conditional expression systems (Tet-on/Tet-off) and their applications are given in section 2.4.

### *3.4. Transgene Stabilization*

Most transgenic insects have been routinely generated using germline transformation procedures and transposable elements as tools to randomly integrate DNA into genomes. To prevent instability or a horizontal gene transfer through the transposon system to other insect species, all commonly used transposons have been split into two non-autonomous parts, one vector encoding the transposase gene (helper), and a second vector containing the inverted repeats flanking the transgene construct to be integrated into the genome. After injection into the germline, the transposase is only transiently expressed from the plasmid that quickly gets lost during cell divisions, while the transgene should be stably integrated into the genome. However, as long as the intact inverted terminal repeats (ITRs) of the transposons flank the transgene

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construct in the genome, it can be remobilized in the presence of a suitable transposase enzyme, which could lead to a loss of function. Thus, endogenous, related cross-mobilizing transposases, could potentially remobilize the construct.

An additional concern has to do with the risk of horizontal gene transfer through the uptake of foreign DNA by other organisms in the field and the subsequent remobilization of transposons contained therein by various means. Although this risk is minimal, the possibility exists. For the use of transgenic insects in SIT projects, this possibility could be investigated by feeding the transgenic organism to other organisms in question and assess potential horizontal transfer events by molecular techniques.

Regarding remobilization, such events could be prevented by removing one or both inverted repeats. Mechanisms described for *D. melanogaster* and *C. capitata* introduce additional ITRs such that additional ITR-flanked cassettes are created at the genomic integration site. A subsequent remobilization experiment either remobilizes the two outermost ITRs, which removes the whole transgene construct (the unwanted side product), or one of the outer ITRs remobilizes with the internal ITR, thus leaving the organism with the third ITR and part of the transgene construct (the desired product). Studies verified that one ITR is not sufficient anymore to remobilize the transgene, which is therefore considered as "stabilized" (Handler 2004; Dafa'alla et al. 2006; Schetelig et al. 2009b; Long et al. 2015).

### *3.5. Quality Control of Transgenic Lines*

The success of large-scale operational programmes with an SIT component depends on the regular monitoring and evaluation of the quality of the strain used (Franz et al., this volume; Parker, Vreysen et al., this volume; Vreysen, this volume). After several decades of successful development and implementation of the SIT, the FAO, IAEA, and USDA have developed standard operating procedures for the evaluation of the biological quality of strains used in mass-rearing and SIT applications (FAO/IAEA/USDA 2019). The most important parameters are rearing efficiency and sterile male performance, including mating competitiveness.

The VIENNA 8 strain is the non-transgenic genetic sexing strain, produced through classical genetics, currently used in large-scale operational Mediterranean fruit fly SIT programmes worldwide (Augustinos et al. 2017; Franz et al., this volume). VIENNA 8 exists in two versions, with and without the inversion D53, which is used to suppress recombination and ensure the genetic stability of the strain under large scale production: VIENNA $8^{D53+}$ and VIENNA $8^{D53-}$ (Augustinos et al. 2017). Its sexing properties are based on two selectable markers, the *white pupae* (*wp*) and the *temperature-sensitive lethal* (*tsl*) genes. A transgenic version of this strain was developed, VIENNA 8-1260, which carries two fluorescent markers, the DsRed in the body and the GFP in the testes (Scolari et al. 2008). In a recent study, the Mediterranean fruit fly VIENNA $8^{D53+}$ strain was compared with the transgenic strain VIENNA 8-1260 with respect to the parameters egg production, egg hatch, and egg sterility under semi-mass-rearing conditions, and male mating competitiveness in field cages (Rempoulakis et al. 2016). VIENNA 8-1260 produced significantly fewer eggs as compared with the classical genetic sexing

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VIENNA $8^{D53+}$ strain, while egg hatch and egg sterility were similar. However, after ten generations under continuous semi-mass-rearing conditions, the male mating competitiveness of the VIENNA 8-1260 strain, when compared with wild-type males, was statistically significantly lower than that of the classical genetic sexing VIENNA 8 strain (Rempoulakis et al. 2016). The VIENNA $8^{D53+}$, VIENNA $8^{D53-}$, and VIENNA 8-1260 strains were also tested on a mass-rearing scale. The analysis showed that the transgenic VIENNA 8-1260 strain had significantly lower yield than both VIENNA $8^{D53+}$ and VIENNA $8^{D53-}$ strains. In addition, the relative sterility index and male mating performance of the VIENNA 8-1260 was significantly lower than those of VIENNA $8^{D53-}$, which is a major disadvantage for its use in large-scale operational SIT programmes (Ramírez-Santos et al. 2017a). Another limitation of this transgenic strain is that it requires higher irradiation doses to eliminate the possibility of the vertical transfer of the fluorescent gene into wild flies and the environment (Ramírez-Santos et al. 2017b). However, the required higher irradiation doses compared with the classical genetic sexing strain VIENNA 8 may further reduce the mating competitiveness of the transgenic strain VIENNA 8-1260 (Ramírez-Santos et al. 2017b; Bakri et al., this volume).

Until now, only a few transgenic strains (and of only two species, *C. capitata* and *Ae. aegypti*) have been evaluated with regard to their potential suitability for use in large-scale open-field releases. In a recent study, a *C. capitata* fsRIDL strain, OX3864A, was assessed under field-cage conditions in comparison with the VIENNA $8^{D53+}$ strain and wild-type males for its male sexual competitiveness (Virginio et al. 2017). For both strains, the mean duration of mating events was significantly lower than for the wild type. Moreover, the latency period, i.e. the time elapsed until mating starts, for transgenic males was significantly longer than for VIENNA $8^{D53+}$. In contrast, there were no differences observed in the proportion of females mated, and in the relative sterility index.

Transgenic RIDL strains of *Ae. aegypti* have been developed and tested for their rearing efficiency and male mating competitiveness. A female-specific RIDL version of an *Ae. aegypti* strain (fsRIDL) was tested in outdoor field cages; it failed to eliminate a target population because of its low mating competitiveness (Facchinelli et al. 2013). The bisexual *Ae. aegypti* OX513A RIDL strain has, in several places, successfully suppressed the population of this major mosquito vector species (see below). It is the only transgenic mosquito line which has been semi-mass-reared for releases of up to 1–1.5 million male mosquitoes per week (Carvalho et al. 2014). However, in addition to its low male mating competitiveness (thus releases were done with very high transgenic to wild male ratios), this RIDL strain also presents additional disadvantages. It is not a sexing strain, meaning that sex separation based on pupal size is very labour intensive and inefficient, thus some fertile females are released into nature; this raises concerns about their role in disease transmission as well as in spreading the transgene and leaving OX513A‘s genetic footprint in the environment (Bourtzis et al. 2016). Moreover, a recent study showed introgression of OX513A’s genetic background into the local population, resulting in hybrids of originally genetically quite distinct populations with potentially increased robustness, and an unclear vectorial capacity (Evans et al. 2019). The rearing of this transgenic *Ae. aegypti* OX513A RIDL strain also depends

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on the use of tetracycline in mass-rearing. The use of this antibiotic may have a negative impact on the mosquitoes' endogenous microbiota -- known to be important in the biology, physiology, and ecology of the host, potentially affecting the rearing efficiency and mating competitiveness of the strain (Bourtzis et al. 2016).

During the past 15 years, numerous so-called landing-site lines for site-specific genome modification have been created in many insects. These lines carry transgene constructs with recombination sites for site-specific recombination systems like Cre/*lox*, Flp/*FRT* or PhiC31/*att* (section 2.2.), and could be used to build transgenic strains for population control programmes. Since all of these lines were created by transposon-mediated transformation resulting in the random transgene integration into the genome, the integration site could have a negative effect on the biological quality of these lines, and thereby on their suitability for large-scale pest control programmes. Therefore, it is crucial to assess the fitness of such lines.

Such assessments have been performed recently for several landing-site lines of the vector *Ae. aegypti* in a comprehensive set of laboratory-scale tests (Häcker et al. 2017). The lines carry a construct for site-specific genome modification via Cre-RMCE. The tests included parameters important for successful and economical mass-rearing, e.g. female fecundity and fertility, and time for larval development. In addition, they included parameters important for releases such as male mating competitiveness and adult male longevity. They were based on a statistical sample size estimation to ensure the detection of differences in quality of up to five percentage points compared with the parental strain Higgs White Eye (HWE). The tests showed that one of the lines, AH0212-F1, with an intergenic integration site of the transgene construct, performed closest to the parental HWE, and thus is the most suitable line for downstream site-specific modifications.

However, the tests also showed that transgene integration at sites annotated as intergenic (DNA sequences located between genes) does not necessarily indicate a "good" integration site regarding fitness costs, as observed for the line AH0212-M1 (Häcker et al. 2017). This line showed strong deficits in several parameters including female fecundity and longevity, and pupation time. This effect is purely due to the integration site since the transgene construct is identical in all lines. None of the lines, however, performed "good" or "bad" in all of the quality parameters, because different integration sites will influence different sets of genes or gene-expression pathways, thus influencing various life traits. Moreover, experiments analysing the larval hatch rate under different food conditions (baker's yeast as minimal *Ae. aegypti* food source in the hatching water versus TabiMin fish food as optimal food source) revealed a strong influence of the chosen experimental conditions on the results of the fitness tests. When using an optimal food source, no significant differences in larval hatch rates between the transgenic lines and the HWE reference strain were observed. However, significantly decreased hatch rates for some lines were revealed when the lines were put under "stress" by hatching them using minimal food (Häcker et al. 2017). All of the above-mentioned assessments were performed under small-scale laboratory conditions. The next step is to evaluate these lines under semi-mass-rearing conditions, and finally the best line will be assessed in a large-scale mass-rearing setting.

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## 4. SUCCESSFULLY APPLYING MOLECULAR TOOLS – FIRST RELEASES OF TRANSGENIC INSECTS

The first open-field tests with transgenic insects were performed in 2007/2008 with the pink bollworm strain OX1138B; this strain carried a transgenic marker for field monitoring. After the first field trials in 2007, when 1.1 million moths were released in Yuma County, Arizona, in 2008 this was increased to over 15 million moths released within the framework of an operational demonstration trial. The performance of the moths was equal to that of the traditional non-transgenic APHIS strain established for the ongoing SIT programme against the pink bollworm, i.e. in terms of mating success, dispersal, and persistence in the release area (Simmons et al. 2011).

The first open-field trials with transgenic *Ae. aegypti* mosquitoes were started in 2009 and 2010 in Grand Cayman (Harris et al. 2011), followed in 2010 by a larger scale release; over a 23-week period, 3.3 million engineered OX513A males were released in three adjacent areas. This trial showed the potential of the transgenic control strategies for mosquitoes in general, and of the OX513A in particular, as it achieved 80% population suppression at an overflooding ratio of 5:1 (Harris et al. 2012). If there is a link between the disease-carrying insects and the epidemiological burden (which should be further evaluated in the future), this reduction in the insect population is most important and valuable. Subsequent to the success of the Grand Cayman study with OX513A, a sustained release of this strain, for a period of more than one year, was done in 2015 in a suburb of Juazeiro, Bahia, Brazil, reducing the local population by 81% (Carvalho et al. 2015). The most recently published study on OX513A additionally addresses the important question of recurrence of mosquito populations following post-release interruption in two distinct release areas in Brazil (Garziera et al. 2017). The study showed that mosquito populations recovered within 17–32 weeks after the end of the releases, demonstrating the necessity of continuous releases for permanent population suppression. Field trials with OX513A are also scheduled for the Florida Keys after the 2016 final decision by the FDA that no significant impacts on the environment are expected, although these have until now not been initiated in view of public opposition.

Field trials with *Wolbachia*-infected *Ae. aegypti* have been successfully performed in Australia, starting in 2011, with a self-sustaining (population replacement) objective (Hoffmann et al. 2011). More releases of *Wolbachia*-infected *Aedes* males are ongoing or planned in Brazil, China, Singapore, and the USA, although mainly as part of self-limiting (IIT) approaches (Callaway 2016; Waltz 2017).

A large-scale performance evaluation is also planned for a transgenic sexing strain of the New World screwworm; it will be tested for its performance in mass-rearing by the *Comisión Panamá - Estados Unidos para la Erradicación y Prevención del Gusano Barrenador del Ganado* (COPEG). An application to conduct open-field trials has been submitted to the government of Panama (Concha et al. 2016).

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## 5. CONCLUSIONS AND FUTURE CONSIDERATIONS

Modern molecular technologies offer many possibilities to address issues and bottlenecks in current insect pest and disease-vector population suppression programmes, e.g. the sexing of insects for male-only releases; for many species this is a critical tool in establishing an efficient large-scale operational programme. In addition, sterilization and marking techniques can be developed and combined to create sterile-male insect strains of good biological quality for population control. These modern biotechnologies permit strains to be tailored to meet the requirements of control programmes, and have the potential to facilitate greatly the transfer of successful strategies to new pest species in just a few years. However, for a species to be effectively targeted with these technologies, the availability of genome sequence data is beneficial, and molecular tools for insect transformation have to be available in the species of interest. For the Mediterranean fruit fly, such a resource has been built up (Papanicolaou et al. 2016), and in combination with a strong understanding of the chromosomal organization resulting from research and development of the SIT for this species, this database and knowledge will help to further identify crucial markers, genes, and targets for related tephritid and other pest species. However, high-quality genomes with mostly manual annotations are still limited, and, although the i5k insect genome project (Robinson et al. 2011) is sequencing multiple insect species, it will take many years before high-quality genomes will be available.

The CRISPR/Cas technology will also benefit from available sequence information, and promises to be a key method for editing genomes in the coming years. Current drawbacks of the method, because of off-target effects, are continuously being addressed, and the system is being improved and refined. CRISPR/Cas is only one of the techniques that can be used for creating strains useful for the suppression of populations of insect pests and disease vectors. There are several other techniques available, with distinct advantages, which can complement those of CRISPR/Cas. Thus, while CRISPR/Cas is highly flexible regarding the choice of the target site, Cre- or FLP-RMCE offer the possibility of successively targeting the same site, permitting the modification and adjustment of existing systems to changing requirements.

Until now, the described population suppression strategies for SIT applications are based on self-limiting genetic systems; they are suitable for both disease vectors and agricultural pests. In contrast, population replacement strategies make most sense in species that are transmitting a disease but are not pests by themselves, e.g. mosquitoes or vectors of plant diseases. In such insects, replacing the natural population with insects refractory to infection with the pathogen could be a viable option for disease control. However, such approaches have the disadvantage that they leave an "ecological footprint", and unexpected complications would have to be controlled by yet another self-sustaining genetic system controlling the problematic first one.

Research on such refractory strains has been performed mostly with regard to dengue and Zika infections in *Ae. aegypti,* using a *Wolbachia*-based replacement approach (Moreira et al. 2009; Hoffmann et al. 2011; Walker et al. 2011; Dutra et al. 2016; Lees et al., this volume). Moreover, trypanosome refractoriness in *Glossina*

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*morsitans morsitans* Westwood was achieved using a paratransgenesis approach (De Vooght et al. 2018). Another strategy uses transgenic approaches to make *Anopheles* mosquitoes refractory to *Plasmodium* infection (Ito et al. 2002; Moreira et al. 2004; Meredith et al. 2011; Isaacs et al. 2012; Smith et al. 2013; Sumitani et al. 2013; McArthur et al. 2014; Gantz et al. 2015). For population replacement approaches, the plan is to use gene drives to spread and establish such a transgenic refractory system in a wild population. Synthetic gene drives, based on naturally occurring selfish genes (the so-called homing endonuclease genes (HEG)), were first proposed in 2003 (Burt 2003). HEGs can be reprogrammed to target other than their natural target sites in the genome, e.g. in the human malaria vector *An. gambiae* (Windbichler et al. 2011). However, reprogramming HEGs is a challenging process, and limits their application in developing new gene drives (Champer et al. 2016).

The discovery of CRISPR/Cas not only provided a universal genome editing tool but also opened the door for the fast development of gene-drive systems. Due to the easy reprogramming of the CRISPR/Cas target site via the sgRNA sequence, the CRISPR-derived gene drives could theoretically be used to engineer almost any trait. In fact, a CRISPR/Cas gene drive with dual anti-*Plasmodium* effector genes has already been created for the malaria mosquito *An. stephensi* (Gantz et al. 2015). In case population replacement is not the desired strategy, gene drives could also be used to temporarily or locally suppress a population, e.g. by targeting female reproduction as shown for *An. gambiae* (Hammond et al. 2016) or by female to male conversion as modelled for *C. capitata* (KaramiNejadRanjbar et al. 2018). The difference to other population suppression strategies, such as the SIT or the RIDL system, is that theoretically the gene drive requires only a single seed release of a small number of individuals spreading into the population in contrast to the necessity of repeated mass-releases of sterile or transgenic males.

### *5.1. Mutations and Resistance Development to Genetic Modifications*

All genetically-based technologies have the risk that mutations and recombinations could alter or inactivate the transgene function. An important quality parameter for strains used in operational programmes is their genetic stability under large-scale rearing conditions. Mass-rearing facilities can produce hundreds or even thousands of millions of insects per week, which significantly increases the probability of the occurrence of these chance events (Dowell et al., this volume; Franz et al., this volume; Parker, Mamai et al., this volume;). Depending on where the mutations occur, they can have a variety of impacts on the transgene. Single-point mutations (which are the most likely mutations to occur) in the promoter or regulatory elements might not abolish the function of the element, but the accumulation of multiple mutations might do so. In contrast, a point mutation in a coding gene, leading to a missense or nonsense mutation, could cause a knockdown or knockout effect. The potential rate of a knockout mutation in a regulatory sequence can be estimated at the rate of $10^{-7}$ per bp or similar to a point-mutation reversion rate in a ~1 kb coding region (Handler 2016), while a knockout mutation in a 1 kb coding region is estimated at a frequency of about 1–5 x $10^{-6}$ (Tobari and Kojima 1972; Neel 1983; Woodruff et al. 1983). Therefore, under mass-rearing conditions (up to

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2 x $10^9$ insects per week), the occurrence of one or more revertant mutations can be expected. Upon release, these mutations might allow survival under restrictive conditions, and might even lead to population replacement or result in the breakdown of the mass-reared strain. Therefore, backup systems will be needed. Moreover, large-scale tests will be required to test for the actual occurrence of revertant mutations, and revertants to be analysed to identify the resistance mechanism. If a transgenic strain is mass-produced on a large scale, and taking into consideration natural mutation rates, a mutation inactivating the transgene resulting in genetic instability, and the breakdown of the transgenic strain, is possible (Handler 2016). This is a significant concern which has urged researchers in the field to consider strategies which can minimize or eliminate the likelihood of resistance development by incorporating, for example, a second selectable marker or effector gene (Eckermann et al. 2014; Handler 2016). However, such transgenic strains have not yet been developed and validated. Currently, filter rearing systems are used in mass-rearing to prevent spontaneous mutations from spreading in the mother colony and being released; each batch of insects is started from a checked population and then mass-reared for the release population (Parker, Mamai et al., this volume).

In the case of gene drives for population suppression or population replacement, it is expected that drive-resistant alleles will emerge for virtually any gene drive described so far (Champer et al. 2017; Noble et al. 2017; Unckless et al. 2017; KaramiNejadRanjbar et al. 2018). Resistance can arise from a variety of mechanisms. The most frequent reason will probably be by the action of the drive itself, when the Cas-induced double-strand break is not repaired by homology-directed repair as intended, but instead by the cell's non-homologous end-joining (NHEJ) repair mechanism, that often introduces small insertions or deletions at the repair site. These base changes will then not be recognized by the drive anymore, thus preventing the construct from spreading to the entire population. This is more likely to happen if the drive cuts only at one site. Other mechanisms of resistance are possible as well but will likely be a less frequent cause, such as standing genetic variation between populations as found for *An. gambiae* mosquitoes in Africa (Miles et al. 2016) or *de novo* mutations in the targeted gene(s), particularly if the target locus is not highly conserved.

Several solutions have been suggested to prevent resistance development against gene drives, e.g. targeting multiple sequences within a gene or targeting different genes at once, so that resistance would have to evolve independently for each site (Esvelt et al. 2014; Champer et al. 2016; Noble et al. 2017; KaramiNejadRanjbar et al. 2018). Moreover, it should be possible to prevent, or at least delay, the emergence of resistance by targeting highly conserved genes for which resistance due to mutation is expected to have a severe fitness cost, or to target a haplolethal gene whose function is only preserved under successful conversion (Esvelt et al. 2014; Champer et al. 2016; Noble et al. 2017). The concomitant expression of a short-hairpin RNA (shRNA) gene to suppress NHEJ repair could also help to prevent resistance development (Chu et al. 2015).

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### *5.2. Regulatory Considerations for the Application of Transgenic Insects*

Most modern molecular techniques applied to insect genomes create transgenic insects. The use of transgenic strains in open-field conditions, and potentially in operational programmes, requires them to be of adequate biological quality, but they also need to have all of the necessary regulatory approvals (Reeves et al. 2012). It is worth noting that the regulatory framework for transgenic insects, developed by the various technologies, is not harmonized within and between different countries and continents, and this is not expected to change in the near future. In addition, scientists and managers planning to use transgenic insects in the field must, in advance, address questions and concerns raised by the general public and stakeholders. In that respect, a decision-making process based on scientific evidence, evaluations, risk assessment studies, and large-scale experiments (in the laboratory, but also in contained and open-field release trials) would be necessary (FAO/IPPC 2004; FAO/IAEA 2006). Thanks to the novel technologies, and because of the extremely different control scenarios unfolding, a clear case-by-case evaluation is needed. A generalized treatment of the different systems and/or insect strains should be avoided. Several efforts have been initiated to include transgenic insects in regulatory frameworks, e.g. by the North American Plant Protection Organization (NAPPO 2007), the European Food Safety Authority (EFSA 2013), and recently by the National Academy of Sciences, Engineering, and Medicine (NASEM 2016). These documents inform in a detailed and scientific way, and hopefully will foster a balanced discussion to improve the decision-making in this exciting but complicated modern biotechnological field.

As an alternative, non-transgenic approaches could be pursued. In addition to classical genetic approaches, CRISPR/Cas and RNAi strategies offer different possibilities. Although organisms created by CRISPR/Cas genome editing will be treated under the GMO directive in the EU, based on a very recent decision of the European Court of Justice, CRISPR mutations that could also have been developed through traditional breeding methods, such as point mutations or small insertions or deletions, are classified as non-GM by the US Department of Agriculture and the Government of Japan (Nature 2018). As the evaluation of the CRISPR technology by governments and authorities is just starting, in future CRISPR mutations have the potential to be classified as non-GM in more countries. Thus, in these countries, strains created by CRISPR mutagenesis would not be affected by the regulations for transgenic organisms. Such mutations could be used, for example, to knock out genes required for sex determination to create non-transgenic sexing strains. RNAi methods also offer a way to work without transgenic organisms, i.e. if the dsRNA is not conditionally or constitutively expressed in the insect, and if it does not persist in the environment.

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### *5.3. Novel Developments in Insect Pest Control: Impaired Male Fertility*

Very recently, a completely new path to population suppression via impaired male fertility has been studied; the idea is based on the fact that mitochondria are inherited maternally. Therefore, mutations on the mitochondrial DNA (mtDNA) that are deleterious to males escape evolutionary selection to a high degree; the mutations don't have negative effects on the females which pass them on across generations (Frank and Hurst 1996; Gemmell et al. 2004; Beekman et al. 2014). By releasing females carrying such mutations into wild populations, these females and their female descendants would continuously produce males with impaired fertility, which should cause perpetual suppression of the target population. This idea was termed the Trojan Female Technique (TFT) (Gemmell et al. 2013). The initial data from different organisms, including *Drosophila* (Yee et al. 2013; Dowling et al. 2015; Patel et al. 2016; Wolff et al. 2016) and the cowpea weevil *Callosobruchus maculatus* (F.) (Dowling et al. 2007), show that such mtDNA mutations indeed can affect male fertility without affecting female fertility, supporting the possibility of using TFT for population suppression. However, there are many factors that could influence the effectiveness of the TFT (Wolff et al. 2016); for example, females could still get enough viable sperm to fertilize the eggs if males are not completely infertile or by remating with another partially fertile male. Moreover, the selective pressure on the males could lead to compensatory mutations, and genetic drift and population dynamics among other factors could lead to the disappearance of male fertility-impairing mutations even if they are not selected against (Rand et al. 2001; White et al. 2008).

An initial study with *Drosophila melanogaster* showed that a male-harming mitochondrial haplotype can indeed decrease the population size under density-controlled conditions over 10 generations by an average of 8% at a 75% haplotype frequency at the start of the treatment (Wolff et al. 2016). However, when the population size fluctuated, no effect was observed, although the TFT haplotype was stably persistent in most cases. Many more studies with different candidate mutations will be necessary to test if such Trojan females can indeed decrease the population size to meaningful levels under natural population conditions and across generations. If a naturally occurring mtDNA mutation was used, TFT would also be considered a non-transgenic approach.

Nevertheless, independent of the technology and strategy used, each product resulting from the application of modern biotechnology should be thoroughly tested and evaluated for functionality, efficacy, safety, and stability of the applied system. The ultimate goal is to select a safe, economical, and ethical strategy to perform environment-friendly and thus sustainable pest control for most of the important insect pest and vector species.

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# CHAPTER 4.7.

# USING GEOGRAPHIC INFORMATION SYSTEMS AND SPATIAL MODELLING IN AREA-WIDE INTEGRATED PEST MANAGEMENT PROGRAMMES THAT INTEGRATE THE STERILE INSECT TECHNIQUE

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## SUMMARY

Geographic information systems (GIS) and spatial modelling are crucial for designing, implementing, and optimizing area-wide programmes of insect and/or disease control. This chapter provides a basic introduction to the science of GIS, Global Positioning System (GPS), satellite remote sensing (RS), and spatial modelling, and reviews the principal ways in which these technologies can be used to assist various stages of development of the sterile insect technique (SIT) as part of area-wide integrated pest management (AW-IPM) programmes — from the selection of project sites, and feasibility assessments and planning of pre-intervention surveys, to the monitoring and analysis of insect suppression programmes, and the release of sterile insects. Potential barriers to the successful deployment of GIS tools are also discussed.

## 1. INTRODUCTION

The success of the sterile insect technique (SIT) and other area-wide interventions, aimed at controlling populations of insect pests, depends to a large degree on appropriate project planning and implementation. More specifically, successful programmes depend on an accurate knowledge of pre-existing distributions of insects in time and space, on the appropriate design of insect suppression strategies and sterile insect release projects, and on the development of suitable frameworks for monitoring and evaluation. Methods and tools have become available that enable the development of models simulating the demography of a metapopulation of insects, thus making the *ex-ante* assessment of various control strategies possible (Peck 2012; Peck and Bouyer 2012).

Geographic Information Systems (GIS), the Global Positioning System (GPS), remote sensing (RS), and spatial modelling are allied technologies that together provide a means of gathering, integrating, and analysing spatial data. These tools are already being used extensively in other areas of agroecological management and epidemiological research (Barnes et al. 1999; Nutter et al. 2002; Moiroux et al. 2013; Tsafack et al. 2013; Dicko et al. 2015), and their use within area-wide integrated pest management (AW-IPM) programmes (Adam et al. 2013; Percoma et al. 2016) including those with an SIT component is growing quickly (Bouyer et al. 2010; Dicko et al. 2014). The tools are becoming increasingly accessible to non-specialists thanks to a wide range of freeware (FAO/IAEA 2006).

The principal aim of this chapter is to present applications of GIS and associated spatial tools within AW-IPM programmes. The first part of the chapter constitutes a short primer on GIS, GPS, and RS technologies. Subsequent sections illustrate the use of these tools in ecological and epidemiological studies, and address issues specific to

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area-wide programmes integrating the SIT, particularly with respect to the use of spatial tools in feasibility assessments, planning and implementing pre-intervention surveys, and guiding the subsequent operational programmes of insect suppression and sterile insect release.

## 2. SPATIAL TOOLS: STATE OF THE ART

### *2.1. Geographic Information Systems (GIS)*

GIS can be defined as computer-based systems capable of capturing, cleaning (checking for errors and gaps), integrating, storing, retrieving, analysing, and displaying spatial data. GIS incorporate spatial data (geographical features) in the form of geographical coverages (maps), and descriptive data (attributes) in the form of relational databases linked to the mapped features (Kitron 1998).

GIS coverages can be developed using information from a variety of sources, including digitized paper maps, field surveys using hand-held GPS receivers, and thematic layers derived from remote sensing. Much of the utility of GIS stems from their ability to combine datasets of different provenances, spatial scales, and data types. Most, if not all, of these applications have become freely available in general-purpose computing environments such as R (Bivand et al. 2008).

In most GIS packages, geographic data are represented by vector and raster data models. In the vector model, geographical features are represented by points, or as lines and polygons made up of points joined by lines (arcs). In the raster model, spatial data comprise a regular grid of cells in which points are represented as single cells, and lines as strings of connected cells. Raster data are better suited to storing and modelling variables that vary continuously in space (Bonham-Carter 1994). Topographic data, for example, are commonly stored as raster grids (digital elevation models). Climate data, which vary continuously in space and time, are also commonly stored as rasterized climate "surfaces" (Hutchinson et al. 1995) or raster stacks combining hundreds or thousands of elementary rasters (Anyamba et al. 2014). Most GIS software packages can handle both vector and raster data, which are complementary and frequently used jointly.

GIS can be used for their mapping and visualization capabilities, or for much more sophisticated forms of spatial and statistical analysis. In this context, spatial analysis refers to the manipulation and transformation of GIS data to extract additional meaning from them. Common examples of spatial analysis include buffering map features (e.g. to define areas of exposure (potential infestation) around insect-breeding sites), interpolating between points (e.g. to produce climate "surfaces" from a network of weather stations), overlaying a number of individual geographical coverages to produce derivative maps, or even spatial modelling of suitable landscapes for target insect species, insect distribution or density. The latter approach can often take the form of "suitability analysis", in which spatial coverages are weighted and combined to identify and display locations that meet specific criteria (Clarke et al. 1996). Later in this chapter, an example is described in which suitability analysis has been used for decision support in trypanosomosis control. Several introductory texts provide more detail on the range of spatial analytical techniques

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available in most GIS packages (Bonham-Carter 1994; Burrough and McDonnell 1998).

GIS software and general-purpose computing environments now commonly include a range of geostatistical commands and specialized add-on packages allowing basic as well as sophisticated spatial analyses, including testing for space-time clustering among point and polygon data (Pfeiffer and Hugh-Jones 2002; Bivand et al. 2008). This type of exploratory data analysis is particularly appropriate for identifying unusual spatial patterns within large datasets, and is often used as a means of hypothesis generation.

The accuracy of the final output from a GIS-based analysis is, to a significant degree, determined by the quality of the data in the GIS. The spatial and temporal resolutions of the data used in GIS need to be appropriate for the application in question. For example, topographic maps at a scale of 1:250 000 would be of little use in a village-scale study. Similarly, a series of annual climate surfaces would be ill-suited to attempts to determine the seasonality of insect populations. Hand-held GPS receivers for ground assessment and validation, and satellite remote sensing for updated information on changes in surface conditions, have become easily accessible for pest control programmes.

### *2.2. Global Positioning System (GPS)*

Hand-held GPS receivers are ideally suited to mapping spatial features where conventional maps are unavailable or inadequate (Thomson and Connor 2000). The basis of the GPS is a constellation of 24 NAVSTAR satellites developed and maintained by the US Department of Defense. These satellites act as reference points, with each satellite transmitting a radio signal in the form of pseudo-random code. On the ground, GPS receivers use this code to determine distances to each satellite ("ranging"), and calculate their position and altitude by "trilaterating" signals from a number of satellites. A European alternative named "Galileo" was launched in May 2016 with two new satellites, and will allow much more accurate positioning, but it will not be available before 2020.

GPS receivers typically achieve a horizontal accuracy in the 5–15 m range. Positional errors arise mainly from atmospheric effects on the GPS signals, from clock errors, and as a result of multipath reflection of signals at ground level. Much of the error due to atmospheric effects can be removed using "differential" GPS techniques, in which positions obtained from a roving GPS are corrected using signals received by a static GPS located at an accurately surveyed position. Horizontal accuracy using differential GPS techniques is usually in the 1–5 m range, although sub-metre accuracy can also be achieved depending on the hardware used. However, in most ecological or entomological survey situations, a positional error of 5–15 m is probably acceptable.

GPS receivers are often used simply to collect spatial data (coordinates) for geographical features, with associated attribute data recorded separately and manually on survey forms. However, in many cases, GPS-receiver software now includes programmable "data dictionaries" which can be used to capture attribute information directly. Alternatively, some GPS receivers can be linked up to other devices such as

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a tablet or a notebook computer. Both approaches greatly increase the speed and efficiency with which GPS data can subsequently be incorporated into existing GIS.

It is also possible to upload the results of spatial modelling, like suitable habitats for insects (see below), using a GPS to guide operational teams toward specific sites used for monitoring (Bouyer et al. 2010) or control (Dicko et al. 2014).

### *2.3. Satellite Remote Sensing (RS)*

Satellite RS is the process of gathering information about the earth's surface using electromagnetic sensors on board satellites. Sensors can be "passive", e.g. Spot, Landsat, and Meteosat satellites, in the sense that they detect solar radiation reflected from the earth's surface, or "active", e.g. radar, which provide their own energy source for illumination, and the reflected radiation is measured by the sensor. The latter have rarely been applied in ecological and epidemiological studies. Data from passive sensors can be used in a relatively raw form, e.g. to derive land-cover classification maps, or can be transformed into indices that constitute direct proxies (substitutes) for environmental variables, such as rainfall, land-surface temperature, and vegetation status (Hay et al. 1996).

The value of satellite RS for ecological research has long been recognized, particularly in terms of its ability to offer objective, up-to-date assessments of surface conditions over large, sometimes inaccessible, areas. Moreover, the repeatability of satellite measurements makes RS particularly suitable for monitoring environmental conditions over time. The applicability of remote sensing to different types of ecological study will, however, depend on both the nature of the study and the spatial, temporal, and spectral characteristics of available image data (Box 1).

Images from different sensors vary greatly in terms of spatial resolution, e.g. with pixel sizes for commonly available products currently ranging from under a square metre to several square kilometres. Similarly, the temporal resolution (or revisit time) of individual sensors can be as little as 30 minutes in the case of geostationary meteorological satellites, or as much as 30 days in the case of some polar-orbiting satellites. Near-polar orbit satellites such as Landsat, Spot, or Moderate-resolution Imaging Spectroradiometer (MODIS) offer intermediary temporal resolutions, and are the most frequently used. In a project that requires local, detailed assessments of land cover, spatial resolution will be the prime consideration when selecting satellite data. If the project is more concerned with changing meteorological and vegetation patterns over time, temporal resolution will be of greater concern.

Species distribution modelling is increasingly based on the combination of high spatial and temporal resolution data, particularly MODIS data (Hartemink et al. 2011; Dicko et al. 2014).

In the context of ecological and epidemiological studies, satellite data have been extensively used to model and predict the distributions of insects and/or associated diseases in time and space (Rogers and Randolph 1986; Rogers et al. 1996; Linthicum et al. 1999; Moiroux et al. 2013; Dicko et al. 2015). Modelling on a large scale has commonly involved using satellite data to delimit specific insect-breeding sites or habitats (Linthicum et al. 1987; Pope et al. 1994; Rejmankova et al. 1995). On a national or regional scale, these distributions are more commonly modelled on the

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basis of proxies for meteorological variables and/or vegetation status (Linthicum et al. 1987, 1990; Rogers et al. 1996; Hay et al. 1998; Brooker and Michael 2000; Randolph 2000). Several general reviews, covering these and other studies, are available (Thomson and Connor 2000; Hay et al. 2006).

*Box 1. Resolution in Satellite Remote Sensing*

*Spatial Resolution (Fig. 1)*

The spatial resolution (pixel size) of various sensors varies enormously: 0.61–2.4 m for QuickBird, 5–10 m for SPOT 5, 15–90 m for ASTER, 15–60 m for Landsat, 250–2000 m for MODIS. The width (swath) of images varies accordingly, e.g. about 25 km for QuickBird, 185 km for Landsat. Polar-orbiting meteorological satellites have relatively low spatial resolutions and large swath widths (1.1 km and about 2400 km, respectively, for AVHRR), while images from Meteosat and other geostationary meteorological satellites have 1–8 km pixels, but comprise an entire earth half-disk.

*Temporal Resolution*

Temporal resolution is defined by the time taken for a satellite to revisit the same point in its orbit (repeat time). Sensors with high spatial resolutions tend to have low orbits and long repeat times, e.g. 16 and 26 days in the case of Landsat and SPOT satellites, respectively. Since over a year's period some satellites sense only a few images for a given locality, obtaining cloud-free data can be problematic. At the other extreme, meteorological satellites have very short repeat times (12 hours for AVHRR, 30 minutes for Meteosat), and obtaining cloud-free data is rarely a problem.

*Spectral Resolution*

Passive sensors detect radiation from the sun that has been reflected by the earth's surface (as well as, in some cases, radiation emitted directly from earth). The amount of reflected radiation depends on the nature of the surface and on the wavelength of the radiation concerned. For example, vegetation reflects most of the radiation it receives in the green (visible) part of the electromagnetic spectrum, but absorbs much of infrared energy. Dry soil, on the other hand, absorbs large amounts of visible light, but reflects a large proportion of near infrared. The ability to use these "spatial signatures" to infer surface properties depends on the spectral resolution of the remote-sensing data being used. Spectral resolution refers to the number, width and spacing of the spectral "bands" used by the sensor. Traditionally, most sensors have included three to seven bands in the visible and near-to-thermal infrared part of the electromagnetic spectrum (0.3–14-mm wavelengths). However some new sensors, e.g. MODIS and ASTER, have many more, and this improved spectral resolution should increase the ability to distinguish between different land-cover types.

Long-standing sensors, such as the National Oceanographic and Atmospheric Administration's Advanced Very-High-Resolution Radiometer (AVHRR), or the MODIS on NASA's Terra satellite, are helping to bridge existing gaps in data availability by providing data at both moderate spatial and temporal resolutions (250–2000 m, and daily, respectively, in the case of MODIS). Another Terra sensor, ASTER, provides imagery with a spatial resolution similar to Landsat and SPOT data (15–30 m in visible and near-infrared bands), but with a vastly superior spectral resolution. Commercial satellites now also provide relatively low-cost data at very high spatial resolutions. For example, data from Digital Globe's QuickBird sensor has a spatial resolution of 0.61 m in panchromatic mode and 2.4 m in multispectral mode. SPOT images offer a good compromise between spatial resolution and coverage. Pléiades 1A (launched in 2011), Pléiades 1B (launched in 2012), and SPOT 7 satellites (launched in 2014) deliver images at very high spatial resolution (0.5 cm and 1.5 m).

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*Figure 1. The significance of spatial resolution in remote sensing. These images represent false-colour composites of data from three satellite-borne sensors: (Left) ASTER (bands 2, 3, 1) with a 15-m spatial resolution (pixel size); (Centre) Landsat ETM+ (bands 2, 4, 3) with a 30-m spatial resolution; and (Right) MODIS (MOD09A1 8-day surface reflectance composite, bands 1, 4, 3) with a 500-m spatial resolution. The images represent an area of approximately 7 x 8.25 km, and cover a portion of the Nile Valley in the vicinity of Merowe, northern Sudan. Field boundaries and roads are clearly visible in the ASTER data, but are less apparent in the Landsat data. At a resolution of 500 m, the spectral properties of the Nile channel and surrounding vegetation are not visually distinct. (Note that data from meteorological satellites (AVHRR and Meteosat) are commonly available at a resolution of 8 km.)*

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By improving the accuracy and coverage, taking into consideration temporal and geographic requirements, current satellite imagery is ideally suited for disease/vector control programmes at regional, national or smaller scale. Thematic layers like hydrographic networks, human or animal densities, tree cover, and vegetation classification are available on the web and can be used for operational programmes (Cecchi and Mattioli 2009; Stevens et al. 2015; Nicolas et al. 2016). However, it is crucial to validate the various layers in the field depending on its intended specific use, particularly in the case of vegetation layers that should be validated depending on the ecology of the target insect species (Guerrini et al. 2008, 2009).

## 3. APPLICATION AREAS FOR GIS, GPS, AND RS IN OPERATIONAL PROGRAMMES

There is enormous potential to use powerful analytical frameworks in spatial decision-support systems in AW-IPM programmes, which can take decision-makers beyond the point of simply possessing data, information, and knowledge.

Individually, GIS, GPS, and RS potentially have several important roles to play at various stages of project planning and implementation. The following sections illustrate how these technologies can be used to prioritize areas for control operations or to optimize these operations. Several key individual stages of project planning and implementation are addressed, from the design of pre-intervention surveys to monitoring and analysing data from insect release programmes.

### *3.1. Planning and Implementing Pre-Intervention (Insect, Disease, Host) Surveys — GIS-Based Modelling of Spatial Distribution of Target Insects*

Insect pest control programmes, integrating a combination of suppression techniques, require accurate, up-to-date information on the spatial and temporal distribution of the target insect population. A spatially-explicit analysis can bring together a wide range of information sources — e.g. climate data, remote-sensing data, land-use and topographic data, historical data on insect distribution and abundance, disease prevalence, etc. — that together can be used to develop modelled or empirical estimates of the temporal and spatial distributions of the pest or disease. The nature of this spatial analysis, and the data sources used for it, will reflect the stage to which pre-intervention planning has developed. At the very early stages of feasibility assessment and planning, for example, spatial modelling will focus on identifying areas of relatively high pest density or areas where intervention programmes have potentially high benefit-cost ratios (Mumford, this volume) at the national or regional level, using low spatial resolution data for climate and medium or high spatial resolution data for land cover in combination with available historical information on the insects and/or diseases. These maps may be adequate for planning purposes in cases where insect intervention programmes are implemented at the national or regional scale. In other cases, it may be more appropriate to use these broad assessments for directing more detailed modelling efforts, using higher-resolution geographic datasets to focus sampling of insects to specific areas of interest.

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*3.1.1. Mapping Pest Distribution on a Regional Scale*

Many published research studies have used GIS and RS to predict the distribution of insects on national to global scales (Hay et al. 2006; Rogers 2006). The discussion here is limited to work most pertinent to using the SIT in the context of AW-IPM programmes, most of which has focused on the spatial prediction of tsetse flies *Glossina* spp.

The use of low-spatial-resolution satellite data to predict insect distributions dates back to attempts in the early 1990s to correlate the distribution of tsetse and the incidence of trypanosomosis to spatial variations in climate and the normalized difference vegetation index (NDVI) (Rogers 1991; Rogers and Randolph 1991; Rogers and Williams 1993). Later models also incorporated surrogates of land-surface temperature from AVHRR satellite data, and a proxy variable for rainfall (cold-cloud duration) from Meteosat data. Rogers et al. (1996), for example, used Fourier-processed satellite data for climate and NDVI in combination with digital elevation data to predict the presence/absence of eight tsetse species in Côte d'Ivoire and Burkina Faso, with an accuracy of 67–100%.

A similar approach, using logistic regression, has also been used to model ranges of tsetse species in East Africa. The modelling process relies on the logistic regression of fly presence against a wide range of predictor variables for a large number of regularly spaced sample points for each area. The predictor variables include remotely sensed (satellite image) surrogates of climate — vegetation, temperature, and moisture, which have been subjected to Fourier processing to provide an additional set of season- and timing-related measures for each parameter. Demographic, topographic, and agro-ecological predictors are also used. These models are then applied to the predictor imagery to produce predicted probabilities of fly distributions at 1-km resolution.

In southern Africa, Robinson et al. (1997) used climate surfaces, together with NDVI and elevation, to model the distributions of three tsetse species in the common fly belt. Maximum-likelihood classification techniques yielded overall correct predictions of 92.8 and 85.1% for *Glossina morsitans centralis* Machado and *Glossina morsitans morsitans* Westwood, respectively. In Togo, Hendrickx et al. (2001) found that discriminant models, based on satellite data, were generally less successful at predicting disease outcomes in cattle (trypanosomosis prevalence or packed-cell volume) than tsetse abundance.

More recently, MaxEnt models have been used extensively to predict tsetse suitable habitats in southern (Matawa et al. 2013) and western Africa (Bouyer et al. 2015; Dicko et al. 2015). MaxEnt, one of the most widely used species distribution models, is a machine-learning method based on the information theory concept of maximum entropy (Elith et al. 2011). MaxEnt fits a species distribution by contrasting the environmental conditions where the species is present to the global environment characterized by some generated pseudo-absence data, also called the background. The logistic output gives us a quantitative indicator of the habitat preferences of the species in the study area (Fig. 2). In West Africa it was used, together with a friction model to predict the resistance of landscape to tsetse dispersal (Krafsur and Ouma, this volume), to identify isolated populations of *Glossina palpalis gambiensis* Vanderplank that could be potential targets for eradication.

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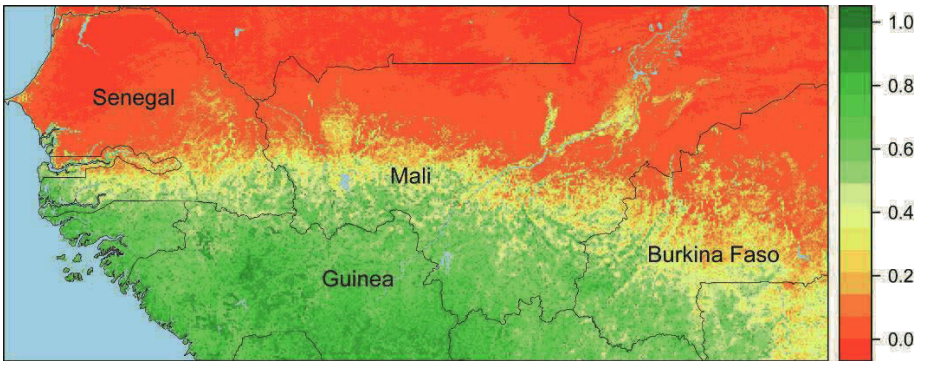



*Figure 2. Distribution of* G. p. gambiensis *in West Africa. Mean habitat suitability index predicted by a MaxEnt model. The index varies between 0 (less suitable, red scale) and 1 (highly suitable, green scale). (Modified from Bouyer et al. 2015, reproduced with permission.)*

High predictive accuracy in prediction models requires recent and well-distributed insect data to train and validate the prediction process, and assess the accuracy of the model output. Models based on entomological data that were collected several decades ago may be used for regional prioritization of tsetse and trypanosomosis interventions but, because of their poor sensitivity and specificity, should be avoided for guiding control programmes (Guerrini et al. 2009).

Data on insect abundance is even more sensitive because the efficiency of traps depends largely on environmental conditions, period of the year, experience of the entomological team, etc. At the continental or regional scale, it could be argued that the relative risk of insect presence is an adequate indicator of abundance. However, this assumption is not valid for smaller areas, where models require up-to-date data from abundance surveys. It is probable that, in these situations, the principal role of RS and GIS is to make prospective surveys more cost-effective (Hendrickx et al. 1999). In Burkina Faso a large grid-based baseline data survey, implemented in preparation for a control programme (L. Percoma, personal communication), deployed 3189 traps in an area of 40 000 km$^2$, and the data were used to develop a spatio-temporal model of African Animal Trypanosomosis (AAT) risk that was validated using parasitological data on cattle (Dicko et al. 2015; Feldmann et al., this volume). The model included the prediction of tsetse densities and their infection rate, and hence such predictions can be very useful to focus the control effort on disease transmission "hot spots".

### *3.1.2. GIS for Decision Support*

Mapping pest distribution on a regional or national scale is an important first step in assessing the feasibility and spatial targeting of the SIT and other area-wide control actions. However, technical and resource constraints may make large-scale operations, over the whole of the identified area of potential pest distribution, to be impractical or uneconomic. Therefore, specific environmental information is required,

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either to identify parameters that are linked to pest presence, or to guide future sampling efforts to address specific questions including levels of genetic variability, similarity, and diversity among target insects.

Recently, the economic benefits to livestock keepers from intervening against AAT were mapped in eastern Africa (Shaw et al. 2014). The study was conducted for six tsetse-infested countries in eastern Africa: Ethiopia, Kenya, Somalia, South Sudan, Sudan, and Uganda. Cattle production systems were mapped, and herd models for each production system were developed, in the presence or absence of AAT. The differences in income between these two scenarios enabled the mapping of the maximum potential benefits that could be obtained from tsetse and trypanosomosis control. The potential benefits ranged from USD 500 per square kilometre to more than USD 10 000. Such models could be used to prioritize tsetse and trypanosomosis interventions on a regional scale when used in combination with other data layers, e.g. tsetse distribution and density. The models also accounted for possible restocking of livestock in neighbouring areas when maximum stocking rates were exceeded.

### *3.1.3. Mapping Pest Density with a Large Spatial Resolution*

The spatial distribution of most insect pests is not uniform but patchy, and there can be localized pockets of high insect density, in spite of a potentially misleading low-overall population density. It is of prime importance, for the development and implementation of an insect control programme, that these pockets or "hot spots" be located (Shiga 1991).

Depending on the spatial scale of the heterogeneity of insect density, the climate and remote-sensing datasets used to predict insect distribution over wide areas may not be appropriate for work on larger scales. AVHRR data, for example, have a native resolution of 1.1 km, but are commonly resampled to derive images with 4×4 or 8×8 km pixels. However, several studies have successfully used high-resolution spatial data from Landsat and SPOT satellites to identify habitats associated with high insect density (Rejmankova et al. 1995; Roberts et al. 1996). For tsetse flies, fine-tuned studies of their habitat (type of vegetation and fragmentation level) at a high resolution (Landsat ETM+ data with a 30-m resolution) successfully predicted their densities at a local scale in Zambia and Burkina Faso (Guerrini et al. 2008; Ducheyne et al. 2009). More recently, MODIS data (1-km resolution) were used to upscale these studies to a national level in Burkina Faso (Dicko et al. 2015).

Since the spatial distributions of insect populations are not constant, but tend to change over time, it is important that, where possible, risk maps be dynamic rather than static. For example, populations of riverine tsetse flies in West Africa commonly expand and contract seasonally along the river vegetation and perpendicular to the tributaries. The use of spatial analysis incorporating multi-temporal remote-sensing data enabled generating a "dynamic population distribution model" to predict these temporal and spatial population dynamics, and to link spatial patterns with heterogeneity of habitat (Dicko et al. 2015). This allows a more efficient and guided (rather than ad hoc) deployment of sampling devices during subsequent surveys, i.e. the sampling devices can be deployed in those areas where there is a high probability of trapping or, alternatively, in areas of low probability to confirm the model). The implication is that, assuming an adequate geo-spatial model exists, an efficient survey

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strategy can be developed largely from the office, and detailed implementation guidelines, regarding where, how, and when to deploy the sampling devices, can be elaborated for the field teams. This would not only ensure adequate sampling coverage in all ecosystems, but also prevent the deployment of too many sampling devices in unproductive or unrepresentative sites, unnecessarily increasing project costs (Bouyer et al. 2010).

The availability of temporal and spatial distribution models of the target insect populations on a large spatial scale has implications beyond the design of efficient sampling frames. In particular, such models can facilitate a more-efficient deployment of suppression tools and also a better-targeted release of sterile insects (Dicko et al. 2014). This would also enable simulating combinations of strategies like successive or simultaneous use of insecticide targets and the SIT to identify the most effective strategy (S. L. Peck, personal communication). This increased efficiency should also translate into considerable economic savings in terms of logistics, personnel and sterile insects.

### *3.2. Development and Implementation of Appropriate Insect Suppression and Sterile Male Release Programmes*

#### *3.2.1. Selection of Appropriate Suppression Methods*

Since the release of sterile insects is only efficient when they sufficiently outnumber the native insects, the SIT is more cost-efficient with a reduced density of the target population (Dame 1971). The density of untreated insect populations is usually too high, and needs to be reduced prior to the mass-release of sterile insects. Depending on the target insect, a variety of pre-release population suppression methods are usually available (Mangan and Bouyer, this volume), but their usefulness, appropriateness, and effectiveness will be determined by the characteristics of each target area or local situation. Pending the availability of suitable data layers (demography, land use/land cover, vegetation classification, distribution of target insect, etc.), spatial analysis can assist with the decision to select the most appropriate suppression method for a given target zone. This is demonstrated by the following examples for the suppression of tsetse fly populations in AW-IPM programmes:

- *Sequential Aerosol Technique (SAT).* The SAT involves the application of non-residual ultra-low-volume insecticides by fixed-wing aircraft or helicopter (or from vehicles using hot and cold fogging). The goal is to kill adult tsetse flies in the first spraying cycle by direct contact with insecticide droplets, and then kill emerging flies in five subsequent application cycles before the emerged flies can deposit larvae (Allsopp 1984). In view of the sensitivity and susceptibility of the SAT technique to topography, wind velocity and direction, temperature inversion, etc., a spatial analysis can provide information on the suitability of the target zone in terms of: (1) topography (the application of the SAT becomes problematic when the terrain becomes mountainous), (2) habitat and vegetation cover (correlation analysis between the vegetation density and the propensity of insecticide droplets to penetrate the tree canopy, to make predictions on the vertical dispersal rate of insecticide droplets in various vegetation types), and (3)

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wind velocity (using climatic models to predict the dispersal and distribution patterns of insecticide droplets in each particular situation). In the eradication programme against *Glossina morsitans centralis* in Botswana (Kgori et al. 2006), GIS not only enabled the close alignment of flight swaths to assure full coverage of the target population in the spraying areas, but were also used to develop the monitoring system that demonstrated the successful outcome of the project.

- *Live-Bait Technology.* In this technique, residual insecticides are applied to host animals that attract tsetse flies, which are killed on contact with the insecticide (Bauer et al. 1992). The technique is efficient and cost-effective, provided the density of the livestock population in the target area (if livestock is the main host) is sufficiently high. The distribution of animal herds is generally heterogeneous in space, and GIS can help identify areas where additional control tactics such as targets and traps are necessary to control the target population (Kagbadouno et al. 2011; Adam et al. 2013).
- *Stationary-Bait Technology (insecticide-impregnated targets and traps).* Tsetse populations can also be suppressed by deploying artificial stationary devices, which attract tsetse flies (Green 1994). The flies will be killed either upon making contact with the surface area of the target/trap (which is impregnated with a residual insecticide) (Laveissière et al. 1980) or when retained in a no-escape device (Dransfield et al. 1990). A spatial correlation analysis, using demographic data layers and tsetse population distribution models, can indicate how efficient or suitable this technology is in specific areas.

### *3.2.2. Models to Predict Outcome of Different Suppression Scenarios*

Each area targeted for control will in most cases be heterogeneous, in terms of composition of the habitat (land use and land cover), vegetation cover and species composition, host (e.g. livestock and agricultural crops) distribution, demography, topography, etc., which will demand an integration of different suppression methods (Vreysen 2001; Mangan and Bouyer, this volume). Spatial analysis can be used to model the effect of various combinations of methods on the insect population, and assist in the decision to select the best combination (Kagbadouno et al. 2011; Adam et al. 2013).

### *3.2.3. Implementation of Suppression and Sterile Male Release Programmes*

Aside from providing decision-support in the development and implementation of surveys, optimizing the suppression phase, and modelling the outcome of various suppression scenarios, GIS tools can assist in the actual implementation of suppression programmes, including sterile insect releases. A geo-spatial model, e.g. developed in the programme against *G. p. gambiensis* in Senegal to guide the distribution and density surveys, can be exploited to inform decisions on how a suppression strategy can be implemented. For example, spatial analysis can provide decision-support to select appropriate sites to deploy insecticide-impregnated traps and targets in tsetse suppression programmes, and ensure that the required target/trap density per surface unit is achieved with respect to vegetation type, topography, insect distribution, and other relevant factors (Fig. 3 and Box 2).

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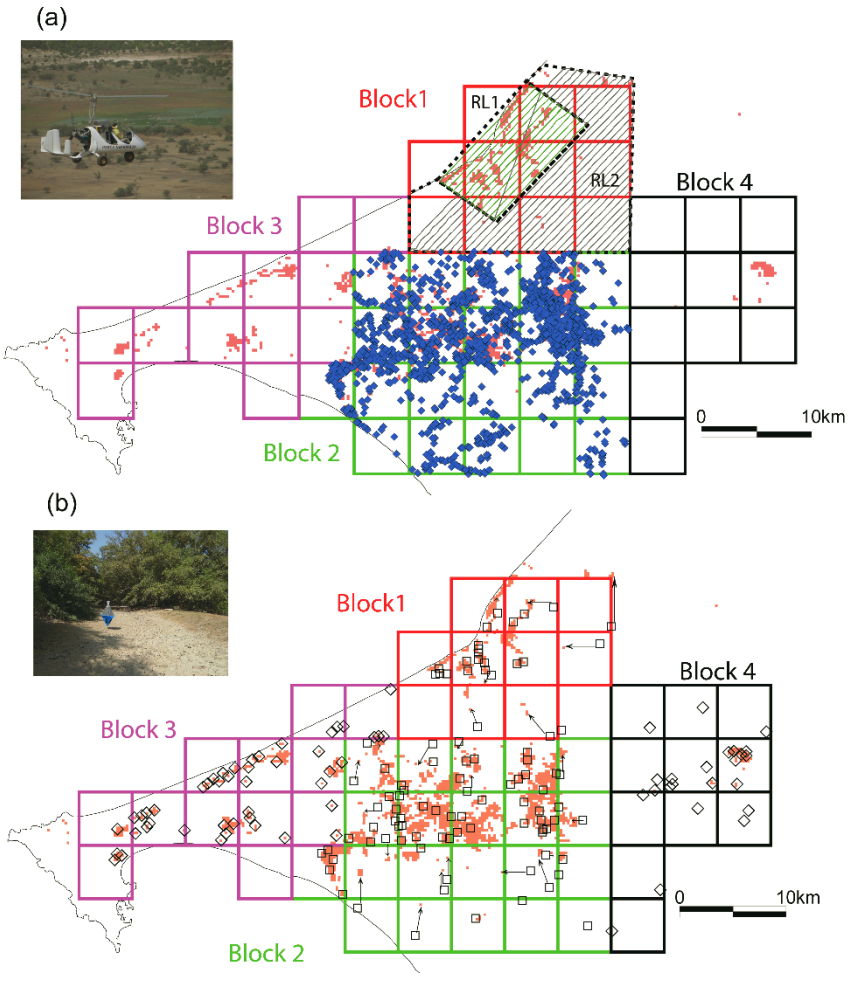



*Figure 3. GIS and spatial modelling to optimize the tsetse eradication project in the Niayes region of Senegal. (a) Optimization of the integrated control strategy using model predictions. A MaxEnt model predicted suitable habitats for G. p. gambiensis (sensitivity 0.96, specificity 0.57). Block 1 - suitable habitats delimited two polygons for aerial releases (RL1 and RL2) where the minimum numbers of sterile males released per km² were 24 and 11, respectively. Chilled adult tsetse flies were released with a Mubarqui smart release machine in a gyrocopter (upper left) from the Kalahari aerodrome. Block 1 - green and grey lines show the track flying records on 14.04.2014 in RL1 and 11.04.2014 in RL2, respectively. Block 2 – for population suppression 1347 insecticide-impregnated traps were set from December 2012 to February 2013 in the predicted suitable sites (blue lozenges). (b) Optimization of the monitoring system (biconical traps, upper left) using MaxEnt model predictions. Blocks 1 and 2 - 23% of the traps (black squares) deployed in the closest suitable patch. Blocks 3 and 4 (monitoring not yet started) - theoretical positions of the traps (black lozenges) suited to model predictions. Black arrows show displacements of traps following model predictions. (Modified from Dicko et al. 2014, reproduced with permission.)*

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*Box 2. Past and Current Applications of GIS in Area-Wide Integrated Pest Management Programmes that Include the SIT*

The GIS/RS, as a decision-support tool in AW-IPM programmes integrating the release of sterile insects, has been used to spatially display data, and to plan, implement and evaluate programmes. AW-IPM programmes using the SIT against fruit flies are among the most successful programmes against major insect pests, and these programmes rely on the advanced use of GIS. In programmes against the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), such as the programme in Argentina, the "Moscamed" programme in Guatemala/Mexico, and the prevention programmes in California and Florida (Enkerlin, this volume), GIS/GPS/RS are mainly used to:

- Provide navigational guidance in the release of the sterile insects, and to provide "real-time tracking" using commercially available satellite navigation/flight recorders.
- Map and visually display the various trapping sites and monitoring routes.
- Select trapping sites, using a grid layer over topographical maps or satellite imagery, and associating the trapping site with host and topographical features and their attributes.

In addition, the "Moscamed" programme in Guatemala/Mexico is using GIS as a major component of various studies on: (1) fly performance in relation to altitude, wind velocity, habitat, etc., (2) "hot-spot" areas (where the pest persists over time despite intense efforts to suppress it), (3) insect behaviour in relation to the timing of release (afternoon, night, or morning), and (4) changes in the dispersal behaviour of sterile insects over time (P. Gomes, personal communication).

GIS/RS were also used in AW-IPM programmes against the New World screwworm *Cochliomyia hominivorax* (Coquerel). In Panama, GIS/RS were used to identify different vegetation types, and correlate the spatial and temporal distribution of screwworms with the various vegetation covers in a tropical environment. After classifying the forests based on tree height, structure, and species composition, the highest screwworm population density was found during the transition period from the wet to the dry season, and in forest habitats as opposed to open areas (Phillips et al. 2004). These data were used to more efficiently deploy monitoring tools in habitats favoured by screwworms, which could lead to earlier detection of low densities of screwworm populations, or possibly earlier control of outbreaks. The study clearly showed that GIS/RS can be used to improve trap placement by identifying areas of high screwworm activity. The method was applied in early 2003 to develop a trapping strategy after the accidental outbreak of the screwworm at the screwworm facility in Chiapas, Mexico (Phillips et al. 2004). Using GIS/RS techniques, optimal trapping sites were selected, which represented a reduction of 79% of the original trapping sites, i.e. considerable savings in terms of personnel and logistics.

A recent example of using GIS and spatial modelling for planning, implementing and evaluating an AW-IPM program with an SIT component is the tsetse eradication programme in Senegal. In this programme suitable tsetse habitat was identified using a supervised classification during the feasibility phase, which reduced by 94% the required sample area to identify the distribution limits of target populations (Bouyer et al. 2010). Thereafter, these technologies were used during the pre-operational phase to assess the survival, mortality, and competitiveness of sterile males during pilot release trials. Finally, the method to identify suitable habitats for *G. p. gambiensis* was optimized and improved by RS techniques that were based on a MaxEnt population distribution model (Dicko et al. 2014). Using this model the number of traps per km² needed to suppress tsetse populations was reduced by >95%. The model was also used to determine the densities of sterile males to be released in the target area, i.e. 10 sterile males per km² if the habitat was unsuitable, 100 sterile males per km² if it was suitable. The releases were done using an automatic chilled-adult release device that was parameterized with the GIS during the flights. Finally, it improved the sensibility of the permanent monitoring system by locating the fixed monitoring sites in the most suitable places (Fig. 3).

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The uniform application of certain suppression measures (e.g. bait sprays for fruit flies, SAT for tsetse flies) over a heterogeneous pest distribution target area can have negative implications, both in terms of cost and environmental impact, since habitats may be contaminated by unnecessary applications of insecticide (Papadopoulos et al. 2003). Navigation/recording systems (such as Trimble's TrimFlight 3 Ag-GPS system or SATLOCK's AirStar system), which guarantee the correct application of the insecticides during aerial application, and ensure that fuel and materials are used efficiently, have been used for years in several fruit fly AW-IPM programmes that release sterile flies. Using these systems facilitates precise guidance, automated recording of covered areas (maximum efficiency, minimum overlap, and skips (omission of areas), identification and remedy of skips before leaving the operational area (avoiding costly call-backs), waypoint navigation, mapping capabilities, and control of insecticide-flow and sterile insect release rates (depending on pest distribution and density) based on ground speed. This eliminates the need for time-consuming and costly ground markers, such as beacons or flaggers. The system is compatible with a wide range of GIS software packages, and enables applications to have better than 1-m accuracy.

In many programmes that include the release of sterile insects, these are released at a constant dispersal rate (i.e. a uniform density per surface unit). As a consequence, insufficient overflooding ratios might result in areas of high native-insect population density, whereas more sterile insects than necessary might be released in areas of low native-insect population density (Vreysen, this volume). GIS and spatial analysis can provide guidance on spatial sterile insect requirements and dispersal patterns, in relation to wild insect population densities, habitat, elevation, etc. in a dynamic way (i.e. data layers on the target insect distribution are updated regularly or spatio-temporal models of insect densities are used (Dicko et al. 2015)). This will result in a much more efficient use of sterile insects. In addition, commercially available satellite navigation/flight recorder systems, e.g. Ag-Nav, provide real-time tracking, and can visually display the areas that were treated with sterile insects (or have been left out), and at which altitude insects were released (to ensure a proper spread). This permits proper feedback to programme managers (Dowell et al., this volume).

An example of a new automatic release system is the Macx System (Mubarqui group); it has been used routinely in fruit fly control programmes. It is linked to GIS software installed on tablets on board the aircraft. These systems ensure an efficient use of the sterile insects available as they automatically start and stop the releases of sterile males when entering or leaving the release areas (Fig. 4a), but also because they can adapt the sterile male release density to that of certain vegetation types, topography or density of the wild populations (Mubarqui et al. 2014). (For example, in Valencia, Spain, the rate of releasing sterile Mediterranean fruit flies is automatically adjusted according to the ripening period of citrus varieties in the target area (R. Argilés Herrero, personal communication)). The software provides automatic reports of the flights by uploading the data to an online relational database once the aircraft is back at the airport, or even in real time using a Global System for Mobile Communications (GSM) connection.

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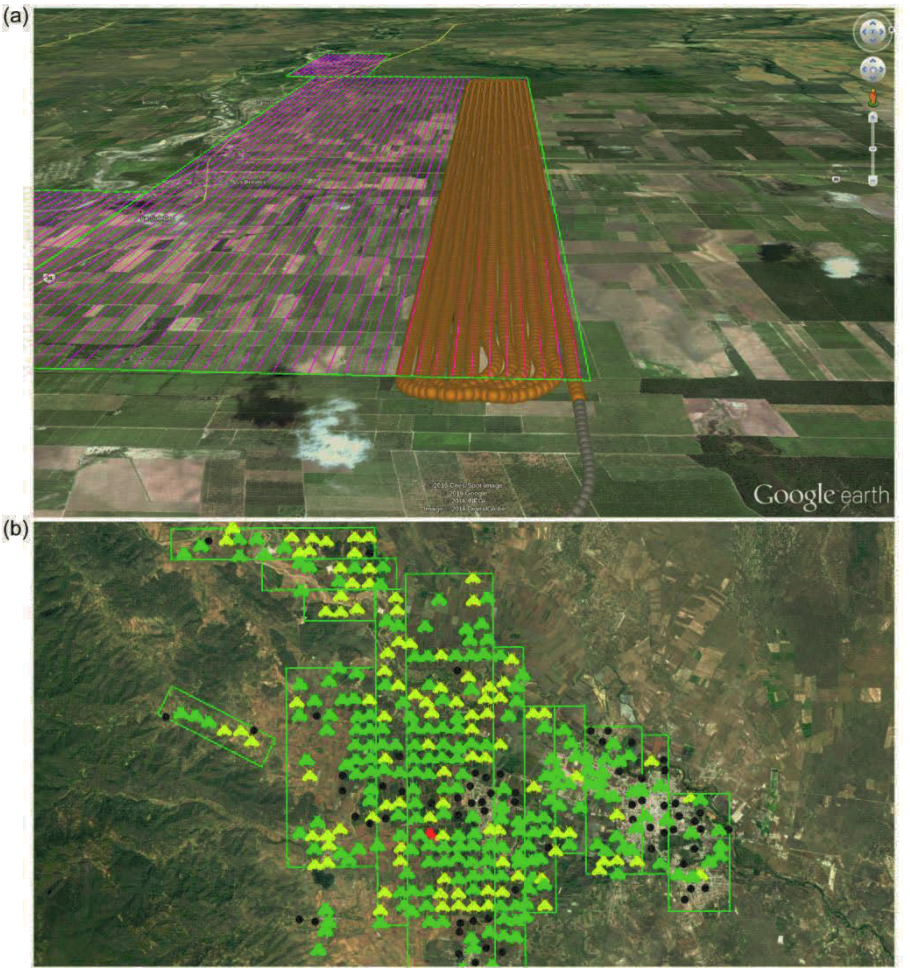



*Figure 4. Optimization of area-wide fruit fly control in Mexico using the Macx System. (a) Parametrization of the sterile male releases of Anastrepha ludens (Loew) near Padilla, Tamps, on 10 July 2016. Release polygons are shown as green rectangles, theoretical release lines as pink lines, and tracks of the actual flying/release lines as orange-coloured spheres. (b) Monitoring results of A. ludens on week 28 of 2016 near Rio Verde, San Luis Potosí. Release polygons are shown as green rectangles, traps with catches of only sterile males as symbolic green flies, traps with catches of mixed sterile and wild males as yellow flies, traps with catches of only wild males as red flies, and traps without captures as black circles. (Reproduced with the permission of the Mubarqui group.)*

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### *3.3. Monitoring Suppression or Release Programmes*

Monitoring is an essential component of any AW-IPM programme (Vreysen, this volume). However, monitoring is time-consuming, and requires considerable funds for materials, logistics, and personnel. A careful balance must be found between "cost-efficiency" and collecting "reliable data". In most AW-IPM programmes, in view of the size of the target areas, monitoring (direct and indirect) must be restricted to carefully selected representative sites. Spatial analysis can assist in identifying and selecting appropriate reference or fixed monitoring sites (FMS). The concept of FMS was developed and used during the monitoring activities of the AW-IPM programme in Zanzibar, Tanzania, against the tsetse fly *Glossina austeni* Newstead, albeit without any GIS support (Vreysen et al. 2000; Vreysen, this volume), and was also used in the eradication programme of *G. p. gambiensis* in Senegal (Box 2).

From a pragmatic standpoint, it is important that all avenues of increasing the efficiency of trap-based monitoring systems are explored. Technologies that assist in the rapid transfer of data from the field to GIS are a key element of such systems. For example, in the codling moth AW-IPM programme in British Columbia, Canada, a GIS-based system is used to monitor a network of georeferenced traps. A bar-coding system is employed, in which the time and date that traps are monitored is automatically recorded, along with data on trap catch entered manually during a trap check (Dyck et al. 1993). These data can then be uploaded to the main project database via modem, facilitating the rapid output of electronic maps showing trap data.

Area-wide pest control activities can also use geostatistical analysis routines to get "better value" out of available trap data. A range of spatial analysis techniques, employing both geostatistics and GIS, may be valuable for analysing insect population processes at a landscape scale (Liebhold et al. 1993). Of these, one possible analytical technique is "kriging" — an interpolation procedure that relies on an autocorrelation function (the variogram) to provide weighting of nearby points used in the estimates. Kriging is ideally suited to the analysis of trap data, with interpolated output taking the form of contour maps or density surfaces of insect densities.

Such an approach has been used to monitor and predict populations of the spruce budworm *Choristoneura fumiferana* (Clemens), an important defoliator of trees in boreal forests in North America. Using kriging as a basis, Lyons et al. (2002) developed a set of software tools to produce interpolated estimates, using data from a pheromone-trapping network covering much of Canada and north-eastern USA. Output from the software system can be reclassified in a variety of ways, using GIS to provide maps that address particular management concerns. For example, maps are routinely produced that display areas where moth densities exceed a critical threshold, and hence where conventional larval sampling activities need to be initiated. Change-analysis approaches are also used to create difference maps for consecutive years which, when reclassified, highlight areas of increasing, decreasing and stable moth populations. Kriged map surfaces have also been used as variables which, when combined with historical data on defoliation and defoliation frequency, have been used to successfully predict levels of defoliation in the following year.

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In the wider context of commercial crop decision-making, this type of approach has been taken a step further. In several states in the USA, integrated systems of insect and weather monitoring have been developed, providing estimates of insect and disease risk in near real-time. These systems, described by Thomas et al. (2002), use a network of georeferenced automated weather stations, which utilize radio telemetry to send data — on temperature, relative humidity, precipitation, and other meteorological parameters — every 15 minutes to a central processing centre. These data form the basis of Internet-based, daily estimates of weather and disease-risk related parameters (including insect degree-day maps), in addition to risk maps for specific diseases such as powdery mildew and *Botrytis* spp.

Another example of the use of GIS for monitoring is the Moscamed Programme in Mexico. This successful containment programme includes a trapping network of 24 760 georeferenced traps (14 710 in Chiapas and 10 050 in the rest of the Mexican States) (Enkerlin et al. 2015). Data of adult captures, including sterile and wild flies, enable the assessment of relative population density and ratio of sterile to wild males in space and time (Fig. 4b). A new system called "sabueso" has recently been developed by the Mubarqui group in Mexico, which represents a relevant advance in technology for capturing the information from the field in real-time from the trapping network. The system is installed in low-cost smartphones, and allows the monitoring teams who are seeking the larvae and outbreaks to report their observations in real-time to the team in charge of mechanical control. The same information is available in maps similar to Fig. 4b, enabling easy queries and analyses by management teams while optimizing economical and human resources.

### *3.4. Data Analysis*

Area-wide insect pest control programmes generate a large amount of entomological and other related data, not only during baseline data collection and feasibility surveys but also during the monitoring of suppression and release activities (Dyck, Reyes Flores et al., this volume; Vreysen, this volume). It is a real challenge to manage data efficiently, analyse and interpret the results in a timely manner, and provide programme managers with consolidated data in a suitable format. The GIS unit of any pest control programme, if linked to a relational database, provides an ideal medium for the storage and analysis of data, and it can greatly facilitate their interpretation. Adequate feedback to programme managers is a prerequisite for sound decision-making. The complexity and diversity of data accumulated during a control programme, requiring proper spatial analysis, are typified in the following examples:

- *Data derived from initial surveys.*
  Temporal and spatial fluctuations in:
  - Population distribution (immature and adult stages)
  - Population density
  - Population structure (composition -- immature and adult stages)
  - Disease prevalence or infestation level in hosts.
- *Data derived from monitoring suppression activities.*
  - Temporal and spatial changes in population distribution due to the application of the suppression methods

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  - Temporal decline in the density of the target pest population in relation to different ecosystems
  - Temporal changes in population structure (due to increase in mortality rates) in relation to different ecosystems.
- *Data derived from monitoring sterile insect releases.*
  - Spatial and temporal fluctuations in the ratio of sterile to wild insects
  - Spatial and temporal fluctuations in the rate of induced sterility in the native insect population.

  Spatial correlation analysis using these variables will contribute to modelling the competitiveness of released insects in relation to habitat, host abundance/distribution, climatic variables, etc. Obtaining spatial and temporal data on sterile insect competitiveness is one of the most important features of any AW-IPM programme that releases sterile insects.
  - Spatial and temporal fluctuations in the recapture rate of insects in traps, damage to hosts, disease patterns, etc.

  These data can be correlated with the release rate of sterile insects over the release grids, and then models developed on the mobility, dispersal characteristics (Vreysen et al. 2013), and spatial occupation of the sterile insects in different vegetation types, etc. It can also be used to assess whether sterile males are aggregating in the same ecological niches as wild males -- to ensure appropriate sterile to wild male ratios throughout the control area (Vreysen et al. 2011).

The results of a spatial analysis of a suppression and release programme can provide answers on issues such as the adequacy of deployment of suppression devices (sufficient number, adequacy of spread, efficiency, level of damage, timeliness of replacement of the suppression methods, etc.), and the appropriateness of sterile insect release operations (e.g. coverage of the proper block, flight at the proper altitude, possibility that insects were blown into nearby bodies of water, influence of wind, delivery of sterile insects so that they find and concentrate at the same locations as wild insects, etc.). They can also be used to implement spatio-temporal models that enable the testing of various control scenarios and then using the most appropriate one (S. L. Peck, personal communication).

### *3.5. Support for Sterile Insect Quality Control*

Frequent monitoring of the performance of sterile insects (i.e. insect quality) in the field is an important, although often neglected, component of AW-IPM programmes that integrate the SIT (FAO/IAEA/USDA 2019). Parameters such as survival, mobility, dispersal characteristics, and spatial occupation of the habitat significantly influence the field performance or the competitiveness of released insects (Lance and McInnis, this volume; Parker, Vreysen et al., this volume; Vreysen, this volume). The values of these parameters may change over time, and are affected by host availability, vegetation cover, vegetation species, altitude, etc. GIS and spatial modelling can be used to predict these temporal and spatial changes in insect quality, and thus assist in developing remedies and timely adjustments in an intervention programme. As an example, swaths of flight release lanes are inherently linked to the dispersal capacity and mobility of the released insects, whereas their average survival

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determines the release frequency (Hendrichs, Vreysen et al., this volume). Models can be developed to correlate sterile insect survival with host availability, vegetation cover, etc. to assist in regularly adjusting the dispersal rate of insects in relation to space (e.g. more frequent releases in those areas where survival is low). In addition, a spatial and temporal analysis of the dispersal characteristics offers opportunities to assess whether this parameter is changing with the length of time that the insects have been colonized in the rearing facility (Parker, Mamai et al., this volume).

There is great potential for GIS to support the assessment of the quality control of released insects, particularly with regard to: (1) comparing the performance (competitiveness, mobility, dispersal, survival) of sterile insects derived from different strains, (2) studying the effects of releasing flies using different release systems, or at different altitudes, over different topographies (e.g. canyons), at different wind velocities, etc., and (3) analysing hot spots or reservoir areas where the pest persists in spite of intense actions to suppress the population.

### *3.6. Barriers to Using GIS*

After the 1990's GIS became more available and accessible to the general public, and the further development and diffusion of GIS have made available a large amount of information from different sources. The cost of acquiring primary data (such as satellite remote-sensing data) has decreased considerably, and many satellite data are now freely available on the Internet. RS images and derivative products (vegetation indices, rainfall indices, etc.) are now available at different levels of geometric pre-processing, thus facilitating their use by non-experts.

Today, the barriers to successful GIS development are significantly lower than they were in the past. The costs of hardware are now relatively low, while most software and data are free. Perhaps most significantly, GIS software is becoming increasingly accessible to non-specialists; non-geographers, after receiving appropriate training, can now carry out many GIS procedures without specialist support (although this does not completely eliminate the need for expert input; note comments below under "GIS expertise").

These advances, however, do not mean that potential barriers or pitfalls no longer exist. Several considerations need special attention, particularly when working in the context of a developing country, if a GIS endeavour is to be successful (BESR 2002). These considerations include:

- *Technical limitations.* Limits in the accessibility of spatial data, such as inadequate telecommunications infrastructure, limited bandwidth or data storage capacities, and low Internet connectivity in certain countries, may hamper efforts to rapidly process and output GIS data.
- *GIS expertise.* Trained and experienced experts in GIS are often in rather short supply, particularly in developing countries. The increasing accessibility of GIS software does not obviate the need for input from appropriately trained operators. Technical issues around data capture and integration can be non-trivial, as are procedures for image processing. Decisions regarding accuracy and permissible levels of data generalization also require relevant experience on the part of the GIS operator.

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- *Maintaining consistency.* In many instances, AW-IPM programmes are implemented over large geographical areas, i.e. on a national or regional scale, employing various field teams in a series of sub-programmes, each attached to a particular country, zone or region. Agreements must be reached on compatible GIS formats, standardized methods of data collection, and data resources. These need to be kept consistent throughout the life of a project.
- *Administrative challenges.* It can be a real challenge to easily gain access to available data because of unawareness about data request procedures on the part of government officials, complicated protocols for requesting government data, a variety of data standards making the sharing of data difficult, and complications due to copyright and distribution issues.
- *Pre-processing GIS and RS data.* This step is often the most time-consuming task in any project involving such data. Based on the data considered in the analysis and particularly for RS data, some pre-processing steps must be applied to make the data usable for further analysis. The most common pre-processing methods for RS data are corrections (radiometric and geometric corrections) that seek to minimize distortion caused by satellite sensors. Furthermore, image enhancement methods such as noise removal or filtering may also be applied to RS data prior to the analysis. Finally, other operations such as georeferencing and mosaicking (combining multiple images) can also be used before analysis.

It is important that, at all stages of GIS development, a dialogue between those responsible for designing and maintaining a GIS system, and those whose principal interest is the output of the system, begins at the start of the planning process, and continues throughout the life of a project. GIS operators need to realize that many decision-makers, in developed and developing countries, have no experience with GIS and other spatial decision-support tools, and thus do not appreciate the potential in using geographic information, or the technical issues involved in setting up and maintaining GIS.

In this context, one essential message must be conveyed — the development of GIS requires careful planning, and often a substantial time-commitment. In a previous section (2.1.) of this chapter, GIS were defined as systems for capturing, cleaning, integrating, storing, retrieving, analysing, and displaying mapped information and data. These functions can be broadly seen as a series of operations that describes the data stream from the original source to a final map or output. The initial stages of this process (up to the point of storing the data) can be extremely time-consuming, and therefore it is important that GIS are designed to meet the needs of the various end users, and to include as little redundant information as possible. Once data have been integrated into GIS, it is possible to perform a wide range of spatial analyses on them at very little cost.

## 4. CONCLUSIONS

In ecology and epidemiology, GIS, GPS, RS, and spatial modelling are increasingly used as a complementary set of spatial tools for project planning, implementation, and evaluation. A GIS-centred approach offers many potential advantages to the area-wide application of the SIT and other control methods, as a decision-support tool in

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the day-to-day management of AW-IPM programmes. In the past, GIS have been applied mainly by academics as an end in itself, or at best as a research tool analysing correlations between different parameters to select priority areas where pest elimination would result in the highest socio-economic impact. More recently, GIS have been used as a tool for planning, implementing, optimizing, and evaluating SIT-based operations.

GIS also facilitate the overlaying of a variety of data coverages, e.g. climate, land use, drainage, etc., to identify factors that may explain the spatial and temporal patterns of insects and/or diseases. Using appropriate spatial analytical approaches, GIS can be used to identify and map the habitat of insect species and their relationship to cropping areas or human and animal populations. In this way, it is possible to generate maps indicating the risk of pests or diseases on a variety of spatial scales, and to monitor, in space and time, integrated information on insect population dynamics, ecological and meteorological conditions, and the incidence of disease or crop damage.

AW-IPM programmes that include the release of sterile insects are, because of the interdependence of their many linked components, inherently complex; the collapse of one component might jeopardize the successful outcome of the entire programme. The success of such a programme depends mainly on aspects related to: (1) the quality of the sterile insect (e.g. sexual competitiveness of the irradiated and released insects, survival, mobility, dispersal characteristics, etc.), (2) the management of the release programme (e.g. timely delivery of insects, appropriate placement of the insects in the natural habitat, uninterrupted supply of sufficient sterile insects, etc.) (Dowell et al., this volume), and (3) the implementation of related programme components (e.g. adequate suppression of the native-insect population, relevant public relation campaigns, ample collaboration with the livestock/crop industry and other stakeholders, etc.) (Dyck, Regidor Fernández et al., this volume; Mangan and Bouyer, this volume). Programme managers need to keep a comprehensive overview of all these essential programme components and their outcomes, almost on a daily basis, and GIS provide an ideal tool to analyse and display data from these multifaceted programmes. Close collaboration between programme entomologists, and SIT and GIS experts and modellers, will be a prerequisite to fully exploit the potential of GIS and spatial modelling as decision-support tools, and to render AW-IPM programmes using the SIT much more efficient and cost-effective.

To make GIS/RS more applicable, programme managers must get access to all available data layers (administrative boundaries, soil types, crops, meteorological data, satellite imagery, vegetation cover, etc.). GIS technicians and modellers can, in many instances, produce data layers that are not available or are missing, e.g. by digitizing topographical maps and implementing supervised classification to map the landscape cover or even insect distribution models. Also, the establishment of global networks to enhance research collaboration, data sharing, and the pooling of common resources, e.g. via the development of special websites, can greatly facilitate the development potential of GIS. Regarding the day-to-day implementation of the various programme components, all data sets on the target insect (survey data, monitoring data, etc.) and related aspects (crop damage, disease incidence, etc.) must be georeferenced and entered into relational databases that are compatible with, and

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can easily be linked to, GIS software (e.g. using ACCESS-based databases rather than EXCEL spreadsheets), allowing straightforward summaries and queries. Finally, standardized data collection, continuous flow of data files to a central location, and increased understanding of the basics of GIS by programme managers, are additional prerequisites to exploit GIS to their full potential in AW-IPM programmes.

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# CHAPTER 5.1.

# DESIGN AND ECONOMIC EVALUATION OF PROGRAMMES INTEGRATING THE STERILE INSECT TECHNIQUE

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SUMMARY

Area-wide integrated pest management is designed for specific purposes, and an economic evaluation should determine its performance against those purposes. The sterile insect technique is used for eradication, suppression, containment and prevention, each of which has different performance measures. Eradication or suppression of insect pest populations using the sterile insect technique (SIT), together with other area-wide control measures, may require significant initial capital investments to achieve long-term returns in subsequent periods. It may also raise questions about the distribution of benefits or the justification of public or private pest control efforts, given long-term uncertainties about the pest challenge and the potential of new control options. A consistent and transparent evaluation is needed to analyse the benefits and costs of such projects and to demonstrate their value, or in some cases to assess appropriate contributions to the costs by the various stakeholders who gain the benefits. Suppression must be compared with expected losses and costs in alternative conditions without SIT application, either from recent experience prior to implementation of the SIT or with similar areas without SIT treatment. Preventive SIT should reduce the frequency of outbreaks compared with experience prior to the application of the SIT or in similar areas elsewhere without SIT use. This chapter outlines the process of benefit/cost analysis, in which itemized future costs and benefits are compared in terms of present values. It also provides a review and examples of the application of benefit/cost analysis to the SIT. A checklist of benefit/cost analysis inputs and some example benefit/cost outputs are also presented.

## 1. INTRODUCTION

The sterile insect technique (SIT) has been used under different circumstances for four purposes: eradication, suppression, containment and prevention (Hendrichs, Vreysen et al., this volume). Eradication has been effective particularly in eliminating new outbreaks in areas with a low probability of reinvasion, or on established populations in isolated areas such as islands (Feldmann et al., this volume). Suppression has been effective for established populations in endemic areas, with buffer zones to limit immigration of mated wild females (Mangan and Bouyer, this volume). Containment has been achieved to avoid the spread of a pest into a free area (Enkerlin, this volume; Vargas-Terán et al., this volume). Prevention has been successful to prevent the establishment of an invasive pest incursion in areas of relatively frequent invasion (Enkerlin, this volume). Economic evaluation of programmes for each of these purposes should focus on indicators of performance relevant to the intended design, and the temporal and spatial scales involved. In each case a form of benefit/cost analysis (BCA) may be appropriate, usually involving probabilistic simulations of scenarios reflecting the pest challenge and control efficacy.

### *1.1. Benefit/Cost Analysis*

The principle of a benefit/cost analysis is to provide a model framework in which all costs and benefits applicable to a programme (targeting eradication, suppression, containment or prevention) can be compared with alternative management options over a specified period of time (Kehlenbeck et al. 2012). This is important for comparing area-wide projects integrating the SIT (which generally have substantial initial costs, but which provide long-term benefits, including in some cases eradication) with individual and short-term localized control (such as by conventional pesticide application). The analysis informs decision-making by

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structuring the estimates of all costs and benefits, including externalities such as environmental and social impacts, but it does not prescribe choices. Ultimately, decisions depend on social, political and commercial judgements, and a BCA is a tool for making that decision process more transparent for governments, investors and beneficiaries.

In a BCA the monetary values of all identifiable benefits and costs are estimated as objectively as possible over the expected period during which the project will operate and be effective. Establishing a reasonable estimate of the relevant time horizon in which to judge a programme is a critical step in such an analysis. Because the benefits and costs are in the future, inevitably there is uncertainty in these estimates. A BCA model needs to be flexible so that the various management options and expected scenarios can be tested, taking into account these uncertainties and demonstrating the sources and influence of the uncertainty. For example, Vo (2000) presented two scenarios for an assessment of the New World screwworm *Cochliomyia hominivorax* (Coquerel) in Jamaica, in which the major uncertainty was programme cost. For some species, e.g. the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), rearing costs are well known (IAEA 2008) but there may be considerable uncertainty about other variables, such as reinvasion frequency, which may change with trade patterns or short-term climate variability. For other species that are less commonly mass-reared, such as the codling moth *Cydia pomonella* (L.), technological improvements can quickly change the costs and effectiveness of reared irradiated insects (Vreysen et al. (2010); Chidawanyika and Terblanche (2011); Parker, Mamai et al., this volume; Simmons et al., this volume). Current small-scale releases of sterile or genetically modified sterile mosquitoes for dengue and Zika management in Brazil (Carvalho et al. 2015) are likely to become much less expensive and more efficient as experience and development continues (Lees et al., this volume). The combination of technological, trade, development and climate change suggests keeping time horizons for a BCA relatively short to avoid levels of uncertainty that could make sound predictions not feasible.

Sensitivity analysis, the process of testing the model with a realistic range of values, is important in benefit/cost analysis to indicate how risks associated with the project could affect decisions. Ideally, the economic framework should be prepared in parallel with the design of technical plans for the project so that each can inform the other. In this way, the final analyses can be efficiently directed to the most effective technical and economically viable plans.

### *1.2. Project Phases*

The initial steps in a BCA include defining the timescale and the likely geographic scope of the project. The time period may include: preparatory phases (research, host surveys, baseline data collection, construction of SIT production facilities, etc.), a control phase (which could include a series of zones through which treatments are applied in succession as eradication, suppression, containment or prevention is achieved), and a reasonable period beyond the control stage (long enough for benefits to establish before there is the inevitable time-related increase in uncertainty about reinvasion, new pest entry or other circumstances that could affect the

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expected benefits or costs). The first two phases can be limited by technical constraints, although there may be opportunities to reduce such constraints by spending more money, whereas managerial and political constraints are more difficult but can be overcome by improving managerial capacities, facilitating the organization of grower associations, and establishing public-private partnerships. The geographic scale may also be determined by technical considerations (islands, topography and limit of host range) or by economic factors (too little return in areas of marginal productivity or lower pest attack).

A cost function is likely to be composed of three parts: variable costs per area to be treated for control and variable costs for all other related management activities (surveillance, follow-up treatment, etc.), along with fixed costs associated with operating the project. The benefits would include a function based on replacing current costs and losses in the area to be controlled, plus any additional market opportunities that may arise through low pest prevalence, pest eradication and/or elimination of pesticide residues. Costs and benefits may need to be attributed to particular production sectors or uses, e.g. production of meat, milk and draught power for tsetse fly control (Feldmann et al., this volume), or to geographic areas, e.g. in selection of individual regions where benefits might be greatest. Environmental costs and benefits, discussed later in this chapter, should be included along with direct monetary values from improved production and cost savings. An increasingly important issue in pest management BCAs is how much of the cost can be recovered from stakeholders (in cash or kind), and how this can be achieved (Waage et al. 2007; Dyck, Reyes Flores et al., this volume).

### *1.3. Project Implementation*

A BCA may be needed both before and after project implementation, first as a design tool to plan the project, and later to evaluate performance and suggest operational improvements (Pereira et al. 2013). Many eradication and suppression projects have been undertaken or proposed, of which most have had either formal or informal benefit-cost analyses. Enkerlin (this volume) summarizes benefits from a wide range of fruit fly programmes. SIT projects and plans have included: Mediterranean fruit fly (California (Dowell et al. 2000; CDFA 2014; USDA 2014), Florida (USDA 2014), Maghreb (Mumford et al. 1995), Eastern Mediterranean (Enkerlin and Mumford 1997), South Africa (Mumford 1997; Barnes 2007), Portugal (Mumford and Larcher Carvalho 2001), Western Australia (Mumford et al. 2001), Chile (UN 1997), Tunisia (Knight 2001), Argentina (De Longo et al. 2000)), tsetse fly (Kabayo and Feldmann 2000; Msangi et al. 2000; Vreysen et al. 2014; Feldmann et al., this volume), New World screwworms and Old World screwworms *Chrysomya bezziana* (Villeneuve) (Tweddle and Mahon 2000; Vo 2000; Wyss 2000; Vargas-Terán et al., this volume), codling moth (Canada (Bloem et al. 2000; Simmons et al., this volume), Syria (Mumford and Knight 1996), South Africa (Mumford 1997)), and *Aedes* mosquitoes (Carvalho et al. 2015; Lees et al., this volume). Cost and general loss estimates for some other fruit fly programmes without an SIT component exist for Egypt (Joomaye et al. 2000), the Indian Ocean

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islands (Price and Seeworoothun 2000) and Pakistan (Stonehouse et al. 1998) for comparison with programmes that integrate SIT management.

The economic conditions that favour an area-wide approach include the integration of efficient and effective techniques such as the SIT, clearly articulated demand by stakeholders, good management capacity (Dyck, Reyes Flores et al., this volume), stakeholder participation, risks that are similar across a broad range of beneficiaries so that economic returns are fairly evenly distributed, a mechanism to capture benefits and recover costs, and, because the SIT is species-specific, it also relies on there being preferably a single dominant pest species (Klassen 2000; Lindquist 2000; Mumford 2000).

## 2. PREVENTION

Prevention of pest establishment using the SIT in pest free areas with a high invasion risk reduces the frequency and cost of outbreaks. It replaces occasional and unpredictable costs with a lower, relatively stable cost of preventive control. It can also reduce the externalities associated with emergency control in a crisis, if widespread pesticide spraying is needed in such a crisis.

The preventive SIT programme against the Mediterranean fruit fly in California is a partnership between the California Food and Agriculture Department and the United States Department of Agriculture. It operates in an area around the Los Angeles basin at a cost of about USD 16 million per year, and has reduced outbreaks from an average of 7.5 per year to less than 1 per year within this area (CDFA 2014). Similar programmes have been run in Florida, with both states using preventive SIT since the mid-1990s. These long-term predictable costs are preferable to relatively frequent and unexpected emergency eradication costs which, together with the temporary loss of exports, can run in extreme cases to over USD 100 million for a single eradication campaign (Box 1)

*Box 1. Preventive Release*

This is an example of a comparison of scenarios for preventive SIT release with an alternative form of periodic pest outbreak control. In this example, preventive SIT reduces the annual probability of a pest introduction becoming established to create an outbreak from 0.3 to 0.1, and the cost of controlling such an outbreak when it does occur from a mean and standard deviation of (USD 50 million, USD 10 million) to a much smaller distribution of costs (mean USD 10 million, standard deviation USD 2 million). In this example preventive SIT has an annual cost of USD 5 million, which would include rearing and release of the sterile insects, monitoring and surveillance, and publicity. The two options can be compared in a series of simulation runs sampled from the probability distributions of outbreaks and costs and a net present value (NPV) cost for each option can be calculated, in this case with a discount rate of 0.03. Multiple runs would provide a distribution of potential outcomes (based on total costs over ten years) so that a comparison of the two distributions could be made. Fig. 1 illustrates one such run. Because the number and cost of outbreaks are probabilistic, the number and timing of outbreaks would be different in each run.

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| | | | Year | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **No control** | | | | | | | | | | | | | | |
| Outbreak frequency | 0.3 | | Outbreak | | | | X | | X | | X | | | |
| Outbreak mean cost | 50 | $ mn | Cost $ mn | 0.000 | 0.000 | 0.000 | 56.578 | 0.000 | 60.198 | 0.000 | 39.814 | 0.000 | 0.000 | |
| stddev | 10 | $ mn | | | | | | | | | | | | |
| | | | | | | | | | | | | | | Net Present Value |
| Total annual cost | | $ mn | | 0.000 | 0.000 | 0.000 | 56.578 | 0.000 | 60.198 | 0.000 | 39.814 | 0.000 | 0.000 | $132.113 |
| **Preventive SIT** | | | | | | | | | | | | | | |
| Outbreak frequency | 0.1 | | Outbreak | | | | | X | | | | | | |
| Outbreak mean cost | 10 | $ mn | Cost $ mn | 0.000 | 0.000 | 0.000 | 0.000 | 9.756 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| stddev | 2 | $ mn | | | | | | | | | | | | |
| Annual SIT cost | 5 | $ mn | | 5.000 | 5.000 | 5.000 | 5.000 | 5.000 | 5.000 | 5.000 | 5.000 | 5.000 | 5.000 | |
| | | | | | | | | | | | | | | Net Present Value |
| Total annual cost | | $ mn | | 5.000 | 5.000 | 5.000 | 5.000 | 14.756 | 5.000 | 5.000 | 5.000 | 5.000 | 5.000 | $51.067 |
| Discount rate | 0.03 | | | | | | | | | | | | | |

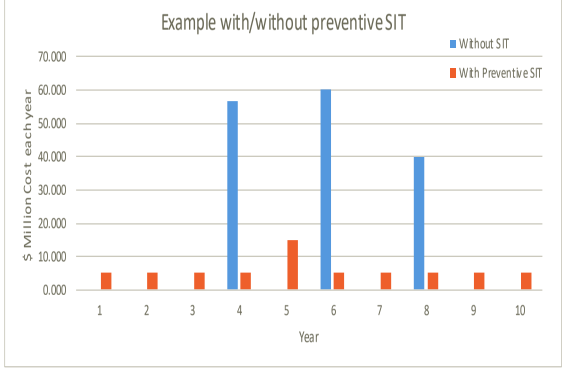



*Figure 1. An example output from a model of outbreak frequency and cost over a 10-year horizon (other runs would be different, but based on the same distributions).*

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## 3. CONTAINMENT

Releases to establish a sterile-insect barrier across the edge of a potentially advancing pest population can have a containment effect, where they are used to avoid the spread of the pest population into an area free of that pest (Enkerlin, this volume; Vargas-Terán et al., this volume). This is in principle similar to a preventive release programme, but one in which the threat comes mainly from natural spread from an immediately adjacent infested area rather than from introductions through trade or travel from a distant source. The economic value of containment is the trade value retained by being able to demonstrate continued pest free status in the uninfested area and the further value of not needing to apply regular pest control measures for the excluded pest. In several respects the conditions for a containment barrier are different from a preventive release programme. In containment the source, population density, movement and destination of the pest are likely to be well known and occur at relatively high pressure along the boundary between the infested and uninfested areas. For preventive programmes, intermittent unpredictable introductions from a range of sources can occur that are likely to result in dispersed isolated outbreaks over large areas of vulnerable territory, depending on where infested material is taken through human-assisted transport. Containment programmes, therefore, are likely to face greater and more sustained pest pressure, but in relatively well-defined areas.

Containment, by some means, would be a form of official control needed to establish and maintain the status of a pest free area. The containment activities would need to be compatible with the surveillance used to demonstrate pest free status. The width and intensity of the containment zone would be the major determinants of the cost of containment, and the extent to which the containment zone extended into areas which otherwise might enjoy pest free status would limit the benefits.

Successful containment may also have an economic option value, keeping open the potential for a future eradication campaign in the infested area.

## 4. ERADICATION

The benefits of SIT-based eradication have been widely described, and vary enormously with the species concerned and the scale and objective of the project. For example, the potential eradication of tsetse species of economic importance throughout Africa anticipated an overall benefit of USD 4500 million/year (of which USD 1200 million/year is in direct losses from trypanosomiasis affected cattle and associated current control costs (OAU 2000)). Vreysen et al. (2014) indicated higher development potential in areas with effective tsetse control, with the proportion of small-scale farmers keeping indigenous cattle increasing three fold to 94% after the elimination of tsetse pressure in Zanzibar. Subsequent to eliminating tsetse, the largest benefit to agriculture in the long-term is the opportunity to introduce more productive cattle breeds (Feldmann et al., this volume). On the other hand, in areas with multiple tsetse species, eradication would entail much higher costs and therefore be less economically feasible (Hargrove 2003). Such analyses could also

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be used to set target cost levels for more efficient sterile insect production (Parker, Mamai et al., this volume) and release (Dowell et al., this volume) to achieve returns comparable with conventional control that also has the capacity to achieve eradication.

Very large-scale eradication projections should take into account uncertainties about the continued political will or institutional capability to see a programme through to its final stages to ensure full realization of the benefits, and prevent reinvasion from the remaining uncontrolled areas. Continental-scale eradication integrating the SIT for the New World screwworm in North and Central America has been possible because of the long-term commitment to maintain and extend the SIT control effort.

Mediterranean fruit fly outbreaks in California threaten losses estimated from USD 1000 million/year (IAEA 1999) to USD 1800 million/year (CDFA 2008). The successful eradication of the Mediterranean fruit fly in Chile in 1995 opened up approximately USD 100 million/year in additional fruit markets (IAEA 1999). Enkerlin (this volume) reviews other examples of significant economic benefits from SIT eradication projects (Box 2).

*Box 2. Eradication*

The Mediterranean fruit fly has been a pest of commercial and backyard fruit throughout much of Western Australia since it was introduced to the state around 1900. It imposes costs on fruit growers who use pesticides for control and through both the presence of the insects themselves and the residues from insecticide. Backyard growers also suffer and get less enjoyment from their fruit trees. It also causes problems for the export of Western Australian fruit internationally and to other Australian states. South Australia, in particular, is faced with costs of quarantine and eradication of frequent incursions or outbreaks due to the Mediterranean fruit fly originating from Western Australia.

A pilot project applying the SIT was conducted at Broom, Western Australia, and showed that SIT-based eradication of the Mediterranean fruit fly was technically achievable in Australia (Jessup et al., 2007). In an analysis of the feasibility of eradicating this pest in Western Australia (Mumford et al. 2001), it was clear that eradication would take several years in a series of geographical zones. The model was based on a concept of summing the individual costs and benefits across each zone, allowing for a phased extension of the eradication across the state with a rolling quarantine to protect the eradication frontier as it progressed. The principal inputs within each zone affecting costs and benefits were the total areas to be treated and the values of losses that would be prevented with eradication. The zone boundaries were selected based on:

- climate (related to the threat from the Mediterranean fruit fly, mainly the effect of winter temperature)
- a phased increase in the treated area to build up expertise and capacity
- treatment areas were determined by satellite imagery of likely host presence and agricultural census data
- a maximum annual area to treat of 1000 $km^2$ (reflecting managerial capacity)
- a phased decrease in treated area as the programme winds down through lower risk areas to maintain capacity in the event of renewed outbreaks in any fly-free areas
- existing local government administrative districts to be used as the basis of both statistics and management.

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## 5. SUPPRESSION

On a much smaller scale, in South Africa grape growers on 4000 hectares in the Hex River Valley were predicted to save over USD 150 per hectare per year in conventional insecticide costs, plus the added value of entering low-residue markets, through using the SIT for Mediterranean fruit fly suppression (Mumford 1997). Subsequent results showed control costs falling from USD 350 000 to USD 130 000 as the SIT replaced most chemical control, and market rejections due to fruit flies were reduced by half (Enkerlin, this volume). While pesticide reduction was not as great as expected, it was still substantial, and illustrates the uncertainties involved in making estimates. Permanent suppression integrating the SIT, rather than targeting eradication, offers advantages for Mediterranean fruit fly control in countries around the Mediterranean where nearby endemic pest populations would result in continual reinfestation and the cost of effective quarantine would be prohibitively high. While suppression does not have the finality that gives eradication such political appeal, it does not have such high costs of certification and quarantine (Mangan and Bouyer, this volume). Furthermore, because there would be an ongoing need for sterile insects, there may be greater potential interest for private investment in sterile insect production facilities and delivery services (Mumford 2000; IAEA 2008). Continued benefit from reduced pesticide use can offer a steady and attractive market for efficient SIT suppression.

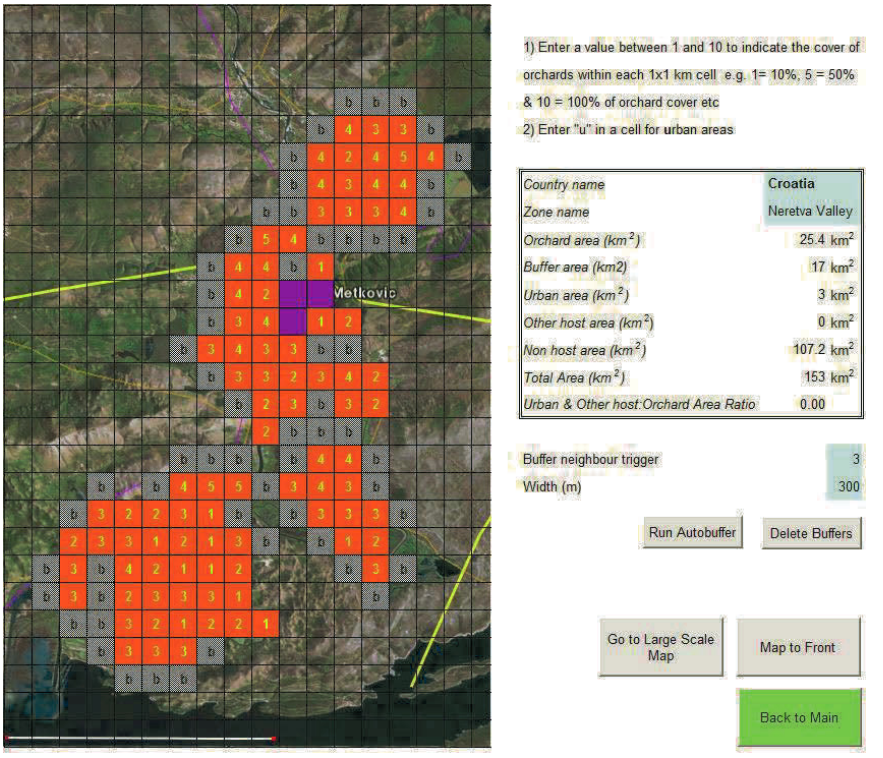
The integration of sterile or genetically modified mosquitoes for suppression of vectors is a new development for the SIT, aimed at more effective control than can be achieved by only applying insecticides or removing breeding sites. Outbreaks of dengue can occur at very low densities of *Aedes* mosquitoes, and control methods with inverse density-dependent performance such as the SIT are more economically efficient in such low-density populations, or after other vector populations have been suppressed by conventional means (Pereira et al. 2013; Häcker et al., this volume; Lees et al., this volume; Mangan and Bouyer, this volume). Effective suppression of vectors may in some cases be sufficient to eliminate a disease in an area, if the vector population can be reduced to a density that does not sustain transmission (Smith et al. 2012).

For a practical decision on the merits of SIT eradication or suppression, the benefits must be set against expected costs, which for many projects integrating the SIT are now well-documented in a range of national circumstances. Issues remain, however, around how to capture the benefits within the various economic sectors that gain from control, and to transfer some of this to the public or cooperative sectors that provide the service. SIT projects have traditionally been public projects, but may increasingly be partly or wholly funded directly by beneficiaries. Payment for the SIT as a service by direct beneficiaries is more likely where ongoing inputs are involved (suppression and prevention) to give relatively immediate returns to those paying for the service (Dyck, Reyes Flores et al., this volume). Because eradication gives benefits in the future, and requires external efforts to prevent reinvasion, it is likely to be less attractive to private subscribers (Box 3).

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*Box 3. Suppression*

Scenarios for suppression can be designed on a spatial grid that considers the costs and benefits per cell each year. The example below, from the CLEANFRUIT project funded by the European Commission (J. D. Mumford and A. W. Leach, unpublished; CLEANFRUIT 2017), illustrates an example of a scenario for a Mediterranean fruit fly suppression programme in Croatia. Each 1-km$^2$ cell on the map has a number indicating the density of citrus production in the cell, which is an indicator of the potential benefit from SIT suppression. Buffer zones are indicated near any cell with a sufficient density of citrus to trigger the need for a buffer. All citrus, urban and buffer cells have a standard cost function for the SIT inputs needed for a cell area of that size. Annual costs and benefits can then be estimated for any scenario in a potential programme design, supporting decisions on where to draw programme boundaries and how to allocate public or subscription costs. For example, private subscriptions may be based on specific cell benefits and public subsidy based on average cell costs.




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## 6. BENEFIT/COST ANALYSIS FORMAT

The output of a BCA is likely to appear as a time profile indicating inputs and outputs by year, location and sector (which could be crop/livestock/human health type, urban/rural, public/private, etc.) depending on the needs of the commissioning agency. A scenario for a programme outlines the conditions for the modelled flows of inputs and benefits as each area reaches the stage assigned for particular management actions. An example of output flow of benefits minus costs appears in Fig. 2, illustrating the time flow of outcomes in an eradication plan.

The model structure has a set of cost and benefit components, specified as a probability distribution for each area and each year. Each of these refers to a standard set of cost, price and production parameters (with associated uncertainties) per area to give the model consistency while allowing the flexibility to analyse different SIT management plans. Different plans could, for instance, include changing the sequence of zones to be controlled, or the number or size of zones eradicated in each year. Values would be expressed with specified levels of uncertainty associated with them. For example, the cost of sterile flies may not be known before a factory is built, but costs from similar factories give a good approximation. Sensitivity analysis would demonstrate the range of outcomes using input values with some variation around the most likely expected values.

Many of the BCA models referred to in this chapter are related to the general format of a generic Mediterranean fruit fly BCA model developed for the IAEA (FAO/IAEA 2007). While each case has specific elements of geography, ecology or market conditions, there are many common principles and a growing globalization of SIT infrastructure. For example, large and efficient production facilities (IAEA 2008) can ship sterile insects to an international market at competitive prices: New World screwworms from Mexico to Libya, and Mediterranean fruit flies from Guatemala to the USA, Israel and South Africa, Spain to Morocco, and Israel to Croatia. Many items of equipment used in rearing and aerial release are now common across many projects. Many components of costs for sterile insects and their application are therefore fairly standard throughout the world, and common tables of such costs and standard application procedures help in planning both publicly and privately financed SIT efforts. However, as noted earlier, costs are not so stable for many species other than the Mediterranean fruit fly. Labour costs vary from country to country, but automated systems can reduce some of these costs.

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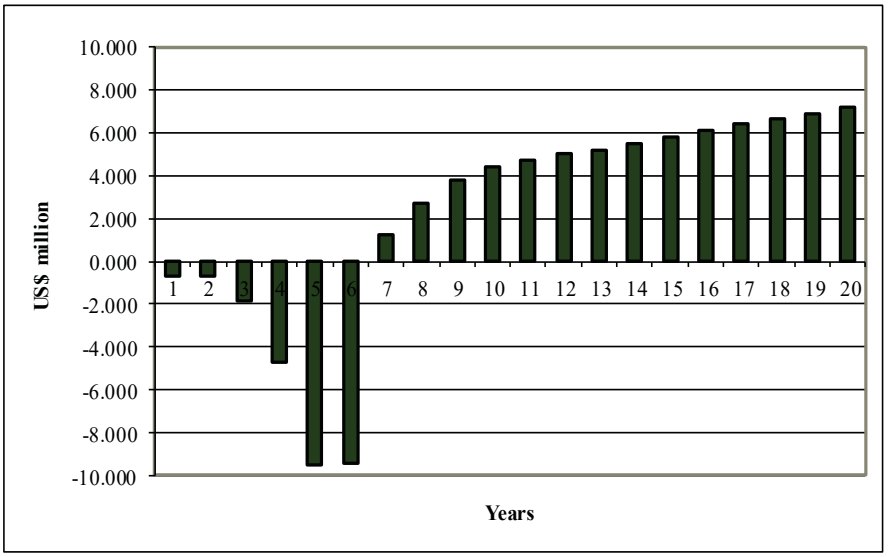



*Figure 2. Annual net projected benefit-cost for one scenario of the Western Australia Mediterranean fruit fly eradication plan before any discount rate was applied (Mumford et al. 2001).*

### *6.1. Time Dimension*

The time dimension for BCA predictions is very important, since there is a trade-off between adding the extra benefits over a longer term after most of the cost has been completed and adding greater uncertainty of further pest invasion, market changes, etc. While eradication may be seen by many as a once and for all achievement with an indefinite stream of benefits, experience shows that reinvasion may occur frequently in some areas of high risk of incursions (for example, the Mediterranean fruit fly has recurred in California and Florida following eradications, although the benefits of even short-term eradication exceeded the costs in such major exporting areas). So, while longer time frames would give a greater apparent return to an eradication programme, assuming the costs of maintaining quarantine or preventive control are not prohibitive, the probability of losing the benefits through a new outbreak increases as more years are added to the anticipated flow. In any event, future discounting reduces the impact of extending the time horizon.

### *6.2. Discount Rate*

Benefits and costs that arise in future years should be compared in terms of their equivalent present values so that all the values are directly comparable, since the same nominal value further in the future is worth less in present terms. The net benefit (benefit minus cost) over the whole project period being considered would

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be expressed as a net present value. The discount rate is used to calculate these present values. The discount rate is a measure of the value that people place on having money now rather than later. It is generally considered to be equal to the interest rate on savings minus the inflation rate, both of which are currently at very low levels in developed economies. At present, in relatively stable economies, the discount rate ranges from about 1–4%, with the US government guideline on discount rate suggesting a central value of 2.9% for public benefit projects with a time period of 10 years (OMB 2015). However, it is likely to be higher in less stable economies (Mumford 2000). While a common discount rate, such as 5%, could be used in all analyses, this would not demonstrate the differences in future values that actually occur in different economies. To calculate present value, the following formula is used:

$$\text{Present value} = \text{Future value}/((1+\text{Discount rate})^{\text{Years}})$$

At a discount rate of 0.05 (5%) this formula indicates that a value of USD 100 in 10 years has a present value of only USD 61, and USD 100 in 20 years is worth only USD 38 in present terms. Therefore, long time frames do not add as much benefit as may be imagined, particularly where discount rates are high, while they add greatly to the uncertainty of the estimates. Because discount rates now used by many governments are much lower than they were in the past, the net present value of area-wide insect control over ten-year periods will be much greater than it would have been in previous decades, when much higher discount rates prevailed.

## 7. MODEL INPUTS

### *7.1. Costs*

The following cost items need to be predicted:

- Pest management treatments (the combination of the SIT and related technologies to be applied based on technical selection and specification of control activities and locations; variable costs to be determined per unit area, plus initial and subsequent annual fixed costs)
- Management area (this is the main driver for costs because most costs are variables based on treatment application per unit area; also consider if particular areas have additional needs that affect costs, due to terrain, remoteness, etc.)
- Cost categories (examples are given for some of the important cost categories, mainly based on values for the Mediterranean fruit fly, one of the most commonly controlled pests using the SIT, but some of the categories are too site-specific to give meaningful examples)
- Pre-treatment preparation (baseline data collection, demonstrations, trials to prove the effectiveness of techniques, and to build technical capacity and public confidence)
- Surveys (pest population, host areas, current control practices and losses)

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- Population reduction needed prior to the SIT (by bait or other treatment) to bring populations to a low enough density for effective SIT control (Mangan and Bouyer, this volume)
  - For the Mediterranean fruit fly, approximately USD 6000 per $km^2$ (Mumford et al. 2001)
- Environmental costs (mainly pesticides used for pre-SIT population reduction):
  - Leach and Mumford (2008) proposed a Pesticide Environmental Accounting value for pesticides which gives an economic value for the environmental damage for many individual pesticides, particularly those used in horticulture. The environmental values are very variable depending on the particular pesticide properties.
  - Environmental and health impacts can also be quantified in monetary units using willingness-to-pay methods or by establishing aspiration levels (Farnsworth 1986; Nagel and Peveling, this volume).
- SIT costs:
  - Production, for the Mediterranean fruit fly, approximately USD 250–500 per million irradiated male pupae at the factory (rate of release would be 100 000–150 000 per $km^2$ per week, numbers may depend on host density) (IAEA 2008)
  - Delivery, reception and release actions are described in detail in FAO/IAEA (2017) (Dowell et al., this volume)
    - Costs are highly dependent on the proximity of the production facility to release points.
    - Aerial release costs depend on the type of aircraft and the numbers released; ground release is only practical for small areas.
- Quarantine (prevention of re-entry and management of outbreaks post-eradication)
- Monitoring (during and post-eradication) and certification (intensive monitoring post-eradication to prove pest free status) (Barclay et al., this volume; Vreysen, this volume)
  - For the Mediterranean fruit fly, a certification trap grid of 10 per $km^2$ inspected fortnightly at USD 2–3 per inspection (Mumford et al. 2001); monitoring during control may be less, e.g. 4 traps per $km^2$ in the preventive release programme in California (Dowell et al. 2000).
- Miscellaneous costs (administration, public awareness, marketing the improved pest free quality of produce from the area)
- Additional management and infrastructure costs to cope with possible increased pressure on land use after pests are eliminated.

Also needed - a time profile of the inputs, with changes over time, a time limit for analysis and an agreed discount rate.

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### *7.2. Management Operations*

The management operations are specified according to the technical needs of the project, for example pre-SIT bait applications. The economic analysis can be used to choose between different technical options, e.g. the order in which zones are treated in a phased eradication may have significant economic implications. There may be technical or managerial limits on the size of treatment zones, which affects the pace of eradication. The release rate of sterile insects, pre-SIT control regimes and standards for monitoring are all based on previous experience in successful eradication projects and the ecological circumstances in the area, or for new SIT species on field research in pilot projects.

The treatment area in each year consists of all of the pest-host area within a zone, along with some additional areas along the edges of host areas, which may result in the infilling of heterogeneous areas depending on the distribution of hosts. The areas may be predicted by land-use images from satellite or aerial photos, and/or from ground surveys. Crop areas, livestock densities and production levels are often available from agricultural statistics, and households can be obtained from census records. Local surveys of vegetation, animals and households may be needed where information is scarce.

Prior to applying SIT treatments there may be a need for trial runs to practise procedures, or to provide some stakeholders with demonstrations of operations and impacts. All subsequent operations can be treated as either direct area functions in a BCA spreadsheet, or as indirect area functions (for instance, environmental costs are likely to be determined by the volume or value of pesticide used, which will itself be area-related).

The timescale for the analysis should include the preparatory phase, the operational phase, and the ongoing period during which benefits and any further costs can be confidently expected to accrue. The endpoint for the analysis should be chosen after consideration of ecological, market and quarantine uncertainties, which increase over time, and the effect of future discounting, which makes long-term future values relatively less significant in present terms.

### *7.3. Benefits*

The following groups of benefits are likely to accrue:

- Reduced direct and indirect costs of current control (this requires technical specification and information on the proportion of users for each current practice, obtained by survey)
- Reduced residual losses to crops or livestock, and reduced disease transmission, due to target pests which an SIT treatment would eliminate (such losses may exist despite current control efforts, either because little or no control is applied in many low-input farms, or because control is often not completely effective even when fully applied). The lack of fully effective controls is often a substantial motivation for an SIT treatment, whether targeting suppression or eradication.

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- For livestock, reduced veterinary, surveillance and treatment costs, and faster time for animals to reach sale weight
- Pest control reduces losses because pest attack lowers resistance and increases susceptibility to further insect and disease attack
- Reduced environmental impact from pests that affect natural vegetation or wildlife, which control would prevent. Mumford (2001) describes the ways in which economic values can be put on non-crop losses in natural environments.
- New market opportunities or improved retention of existing markets, e.g. due to reduced pesticide residues on produce or certified disease or insect free status
- Additional development potential in areas under effective pest management, e.g. see Vreysen et al. (2014)
- Greater impetus to invest in agriculture in areas in which pests have been controlled.

A time profile of benefits and their distribution (geographical, sectors, etc.) would be needed, along the same lines as for costs.

### *7.4. Input Format*

A typical spreadsheet for a BCA would consist of the following example data pages: a delineation of areas; a catalogue of data on $km^2$ per district (total area and area for particular pest hosts, or density of hosts); SIT treatment areas by zone (excluding areas the target pest would not inhabit due to a lack of hosts or to climatic conditions); potential and residual losses due to the pest (affected by productivity in the area, climate and susceptibility of hosts to pest); current control costs, including social and environmental costs of current control practices, lost market opportunities (due to residues, residual pest damage or quarantine exclusion), SIT treatment costs, including additional monitoring and quarantine costs and pre-treatment preparation, and a discount rate for the country; and the input summaries for each of the scenarios being compared.

## 8. MODEL OUTPUTS

Model outputs should indicate: summaries of costs and benefits over a time line agreed for the analysis of the scenario, and economic indicators (such as net present value, payback period, and internal rate of return) for each proposed scenario. The net present value is the sum of the present values of future net returns, using the discount rate to calculate back to the present from the expected future nominal values. The payback period is the number of years before the cumulative benefits exceed the cumulative costs, which is a measure of the riskiness of a project. The internal rate of return is the discount rate that would give a net present value of zero to the stream of net benefits from the project. The project would exceed the break-even point if actual discount rates are expected to be below this value.

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### *8.1. Interpretation*

The output of the BCA provides a comparison of the stream of net benefits, expressed in present values. Scenarios with higher net present values are preferred, although sensitivity analysis may indicate that some high returns are associated with greater risk. Eradication requires a long-term commitment to ensure the investment in eradication is protected, and this can add considerable cost. The net present value displayed is calculated using the average of each input value, but each of these inputs may be uncertain. Where probability ranges have been estimated for various input values it is possible to use simulation software such as @Risk™ to calculate the range and frequencies of output values.

The distribution of benefits can be apportioned by sector (e.g. commercial versus backyard), by geographical zone, by public/private finance, etc. This has important implications for the political desirability of a project, the relative role of various stakeholders and the potential for cost recovery. The initial benefits are likely to go to commercial producers for projects involving fruit flies, codling moths or other agricultural pests. Consumers may subsequently benefit from lower prices if production becomes more efficient later. Also, inhabitants in an area with lower insecticide use will benefit, justifying their financial support to an SIT project (Cartier 2014; Dyck, Reyes-Flores et al., this volume; Simmons et al., this volume).

### *8.2. Cost Recovery*

Many countries now have policies that require government to seek to recover costs, wherever possible, from public projects such as insect eradication (Waage et al. 2007). A BCA could form the basis for determining not only what expenditure will be needed for a successful project but, through the assessment of the benefits, how that expenditure should be shared. However, identifying benefits does not directly indicate who should pay. Some costs, for instance non-monetary environmental costs, are difficult to recover, and may only be practical for society as a whole to bear or to claim compensation from government (Mumford 2001). In other cases, many beneficiaries may be involved, making it difficult to collect voluntary payments for area-wide control individually, e.g. when urban householders benefit. In such cases collective payments are more efficiently made, as in the Sterile Insect Release Program in Canada, which has been supported partially by urban residents (through property taxes) for more than 20 years (Cartier 2014; OKSIR 2020; Dyck, Reyes-Flores et al., this volume; Simmons at al., this volume). Such support may need the mandate of a local referendum, or election, for it to be imposed on all those required to pay.

Any area-wide integrated pest management programme (AW-IPM) will encounter the issue of free-riders who do not contribute directly (Lindquist 2000). Where these benefits contribute to the broader public good it may be more efficient to fund projects centrally from government and spread the cost through general taxation. Where the benefits are geographically isolated and beneficiaries are few in number and well-organized, such as in the Hex River Valley Mediterranean fruit fly control project in the South African table grape industry (Barnes 2007), a levy on

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growers or a cooperative subscription system is a practical and fair way to pay for part of the costs. In Brazil, the city of Piracicaba has contracted a commercial supplier to rear and release sterile *Aedes* mosquitoes in a municipal vector control programme (Le Page 2016), along with enforcement efforts to reduce mosquito breeding sites.

## 9. BENEFIT/COST ANALYSIS CHECKLIST

The following bullet points provide a checklist for decisions on benefits and costs; further examples related to control by more general means can be seen in Kehlenbeck et al. (2012):

- Planning and feasibility studies (technical and economic) that provide initial descriptions of inputs, and estimates of costs and effectiveness
- Current losses due to pests (without control and even with control, for crops or livestock over several seasons) or disease transmission by insect vectors
- Market exclusion due to pest presence, damage or pesticide residues
- Current control practices (area treated, effectiveness, cost, environmental impact)
- Area to be treated overall, including necessary buffer areas beyond infested host areas to prevent reinvasion
- Area within the overall treatment area that contains non-productive land
- Pre-project baseline data collection and monitoring for hosts and populations
- Publicity to make the public aware of an area-wide programme (Dyck, Regidor Fernández et al., this volume)
- Regulatory controls (such as hygiene, local quarantine inspections, reporting of pest occurrence)
- SIT costs:
  - Pre-release insecticide baiting (or alternative population reduction practices)
  - Sterile insect production (Parker, Mamai et al., this volume)
  - Sterile insect storage and transport
  - Sterile insect release (Dowell et al., this volume)
  - Field monitoring (for operational management) (Vreysen, this volume)
- Field monitoring for pest free certification (Barclay et al., this volume; Vreysen, this volume)
- Post-control area quarantine
- Marketing to capture benefits of pest and pesticide reduction/elimination
- Additional development potential in controlled areas with effective pest management
- Agreed project timescale and applicable discount rates.

This checklist provides guidelines on the basic information that would ideally be used in a BCA. More precise information will give more confidence in the analysis, but may be expensive to obtain. Therefore, some compromises between uncertainty and cost may be required. The goal is to provide transparent comparisons of specified scenarios with as much objectively agreed information as possible, with any uncertainties explicitly identified and included.

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# CHAPTER 5.2.

# ENVIRONMENT AND THE STERILE INSECT TECHNIQUE

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SUMMARY

The sterile insect technique (SIT) is an exceptionally promising pest control method in terms of efficacy and environmental compatibility. Assessments of environmental risks vary according to the status and origin of the target pests. The suppression or eradication of non-native pest populations with the sit raises few environmental concerns, and these are related mainly to some pre-release suppression techniques. However, the elimination of native species, or at least populations of native species, requires more detailed and complex assessments of ecological effects and consequences for biodiversity conservation. Eradication programmes provide opportunities to study these topics within the scope of both environmental impact assessments and operational monitoring programmes.

## 1. INTRODUCTION

The sterile insect technique (SIT) is a species-specific control method that may be applied in the area-wide integrated pest management (AW-IPM) of insect pests of medical, veterinary, and agricultural importance. Contrary to chemical or other products that are intrusive, toxic, pathogenic or otherwise destructive entities, or to most other biological agents whose establishment is irreversible once they are released, sterile insects are non-invasive agents that leave no "ecological footprint" because they cannot become established due to their sterility. Consequently, sterile insects have been recognized among *beneficial organisms* by the International Plant Protection Convention to which most countries are parties (IPPC 2017). Therefore, the SIT alone poses *a priori* an exceptionally low risk to the environment (Müller and Nagel 1994). Ecologically, the release of sterile insects represents an input into ecosystems of allochthonous, living yet non-reproductive biomass readily integrated into and processed within food webs. Thus, if there are any direct adverse impacts of sterile insects on non-target biota, they are most likely related to changes in interactions among species.

The SIT is not a stand-alone technique, however, and in most situations, to be effective and economically viable, the SIT requires pre-release suppression of the pest population with conventional control techniques. These encompass simple devices such as sticky traps, as well as large-scale aerial treatments with insecticides. Therefore, environmental hazards range from negligible to high, depending on the actual type and combination of suppression agents and tactics. When target population densities are low, less hazardous control techniques can be employed to reduce target populations. For example, the initial population densities of the tsetse fly *Glossina austeni* Newstead in Unguja Island, Zanzibar, were already low due to previous habitat destruction by farmers. Thus, suppression was achieved with pour-on pyrethroids sprayed on cattle (living targets) in pastoral areas, and with insecticide-impregnated blue cotton screens (targets) deployed in areas where cattle were scarce (Vreysen et al. 2000), posing a low risk to the environment (Nagel 1995). An even lower risk is estimated from the use of chemical larvicides to control the New World screwworm *Cochliomyia hominivorax* (Coquerel); they are applied directly to the wounds of infested livestock, posing no hazard to treated animals or components of the environment (USDA 2001a).

Area-wide control targets the entire population of a specific pest in a particular area (Lindquist 2000; Hendrichs et al. 2007; Klassen and Vreysen, this volume). Therefore, it is more cost-effective than local control which only targets a portion of

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the pest population and permits continuous pest movement from non-treated neighbouring areas into the treated areas (Klassen 2000; Hendrichs et al. 2007).

Most suppression or eradication programmes with an SIT component were directed against populations of introduced (non-indigenous) species, and did not aim explicitly at eradicating a pest species from its entire natural range. The risk assessment of full eradication strategies requires in-depth investigation of the ecological role of target indigenous pests in ecosystems and food webs, and the possible consequences of their disappearance.

This chapter discusses environmental issues related to different steps in the SIT strategy and process, from sterile insect production to release, and from direct to indirect ecological effects. Given that suppression and/or eradication programmes may eventually extend from area-wide to range-wide, in other words from population eradication to species extinction in the wild, trophic and other ecological interactions among target insects and non-target fauna and flora will also be addressed. While evaluating environmental risks associated with the SIT in a more general sense, particular emphasis is placed on the environmental effects of tsetse fly *Glossina* spp. control in Africa.

## 2. MASS-REARING AND STERILIZATION

### *2.1. Mass-Rearing*

Insect-rearing facilities pose a low environmental risk if designed using current biosafety principles, and if managed according to best practices and standard operating procedures (FAO/IAEA 2008; Parker, Mamai et al., this volume). However, given that large numbers of insects are reared at a single site, they are a potential source of unintentional release of fertile insects into the environment. This might cause limited damage if facilities are located within the natural range of the native pest species, but potentially more serious hazard if environmental conditions are suitable for the establishment of an invasive species, although such a situation could immediately be mitigated by releases of sterile insects of that same species. Therefore, setting up facilities within the potential habitats of the target insect requires adherence to strict quarantine and security guidelines during all steps of insect production and shipping. To reduce this risk, sterile fruit fly production in the United States is only allowed in areas where the respective species is established or where environmental conditions are not suitable for establishment (USDA 2001b). Thus, production facilities may be located far away from the release area, but in many programmes that release sterile insects, mass-production takes place in their vicinity to minimize shipping costs.

Insect mass-rearing facilities can pose occupational health risks. Exposure to frass, hair, and scales may induce allergenic responses in humans (Parker, Mamai et al., this volume). Another risk arises from exposure to microbials (fungi, bacteria). Therefore, rearing facilities must be equipped with effective air filtration systems to minimize the aerial concentration of allergenic particles and potential pathogens, and staff frequently exposed to these agents should wear protective clothing and masks.

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This may be problematic in countries with insufficient occupational health standards and capacities for industrial processes in general.

Another problem is the disposal of large quantities of liquid and solid organic wastes (Parker, Mamai et al., this volume), which can carry immature stages of the insect as well as various microbial species, e.g. *Escherichia coli* (Migula). Effective waste-treatment facilities are needed to prevent the release of insects and pathogens into the environment, and to prevent the direct infection of livestock if solid waste is processed further into animal feed.

RIDL (release of insects carrying a dominant lethal), a genetic control method based on modern biotechnology, is being applied using transgenic strains that require tetracycline in their larval diet to suppress lethality during mass-rearing (Alphey 2014). The disposal of such diet will require special procedures to prevent this broad-spectrum antibiotic from ending up in animal feed or the environment. Also, the potential effects of continuous exposure of mass-rearing staff to such antibiotics in the production facility needs to be assessed. Moreover, according to Leftwich et al. (2016), the permanent exposure of mass-reared colonies of RIDL strains to antibiotics is likely to (1) alter the composition of gut bacterial communities through a reduction in gut bacterial diversity, and (2) select for tetracycline-resistant gut bacteria. The effects on sterile insect fitness of gut bacterial communities that are altered in these ways, or their environmental impact, are not yet known (Augustinos et al., this volume).

Insects mass-produced for the SIT are reared on natural or artificial diets. For most species, no problems arise from using these techniques and diet ingredients. However, in the early days of tsetse mass-rearing, living mammals were used as hosts to feed the flies. This procedure was neither practical nor compatible with animal welfare. It was eventually replaced with a silicone-membrane feeding system simulating the skin of a host, and using warmed blood collected from abattoirs (Bauer and Wetzel 1976).

### *2.2. Irradiation*

The radioactive isotope Co-60 is the most commonly used isotope for the sterilization of pupae or adults of target insects (Bakri et al., this volume). In the past, some radiation sources used Cs-137; this is problematic because Cs-137 in nature can move easily due to the high water-solubility of caesium's most common chemical compounds. Therefore, its use has been discouraged for insect sterilization. Nevertheless, also facilities containing Co-60 sources have to be earthquake-safe and operated according to nuclear safety standards. These are part of the standard operating procedures for sterile insect production (FAO/IAEA 2008). Further guidance is given by the International Atomic Energy Agency (IAEA), which supports many AW-IPM programmes that use the SIT. Thus, in countries with a proven record of the safe use and correct disposal of hazardous substances, a risk associated with the regular use of irradiation sources is not anticipated. According to IAEA norms, irradiation facilities cannot be set up in countries with insufficient nuclear safety legislation and infrastructure. In situations of political instability,

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contingency plans must foresee that irradiators and associated technology are secured.

Recently, the development of X-ray machines for insect sterilization is being encouraged because they have the advantage of requiring access only to electricity, and do not involve the complex and costly shipment of radioactive sources across international borders, as well as their eventual problematic disposal (IAEA 2012, 2017).

### *2.3. Autosterilization*

Autosterilization in the field has been proposed as a complement to the release of reared sterile insects. This technique relies on attracting target insects to baits or trapping devices and exposing them to chemosterilants which they then spread to other individuals of the target population. Various chemosterilants have been tested for tsetse control, including metepa, bisazir and insect growth regulators (IGRs) such as triflumuron, a chitin synthesis inhibitor, and pyriproxyfen, a juvenile hormone mimic. They aim at female flies, leading to the sterilization of females and a gradual suppression of the tsetse population (Knipling 1999; Oloo et al. 2000). Metepa and bisazir are carcinogenic, and may pose a risk to operators and trespassers. Therefore, these chemosterilants have never been used in operational control programmes. The other agents are less harmful, but lack clear evidence of efficacy. The environmental risk of chemosterilants such as IGRs is similar to the risk associated with stationary attractive devices (section 3.3.). Field work in Spain has indicated some potential for this technique (Navarro-Llopis et al. 2004).

## 3. SUPPRESSION OF TARGET POPULATION

### *3.1. Overview*

Although large numbers of sterile insects can be produced in modern rearing facilities, achieving adequate overflooding ratios with competitive sterile insects is essential for effective SIT application. Depending on the target species, minimum ratios of sterile to wild fertile insects during the first release cycle vary from 2:1 to 15:1 (Feldmann and Hendrichs 2001), but may reach 100:1 (Drew et al. 1982). Therefore, applying additional suppression measures against the wild population remains a prerequisite for many AW-IPM programmes that release sterile insects. However, not all such programmes need to be preceded by sprays of synthetic insecticides to suppress the target population. Integrating pest management strategies by combining different techniques, both before and during sterile insect releases, can optimize control efficacy and minimize adverse environmental effects (Mangan and Bouyer, this volume).

One approach is to make releases when the target wild population is naturally low, as generally occurs in winter or dry seasons in sub-tropical and temperate climates, or during periods of low natural host availability. Such strategies often include using non-insecticidal suppression methods such as those based on cultural and mechanical controls, but can also include mating disruption, augmentative

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release of natural enemies and pathogens, baits and biopesticides, or even genetically modified crop plants that express a toxin (Suckling et al. 2012).

Sometimes, insecticide-based methods are applied to suppress target populations before SIT application; they fall into two broad categories: insecticide spraying and artificial attractive devices. Other methods are employed against particular insects, e.g. insecticide treatment of livestock (pour-ons) against tsetse flies, wound treatment against screwworms, and soil-drench treatment against fruit flies.

### *3.2. Insecticide Spraying*

Insecticides are applied with ground sprayers or aircraft, depending on the type, accessibility, and size of the target area. Aircraft are often used to apply larvicides to bodies of water to control mosquitoes and black flies. Ground applications include spot as well as small-scale, full-cover treatments, and are more focused than aerial applications. Many AW-IPM programmes, however, extend over large areas where ground access is very limited or even impossible, and reliance has then to be placed on aerial spraying as the main tool to reduce a population. Chemical pesticides are selected mainly based of their efficacy and friendliness to the environment. Furthermore, spraying techniques and cycles are adapted to specific behavioural and life-history traits of the target. Thus, environmental effects depend not only on the type of insecticide but also on the actual use pattern. Two examples of the environmental impact of aerial spraying for population suppression are given below.

#### *3.2.1. Fruit Flies*

Aerial bait-spray applications have been an effective tool as part of eradication campaigns against large outbreaks of non-native fruit flies (USDA 1993, 2001b), although their use is increasingly avoided in view of growing public opposition. The spray is used alone, or in advance of SIT application, to suppress or eradicate fruit flies. In the past, the sprays consisted mainly of the organophosphorous insecticide malathion mixed with a protein hydrolysate acting as an attractant and feeding stimulant. The bait, applied in larger droplets, improves efficacy because the insecticide uptake by attracted flies is then higher (via ingestion and the integument). Therefore, aerial sprays can be applied at ultra-low volume dose rates, lower than necessary for contact applications. Malathion is highly toxic to terrestrial and aquatic non-target invertebrates. Aquatic fauna are exposed via direct over-spray of water bodies or via run-off. Terrestrial insects, which are also attracted to the bait, are particularly at risk, including ground beetles (Carabidae) and many other beneficial organisms. In fact, a malathion bait spray may disrupt a substantial portion of natural biological control agents (USDA 1993, 2001b). Native fruit flies may also be adversely affected, resulting in shifts in community structure and perturbations of ecological services (section 5.5.). Possible hazards further up the food chain include secondary poisoning or food deprivation of insectivorous vertebrates.

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Fortunately, malathion has been largely replaced by new and more environment-friendly insecticides in the bait, in particular spinosad (naturally produced bacterial (actinomycete) compounds) and SureDye® (xanthene dye) (Vargas et al. 2002; Mangan 2014; Mangan and Bouyer, this volume). The toxicity of these agents to non-target biota is much lower than that of malathion, and fewer adverse effects have been recorded (USDA/APHIS 2003). Acceptable spinosad formulations, certified as organic, have been developed and are being used extensively, e.g. in pest hot spots in the Mediterranean fruit fly programme in Guatemala and Mexico, before sterile male releases (USDA/APHIS/PPQ 2000; Enkerlin et al. 2017).

### *3.2.2. Tsetse Flies*

The second example is from tsetse fly control. The pre-release suppression of tsetse, in some cases, has involved sophisticated aerial spraying techniques. Sequential aerosol drift application with fixed-wing aircraft, which previously relied on endosulfan with sometimes negative ecological impact especially on fish in shallow water, is now based on pyrethroids, in particular deltamethrin. This insecticide is one or two orders of magnitude more toxic to tsetse than to house flies and honey bees, respectively (SWRC 2001). Therefore, field rates are exceptionally low, posing a low risk to terrestrial non-target invertebrates. Furthermore, application parameters such as height, time and sequence of spraying are adapted specifically to behavioural and life-history traits of tsetse flies, reducing the risk to terrestrial organisms even further. Finally, as deltamethrin has low persistence, it does not accumulate in food chains. Nevertheless, adverse effects may occur temporarily in terrestrial environments, e.g. effects on certain spiders, and in particular in aquatic environments (Peveling and Nagel 2001). For example, diving beetles and decapod crustaceans are highly susceptible to pyrethroids. A reduction of aquatic and semi-aquatic macroinvertebrates may translate into food shortages for fish and reduce the survival of fry, which in turn may adversely affect local fisheries.

Another possible risk arising from broad-scale sequential applications of low-dose pyrethroids is that the selection for resistance in other insect pests may be enhanced. For example, bednets impregnated with pyrethroids are widely used for mosquito control to prevent malaria in tropical areas. Selection for resistance in malaria vectors, enhanced through insecticide use in AW-IPM programmes that release sterile insects against agricultural pests, would reduce the efficacy of this important control method. This externalized consequence of population suppression for the SIT would be expected mainly in rural areas with a high human population density, where pesticides are used for domestic and/or agricultural purposes.

Treatments with deltamethrin, using the sequential aerosol technique (SAT), have been used mainly in combination with trapping and baiting devices for tsetse suppression (section 3.3.). In a tsetse eradication programme in the Okavango Delta, Botswana, the technique was successfully applied for eradication (Allsopp and Phillemon-Motsu 2002; Feldmann et al., this volume). Preliminary assessments showed that adverse effects on terrestrial invertebrates were minimal (Biotrack 2003). Another insecticide widely used for sequential aerosol applications in the past is endosulfan, a chlorinated hydrocarbon. Tsetse flies are three orders of magnitude more sensitive to this chemical than honey bees (SWRC 2001). However,

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endosulfan is highly toxic to fish and other non-targets, including the Salvinia weevil *Cyrtobagous salviniae* Calder and Sands, a classical biological control agent introduced successfully to contain the invasive aquatic fern *Salvinia molesta* Mitchell (SWRC 2001). In view of these risks, the endosulfan option for Okavango was discarded in favour of deltamethrin.

All available evidence suggests that the effects of the SAT with deltamethrin on most terrestrial non-target arthropods are of short duration. Nevertheless, environmental monitoring in Zimbabwe revealed long-term adverse effects on populations of certain lycosid spiders, *Hippasa* sp. (SEMG 1993). Given that little is known about the resilience of affected populations in natural or semi-natural ecosystems, the SAT should be used only temporarily for pre-release suppression. More selective techniques, such as stationary targets (section 3.3.), should be given priority unless they are logistically too demanding for large-scale operations or do not yield sufficient pest population suppression. Using a four-scale risk classification scheme (negligible, minor, serious, severe), Grant (2001) classified the environmental impact of sequential aerosol spraying as "minor". In principle, this classification is acceptable, but should be taken with caution. The resilience of small and often highly fragmented non-target populations, to tolerate incremental insecticide stress in a multiple stressor environment, declines (Peveling 2001). Therefore, environmental impact assessments should be conducted with respect to site-specific features, such as the prevailing land-use patterns or the presence of vulnerable species. As one measure to mitigate adverse effects, Nagel (1995) proposed patchwork applications of different insecticides. This tactic would also reduce the above-mentioned risk of resistance development in other pests (Hoy 1998).

Low-flying spray aircraft may disturb domestic animals and wildlife, visually and by noise. Nesting birds are particularly vulnerable to this combination of disturbances. Although adverse effects such as increased susceptibility to predation should be temporary, nest abandonment may occur in the more sensitive species (USDA 2001b). A critical situation may arise in tsetse spraying, which relies on nocturnal treatments for optimal efficacy, using low-flying aircraft equipped with bright spotlights. Scared birds are unlikely to recover their nests during the night, leading to increased egg or nestling mortality. In Botswana, for example, there was concern that the wattled crane *Grus carunculatus* (Gmelin), an endangered species breeding in seasonally flooded marshes in the Okavango Delta, might experience reduced breeding success following perturbations by spray aircraft and insecticide-induced food shortages. A pilot study found high levels of nest attrition due to predation and other factors in both sprayed and unsprayed areas, but no indication of breeding failure due to tsetse spraying (Allsopp and Phillemon-Motsu 2002; BBCWG 2002). However, pre-spray observations were lacking, and follow-up studies are necessary before final conclusions can be drawn.

### *3.3. Artificial Attractive Devices*

A variety of traps and targets (devices attracting insects without necessarily trapping them) have been developed for the detection, monitoring or control of insects. Tsetse

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flies are attracted by odours (ketones, octenols, phenols, acetone, carbon dioxide) and by visual cues (colour, shape). Some odours induce host searching behaviour while others promote landing on the device or entering the trap (Vale 1993). Fruit flies and screwworms respond to olfactory cues, such as pheromones, parapheromones, host odours or food baits. Devices such as odour-baited targets for tsetse flies, and coloured baited traps or bait stations for fruit flies, combine visual and olfactory stimuli to enhance trapping efficacy (Piñero et al. 2014; FAO/IAEA 2018). Insects lured to traps or targets become stuck to glue (sticky traps), or are exposed to insecticides applied to the material. Traps or sticky panels for fruit fly suppression use synthetic lures (trimedlure, ceralure, cuelure) applied directly to the panels or to wicks attached to the traps or panels (USDA 2001b). These devices are deployed from the ground by placing on host trees, or nailing to posts. Alternatively, insecticide-treated baited blocks (wood chips) or wicks (so-called cordelitos) are released from aircraft for population suppression. The parapheromone methyl eugenol is particularly attractive to males of some species of *Bactrocera* fruit flies (Shelly et al. 2014), and is used in male-annihilation technique (MAT) programmes, either alone, or prior to or simultaneous with the release of sterile males (Barclay et al. 2014; Vargas et al. 2014). Baited traps for screwworm flies are employed only for detection and monitoring purposes (USDA 2001a); population suppression is achieved effectively by treating livestock.

The densities of traps or targets deployed for suppression vary depending on the insect species and its potential growth rate (Weidhaas and Haile 1978), the type of device, and features of the landscape. In the case of fruit fly suppression, trap densities must be very high to be effective, and mass-trapping is, therefore, not practicable over larger areas (USDA 2001b; Navarro-Llopis and Vacas 2014). For example, more than 1000 traps per square kilometre were deployed in an oriental fruit fly *Bactrocera dorsalis* (Hendel) eradication programme in Mauritius (Seewooruthan et al. 1997). In contrast, suppression of certain tsetse fly populations can be achieved with as few as 2–4 targets per square kilometre (Dransfield and Brightwell 1992).

The deployment and maintenance of trapping devices requires a network of tracks and roads. Servicing activities may disturb wildlife, especially in wilderness and/or sensitive areas. Thus, two issues have to be addressed with respect to the risk assessment of insect trapping techniques, particularly when deployed at higher densities for population suppression: (1) direct effects on non-target animals, and (2) indirect effects related to trap placement and servicing.

### *3.3.1. Direct Effects on Non-Target Animals*

Traps using species-specific pheromones pose a low risk to non-target organisms. Certain species may be attracted coincidentally, especially potential predators and scavengers of target insects. However, local populations are unlikely to be affected. Ecologically relevant adverse effects are most likely to occur with non-target animals from the same genus or family as the target insects, even though other non-target species may sometimes be collected in high numbers. For example, mass-trapping of fruit flies, using parapheromones as attractants, may also have an effect on non-pest fruit flies. Some species are pollinators, thus, there may be a risk of

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reduced pollination and hence fruit-set of certain plants at high trap densities (Tan 2000) (section 5.5.). Obviously, the risk to non-target organisms rises with increasing trap density, yet the risk is expected to be lower than that associated with aerial bait spray or with the application of insecticide-treated cordelitos dispensed in high numbers from aircraft (USDA 2001b). Suda and Cunningham (1970) reported that lacewings (beneficial predators) were caught unintentionally in methyl eugenol-baited traps or sources (fibreboard blocks, cotton strings or viscous slow-release paste) applied by ground or air in some MAT programmes against *Bactrocera* spp. fruit flies (Koyama et al. 1984; Vargas et al. 2014). However, Vayssières et al. (2007) provided evidence that the area-wide release of methyl eugenol-impregnated blocks had no greater impact on non-target insect populations than the non-impregnated blocks.

Contrary to fruit flies, tsetse fly trap densities are much lower, and the chances of incidental encounters of non-target organisms with stationary targets deployed for tsetse suppression are scarce. The degree of species-specificity depends on the combination of visual traits (shape, colour) and odours (octenols, phenols), as well as on the mode of placement. Blue and white targets attract more insects, including pollinators, than odour-baited black screens, which attract mainly stable flies (Stomoxyinae), non-biting Muscidae, and horse flies (Tabanidae). Some of these flies are possible vectors of livestock and human diseases, including trypanosomosis (Sumba et al. 1998), and can be considered as pests, but others such as male tabanids are beneficial flower-visitors and pollinators. Even though the number of studies is limited, there is as yet no clear indication that these taxa are adversely affected at the population level (SEMG 1993; SWRC 2001; Ciss et al. 2019). Likewise, insecticides such as deltamethrin and α-cypermethrin, used to impregnate the fabric of targets, pose a low risk. During the rainy season, some active ingredient may be washed off, but the contamination is negligible compared with full-cover spraying in agriculture and vector control, and the effects are restricted locally (Cuisance et al. 1984; Müller et al. 1984).

### *3.3.2. Indirect Effects on Non-Target Animals*

The deployment and servicing of large numbers of traps and targets are logistically feasible and environmentally sound in agricultural areas with existing road networks. These conditions prevail in some areas subjected to fruit fly suppression or eradication. Therefore, disturbances of wildlife through the placement and maintenance of traps are expected to be minimal when compared with those induced by other agricultural activities such as planting, irrigation, and harvesting. However, disturbances may become more critical in landscapes composed of mosaics of agricultural and natural land.

This is particularly true for tsetse control, which is often conducted in protected and/or wilderness areas. Opening vehicle tracks for the deployment and maintenance of stationary targets can cause erosion, and provide access, not only to control operators and park rangers, but also to poachers (Grant 2001). It also facilitates unauthorized logging activities and firewood collection. Such illegal activities may disturb wildlife. A study in Kasungu National Park, Malawi, found significantly reduced numbers of small antelopes and certain birds on transects with odour-baited targets (Cheke et al. 1997; De Garine-Witchatitsky et al. 2001). For birds, the effect

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was linked to the possible decline in the number of horse flies, affecting the insectivorous little bee-eater *Merops pusillus* Muller, and, as a consequence, reduced pollination and fruit-set of flowering plants, affecting nectarivorous sunbirds and the frugivorous bulbul *Pycnonotus barbatus* (Desfontaines). However, as no pre-treatment observations were made, follow-up studies are needed to validate these results. In West African gallery forests, insecticide-impregnated blue screens along river courses are sometimes deployed at distances of only 100 m. Studies in West Africa on invertebrates and insecticide residues in soil and wildlife showed at most negligible ecological effects (P. Müller et al., unpublished data). It is not known if there are further studies on indirect ecological effects resulting from such high screen densities, but assumedly the effects are of minor importance compared with other anthropogenic disturbances encountered in West African river systems.

### *3.4. Other Methods*

A range of alternative methods to suppress fruit fly populations in AW-IPM programmes has been reviewed in environmental impact assessments, including mechanical, cultural, biological and biotechnological methods (USDA 2001b). A detailed discussion of these methods is beyond the scope of this chapter, but one method, soil treatment with organophosphorous insecticide, deserves mention because it may cause serious hazards. It consists of drench treatments within the dripline of infested host plants to control larvae entering the soil and adults emerging from the soil. Soil drenches with organophosphates, such as chlorpyrifos, fenthion and diazinon, are highly toxic to soil fauna, and may adversely affect key ecological processes, such as the breakdown of organic matter and nutrient cycling. Individual vertebrates are also at risk, even though the limited use of soil drenches (usually less than 1% of the total surface), as a whole, provides protection of populations.

Pour-on treatments of livestock (living targets) with insecticide, for population suppression of tsetse flies and other parasites, are widely used in Africa. Livestock are treated in dip tanks or by applying pour-on formulations. Pyrethroids, such as deltamethrin, α-cypermethrin and flumethrin, are used for this purpose. Originally, pyrethroids were applied mainly to control ticks, but it was found that they were equally effective against tsetse flies.

Dip and pour-on techniques may have adverse effects on non-target insects, including blood- and dung-feeders, as well as on livestock-associated insect-eating birds. Insect species attracted to livestock are often the same as those attracted to stationary targets, but there is an elevated risk since livestock densities are often much higher than stationary target densities. Adverse effects to other dipteran pests are highly desirable, from an animal health perspective. However, a risk to birds cannot be dismissed. For example, the decline or local extinction of the oxpecker *Buphagus africanus* L. in South African rangelands has been associated with the use of arsenic pesticides and organophosphates in cattle dips for tick control (Mundy 1991). These compounds were eventually replaced with insecticides posing a low risk of acute poisoning in birds. Nonetheless, little is known about sub-lethal effects or indirect effects due to food deprivation.

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Dung beetles (Scarabaeidae) are important decomposers of animal dung, and benefit soils and plants by improving soil fertility. They are also important food items for a wide range of invertebrate and vertebrate predators. Similar to parasiticides such as avermectin (Petney 1997; Steel and Wardhaugh 2002), faecal residues of pyrethroids are toxic to dung beetles and other dung fauna such as muscoid larvae (Krüger et al. 1999; Grant 2001; Vale 2002), thereby impeding the decomposition of pats and depriving predators of their prey.

Inappropriate disposal of used pesticides is another serious risk of the insecticide-dipping technique. Dips are often disposed of on open ground, resulting in soil contamination. In other cases, dips are dumped in pits and covered with soil; this may lead to the contamination of groundwater.

### *3.5. Environmental Effects of Some Suppression Techniques — Conclusion*

Each population suppression technique incorporated into AW-IPM programmes is associated with particular environmental risks. The optimum combinations of control methods, that result in the lowest cumulative risk but achieve the highest possible effect on target populations, vary among species, regions and ecosystems. Therefore, environmental assessments may come to different conclusions in different environmental settings. Nevertheless, some general conclusions can be drawn. Aerial spraying, depending on the form of application, is usually more harmful to non-target biota than ground spraying (which is often more focused and limited in scale) or attraction techniques using traps, bait stations or targets. One exception is residual ground application against tsetse flies; due to relatively high insecticide doses and persistence, it may be more harmful than aerial sequential aerosol applications as shown by the environmental recovery monitoring in Botswana, which indicated that most non-target species recovered to pre-spraying levels (Perkins and Ramberg 2004). Stationary attractive devices are normally the least harmful method (Nagel 1995, 1996), but the direct and indirect impact of generating and maintaining access (tracks or transects) to service them over large areas is usually underestimated.

In general, treatments in protected and/or wilderness areas require more careful approaches and risk mitigation measures than those conducted in croplands. This is because protected/wilderness areas are more complex in terms of biodiversity and interactions among biota, and hence less predictable in their responses to disturbances. Also, natural systems lack the adaptation of agroecosystems to human impact manifested in the dominance of ubiquitous or euryoecious species.

## 4. STERILE INSECT RELEASE

In AW-IPM programmes integrating the SIT, sterile insects are released from vehicles or aircraft (Dowell et al., this volume). Ground release requires appropriate road networks. This may be critical in sensitive areas, posing similar risks to non-target organisms as trap or target deployment and maintenance (section 3.3.2.) (Vreysen, this volume). Likewise, sensitive species, or life stages such as nesting birds, may be disturbed by the sight of, and noise from, aircraft dispensing sterile

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insects (USDA 2000b; SWRC 2001). However, such disturbances are expected to be lower than those caused by low-flying spray aircraft because sterile insects are released at higher altitudes, e.g. greater than 200 m for tsetse flies (Vreysen et al. 2000) and about 600 m for Mexican fruit flies *Anastrepha ludens* (Loew) (SIRF 2001; FAO/IAEA 2017).

Aerially applied sterile insects are released directly into the air from temperature-controlled containers (chilled flies) or indirectly from cardboard boxes or paper bags that open once thrown out of the aircraft (FAO/IAEA 2017). In the latter cases, debris from the releases may be a visual disturbance, but such disturbance is transient since the materials used are biodegradable.

Sterile insects released in large numbers may become a nuisance to potential host animals, or may damage plants or fruit. Moreover, natural trophic interactions within communities, such as host or prey detection, may be disturbed by the sheer abundance of sterile insects. However, this is unrealistic, as in the case of the Mediterranean fruit fly, the insect with the highest release rates, approximately only one sterile male is released per 10 $m^2$. Nevertheless, little research has been done on this subject. In the case of tsetse control, sterile males are released at the lowest rates (approximately 20–100 sterile insects per $km^2$). Since this may increase the vector load in the target area, leading to a temporary increased risk of trypanosomosis transmission, only sterile males that have been pre-fed with a trypanocide-treated blood meal are released (Dyck et al. 2000).

Biosecurity concerns may arise in the case of releases of sterile insects of species outside their natural range (section 2.1.). Nevertheless, unlike classic biological control, where released non-native agents are fertile and become established with sometimes irreversible negative consequences (Simberloff and Stiling 1996; Follett and Duan 2000), any adverse effects of released sterile insects are short-lived because of their high natural mortality, and because they cannot reproduce. No such concerns exist if AW-IPM programmes using the SIT are directed against native species. In this case, the SIT can be compared with augmentative biological control, in which native species are mass-produced and released in their natural range.

## 5. ECOLOGICAL CONSEQUENCES OF ERADICATION

Many area-wide SIT applications aim at suppression, containment or prevention, not eradication (Simmons et al., this volume; Enkerlin, this volume; Hendrichs, Vreysen et al., this volume; Klassen et al., this volume). However, if eradication is the goal (Feldmann et al., this volume; Vargas-Terán et al., this volume), the following issues are relevant.

### *5.1. Risks and Benefits*

Populations living on remote islands are more vulnerable to extinction than those living on continents. This also holds for pest species targeted by area-wide control programmes. For example, the coconut moth *Levuana iridescens* Bethune-Baker, a widespread local pest in Fiji, is thought to have been eradicated by the tachinid fly *Bessa remota* (Aldrich) during a classical biological control programme in the 1920s

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(Howarth 1991). However, in the history of economic entomology, there is only one known case of a continental agricultural insect pest becoming extinct. The Rocky Mountain grasshopper *Melanoplus spretus* (Walsh) was a major pest of crops in North America in the nineteenth century. The demise of this species was the result of the anthropogenic destruction of breeding habitats in river valleys (Lockwood and Debrey 1990). This shows that numerical abundance alone does not assure survival (Lockwood 2002). On the other hand, even the most intensive use of chemical insecticides has not threatened the survival of any major insect pest. Only area-wide control strategies incorporating the SIT or modern biotechnologies offer the opportunity to eradicate entire pest populations from selected areas. Eradication is most effective when directed against isolated populations on islands and in isolated habitats separated by natural barriers from the main range. However, the eradication of the New World screwworm populations in North and Central America shows that a pest can be eliminated even from continuous continental areas (Vargas-Terán et al., this volume).

Thus, the extinction of whole pest species from the wild seems possible in the longer term. It follows that ecological consequences must be evaluated thoroughly and impartially. What are the benefits and risks associated with the area-wide elimination of major pests? As for the benefits, these are beyond dispute. An immediate economic as well as environmental benefit is the enormous reduction in pesticide use (Kinley 1998; CDFA 2001). For example, to contain and mitigate permanent Mediterranean fruit fly establishment in California with conventional methods, more than 2 million kg of active ingredient would be needed annually (CDFA 2001; Enkerlin, this volume). The benefits arising from the eradication in 1991 of the New World screwworm in Libya are immeasurable (Reichard 2002; Vargas-Terán et al., this volume). Its spread over the entire African continent and Mediterranean region would have been an environmental, social and economic disaster, possibly comparable with the impact of rinderpest in the 19$^{th}$ century.

The environmental risks of eradicating indigenous populations are much more controversial (Allsopp 2001; Feldmann and Hendrichs 2001; Grant 2001; Vargas-Terán et al., this volume). In view of the devastating economic and/or medical impacts of the prime targets of the SIT, most notably the huge economic losses caused by fruit flies, mosquitoes, moths, screwworms and tsetse flies (Mumford 2000; Enkerlin, this volume; Vargas-Terán et al., this volume), the suffering of people and livestock from tsetse-transmitted trypanosomosis in Africa (Grant 2001), and the threat to the biodiversity of island faunas by non-indigenous invasive species which could be effectively controlled with the SIT (Suckling 2003), one might even question the justification for raising the risk issue at all. Nonetheless, it is important that possible risks, however low, be brought into perspective too, irrespective of the overwhelming environmental, economic and health benefits; only then can risks be managed adequately. The specific goal of this section is to elucidate ecological roles of certain target insects in their respective ecosystems, and to identify research themes and topics that should be addressed when embarking on future eradication programmes.

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### *5.2. Loss in Genetic Diversity of Target Species*

An area-wide programme that targets the eradication of populations of the pest insect leads inevitably to a decline in the genetic diversity of that pest species. For various reasons (section 5.3.) this may not be generally acceptable. Therefore, in the unlikely case of eradication from the whole range, a certain level of genetic diversity of the target insect can be maintained in laboratory colonies. These could be used, and their genomes explored, for future research in the biological and life sciences. It would be impractical, of course, to rear strains from all populations representing discrete demes within metapopulations. Yet, as a minimum requirement, voucher specimens from all target populations must be deposited in scientific collections, including all development stages and both sexes. One part should be mounted, and others preserved deep-frozen and in alcohol (various concentrations between 60–100%). The preservation of insects will be greatly facilitated by using the cryopreservation techniques for embryos (long-term storage at liquid-nitrogen temperatures), which are already available for a number of insects (Leopold 2000; Parker, Mamai et al., this volume).

### *5.3. Eradication versus Conservation*

The prospect of the eradication of native species from their complete natural range is largely theoretical at this point. Nevertheless, it raises some general questions with respect to conservation. On the one hand, the planned extinction of a limited number of pests appears to be a negligible issue in view of the scale and dynamics of the speciation and extinction processes in the evolutionary history of the earth. However, in modern times, human activities have become the major cause of extinction, leading to a strong imbalance of speciation/extinction rates towards extinction. Given the dramatic decline of species worldwide, biodiversity conservation — as outlined in the Convention on Biological Diversity (CBD) — has become a global priority. This was motivated partly by the prospect that biodiversity will provide enormous resources for future uses, as well as by fears that ecosystems might be destabilized if extinction continues unabatedly. The latter notion leads to the introduction of the *precautionary principle* into decision-making (Cartagena Protocol on Biosafety (CBD 2018). Precautions must be taken whenever we are uncertain about the ecological consequences of our activities. This principle, however tentatively, also applies to pathogens and pests. Only if ecological consequences could be assessed and predicted conclusively would we dare to signal the deliberate elimination of a species. Such safety of judgement did not even exist for the smallpox virus, which is still being preserved for possible future medical research, including drug development (Joklik et al. 1993).

In view of our limited knowledge about the consequences of eradication, strategies that suppress populations of native pests, without necessarily eradicating them from their entire natural range, are preferred. Suppressing a key insect pest population by the integration of the SIT with other environment-friendly methods often also leads to decreases in secondary pest populations once overall insecticide treatments are minimized, which in turn allows even further reductions in insecticide

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use. For example, as a result of the codling moth sterile insect release (SIR) programme in Canada, leafroller populations (*Spilonata ocellana* (Denis and Schiffermüller), *Choristoneura rosaceana* (Harris) and others) appear to be on the decline (Nelson et al. 2021). This is similar to what organic growers have experienced when natural enemy populations are allowed to increase once insecticide use is reduced (Leach and Mumford 2008). Furthermore, alternative approaches, such as transgenic technologies introducing and expressing foreign genes with antipathogenic properties that interfere with pathogen viability, development or transmission, rather than vector population elimination, should not be ignored (Hao et al. 2001; Van Den Abbeele et al. 2013; Gilbert et al. 2016). These options obviously require that genetic resources be preserved in a viable state.

The precautionary approach does not apply, in the same way, to the elimination of populations of invasive species that have invaded areas outside their native range (Hendrichs, Enkerlin et al., this volume). Application here of the precautionary principle implies the imperative that outbreaks of non-indigenous pests be eliminated to prevent adverse effects on native communities (CBD 2000, 2001). Even then, care should be taken to minimize destabilizing impacts on other species as the non-native one is progressively removed.

It is beyond the scope of this chapter to discuss these hypothetical possibilities in more detail; doing so would involve ethical, philosophical, biological, and ecological discourses. Nevertheless, it is important to point out that these aspects should be considered when designing strategies that attempt the eradication of native species throughout their whole range.

### *5.4. Release of Wildlife from Disease*

Diseases induced by tsetse flies, screwworms, and other insect vectors can adversely affect the health of wildlife, and reduce their survival rates. Conversely, free-ranging wild animals may benefit from the eradication of disease vectors and/or parasites, and increase the size of their populations.

If tsetse flies were eliminated, the immediate benefit to wild mammals would be only moderate, since African wildlife is trypanotolerant to at least those trypanosomes to which they are continuously exposed (Reichard 2002). Yet wildlife could benefit indirectly by spending more time in habitats previously avoided when fly densities were high. For example, the presence of tsetse can deter elephants from riverine forests (Bond 1993). These habitats would become fully available after tsetse eradication, but to the possible detriment of the woody vegetation. Nevertheless, experience has shown that forests are exploited and devastated whenever elephant populations exceed the carrying capacity, irrespective of the presence or absence of tsetse flies (Nagel 1995).

The impact of screwworms on wildlife can be much more pronounced. Prior to the eradication of the New World screwworm from the southern United States, a wide range of mammals was attacked by this parasite. White-tailed deer *Odocoileus virginianus* (Boddaert) populations were highly susceptible to myiasis, with fawn mortality reaching 80% (Reichard 2002; USDA/APHIS 2017). White-tailed deer are hosts to more than 100 disease agents (Schaefer and Main 2001). While these agents

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are seldom, by themselves, fatal to deer, interactive effects may, in periods of stress and malnutrition, weaken animals and cause substantial mortality. After the eradication of the screwworm, a dramatic surge in deer numbers was observed, and this was welcomed by hunters and the game-ranching industry (Reichard 2002). However, it caused a new problem for cattle — they became heavily infested with the deer-parasitizing Gulf Coast tick *Amblyomma maculatum* Koch (Kettle 1993). Moreover, in marginal production areas, increased numbers of deer increased competition for pasture. Overall, however, both domestic and wild animals benefited tremendously from screwworm eradication. This includes the endangered Florida or dwarf key deer subspecies *Odocoileus virginianus clavium* Barbour and G. M. Allen. Its population has stabilized to several hundred animals from a low of fifty in the 1930s (Schaefer and Main 2001). Likewise, the endangered Florida panther *Puma concolor coryi* (Bangs), the bobcat *Lynx rufus* (Schreber), and other predators profited from the increase in the numbers of deer and wild boar *Sus scrofa* L.

Similar effects may occur in other areas subjected to screwworm eradication, yet with different ecological consequences and management implications. The endemic Jamaican hutia or cony *Geocapromys brownii* (J. Fischer) is highly endangered due to predation by the invasive small Indian mongoose *Herpestes javanicus* (E. Geoffroy Saint-Hilaire), as well as stray domestic cats and dogs. Stray dogs, with a screwworm infestation rate of 40% (Hoelscher 1999), are to some extent kept in check by this pest. Releasing cats and dogs (mongoose seems to be less susceptible) from the disease may translate into increased predation on conies, and, unless predator control measures are intensified, upset conservation efforts. Likewise, free-ranging introduced deer, pigs, and goats can be expected to increase in numbers in the absence of myiasis, threatening crops and remnants of natural vegetation. These examples show that the risk of area-wide control differs among ecosystems. Island ecosystems are highly susceptible to the addition or elimination of elements of native food webs. Pest management solutions must, therefore, be adjusted to recognize these specific risks.

### *5.5. Pollinator-Plant Interaction*

Pollination is the most important ecological service provided by insects to flowering plants. Pollinator assemblages comprise a wide range of insects, including blow flies (Calliphoridae) and fruit flies (Tephritidae) (section 3.3.1. regarding male horse flies). Most species in these families are opportunistic, non-specific pollinators. Therefore, the removal of the population of a single species is unlikely to impede pollination. Among the main dipteran pests targeted by the SIT, only some fruit flies comprise obligate pollinators. A mutualistic relationship has coevolved between species of *Bulbophyllum* orchids and a guild of *Bactrocera* spp. fruit fly species, including pest and non-pest species (Tan 2000). Male *Bactrocera* are attracted to orchid flowers by synomones, i.e. chemicals benefiting both the plant and the fly. These are consumed (pharmacophagy), and used further by the males as aggregation and sex pheromones to attract females. Apart from orchids, some species of the Araceae and Lecythidaceae are also pollinated by male fruit flies. It is evident that perturbation of species-specific flower-pollinator systems may result in reproductive

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failure of the plant. In this case, applying the SIT against pollinator fruit flies within their natural range may not be an environmentally acceptable control option. However, apparently, no specific flower-pollinator relationships exist for the principal SIT targets. Furthermore, the Mediterranean fruit fly, the oriental fruit fly, the Solanum fruit fly *Bactrocera latifrons* (Hendel), and the melon fly *Zeugodacus cucurbitae* (Coquillett) are often controlled as non-native pests mainly outside of their natural range.

### *5.6. Predator-Prey and Host-Parasitoid Interaction*

There are apparently no obligate predators of insects targeted by the SIT (Laird 1977; Nagel 1988, 1995). In fact, most predators have opportunistic feeding habits. Therefore, ecologically relevant effects on predator populations are unlikely. The role of parasitoids, however, is much more specific, and needs to be discussed in more detail.

Many hymenopteran larval and egg parasitoids attack fruit flies. Their potential as biocontrol agents has been explored on various scales in several classical and augmentative biological control programmes (Montoya and Liedo 2000). The host range of parasitoids among tephritids varies, but none seems to be host-specific. Therefore, a risk to parasitoids from fruit fly eradication, resulting from an AW-IPM programme using the SIT, is not anticipated.

Contrary to the species-rich Tephritidae and Calliphoridae, the Glossinidae comprise only 23 species. The most frequent parasitoids of tsetse puparia are *Chrestomutilla* spp. (Mutillidae) and *Exhyalanthrax* spp. (Bombyliidae). In tsetse flies, about 40 parasitoid species have been reported, some of which appear to be hyperparasitoids (Greathead 1980). *Exhyalanthrax* spp. have a wide host range among tsetse flies and other dipterans. Some species have been reared from only one tsetse species (Greathead 1980). *Chrestomutilla glossinae* (Turner) appears to be restricted to *Glossina* spp., while *Nesolynx glossinae* (Waterston) (Eulophidae) is believed to have hosts other than tsetse (Greathead 1980). The rate of natural parasitism rarely exceeds 20%, and often is considerably lower. Although some speculation remains with regard to host specificity, experience from other taxa suggests that several parasitoids are specific to certain tsetse fly species (but only about 5–7 tsetse species are of economic importance). Therefore, the possibility remains that parasitoid species would be eliminated along with their hosts resulting in a loss of biodiversity and, possibly, in a diminution of natural control in case of tsetse fly reinvasions.

Females of *C. glossinae* are long-lived, and have exceptional host-finding ability. These traits can be used in biological control approaches. Knipling (1999) proposed tsetse eradication tactics that combine augmentative releases of *C. glossinae* with the SIT. The logical end, however, should the eradication of all hosts from their whole range be achieved, would be the demise of the parasitoid. Even though, at this stage, this scenario is merely hypothetical, measures to conserve mutillid parasitoids should be components of full-range eradication programmes. Conservation can be achieved by establishing and maintaining combined tsetse/parasitoid rearing facilities. This would also provide insurance against the possibility that mutillids or

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other parasitoids are eliminated but not their hosts. Such undesirable effects have been observed in previous tsetse programmes targeting eradication at the population level (Fiedler et al. 1954). In this case, parasitoids could be released to re-establish natural biological control.

### *5.7. Host-Vector Interaction*

Tsetse flies are the main vectors of African trypanosomes. Metacyclic (infective) forms of the parasites colonize the salivary glands, and are transmitted during blood meals. Apart from trypanosomes, saliva may also contain viruses (Sang et al. 1999), bacterial symbionts (Beard et al. 1998), and rickettsia-like organisms (Weyda et al. 1995). This suggests that tsetse could also transmit pathogenic bacteria and viruses, or antigenic fragments of them, to their hosts, evoking concomitant immuno-responses. Therefore, wildlife immunization against bacterial or viral diseases may be enhanced by exposure to pathogens and/or antigens transmitted by tsetse (and other blood-sucking insects), thereby improving the health and fitness of wildlife. However, there is as yet no evidence on which to base such a hypothesis. Some salivary viruses are known to be pathogenic to tsetse flies (Sang et al. 1997; Abd-Alla et al., this volume), but their pathogenicity in vertebrates is unknown. Even if wildlife pathogens and/or antigens were transmitted by tsetse, their contribution to the overall load of transmission would probably be low compared with more abundant blood-sucking species such as horse flies or stable flies *Stomoxys* spp. (Muscidae). So "vaccination services" provided by tsetse may be seen as an odd and far-fetched scenario. Nevertheless, as long as epidemiological data are insufficient to unambiguously dismiss this hypothesis, this topic should be put on the research agenda.

Pre-release immunizations against viral and bacterial diseases are important steps in programmes of wildlife translocation and release. If the animals are to be released in high-density tsetse areas (Woodford 2000), existing protocols even suggest moderate pre-release exposure to vectors of trypanosomes and other parasites to enhance immunization. Allsopp and Phillemon-Motsu (2000) predicted that the eradication of a tsetse population in a given area may cause a temporary rise in endemicity of trypanosomosis. If tsetse disappear from areas in which trypanotolerant breeds were reared, the more productive trypanosusceptible Zebu cattle might be introduced and the genetic trait of trypanotolerance lost. In the event of a reintroduction of tsetse, this loss would seriously disrupt livestock production. These examples show that host-vector interactions should be evaluated beyond established views.

This is substantiated by experiences in the temperate zone, showing that management traditions may be ill-founded. In areas in the UK, with high bovine tuberculosis infection rates, it has been a long-standing practice to cull Eurasian badgers *Meles meles* (L.), the main non-bovine reservoir of *Mycobacterium bovis* Karlson and Lessel. Surprisingly, field trials revealed that culling increases rather than controls the incidence of bovine tuberculosis in cattle, indicating that transmission pathways and dynamics are highly complex and not fully understood (Donnelly et al. 2003).

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These arguments are not raised against livestock-insect pest eradication programmes. Instead they aim to encourage and extend epidemiological research to non-haemoparasitic diseases in eradication zones, and to emphasize the importance of trypanotolerance in wildlife populations and livestock breeds.

### *5.8. Effects of Tsetse Eradication on Land Use*

The area-wide control of disease vectors and parasites may cause a strong increase in the number of domestic and wild herbivores, resulting in significant changes in land-use dynamics and patterns. This issue is particularly controversial with respect to tsetse flies (Grant 2001; Peveling and Nagel 2001). On the one hand, it has been suggested that tsetse flies provide insurance against human encroachment of wilderness areas (Jordan 1986; Chater 2003), and against environmental degradation caused by cattle overgrazing in the ecologically vulnerable Sahel zone (Ormerod 1990). Grzimek and Grzimek (1960) wrote about the tsetse fly:

> It is the only true friend the elephants and zebras have left, for it makes their homeland uninhabitable for men.

Other authors dismiss these notions on the grounds that it is the enforcement of regulations and not tsetse that protects wildlife areas (including gallery forests and dense woodland), and that new areas are opened up to cultivation as a result of human population pressure, independent of tsetse presence or absence (Nagel 1994; Kinley 1998; Feldmann and Hendrichs 2001). Furthermore, African landscapes now plagued with tsetse were thriving pastoral lands rather than wildlife havens until the rinderpest pandemic swept over the continent in the 19$^{th}$ century, depriving once prosperous pastoral societies of their livelihood (Pearce 2000; Feldmann and Hendrichs 2001). From this perspective, fighting tsetse flies means reclaiming land that had already been used by humans for thousands of years. Arguments can be raised in favour of either standpoint (Feldmann et al., this volume).

Pearce (2000) holds rinderpest responsible for the spread of tsetse flies in the 19$^{th}$ century — the demise of cattle led to the transformation of pastures into savannah woodland, providing new habitats for tsetse flies. This mechanism, however, is hypothetical. Typically, cattle promote bush encroachment by selectively exploiting the grass biomass, which in turn favours the growth of tree seedlings and shrubs. Furthermore, because the fuel load of the standing grass crop in heavily grazed savannahs is low, seasonal bushfires are less destructive to the woody vegetation. This idea, that tsetse flies benefited from land-use changes, was already put forward by Ford (1971). In his view, these changes resulted from activities of cultivators and pastoralists who transformed forests into savannah woodland long before trypanosomosis and rinderpest struck the area.

Historical evidence suggests that tsetse flies prevented the migration of early pastoralists into the tsetse "belt" of Africa. Surely tsetse flies and trypanosomosis were a constraint to the colonization of African landscapes for animal husbandry and agriculture, but their presence did not halt this process. For example, a study in Côte d'Ivoire found no indications that recent land-use changes and deforestation were governed by tsetse control (Erdelen et al. 1994; Nagel 1994). Studies in Ethiopia

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found expanding agriculture in areas where tsetse suppression was successful, but overall changes in vegetation cover and structure were low (Reid et al. 1997; Wilson et al. 1997). In other parts of Africa, tsetse suppression may have had a greater impact on rural development (Jordan 1986; Stevenson 1988; Bourn et al. 2001). Conversely, rural development may affect the distribution of tsetse flies. Some species have disappeared due to the expansion of cropland, and the destruction of woodland and riverine forest habitats. Others have adapted to rural landscapes, where they feed mainly on domestic animals (Jordan 1986; Dye 1992).

It has been proposed that the eradication of economically important species of tsetse flies may enable a more-even distribution of livestock, thereby reducing over-grazing and erosion in the labile Sahel and highlands, and may also reduce the poaching intensity in national parks and wilderness areas since eliminating wildlife reservoirs of trypanosomes would no longer be practiced (Feldmann and Hendrichs 2001). This is a desirable but unlikely scenario. The following issues are considered to be equally important determinants of resource allocation and exploitation: food supply and security, land tenure, access to regional and global markets, and political and socio-economic stability. Decisions by people are based on the specific combination of these factors (Jordan 1986, 1992). The key to the sustainable use of natural resources is strategic land-use planning and implementation that mitigate deleterious changes (Grant 2001). This is true irrespective of the severity and extent of tsetse infestations.

In conclusion, it is true that wilderness and protected areas in Africa may harbour dense populations of tsetse flies. However, the conservation of many of these areas is also due to the fact that they are unsuitable as arable land. Moreover, some tsetse species have become resident in rural agricultural areas. Thus, the notion that tsetse flies are key protectors of wilderness areas cannot, as a general rule, be maintained. Finally, human/livestock health and wildlife conservation should not be traded against each other. Both are objectives in their own right, and they require concerted efforts to realize one without hampering the other.

## 6. CONCLUSIONS

In this chapter the principal environmental risks of SIT application and related activities have been reviewed. Some of them have been substantiated by reliable scientific evidence, while others are merely hypothetical and warrant further investigation.

SIT-related activities *sensu stricto* comprise the mass-rearing, sterilization, and release of sterile insects. Few environmental risks are associated with these activities, as long as safety standards and good field practices are guaranteed. However, SIT implementation also involves other supporting activities, and some of these may have adverse effects on ecosystems.

The first relates to pre-release population suppression. Insecticide applications may be harmful to the environment. In many situations, suppression can be achieved with more selective insecticides, some of which have been certified as organic, as well as ecologically sound techniques, such as traps and targets. These can be used alone or in combination, and may provide an environmentally acceptable alternative

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or supplement to spraying non-persistent insecticides. However, adverse effects on selected biota or ecological processes and interactions cannot be ruled out for either method.

Secondly, area-wide control leading to the suppression or eradication of pest populations may have adverse indirect effects. These are often related to insufficient land-use planning and/or the lack of appropriate means of implementation, which in turn may lead to the unsustainable use of natural resources.

The third aspect is of particular importance if native species are targeted. Eradication of a species throughout its range may lead to a loss in species diversity of target and non-target species, including parasitoids and hyperparasitoids. Thus, the SIT can adversely affect host-parasitoid systems. Another aspect refers to the risk to pollinators, which has to be taken into account in all AW-IPM programmes that integrate the SIT. Finally, the immunological and epidemiological consequences of the transmission of non-haemoparasitic disease agents by vector insects, and the consequences of a loss of trypanotolerance in wildlife and livestock populations in tsetse infested areas, are open questions.

Optimum combinations of different control methods, resulting in the lowest cumulative risk, while achieving the highest possible effect on target populations, vary among species, regions and ecosystems. Thus, environmental assessments may reach different conclusions in different environmental situations, and pest management solutions must be adjusted accordingly.

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# CHAPTER 5.3.

# MANAGEMENT OF AREA-WIDE PEST MANAGEMENT PROGRAMMES THAT INTEGRATE THE STERILE INSECT TECHNIQUE

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## SUMMARY

Effective management of area-wide integrated pest management (AW-IPM) programmes that integrate the sterile insect technique (SIT) is key to their success. Programme planning includes collection of baseline data and technical and financial feasibility assessments. Programmes should be initiated only if there is strong stakeholder support and political long-term commitment. The optimal management structure permits the programme to be implemented effectively and flexibly, as independent as possible of government politics and routine bureaucracy that can impede timely goal achievement. At the same time, high-level oversight is required to avoid corruption and to benefit from expert committees that provide independent technical advice. Ideally, programmes include a mixture of public management and the outsourcing of some routine tasks to private management. They require strong and steady financial support. Governments and donors are the most common sources of funds, but a mixture of public, community, and private funds is now the trend. Interrupted cash flow severely restrains programme performance. Physical support of programme infrastructure and operations must be reliable, and led by a maintenance professional. It is essential to have full-time, well-paid, and motivated staff led by a programme manager with technical and management experience. Poor management and inadequate support often result in programme failure.

## 1. INTRODUCTION

Even though the technical elements of area-wide integrated pest management (AW-IPM) programmes that include the sterile insect technique (SIT) are critical to their success, the management components are of equal importance. However, more emphasis is usually placed on getting the "science right" than getting the "management right". Failure of an area-wide programme is usually due to poor management and inadequate support and not to how well the scientific technology is implemented.

Management is important for AW-IPM programmes with an SIT component because, firstly, these programmes affect large segments of the community, as do other more generally accepted area-wide activities such as police protection, electricity supply, or other services, where virtually everyone within the concerned area is involved (Knipling 1980; Lindquist 2001; Hendrichs et al. 2007; Klassen and Vreysen, this volume). This necessarily involves public financing, public accountability, legislation, enforcement, community participation, substantial infrastructure and organization, long-term support, and high levels of managerial skill. Secondly, as the SIT requires the successful handling of live insects, which have a very short "shelf life", as well as a number of other activities that need to be carried out in a defined time and space sequence, flexibility and high levels of technical responsibility to conduct and coordinate operations effectively and in a timely manner are essential.

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Reyes et al. (1988) described the general organization and structure of an AW-IPM campaign involving the use of the SIT. This chapter discusses generic aspects of programme management and its impact on programme success or failure.

## 2. FEASIBILITY ASSESSMENT

Technical and economic feasibility assessments are essential, e.g. DeBiasio (1988) on the codling moth *Cydia pomonella* (L.). They address financial issues such as funding and budgets (costs and benefits) (Rhode 1975; Mumford, this volume), expected benefits other than financial, the biological and practical feasibility of achieving the goal of pest suppression or eradication (Rhode 1970), details of the proposed organization and administration, and activities needed to conduct the programme. Also, the pest situation, current control methods, impact of the pest on agriculture, the environment and human health, and the complexity of the human, biological and physical environments must be assessed. An assessment of the political and fiscal stability of a country, government priorities, governance, public and programme security, social and economic values, and economic development all impact on how feasible it would be to conduct an AW-IPM programme that applies the SIT.

The baseline data needed for a feasibility assessment are: (1) data on the area and commodities (for domestic consumption or export) or livestock/human population to protect, (2) data on population ecology, dynamics, and distribution of the insect pest in the proposed area using geo-referenced data, and an assessment of potential immigration of the insect into the area, (3) assessment of the pest problem (Tween 1993) — losses, both direct and indirect, caused by the pest, and the impact of the pest on human health, agriculture and the economy, (4) cost and effectiveness of current pest control methods, and their disadvantages, and (5) impact of the pest and of current control methods on the environment (Tween 1993; Vreysen et al. 1999; Nagel and Peveling, this volume; Vreysen, this volume). Decision-support tools, such as geographic information systems (GIS), remote sensing (RS), population genetics (Krafsur and Ouma, this volume), and mathematical modelling (Bouyer et al. 2010; Barclay, this volume; Bouyer et al., this volume) can greatly assist in feasibility assessment and planning of these programmes.

## 3. PROGRAMME PLANNING

A programme can be planned properly only if the baseline data are available, and all required feasibility assessments have been carried out.

If such assessments show that a programme is technically feasible, economically viable, and socio-politically acceptable, detailed plans can then be made and incorporated into a programme document or business plan (FAO/IAEA 2008). Such a document provides details of programme strategy, milestones, operational procedures (including standard operational procedures (SOPs)), budgets, plan for public relations activities, and management policies and procedures.

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A legal agreement between the programme organization and commercial farmer associations, other stakeholder groups, and local and regional authorities, may be needed (Lindquist 2000). This agreement would describe responsibilities and authorities of all parties, in terms of financial and in-kind contributions, monitoring activities, the required level of pre-release suppression, sterile insect quality, expected insect deliveries, identification and handling protocols, expected degree of insect control, penalties for lack of compliance or poor results, and insurance coverage (Nelson et al. 2021).

Unfortunately, there is a tendency to begin implementation before planning is complete (Vreysen et al. 2000; Itô et al., this volume). A premature start can easily cause the programme to fail, or at least perform poorly, leading to discouragement, and possibly to abandonment of the programme. All components of the programme must be ready and harmonized, including a carefully selected and properly trained staff, before operations can begin. Sometimes programmes can begin on a pilot scale, to provide hands-on training for staff and to test all components, or start with areas in which success can be more easily achieved, and then move on to larger or more difficult areas. The lessons learned in the pilot programme are useful in making plans for the large-scale programme, and in any case flexibility (Norton 1986) is always needed as programmes proceed. In cases where a pest emergency occurs, such as an invasion or introduction of a new pest (Patton 1984; Hendrichs, Enkerlin et al., this volume), a rapid response is essential and proper planning is usually not done, and ad hoc procedures have to be adopted with the attendant high risk of failure. However, in spite of the crisis of the New World screwworm *Cochliomyia hominivorax* (Coquerel) invasion into Libya, the appropriate time was still taken to develop a plan of action for an AW-IPM programme (Lindquist et al. 1992).

If programmes are aimed at eradication, especially of pests that attack export crops, plans to continue the appropriate activities indefinitely, e.g. pest monitoring, quarantine, and contingency plans and readiness to re-eradicate outbreaks if needed, should be included (Krafsur et al. 1987; Reyes et al. 1988; Lindquist et al. 1992; Barclay et al., this volume; Hendrichs, Vreysen et al., this volume; Vreysen, this volume).

## 4. MANAGEMENT STRUCTURE

AW-IPM programmes tend to be large in size, complex, comprehensive, flexible, dynamic, intense, and demanding, and are relatively long-term. They require multidisciplinary teams of technically knowledgeable people with special skills to integrate different control methods, an organization involving whole communities and thus needing public and political support at many levels, and significant facilities and equipment. It is a real challenge to conduct such programmes, and both public and private sectors of society need to be educated and encouraged to play their essential roles in the programmes (Dyck, Regidor Fernández et al., this volume; Enkerlin, this volume).

AW-IPM programmes cannot be conducted by individual beneficiaries such as a farmer on a single farm or one individual in a community. Instead, an organized

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group, in the area defined by the distribution of the pest population, must be formed that coordinates the implementation of harmonized activities on an area-wide basis (Tweddle 2002; Vreysen et al. 2007; Klassen and Vreysen, this volume). Therefore, it is essential that coordination among all stakeholders is effective, and that tasks and procedures are well described and clear responsibilities shown.

### *4.1. Government Management*

Traditionally, SIT-related programmes have been managed "top-down", where the planning and directing is done by hired programme managers, usually government employees such as was the case in the melon fly *Zeugodacus cucurbitae* (Coquillett) programme in Japan (Yamagishi et al. 1993), where national and prefectural technical specialists directed the activities. The eradication programme for the New World screwworm in Libya (FAO 1992) was strongly supported by the national government and international donors, and managed by a team of international technical specialists and their Libyan counterparts. Strong support for a programme from its stakeholders is essential for its success (Hendrichs 1986).

A government-led programme has the advantage of political, social, and legal authority in the programme area, and therefore persons in the public sector appropriately may be programme staff members. This may involve provincial and/or federal laws which can authorize the appropriate office and personnel to direct the programme, and also provide a revenue source and budget. For example, in Mendoza, Argentina, the provincial law number 6333 (1995) determines responsibility for plant and animal protection. A self-governing institute, ISCAMen, was created to deal especially with the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann). Each year ISCAMen receives a budget provided by law through the Provincial Congress; its economic independence permits funds to be dispersed without diversions elsewhere and enable it to fulfil its legal commitments.

Political independence and power in the hands of a few managers, even though usually an advantage for an action programme where some flexibility in decision-making is essential and operations are time-critical, can nevertheless under certain conditions become a disadvantage. Empowerment of people can become a real detriment to success if the programme then becomes burdened by political concerns and vested interests. Those not accustomed to having access to large amounts of money and authority may be tempted to use it unwisely, jeopardizing the programme. Therefore, empowerment needs to be accompanied by some oversight regime and should not be granted unilaterally, but only after individuals and groups have demonstrated the ability to manage resources in the best interests of the programme.

However, AW-IPM programmes almost always require both "top-down" and "bottom-up" elements for effective management. The local community and direct clients can influence the programme from the "bottom-up", such as the initiative shown by ranchers in the USA to get screwworm flies eradicated (Baumhover 2001), and by fruit growers in Canada to study the feasibility of a sterile insect release programme for the codling moth (Dyck et al. 1993). Community participation, at some level, is also essential to obtain the cooperation of farmers

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(and sometimes the community at large) for pest suppression activities prior to the release of sterile insects, pest monitoring, quarantine, and publicity. Where pest suppression has to be carried out in residential areas, e.g. in the case of *Aedes* spp. mosquitoes, the active participation of homeowners is essential. When an outbreak of the New World screwworm was discovered in the Florida Keys, the travelling public was required to stop at roadside checkpoints to permit pets to be inspected in case they were infected (Robles 2016; USDA/APHIS 2017).

Nevertheless, the area-wide nature of programmes, and the technical activities of sterile insect production and release, almost always need a centralized organization led by experienced and knowledgeable managers. For transboundary programmes involving more than one country (where the pest distribution range includes areas in two or more adjacent countries), a multinational or international organization is required to coordinate the activities (FAO 1992). Such an organization may introduce cost savings that are not feasible if there is only a national programme, and a regional approach to pest control often has technical benefits (Enkerlin et al., 2017). Selection of a management structure for a programme must be made carefully since, once it has started, it will probably be impossible to change it.

### *4.2. Private Management*

Ideally, AW-IPM programmes include a mix of public and private management, but the balance depends on the local situation, and each case is different. Private companies are often cost-effective and dependable for routine tasks, e.g. maintenance and security of a rearing facility, purchase of materials, insecticide applications, aerial release of insects, and research contracts, but supervision of any out-sourced activity is essential to maintain the quality of the programme (Barnes 2007; Bassi et al. 2007; T. Matsuyama, personal communication). However, the final cost to the community may be high if there is not enough competition among companies, and private firms may take a large profit. In Mexico, an international institute contracts with the government to provide administrative services (with an 8% overhead charge) in the *Anastrepha* spp. rearing facility, resulting in an efficient work force and no labour union conflicts. In Argentina, a public-private foundation, hired by ISCAMen, provides contracts to personnel for the programme.

Another option is for a private firm to manage a programme, especially a permanent suppression programme, and where there is a continuous demand for sterile insects. A private company in The Netherlands (de Groene Vlieg Bio Control b. v.) (DGVBC 2018) produces and releases sterile *Delia antiqua* (Meigen) flies as a suppression pest control method for onion growers who request this; these growers pay for the monitoring and release of sterile flies on their fields (Loosjes 2000; DGVBC 2018). This programme now covers about 40% of the onion production area in The Netherlands (M. Loosjes, personal communication). In South Africa, FruitFly Africa (Pty) Ltd. handles insect rearing, sterile fly distribution to release areas, coordination of aerial baiting, and technical support for a Mediterranean fruit fly suppression programme (Barnes et al. 2015; Barnes 2016), and another company, Xsit (Pty) Ltd., was created to produce and release sterile false codling moths *Thaumatotibia leucotreta* (Meyrick) (Hofmeyr et al. 2015). A private company

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established to produce and release sterile codling moths in South Africa operated on a pilot scale for several years, but closed down in 2014 (Barnes et al. 2015).

Interest is increasing for private companies to supply sterile Mediterranean fruit flies in Israel and Croatia (FAO/IAEA 2012a; Bio-Fly 2018), and tsetse flies *Glossina* spp. in Slovakia. The risks of a business based on biology, and of matching correctly the demand for, and production of, sterile insects, are a significant challenge for private business (Barnes 2007; Bassi et al. 2007). Production contracts must specify the required quality of the insects. Nevertheless, commercialization of sterile insect production, with the potential cost reductions (as already shown by successful companies producing biological control agents), is likely to become increasingly common (Quinlan and Enkerlin 2003; FAO/IAEA 2008). Companies involved in the SIT strengthen the local economy because they produce value-added products and create jobs (Hofmeyr et al. 2015). However, the area-wide nature of SIT field operations, with the consequent involvement of various stakeholders and the public, complicates privatization of these activities. To attract private investors, the industry supporting an AW-IPM programme must be robust and strong.

If insect rearing and field operations are managed by two or more different organizations, it is important that misunderstandings and poor coordination between them do not develop. A lack of coordination would be awkward at best, and disastrous at worst, for a programme. One organization with all programme components is the easiest to manage — the programme manager has authority over, and can coordinate, all components. However, when this is not the case — such as a programme that purchases sterile insects from another, possibly distant, organization, and a third one is contracted to release them (Quinlan and Enkerlin 2003; Cayol et al. 2004; Enkerlin and Quinlan 2004; Dowell et al., this volume) — ways must be found to ensure strong cooperation between the organizations. If a programme is implemented by more than one agency in a joint undertaking, the roles of each agency must be clearly defined. To maximize effectiveness and efficiency, it is essential that the authority for day-to-day operations be held by the executing organization (Rhode 1969).

Corruption can be a significant problem for programme managers, and AW-IPM programmes with large budgets, such as those that use the SIT, are especially vulnerable to it. Examples of corruption are: (1) diversion of funds and materials for personal use or for other units of government, (2) personal use of equipment such as vehicles, and (3) personal or political favouritism or nepotism in selecting or rewarding staff, and in selecting (or obtaining kickbacks from) suppliers or companies providing services. Paying bribes, e.g. to customs personnel, cannot be done officially, but sometimes for the sake of programme efficiency it is possible to reward cooperators in a culturally acceptable way. Political disturbances and civil unrest are also a threat to programme operations (Rhode 1969).

## 5. OPERATIONAL FLEXIBILITY AND INDEPENDENCE

Due to the rather unique challenges of managing an AW-IPM programme, there will almost always be better results if the organization is politically and financially autonomous, and independent of the usual government bureaucracies, and politics,

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and sometimes corruption, that reduce efficiency and block progress (Hendrichs 1986; Box 1). Insufficient operational independence, and the burden of stifling administrative restrictions, can cause programme failure.

*Box 1. Tsetse Eradication Programme in the Niayes Region of Senegal (Vreysen et al. 2021)*

The programme adopted an "adaptive management" approach (which included monthly project coordination meetings with the various stakeholders, including researchers) that, as stated in EXPO 2015 (2015), ensured transparency and decision-making by consensus. Decisions were based on scientific (not political, personal or emotional) principles, and were guided by analysed field or other data. Day-to-day operational and financial problems were openly discussed and solutions found. Any decision that required follow-up action by the different actors was immediately implemented according to project plans. The collaboration between internal stakeholders and international partners, and a policy of "non-interference" by government ministries, enabled a smooth implementation of the programme.

Operational independence, with a focused and goal-directed agenda, can produce stability and remarkable achievements. The melon fly programme in Japan was conducted through a special organization, the Okinawa Prefectural Fruit Fly Eradication Project Office. This office was independent, like a trust, and had considerable flexibility and freedom to conduct operations and follow procedures conducive to programme success. The prefecture also provided administrative support. The Mexico–United States Commission for the Eradication of the Screwworm, established in 1972 for the AW-IPM programme on screwworm flies (Krafsur et al. 1987; Vargas-Terán 1991; Wyss 2000; Baumhover 2001), represented such an independent but legal body that had the authority but also the flexibility to "get the job done" effectively and efficiently. This commission, or its current follow-up, the Panama-United States Commission for the Eradication and Prevention of the Screwworm (COPEG), was exempt from the usual bureaucratic rules, and decentralized from government with an independent management, with its own regulations, accountability, and financial audits (Tweddle 2002). Another example is the Programa Moscamed, which implements a trinational (Guatemala/Mexico/USA) Mediterranean fruit fly programme (Enkerlin et al. 2017). The eradication programme for the New World screwworm in Libya (FAO 1991, 1992; Lindquist et al. 1992) was operated under a specially created organization, Screwworm Emergency Centre for North Africa (SECNA), which had the required independence and authority to achieve its objectives. The same is true for programmes operated by ISCAMen in Argentina – Mediterranean fruit fly, codling moth, European grapevine moth *Lobesia botrana* (Denis and Schiffermüller), and other species (section 4.1.).

On-site programme managers have not only the responsibility to make a programme successful, but also require the authority to make management decisions in a timely manner so that the programme can achieve its goals. This should include authority to dismiss an employee for unproductive or disruptive performance (Rhode 1969).

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## 6. PROGRAMME STAFF

An adequate number of qualified, competent, responsible, goal-oriented, motivated, dedicated, and hard-working staff members, having a high level of performance and morale, are essential to programme success (Lorraine and Meltsner 1987). Employees must have "can do" and "do it today, not tomorrow" attitudes. Staff recruitment should be done well in advance of the time when employees will be needed.

Staff, especially the managers, ideally must be full-time workers to encourage a high level of commitment and provide strong leadership, preventing other interests or responsibilities from diverting attention away from the programme (Hendrichs 1986). In the past, some programmes have suffered greatly because the leading professional staff could not spend the required time on the programme due to other duties or insufficient government salaries (Box 2). In the programme in Senegal (Box 1), project staffing (managers and technical personnel) was stable, with little turnover in 12 years. This created a staffing culture of reliability, transparency, and trust, ensuring the necessary institutional memory (Vreysen et al. 2021). To prevent the loss of accumulated knowledge about programme operations, competent staff must be encouraged to remain in the job and not be subject to staff rotation for bureaucratic reasons.

It is important to provide incentives for staff to maximize job commitment and continuity, and to improve their performance on the job. For example, higher than normal salaries, or special incentive pay based on performance, can be given to prevent staff taking on competing extra jobs to earn more money. High salaries also attract the best workers and discourage resignations to take more lucrative positions elsewhere. Staff members taking higher risks than usual, such as persons supervising the release of sterile insects from aircraft, should receive appropriate compensation and life insurance coverage. If the organization is unique or independent of government, promotion and the accumulation of pension benefits may not have much relevance to employees of the programme, and therefore a high salary is justifiable.

Job security and opportunities for promotion help to maintain job satisfaction. To make workers feel comfortable and able to concentrate on their tasks, safe practices in the work environment should always be a priority. It is vital that there be a high level of *esprit de corps*, using various forms of recognition and reward for good performance to encourage employees and make them feel proud to be a part of the programme; a positive work environment also discourages thoughts of sabotage. A biological programme has a high degree of dependence on the good performance of the programme's workers; sloppy work from one employee can negatively impact the whole programme.

The appropriate expertise needed for all of the various operations must be available among the programme staff, not only biological and scientific but also engineering and managerial expertise. This is especially true of the management of insect production (Fisher 1984; Leppla 1984; Schwalbe and Forrester 1984; Fisher 2009). If the appropriate persons needed to operate a programme are not available, it

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is better not to start than to start and fail. Incompetent management leads to programme failure.

*Box 2. New World Screwworm Eradication Programme in Jamaica — Lessons Learned (Vreysen et al. 2007)*

The programme began releasing sterile flies in 1999, but by mid-2004 little progress had been made. A special management configuration, which could operate independently of existing inflexible regulations of the government, was never established. Instead, the programme was embedded in existing government structures, and senior staff continued with their normal animal health responsibilities. It was technically and financially supported by several "outside" stakeholders. The Government of Jamaica purchased the sterile flies from the Mexico-US Commission that, at that time, operated the only screwworm mass-rearing facility in the world (located in Tuxtla Gutiérrez, Mexico).

The programme was implemented on the premise that the screwworm SIT technology was infallible. Consequently, emphasis was placed on operational procedures rather than on strategic considerations that took local conditions into account. Most of the problems encountered in the programme were not related to the SIT technology *per se,* but were due to a reluctance to address problems in a scientific way. Although there were many factors that contributed to the lack of programme progress, the following were the most significant:

- The importance of collecting appropriate and sufficient baseline data on screwworm population ecology and dynamics prior to the initiation of sterile insect releases was not recognized. Sterile insects were dispersed using long-established protocols (which proved their usefulness in other countries under different environmental conditions) that did not take into account the distribution, and spatial and temporal fluctuations in density, of the local screwworm population. Consequently, the density of the screwworm population was greatly underestimated, and insufficient attention was given to adequate population suppression prior to and during the releases. The programme adhered to a dogmatic belief in the "supremacy" of the sterile flies, and ignored an important lesson from history, i.e. screwworm SIT cannot succeed without efficient field operations that continuously suppress the native fly population.
- Appropriate field data to monitor programme progress (Vreysen, this volume) were not collected. Evaluation of the programme was based solely on the number of reported positive screwworm cases, a very crude parameter influenced more by the willingness of farmers to collaborate and submit samples than by the effect of sterile flies. No attempts were made to systematically collect information on mating frequencies between sterile male and wild female flies. It was always assumed that the released sterile insects would perform adequately, and any potential inferior competitiveness was not considered. Many decisions were based on assumptions, the underlying causes of the lack of progress were never identified, the real problems were never rectified, and the programme was allowed to "drift along" for several years.
- Privatization of the veterinary services created a conflict of interest for veterinarians working for the screwworm programme. The more animals they treated against screwworms, the more money they received; this discouraged the veterinarians from working towards screwworm eradication.
- AW-IPM programmes that integrate the SIT are inherently complex, and require flexibility to react swiftly to changing pest population distributions and densities, and to solve emerging problems. A flexible and proactive management style is required to ensure continuity in the implementation of all important programme components. Any interruption in a critical component will automatically result in severe setbacks. This is especially true in programmes against the New World screwworm fly (which has a very short life cycle — 21 days in optimal conditions). Fig. 1 shows that, for the Jamaica programme in 2003, optimal implementation conditions, i.e. continuity in all important components, were achieved only 52% of the time (during 27 of 52 weeks). Although some of the problems such as the interruption in the dispersal of sterile flies, or the accidental release of fertile flies, were beyond the control of the programme, the malfunctioning of the chilled fly unit (which reduced both fly quality and the number of sterile flies released), and the interruption in the employment of all or at least some temporary field inspectors, could have been avoided.

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*Box 2. Continued*

- The lack of an independent centralized management structure, with the responsibility, authority and flexibility to implement all programme components, was a major constraint and at the core of many persistent problems:
  - Programme staff members were usually civil servants, and especially the senior persons were assigned many other duties and responsibilities outside of the screwworm programme. The complex nature of AW-IPM programmes demands the full commitment and complete dedication of senior staff, and the many diversions caused by the other duties were detrimental to the programme.
  - Due to the absence of locally available SIT-related expertise at the onset of the programme, expert advice had to be provided continuously by internationally recruited technical advisors. However, these scientists were authorized only to give advice and thus could not play a significant role in programme decision-making. Many management decisions were unduly influenced by political concerns instead of scientific principles and field data.
  - Government regulations, that imposed severe restrictions on programme implementation, had to be followed, e.g. the programme could not employ temporary field staff according to the changing needs of the programme over time.
  - Some critical components of the programme were beyond the control of the managers. For example, there was only one source of sterile screwworm flies, the screwworm production facility in Mexico. Delivery of sterile insects to Jamaica was interrupted twice due to labour unrest at the facility. The second interruption (in 2001) lasted for 38 days, a time equal to almost two screwworm generations, and as a result all progress made earlier was lost. Another catastrophe occurred in January 2003 when, due to a malfunctioning irradiator at the production facility (Merrell 2003), a batch of at least 400 000 fertile insects (potentially 200 000 fertile female flies) was delivered to Jamaica. The upsurge in screwworm cases after this incident is hardly surprising in view of the very large potential for female screwworm flies to proliferate (each fertile female can, in her lifetime, produce several egg masses, each with up to 400 eggs). A third disaster occurred when a hurricane passed through the area, causing damage to the fly release facility and interrupting sterile fly releases.
- The importance of culturally determined factors, such as the attitude of the public and farmers towards animals, was severely underestimated. The rough handling of pets and livestock, creating small wounds (and oviposition sites for flies), generated ideal and persistent opportunities for the fly population to sustain itself. Obviously, the public education activities were not enough to change this attitude. This problem could have been circumvented by not relying on farmer participation, but instead employing more permanent or temporary field staff to proactively screen animals and treat both infested and uninfested wounds with insecticide; government regulations and a tight budget prevented such actions.
- The programme was impaired by having too many stakeholders, each with a slightly different interest and agenda. Often the result was political and diplomatic dithering rather than firm and focussed decision-making. As a consequence, an attitude of "getting the job done", a prerequisite for programme success, was never developed.

If there is a conflict of interest, such as between insect production and field work, or between insect production and quality control, the lines of authority must permit staff to report their results to managers who will not be biased, e.g. product quality control activities should be done by an organizational unit that is not responsible for sterile insect production (FAO/IAEA/USDA 2019; Parker, Mamai et al., this volume; Parker, Vreysen et al., this volume).

In addition to producing a quality product, applying quality management practices in field operations, releases and administration, based on the implementation and certification of standards from the ISO (International Organization for Standardization), ensure the best and systematic procedures

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without using subjective standards. Therefore, written procedures for all strategic steps in the various processes should be followed, e.g. field operations, mass-rearing, sterile insect release, etc.

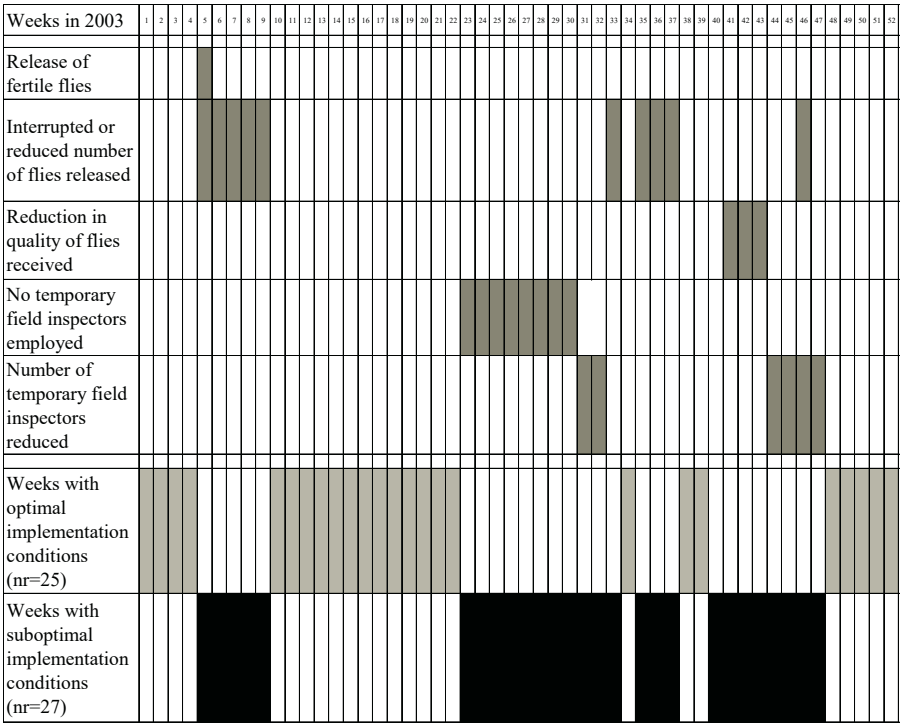



*Figure 1. Diagram showing, during one year, the weeks with suboptimal and with optimal implementation of the sterile fly releases and field activities in the New World screwworm programme in Jamaica.*

Labour-management relations must be kept positive to maintain staff morale (H. Hofmeyr, personal communication). Refusal to accede to union demands can lead to strikes or work sabotage, while capitulation results in the programme becoming a hostage and subject to blackmail. A labour strike, and the consequent failure to operate a biologically robust programme, can severely retard and even jeopardize the programme's success. Labour strikes at the New World screwworm production plant in Tuxtla Gutiérrez, Mexico, caused delays in the progress of the Jamaican screwworm eradication programme because it depended on this sole source of sterile screwworms (Box 2).

Especially if staff motivation is low, negative personal habits, attitudes, and values, and even local customs, can create significant problems. If not taken into account and creative solutions found, holidays, religious practices, personal behaviour patterns and conflict among staff members can reduce programme efficiency, and lead to programme losses. Rearing and handling sterile insects is a

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24-hours-per-day and 365-days-per-year job. In ways that do not offend individuals and local customs, the insects must somehow be given the first priority. After a clear explanation of the reason for timely actions, properly motivated workers will usually respond with the kind of behaviour that is good for the programme, especially if recognition and compensation for exceptional work are being given.

The need for timely action is not only true for the biological elements of a programme, but managerial actions must also be carried out at the appropriate time (Kakinohana et al. 1993). A delayed management decision, just like a delayed biological activity, could result in programme termination. In the Jamaica New World screwworm eradication programme (Grant et al. 2000), interruptions in critical programme components, such as the employment of temporary field inspectors, resulted in severe programme setbacks (Box 2, Fig. 1).

In addition to holding training courses on the specific skills needed in the various tasks in the programme, e.g. field operations, mass-rearing, sterile insect release, etc. (section 6.1.), a Human Resource Management team in the programme or "parent institution" is needed to provide onsite training on all aspects of management and decision-making.

Due to a shortage of funds, or for convenience, volunteers such as local farmers may be recruited to perform certain tasks, e.g. set up insect traps or distribute a lure-toxicant (Teruya 2000; T. Matsuyama, personal communication). The screwworm programme in Central America developed an honorary-inspector scheme that effectively complemented the activities of programme field workers. However, experience has shown that sometimes volunteers do not perform properly, and therefore close supervision is indispensable; the critical operations of a programme should not be assigned to volunteers.

### *6.1. Training*

Specialized training for the various tasks related to SIT implementation is essential; it improves performance directly, and also represents an incentive for staff. It is important to train back-up employees so that the work usually done by a person who is absent can continue.

There are many national training programmes on insect pests, e.g. Florida State Agricultural Response Team (FSART 2012), as well as degrees in entomology offered by universities, but virtually none focuses on aspects of area-wide pest control and SIT application (one example is the specialized course "International Insect Rearing Workshop" (MSU 2018) offered periodically by Mississippi State University). Therefore, on-the-job training often has to be provided internally by the programmes themselves. For example, in the tsetse mass-rearing insectary in Ethiopia, the colony's performance improved substantially after the implementation of a permanent training programme. This programme was conditioned on the performance of the project and aimed at motivating the staff in two ways: by delivering specialized training on the SOPs in the different activities of the project, and by stimulating the staff to attend training sessions after the regular working time with an overtime compensation fee. Another powerful motivation tool is to offer deserving staff (those who have shown an outstanding aptitude and level of

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performance) the possibility of enrolling in flexible postgraduate studies (MSc, PhD) in local universities, as long as this doesn't interfere in the performance of their daily activities in the programme and the topic of the research component is on an operational aspect of the programme. This requires an additional effort on the part of the student and on that of the programme, but benefits both the student's personal career and the programme's technical capacity (R. Argilés Herrero, personal communication).

Over many decades the FAO/IAEA (Food and Agriculture Organization of the United Nations/International Atomic Energy Agency) has been providing specialized national and regional training courses on various aspects related to implementing the SIT. Such courses provide opportunities to accommodate specific language and local issues, e.g. training courses on applying the SIT against insect pests in Brazil, Asia and Africa (IAEA 2016). Training courses may include a wide range of subjects: insect biology and mass-rearing, induction of sterility, field operations -- monitoring and sterile insect release, and management issues such as economics, financial planning, staff management, project implementation, public relations (Nature 1975; Jayaraman 1997; Dyck, Regidor Fernández et al., this volume), import/export requirements, and risk management. Most importantly are specialized training opportunities and in particular comprehensive interregional training courses for managers that are organized periodically by the FAO/IAEA at sites of operational programmes (FAO/IAEA 2018a).

If a programme results in pest eradication, there would appear to be little need in the future for the acquired knowledge and skills on that pest in that area. Nevertheless, experience shows that the knowledge and generic skills on AW-IPM acquired in a successful programme can be used by animal and plant health authorities in area-wide programmes against other pests or the same pest in other areas.

### *6.2. Risks*

There are significant risks associated with the manned aerial release of sterile insects. Every effort must be made to employ (or contract companies with) experienced and skilled pilots, and competent maintenance workers, and to use the best available equipment and facilities. Nevertheless, aircraft accidents do occur.

There were ongoing fatalities due to aircraft accidents in the New World screwworm eradication programme in Mexico and Guatemala from 1972 to 1992. However, for more than 25 years now, there have been no aircraft-related fatalities during the Central America and Panama programmes (J. H. Wyss, personal communication). Also, the aerial release of sterile false codling moths using gyrocopters in South Africa was not accident-free -- three pilots were lost in accidents. Subsequently, releases were made using fixed-wing aircraft (S. Groenewald, personal communication). To avoid risk to the lives of pilots and accompanying personnel, a significant effort is now being made in several programmes to test remotely operated drones for releasing sterile insects; this is a welcome development (PFR 2018; Boersma 2021; Dowell et al., this volume).

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Also, workers using irradiators must face risks, even though extensive safety features are built into modern irradiation equipment (Bakri et al., this volume). In most countries, the use of irradiators is not permitted by national nuclear authorities unless programme operators have been thoroughly trained in regulatory and safety procedures, using written guidelines and supervised by an instructor certified by the national nuclear authority.

## 7. PROGRAMME MANAGER

The management of AW-IPM programmes that integrate the SIT is multifaceted and intensive. Administrative tasks can be done by persons with a business orientation, but the senior managers have a much larger responsibility — to give overall leadership to the programme! Ideally, besides being able to manage personnel, infrastructure, finances, and the political side of the programme, and to make difficult decisions, managers should also have theoretical and practical knowledge about relevant technical matters, and have experience in conducting area-wide and complex insect control programmes. Many of the day-to-day decisions in a programme require sensitivity to critical biological issues, including environmental issues (Kinney 1993). Managers must be convinced that the technology being used will achieve the programme's objectives. Area-wide programmes require strong and persuasive leadership. Managers must be able to communicate effectively with the programme's stakeholders (Patton 1984; Dyck, Regidor Fernández et al., this volume), but still promise only realistic deliverables (Barnes et al. 2015).

An "ideal programme manager", i.e. a person who has all of the qualifications needed in a programme manager, is difficult to find. In science-related institutions the senior manager is usually a scientist, and all of the other needed skills and knowledge are acquired secondarily. However, sometimes the senior manager is an administrator, and the scientific skills and knowledge are acquired secondarily. Both situations can function satisfactorily, but most often there is a need for additional personnel to be hired to complement the programme manager in the subject areas that need strengthening, e.g. administration or technical matters. Such a team-approach is the one most likely to manage a programme successfully.

Quite often the nomination of programme managers is done at the political level. Although there is an undeniable political dimension to large operational programmes, and strong links to political authorities is an important asset, it is also true that such programmes can be logistically complex and technically demanding, requiring an adaptive management to be successful. Therefore, it is essential to have a technical manager with knowledge and experience in all SIT-related activities (including field activities, sterile insect releases and mass-rearing) and with an executive capacity for decision-making at the technical level (R. Argilés Herrero, personal communication).

The programme manager, and similar senior positions in the management structure, must be chosen subsequent to official competitions that really provide for fair, thorough, and unbiased assessment and selection procedures.

Since there are relatively few persons in the world who have all of these required qualifications, hiring an experienced manager from outside the country may, at least

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initially, be appropriate (FAO/IAEA/TANZANIA 1994). In fact, due to cultural norms, an "outsider" can sometimes achieve things more efficiently than a local person. However, for a number of local reasons, an external expert is often only accepted as a co-director (FAO 1992) or as an advisor to the national director, which can have negative consequences (Box 2). Normally, a programme manager operates under the guidelines and oversight of a board drawn from the official supporting agencies.

If a government takes a leadership role in programme management, it can produce mixed results, since standards of good governance vary tremendously among countries. A risk of government-run programmes is that senior management positions may be seen as political rewards, independent of managerial and technical competence. Also, if a government changes, there is the potential for staff changes, and this lack of continuity could disrupt a programme (Rhode 1969).

The suggestions made by Lorraine and Chambers (1989) in the context of the 1980–1982 California Mediterranean Fruit Fly Programme are worth noting. In urban entomology, with today's increased participation of community activists and special-interest groups, it is a major challenge to deal with complex public policies and programme activities that are not derived from experimental sciences. In modern life, there is no longer a "best solution" but only bargained negotiated compromises that more or less balance competing interests. Coping with the public's desire to participate in decision-making, and even having public confrontations over technical issues, are rather new experiences for most managers of AW-IPM programmes.

## 8. FINANCIAL SUPPORT

AW-IPM programmes using the SIT may operate over large areas, and sometimes involve major facilities and equipment. The financial resources required may also be large. Even though a programme might be economical on a benefit/cost basis (Mumford, this volume), it is not always affordable, and obtaining start-up and operating funds can be the most important issue facing a programme. The type of financing used affects programme strategy and operations, the duration of programme support, and the reliability of this support.

Enkerlin (this volume, section 3.1.) provides an extensive summary of various funding options for AW-IPM programmes that apply the SIT, and discusses the strengths and weaknesses of the various options. Table 1 shows the various complexities of different funding arrangements (J. Hendrichs, personal communication).

### *8.1. Financial Support from Various Levels of Government and/or Private Sources*

AW-IPM programmes can be financed through a variety of funding sources and financial operating systems. Many programmes are funded from one or another level of government, or a mix of levels, ranging from national governments, state/provincial governments, and regional/district/local governments. In many cases this support is essential to the stability and success of the programme, e.g. melon fly

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in Japan (Kakinohana et al. 1993), New World screwworm in the USA, Mexico and Central America, oriental fruit fly *Bactrocera dorsalis* (Hendel) in Thailand, Mediterranean fruit fly in Latin America (Rhode 1975; Patton 1984; Dowell et al., this volume), and codling moth in Canada (Nelson et al. 2021). However, sometimes government support is unreliable or not delivered in a timely manner; delays create uncertainty for managers (especially if political considerations become dominant), unnecessary repetition of work, and even programme failure (Hendrichs 1986). Convincing government officials (who may reside far from, and have very different concerns than, the people in the programme area) to support a programme is often a huge challenge.

*Table 1. Funding Situation for Various Types of Programmes*[1]

| Funding Situation | AREA-WIDE PROGRAMMES (with central coordination and government involvement) | | NON-AREA-WIDE PROGRAMMES |
|---|---|---|---|
| | Federal and State Government Programmes | Local Community Government Programmes | Local Individual Stakeholders Programmes |
| Programme managed and implemented as government service (including contracting out some services) | Yes | Yes | No |
| All citizens, including non-beneficiaries, contribute indirectly to programme funding through generic government taxes | Yes | No | No |
| Only local beneficiaries contribute directly to the programme, mostly through local taxes, and sometimes also through other in-kind or cash contributions | No | Yes | No |
| Local beneficiaries are involved in the decision-making on programme funding and governance | No | Yes | Yes |
| Beneficiaries contract and fund services individually, without any local coordination or government involvement | No | No | Yes |

[1] Source: J. Hendrichs (personal communication)

In one way or another, taxation provides the resource that governments use to support their activities. Sometimes citizens have no direct influence on how money is spent. However, all government-based funding systems have some level of accountability to the stakeholders, and if a programme is largely funded by a local government, then the stakeholders will probably have a greater influence on the programme activities than a programme funded by a national or state government.

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Community involvement usually benefits a programme; stakeholder participation can improve programme delivery and effectiveness. If the tax base used to support a programme is drawn from a specific community that includes programme beneficiaries, e.g. farmers or members of a larger community (Dyck et al. 1993; OKSIR 2018; Seymour 2018; Nelson et al. 2021; Mumford, this volume; Simmons et al., this volume), then residents of a community can influence local government decisions, e.g. decisions on support for an AW-IPM programme, through elections and support of, or opposition to, a particular feature of the programme or a change in the tax rate. Mosquito abatement districts are the most common examples of such locally organized, managed and taxed programmes, with Florida alone having currently 61 local mosquito control programmes (UF 2018). This involvement of the community creates an opportunity to obtain broad public support, but also for uninformed people to influence the programme; therefore, the role of public education becomes very important in such cases (Dyck, Regidor Fernández et al., this volume).

If governments provide the finances to operate a programme, this can foster a culture of dependence and reduce the incentive for stakeholders, e.g. farmers in an agricultural setting, or citizens in public health campaigns, to be involved actively. If stakeholders do not contribute in some way to the pest control programme, e.g. managing pest "hot spots", eliminating mosquito breeding areas, or checking insect traps or pest damage to crops, their lack of supportive actions could reduce the effectiveness of the whole programme. Therefore, to ensure programme success, it is usually essential to keep stakeholders involved in programme activities, even if most funding comes from governments or donors (I. Pla Mora, personal communication). In area-wide programmes, the participation of farmers is essential in view of their first-hand knowledge of the pest situation on their farms, and their cooperation is necessary even if they are reluctant, for whatever reason, to take advice from programme staff.

Direct programme beneficiaries, such as growers, orchardists, and ranchers, are goal-oriented, and the pest or its control directly affect their livelihoods, environment, or even health. They have a personal stake in the programme's success. Such stakeholders are usually supportive of the programmes since they will reap many of the benefits of success, but often are also "cash poor", even if sometimes "asset rich", and tend to offer quite limited financial resources to a programme. Those farmers whose products for export must be residue-free, or even completely free of quarantine pests, are often very supportive. Even though in some programmes there is little or no contribution from the direct beneficiaries, many programmes obtain contributions "in cash" or "in kind" from stakeholders, e.g. fruit growers in Argentina and South Africa (Barnes et al. 2004, 2015) support the field operations of Mediterranean fruit fly programmes, cotton growers in California, USA, helped fund the pink bollworm *Pectinophora gossypiella* (Saunders) eradication programme, and cattlemen in the south-western United States made contributions to a New World screwworm programme (Wyss 2000; Baumhover 2001).

Support from programme beneficiaries is good from an operational point of view, but the financial aspects are often less stable than government funding.

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Contributions need to be reliable, and therefore probably compulsory. If market returns are low, or the economic health of an industry is poor, the participants in that industry will be reluctant to continue supporting a programme. In addition, as in many AW-IPM programmes, not all beneficiaries tend to be supportive and educated in technical matters. They are also sometimes poorly organized, and reluctant or even opposed to cooperating in joint area-wide activities.

There are good reasons for a community to contribute financially to an AW-IPM programme that applies the SIT, especially if the insects for release are reared locally -- quantifiable economic and social benefits to the region, e.g. employment, purchase of locally produced materials, increased tourism because of a safer environment, and a potential for higher market returns of the commodity, such as fruit, being produced largely insecticide-free (Mumford, this volume).

A mix of public and private funds seems to work best in some programmes, and this is now the trend (Barnes et al. 2015; IAEA 2015; I. Pla Mora, personal communication). For example, the successful *Anastrepha* spp. fruit fly programme in northern Mexico is funded one-third by grower associations, one-third by state authorities, and one-third by the national government. However, the larger the number of funding sources, the greater the complexity of financial management, but probably also the greater the funding stability.

A few programmes are largely or completely operated through the direct payment by stakeholders and members of a community to a commercial company. The onion maggot programme in The Netherlands is conducted entirely with funds from individual growers that contract for the service of pest monitoring and suppression in their onion fields, including the release of sterile flies (Loosjes 2000). In the case of mosquitoes, it is quite common for citizens of a county or community to fund their mosquito-abatement districts, including the recruitment of professional managers and entomologists. However, in cases where individual farmers have sterile insects applied but not as a part of a coordinated area-wide approach, as in the example of the onion maggot, such support would mainly benefit the farmers who contracted to have the SIT applied. Neighbouring farmers, who are “free riders”, obtain benefits from dispersing sterile flies without paying for them, while farmers that pay for the SIT suffer when mated fertile female flies enter their land from nearby farms that are not part of the programme (Klassen et al., this volume).

### *8.2. Financial Support from Donors and Foreign-Aid Programmes*

In developing countries, it is common for donor organizations and government-sponsored foreign-aid programmes to provide some support to national governments in area-wide pest control programmes, e.g. the New World screwworm programme in Libya (FAO 1992; Lindquist et al. 1992), and the tsetse fly *Glossina austeni* Newstead programme in Unguja Island, Zanzibar, United Republic of Tanzania (FAO/IAEA/TANZANIA 1994; Dyck et al. 1999). In Ethiopia, non-governmental organizations (NGOs) provide some financial support for the control of tsetse flies, although most funds are received from the government. It is also common, at the request of developing countries, for international organizations to provide some multilateral support to national governments (LaChance 1993), such as in the

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screwworm programme in Libya (FAO 1991, 1992; Lindquist et al. 1992), and the tsetse fly programme in Zanzibar (FAO/IAEA/TANZANIA 1994; Dyck et al. 1999). Most such support is provided "in kind", and focused on important expert services for training, capacity building, and feasibility studies, as well as the provision of special equipment. However, sometimes the equipment provided is not appropriate to the practical operations, or difficult to maintain locally, and may therefore become unusable. Thus, some "in cash" support, although not common, is also very useful to a programme for some local purchases and to overcome local constraints and cover needs not foreseen in inflexible government budgets.

In many countries the FAO/IAEA has provided financial support for some applied research activities, capacity building, and pilot operational programmes related to applying the SIT, e.g. South Africa (Barnes et al. 2015).

## 9. MANAGING FINANCES

Often the biggest problem in managing finances is interrupted cash flow due to delays in receiving funds from financial supporters of a programme, be it from government or other sources. Budget cuts, caused by economic downturns or political decisions, especially if unexpected, have a strong negative impact on the progress of a programme (Patton 1984). Sometimes a shortage of money is caused simply by bureaucratic delays. If there is no money or money is delayed, usually the programme activities have to be restructured, curtailed, or even stopped, and this can be disastrous for a biological programme that requires continuity and where "timing is everything" (Lorraine and Meltsner 1987). Insect rearing and release activities cannot be "put on hold" and resumed later when money becomes available. Failure to sustain activities can result in a total loss of previous achievements and the necessity to start the programme again (Box 2). On the other hand, if a programme suddenly receives an excess of funds shortly before the end of the fiscal year, panic spending could lead to the inefficient use of money. One way to minimize the problem of local cash flow is for the funding organization to regularly advance money to the programme manager, who can anticipate expenses and budget these funds accurately.

A back-up plan must be included in the programme planning document so that, when a programme supporter fails to deliver promised money, funds can still be made available, and the programme can proceed on schedule. A possible mechanism to solve this problem would be for one financial supporter to agree, in advance, to provide contingency funds. In any case, a contingency fund is needed to cope with emergencies.

Reliability in funding and cash flow, and also the provision of larger amounts of money at those times of year when biological operations require it, are essential for an AW-IPM programme involving the SIT. In the case of seasonality of programme implementation, financial planning is further complicated. This emphasizes the need for a separate organization (section 5.), located at the site of the programme, with its own financial accounting system and audits, which can control cash flow and prevent unauthorized use of money.

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Especially in poor countries, payment of incentive bonuses to complement on a regular basis the low staff salaries is important for maintaining staff morale and a high level of performance, both of which are essential for effective field operations and the production of high-quality sterile insects for release.

Declining exchange rates between local and foreign currencies can be a major problem for programmes that have some of their expenses in foreign currencies. Under these conditions the conversion of funds has to be carefully managed, as a rapid decline makes it more difficult for a programme to purchase foreign-produced goods such as technical equipment or aircraft services.

## 10. LOGISTICAL AND PHYSICAL SUPPORT

Even though the quality of the human input into an SIT-related AW-IPM programme is a top priority, programme staff cannot do the work alone, and logistical support and the physical infrastructure are essential to achieve a programme's objectives. Materials and equipment must be of appropriate specifications and quality, cost-effective, procured in a timely manner, and properly maintained. A well-executed procurement plan should take into account that biological processes, once started, must be completed, and that the lack of even one item could mean disaster. Stability in the supply of critical materials, and an adequate stock of essential items, are required. For example, in mass-rearing, tested diet ingredients for at least 3–6 months operation are required, and warehouses have to be sized accordingly (Parker, Mamai et al., this volume).

Local rather than foreign procurement would appear, at first glance, to be the best policy, and can be advantageous in terms of availability, lower costs, and reduced transport expenditures. However, purchasing products from another country may be necessary in some cases to obtain a high-enough quality or items that simply are not available locally. Flexibility in procurement procedures is required to enable the correct materials to be purchased, and to permit quick action when biological events demand it. The lowest bid on an item is often not the correct criterion on which to base a purchase decision. Diet ingredients require especially stringent quality control procedures (Parker, Mamai et al., this volume).

The location of a mass-rearing facility has an impact on operational efficiency, and on the availability of materials and appropriate staff (Rhode 1969; Hendrichs 1986; Phillimore 2002; FAO/IAEA 2004, 2012b; Cáceres et al. 2012; Dowell et al., this volume; Parker, Mamai et al., this volume). If the programme is located in a difficult-to-access place, procurement must be done long before items are needed. This applies especially to insect rearing and release materials and equipment, and to field monitoring supplies.

To protect the environment and maintain respect for nearby communities, diet should be biodegradable and the disposal of spent diet through treatment of waste solids and water is an essential activity in facilities mass-rearing fruit flies, moths and screwworms, requiring appropriate infrastructure (Wyss 2002; Nagel and Peveling, this volume).

The production and release of a perishable product like sterile insects need a strong support system to ensure that investments are not wasted by the absence of,

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or having low-quality, materials and equipment. Proper maintenance of equipment is essential so that mechanical failures do not result in the production of fewer or low-quality insects. Equipment should not be more complex than necessary (Rhode 1969), but of good quality to minimize breakdowns that lead to inefficient operations. Back-up equipment and plans for all critical operations, and a stock of spare parts for essential equipment to minimize equipment downtime, are necessary. Accidents and emergencies do happen, and therefore redundancy is needed to avoid negatively affecting field operations or to prevent declines in quality insect production. For example, even though several smaller independently operated rearing rooms cost more to build and operate than one large room, the smaller rooms provide much more production security than one large room (Tween 1987; Dyck et al. 1993; Phillimore 2002; Dowell et al., this volume; Parker, Mamai et al., this volume). For the sake of maximizing efficiencies and productivity, competition among workers responsible for the performance of different rooms can be fostered.

Ideally, utilities must be reliable, but back-up systems, e.g. electricity generator, should always be available so that temperature controls and other essential activities can continue operating.

Vehicles, including release aircraft, must be adequate in number and maintained properly to provide reliable and safe service. The aerial release of sterile insects has sometimes been done from aircraft owned by the programme. However, it may often be appropriate to contract out aerial release activities to private or government aircraft companies (Dowell et al., this volume). Nevertheless, to maximize programme effectiveness, programme staff must still supervise release operations. The high cost of aerial release necessitates a careful assessment of operational procedures to accommodate any concerns with economic, efficiency or technical issues (Teruya 2000).

In selected cases it may be realistic and appropriate for a large regional mass-rearing facility to produce enough sterile insects for the needs of a whole region, from where sterile insects are sent to the various emergence and release centres for handling and release. Sometimes it is more efficient and economical to construct a regional facility than for each programme to have its own facility (Dowell et al., this volume). However, this is risky in view of unforeseen catastrophic or political events, and can lead to complete dependence on the supply of insects, especially if there is only one production facility in the world, as is currently the case for the New World screwworm, currently in Panama (Vargas-Terán et al., this volume). Such a monopoly situation had a negative impact on the Jamaica New World screwworm programme (Box 2). In the absence of competition, dependence on the operating procedures and policies of, and the cost charged by, the only external source of sterile insects might not be in the interest of a particular programme; therefore, such a system is “the exception rather than the rule”.

## 11. EXPORTING/IMPORTING STERILE INSECTS

Sterile insects have routinely been shipped across borders for decades (see Table 2 for history of transboundary shipments), which is facilitated by the fact that sterile insects are non-invasive agents that leave no “ecological footprint” because they

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cannot become established due to their sterility. In 2005 the shipment of sterile insects across national borders was facilitated further by a decision of the International Plant Protection Convention (to which most countries are parties), formally categorizing sterile insects among beneficial organisms (IPPC 2017; Klassen et al., this volume).

*Table 2. History of transboundary shipments of sterile insects*[1]

| Year | Tephritid species | Site of production | Approximate amount shipped (million pupae) | Recipient | Observations |
|---|---|---|---|---|---|
| 1963–1990 | Mexican fruit fly *Anastrepha ludens* | Monterrey, Mexico | Unknown | Texas, USA | |
| 1970/1971 | Mediterranean fruit fly | Seibersdorf, Austria | Unknown | Procida, Italy, and Greece | Relatively small amount since sterile flies were used for field trials |
| 1970 | Mediterranean fruit fly | Costa Rica | Unknown | Nicaragua | Relatively small amount since sterile flies were used for field trials |
| 1975–1977 | Mediterranean fruit fly | Madrid, Spain | 302 | Canary Islands | |
| 1978 | Mediterranean fruit fly | Seibersdorf, Austria | Unknown | Guatemala | Sterile pupae shipped from the IAEA laboratories (Seibersdorf) to a packing and emergence facility in Guatemala for field trials and staff training in SIT techniques |
| 1979–2017 | Mediterranean fruit fly | Metapa, Chiapas, Mexico | 357 600 | Guatemala | Transboundary shipments have been carried out periodically for the past 36 years |
| 1989–1994 | Mediterranean fruit fly | Metapa, Chiapas, Mexico | 6670 | California, USA | To assist the CDFA in eradication of medfly outbreaks |
| 1989–1990 | Mediterranean fruit fly | Seibersdorf, Austria | Unknown | Israel | Pilot trials |
| 1990 | Mediterranean fruit fly | Metapa, Chiapas, Mexico | 552 | Chile | Sterile flies donated by the Mexican government to Chile |
| 1990–1991 | New World screwworm (NWS) | Tuxtla Gutierrez, Chiapas, Mexico | 1800 | Tripoli, Libya | To assist the eradication of a NWS outbreak |
| 1993–2006 | New World screwworm | Tuxtla Gutierrez, Chiapas, Mexico | 107 000 | Central American countries | To assist NWS eradication in Central America |

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*Table 2. Continued*[1]

| Year | Tephritid species | Site of production | Approximate amount shipped (million pupae) | Recipient | Observations |
|---|---|---|---|---|---|
| 1990–1998 | Tsetse fly *Glossina* spp. | Seibersdorf, Austria | 8 | Tanga, Tanzania | To assist tsetse eradication in Zanzibar |
| 1994 | Mediterranean fruit fly | Seibersdorf, Austria | 60 | Tunisia | Pilot trials |
| 1996–2000 | Mexican fruit fly | Metapa, Chiapas, Mexico | 2511 | California, USA | To assist the CDFA in eradication of Mexican fruit fly outbreaks |
| 1994–2017 | Mediterranean fruit fly | El Pino, Guatemala | 152 100 | California, USA | Preventive Release Programme and to eradicate medfly outbreaks |
| 1997/1998 | Mediterranean fruit fly | Madeira, Portugal | 206 | Israel | In support of pilot suppression programme |
| 1997–2000 | Mediterranean fruit fly | El Pino, Guatemala | 3700 | Israel | In support of pilot suppression programme |
| 1998–2017 | Mediterranean fruit fly | El Pino, Guatemala | 97 720 | Florida, USA | Preventive Release Programme and to eradicate medfly outbreaks |
| 1999–2000 | Mediterranean fruit fly | El Pino, Guatemala | 720 | South Africa | In support of pilot suppression programme |
| 2003–2006 | Mediterranean fruit fly | Mendoza, Argentina | 1681 | Valencia, Spain | In support of suppression programme |
| 2011–2013 | Mediterranean fruit fly | Valencia, Spain | 425 | Croatia | In support of suppression programme |
| 2008–2017 | Mediterranean fruit fly | Bio-Fly Israel | 650 | Jordan | In support of suppression programme |
| 2010, 2012, and 2014 to 2017 | Mediterranean fruit fly | Bio-Fly Israel | 3289 | Croatia | In support of suppression programme |
| 2010–2018 | Tsetse fly | Slovakia | 9 | Senegal | To assist tsetse eradication in Senegal |
| 2014–2017 | Mediterranean fruit fly | Valencia, Spain | 600 | Morocco | In support of suppression programme |
| 2015–2017 | Mediterranean fruit fly | El Pino, Guatemala | 4000 | Dominican Republic | In support of eradication programme |
| 2016–2017 | New World screwworm | COPEG, Panama | 194 | Florida, USA | To assist the eradication of a NWS outbreak |
| 2008–2017 | Mediterranean fruit fly | El Pino, Guatemala | 260 000[2] | Metapa, Chiapas, Mexico | In support of eradication and containment programme |
| | TOTAL | | 1 001 797 | | |

[1] Data from FAO/IAEA (2018b), reproduced with permission
[2] Only in this case, insects shipped as eggs

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There is a long history of successful shipments of live sterile insects across national borders, especially insects in the pupal stage, e.g. Mediterranean fruit fly, New World screwworm. In future, there is no doubt that this practice will continue (Dyck 2010; Blomefield et al. 2011; Seck et al. 2015; FAO/IAEA 2017). As stated in FAO/IAEA (2018b),

> The transboundary shipment of sterile insects has taken place on a continuous basis for 55 years. The total number of sterile insects shipped has been estimated at more than 1 trillion [thousand thousand million] in thousands of shipments across borders to 23 recipient countries from 50 sterile insect factories in 25 countries. During this long period and many precedents, no problems associated with possible hazards have been identified, and thus the shipment of sterile insects has never been subjected to any regulatory action. The table shows the history of transboundary shipments which started in 1963 with the shipments of sterile Mexican fruit flies *Anastrepha ludens* (Loew) from Monterrey, Mexico, to Texas, USA.

The Okanagan-Kootenay Sterile Insect Release (OKSIR) Program in British Columbia, Canada (OKSIR 2018), has during the winter season in Canada shipped sterile adult codling moths to South Africa and New Zealand for use in both research and field control programmes (Taret et al. 2010; Blomefield et al. 2011; Horner et al., 2016; Suckling et al. 2017; Fries 2018; Nelson et al. 2021). However, recently there was a delay in shipments to New Zealand because of quarantine concerns (in New Zealand an Import Health Standard and permit to import live animals is necessary under the Biosecurity Act) (R. M. Horner, personal communication). Eventually, the requirement for a veterinary certificate was revised; an official letter of verification from the OKSIR (declaring that the shipped live insects are sterile codling moths produced at the OKSIR mass-rearing facility) was deemed acceptable, and shipments were allowed to resume. Nevertheless, even though such problems can usually be resolved, this illustrates the potential for difficulties in shipping sterile insects across borders (Quinlan and Larcher-Carvalho 2007; C. Nelson, personal communication; Klassen et al., this volume).

Shipments from the OKSIR to researchers in the USA, which initially would appear to be a simple matter given the proximity of supplier to the users, has become complicated. Hand-carried shipments work well, but such arrangements are always ad hoc and not sustainable. Alternatively, import fees, official inspections, and difficulties in finding carriers that can transport live insects quickly can be barriers to cross-border shipments. Export shipments sold at a profit are a vital component of the financial plan of mass-rearing facilities that operate year-round but need the produced sterile insects only during the growing season (Fries 2018; C. Nelson, personal communication).

The Nagoya Protocol (2018) was established in 2014 under the Convention of Biological Diversity as an organized system to permit the fair exchange of biological control organisms among countries, and to share the benefits of such exchanges with all involved. This system now exists in principle, but it is so restrictive that it has recently brought about an almost complete stop to natural enemy exploration for classical biological control programmes (van Lenteren 2021). In future, it could affect the free movement of sterile insects across some national borders.

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## 12. PROGRAMME EVALUATIONS AND INDEPENDENT ASSESSMENTS

Programme managers, and even standing advisory committees and associated research scientists and institutions, need regularly to invite independent external experts and consultants to evaluate a programme and then give constructive advice and guidance. Both national and international consultants play valuable roles. External reviews of a programme reduce the likelihood of failure due to the "cannot fail syndrome", the unreasonable confidence that a process done correctly cannot fail to achieve the goal, and success does not need to be monitored (Dowell and Wange 1986). Experience gained in other programmes using the SIT can provide invaluable information and ideas to resolve problems and help achieve the programme's goals as efficiently as possible. Advice is often available, but usually at a cost, from university professors and other technically trained persons that are not affiliated with the programme's organization. The advice should be impartial, unbiased, and given "at arm's length". Sometimes it is appropriate to bring in full-time experts to advise the programme manager, especially on technical issues. If a programme claims to have eradicated an insect pest in a given region, it is important to obtain an in-depth assessment from a group of experts (Barclay et al., this volume).

There are numerous examples of good and helpful advice obtained from short-term consultants in many AW-IPM programmes. One is the international Technical Advisory Panel that recommended in 1994 an area-wide preventive release programme to deal with the recurring Mediterranean fruit fly outbreaks in the Los Angeles basin (Dowell et al. 2000). A more recent example is the Technical Advisory Committee (also composed of international experts) that advised the Dominican Republic on eradicating the Mediterranean fruit fly (Zavala-López et al. 2021).

However, there are also examples of bad advice that have damaged programmes (Box 3) (Klassen et al., this volume). Some caution in selecting consultants is needed since persons with no stake in a programme might in fact be biased, unrealistic, or too theoretical. External reviewers are not always correct in their assessments or recommendations, but it is extremely difficult politically for a programme manager to reject them. It is also difficult for non-technical managers, and representatives of donor organizations who hire technical consultants, to know if a consultant is biased or not, and has the required practical field experience. Therefore, external reviews need to be managed carefully, but on balance in most cases they are very valuable.

## 13. PROGRAMME SUCCESS OR FAILURE

Even though often blamed for programme failure, problems with technical issues in fact usually only delay the successful achievement of a programme's objectives, and rarely lead to failure. However, several programmes have failed because of bad management, and inadequate funding and political support.

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*Box 3. Mediterranean Fruit Fly Invasion of Central America*

In Costa Rica, the Mediterranean fruit fly was first found in 1955, and by 1962 it had spread to southern Nicaragua. Field trials to contain the northwards invasion of the fly demonstrated that the technology (including the use of the SIT) was adequate, but that more funds, a vision to achieve the goal, and an organization were required (FAO/IAEA 1970). However, the advice given in 1970 by a three-person review panel of university professors (with no experience in managing area-wide programmes) was to abandon any containment efforts in Nicaragua. The panel did not accept that eradication should be the goal, questioned the cost and feasibility of the programme, and instead recommended to "live with the pest" with a focus on enhancing biological control. Preconceived conservative notions against AW-IPM, and the economics and politics of the situation, evidently "ruled the day"; an opportunity to eradicate, or at least contain, the invasive fly was lost. Abandoning all containment efforts resulted in the fly rapidly invading the next three neighbouring countries (Honduras, El Salvador, and Guatemala), and reaching southern Mexico by 1977. A costly trinational emergency programme had to be initiated to prevent the Mediterranean fruit fly from invading Mexico and the USA (Enkerlin et al. 2017). Since then, the great amount of effort and money that has had to be spent each year (currently over USD 40 million), to prevent this invasion from continuing and to reverse the trend, is at least an order of magnitude larger than the budget (USD 1.5 million for 4 years) that the experts had rejected for the Nicaragua programme (FAO/IAEA 1970). The economic problems caused by the fly invasion are still present in Central America, with significant losses and conventional control costs, and representing the major limitation of countries in the subregion, i.e. developing a tropical fruit industry and access to the nearby US export market. In comparison, Mexico recently celebrated 40 years of keeping the country free of the Mediterranean fruit fly, as a result of which the fruit and vegetable exports have exploded to over USD 4000 million per year. Thus, in the case of the Central American region, the recommendation of the external panel turned out to be bad advice. Meanwhile, the trinational Moscamed Programme has resulted in major economic and other benefits for Belize, Guatemala, Mexico, and the USA (Enkerlin, this volume).

Many lessons about programme management have been learned from past and ongoing AW-IPM programmes that integrate the SIT (Vreysen et al. 2007). Programmes have a high probability of success if:

- Thorough baseline data collection and feasibility studies are implemented for proper programme planning.
- They are managed by a flexible, independent and efficient management structure.
- They have carefully selected technical staff that work full-time for the programme, and also offer specialized training for the different tasks related to implementing the SIT.
- They have strong and reliable financial support, and also strong political stakeholder support.
- They have adequate and appropriate logistical support and physical infrastructure.
- They seek expert advice, especially technical advice where needed. Even when pilot programmes encounter problems, eventual success can be achieved if adequate applied research support (probably provided from outside the operational AW-IPM programme), good cooperation, and international technical support and consultation are available.

However, other lessons show that programmes will risk failure, or have significant difficulties, if:

- They have unrealistic objectives and expectations.

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- They are immature, based on inadequate knowledge, patterned after other programmes without recognizing unique local characteristics, or are started too soon or with too little advance planning.
- They lack operational flexibility and independence, and carry the burden of stifling administrative restrictions.
- Their managers have inadequate (especially scientific and SIT-relevant) knowledge and experience, have a predominantly profit-oriented attitude (where the programme is a commercial enterprise), or do not work full-time in the programme.
- They have inadequate funding (Chinvinijkul et al. 2016), and staff lacks training or technical support.
- They have inadequate communications with the stakeholders, political leaders and the general public (Dyck, Regidor Fernández et al., this volume).

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# CHAPTER 5.4.

# COMMUNICATION AND STAKEHOLDER ENGAGEMENT IN AREA-WIDE PEST MANAGEMENT PROGRAMMES THAT INTEGRATE THE STERILE INSECT TECHNIQUE

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## SUMMARY

Effective stakeholder communication and engagement is an essential component of area-wide integrated pest management (AW-IPM) programmes that integrate the sterile insect technique (SIT) because it has a large impact on programme success. Full-time professionals should direct communication activities and help secure vital support and "goodwill" from governments and community organizations. Good and frequent communication among programme staff, and between programme staff and stakeholders, is required to maintain engagement and support, and to keep the work goal-oriented even when some programme activities are controversial. The importance of engaging stakeholders in the programme is emphasized. The media can be valuable and effective partners by informing stakeholders and the general public about the facts and activities of a programme, especially if this is done in a non-technical and straightforward way. Methods of communication, including the use of videos and social media platforms, are described. Ongoing research helps overcome controversial aspects of programme technologies. Programme failure can result from poor communication and inadequate stakeholder support.

## 1. INTRODUCTION

Effective communication in area-wide integrated pest management (AW-IPM) programmes that include the sterile insect technique (SIT) is critical to success. The overall communication of programme goals, objectives, and activities is often not given as much attention as are the technical aspects of a programme, and inadequate stakeholder support can be a cause of failure. Good stakeholder communication is just as important for AW-IPM programmes as is good management (Dyck, Reyes Flores et al., this volume) because these programmes affect whole communities (Lindquist 2000, 2001; Pennington et al. 2021; Klassen and Vreysen, this volume).

AW-IPM programmes are visible; they may involve large communities or even several contiguous communities. The participation, support, and possibly the financial contributions of many, if not all, members of these communities, even if disparate, are essential for success. When urban areas have to be included, it is also necessary to inform this more complex segment of the community about the need for the programme, its objectives and progress. Quarantine checkpoints established to maintain pest free areas affect travellers who may not live within the programme area (Hendrichs, Vreysen et al., this volume). Since it is environment-friendly, the SIT technology is likely to be accepted by the public when properly informed. However, sometimes problems arise from the field and regulatory activities of AW-

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IPM programmes, and these need to be resolved in a transparent manner (Reyes et al. 1988).

Stakeholders need to be pro-actively informed about area-wide programmes, with emphasis on the benefits that a programme brings to the community and country (Lindquist 2000, 2001; Mau et al. 2003). Communication activities require a strategy, implementation plan, and a budget just like other components of a programme; they are an integral part of it. Essential equipment and staff for these activities must be available at the beginning of a programme.

Before a programme is initiated, a survey should be made to identify the important stakeholders, the segments of the community that will be affected by the programme, and their likely primary concerns. A strategy of communication and engagement, and a plan for appropriate activities for each segment of the community, can then be developed and implementation started before initiating programme activities.

The details of the communication plan, and messages to the audience, will be unique to each programme. The type of media used should be suited to the communication plan and the situation (P. Rendón, personal communication). Translation of the messages into local languages may be necessary, and certainly the content, style, and format should fit the intended audience (Schwarz-Gehrke 1983). A periodic evaluation of the stakeholder communication programme is a valuable way to monitor and if necessary improve the communication strategy and activities.

Since technical specialists are often not good communicators, a full-time professional, with appropriate training and experience in communication, and a good knowledge of all aspects of the programme, should be appointed to lead the stakeholder communication activities (Dyck, Reyes Flores et al., this volume). Several persons together could form a communications team. Good interpersonal skills will help ensure an effective communication programme delivery. Consideration should be given to contracting public companies that can organize major media campaigns.

## 2. COMMUNICATING WITH STAKEHOLDERS

Regular and timely communication with a programme's stakeholders about programme objectives and benefits is an indispensable prerequisite to obtaining their support and participation. However, educating the public at large is difficult, and requires considerable effort and resources. Besides informing people about various aspects of AW-IPM and the SIT, using creative and effective methods is necessary to pre-empt the development of resistance that often develops when area-wide activities are initiated (Klassen and Vreysen, this volume).

Information provided on programme progress must be clear, reliable, up-do-date, relatively simple, practical, and sensitive to people's concerns. An opportunity for feedback should be provided. It must be recognized that perceptions among the various stakeholders will not be the same. The same issue may require more than one explanation, depending on the audience. Issues will relate to both technical and administrative matters. If prejudices or resentments are involved, not all issues may be solved satisfactorily.

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Potential urban-rural conflicts, such as in an agricultural programme involving insects attacking tree fruits, can often be alleviated through information exchange and one-on-one meetings with antagonists. Obtaining the early support of labour organizations is highly desirable, and often essential, to assist in informing their members. Indigenous people should be informed about a programme and, where relevant to area-wide activities, their permission obtained to enter traditional or sacred areas.

Obtaining area-wide community support and cooperation is perhaps the most difficult aspect of stakeholder relations (Patton 1984; Tween 1993; Pereira 2001; Allwood and Vueti 2003). Even when the majority of stakeholders has understood a programme's approach and benefits through an education campaign, there are always some individuals who are not supportive of a public good that is beneficial to the community as a whole. In Canada, the most difficult group (that needed to understand the value of area-wide codling moth control) was the urban property owners who had a few apple or pear trees in their backyards. Some of these persons did not want to cooperate with the programme, or cooperated reluctantly, and in an area-wide programme it is essential that all cooperate. In urban areas where there are no direct stakeholders or beneficiaries, e.g. programmes against pests that attack fruit, activities of AW-IPM programmes applying the SIT may appear to be strange, intrusive, or even annoying, e.g. aerial release of sterile insects in bags or boxes, or formerly the application of aerial bait sprays over the Los Angeles basin in California, USA (Lorraine and Chambers 1989; Mangan and Bouyer, this volume).

In the case of insect pests of animals, and where the programme may include urban animals such as pets and stray dogs, it is often difficult to get urban residents to inspect and treat these animals (Dyck, Reyes Flores et al., this volume). In 2016 travellers on roads in the Florida Keys, USA, were required to pass checkpoints so that any pets being transported in private vehicles could be inspected by government personnel for possible screwworm infestation (Staletovich 2016; USDA/APHIS 2017a, b).

Obtaining formal support from stakeholders may require endorsement through local referenda or the passing of legislation by public bodies (Dyck et al. 1993; El-Lissy and Grefenstette 2007). Prior to a public vote or formal response to a programme's plans, a thorough information campaign will contribute to ensuring that the public has a good understanding of the issues at stake, especially if public funds or taxes are involved.

It is exceptionally difficult to conduct a programme in areas of political or civil unrest. Under these conditions, programme staff members who live in the area should be the ones contacting the local people -- they are probably more acceptable to the local population, and more aware of sensitivities, in finding ways to address the specific concerns of the people.

Local organizations, such as farmer associations and citizen groups, special-interest or donor groups, non-governmental organizations (NGOs), and industry-related groups, can be important supporters of a programme, and may even be helpful in addressing protests that impede programme operations. Such organizations are in a position of influence in a community, and can use this position to communicate information to particular stakeholder groups. If the livelihoods of

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local stakeholders are at risk, e.g. being able to export their produce, they can help convince governments to support a programme (Dowell et al., this volume). They often care deeply about the specific issues that support the use of the SIT, such as environmental concerns. Nevertheless, local organizations may be small and therefore can be influenced by a few vocal or persuasive individuals; if they decide to oppose a programme, it will have a significant negative impact.

Admittedly, explaining technical issues to stakeholders is difficult. Technical experts have to realize that technical advice may not always be value-neutral (Lorraine and Chambers 1989). The public does not easily understand the concepts of AW-IPM and the SIT, and may be sceptical (especially about the need to control established pests). Special efforts have to be made to communicate the facts simply but effectively, using a variety of formats to ensure clarity. The public may require proof of the effectiveness of the technology. If not explained properly, using radiation to sterilize insects is easily misunderstood. Objections to releasing irradiated insects, which may be thought to be radioactive, must be handled carefully so that the facts are made clear and fears alleviated (Whitten and Mahon, this volume).

The response in Costa Rica to naturalists who objected to the eradication of the New World screwworm *Cochliomyia hominivorax* (Coquerel) was a public relations campaign called "The Ecological Fly"; it emphasized the value of using the SIT to help preserve endangered species in the forest.

Mass media can have a powerful effect on a programme. For example, the programme in India supported by the World Health Organization (WHO), where research on using sterile mosquitoes for control of the vectors of dengue haemorrhagic fever was conducted, had to be terminated by Indian authorities after severe negative publicity, wherein the programme staff was falsely accused by journalists of working on developing biological warfare. A timely rebuttal in the international media, to openly defend the programme and clarify the scientific issues, should have been made (Nature 1975), but this was not done properly, and the true facts were not made public until a clear rebuttal was published much later (WHO 1976; Jayaraman 1997).

Probably the hardest task for a programme manager, in regard to the media, is handling criticism, both fair and unfair. Much criticism arises from incorrect information or a lack of information (Klassen and Vreysen, this volume). However, sometimes there are significant issues at stake. It is important to keep stakeholder support for a programme strong; admitting to failure could weaken that support. Temporary programme setbacks need to be explained within the perspective of the overall goals. It is not helpful to become too technical; people will be more supportive if they understand the basics of the programme. For example, if people object to regular flights (for sterile insect release) over their homes, the need for these flights should be explained in a simple way. It is important to be positive, sincere, and timely, and to prevent small issues from becoming large issues. It is also vital to describe how the programme impacts on the residents of the community, the benefits people will get from it, and how they can contribute to it, e.g. by controlling pests on their own property.

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It is usually an advantage to receive support from an international organization. Such a body lends stature to a programme. International support can stabilize a programme even if local support fluctuates or diminishes, but it cannot replace local commitment and "ownership". The FAO/IAEA has a long history of providing some support to AW-IPM programmes that apply the SIT (Barnes et al. 2015).

Financial supporters require regular reports on the financial status of a programme. However, in addition, donors and governments need to know that there is public support for the programme. Articles in the national press, or items on television and radio, provide indirect support.

Effective communication among programme staff is essential. Programa Moscamed, which involves three countries (Guatemala, Mexico, and USA) and includes several mass-rearing facilities and field centres distributed over vast territories in southern Mexico and Guatemala, uses an internal website to improve the information flow and allow programme staff to have instantaneous access to an enormous amount of data. Having the appropriate information helps to keep workers motivated, focused, coordinated, and productive. Opportunities should always be given to staff members to express their ideas on how the programme is progressing, offer suggestions for improvement, and participate in planning, assessment, and decision-making. A team that is informed encourages cohesive behaviour and mutual support.

To facilitate operational efficiency and effectiveness, a reliable communications system between insect-production facilities and field operations is needed (Rhode 1969; Dyck et al. 1999). The rapid sharing of data among staff members is essential for appropriate and timely decision-making, and fostering good cooperation and coordination (Vreysen, this volume).

Field staff members at all levels must be well-informed about programme goals and activities; they interact directly with the community, and therefore play an important role in informing stakeholders about the programme.

## 3. COMMUNICATION AND STAKEHOLDER ENGAGEMENT

Not only do the biological and geographical characteristics of an AW-IPM programme impact the required features of an effective stakeholder communication programme, but so do the sociology of a community, the type of inhabitants, culture, traditions, economics, politics, and even the security concerns involving rebel groups and the military.

Barnes et al. (2015) pointed out that the beneficiaries of a programme need to cooperate actively, e.g. implement effective sanitation in their backyards, fields and orchards, carry out husbandry and other cultural controls in a standardized and timely manner, contribute to accurate field data recording, have realistic expectations of what the deliverables will be, and fully understand how they need to be involved. As Dyck (2017) asserted in another context – stakeholder involvement matters!

A participatory approach to extension methods is appropriate for knowledge-intensive technology. Stakeholders learn more when they are involved and engaged. Field days with "hands on" demonstrations, "farmer field schools", visits to "model

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farms" where the SIT is being applied, and "open house" days at project facilities are all helpful extension tools (Peshin and Dhawan 2009). Stakeholder meetings, e.g. meetings of farmer cooperatives and associations (Kakinohana et al. 1993), are useful for informing all interested parties of plans, progress, and problems. If there are language barriers among stakeholders, meetings provide opportunities for multi-language explanations and discussion (Rhode 1969).

A detailed example of involvement with the community is described by Bustamante et al. (2014), De Urioste-Stone et al. (2015), and Sommerfeld and Kroeger (2015) in connection with a programme to control Chagas disease in Guatemala. Researchers spent considerable time living in the communities that were being studied in relation to the control of the vector; the communities actively participated in proposing solutions to the pest and disease problem (community-based participatory and partnership strategy) that led to empowerment; "bottom-to-top" and "top-to-bottom" strategies were combined, and community volunteers were trained and supervised in applying cultural and other pest-control measures.

In preparation for an experiment in which transgenic mosquitoes were released in an urban area of Brazil, a major public awareness and engagement campaign was conducted (Capurro et al. 2016), emphasizing participatory action and a community-based programme involving frequent communication with local, state and national leaders. The social aspects of regulatory issues of the releases and project activities were assessed, including people's perceptions after releases stopped, and then followed by feedback and joint evaluation. The procedures in this study provide an example of methods that can be used to prepare for and measure the social impact of mosquito releases on an urban human population.

In a similar project on dengue in Australia, the effectiveness of community engagement was studied; stakeholder acceptance of the open-release field trials (release of *Wolbachia*-infected mosquitoes) was essential for the project to proceed (Kolopack et al. 2015). Note a similar project in Singapore (Liew et al. 2021).

As a result of the long history of applying the SIT in Okinawa, Japan, public awareness is high. Local people cooperate readily in assisting field operations. However, a lack of risk awareness in relation to maintaining the pest-free status can result from the long-standing familiarity with such projects (T. Matsuyama, personal communication).

## 4. METHODS OF COMMUNICATION AND STAKEHOLDER ENGAGEMENT

AW-IPM programmes need to communicate with the public! It is vital to build good relations with community leaders, as well as several media outlets and people in the media business, so that opportunities to communicate with the public become available, and the best way of reaching the right audience is found. The appropriate media outlets must be carefully discerned to maximize the desired communication to the target audiences.

Information provided to leaders and the media must be correct, authoritative, and also realistic about both the problem and the solution so as not to oversell a programme (Barnes et al. 2015). When the message is clear and straightforward, the SIT and related activities can be promoted effectively through the media.

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When the various groups of stakeholders are identified, methods of communication can be selected that fit the groups. For example, programmes in Mendoza, Argentina, used television and radio broadcasts of sporting events to communicate short key messages about their activities. Press kits may be a useful way of giving information to journalists. Sometimes communication in more than one language is required, but always in a format and style appropriate to the audience. Monitoring media coverage reveals which issues interest the public.

Drawings of the basic concepts of the SIT (sequence of insect rearing, sex separation, irradiation, release of sterile insects, and monitoring) are shown in many outreach materials, enabling everyone to understand the processes involved. As an example, a simple and humorous cartoon used in Thailand (based on an original cartoon from MOSCAMED, Guatemala) is shown in Fig. 1. Such drawings get the message across in an easy-to-understand manner. Another drawing is given in Broll (2016), illustrating the application of the SIT against mosquitoes. The basics of the SIT are described on the FAO/IAEA website (FAO/IAEA 2018e). The FAO/IAEA also produced a tri-fold leaflet with explanatory text about the SIT (FAO/IAEA 2018f). The SIT is also explained in simple terms in many of the videos and photo essays listed in section 5., e.g. FAO/IAEA (2015a; 2016a, e); other examples are in Engelking (2017) and Viegas and Gil (2018).

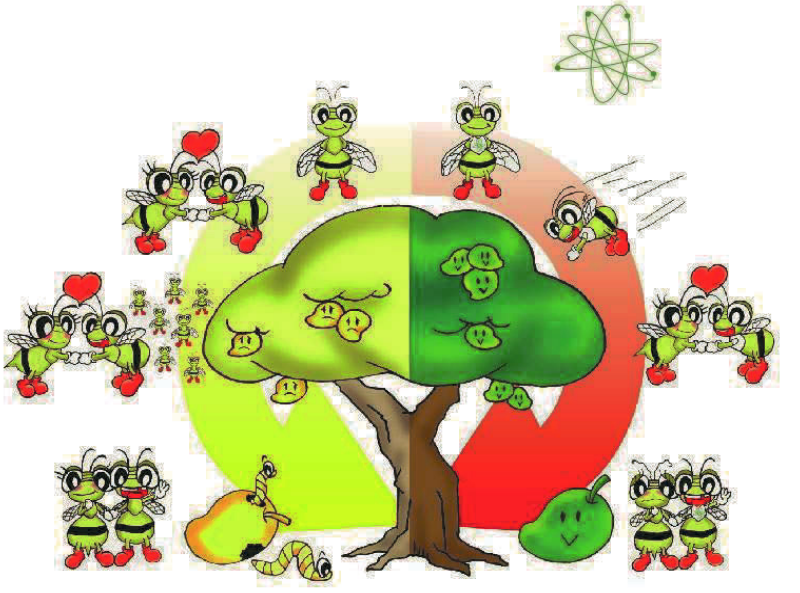


*Figure 1. Cartoon illustrating the basic principles of the SIT applied against fruit flies. (Cartoon from Thailand, reproduced with permission from Watchreeporn Orankanok.)*

Exhibits in public places and fairs are useful to create awareness of a programme. A display about the Okanagan Kootenay Sterile Insect Release (OKSIR) Program in Canada was set up in a museum (on the history of the fruit industry in the region), attracting local people and tourists.

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House-to-house visits are often a good way to ensure that residents are informed about the programme and the need for their cooperation. Communication through the mail, telephone messages or public meetings is useful, but in many cases there should be direct communication at a personal level (P. Rendón, personal communication).

Opportunities can be created in rural areas — informal meetings and using speakers on vehicles — to communicate with farmers and those who do not readily get messages through large-scale media services (Patton 1984).

A toll-free telephone number should be available for persons to ask questions about the programme and to report on related activities. Also, a "Frequently Asked Questions" (FAQ) or "Questions and Answers" (Q&A) item on a programme website is useful for the general public and other stakeholders (OKSIR 2018a; OPG 2018).

Universities are expanding the teaching of the principles of AW-IPM and the SIT (Lindquist 2001). In Madeira, Portugal, Caldeira (2001) emphasized the need to use both technical and non-technical materials, in a variety of formats, and in various public settings, to inform urban dwellers, including school children, of the objectives and activities of the Mediterranean fruit fly programme. Informing members of school clubs in Ethiopia stimulated cooperation in setting up tsetse fly traps. Students in South Africa and Argentina (Box 1) learn about AW-IPM programmes through official additions to the school curriculum. In Argentina, an interactive computer game helped teach the basics of the SIT to children, allowing players to release bags of sterile insects to hit target infested trees displayed on a screen (P. Rendón, personal communication).

*Box 1. Students in Argentina Learn about the Sterile Insect Technique*

The BioContainer Programme of ISCAMen, Argentina, was developed for 6th-degree school students (12–13 years old) to study the biology of the Mediterranean fruit fly, its role as a fruit pest, and the eradication programme (R. Mellado, personal communication). The BioContainers (Fig. A) are paper boxes containing sterile male pupae, provided by de Biofactory of Mendoza. Boxes include written information and instructions about the fly's life cycle, and how sterile insects control the pest. The emergence of adults from pupae can be observed through a window in the box. After flies have emerged, students are asked to release them on host fruit plants in their own or someone's backyard, assisting local pest control, and engaging the students and their families in the control process in urban areas. In the past 5 years the Programme has given boxes to 108 050 students, leading to an estimated 432 200 personal contacts, e.g. parents, siblings, and friends.

Before boxes reach students, school teachers are trained in the biology of the pest and the use of the BioContainer. In 2018, 1580 school teachers participated in the BioContainer Programme.

After using a BioContainer, students have the opportunity to visit de Biofactory. The factory has a circuit of glass bridges from which one can see the whole process of raising insects from egg to pupae, and the subsequent irradiation process, without actually entering the plant. In the past 5 years, 2650 students have visited de Biofactory.




*Figure A. Left: BioContainer logo. Centre: BioContainer containing living sterile male fruit flies. Right: Opened BioContainer showing emerged flies. (Photos from R. Mellado, reproduced with permission.)*

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### *4.1. Press Releases*

Below are some examples of press releases related to AW-IPM programmes:

- International Atomic Energy Agency (IAEA 2017). 27 April 2017 – Atoms for peace and development in the 21st century
- New Zealand: Plant and Food Research (PFR 2018a). 14 March 2018 - Scientists discuss sex-less insects; 22 March 2018 - Dropped in for fruitless sex
- International Atomic Energy Agency (IAEA 2018a). 19 April 2018 – IAEA conducts successful test of drones in fight against disease-transmitting mosquitoes.

### *4.2. Web Stories and Web Audio Interviews*

Below are examples of web stories:

- Gaspar (2016a), IAEA, 7 June 2016 – South African experts advance in researching nuclear technique to fight malaria, sugarcane pest
- Gaspar (2016b), IAEA, 28 June 2016 – How a nuclear technique helped save the Western Cape's orange industry
- Gil (2017a), IAEA, 14 December 2017 - Argentina uses nuclear technology to control insect pests
- Jawerth (2018), IAEA, 5 September 2018 – Irradiated fruit flies: the secret to protecting Thailand's premium fruit exports
- Marais (2019), IAEA, 14 March 2019 – Integrated pest management to boost dragon fruit production in Viet Nam
- Rawat (2019), India Today, 24 January 2019 – Horny mosquitoes in high demand: why scientists are developing sexually active mosquitoes (contains links to videos describing the SIT).

Below is an example of a web audio interview:

- UN News (UN 2018), 20 April 2018 – Breakthrough drone-powered mosquitoes: "absolutely no impact" on other species.

### *4.3. Other Methods of Communication*

- Engaging directly with stakeholders -- personal visits of programme staff to project sites, e.g. farms, to hold discussions with the property owners
- Stakeholder engagement survey, e.g. OKSIR (2018d)
- Meetings and presentations to farmer cooperatives and grower associations, citizen groups, schools, and the general public
- Articles in newspapers, books, and magazines, e.g. Gil (2017b); Paquette (2019)
- Newsletters, e.g. OKSIR (2016), brochures, fact sheets, and calendars
- Manuals such as those produced by the FAO/IAEA (2018b, f)
- Expositions, public displays and demonstrations, and giving away key chains with a project logo (A. Bello Rivera, personal communication)
- Postage stamp, e.g. a stamp featuring the SIT and Dieter Enkerlin, a pioneer in applying the technology in Mexico (Fig. 2)

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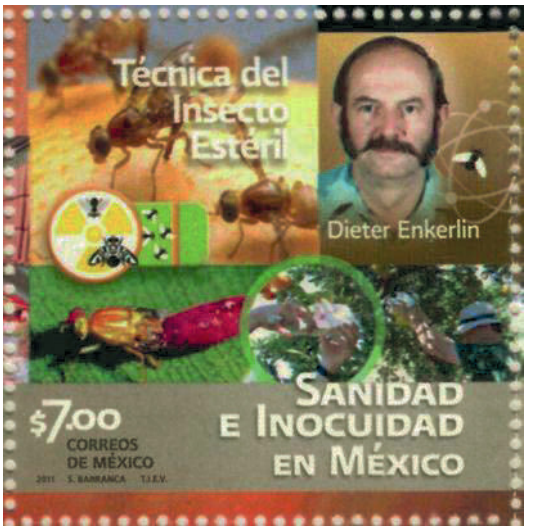



*Figure 2. Postage stamp issued in Mexico in 2011 featuring the SIT and Dieter Enkerlin.* (*Stamp reproduced with permission from W. R. Enkerlin.*)

- In Okinawa, local advertisements (including in airports and seaports) of project activities, and flyers and newspaper inserts, are widely distributed (T. Matsuyama, personal communication)
- The Moscamed Programme in Guatemala promotes bee-keeping; this enables growers to see for themselves that aerial sprays applied to suppress the Mediterranean fruit fly in pest hot spots do not kill bees (P. Rendón, personal communication)
- Programme logo – using an attractive, simple but self-explanatory logo gives a programme an identity and an enhanced public image, and encourages staff to be proud of the programme (Fig. 3)

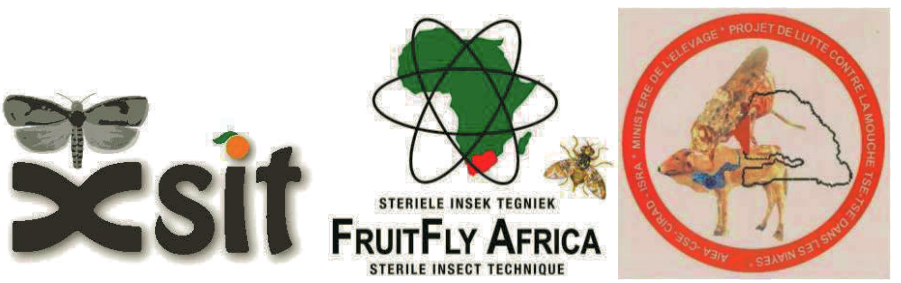



*Figure 3. Examples of programme logos: Left - Xsit in South Africa; Centre - FruitFly Africa in South Africa; Right – Senegal tsetse fly eradication project in the Niayes region (Senegal 2018).* (*Reproduced with permissions from T. Lombard, N. Baard, and B. Sall, respectively.*)

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- Online information sources, e.g. project websites -- providing programme information, publications for download, links, and videos, e.g. OKSIR (2018a, c), OPG (2018). Real-time data and information can be provided, e.g. the weekly area-wide distribution and population densities of the pest based on results from trap and other monitoring, and assisting farmers with timely advice on field operations (Nelson et al. 2021)
- Online forums; blogs maintained by interested stakeholders
- Email, electronic messages on smart phones
- Telephone calls, call centre.

## 5. VIDEOS AND PHOTO ESSAYS

In recent years there has been a large increase in the number of videos and photo essays available online - for viewing and sometimes for downloading. A partial list of those available through the FAO/IAEA is provided in FAO/IAEA (2016a, 2018a).

### *5.1. The SIT and AW-IPM*

- Science, sex, superflies (FAO/IAEA 2007a)
- Using nuclear science to control pests (FAO/IAEA 2015a)
- The SIT - an environment-friendly method of insect pest suppression and eradication (FAO/IAEA 2016a)
- How a nuclear technique is helping the Dominican Republic win the war against the Mediterranean fruit fly (FAO/IAEA 2016c)
- Using nuclear science to control mosquitoes (FAO/IAEA 2016e)
- Using nuclear science to control pests (FAO/IAEA 2017a)
- Area-wide management of insect pests (FAO/IAEA 2017b).

### *5.2. Fruit Flies*

- The Middle East's fruitful valley – Israel (FAO/IAEA 2007b)
- Olive fly: nuclear technology to protect an ancient fruit (FAO/IAEA 2009)
- Medfly eradication: what to expect (FAO/IAEA 2011)
- Better fruit for Neretva Valley (FAO/IAEA 2012)
- Nuclear techniques keep insects at bay in Croatia's Neretva Valley (FAO/IAEA 2015b)
- Tackling fruit flies with nuclear technology in the Dominican Republic (FAO/IAEA 2016b)
- How a nuclear technique is helping the Dominican Republic win the war against the Mediterranean fruit fly (FAO/IAEA 2016c)
- How a nuclear technique helped save the orange industry in Western Cape, South Africa (FAO/IAEA 2016g)
- Using nuclear technology to control pests (FAO/IAEA 2017a)

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- Nuclear technology helps the Dominican Republic fight insect pests (FAO/IAEA 2017c)
- Nuclear technique helps prevent insects from harming your coffee beans (FAO/IAEA 2017d)
- Successful control of the Mediterranean fruit fly in Jordan (FAO/IAEA 2017e)
- Argentina tackles fly pests using nuclear technology (IAEA 2018b)
- Irradiated fruit flies: protecting Thailand's fruit exports (FAO/IAEA 2018c)
- Video Programa Moscamed Guatemala (PMG 2018a)
- Video Programa Moscamed (SS 2013, 2018a, b)
- Web novela: Misión Moscafrut (SS 2018c)
- Control y erradicación de la mosca de la fruta (SS 2018d).

### *5.3. Mosquitoes*

- With IAEA support, Sudan suppresses mosquito populations (FAO/IAEA 2015c)
- Preventing procreation: IAEA and mosquito control (FAO/IAEA 2015d)
- Zika crisis – the IAEA responds (FAO/IAEA 2016d)
- Using nuclear science to control mosquitoes (FAO/IAEA 2016e)
- Drones for good 2016: FAO/IAEA's ROMEO system for aerial release of sterile male mosquitoes (FAO/IAEA 2016f)
- 20 million sterile mosquitoes for release - California (Haridy 2017; Debug 2019)
- Video on rearing mosquitoes in Singapore (Co 2019)
- Nuclear technique helps fight mosquito-borne illnesses (FAO/IAEA 2019a)
- Technique de l'insecte stérile, Le moustique tigre. Videos, in French (TIS 2020).

### *5.4. Moths*

- Pink bollworm eradication program: informational video (NCCA 2009)
- Photo Essay: How a nuclear technique helped save the orange industry in Western Cape, South Africa (FAO/IAEA 2016g)
- Falling Moths. Unmanned aerial vehicle (UAV) releasing codling moths in New Zealand (PFR 2018b)
- Palomilla del Nopal (*Cactoblastis cactorum*) (SS 2018e)
- Accessing SIR trap counts online (OKSIR 2018b)
- False codling moth in South Africa (Xsit 2018, 2019).

### *5.5. Tsetse Flies*

- Photo Essay: Eradicating flies to improve lives -- IAEA helps countries in Africa to combat tsetse fly (FAO/IAEA 2013)
- Paving the way for tsetse eradication – Ethiopia's journey (FAO/IAEA 2014)
- A farewell to tsetse (FAO/IAEA 2016h)
- The IAEA and food – tsetse fly eradication – Senegal (FAO/IAEA 2017f).

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### *5.6. Screwworms*

- SIT pilot test against New World screwworm (*Cochliomyia hominivorax* Coquerel) in Artigas, Uruguay (FAO/IAEA 2018d).

## 6. TECHNICAL INFORMATION ON AW-IPM AND THE SIT

A source of technical information on AW-IPM and the SIT is the Insect Pest Control Section at the FAO/IAEA (FAO/IAEA 2015a; 2016a; 2017b; 2018a, b, e, f, g):

- Information on AW-IPM and the SIT (FAO/IAEA 2018e; Wikipedia 2018)
- Publications arising from major conferences and symposia on insect pests (FAO/IAEA 2018h), e.g. FAO/IAEA (2000, 2007c, 2021)
- Publication of results from research conducted by entomologists at FAO/IAEA (lists of scientific papers and annual reports) (FAO/IAEA 2018b, i, m)
- Coordinated Research Projects (CRPs) -- results are published in peer-reviewed journals (FAO/IAEA 2018j), e.g. ZK (2015), FE (2016)
- Special issues of journals on research related to the SIT, e.g.: EEA (2017)
- Technical reports (consultant and advisory group meetings) (FAO/IAEA 2018k)
- Manuals and protocols (FAO/IAEA 2018l)
- Newsletters and annual reports (FAO/IAEA 2018m)
- Thematic plans (FAO/IAEA 2018n)
- Information and knowledge management (FAO/IAEA 2018o)
- Forthcoming meetings (FAO/IAEA 2018p)
- FAO/IAEA technical cooperation projects (FAO/IAEA 2018q)
- News from the FAO/IAEA on the SIT (FAO/IAEA 2018r)
- Regularly updated databases, e.g. IDIDAS, DIRSIT, TWD, IDCT (Bakri et al. 2016; FAO/IAEA 2018b; TWD 2018)
- Online glossary of SIT-related terms with definitions, references, and a search function (FAO/IAEA 2018g)
- E-learning course available online (FAO/IAEA 2019b).

## 7. SOCIAL MEDIA

Social media are interactive computer-mediated technologies that facilitate the creation and sharing of information and other forms of expression via virtual communities and networks. Social media platforms, e.g. Facebook, Twitter, YouTube, and LinkedIn (Fig. 4), are now a part of everyday communications. More than 100 000 000 registered users in the world have social media accounts. However, as is seen in recent times, precautions are needed when using social media to protect the privacy of stakeholder data, and to prevent the misuse of data.

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**FACEBOOK TWITTER YOUTUBE LINKEDIN**

*Figure 4. Some popular social media platforms.*

The trend in the increasing use of social media also includes some AW-IPM programmes that apply the SIT:

- Guatemala - Programa Moscamed Guatemala (PMG 2018b)
- Mexico - Campaña Nacional Contra Moscas de la Fruta Nativas (CNCMFN 2018), SENASICA SAGARPA (SS 2018f)
- South Africa - FruitFly Africa (FFA 2018)
- USA - Animal and Plant Health Inspection Service (USDA/APHIS 2018)
- Plant and Food Research, New Zealand (PFR 2018c)
- Tephritid Workers Database (TWD), managed by the FAO/IAEA, invites workers to follow the TWD on Facebook (TWD 2018).

Most of the above programmes are associated with "parent organizations" that have social media accounts, e.g. the Insect Pest Control Section at the FAO/IAEA (FAO/IAEA 2018b). However, a major exception is Programa Moscamed Guatemala as described below.

### *7.1. Programa Moscamed Guatemala*

Programa Moscamed Guatemala has its own website (PMG 2018b), and has social media accounts – Facebook, Twitter, and LinkedIn.

In a review of these social media at Programa Moscamed Guatemala (social media are part of its regular communication activities), the following points were made:

- The programme website was created in 2008. According to Google Analytics, from 2011 to 2017 the programme website was visited about 13 000 times per year. In 2018, website visits originated in 42 countries (71% of visits from Guatemala), 85% of which were made by new visitors. The majority (60%) of visitors were quite young – 18–34 years of age.
- In 2015 a space in the digital encyclopaedia Wikipedia was opened, and a link to the platform LinkedIn was initiated.
- Facebook is the most popular platform used in the programme; people from both urban and rural areas (including people on farms), and also people from various organizations and institutions, use Facebook.
- Facebook and Twitter are used to convey technical information on the community-support actions and activities of the programme, and on the availability of technical assistance and training, but also to obtain feedback from stakeholders. Most communication involves requests for clarification on programme activities and technical assistance, and for advice on agricultural

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issues. Comments and questions from the public are mostly positive. However, a few negative and critical comments are also received, and these need to be handled promptly; such comments often relate to the environmental impact of aerial release and the chemicals applied in ground control of hot spots.

- The programme recommends that an area-wide programme should have a well-maintained website, use social networks, and set up information repositories such as databases and pages in Wikipedia. Each programme should have a full-time professional communicator to supervise the handling of information and communication with stakeholders. Support from programme leaders is essential.

## 8. SUPPORT FROM GOVERNMENTS

Obtaining the support of national, regional or local governments is dependent largely on the perceived importance of the commodity at stake, e.g. plant products for export (Buchinger 1993; Kinney 1993), health of livestock that are vital to the economy of the region, survival of subsistence farmers, human health threatened by insect vectors of disease, and recreational areas that require no or few environmental hazards such as insecticides that discourage tourism.

It is often difficult to obtain government support and the legislation and regulations needed to operate a programme. Considering the competing demands on financial resources, governments may regard pest control programmes as a low priority because they usually benefit only some, not all, segments of a community. Positive examples of exceptional support from national governments are the fruit fly programmes in Chile, Japan and Mexico. These successful programmes resulted in the opportunity to export crops to previously closed markets (MAG/SAG 1995; SAG 1996; Reyes et al. 2000; Teruya 2000; Enkerlin et al. 2015; Enkerlin, this volume).

Legislation and regulations are usually required to support the implementation of complex area-wide programme activities. Programme staff and others, possibly perceived as plant or animal health inspectors, will need to enter private property, monitor the pest population, inspect and treat crops or livestock, possibly remove derelict host plants, and enforce treatment and quarantine activities. Legislation to collect funds from the community, and to obtain enforcement authority, may be needed, but outreach activities to educate stakeholders and increase public awareness tend to reduce the need for enforcement actions (Dyck et al. 1993; Seymour 2018; Nelson et al. 2021). The approval of legislation and enforcement is an indication of government support for an AW-IPM programme. For example, legislation that regulates the culture of alternate hosts of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) was recently passed in Spain to support an AW-IPM programme to control this major pest of citrus (W. R. Enkerlin, personal communication). Also, in California, USA, where fruit infested by this fly is sometimes smuggled into the state, legislation was passed to try to prevent this, and to levy fines against those who are caught smuggling banned fruit (CDFA 2005).

Besides fines or other negative enforcement actions, positive incentives can also be provided to obtain the cooperation of people living within the area of an AW-IPM programme, e.g. trees for urban property owners to replace those that are

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susceptible to the target pest (OKSIR 2018a), and free insecticide for farmers to treat cattle against pests (Dyck et al. 2000).

Strong government support helps to stabilize, legitimize, and empower a programme so that it can operate effectively. Ideally, governments have a long-term view, and an all-embracing view, of a whole community's welfare; this is needed to support the characteristically long-term AW-IPM programmes that apply the SIT. Endorsement of a programme by a government helps encourage the provision of the necessary resources, e.g. skilled personnel and facilities to rear and irradiate insects. Also, programme endorsement by a government, offer of technical assistance, or other kinds of incentive from another country that may represent a potential but important export market, will encourage programme success.

Nevertheless, political conditions change over time, and can change frequently (Patton 1984). Some politicians may have other objectives. Long-term support from a government is never a certainty. A programme needs to be protected from changes in government so as to provide the stability required to attain its goals.

In government programmes, the assignment of new key personnel can radically change the support given to a programme. Therefore, new people appointed to key positions need to be informed quickly about the programme. Political leaders are usually not technical experts. Therefore, efforts should be made to educate influential people.

Political sensitivities (Patton 1984) can negatively affect a programme. Political uncertainties and civil unrest have impaired the implementation of SIT-related programmes, e.g. in the case of the Mediterranean fruit fly, the area-wide eradication programme in southern Mexico and Guatemala, or the transboundary suppression programme in contiguous areas of Israel, Jordan and the Territories Under the Jurisdiction of the Palestinian Authority (Enkerlin, this volume; Hendrichs, Vreysen et al., this volume).

A high level of support from governments means the involvement of high-level officials, both decision-makers in the executive branch and those who approve programme funds or appropriations in the legislative branch of government. It is useful to identify a knowledgeable "champion" of the programme who can highlight the programme's benefits vis-à-vis politicians. Obtaining the authority and support from a senior government official, who understands the programme and its objectives, is a big advantage.

One of the important roles of government in successful pest eradication programmes is the official declaration that a particular species has indeed been eradicated in a certain area (Teruya 2000; Barclay et al., this volume; Vreysen, this volume).

## 9. TECHNICAL SUPPORT

AW-IPM programmes need the support of local, national, and international technical experts. This gives a programme technical credibility. Opposition from scientists, for whatever reason, tends to discredit a programme (Winston 1997; DFID 2002; Whitten and Mahon, this volume). Papers in technical journals, presentations at scientific meetings, and participation in training programmes and collaborative

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research projects are essential to foster an open and fair exchange of information (P. Rendón, personal communication).

Frequent communication between programme staff and the scientific community is essential to generate "programme ownership" and "goodwill", as well as to interest and involve researchers in improving technology (and thereafter possibly obtain the new technology for use in the programme). There are costs and operational limitations to adopting new technologies frequently, but programme managers must always be open to improve the techniques being used. Therefore, the technical and operational aspects of a programme, especially a long-term programme, needs to evolve over time (Buchinger 1993; Kinney 1993).

Applied research on various aspects of a programme conducted by a supporting technical unit, often called "methods development", helps to ensure its success (Lindquist et al. 1992; LaChance 1993; Tween 1993; FAO/IAEA 2018j). The melon fly *Zeugodacus cucurbitae* (Coquillett) programme in Japan received very strong support through basic and applied research conducted at national institutions and agricultural experiment stations by entomologists in the country; certainly this contributed to the programme's success (Kakinohana et al. 1993; Yamagishi et al. 1993; Itô et al. 2003; Koyama et al. 2004).

## 10. PROGRAMME SUCCESS OR FAILURE

Even though adequate internal and external technical support is fundamental to progressively increasing programme effectiveness and efficiency, technical issues are rarely the main reason for programme failure. Some AW-IPM programmes with an SIT component have failed, or achieved limited success, because of poor communication and engagement with stakeholders and inadequate public support.

Many lessons regarding stakeholder communication, engagement and support have been learned, some positive and some negative (Vreysen et al. 2007; Dyck, Reyes Flores et al., this volume):

- Stakeholder support at various levels is vital for the operation of an AW-IPM programme.
- Communication among programme staff, with the programme's stakeholders and its financial supporters, and with the general public, is essential for a programme to receive the support it needs.
- Leaders in a community are role models; they are particularly influential in spreading the adoption of new technologies, and are important for promoting the acceptance of AW-IPM programmes.
- Continuing input from technical experts is needed to keep programmes up-to-date with the latest research findings, and to maintain technical credibility.
- Even though potentially costly and often not recognized by financial supporters, communication and engagement with stakeholders and the public is as important to a programme's success as is effective management (Dyck, Reyes Flores et al., this volume).

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# CHAPTER 6.1.

# STRATEGIC OPTIONS IN USING STERILE INSECTS FOR AREA-WIDE INTEGRATED PEST MANAGEMENT

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## SUMMARY

The four strategic options, "suppression", "eradication", "containment", and "prevention", in which the sterile insect technique (SIT) can be deployed as part of area-wide integrated pest management (AW-IPM) programmes, are defined and described in relation to the contexts in which they are applied against non-native invasive or naturally occurring major insect pests. Advantages and disadvantages of these strategic options are analysed, and examples of successful programmes provided. Considerations that affect decision-making in relation to the selection of a strategic option, e.g. pest biology, whether the pest is a disease vector or agricultural pest, the pest's status in the target area, and the target market for the produced crop or livestock commodities, are reviewed and discussed in terms of a phased conditional approach to programme planning, preparation, and implementation. If an option is changed during implementation of the SIT, unrealistic expectations often result, leading to high political costs and the discrediting of the technology. Before embarking on an AW-IPM programme with an SIT component, the choice of a strategic option needs to be assessed carefully, and preparations should be supported by considerable baseline data, technical and economic feasibility assessments, and detailed planning.

## 1. INTRODUCTION

E. F. Knipling developed a theoretical model of the sterile insect technique (SIT) in the early 1940s (Klassen 2003; Klassen et al., this volume), but it was not until 1954 that the technique was successfully demonstrated with the elimination of the New World screwworm *Cochliomyia hominivorax* (Coquerel) from the island of Curaçao following continuous releases of sterile insects for 6 months (Baumhover et al. 1955). Since then, in line with Knipling's basic model, the SIT has on numerous occasions been confirmed in field programmes as an effective and very powerful method of insect pest management. In spite of this history of successful SIT applications, the existence of puzzling terminology, conflicting definitions, and inappropriate utilization of concepts/strategies continue to cause confusion.

Baumhover et al. (1955) referred to the strategy applied in the Curaçao experiment as screwworm "control". Both Knipling (1955) and Lindquist (1955) proposed "control" and "eradication" as possible strategic options in using the SIT (Lindquist et al. 1990). While Lindquist (1955) used the term "control" to refer to the general use of the SIT, Knipling (1969) defined for the first time "eradication" and "suppression" as the two major strategic options. Knipling suggested that the terms "suppression" and "management" could be used interchangeably (Knipling 1979). During the decade that the screwworm programme in the south-west of the USA was maintaining a "containment" buffer along the Mexican border, he explained that it was erroneously referred to as an "eradication" programme, and argued that, since long-range migrating flies reinvaded the area every year, the term eradication was misleading in this context when compared with the Curaçao and Florida programmes.

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Referring to programmes such as the one against the Mexican fruit fly *Anastrepha ludens* (Loew) in southern California, Knipling (1979) described "prevention" as another possible strategic use of the SIT:

> As more experience is gained in the use of sterile insects for insect suppression and with greater confidence in the value of this technique, releasing sterile insects routinely in certain areas may be more expedient to prevent establishment of major pests than eliminating them after they become established.

Since 1996, this principle has been applied in the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) Preventive Release Program in the Los Angeles Basin, California, USA (Dowell et al. 2000) (section 4.1.).

In 2005, the International Plant Protection Convention (IPPC) adopted the revised International Standard of Phytosanitary Measures (ISPM) 3, "*Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms*"; for the first time sterile insects were included among beneficial organisms (FAO 2017a). The guidelines promote the safe use of biological control agents and beneficial organisms such as sterile insects, but do not involve special regulatory requirements such as those needed for the release of genetically modified insects. The inclusion of the SIT in the revision of ISPM 3 was a consequence of the growing acceptance of its application as part of an area-wide integrated pest management (AW-IPM) approach against key non-native or naturally occurring insect pests. This change in the mindset of decision-makers and stakeholders is driven mainly by the demands of large retailers (who are responding to consumer requests for cleaner food and a safer environment). It is also driven by the need for alternatives to the indiscriminate use of insecticides, and by the increase in globalization and concomitant international agricultural trade, which requires enforcement, through national animal and plant protection organizations, of the Agreement on the Application of Sanitary and Phytosanitary Measures (the "SPS Agreement") under the World Trade Organization (WTO 1994).

The programmes applying the SIT tend to become grander in scale and scope, thereby increasing their commercial impact and simultaneously reducing their relative operational costs, mainly due to economies of scale and improvements in the technology of rearing and releasing large numbers of sterile insects (Hendrichs 2000). An extreme example of a very large programme is the Moscamed Mediterranean fruit fly containment effort in Guatemala and southern Mexico, which operates on an annual budget of ca. USD 30 million (data from 2003), (Enkerlin et al. 2015, 2017). Within such contexts and under these pressures, the authorities deciding on AW-IPM campaigns are facing crucial choices regarding the inclusion of the SIT, and which strategic option to apply. In fact, as noted by Klassen (2000), "the terms of reference and contingencies have a tremendous influence on the selection of strategies", which then are "the real decision-makers".

The objectives of this chapter are to review the terminologies, the main characteristics and strengths and weaknesses of the various strategic options where sterile insects can be used, and to propose definitions in accordance with international agreements. In addition, the chapter reviews briefly the phased conditional approach to programme implementation, and addresses important species and case-specific issues, e.g. biology of the target insect, buffers, release densities, topography, etc., that

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require careful analysis before selecting one of the four strategic options under which to integrate the SIT into AW-IPM programmes.

## 2. FOUR STRATEGIC OPTIONS: DEFINITIONS AND DESCRIPTIONS

The Food and Agriculture Organization of the United Nations (FAO), through its IPPC, whose international phytosanitary standards are accepted by signatory countries under the SPS Agreement of the WTO, defines "control" of a given plant pest (FAO 2017b) as encompassing:

> Suppression, containment or eradication of a pest population.

These three strategies would apply to most AW-IPM programmes with an SIT component, including those against insect pests of medical and veterinary importance. However, the most efficient and cost-effective "control" programme is the one that aims at *preventing* the entry of a pest (movement of a pest into an area where it is not yet present (FAO 2017b)) rather than at dedicating resources to suppress, eradicate or contain an *introduction* (the entry of a pest resulting in its establishment (FAO 2017b)) once it has occurred (Knipling 1979). On this basis, four strategic options, i.e. "suppression", "eradication", "containment", and "prevention", will be discussed.

### *2.1. Suppression*

The FAO (2017b) defines suppression as:

> The application of phytosanitary measures in an infested area to reduce pest populations.

Thus the main objective of a suppression programme is to maintain the pest population below an agreed and acceptable economic injury level and/or prevalence level. Until the early 1990s, the SIT was generally considered an appropriate technique for eradicating certain insect pests. This view largely resulted from the high visibility of the successful screwworm eradication programme in North and Central America (Baumhover 2002; Klassen et al., this volume), and the assumption that the SIT was too expensive to compete with other control methods for routine pest management. In recent years, however, suppression of a pest population is increasingly being viewed as a suitable strategy to apply the SIT as part of an AW-IPM approach for some pests of agricultural importance (Hendrichs et al. 2007) due to: (1) crucial improvements made in the rearing and release techniques for some key insect species, which have significantly improved the cost-effectiveness of SIT application (Caceres et al. 2004; FAO/IAEA 2017; Parker, Mamai et al., this volume), (2) increased restrictions imposed on the use of insecticides (Matteson 1995) and more stringent regulations on their residues, (3) increasing development of insecticide resistance in arthropods (Whalon et al. 2008), (4) increased intermingling of commercial production areas and human settlements, which complicates the routine use of insecticides, (5) increased customer health consciousness and the demand for low-residue or even "organic" products (Economist 2001; Greene et al. 2017), and (6) difficulties in maintaining effective quarantines to keep an area pest free (Mumford, this volume).

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One major advantage of applying a suppression strategy is the significantly lower investment needed to monitor the pest population as compared with the more intensive monitoring required during and after an eradication campaign (Table 1). Moreover, the set-up and rigorous implementation of effective quarantines (which demand legislation and considerable investment) to protect a pest free area (FAO 1999) are not needed or demand less attention and resources when a suppression strategy is applied (Table 2). Another strength of the suppression strategy is the focus on environmental benefits compared with conventional control methods (Table 1). These trends have culminated in the implementation of suppression programmes using the SIT as an environment-friendly replacement for the use of insecticides (no longer disrupting pollination and the biological control of secondary pests) (Hendrichs et al. 1995; Enkerlin et al. 2003). This strategy has gained acceptance mainly for pest insects of phytosanitary importance, since a certain level of "crop damage" to agricultural commodities is usually acceptable. However, this concept has in general been considered less applicable to insect pests of veterinary, and particularly medical, importance. However, a suppression strategy against disease transmitting mosquitoes would be feasible, provided that only sterile males are released, without sexing errors, as the males are not the disease transmitting sex (Bourtzis et al. 2016).

It should be emphasized that implementing a suppression strategy in an area does not preclude exporting agricultural commodities from that area to countries that have a pest free status. Export markets that accept only pest free commodities can be accommodated within the context of a "systems approach" (FAO 2017c), whereby an effective preharvest suppression programme with an SIT component can be integrated with other efficient pest risk-mitigation measures (for example, postharvest treatments) to guarantee pest free agricultural products. This strategic approach is successfully applied, e.g. in the Arava Valley of Israel, where the use of greenhouses, as an additional risk-mitigation measure, has allowed the export of Mediterranean fruit fly-free vegetable commodities from this area to the USA (Cayol et al. 2004).

Whereas eradication aims at eliminating the last individual of a population in the target area, a suppression strategy can tolerate a certain residual pest population, and lead to the establishment of an "area of low pest prevalence" (FAO 2016b). Suppression can be achieved much more quickly than eradication, is less complex, resource demanding, and management intensive, and therefore less expensive in the initial years (Enkerlin and Mumford 1997; Mumford, this volume). Nevertheless, a suppression strategy requires continuing releases of sterile insects, often combined with other suppression measures, to maintain a low pest population level (Table 1). However, adopting a suppression or other strategy does not exclude eventually progressing towards an eradication strategy once low pest populations are maintained sustainably and new situations arise (Suckling et al. 2014; Staten and Walters 2021).

On the other hand, while the permanent application of a suppression strategy, including continuing releases of sterile insects, could be considered disadvantageous when compared with the sustainable elimination of a pest from an area, this permanent need for sterile insects could stimulate and promote investment in, and the commercialization of, the mass production of sterile insects (Enkerlin and Quinlan 2003) (Table 2). A similar demand has already resulted in a rapidly growing augmentative biological control industry (ANPB 2005; IBMA 2017).

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*Table 1. Strengths and weaknesses of the four strategic control options applied in AW-IPM programmes integrating the SIT, depending on the type of area*

| Control Strategy | | | | |
|---|---|---|---|---|
| Suppression (infested area) | Eradication (infested area) | Containment (pest infested and pest free area) | Prevention (pest free area) | Eradication (introduction into pest free area) |
| **Strengths** | | | | |
| Decreased pesticide use<br>Lower investment in long-term monitoring and in short-term capital and operational costs<br>No need for quarantine measures<br>Access to markets discriminating for low pesticide residues | Insecticide use eliminated eventually<br>Access to specific pest free export markets[1] as well as to markets discriminating for low pesticide residues without pre-export treatment<br>Most activities and costs limited in time<br>Strengthened quarantine infrastructure also against other pests<br>Strong public and political support for disease vectors | Protection of neighbouring pest free areas<br>Environmental and economic benefits for protected area<br>Pest free area can be expanded gradually<br>Threat of further pest expansion helps to obtain public and political support for activities<br>Maintains export markets | Proactive rather than reactive approach (risk mitigation)<br>In case of introduction, effective sterile to wild insect ratios (low wild population density)<br>No trade disruption in case of outbreak in release area<br>Infrastructure for SIT is in place in case of other outbreaks in the region<br>Maintains export markets | Establishment of pest precluded (if action taken immediately)<br>Much cheaper in medium and long term than having to "live with" new pest forever<br>Infrastructure and experience gained can be applied against other invasive pests<br>Success in avoiding pest establishment promotes preparedness plans for other invasive pests<br>Maintains export markets |

[1] Only for agricultural pests, excluding insect pests of medical importance

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*Table 1. Continued*

| Control Strategy | | | | |
|---|---|---|---|---|
| Suppression (infested area) | Eradication (infested area) | Containment (part infested and part pest free area) | Prevention (pest free area) | Eradication (introduction into pest free area) |
| **Weaknesses** | | | | |
| Permanent control activities<br>Post-harvest treatments required for export<br>More active participation and better organization of grower associations needed | High short-term investment<br>Intensive public relations campaign<br>Long-term investment in permanent monitoring networks<br>Continuous rigorous quarantine set-up | Cooperation between infested and non-infested areas<br>Intensive public relations campaign<br>Disruption of trade and free movement of commodities between infested and non-infested areas | Investment while the targeted pest is not present (political aspect)<br>Not all areas at high risk of introduction can be protected<br>Difficult to appreciate benefits of programmes that run for long time frames and thus susceptible to budget cuts when financial situation becomes difficult | Species-specific preparedness needed (plan of action, SIT technology and sterile insect supply, etc.)<br>Early detection and quarantine to delimit and isolate incursions<br>Long-term investment in permanent monitoring networks<br>In some situations, temporal disruption of trade. |

While insecticide costs have been increasing rapidly following the removal of many older products from the market, and crop protection companies facing higher costs and steeper regulatory hurdles for launching new pesticide products (CropLife 2016), using sterile insects as part of a suppression strategy for some key fruit fly and moth pests has become cost-competitive with conventional or other population reduction methods (Pereira et al. 2013; Vreysen et al. 2016) (Table 3). Examples of suppression programmes are: Mediterranean fruit fly in Israel and Jordan (Bassi et al. 2007), Croatia (Bjelis et al. 2016), South Africa (Venter et al. 2021), and Valencia, Spain (Primo 2003); codling moth *Cydia pomonella* (L.) in British Columbia, Canada (Bloem et al. 1998; Nelson et al. 2021); false codling moth *Thaumatotibia leucotreta* (Meyrick) in South Africa (Boersma 2021); and some are under development for other species of Diptera and Lepidoptera. These programmes are driven by the need to decrease insecticide use to comply with minimum residue levels required for export and/or local markets and communities, or to offer an alternate method of control for pests that have become resistant to many available insecticides, such as the false codling moth and diamondback moth *Plutella xylostella* (L.).

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*Table 2. Characteristics of the major strategic control options applied in AW-IPM programmes integrating the SIT, depending on the type of area*

| Adjunct Issues | Control Strategy | | | | |
|---|---|---|---|---|---|
| | Suppression (infested area) | Eradication (infested area) | Containment (part infested and part pest free area) | Prevention (pest free area) | Eradication (introduction into pest free area) |
| Quarantine | No | Yes | Yes | No | Yes |
| Investment in monitoring | Low | High | Varies along gradient (Fig. 1) | Medium | High |
| Investment / operational costs | Medium | High | High | Medium | Low |
| Trade advantage | Low (but postharvest treatments can guarantee pest free products) | High | Medium | High | High |
| Demand for sterile insects | Continuous | Short term[1] | Medium term (but can be continuous) | Continuous | Short term |
| Potential for commercialization of SIT | High | Low[1] | Medium | Medium | Low |

[1] Nevertheless, where the target area is very large, requiring a division into many blocks or phases, there could be a long-term demand for sterile insects and increased potential for commercialization

## *2.2. Eradication*

Eradication is defined (FAO 2017b) as:

> Application of phytosanitary measures to eliminate a pest from an area.

This definition, which is accepted and used by the agricultural community, clearly implies the *elimination of an isolated local population* of a pest. Nevertheless, for public health (used by the World Health Organization (WHO) mostly for human diseases), the term eradication is restricted to *global extinction* of a pest at the species level (WHO 2001). Classical examples are the eradication of small pox (Fenner et al. 1988), rinderpest (OIE 2011), or the current worldwide efforts to eradicate polio (Lahariya 2007).

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*Table 3. Examples of AW-IPM programmes that have applied the SIT according to major control strategies in relation to the type of area*

| Control Strategy | | | | |
|---|---|---|---|---|
| Suppression (infested area) | Eradication (infested area) | Containment (part infested and part pest free area) | Prevention (pest free area) | Eradication introduction into pest free area) |
| Carob moth[1]: Tunisia; Diamondback moth[3]: Mauritius | New World screwworm[2]: N. and C. America (1958–2003) | New World screwworm: Panama (2004–present) | | New World screwworm: Libya and the Florida Keys |
| Mexican fruit fly[4]: North-east Mexico | Mexican fruit fly and West Indian fruit fly[5]: North-west Mexico | Mexican fruit fly: Sinaloa, Mexico | Mexican fruit fly: Baja California and Rio Grande Valley (Mexico-USA border) | Codling moth: Brazil |
| Codling moth[6]: British Columbia, Canada | Pink bollworm[7]: So. USA and northern Mexico (2001–2012) | Pink bollworm: San Joaq. Valley in California USA (1969–2000) | | Cactus moth[8]: Yucatán peninsula, Mexico |
| Mediterranean fruit fly[9]: Brazil, Croatia, Ecuador, Israel, Jordan, Morocco, South Africa, Spain | Mediterranean fruit fly: Argentina (Patagonia) (1992–present), Chile (1992–1995), Mexico (1978–1982), southern Peru 2002–2008) | Mediterranean fruit fly: Guatemala-Mexico (1983–present), Peru-Chile (1996–2002) | Mediterranean fruit fly: Los Angeles Basin in CA, USA (1996–present), Tampa-Miami in Florida USA (1998–present), Chile-Peru border (2008–present) | Mediterranean fruit fly: Los Angeles Basin in California USA (1980s-1996), Southern Australia, Dominican Republic (2015–2017) |
| Oriental fruit fly[10]: Thailand, Vietnam | Melon fly[11]: Japan (1982–1994) | | Melon fly: Japan (is. near Taiwan) (1995–present) | Painted apple moth[12]: New Zealand |
| Onion maggot[13]: The Netherlands | Sweetpotato weevil[14] and West Indian sweetpotato weevil[15]: Japan | Queensland fruit fly[16]: South-eastern Australia | | Queensland fruit fly: Western Australia |
| Greenhouse whitefly[17], sweetpotato whitefly[18], and Amer. serpentine leafminer[19]: Europe, USA (greenhouses) | Tsetse fly: Unguja Island[20] (Zanzibar) (1993–1998), Niayes[21], Senegal | | | Old World screwworm[22]: Australia[23] |
| False codling moth[24]: South Africa | | | | |

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*Table 3. Continued (footnotes)*

[1] *Ectomyelois ceratoniae* (Zeller)
[2] *Cochliomyia hominivorax* (Coquerel)
[3] *Plutella xylostella* (L.)
[4] *Anastrepha ludens* (Loew)
[5] *Anastrepha obliqua* (Macquart)
[6] *Cydia pomonella* (L.)
[7] *Pectinophora gossypiella* (Saunders)
[8] *Cactoblastis cactorum* (Berg)
[9] *Ceratitis capitata* (Wiedemann)
[10] *Bactrocera dorsalis* (Hendel)
[11] *Zeugodacus cucurbitae* (Coquillett)
[12] *Teia anartoides* Walker
[13] *Delia antiqua* (Meigen)
[14] *Cylas formicarius* (F.)
[15] *Euscepes postfasciatus* (Fairmaire)
[16] *Bactrocera tryoni* (Froggatt)
[17] *Trialeurodes vaporariorum* (Westwood)
[18] *Bemisia tabaci* (Gennadius)
[19] *Liriomyza trifolii* (Burgess)
[20] *Glossina austeni* Newstead
[21] *Glossina palpalis gambiensis* Vanderplank
[22] *Chrysomya bezziana* (Villeneuve)
[23] Although free of the Old World screwworm, Australia maintains a contingency plan against potential incursions of this pest (AFF 2002)
[24] *Thaumatotibia leucotreta* (Meyrick)

An eradication strategy also leads to a reduction in the use of insecticides that is often larger and longer-term (once eradication is eventually achieved) as compared with a suppression strategy, although pre-release population reduction and "hot-spot" treatments may temporarily require increased localized use of insecticides. In addition, eradication allows the establishment of internationally recognized "pest free areas" (Malavasi et al. 1994; FAO 2016a, 2017b) which can permit access to otherwise closed export markets. These trade advantages are often the major motivation for eradication programmes against insect pests of agricultural importance. The eradication of the Mediterranean fruit fly in Chile (SAG 1996) opened trade opportunities annually worth several thousand million USD, and the eradication of the Mexican fruit fly and the West Indian fruit fly in north-western Mexico allows fruit trade with the USA without the need for costly postharvest treatments (Reyes F. et al. 2000; Enkerlin, this volume).

In the past, most AW-IPM programmes integrating the SIT aimed at eventual eradication of the target population, and sterile insects were often released only during the last phase of the programme (section 3.). The eradication strategy is applied mainly in two different situations (Tables 1 and 2):

- Eliminating an established pest population, e.g. the tsetse fly *Glossina austeni* in Unguja Island (Zanzibar) (Vreysen et al. 2000),

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- Eliminating outbreaks of an invasive species before full establishment of the pest can occur, e.g. the painted apple moth in New Zealand (Suckling 2003) (additional examples in Table 3).

The second situation is likely to increase, with more pest introductions due to globalization, pest survival in previously inhospitable climates due to climate change, and the growing awareness by governments of the need for monitoring networks for early detection to facilitate eradication (hendrichs, enkerlin et al., this volume). Once the target pest has been eliminated from a given area, it is imperative to maintain this area pest free. This will require efficient, permanent, and stringent quarantine procedures to preclude reinvasion.

To declare eradication, two very important decisions (which have significant economic implications) have to be made: (1) the period of time in which releases of sterile insects should continue after the last wild insect has been detected (Vreysen, this volume), and (2) the duration of continued monitoring after releases have stopped, to be able to declare with sufficient confidence the status of eradication (Barclay et al., this volume). When creating a new pest free area, this period of no detections can last up to a whole year or longer for some insect pests such as tsetse flies (Vreysen et al. 2000). For the eradication of fruit fly pest outbreaks in free areas, ISPM 26 established a period of zero detections equivalent to a *minimum of three life cycles* after catching the last wild fly (FAO 2016c). This approach has been criticized as unscientific (Carey et al. 2017), but historically has proven effective (McInnis et al. 2017; Shelly et al. 2017). Moreover, in practice and for trade purposes, the actual period required is often established bilaterally between trading partners.

Various mathematical models have been developed that, given the biology of the specific pest species, and the efficiency and density of the deployment of monitoring tools, determine the time required to ascertain, within certain confidence limits, the probability that the target population has been eliminated. These models, which are important complementary decision-making tools, have confirmed the overall effectiveness of the three-life-cycle period (Manoukis and Hoffman 2014; Collier and Manoukis 2017). Agent Based Simulations are a very recent approach to model invading pest populations such as the MED-Fly Outbreak and Eradication Simulation (MED-FOES) that aims at modelling invading Mediterranean fruit fly populations to determine the effective duration of the quarantine and other eradication measures following the last detection of an incursion (Manoukis and Collier 2021).

The decision to establish a pest free area (and then maintain its free status) is sometimes questioned in view of the permanent threat of reinvasion, which would result in a loss of the investment. The likelihood of reinvasion of a pest insect is related to its biology, its geographic distribution, the volume of travel and trade into the free area (Liebhold et al. 2006), the efficiency of the quarantine infrastructure, and the location and size of the pest free area. Therefore, the selection of an eradication strategy will also be influenced by these parameters.

The reinvasion potential of a species in a given area is also inherently linked to the economics of the various strategic options, which will therefore significantly influence the decision to select a suppression or eradication strategy. "*Sequential eradication*" in a target area can still be economically beneficial, notwithstanding periodic reinvasion, if the average period between reinvasions is long enough so that the economic and

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environmental benefits of each pest free period between reinvasions exceed the costs of having to "live with" the pest and the effects of the continuous control.

J. Mumford (personal communication) proposed the concept of "*serial eradication*", which entails the elimination of a pest population in a certain target area without heavy investment to prove the "status of eradication". If the area is reinvaded, the eradication effort is simply repeated. Not having to constantly "demonstrate the pest free status" could save considerable sums of money, although the trade benefits of a certified pest free status would be continuously in jeopardy and therefore not fully available, similar to situations of "near zero" or "low prevalence" populations (FAO 2016b).

Serial eradication could be applied in conjunction with the concept of "pest free place and site of production", where the pest free status needs to be maintained only for a fixed period, for example the harvest season (FAO 2016a). To illustrate, melons from Honduras are being exported to Taiwan from registered and qualified places of production maintained pest free only during the harvest season (IAEA 2017). This concept is normally associated with a situation where the commodity is considered a poor or only "conditional host" of the regulated pest (FAO 2017d). However, when the commodity of interest is considered a "good host", to obtain the trade advantages, the concept of serial eradication would require a "systems approach" (FAO 2017c), which includes a combination of low-cost monitoring, postharvest and other treatments, and occasional intensive area-wide suppression actions.

The decision to adopt a strategy of permanent suppression or periodic eradication is, however, based not only on economics and trade. The affordability of "living with" a disease vector population, even when suppressed, also needs to be considered. Eradication of major vectors of human or livestock diseases (or potentially even plant diseases) is often the preferred option, since a low density of the pest population does not necessarily lead to low levels of disease transmission (Otieno et al. 1990; Feldmann and Hendrichs 2001).

### *2.3. Containment*

Containment is defined (FAO 2017b) as:

> Application of phytosanitary measures in and around an infested area to prevent spread of a pest.

Containment programmes are adopted to avoid the spread of invading pests that have become established in part of an area, or to consolidate progress made in an ongoing eradication programme. Several examples are given in Table 3. Some of these programmes are largely stationary and thus become permanent containment efforts (e.g. the Mediterranean fruit fly programme on the border between Mexico and Guatemala, or the screwworm programme in eastern Panama), whereas others successfully advance or gradually retreat and eventually collapse. An example of the latter is the Queensland fruit fly Tri-State Fruit Fly programme. It was conducted since 1988 in eastern Australia to protect an area that contains much of the horticultural production areas of southern New South Wales, northern Victoria, and eastern South Australia (Jessup et al. 2007), but eventually had to be converted into an area-wide

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suppression programme due to the expanding range of the Queensland fruit fly. On the other hand, the pink bollworm programme in California is an example of a containment programme that, after many decades of successfully protecting cotton production in the San Joaquin Valley, eventually advanced towards successful eradication of the pest from south-western USA and north-western Mexico once technological change (i.e. *Bt*-cotton), the political situation, and stakeholder commitment favoured it (Henneberry 1994; NCCA 2001; Staten and Walters 2021).

A typical containment situation is illustrated spatially or geographically in Fig. 1, showing a gradient in the density of the pest population from an infested area (NAPPO 2004) on the left to a pest free area on the right. In between there is a relatively steep wild population decline across the various operational areas where the pest is being contained.

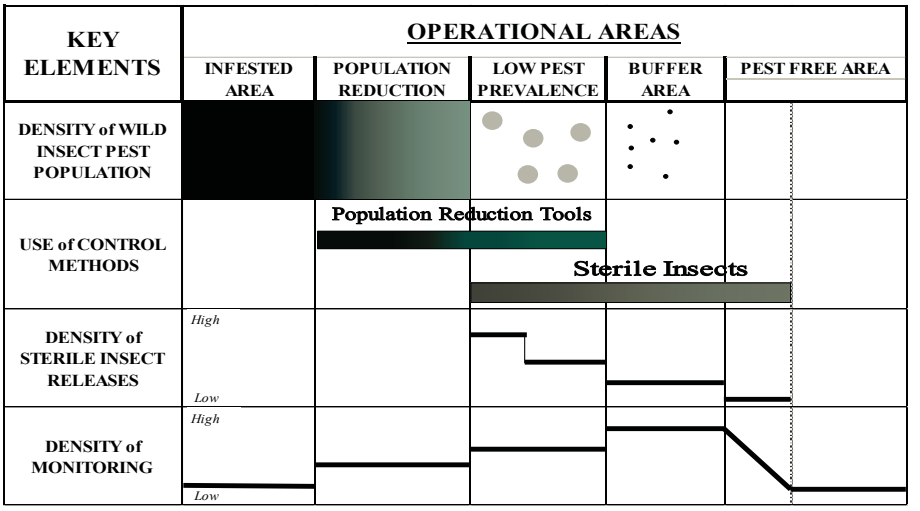



*Figure 1. Schematic representation of containment (and also rolling-carpet approach to eradication in large blocks, section 7.1.), illustrating geographically the gradient in pest population density and degree of activities in the different operational areas, including some preventive sterile insect releases in the pest free area closest to the buffer area.*

Various population reduction tools have to be integrated in areas and hotspots where pest levels are too high for sufficient numbers of sterile insects to be released, whereas the SIT is increasingly implemented over lower population levels, mainly low pest prevalence areas with some pest remnants or *incursions* (FAO 2017b)). Sterile insects are particularly effective in adjacent areas that already are largely pest free, but that are subject to regular pest *entries* (FAO 2017b). Ideally, sterile insects are also released as a safety measure in a buffer zone and over parts of the contiguous pest free areas, in view that the pest may occasionally be moved by the transport of infested host material. The degree of population reduction, sterile insect release, and monitoring efforts along this gradient are also indicated in Fig. 1, reflecting requirements to maximize their effectiveness. For example, to be able to detect as

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early as possible any entry or incursion, monitoring activities have to be the highest in the buffer zones and adjacent pest free area.

### *2.4. Prevention*

The prevention strategy is defined here as the "application of phytosanitary measures in and/or around a pest free area to avoid the introduction of a pest" (however, it applies also to the preventive application of sanitary measures against animal pests). Preventive sterile insect releases have been applied effectively where the invasion pressure is very high, and quarantine activities are not sufficient to maintain the area pest free (table 3). An example is the permanent release of sterile melon flies over the okinawa islands closest to taiwan (kuba et al. 1996). In situations where the invasion risk is not very high, sequential or serial eradication approaches are probably more viable economically (section 2.2.).

For agricultural trade, the strategic options of containment and prevention are designed to maintain market accessibility, although the prevention option is most desirable in terms of cost, as it is always cheaper to prevent a pest problem than to deal with it later (Knipling 1979). (Enkerlin (this volume) provides a more detailed explanation of the costs of containment and prevention.)

## 3. TEMPORAL PHASES OF AREA-WIDE IPM PROGRAMMES INTEGRATING THE SIT

Irrespective of the strategic option selected, AW-IPM campaigns that include the SIT will likely have similar basic elements and be implemented following distinct temporal phases, i.e. from research to feasibility assessments and pilot studies, all the way to operational field implementation (Hendrichs et al. 2007):

- *Pre-intervention Phase*
  a. The collection of baseline data on the distribution, dispersal, and population dynamics of the target species in space and time (Itô et al., this volume; Vreysen, this volume),
  b. Research and development, evaluation and validation of control methods to be integrated,
  c. Technical feasibility and benefit-cost assessments (Mumford, this volume), and adequate programme planning, including obtaining external technical expert advice, and
  d. Assuring political commitment and support from major stakeholders and community leaders, and mobilizing adequate financial resources (Dyck, Reyes Flores et al., this volume).
- *Preparatory Phase*
  a. Staff training and human capacity building, including stakeholders that need to be involved in some activities,
  b. Launching a public relations campaign (Dyck, Regidor Fernández et al., this volume),

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c. Establishment of funding mechanism, organizational structure and semi-autonomous institutional set-up under public-private operation and oversight,
d. Development of physical infrastructure (including the mass-rearing and sterilization facility, emergence and release centres) or contractual arrangements made to procure sterile insects from established mass-rearing facilities and to outsource the release of sterile insects (Dowell et al., this volume; Parker, Mamai et al., this volume),
e. Implementation of small pilot tests to validate the approach and train staff, and
f. Review by independent external experts to confirm readiness to embark on the operational phase (can also provide periodic reviews during this phase).

- *Operational Phase*
  a. Population reduction. Control measures are applied, with varying intensity depending on the strategy and seasonal variation in pest population levels, to reduce the density of the target population prior to the release of sterile insects. Various population reduction methods are available, depending on the target insect (Mangan and Bouyer, this volume). However, these are not always required, e.g. when the pest emerges in low numbers after a winter, or when the population undergoes a natural decline as a result of environmental conditions.
  b. Release of sterile insects. The systematic release of sterile insects over the target area to reduce the target population to a pre-determined level (suppression strategy), to drive the target population to extinction (eradication strategy), or to avoid pest establishment (containment and prevention strategies). This second part of the operational phase can in some instances overlap with continued population reduction, e.g. combining larval suppression and the use of insecticide-impregnated bednets to kill biting mosquito females with the release of sterile males (Mangan and Bouyer, this volume); concurrent treatment of wounds in livestock with insecticides and the release of sterile insects in screwworm eradication campaigns; or the simultaneous application of the male annihilation technique and the SIT as part of a male replacement approach for *Bactrocera* fruit flies (Barclay et al. 2014).
  c. Maintenance of control. To sustain the low prevalence status (suppression strategy) or the pest free status (containment and prevention strategies) through the permanent implementation of activities, or
  d. Verification and preservation of pest free status. To confirm and maintain the pest free status (containment and eradication strategies) through permanent implementation of quarantine and early detection and response activities (Barclay et al., this volume).

Progressing from each of these major phases to the next should ideally follow a *phased conditional approach*, e.g. fulfilment of the activities and requirements of a phase as the basis and precondition for entering the subsequent one. It is particularly important when deciding to embark on the operational or programme implementation phase that all previous pre-intervention and preparatory activities have been completed successfully. Preferably this readiness is confirmed by an independent review by a team of external experts (Vreysen et al. 2021).

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## 4. STRATEGIC CONSIDERATIONS IN RELATION TO PEST STATUS AND TARGET MARKETS

The selection of a control strategy is influenced significantly by whether the pest is a disease vector or an agricultural pest. For major vectors of plant, animal or human disease the relevant issue is whether a suppression strategy (releasing only sterile males) can largely maintain the population below a transmission threshold, or whether even low pest populations can result in considerable disease transmission, requiring the eradication option.

For agricultural pests two main factors are of relevance in decision-making: the status of the pest in the target area, and the target market for the produced crop or livestock commodities.

### *4.1. Target Area and Pest Status*

Pest status depends not only on whether the insect is present or absent in the target area, but also on the specific situation and national plant and animal health legislation of the importing and exporting countries in relation to that pest; thus, a pest can be *non-regulated* or *regulated* (both *quarantine* and *regulated non-quarantine* pests (FAO 2017b)). The pest status in a given area, together with the specific situation of the pest (non-regulated or regulated) (Tables 1, 2 and 3), are the primary factors (Klassen's "decision-makers") that will influence the type of strategy(ies) that are economically viable and feasible to be developed and implemented:

- An area is considered as *infested* (NAPPO 2004) when the pest is naturally occurring or non-native pest *establishment* (perpetuation, for the foreseeable future, of a pest within an area after entry (FAO 2017b)) has taken place and the pest has *spread* (expansion of the geographical distribution of a pest within an area (FAO 2017b)). A suppression or eradication strategy can be applied under these circumstances depending largely on the economics as well as isolation and ease of establishing/maintaining quarantine regulations, e.g. a suppression programme against the Mediterranean fruit fly in Valencia, Spain (Primo 2003), and eradication of the same pest in temperate fruit-production areas in Mendoza and Patagonia, Argentina (De Longo 2000).
- Situations of multiple presence of major pest species in a target area necessarily complicate strategic considerations, although there are some suppression or eradication programmes where more than one species have been simultaneously addressed. Examples are the eradication of both the Mexican and the West Indian fruit flies from north-western Mexico (Reyes F. et al. 2000), or the eradication of three tsetse populations (*Glossina p. gambiensis*, *Glossina tachinoides* Westwood and *Glossina morsitans submorsitans* Newstead) from 3500 km$^2$ in Burkina Faso (Politzar and Cuisance 1984; Clair et al. 1990).
- An area is considered *pest free* when the insect pest is not present in that area (FAO 2017b). If there are frequent *entries* (FAO 2017b) of a major invasive pest, and the area is *endangered* (area where ecological factors favour the establishment of a pest whose presence in the area will cause economically important loss (FAO 2017b)), the most cost-effective strategy to protect the area from any potential

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introductions will often be preventive releases of sterile insects, a successful example being the Preventive Release Programmes in California (Dowell et al. 2000; Enkerlin, this volume). About 150 million sterile male Mediterranean fruit flies are being released per week in the Los Angeles Basin, a "high-risk" area for introductions of this pest because of favourable ecological factors, the huge amount of international trade, the substantial volume of international postal shipments with infested fruit, high traffic in contraband fresh commodities, and the large number of fruits carried illegally by tourists (Liebhold et al. 2006).

- Simultaneous to strengthening quarantine measures, the threat of entry and introduction into a pest free area can be reduced by requesting postharvest treatments and pre-clearance actions, as well as proactively supporting *offshore pest risk-mitigation* activities in places where the pest originates (i.e. neighbouring countries or regions), e.g. Chile's support to the release of sterile Mediterranean fruit flies north of and along the border between Chile and Peru to avoid reinvasion of this pest from Peru into Chile (Enkerlin et al. 2003). Another example is the USA's support of the Moscamed programme along the Guatemala/Mexico border.
- A pest free area, contiguous to an infested area, can be protected by a containment strategy, e.g. the release of sterile pink bollworms over decades to prevent the spread of this pest from infested areas into the cotton production areas of the San Joaquin Valley in California, USA (Henneberry 1994); more recently this programme successfully shifted to an eradication strategy (Staten and Walters 2021).
- When establishment of an invasive insect pest has taken place recently, and the *outbreak* (a recently detected pest population, including an incursion, or a sudden significant increase of an established pest population in an area (FAO 2017b)) has not yet spread significantly out of the outbreak area, the recommended strategy is a rapid response to achieve eradication as soon as possible, e.g. elimination of the New World screwworm in Libya (Lindquist et al. 1992; Krafsur and Lindquist 1996; Vargas-Terán et al., this volume) or the Mediterranean fruit fly in the Dominican Republic (Zavala-López et al. 2021). Containment is the second-best strategic option for this situation, but only when immediate eradication is no longer feasible or has failed.

### *4.2. Requirements of Target Market*

For agricultural pests, the type of market targeted for the commodity produced plays a major role in selecting a control strategy. There are four major potential markets that can be accessed (IAEA 1995) (Table 4):

- *Domestic markets* are normally the least demanding, but also the least lucrative, and a suppression strategy using sanitation and other conventional methods is normally sufficient. Only when there are important local markets having special requirements in terms of low pesticide residues is the use of more environment-friendly pest control methods warranted.
- *Non-discriminating export markets* also often have no special requirements, and thus may be similar to domestic markets. However, increasingly, as a result of globalization, these markets tend to decrease in importance, whereas export

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markets demanding lower pesticide and/or pest presence in commodities are growing.

- *Low-residue export markets* are typically in developed countries that have demanding supermarket chains (in terms of maximum allowed residue levels) and a growing demand for such products. These markets either already have the pest or have climates in which the pest cannot become established. A suppression strategy involving application of more environment-friendly methods is suitable to gain access to these markets.

*Table 4. Access to potential markets using available control strategies*

| Control Strategy | Potential Markets | | | |
|---|---|---|---|---|
| | Domestic markets | Non-discriminating export markets | Low-residue export markets | Pest free export markets |
| Suppression (infested area) | | | | |
| Conventional control | Yes | Yes | No | No |
| Low pesticide including the SIT | Yes | Yes | Yes | No |
| Low pest prevalence and systems approach | Yes | Yes | Yes | Yes[1] |
| Eradication (pest free area developed) | Yes[2] | Yes[2] | Yes[2] | Yes |
| Containment (pest free area protected) | Yes[2] | Yes[2] | Yes[2] | Yes |
| Prevention (pest free area maintained) | Yes[2] | Yes[2] | Yes[2] | Yes |

[1] Only when combined with complementary measures, such as post-harvest treatments, except for marginal hosts of the pest

[2] Development, protection or maintenance of pest free areas, involving quarantines and intense monitoring, is usually not cost-effective for these markets unless they target mainly pest free export markets

- *Pest free export markets* are the most demanding, with zero tolerance for the presence of the pest in the export commodities. Accessing these markets requires an eradication strategy to establish certified pest free areas. Alternatively, a suppression strategy can be followed to establish a low prevalence area, but this strategy demands in addition a systems approach involving integration with complementary pest risk-mitigation measures such as, in some instances,

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postharvest treatments to guarantee pest free commodities. In situations where the exporting areas are already partially or fully pest free, the logical approaches to protect or maintain these markets are containment or prevention strategies, respectively.

## 5. STRATEGIC CONSIDERATIONS IN RELATION TO PEST BIOLOGY AND ECOLOGY

There are several factors related to the biology and ecology of the pest that influence the selection of a control strategy, and affect both planning and implementation, e.g. Pest mobility, pest densities, average lifespan, potential for direct and indirect damage, and ecological aspects such as topography, vegetation, and climate, as well as the capacity to monitor pest presence, together with gene flow, to determine the degree of isolation of the target population.

### *5.1. Pest Dispersal*

One of the most critical aspects is the degree of isolation of the target population and, associated with it, the dispersal ability of the target insect (Vreysen, this volume). Knowledge about dispersal ability is essential to assess the likelihood of invasion or reinvasion, which remains an important consideration in selecting and developing a strategy. Highly mobile insects can be advantageous for programmes that release sterile insects, as this trait will increase the probability of finding and mating with virgin wild female insects. However, at the same time, the greater the dispersal of the wild insect, and in particular wild females, the more difficult it will be to protect pest-cleared areas (Lance and McInnis, this volume).

Mark-release-recapture studies, using both wild and sterile insects, can also be used to study dispersal, but this approach, although valid, can be expensive and cumbersome (Itô et al., this volume).

An alternative is the analysis of the gene frequencies of target and neighbouring populations as an indirect approach to dispersal (Krafsur and Ouma, this volume; Vreysen, this volume). Pest dispersal ability is a decisive component in strategy selection, in assessing the minimum size of an intervention block where the SIT can be effectively applied (section 8.; Barclay et al. 2011; Klassen and Vreysen, this volume), and in determining the need for temporary and permanent buffer zones (section 9.).

### *5.2. Release Density*

Itô et al. (this volume) provide detailed mathematical guidance on estimating absolute densities of insect populations and growth under different situations and for pests with different biology. An estimate of these parameters of the pest population in the target area is key to determining the need and extent of population reduction required before the application of the SIT, and to make accurate estimates of the required number of sterile insects. The latter will obviously influence the total cost of the programme, and

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will represent a major consideration when selecting a strategy (Mumford, this volume).

The cost of the SIT component of AW-IPM programmes has traditionally been calculated using data from the production of sterile insects, and the cost associated with handling and release operations. Even though very large variations in the production and release costs of different insect pests exist, i.e. from only USD 0.25 to produce 1000 Mediterranean fruit flies to USD 85 to produce the same number of tsetse flies (Table 5), the ultimate cost of an SIT operation will also be determined by the number and frequency of sterile male insects per unit of surface that are needed to obtain adequate sterile to wild male overflooding ratios. A comparison of costs per surface and time unit for different pest species is presented in Table 5.

*Table 5. Comparison of costs of AW-IPM programmes that release sterile insects (data for codling moth calculated from information provided by R. Fugger and L. Tomlin, Sterile Insect Release Program, Canada, and Bloem et al. 1998)*

| Pest species | Cost of producing sterile male insects (USD per 1000 insects) | Cost of handling and releasing sterile males (USD per 1000 insects) | Total cost (production and release) (USD per 1000 insects) | Release rate (number of sterile males per $km^2$ per week) | Cost of sterile male production and release (USD per $km^2$ per year) |
|---|---|---|---|---|---|
| Codling moth[1,2] | 1.9 | 0.65 | 2.55 | 100 000 | 5167 |
| Mediterranean fruit fly | 0.25 | 0.15 | 0.4 | 200 000 | 4160 |
| *Glossina austeni* (savannah tsetse species) | 85.0 | 33.0 | 118.0 | 80 | 491 |
| New World screwworm[2] | 1.0 | 0.4 | 1.4 | 2500 | 182 |
| *Glossina p. gambiensis* (riverine tsetse species) | 85.0 | 96.0 | 181.0 | 15 | 141 |

[1] Data for codling moth based on releases for only 20 weeks per year
[2] Both sterile males and females are released

For example, following application of population reduction measures, only 15 sterile male *Glossina p. gambiensis* (riverine species of tsetse) are required per $km^2$ per week, whereas for the Mediterranean fruit fly, ca. 200 000 sterile males per $km^2$ per week are needed. Consequently, although the production and release costs of Mediterranean fruit fly are very low and for tsetse very high, the costs per $km^2$ per week of the SIT operation are much higher for Mediterranean fruit flies (USD 80) than for riverine species of tsetse (USD 2.7). Therefore, considering the cost of the sterile

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fly requirements per surface and time unit provides a much more accurate indicator of the economic implications of SIT operations.

### *5.3. Pest Aggregation*

Aside from the absolute population density, the degree of population aggregation or dispersion is important. Sterile insects are usually released by air, and are thus distributed fairly homogeneously over the target area, irrespective of whether the target pest is distributed evenly or clumped. Pest insects with a clumped distribution require effective hotspot detection and suppression (Shiga 1991), as well as higher release rates as compared with those with a homogeneous distribution, to obtain the required sterile to wild male ratios (Barclay, this volume), and thus pest aggregation also affects strategy selection and its cost. Only if the released insects can find the same aggregation sites and aggregate in a similar manner as wild insects (Vreysen et al. 2011), so that adequate sterile to wild male overflooding ratios are obtained in those sites, is there no need to increase release rates to compensate for such clumping (Vreysen, this volume).

### *5.4. Sterile Male Longevity*

The density of the sterile male population in the field, which fluctuates in relation to the release frequency and the sterile male mortality rate, should not decrease below that needed to maintain the critical ratio (Fig. 2, upper graph) (Kean et al. 2007; Barclay, this volume). Therefore, the frequency and number of sterile males released has to be carefully assessed in relation to the average longevity or survival of the sterile males, to effectively avoid periods when insufficient sterile males are present in the field (Fig. 2, lower graph).

As generations normally overlap in multivoltine species, releases have to be continuous, with survival determining whether they have to occur on a weekly (New World screwworm), twice a week (Mediterranean fruit fly, tsetse), or even daily basis (*Drosophila suzukii* (Matsumura) in greenhouses). The importance of assessing the survival of sterile male insects in the natural habitat must be emphasized here (Vreysen, this volume), as their actual survival in open field conditions is often drastically lower than in protected field-cage situations, where sterile males have easy access to food and are protected from predation (Hendrichs et al. 1993). In addition, mass-rearing conditions often inadvertently select for short-lived individuals (Cayol 2000). A shorter sterile male lifespan, although not directly representative of competitiveness, often requires higher release frequencies, and thus can significantly increase programme costs compared with longer-lived sterile insects.

### *5.5. Topography and Other Conditions of Target Area*

The topography of the target area, combined with the density of roads, has major implications for programme implementation and the selection of an intervention strategy. A flat terrain and a well-developed road network will facilitate most field

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activities (including ground release in some cases), whereas mountainous areas, dense vegetation, and the absence of roads will make implementation more challenging. In most of the larger programmes, releases and some of the population reduction activities use fixed-wing aircraft (Vreysen et al. 2000; Dowell et al. 2000), helicopters (Koyama et al. 2004), gyrocopters (Vreysen et al. 2021), or more recently even unmanned aerial vehicles (Benavente-Sánchez et al. 2021; Dowell et al., this volume), and the topography and presence/absence of a road network are less critical.

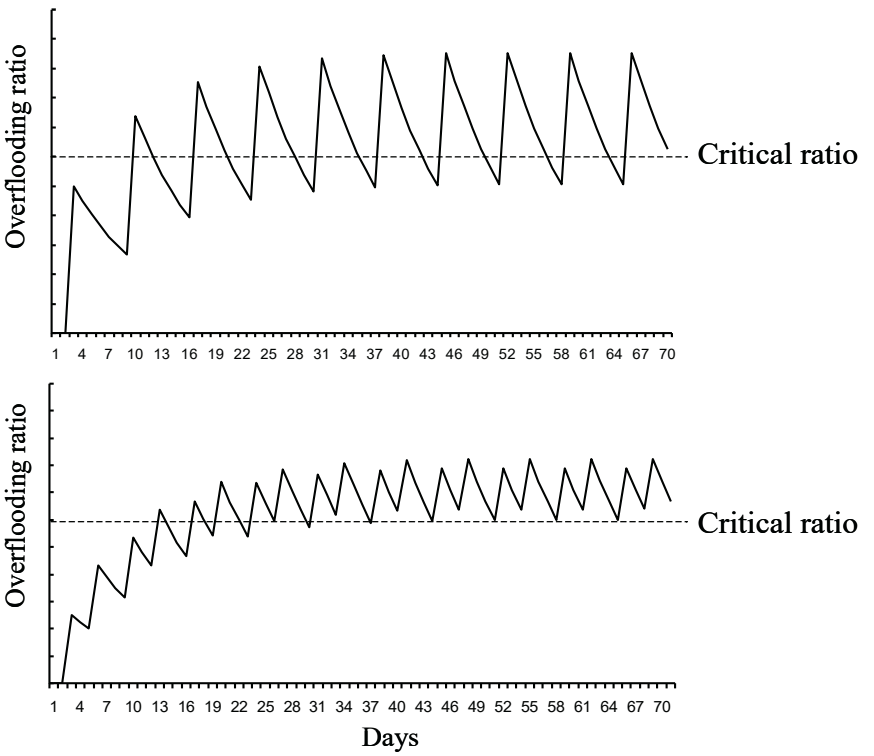



*Figure 2. Effect of sterile insect longevity (assume daily mortality rate of sterile males is 0.1) on sterile to wild overflooding ratio. Upper: Due to only weekly releases, sterile insect population routinely decreases significantly below the critical overflooding ratio; Lower: twice-a-week releases overcome this problem.*

Actually, the absence of a good road network is often advantageous for the establishment of efficient quarantine procedures in support of an eradication strategy. Monitoring, however, is mostly ground-based, and extreme terrain conditions make eradication campaigns (which have a more intensive monitoring component) much more complex and costly than programmes following a suppression strategy (which have less intensive monitoring activities). Likewise, topography influences the requirements of sterile insects or bait sprays, e.g. mountainous areas have a larger surface area per square kilometre as compared with flat, two-dimensional conditions,

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demanding higher sterile insect release rates. Furthermore, helicopters, which are more expensive to operate than fixed-wing aircraft, are often needed in difficult terrain for safety reasons and to properly treat narrow valleys.

### *5.6. Capacity to Monitor and Delimit Pest Presence*

The distribution of the target population has to be known accurately; direct and indirect monitoring methods are usually available to assess this for the different target species. Each monitoring tool, however, has its limitations, and none is perfect. Knowledge about the efficiency and limitations of the monitoring methods is essential to assess the accuracy of, and the ability to demarcate, the total distribution of the target population (Vreysen, this volume).

The different phases of programme implementation require specific adjustments to the monitoring system according to the pest density (Fig. 1). The intensity of deploying monitoring tools, or of indirect surveys, gradually changes from the initial relatively low monitoring effort during the baseline data collection to increased activity during the initial population reduction, low-prevalence, and eradication activities (highest need for detection capability), and will decrease again during maintenance/verification (FAO/IAEA 2018). Therefore, along the gradient in pest population density, the density of monitoring tools will also vary according to their efficiency in the different operational areas. For an eradication strategy, very efficient monitoring tools are particularly important once populations have decreased to very low densities; the same is true for population remnants or entries and incipient incursions (Fig. 1). In pest free areas, monitoring efficiency is important also for early detection of, and delimiting, the population of a new introduction. Inefficient monitoring tools to assess the status of eradication, or for delimiting purposes, will increase the cost of programmes — high deployment densities are required to compensate for the lower efficiency, and longer deployment times to obtain the required confidence limits (McInnis et al. 2017).

## 6. STRATEGIC CONSIDERATIONS IN RELATION TO PEST DISTRIBUTION

The characteristics of the spatial distribution pattern of the target population will be a crucial factor when selecting a strategy and developing an implementation plan. An insect population can be confined to an island, or have a fragmented or continuous distribution on a continent.

### *6.1. Island Distribution*

Targeting a pest population confined to an island is the ideal and simplest situation for which to develop an AW-IPM programme, e.g. New World screwworm on various islands in the Caribbean, cactus moth on islands off the Yucatán Peninsula in Mexico (Bello-Rivera et al. 2021), and melon flies or sweetpotato weevils on islands of the Okinawa archipelago (Kohama et al. 2003; Koyama et al. 2004), etc. Islands can be relatively small, making area-wide intervention easier, and, if the size is not too large,

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the insect population can be tackled at one time. Typically, insect populations on islands are isolated, with the ocean being an ideal natural barrier. Sustaining such areas pest free after eradication is only a minor concern for species such as the tsetse fly *G. austeni* which was eliminated from Unguja Island of Zanzibar, whereas for other species the establishment of quarantine procedures is facilitated by the island situation. However, migration over considerable distances has also been reported for species such as the melon fly (Koyama et al. 2004), where movement between Taiwan and islands in southern Japan has been recorded routinely (Kuba et al. 1996).

### *6.2. Fragmented Distribution*

Insect populations on continents are fortunately not always distributed continuously, but often appear to be fragmented and confined to "ecological or population islands". Examples are the carob moth in date palm oases in the Mediterranean Basin and the Near East, the mosquito *Anopheles arabiensis* Patton confined to isolated mosquito habitat along the Nile River in Sudan (Malcolm et al. 2009), and various tsetse species confined to valley systems in south-western Ethiopia, fragmented habitat in the Niayes of Senegal (Bouyer et al. 2010), and in river basins in West Africa (Bouyer et al. 2015).

Although the distribution of insect populations is sometimes perceived as vast and continuous, topographical features (e.g. mountains, lakes, deserts), and factors such as host availability, climate, and vegetation, often result in the division of populations into smaller population units. This subdivision occurs more easily at the edge of the distribution, where the insects are confined to those areas with favourable conditions. Field surveys assisted by a GIS-based analysis, supported by satellite imagery and population genetics studies (Feldmann and Ready 2014; Bouyer et al., this volume; Krafsur and Ouma, this volume), are needed to determine the degree of reproductive isolation between such smaller population units.

Since detailed data on the distribution of the target population are often not available when needed, satellite-derived parameters are used increasingly to develop models to predict the probability of presence or even abundance of a certain pest in a given target area (Bouyer et al., this volume). These prediction models can greatly facilitate the demarcation of the pest's distribution, and provide the foundation for developing an adequate intervention strategy, as was done for *G. austeni* and *Glossina brevipalpis* Newstead in KwaZulu Natal, South Africa, and Matutuine Province, Mozambique (Kappmeier Green et al. 2007) (Fig. 3). However, the fragmented nature of the distributions of the two species predicted by the distribution prediction model (AVIA-GIS 2002) was later refuted through more extensive surveys that sampled both species where they had not been found before (de Beer et al. 2016).

Insect populations are rarely stable in space and time, and temporal changes in their dispersal potential can significantly affect the isolated nature of seemingly distant population pockets. Seasonal changes in vegetation cover can be particularly dramatic in subtropical and tropical environments, with important consequences for insect dispersal, e.g. populations can be confined to "vegetation islands" during the dry season, when conditions for survival are harsh, but during the rainy season dispersal potential increases, spatial distribution expands, and larger distribution areas are

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created. Likewise, some moths and other pest populations are confined to limited overwintering areas in the subtropics from where they migrate each spring covering vast temperate areas (Wu 2007).

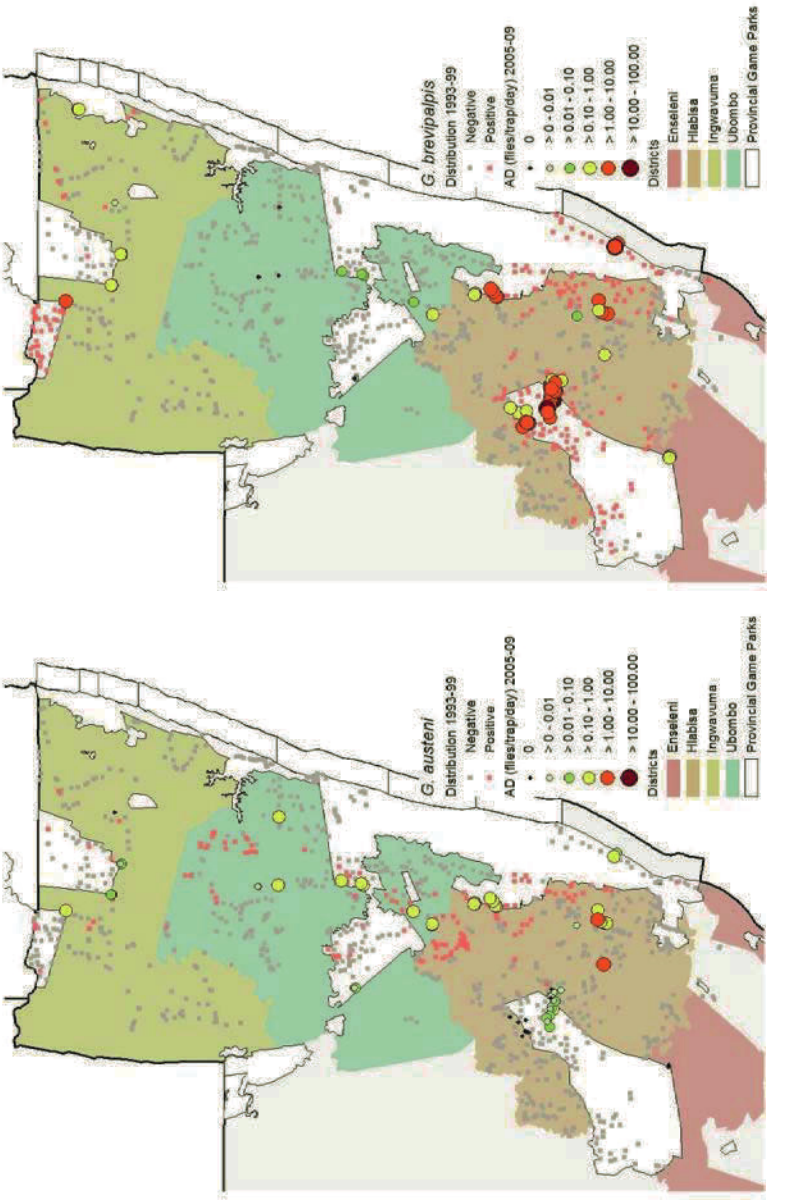



*Figure 3. Distribution of* Glossina austeni *(left) and* Glossina brevipalpis *(right) in KwaZulu-Natal in South Africa, derived from trapping data from the 1993–1999 and 2005–2009 surveys. (Maps reproduced with permission from C. de Beer.)*

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### *6.3. Continuous Distribution*

The most complex scenario is when insects are distributed continuously over very large areas. For both suppression and eradication strategies, a vast distribution of the pest will make it technically, logistically and economically impossible to tackle the entire infested area at one time. Consequently, the AW-IPM programme has to be developed and implemented in different stages, and thus the target area has to be subdivided into "intervention blocks". The appropriate block size will depend on the sterile insect production capacity and the biology of the particular species (section 8.), but has to be large enough to preclude migration of mated fertile females into the core area of each block.

Determining the exact size and precise demarcation of the blocks is a very challenging task, as it could determine success or failure (Barclay et al. 2011). Topographical and ecological features (e.g. major cities, mountain ranges, rivers, non-host areas, and arid or open areas of unsuitable habitat), which constitute natural barriers for the target insect, should separate to the maximum possible extent adjacent intervention blocks. In principle, to reduce the probability of migration among blocks, the size and number of temporary buffer zones between the blocks should be limited.

## 7. STRATEGIES FOR CONTINUOUS DISTRIBUTION

For large target populations that are distributed continuously, the "rolling-carpet principle" or the "wave principle" can be used to plan and implement AW-IPM programmes. Intervention according to the rolling-carpet principle entails advancing along a unidirectional front (Figs. 1 and 4), whereas for a bidirectional or multidirectional front the intervention follows the wave principle (Fig. 5). The different blocks have to be demarcated and selected in such a way as to minimize the invasion pressure from various directions. Multiple fronts complicate a programme, and necessitate the establishment of temporary buffers, which obviously can reduce the probability of success. In both approaches, it is of prime importance that the starting point of operations be selected carefully. Once the programme has started, it must be continued in view of the continuous pest distribution, and cannot be interrupted until a pest free area (eradication strategy), or a low pest prevalence area (suppression strategy), has been obtained in all blocks. Ultimately, in most cases, a permanent containment barrier will need to be established in a block at a more easily defensible location, e.g. Darien Gap for the New World screwworm programme (Klassen et al., this volume; Vargas-Terán et al., this volume).

### *7.1. Rolling-Carpet Principle*

The rolling-carpet principle is dynamic. The basic activities described in section 3. (baseline data collection as part of the pre-intervention phase, and population reduction, releases of sterile insects, and maintenance/verification of low prevalence/pest free areas as part of the operational phase) are carried out simultaneously in a progressive manner along the adjacent blocks (upper part of Fig. 4). Obviously, this approach with overlap of releases in adjacent blocks is more time

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and cost-efficient than without an overlap, in which each of these interventions would be implemented only in a given block before proceeding to the next block.

The diagram in the lower part of Fig. 4 shows the implementation of the rolling-carpet principle in space, i.e. the programme is initiated with the collection of baseline data and capacity building in block 1 (stage 1), and these activities are progressively shifted to adjacent blocks. Simultaneously, reduction of the population, release of sterile insects, and maintenance/verification of low prevalence/pest free areas will be carried out in those blocks where the previous activities have been completed. The approach in stages will entail a gradual increase in operational activities (and operational complexity) during the first four stages, with expanding needs for funding, personnel, and logistics.

Since the duration of each new operational stage can be different, the different stages of the rolling-carpet principle should be considered not only in space, but also in time (upper part of Fig. 4), i.e. baseline data collection (stage 1) usually has to take into account temporal changes in the density, distribution, and structure of insect populations, and often takes at least one full year to complete, whereas reduction (stage 2) of the target population is often implemented in shorter periods.

To achieve the desired population reduction, sterile insects must be released for a period of several generations (Knipling 1979). The duration of sterile insect releases to obtain a pest free area (eradication strategy), or to establish an area of low pest prevalence (suppression strategy), will depend on the reproductive rate and the generation time of the target species. For species such as tsetse flies, which have a long lifespan and long generation times, the release stage can take up to 18 months. Theoretically, this will be less for insects that have shorter generation times, like mosquitoes or fruit flies, but their high rates of reproduction can be a complicating factor.

The exact timing and initiation of each activity will be crucial for efficient implementation of the programme, e.g. a too-early start of the population reduction stage could result in a reduction of the target population before sterile insects are available for release. Therefore, the availability of sufficient sterile males should be taken as the "baseline", and all other activities should be scheduled in relation to the releases. These, in turn, depend largely on the production capacity of the mass-rearing facility. The overlap of releases in certain blocks (upper part of Fig. 4) has important implications for the cost of the programme, the time to reach programme goals, and the need to establish protective buffer zones (section 9.).

For example, in the hypothetical case with an overlap of releases for 12 months in two adjacent blocks (and even an overlap of 6 months in three blocks), the maintenance/verification period in the last low prevalence or pest free block can be initiated in the third quarter of year 5, whereas if there is no overlap of releases in adjacent blocks, this is delayed until the third quarter of year 8. In addition to the more advantageous implementation time, the first example offers a much more effective operation when compared with the second example, since the insect population in the block adjacent to the release area is always under active population reduction that minimizes the dispersal of gravid females into the release area (Fig. 1).

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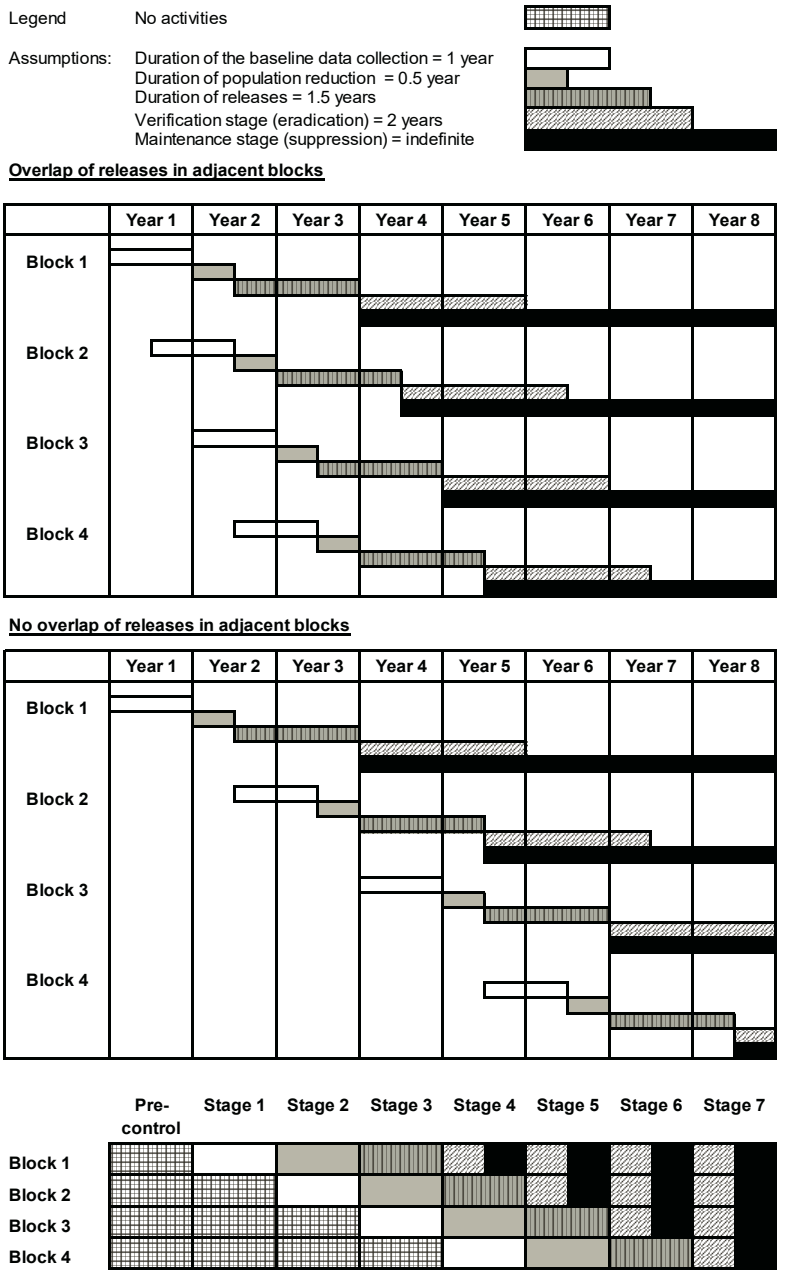



*Figure 4. Example of temporal (upper, with or without overlap of releases in adjacent blocks) and spatial (lower) diagrams of the "rolling-carpet principle" applied in four intervention blocks using eradication or suppression strategic options against a pest population distributed continuously.*

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On the other hand, in the second example the population reduction activities in block 2 are only initiated 12 months after the initiation of releases in block 1, requiring the establishment of temporary buffer zones to prevent the migration of females from the still "untreated" block 2. It is equally important that the contiguous part of the area that is already pest free be treated preventively with sterile insects as a biological buffer zone (Fig. 1), an ultimate insurance that individual entries or incipient incursions cannot become permanently established in the freed area.

### *7.2. Wave Principle*

The wave principle entails an expanding operational block size with each new operational stage (Fig. 5), and this must be considered when designing a production facility. For example, if an intervention were initiated in a 10 x 10 km (100 $km^2$) area, and in each stage expanded by 10 km to the east, west, and south, the total intervention zone would expand from 100 $km^2$ in stage 1 to 6600 $km^2$ in stage 6 (Fig. 6).

In an eradication strategy, the release area (shaded area in Fig. 6) would expand from 100 $km^2$ in stage 1 to 500 and 2100 $km^2$ in stages 2 and 6, respectively. If the release density of sterile males were 200 000 per $km^2$, the sterile male requirements in each stage would increase linearly by 80 million insects, i.e. from 20 million sterile insects in stage 1 to 420 million in stage 6.

In a suppression strategy, however, the sterile insect requirements would be different. If sterile males were released at a density of 200 000 per $km^2$ during the release stage (shaded area), and at a density of 50 000 per $km^2$ during the maintenance stage of low prevalence areas (white area), the sterile insect requirements would increase more dramatically with each stage when compared with an eradication strategy, and are best described by the following equation:
$y = 2E + 0.7x^{1.9119}$ (Fig. 6).

The number of sterile males needed for each stage is also very different in a strategy using the wave principle compared with that using the rolling-carpet principle. In the rolling-carpet principle, subsequent intervention blocks are often approximately the same size (Fig. 4). In the wave principle approach, the requirements of sterile males and other resources will increase with each stage; therefore, there will be a limit beyond which the operations cannot be sustained. At a given point, the programme will need to advance in one direction (for example south), and temporary or permanent buffer zones have to be established in the west and east to protect the low prevalence or pest free zones achieved (Fig. 5).

A mobile, modular insectary approach, e.g. containers as production modules, would in some situations have the advantage that, if the target area were expanding, modules could be added relatively easily (Tween 2004). Furthermore, the entire insectary could be moved with the expanding eradication or low pest prevalence front. This would reduce the transport time and cost between a stationary factory and the release area (in the case of an eradication strategy, the distance between the target zone and the factory increases with each new operational stage).

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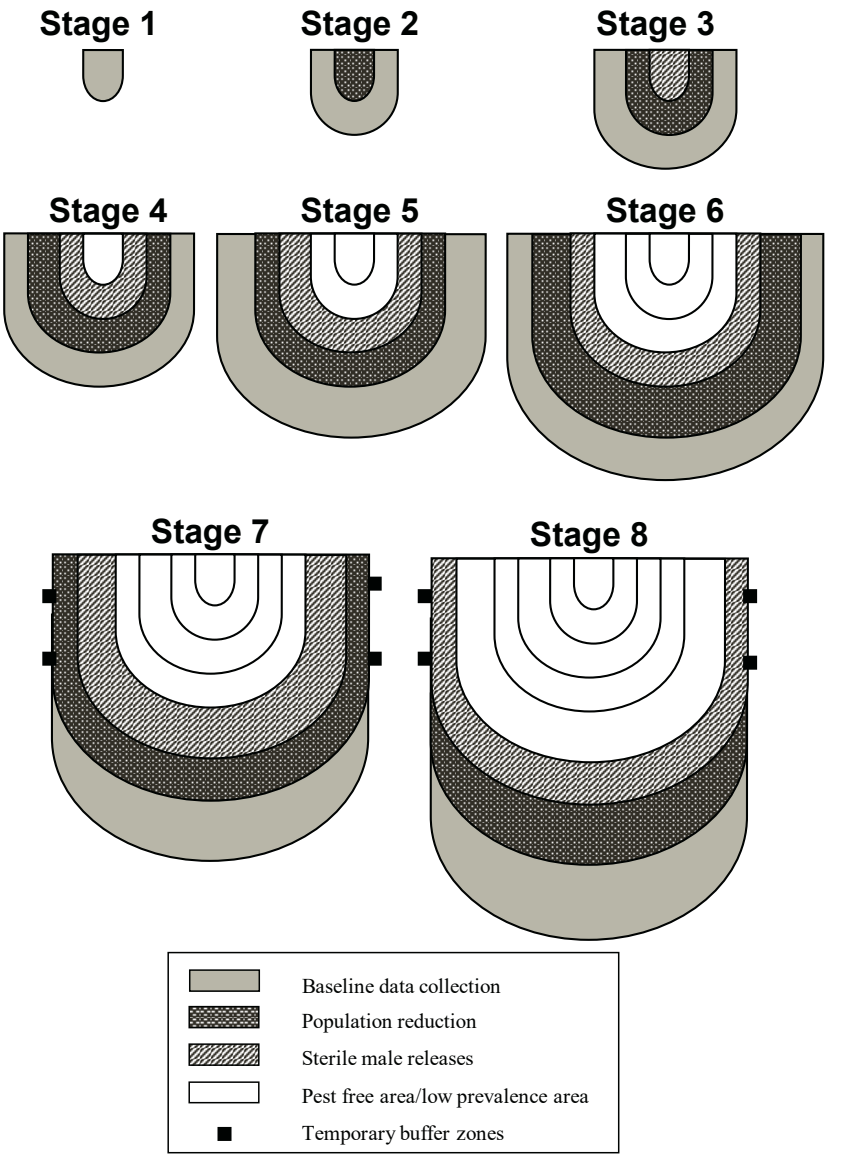



*Figure 5. Diagram of different activities and stages of an AW-IPM programme using the SIT according to the "wave principle" against a pest population with a continuous distribution. In this theoretical example, the intervention starts at the edge of the pest distribution and develops along a multidirectional front in the first 6 stages, until full production capacity of sterile males is reached. Beginning in stage 7, the intervention continues along a one-directional front, having either reached natural barriers or requiring the establishment of temporary buffer zones that includes suppression and quarantine activities as well as the release of sterile flies.*

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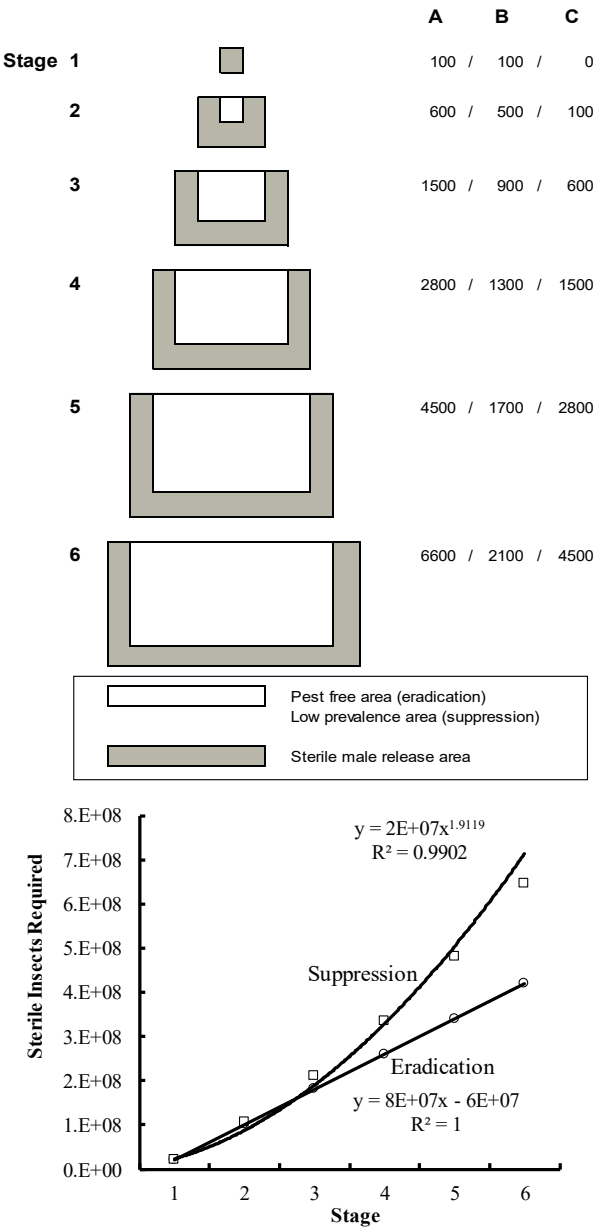



*Figure 6. Diagram of the wave principle (upper drawing) and sterile male requirements (lower graph), with an expanding intervention front of 10 km south, east, and west, and each stage using eradication and suppression. Numbers indicate:* ***(A)*** *total surface area in km² /* ***(B)*** *area in km² where sterile males are released /* ***(C)*** *pest free area in km² (eradication) or low pest prevalence area (suppression). (More details in the text.)*

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A mobile insectary could be relocated occasionally to areas where the pest is still present, and, in the case of eradication, expensive insect-proofing systems would not be needed. However, the relative merits and economics of insect-proof systems and increasing transport time versus relocation costs of the modules need to be considered. An example was the New World screwworm mass-rearing facility in Tuxtla Gutiérrez, Chiapas, Mexico. As the programme succeeded and then advanced southward into Central America, the factory remained in an area now free of screwworms, requiring expensive and cumbersome biosecurity measures. These included the continuing release around the facility of several million sterile insects per week to deal with any fertile flies that might have escaped accidentally from the facility. Eventually, a new facility was established in Panama and the Tuxtla Gutiérrez facility closed (Vargas-Terán et al., this volume).

## 8. SIZE OF INTERVENTION BLOCKS FOR AW-IPM PROGRAMMES

At what spatial scale is an area-wide approach no longer technically and economically feasible? Is there a minimum size of the target area below which effective implementation of AW-IPM programmes using the SIT becomes technically impossible and economically unjustifiable (Barclay et al. 2011)? These questions are of major importance since the SIT, similar to mating disruption (Cardé 2021), does not kill the pest and hence is particularly susceptible to immigrating gravid female insects, which are largely unaffected by the sterile males, although in some target pest species females do remate (Von Borstel 1960).

Intervention blocks are composed basically of two distinct areas, i.e. the core and the edge areas. The core area contains the commodity that is being protected (e.g. crop, cattle, humans in urban area, etc.), whereas the edge area, although included in the treatment zone, is defined by the edge effect: a certain level of infiltration by gravid wild females from the surroundings into the area under treatment (Fig. 7). In some situations the edge area completely surrounds the core area, often requiring the establishment of a protective buffer zone (section 9.) to minimize this edge effect.

The size of both core and edge areas is determined mainly by the combined effects of the dispersal potential of the pest species and the degree of protection required for the target area, which depends on the strategic objective of the programme: higher for eradication, lower for suppression. Other biotic and abiotic factors, such as presence of natural barriers, ecological and topographic characteristics of the area, tolerable nuisance or damage level, acceptable economic threshold, and managerial-logistic capacity, are likewise of relevance.

If the selected target area were completely isolated, e.g. an island, there is no need to establish protective buffer zones unless the pest can reach the island from other areas (Kuba et al. 1996). However, when a continuous pest population surrounds or is adjacent to the selected core area, the size of each block must be much larger to absorb the edge effect without affecting progress in the core (Fig. 7). For pests having a high dispersal rate, individual blocks will have to be larger (Barclay et al. 2011).

The size of the blocks will also affect the strategic approach and the economics of each approach. If the goal is to eradicate the pest and to maintain an area pest free, larger blocks will most likely be required to reduce the relationship of edge to core

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area (Fig. 7). However, if pest suppression is the strategic goal, a certain damage threshold is usually acceptable, thus relatively smaller blocks and smaller protective buffers are acceptable (Larcher-Carvalho and Mumford 2004; Barclay et al. 2011). This confirms earlier observations that, by nature, eradication is a more extensive and intensive strategy when compared with suppression (sections 2.1. and 2.2.).

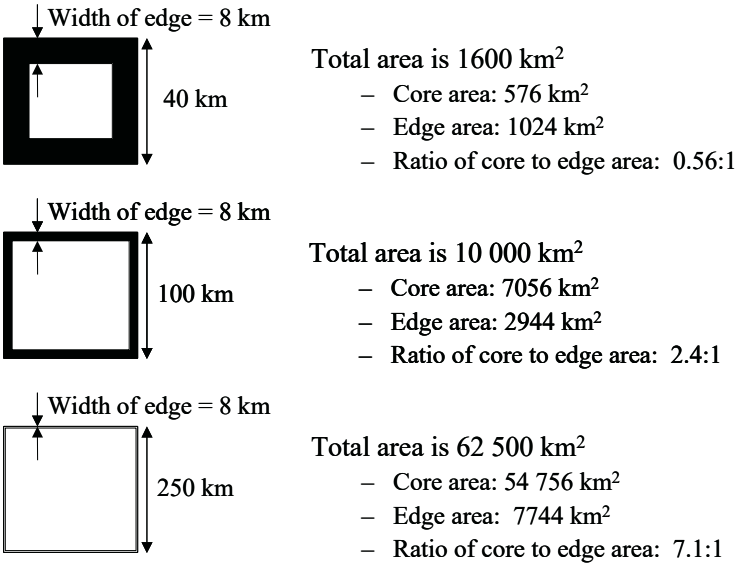



*Figure 7. Relationship of core area to edge area at three different scales of block size. An edge effect based on pest dispersal of 8 km is assumed, and square core areas for either pest free (containment and eradication) or low pest prevalence (suppression) area.*

The ratio between the size of the core and edge areas is related to this, i.e. the larger the core area, the smaller the edge area in relation to the whole block surface. Also, the relative effort needed to maintain protective buffer zones becomes proportionally smaller with larger block areas. The following example illustrates this point. Assuming that the core areas are squares, and that dispersal can occur into an edge with a width of up to 8 km (Fig. 7), the percentage of the total surface area represented by the edge area decreases from 64 to 29 to 12% for a core area of 1600, 10 000, and 62 500 km$^2$, respectively. For the small block of 1600 km$^2$, the effort going into the edge area is relatively larger than the one going into the core area. Such a situation could only be justified if the value of the commodity being protected outweighs the costs incurred to maintain protective activities in the relatively large edge area.

The scale of application will also influence programme economics, especially when it includes the initial capital investment of a mass-rearing and sterilization facility, as well as emergence and release facilities. Particularly in those cases, the programme needs to be applied on a scale large enough to become economically

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viable and competitive with alternative control options (which operate at real economies of scale, i.e. production of insecticides). Therefore, larger blocks incur a lower cost per surface unit or per unit of the commodity that is being protected. This relates directly to the size of the mass-rearing facility; experience has shown that the cost of a unit of produced sterile insects is inversely related to the level of mass production (Enkerlin 2003). Higher fixed costs are associated with factories designed to produce relatively small numbers of insects.

The size of blocks has ranged from tens of square kilometres, as was the case of the oriental fruit fly pilot suppression programme in Thailand (Sutantawong et al. 2004), to thousands of square kilometres in the case of large programmes such as the New World screwworm eradication campaign, where each treatment block encompassed at least one and a half of the Central American countries (Vargas-Terán et al., this volume).

A conceptual model was developed for assessing the minimum size area for AW-IPM programmes (Barclay et al. 2011). It is primarily based on a diffusion coefficient that uses the dispersal rate of the target insect, and considers other main variables such as the fertility rate and mortality involved to produce estimates of minimum-size intervention blocks for different pests and programme objectives. It can be used as a guideline to recommend quantitatively the minimum intervention size for the range of different conditions where sterile insects can be released.

## 9. BUFFER ZONES

To address the edge effect as described above, there is often a need to establish buffer zones (FAO 1999, 2005) to protect containment or eradication efforts, or to maintain low prevalence commercial production areas. This may necessitate the application of conventional control methods or the release of sterile insects or both in the buffer zone, and may be either temporary or more permanent.

### *9.1. Temporary Buffer Zones*

Eradication programmes that proceed in stages or blocks, irrespective if a rolling-carpet or wave principle is adopted, might require buffer zones between the intervention blocks to temporarily protect achievements made in each new operational stage of the advancing programme. These temporary buffer zones have two main objectives:

- To prevent a massive influx of the pest from areas where it is still uncontrolled to blocks where sterile insects are being released. Population reduction activities, normally implemented in blocks adjacent to the release blocks, often serve as temporary buffer zones (Figs. 4 and 5).
- To consolidate progress in areas where the pest has already been eliminated. Preventive sterile insect releases and other control activities are implemented in these areas adjacent to the population reduction and low prevalence areas (Fig. 1). Hence, these buffer zones attempt to ensure that any migrating gravid females, or

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those that are transported across a permeable quarantine, cannot re-establish new populations.

Temporary buffer zones were established during the progressive eradication campaign of the New World screwworm in Mexico and Central America (Vargas-Terán et al., this volume). The programme was implemented according to the rolling-carpet principle, and always included (at the back end of the moving eradication front) a large screwworm-free buffer area in which sterile insects were released as an insurance in case screwworm-infected cattle was moved into the pest free area.

Such buffer zones are only established temporarily in the dynamic rolling-carpet or wave-principle approaches, and are progressively moved from block to block with the advancement of the eradication campaign (Fig. 4). Occasionally, special buffers are needed when the increasing size of the intervention front of an expanding programme exceeds the available resources, e.g. at a given stage, the multidirectional moving front of a wave-principle strategy will be shifted to a front that proceeds temporarily in only one or two directions (Fig. 5). The areas where such buffer zones have to be established should be identified during the collection of baseline data and programme planning activities of the pre-intervention phase.

### *9.2. Permanent Buffer Zones*

Permanent buffer zones are often established for containment (Fig. 1). These buffers should have a width sufficient to intercept any immigrating insect and the capacity to deal with progeny of any gravid female that enters the area. In tsetse flies, for example, which are relatively poor fliers and have no free-living immature stage, the dispersal potential is much lower (Feldmann and Jannin 2001) than that of new world screwworms (Lance and McInnis, this volume). However, the potential for reinvasion of screwworm flies is much lower than that of polyphagous fruit flies, which are present in innumerable small hosts that can contain larvae, and are easily transported by travellers or postal shipments. Therefore, in addition to buffer zones, rigorous quarantine measures/procedures have to be established to intercept any insect that is transported passively with animal or plant commodities, e.g. fruit fly larvae in fruit, screwworm larvae in livestock, pets, and humans, codling moth pupae in packing boxes, and tsetse flies resting on vehicles.

A good example of a permanent buffer zone is the one that has been established permanently in eastern Panama as part of the New World screwworm programme to prevent this pest from dispersing from Colombia to the screwworm-free areas of Central and North America (Fig. 8). Aircraft release ca. 15–20 million sterile insects per week over a 30 000-km$^2$ area bordering with Columbia (APHIS/USDA 2001), and adjusting release densities based on risk areas within the barrier (3000 sterile flies per nautical mile$^2$ in low-risk areas and 6000 in high-risk areas). The large size of the Darien buffer zone is required because of the high mobility of screwworms; individual flies can cover distances up to 290 km (Hightower et al. 1964).

Another example is the sterile Mediterranean fruit fly buffer zone that has been maintained for approximately 40 years between Mexico and Guatemala as part of the Moscamed containment programme (Villaseñor et al. 2000; Enkerlin et al. 2015, 2017). The requirements and optimal dimensions for efficient buffers are often

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underestimated and, when combined with insufficient resources, frequently result in the establishment of localized inefficient buffers that "leak".

Permanent buffer zones are also needed around low pest prevalence commercial production areas where suppression, rather than containment or eradication, is the strategic goal. The objective is to reduce the impact of gravid females moving into such areas by applying conventional methods of pest control or extending the sterile insect releases beyond the core commercial production areas. Once low pest prevalence has been achieved, releases could be decreased in the core area, while the buffer would continue with higher release rates and other population reduction activities (Larcher-Carvalho and Mumford 2004). In cases where the core area is well managed so that it does not contribute to the pest population (i.e. growers routinely remove all infestation sources of fruit flies such as fallen fruit, or citizens eliminate all *Aedes albopictus* (Skuse) mosquito breeding sites in an urban area), area-wide releases can focus mainly on the sources of the pest in the surrounding natural vegetation areas from where the pest reaching the core area originates (Kovaleski and Mastrangelo 2021; Liedo et al. 2021).

Since suppression is the strategic objective in these situations, these permanent buffer zones do not require the establishment of rigorous quarantine procedures, and can be much more modest than those required for containment or eradication programmes. Nevertheless, the width of these buffers also needs to be determined, taking into account pest mobility, host density, tolerable damage threshold, and population pressure from the surrounding areas (Barclay et al. 2011).

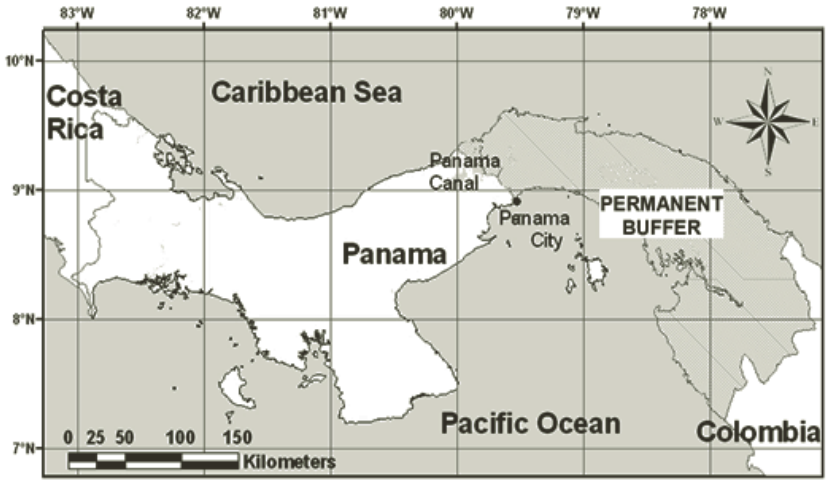



*Figure 8. The permanent buffer zone in the Darien Gap in Panama, consisting of the weekly release of sterile New World screwworm flies. (Map from R. B. Matlock, adapted.)*

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## 10. CONCLUSIONS

Insect pest control using an AW-IPM approach with an SIT component is more complex and management-intensive than applying simpler control methods, and hence requires more initial baseline data collection, feasibility assessments, careful planning, and stakeholder involvement to ensure effective implementation. The SIT is a very powerful technique; for more than a half century it has been applied successfully for the eradication, suppression, containment or prevention of major insect pests. Unfortunately, too often it has been promoted as a "silver bullet", resulting in unrealistic expectations that then often cannot be fulfilled.

Programmes that attempt to eradicate a target insect population are particularly ambitious and challenging, but in reality are often poorly prepared, frequently underfunded, and lacking in public and in regulatory support to achieve their objective. In some cases, such programmes are operating without even the indispensable establishment of quarantine measures to protect a pest free area. In some of these situations, changing the strategy from eradication to containment or suppression is a more realistic option, but changes during ongoing implementation are politically costly, even if eventually successful. More modest initial goals, starting with a suppression objective to reduce insecticide use, or a containment effort to stop the spread of a pest, are often more realistic. If successful, such strategies can, under more favourable political, financial or technical situations, eventually be upgraded to an eradication strategy (Staten and Walters 2021).

Before embarking on an AW-IPM programme with an SIT component, the choice of strategy must be carefully assessed and prepared during pre-intervention and preparatory phases, ideally following a phased conditional approach, so that the programme is based on a thorough understanding of the target pest population as well as on adequate planning and implementation of the operational phase.

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# CHAPTER 6.2.

# INVASIVE INSECT PESTS: CHALLENGES AND THE ROLE OF THE STERILE INSECT TECHNIQUE IN THEIR PREVENTION, CONTAINMENT, AND ERADICATION

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## SUMMARY

The rapid expansion of international travel and trade are facilitating the spread of non-native species, while climate change is creating new ecological niches enabling the establishment of pests in previously inhospitable regions. The temporary large-scale application of insecticides and "scorched earth" approaches are often indispensable to deal effectively with such outbreaks of invasive pests. However, even when the political will is there to implement the required systematic eradication efforts, they often result in public resistance to drastic actions such as the destruction of infested crops, herds, and orchards, and the indiscriminate insecticide spraying that raises concerns about damage to the environment and human health. Furthermore, such campaigns can still fail to achieve final eradication of all remnants of incipient populations. Therefore, and also in response to the enactment of environmental laws and regulations, increased efforts are needed to develop new and effective early detection and eradication tools that can prevent the establishment of a new pest in a less disruptive and more environment-friendly way. The sterile insect technique (SIT) is a proven method to prevent, contain, and eradicate outbreaks of invasive insect pest populations. It is an effective and ecology-friendly method that, unlike insecticides and other control methods, acts in an inversely density-dependent manner, and as a result increases its efficiency with decreasing population density. Therefore, it is particularly well-suited to eliminate incipient invasive-pest introductions and outbreaks when applied as part of an area-wide integrated pest management (AW-IPM) approach. When flooding a target pest population with sterile males, the wild virgin females (that are not controlled with other methods) are actively located and mated by the sterile males, producing no offspring. Some examples of eradication include incipient invasive populations of the painted apple moth *Teia anartoides* Walker in New Zealand, and the cactus moth *Cactoblastis cactorum* (Berg) in Mexico; major outbreaks (*Cochliomyia hominivorax (Coquerel)* in Libya); and all the way to fully established invasive pest populations (pink bollworm *Pectinophora gossypiella* (Saunders) in the southern USA and northern Mexico, and melon fly *Zeugodacus cucurbitae* (Coquillett) in the archipelagos of southern Japan). The SIT is also being used to contain invasive pest populations, such as *Ceratitis capitata* (Wiedemann) in southern Mexico, and preventively by releasing sterile insects over areas with a historically high risk of invasive pest incursions (*C. capitata* in California and Florida). There is great potential to expand the SIT application to similar situations against other major invasive insect pests. This chapter compares some eradication campaigns in the pre-SIT era with those integrating the SIT to eradicate, contain or prevent the establishment of non-native insect pests, and discusses the constraints and needs for contingency planning, including a proactive approach to develop the SIT package for other potential major invasive insect pests.

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# 1. INTRODUCTION

## *1.1. Biological Invasions and their Cost*

Among the urgent major steps required to safeguard the imperilled biosphere, contained in a warning to humanity by more than 15 000 concerned world scientists from 184 countries, is the need to constrain the spread of alien invasive species (Ripple et al. 2017). These can interfere with ecosystem services or disrupt whole ecosystems, and cause the decline of many of the native species that are now listed as endangered or threatened (IUCN 2000; Charles and Dukes 2007; CBD 2018). When such invasive species no longer face the natural enemies and other direct competitors with which they evolved, they may breed without restraint, an "ecological release" that can have devastating effects on agriculture, public health, and the environment. The impacts of invasive species are second only to habitat destruction as a cause of global biodiversity loss (Pimentel 2002).

The global movement of non-native species is one of the costliest, most underestimated, and least known aspects of globalization (Bradshaw et al. 2016), resulting in massive ecological destruction and the spread of diseases, costing hundreds of billions [hundreds of thousands of millions] of US dollars a year to countries' plant and animal resources (CBD 2018). In North America, species of foreign origin constitute approximately 40% of the 600 serious arthropod pests which warrant control measures, and cause as much as 50% of the total economic losses by pest species (Klassen 1989; Kim 1991). Pimentel (2002) estimated the damage to the world economy inflicted by invasive alien species to be about 1.4 trillion [thousand thousand million] US dollars, which in 2002 represented about 5% of the total world economy. These significant costs of invasive-pest control and forgone output are an international "weakest link" public good, requiring effective regional and international coordination (Perrings et al. 2002).

## *1.2. Increasing International Travel, Trade, and Invasive-Pest Introductions*

The rapidly increasing international travel, transport and trade, moving people, agricultural commodities and animals as never before among geographic regions, has also significantly increased the probabilities of introducing invasive pest species into new regions (Lock 2019). Invasions are exacerbated by rising human populations, movement, wealth and international trade (Bradshaw et al. 2016). The rising volumes of shipped goods, the speed and containerization of transport, the high-volume air freight, and the establishment of trade agreements that facilitate international movement of goods, are all important components of globalization, which is directly linked to the fast spread of alien invasive species (Bright 1999). The rate of non-native species introductions has been growing in the past 200 years, from an average of 7.7 per year between 1500 and 1800 to a record 585 in 1996 (Hulme 2009; Nature 2017). For example, in Europe, the pattern of plant pest introductions since the early 1900s has increased steadily, with 67% of the introductions occurring in the second half of that century (Waage et al. 2008). Over time, arthropods and other invertebrates have increasingly become the most effective invaders (Fig. 1).

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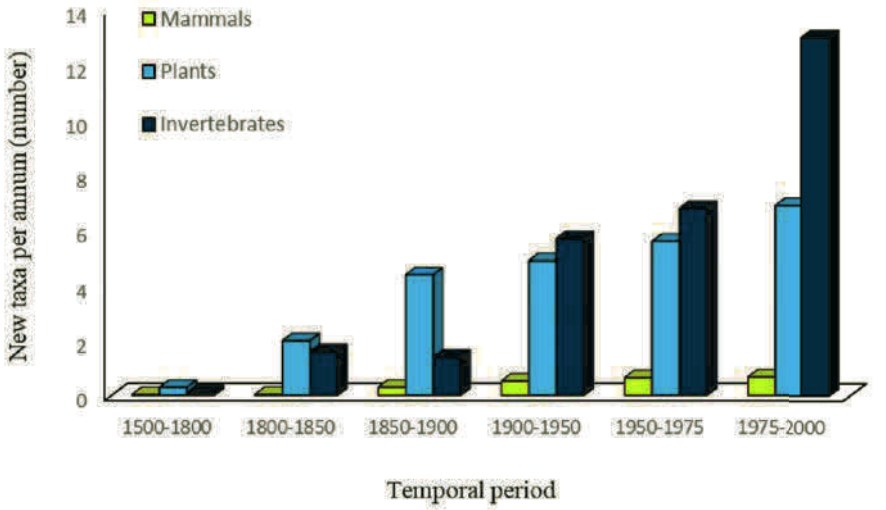



*Figure 1. New invasive taxa introduced per annum into Europe. (Modified from Hulme 2009, used with permission from Wiley & Sons.)*

Managing invasion pathways and this surge of exotic plant diseases and other potential pests is an ongoing challenge for many countries (VanDersal 2007; Hulme 2009). In spite of the application of more resources and improved technologies, inspection capacities for incoming cargo and passengers at ports of entry, the primary exclusionary strategy, are being overwhelmed in most countries in view of the rapidly rising flow of international passengers and cargo (Liebhold et al. 2006). Consequently, safeguard systems are often breached with potential for permanent, unintended, and often unpredictable disruption of ecosystems and economic damage.

Unfortunately, in relation to new pest invasions, the future is one of further increases in international travel and trade (Hulme 2009), while at the same time there is pressure to decrease insecticide residues and to liberalize and deregulate some sanitary and phytosanitary barriers to international trade (Perrings 2002; WTO 2018). Therefore, more invasive-pest introductions can be foreseen even if exclusion programmes are fortified based on new and improved technologies, stronger quarantine regulations, and less-lax enforcement of them at ports of entry, as well as the establishment of offshore risk-mitigation strategies and measures that are increasingly being implemented by some developed countries (Klassen et al. 2002; VanDersal 2007).

### *1.3. Interaction of Globalization and Climate Change*

Changes in climate due to the emission of anthropogenic greenhouse gasses are not only inducing changes in the distribution of many species, but are also facilitating increased survival of invasive pests in previously inhospitable regions (Ziska and Dukes 2014). Whereas the increase in movement and exchange of goods is

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accelerating the redistribution of many insect pests, climate and related land-use changes will likely create new ecological niches, enabling the establishment of new pests in new territories and significant species-range shifts. Climate change projections to 2050 predict a net average increase of 18% in the area of occurrence of arthropod invaders (Bellard et al. 2013). This expansion applies not only to plant pests, but is also very relevant for vector-borne diseases (Higgs 2018), affecting not only the spatial-temporal distributions and population dynamics of the vectors, but also speeding up their life cycles, including those of parasites, their mode of transmission, opportunities for development in new hosts, etc. Higher temperatures can increase the rate of development of pests and pathogens, shortening their generation times and increasing the number of generations per year, which can lead to higher pathogen/parasite population sizes (Harvell et al. 2002; Barber 2016). To mitigate or address these climate- and globalization-induced new pest problems, new legislation and policies are required to manage the public good (Perrings et al. 2002, 2005). Among these is the need to support the development of innovative control approaches to mitigate and manage these biological incursions, and to contain the geographical expansion of non-indigenous pest populations.

### *1.4. Deliberate Introductions of Non-Native Species*

Not all introductions of non-native species into new regions are accidental; some occur with intent (Thomas 2002; Worner and Gevrey 2006). All introductions in support of classical biocontrol are deliberate to manage invasions mainly of arthropods or weeds that have succeeded to invade and have already become established. Overall, classical biocontrol has been a remarkable success, and it is so attractive because it is self-perpetuating in all the successful cases (Simberloff 2008). However, unforeseen environmental effects resulting from such deliberate introductions have also been recorded, including non-target species impacts and even extinctions, as well as numerous cases of biological control agents becoming direct pests (Howarth 1991; Simberloff and Stiling 1996; Follett and Duan 2000; Wajnberg et al. 2001).

The above cases highlight the risks and the need for a cautious approach when assessing the introduction of more non-native species to reduce the adverse impact of past introductions (Myers et al. 1998), although on the other hand excessive regulation has recently brought about an almost complete stop to natural enemy exploration for classical biocontrol programmes (van Lenteren 2021).

Non-indigenous species have been moved, and are also being moved, intentionally across regions for other purposes. Two examples are the introduction of the African honeybee into Brazil, and the subsequent spread of an Africanized hybrid in the Americas, or the release and establishment of dung beetles to reduce accumulation of bovine dung in pastures and rangeland, thereby seeking to reduce livestock disease vectors and parasites (Thomas 2002). Establishment of others was the consequence of escapes from other deliberate introductions, such as the gypsy moth *Lymantria dispar* (L.) (Klassen 1989), or the ongoing lucrative international trade in non-native pets, aquarium and terrarium species, and live bait and live food (Thomas 2002; CBD 2014).

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Unfortunately, such "biological pollution" is worse than other pollution; when such deliberate introductions have gone wrong, they are essentially irreversible (Campbell 1993). Once non-indigenous species are established, they become permanent in space and time, unless serious efforts are made to eliminate such unwanted populations (Simberloff 2008).

Finally, there is the potential risk of the intentional release of major non-indigenous pests and diseases as weapons of agricultural warfare or bioterrorism to cause major socioeconomic damage. Also, there were allegations that some Mediterranean fruit flies *Ceratitis capitata* (Wiedemann) were deliberately released in California by opponents of aerial spraying used as part of eradication campaigns (Bonfante 1990; Cameron et al. 2001). Any such intentional events will also require early detection and rapid response capacities to be mitigated or eliminated.

In this chapter we describe how the sterile insect technique (SIT) (Knipling 1960), deployed as an important component of an AW-IPM approach (Hendrichs et al. 2007), is particularly suited for prevention, containment, and eradication of the described invasive insect pest problems (Knipling 1966), offering considerable social and environmental advantages over area-wide aerial spraying of insecticides and other drastic "scorched earth" interventions, which are increasingly no longer accepted by the public and strictly limited by environmental regulation.

## 2. MULTILATERAL TREATIES AND INVASIVE SPECIES

### *2.1. SPS Agreement and the WTO*

The Agreement on the Application of Sanitary and Phytosanitary Measures (the "SPS Agreement") entered into force with the establishment of the World Trade Organization (WTO) in 1995. It sets out the basic global rules for food safety, and animal- and plant-health standards, which should be applied only to the extent necessary to protect human, animal or crop life or health. Harmonized regulations must be based on science, and should not arbitrarily or unjustifiably discriminate among countries as an excuse for protecting domestic producers (WTO 2018).

The WTO's objective is to ensure that international trade flows as smoothly, predictably, and freely as possible (WTO 2018), and its SPS Agreement has been relatively successful in its intended deterrence of regulatory protectionism, while significantly increasing safe trade opportunities (Heather and Hallman 2008). However, this objective, at least in some countries or regions with poor animal health and plant protection infrastructure, may enable the spread of invasive species. Nevertheless, even though the WTO is largely focused on overcoming and minimizing protectionist barriers to trade, the SPS Agreement does support the enforcement of measures, such as postharvest treatments and other exclusion and risk-management approaches, to avoid the entry or establishment of non-indigenous pests and diseases of animals and plants.

This is the mandate of the World Animal Health Organization (Office International des Epizooties, OIE) and the International Plant Protection Convention (IPPC) based at the Food and Agriculture Organization of the United Nations (FAO); they safeguard world trade in support of the SPS Agreement by harmonizing sanitary

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and phytosanitary measures, respectively, so that countries apply them in a transparent way based on international standards. Most countries are parties to these organizations, and are organized into regional animal health and plant protection organizations (Heather and Hallman 2008).

As relevant as the above organizations in terms of international standardisation is the Convention on Biodiversity (CBD), established by the United Nations Environmental Programme (UNEP) in the early 1990s, whose mandate is protecting biodiversity and the environment, and thus assessing the risks associated with the introduction of invasive species (CBD 2014, 2018). The CBD is supported by funding mechanisms such as the Global Environment Facility (GEF) and international partnerships such as the Global Invasive Species Programme (GISP), and the International Union for the Conservation of Nature (IUCN) with its Invasive Species Specialist Group (ISSG), which is a global network of scientific and policy experts on invasive species (IUCN 2000).

### *2.2. Terminologies and Definitions*

Terminologies and definitions used in the different agreements and conventions in relation to invasive species vary considerably. Most relevant are those available from the CBD, the IPPC, and the OIE. Besides varying, they often have distinct meanings (Box 1). This is understandable because the focus of the CBD is on protecting biodiversity and the environment, but the mandates of the OIE and the IPPC are more oriented towards protecting commercial agriculture and related trade, respectively, covering sanitary and phytosanitary issues in support of the SPS Agreement.

For example, for CBD purposes, an *alien species* is already present in the area that is not within its native distribution, whereas the IPPC is more concerned with organisms that are not yet present in the area of concern, i.e. *quarantine pests*. Thus, for IPPC and OIE the term "*alien*" is not appropriate. Even though terms such as "*exotic*", "*non-indigenous*" or "*non-native*" have been used in the International Standard for Phytosanitary Measures (ISPMs), the term "e*xotic*" is not considered suitable because it presents translation problems (IPPC 2018); therefore "*non-indigenous*" or "*non-native*" are favoured by IPPC and OIE (Box 1).

Another difference is that for CBD a species that is non-indigenous and has entered an area through natural means is not an alien species; it is simply extending its natural range. On the other hand, for IPPC purposes, such a species could still be considered as a potential *quarantine pest* (IPPC 2018).

Other relevant terms used in relation to invasive species and responses to them can also differ significantly (Box 2). For example, whereas *eradication* is complete and permanent at the species level *worldwide* for WHO, for IPPC and OIE it is the local *elimination* of a pathogenic agent or pest population from *a country, area* or *zone* (Heymann 2006). On the other hand, *elimination* is the term used by WHO for the local reduction to zero (or a very low defined target rate) of new cases of an infectious disease, requiring continued measures to prevent re-establishment (Heymann 2006).

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In view of the definitions in Boxes 1 and 2, since SIT implementation in relation to invasive species is applied mainly against insect pest populations in the context of agriculture and agricultural trade, or against populations of disease vectors in the context of public health, primarily the IPPC and OIE terms will be used in this chapter.

*Box 1. Definitions of Terms Related to Invasive Species*

*Alien Species (= Non-Native Species, = Foreign Species)*

A species, subspecies or lower taxon, introduced outside its natural past or present distribution; includes any part, gametes, seeds, eggs, or propagules, of such species that might survive and subsequently reproduce (CBD 2018). "Alien" refers only to the location and distribution of an organism compared with its natural range. It does not imply that the organism is harmful (IPPC 2018).

*Alien Species*

Alien species are taxa that are introduced outside of their natural range, either intentionally or unintentionally (IUCN 2000).

*Exotic Species (= Introduced Species)*

A species occurring in an area outside of its historically known natural range as a result of intentional or accidental dispersal by human activities (IUCN 2000).

*Hitchhiker Organism*

Organism that has an opportunistic association with a commodity or vehicle/vessel or container, and which may be transported unintentionally to a new environment (OIE 2012).

*Introduced Species*

An introduced, alien, exotic, non-indigenous, or non-native species, or simply an introduction, is a species living outside its native distributional range, which has arrived there by human activity, either deliberate or accidental (Wikipedia).

*Invasive Species*

Species that tend to spread beyond their native range to new areas. Invasive species are those plants, animals, and microbes not native to a region which, when introduced either accidentally or intentionally, cause economic or environmental harm or harm to human health (NAL 2008).

*Invasive Alien Species (= Alien Invasive Species*)

An *alien* species whose introduction and/or spread outside its natural past or present distribution threatens biological diversity and whose impact involves significant harm (CBD 2018).

*Invasive Non-Native (or Invasive Alien) Animal*

Animal that has been introduced and subsequently become established and spread outside its native distribution area and caused harm to the environment, animal or human health, or the economy (OIE 2012).

*Native Species (=Indigenous Species)*

Species that have historically occurred as part of an ecosystem in a specific location (Wikipedia).

*Non-Indigenous Species*

An *invasive alien species* (CBD) is an exotic or non-native species that by its establishment or spread has become injurious to plants, or that by risk analysis is shown to be potentially injurious to plants (IPPC 2018). For CBD a non-indigenous that has entered an area through natural means is not an alien species; it is simply extending its natural range.

*Quarantine Pest*

A pest of potential economic importance to the area endangered thereby but not yet present there, or present but not widely distributed and being officially controlled (IPPC 2018**).**

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*Box 2. Definitions of Other Relevant Terms in Relation to Invasive Species*

*Containment*

Application of phytosanitary measures in and around an infested area to prevent spread of a pest (IPPC 2018).

*Elimination*

Reduction to zero (or a very low defined target rate) of new cases of disease or infection in a defined geographical area as a result of deliberate efforts; continued measures to prevent re-establishment of transmission are required (WHO, Heymann 2006).

*Entry*

Movement of a pest into an area where it is not yet present, or present but not widely distributed and being officially controlled (IPPC 2018).

*Eradication*

The complete and permanent *worldwide* reduction to zero new cases of an infectious disease through deliberate efforts; no further control measures are required (WHO, Heymann 2006).

*Eradication*

Application of phytosanitary measures to eliminate a pest from an *area* (IPPC 2018).

*Eradication*

The elimination of a pathogenic agent from a country or zone (OIE 2012).

*Establishment*

Perpetuation, for the foreseeable future, of a pest within an area after entry (IPPC 2018).

*Establishment*

The process of an alien species in a new habitat successfully producing viable offspring with the likelihood of continued survival (CBD 2018).

*Incursion*

An isolated population of a pest recently detected in an area, not known to be established, but expected to survive for the immediate future (IPPC 2018).

*Introduction*

The entry of a pest resulting in its establishment (IPPC 2018).

*Introduction*

The movement by human agency, indirect or direct, of an alien species outside of its natural range (past or present). This movement can be either within a country or between countries or areas beyond national jurisdiction (CBD 2018).

*Intentional Introduction*

The deliberate movement and/or release by humans of an alien species outside its natural range (CBD 2014).

*Outbreak*

A recently detected pest population, including an incursion, or a sudden significant increase of an established pest population in an area (IPPC 2018).

*Phytosanitary Measure*

Any legislation, regulation or official procedure having the purpose to prevent the introduction and/or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests (IPPC 2018).

*Point of Entry*

Airport, seaport, land border point or any other location officially designated for the importation of consignments, or the entrance of persons (IPPC 2018).

*Spread of a Pest*

Expansion of the geographical distribution of a pest within an area (IPPC 2018).

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### *2.3. Three-Stage Hierarchical Response, and Principle of Regional Solidarity*

Although legally non-binding, the above multilateral conventions prescribe that countries shall, as far as possible and where appropriate, follow a "three-stage hierarchical response approach" to mitigate the risk of non-native species invasions (CBD 2018):

#### *2.3.1. Prevention of Introduction*

Priority should first be given to exclusion to prevent the entry of harmful non-native species which threaten ecosystems or livelihoods. Prevention is generally far more cost-effective and environmentally desirable than measures taken following introduction and establishment of a non-native invasive species (CBD 2018).

The cornerstone of safeguarding systems is exclusion based on effective quarantine procedures and thorough inspection at ports of entry. Nevertheless, these can only minimize entries, but are not the most reliable line of defence for exclusion (Liebhold et al. 2006, 2016). Therefore, at least for trade in agricultural commodities, pest risk-mitigation measures at the points of origin are among the more viable approaches to strengthen pest exclusion, as well as identifying paths of high risk to develop improved preventive measures (Klassen et al. 2002).

To mitigate the arrival of invasive-pest risks, the USA has been focusing on offshore strategies. One of these is the Offshore Pest Information System (OPIS) -- a secure web-based information sharing tool that allows users to communicate in an organized manner about offshore plant and animal health events and issues. The OPIS was designed to allow the USA to respond quickly and effectively to offshore plant pests that could potentially threaten the USA (VanDersal 2007).

#### *2.3.2. Prevention of Establishment*

In case the quarantine system is breached and incursion of an invasive pest has taken place, the next priority should be eradication over control (containment or suppression) to eliminate the invasive population as soon as possible. Where it is feasible, eradication is often the best course of action to deal with the introduction and establishment of invasive species (CBD 2018). Thus, the CBD *precautionary principle* does not apply, in the same way, for the elimination of populations of species that have invaded areas outside their native range (CBD 2018).

The best opportunity for eradicating invasive species is in the early stages of invasion, when an outbreak has occurred and incipient populations are still relatively small and localized. This requires early detection systems focused on high-risk points of entry, and availability of contingency plans (see USDA (2015) for examples of action plans) and emergency response funds, to allow rapid action against the invasive populations. This is the preferred response because preventing establishment is a much more viable and cost-effective approach than having to permanently "live with" a new pest. However, appropriate technology specific for the invading species, required for early detection and eradication, is often lacking, causing delays in the initiation of response actions (Liebhold et al. 2006). On the other hand, generic eradication tools such as insecticides or systematic elimination of plant or animal

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hosts are generally less effective to achieve eradication at the low pest densities common at early stages of invasion. Therefore, more eradication tools are needed that are environment-friendly and effective at low to very low densities.

### *2.3.3. Prevention of Spread and Damage*

The third priority is *containment*, if possible to prevent or slow the spread of the pest into free areas. It involves the application of *sustainable long-term control measures*, including the establishment of containment barriers (Barclay, this volume), based on the analysis of benefits and costs (environmental, economic and social). However, this third response in the three-stage hierarchy should be chosen only in case eradication is not technically feasible or resources are not available for eradication. Control measures within the containment barriers should focus on reducing the damage caused as well as the numbers of the invasive species population. Effective control will often rely on a range of integrated management techniques, implemented according to existing national regulations and international codes.

### *2.3.4. Principle of Regional Solidarity*

According to the SPS Agreement, countries are only technically justified in protecting plant and animal life and health on their own territories. Thus, invasive-pest introductions into a region generally lack regional cooperation, and are managed at the national level, if at all.

However, most countries have contiguous territory with neighbouring countries and are not isolated islands. Therefore, it is in their interest, under "the principle of regional solidarity" (EPPO 2003), to proactively cooperate directly in support of weaker neighbouring countries, or to join forces through regional animal health and plant protection organizations, to prevent pest establishment anywhere within a region, and not only when the introduction reaches their own territory.

Equally, if the establishment of a major invasive pest in one country presents a risk of natural spread to neighbouring countries, these countries also need to act in regional solidarity, even if they face lesser risks than other countries in the region, by taking all necessary measures to prevent the spread of the pest beyond their territories, and to accept support from neighbours and regional partners to eliminate the outbreak (EPPO 2003).

Moreover, according to IPPC Article VIII, contracting parties should cooperate with one another to secure coordinated effective action to prevent and control the introduction and spread of pests that can affect agriculture, food security, and biodiversity (IPPC 2012). The regional plant protection organizations (RPPOs) are important partners to the IPPC in terms of developing concrete actions to fulfil its objectives including the implementation of capacity-building programmes.

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## 3. "SCORCHED EARTH" APPROACHES IN INVASIVE-PEST ERADICATION CAMPAIGNS

### *3.1. "Scorched Earth" Eradication Approaches*

Success is not guaranteed in campaigns that attempt eradication of invasive-insect pest introductions and outbreaks, even when the political will and resources are available to implement the required eradication efforts. In addition, such campaigns often lack the tools to surgically remove all individuals of a target population and must rely, once the decision to eradicate has been made, on what has been called a "scorched earth" approach (Ayers 1957; Griffing et al. 2015). This usually entails the large-scale application of insecticide sprays, often indiscriminately affecting target and non-target organisms, as well as the drastic removal of all infested animals and plants, or even the massive destruction of whole herds, crops, and orchards. Even though generally very unpopular, such large-scale application of insecticides and other "scorched earth" interventions and their temporary negative effects must often be accepted when no better tools are available in dealing with outbreaks of major pests that warrant no compromise (Klassen 1989). This applies not only to insect pests but also to other pests, both in agriculture and increasingly in conservation; therefore, the development of new and more species-specific eradication techniques is essential (Owens 2017). Examples of such "scorched earth" eradication campaigns are described below.

#### *3.1.1. Mediterranean Fruit Fly Eradication in the Pre-SIT Era*

When the Mediterranean fruit fly invaded Florida in the early twentieth century, there was a complete absence of methods for its detection and control. Ayers (1957) described the 1929 *C. capitata* eradication programme in Florida as

> based on a veritable scorched earth policy, in which host plants and produce were destroyed in eliminating all possible breeding sites for the Mediterranean fruit fly. A huge field force of more than 5000 men was needed to carry on this programme, gathering host fruits and vegetables, uprooting many host plantings and spraying host trees with a baited insecticide

(which consisted of lead arsenate with syrup, molasses, and sugar). National Guardsmen manned quarantine roadblocks to limit the movement of infested plants and produce. Nevertheless, the 1929 invasion still moved into 20 counties before it was finally eliminated using this drastic approach.

In the 1956 Florida outbreak, the pest invaded 28 counties, and it took 21 months and another major campaign to finally achieve eradication (Ayers 1957). This time no host trees were destroyed, although mass-trapping was applied, together with aerial malathion bait sprays, with full coverage of the huge infested area that included Miami, as well as south and central Florida. The aggregate total area covered was more than 2.6 million hectares sprayed. In addition, extensive dieldrin treatments were made to the ground to kill larvae and pupae.

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The Mediterranean fruit fly invaded the island of Bermuda around 1860, and was established there for the next 100 years (Hilburn and Dow 1990). The first eradication programme, started in 1907, and based mainly on eliminating all major host trees and stripping unripe fruit from other hosts, eventually failed. A second attempt was initiated in 1957 in the wake of the recent successful programme in Florida. It was also based mainly on intensive trapping and malathion bait sprays applied in orchards every 8–10 days, augmented with sprays of dieldrin to the ground. These activities continued until 1962 when eradication was achieved.

The approach followed in these large Mediterranean fruit fly programmes in the pre-SIT era, when only very crude methods were available that significantly inconvenienced growers and the public, confirmed the need to develop more species-specific, and therefore more environment-friendly, eradication tools.

### *3.1.2. Codling Moth Eradication in Southern Brazil*

Another example from plant pests is the codling moth *Cydia pomonella* (L.), first detected in the early 1990s in mostly urban areas of apple-growing regions in southern Brazil. A National Programme for the Eradication of the Codling Moth installed and monitored more than 10 000 traps and destroyed close to 100 000 host and potential host trees in Santa Catarina and Rio Grande do Sul, states that produce 95% of Brazil's apples. Social mobilization was also required to overcome public opposition to these eradication measures. For example, the owners in urban and suburban areas, by agreeing to the removal of all their host trees, received in exchange for uprooted plants the same amount of non-host plant species. The eradication of the isolated populations of the codling moth in the four infested urban settings was concluded in 2011 (Kovaleski and Mumford 2007; Capra 2014).

### *3.1.3. Tsetse Eradication in Principe Island*

One example in the livestock area is the control of tsetse flies (*Glossina* spp.) in sub-Saharan Africa. During colonial times, one component of early tsetse eradication programmes involved complete removal of bush and woody vegetation from target areas, as tsetse tend to rest most of the time on the trunks of trees. This destruction of the flies' habitat made an area inhospitable to them, and protected humans and livestock from the tsetse-vectored diseases (Leak 1999; Bouyer and Vreysen 2018). This strategy is no longer accepted, although rapid human population growth, resulting in the opening of new agricultural lands, is having a similar effect.

Another early technique used in tsetse eradication included slaughtering all the game and wildlife that tsetse fed on, leading to the disappearance of the fly through starvation. For example, the Portuguese island of Principe in West Africa, where tsetse apparently only invaded in 1825 with the importation of cattle (trypanosomosis arrived only in 1877), was entirely cleared of feral pigs and dogs in the 1930s which, together with other measures, led to tsetse elimination (Leak 1999).

### *3.1.4.* Anopheles gambiae *Eradication in North-Eastern Brazil*

An example in the public health area is the eradication of *Anopheles gambiae* Giles in north-eastern Brazil. This vector of malaria was introduced to Brazil in 1930 from

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Senegal, facilitated by the establishment of mail service by seaplanes between Dakar and Natal that started in 1928 (Griffing et al. 2015). In 1931 in Natal there were already 344 deaths, and in response the Rockefeller Foundation's yellow fever service helped the Brazilian government eradicate the Natal outbreak using Paris green (copper acetoarsenite). Nevertheless, *An. gambiae* still spread along the coast, at an average speed of 64 km per year, and by 1938 reached the north-west of Natal, where in some areas 10% of the population died, and crops were no longer planted.

The Government and the Rockefeller Foundation decided that an autonomous, well-funded and highly trained organization needed to be created to eradicate *An. gambiae*. In 1939 President Getúlio Vargas decreed the creation of the North-East Malaria Service (*An. gambiae* having spread 483 km to the west of Natal -- more than 30 000 $km^2$). As it raised the spectre of eventually reaching the Panama Canal, the Rockefeller Foundation assisted in the financing and organization of the new service, using the infrastructure created for the yellow fever service. Frederick Soper directed this project from 1939–1941, managing these war-like efforts with a "scorched earth" approach to a 16-km perimeter around *An. gambiae* range limits (Griffing et al. 2015). The entire area was mapped from the air to ensure no collections of water were left untreated with larvicides within the perimeter or the infected zone. Homes were sprayed with insecticides, and every car and train that left the area was fumigated, as well as every boat and plane disinfected before leaving for uninfested ports. The last *An. gambiae* larva was reported in 1940. Eradication success was aided by the indoor-biting behaviour *An. gambiae*, the use of staff that had previously worked on the *Aedes aegypti* (L.) campaign, and a militaristic approach to programme management. The success in eradicating *An. gambiae* rehabilitated the concept of malaria eradication through vector control (Griffing et al. 2015).

### *3.2. Unsuccessful "Scorched Earth" Programmes and Public Outrage*

It is no surprise that such "scorched earth" measures increasingly resulted in significant public resistance. Most resented were the indiscriminate insecticide applications by air that raised public concern and outrage about possible damage to the environment and human health. As described dramatically in Rachel Carson's *Silent Spring* (Carson 1962), which called for more applied ecology, the early synthetic insecticide era led to several ill-conceived invasive-pest eradication campaigns, sometimes promoted by vested interests. These relied excessively on large-scale aerial sprays of highly residual insecticides, with broad-spectrum action, applied repeatedly with devastating impact on wildlife and the environment, but often also resulting in the development of resistance before achieving eradication.

Referring to such unsuccessful large-scale eradication campaigns, van den Bosch (1978), in his book "The Pesticide Conspiracy", was very critical of some APHIS-USDA programmes (Animal and Plant Health Inspection Service of the Unites States Department of Agriculture) in the 1950 to mid-1970's, calling it the "scorched earth insect eradication agency". His book is a story of insecticide industry-driven eradication programmes, greed and politics, that led to the so-called "pesticide treadmill" during the 1950–1970s, but likewise the surge of opposing forces that promoted more ecologically based approaches and the beginning of the IPM era.

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Also, Ehrlich (1979) referred to "numerous scorched-earth spray campaigns in which huge areas were doused with biocides in unsuccessful major efforts to eradicate pests such as the Japanese beetle *Popillia japonica* Newman, spruce budworm *Choristoneura* spp., and the red imported fire ant *Solenopsis invicta* Buren". According to Metcalf (1996), these failed and thoroughly detrimental invasive-pest eradication programmes were responsible for much of the ensuing public disenchantment with entomologists and insect control.

Among the largest of these programmes, exemplifying how a lack of suitable eradication tools can doom a programme, even when there is strong political will to succeed, was the one against red imported fire ants. This invasive pest was introduced from South America into the south-eastern USA in the 1930s, and eradication was attempted in various campaigns, the largest in the 1950s, involving the aerial dusting and spraying of millions of hectares of urban, agricultural, and natural land in the south-eastern United States. All areas within the vast fire ant control region, whether treated with heptachlor or dieldrin, reported major collateral damage to wildlife and domestic animals, and disastrous effects on aquatic life, while success was not achieved (Carson 1962). A follow-up campaign between 1962 and 1975, this time using mirex, another very toxic, residual, and lipophilic hexachlorocyclopentadiene, applied approximately a quarter of a million kilograms of this product in the form of baits, again with very negative environmental impact. The programme was finally suspended without succeeding, and the use of mirex was banned in 1978. On the other hand, the cost of the ecological and financial failure has been the continued spread of this major pest throughout the southern USA, also reaching southern California. Furthermore, the USA is now spending more than USD 5.5 billion (thousand million) annually to manage and "live with" this agricultural, recreational, and public health problem (Klassen 1989; Vander Meer et al. 2007).

### *3.3. Boll Weevil Eradication Programme*

The above cases of unsuccessful, invasive-pest eradication programmes, relying excessively on synthetic insecticide sprays, unfortunately left in academic circles and the public a negative disposition towards large and controversial eradication campaigns, even though the described situation resulted in the establishment of environmental legislation and its strict application in such programmes by USDA and other governments. Nevertheless, Myers et al. (1998) still concluded that eradication programmes have largely been ecological and financial disasters, while the benefits of eradication are generally overestimated, and the costs are understated. Nonetheless, often the careful elimination of an invasive pest, despite some temporary ecological disruption, can in the end be more environment-friendly than forever having to "live with" a new major pest that requires permanent and significant additional insecticide use.

A recent example of a more carefully executed large eradication programme is the boll weevil *Anthonomus grandis* Boheman that invaded the USA in the late 19th century from its native distribution in Central Mexico. By the 1920s it had infested all USA cotton-growing areas, devastating the industry with significant social impacts (such as mass-migration of farm labourers to industries in the north), and resulting in

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a drastic increase in insecticide use in cotton cultivation. This in turn caused several species of moths and other insects to develop into important secondary pests because of the elimination of their natural enemies, which in turn resulted in even more insecticide application in spite of major efforts to develop IPM programmes.

Following the above negative cases, there was understandably much opposition in academia to embarking on a large boll weevil eradication campaign. Nevertheless, in view of the economic and environmental problems posed by this pest and its control, and strong cotton industry and USDA leadership, successful pilot projects were carried out in the late 1970s showing the potential of combining area-wide application of cultural and quarantine practices with limited insecticide applications to achieve eradication. Even though there was disagreement with academia on the outcome, in 1983 the boll weevil eradication programme was started (Klassen 1989; El-Lissy and Grefenstette 2007). Initiation of the programme in each new county was preceded by grower referendums (2/3 of the vote required) to obtain the growers' agreement and commitment. Grower funds, with some state support, accounted for over 70% of the programme operational budget, with less than 30% of funds provided by the federal government (Kazmierczak and Smith 1996).

The eradication programme was based on thorough mapping of host areas, using pheromone-baited traps for detection, and sound cultural practices (uniform times for planting and harvesting, and residue destruction) applied rigorously on an area-wide basis, together with carefully timed aerial applications of malathion ultra-low volume in the spring. Releases of sterile *A. grandis* were not included because of the midgut damage caused by sterilizing radiation doses (Riemann and Flint 1967).

In some regions, sprays temporarily reduced seasonal mean densities of predators, with populations of beet armyworm *Spodoptera exigua* (Hübner), heliothine and other lepidopteron larvae, and the cotton aphid *Aphis gossypii* Glover, often increasing substantially in cotton fields under boll weevil eradication (Knutson et al. 2011). Nevertheless, the programme was a major success with the boll weevil eradicated from all USA cotton regions (from Virginia to California, as well as a portion of north-western Mexico, except for an area still under treatment in Texas and infested areas of northern Mexico), covering many millions of hectares of cotton production, and resulting in increased yields and much declined pest control costs. Most important are the ensuing environmental benefits in view of the dramatic and sustained reduction in overall insecticide use, largely sparing biological control agents that control *Helicoverpa* spp. and other secondary cotton pests (Klassen 1989; El-Lissy and Grefenstette 2007). This programme, together with the New World screwworm *Cochliomyia hominivorax* (Coquerel) eradication programme (Klassen et al., this volume; Vargas-Terán et al., this volume), are among the biggest and most successful insect control programmes in history.

## 4. STERILE INSECT TECHNIQUE, AN ENVIRONMENT-FRIENDLY ERADICATION TOOL

In contrast to the above described approaches, the SIT is a proven, clean technique that can eradicate, contain or prevent the establishment of an invasive population without raising public opposition or leaving an "ecological footprint". Since it is

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species-specific, and therefore does not affect beneficial and other non-target organisms, it is an ideal tool to eradicate invasive insect pest populations. Its integration, especially in the later phases of eradication campaigns, is particularly effective in view of its inverse density-dependence: the lower the target population, the sooner it will reach the objective of eradication. It also integrates well with other biologically based suppression methods. It can be applied by air, and consequently is effective over irregular topography and areas with limited access, which are often major challenges in eradication campaigns that need to address the invasive pest population on an area-wide basis (Dowell et al., this volume; Hendrichs, Vreysen et al., this volume; Klassen and Vreysen, this volume; Klassen et al., this volume; Mangan and Bouyer, this volume).

### *4.1. Successful Cases of SIT-Based Eradication of Invasive-Pest Outbreaks*

Below several cases exemplify the successful integration of the SIT as an environment-friendly eradication tool, starting with the elimination of:

a) incipient populations in beachheads or bridgeheads and small areas/islands (gypsy moth in parts of the USA (Simmons et al., this volume); painted apple moth in New Zealand; cactus moth in Yucatán, Mexico; melon fly *Zeugodacus* (= *Bactrocera) cucurbitae* in the Mariana Islands; Mediterranean fruit fly in California, Chile, South Australia; solanum fruit fly *Bactrocera latifrons* (Hendel) in Yonaguni Island, Japan; Queensland fruit fly *Bactrocera tryoni* (Froggatt) in South Australia and Tasmania; sweetpotato weevil *Cylas formicarius* (F.) in Kume Island, Japan; etc.); to
b) major outbreaks over larger areas (*C. hominivorax* in Libya; *C. capitata* in the Dominican Republic, in southern Mexico; etc.); all the way to
c) fully established invasive pest populations in whole regions (pink bollworm in south-western USA and north-western Mexico; melon fly in all archipelagos of southern Japan; etc.).

#### *4.1.1. Painted Apple Moth in New Zealand*

The Australian painted apple moth is a polyphagous pest that is native to south-eastern Australia and Tasmania. An incursion of this moth into New Zealand in May 1999, infesting an area of ca. 12 000 hectares in West Auckland, threatened the country's natural ecosystems, as well as its horticulture crops and plantation forestry (Suckling et al. 2007).

The presence of this non-native pest triggered the establishment of an area-wide eradication programme managed between 1999 and 2006 by the New Zealand Ministry of Agriculture and the Forestry Biosecurity Authority. The programme integrated ground searches, the deployment of irradiated sentinel virgin females within traps (there was no effective pheromone), host removal where possible, ground and some aerial applications to host trees in hot spots using chlorpyrifos, deltamethrin and the biopesticide *Bacillus thuringiensis* (Berliner) var. *kurstaki* (*Btk*), and the first use of the SIT against this pest. The SIT was favoured because of its effectiveness as an "end game tactic" at very low population levels, and the strict environmental

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requirements in New Zealand that seriously limited the application of other eradication tools (Suckling et al. 2007).

Development of mass-rearing provided the sterile females for an array of up to 2000 georeferenced traps that were serviced weekly. In addition, marked sterile males were also used for dispersal studies. The lower irradiation dose chosen (100 Gy), needed to induce inherited sterility, offered the best trade-off between sterility and male competitiveness (Suckling et al. 2005). Irradiated males were recaptured in the field up to 10 km away, showing good flight capacity, although they were only 66% as successful as non-irradiated males in seeking females (but their ability to copulate was not affected).

After delimitation of the infested area in 1999–2000, ground applications in 2001–2002 were followed by aerial *Btk* sprays. After suppressing the pest population to ca. 1% of the original population level, releases of sterile insects started in January 2003 and lasted until April 2004 to eliminate remnants of the population. Achieving overflooding ratios up to 100:1 (based on trapping data) drove the wild population to extinction (Suckling et al. 2005). Small-scale releases were reinitiated in 2005 and 2006 because of captures of several males (genetic analyses indicated that they originated from separate incursions) (Suckling et al. 2007).

If no action had been taken to eradicate the invasive painted apple moth populations, the economic and ecological impact of the moth's incursion was estimated at approximately USD 30–213 million over 20 years (Suckling et al. 2007). The authors have called such cost-effective small-scale programmes, targeted at eradicating early detected populations, where only limited numbers of sterile insects must be released each week, as “boutique” SIT.

#### *4.1.2. Cactus Moth in Yucatán*

*C. cactorum* occurs naturally in Paraguay, Uruguay, northern Argentina, and southern Brazil. Considering its wide host range within the Opuntioidea, it represents a great threat to important *Opuntia* ecosystems and cultivation in North and Central America. In Mexico, Cactaceae have the greatest diversity, and *Opuntia* cacti are viewed as having major ecological, socio-economic, and cultural importance (Zimmermann et al. 2007).

The cactus moth is regarded as one of the most successful classical biological control agents. For example, in Australia, the cactus moth effectively controlled approximately 25 million hectares of non-native *Opuntia* that had invaded and densely colonized cattle-grazing lands (Dodd 1940). In the 1950s the cactus moth was released for this same purpose in some Caribbean islands, but has since then been expanding its range in this region. From there it subsequently invaded Florida in 1986, and has been gradually advancing along the Gulf of Mexico coast of Alabama, Mississippi, Louisiana, and more recently Texas, increasingly representing a serious threat to the semiarid ecosystems in the south-western USA and Mexico (Bloem et al. 2007; Hernández et al. 2007).

In view of this threat, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture encouraged the Mexican Ministry of Agriculture to establish a permanent monitoring programme for the cactus moth in risk areas throughout Mexico (IAEA 2003). This trapping network detected outbreaks of the pest in Isla

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Mujeres in July 2006, and in Contoy Island National Park in May 2007, as well as moth presence on the Quintana Roo mainland near Cancún in January 2007. In response, a Programme for the Eradication of the Cactus Moth in the Yucatán Peninsula was launched, involving coordinated efforts among the Mexican Ministry of Agriculture, USDA, and the Joint FAO/IAEA Division (Bello-Rivera et al. 2021). To eradicate these incipient pest populations in Mexico, the outbreak areas were delimited through increased surveillance, and an AW-IPM approach was implemented -- involving the establishment of sentinel sites, moth egg-stick removal and host removal in some areas, followed by the release of sterile moths produced by USDA in Tifton, Georgia (Hight et al. 2005; Bello-Rivera et al. 2021).

Following a two-year campaign, the outbreaks were officially declared eradicated in February 2009 after three biological cycles without any pest detection since the last moth find (Barclay et al., this volume; Vreysen, this volume). Significant damage was avoided to the economically important Mexican cactus and prickly pear industries, as well as to whole ecosystems based on *Opuntia*. Routine surveillance has continued, and through the project Mexico developed the capacity for early detection and eradication of the cactus moth from its national territory (Bello-Rivera et al. 2021).

*4.1.3. Melon and Oriental Fruit Flies in Mariana Islands and Okinawa Archipelago*

The first successful use of the SIT against an insect pest, other than the New World screwworm, was the eradication in 1963 of the melon fly in Rota in the Mariana Islands (Steiner et al. 1965). Considered native to India, this very destructive pest of cucurbit crops and other fruits has spread to many regions and is now found in more than 40 countries in South and Southeast Asia, sub-Saharan Africa, and the Pacific region (CABI 2017). It reached the islands of Rota, Saipan, and Tinian by 1943, interfering with the export of fruits and vegetables that the farmers in these islands had been commercially producing for many years, and resulting in the need for costly phytosanitary treatments to permit the continuation of export shipments.

The eradication campaign conducted in Rota involved first surveillance (1960–1962), followed by bait sprays in host areas to suppress the melon fly population by ca. 75%, and then weekly releases of between 5 and 11 million sterile flies by ground and air (1962–1963). These were mass-reared in Honolulu and shipped by commercial flights to Guam, where some were emerged and then shipped to Rota for aerial release, while others were transported directly to Rota where they emerged in the field. Overall 257 million sterile flies were released, and eradication was achieved in ca. six months, although releases continued for some months after that (Barclay et al., this volume; Vreysen, this volume). In 1963, the neighbouring islands of Saipan, Tinian, and Aguigan were also freed of the melon fly. Rota, however, being the closest island to Guam, experienced reintroduction of melon flies eight times up to June 1971. Each time the development of a melon fly population was prevented by prompt releases of sterile flies (Chambers et al. 1970).

Rota and the other Mariana islands were also freed of the oriental fruit fly *Bactrocera dorsalis*, another major invader, although mainly by area-wide application of the male annihilation technique (MAT), deploying toxic methyl eugenol baits. In the case of Guam, however, *B. dorsalis* eradication was achieved by the weekly release of 16 million sterile flies between September 1963 and February 1964, also

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shipped from Hawaii, but only after a hurricane had drastically reduced the wild population (Steiner et al. 1970).

In the archipelagos of southern Japan, the oriental fruit fly was eradicated based only on MAT implementation (1968–1986), but not in the Ogasawara Islands where two years of area-wide MAT application failed to eradicate the *B. dorsalis* population. This lack of success appeared to result from males evolving a non-responsive behaviour to the methyl eugenol baits. Therefore, the SIT was added after suppressing the population with the MAT along with other methods, enabling the eradication of *B. dorsalis* also from these islands (Habu et al. 1984).

A much larger undertaking was the Japanese melon fly eradication campaign (Table 1). Between 1919 and 1977, *Z. cucurbitae* gradually invaded all the archipelagos of south-western Japan, where populations became fully established, causing major damage and the loss of cucurbit vegetable and fruit exports to mainland Japan, as well as threatening islands further north. Based on the positive experiences gained in Rota and other Pacific islands, in 1972 a major eradication programme was launched to gradually free each of the island groups that the melon fly had invaded.

*Table 1. Island groups in south-western Japan that* Zeugodacus cucurbitae *invaded and from where it was eventually eliminated following a major SIT-based eradication campaign between 1972 and 1993*

| Island group | Number of islands | Approximate total surface ($km^2$) | Year of melon fly invasion | Year of start of SIT releases | Year of final eradication |
|---|---|---|---|---|---|
| Yaeyama/Yonaguni | 32 | 586 | 1919 | 1989 | 1993 |
| Miyako Islands | 8 | 227 | 1929 | 1983 | 1987 |
| Kume Island | 1 | 60 | 1970 | 1972 | 1978 |
| Okinawa Islands | 9 | 1439 | 1972 | 1986 | 1990 |
| Okierabu Islands | 2 | 94 | 1973 | 1985 | 1987 |
| Amami Islands | 6 | 936 | 1974 | 1985 | 1987 |
| Daito Islands | 3 | 44 | 1977 | 1986 | 1990 |
| Total Region | 61 | 3386 | 1919 | 1972 | 1993 |

At first, important basic and applied research was carried out, including a pilot project in Kume island (1972–1978), to improve and refine integrated SIT applications, and to assure the success of the eradication project (Itô et al. 2003; Koyama et al. 2004). Following population suppression by the aerial distribution of cotton strings soaked with cuelure, a male attractant, and naled, the SIT was then systematically applied to the different island groups to eradicate the melon fly. In 1993 the impressive goal of complete eradication was achieved in all of the archipelagos of southern Japan, all the way to the Yaeyama islands closest to Taiwan (Kakinohana et al. 1997).

### *4.1.4. Mediterranean Fruit Fly in the Dominican Republic*

The Mediterranean fruit fly originated in East Africa, from where it has spread to many regions of the world. However, the Caribbean region was still free of this major horticultural pest until its presence was reported in March 2015 in the Dominican

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Republic, where this polyphagous pest had already invaded more than 2000 km$^2$ in the eastern part of the country. The outbreak was centred around the Punta Cana region, a major tourist destination, suggesting that the pest was introduced by tourists carrying infested fruits. Trading partners responded by establishing an immediate ban on exports of most fruits and vegetables from the Dominican Republic, causing a loss of USD 40 million in the remaining nine months of 2015, and putting 30 000 jobs at risk. Moreover, the whole Caribbean region and its trading partners were at risk of significant direct losses and the loss of their export markets (Zavala-López et al. 2021).

As an emergency response, the government, through its Ministry of Agriculture, established the Moscamed Programme in the Dominican Republic, providing the required financial and operational support to perform all required surveillance and eradication activities. FAO, IAEA, and USDA guided and assisted the country in establishing a national monitoring network to delimit the distribution of the outbreak and contain its spread, as well as to initiate an eradication campaign with support also from regional organizations such as OIRSA and IICA. The Guatemala-México-USA Moscamed Programme assisted through staff training, technology transfer, and the provision of sterile flies. Even though the pest had already spread to such a large area, an external Technical Advisory Committee confirmed that eradication was still feasible, and recommended the implementation of an AW-IPM programme integrating the SIT with quarantines and other controls (Zavala-López et al. 2021).

Once the extent of the infestation was well defined, suppression activities were intensified, including some host-tree pruning and fruit destruction in hot spot areas, ground and in some cases aerial bait sprays, and placement of bait stations in inaccessible or sensitive tourist areas. After pest population suppression in hot spot areas, SIT application was started in October 2015 and continued until April 2017, three months after the last wild fly was detected in January 2017. An average of 72 million sterile males of a genetic sexing strain were released per week by air, covering a total of 42 000 ha in the provinces of La Altagracia and La Romana (Dowell et al., this volume). In addition, an average of 15 million sterile flies was released per week by ground along the coast in touristic zones subject to considerable drift due to coastal winds. In total, more than 4 thousand million sterile males were released over the affected areas (Zavala-López et al. 2021). After two years of intense eradication efforts, involving up to 300 staff, in July 2017 official eradication was declared by the government (IAEA 2017). Intensive trapping and fruit sampling continued for another six months after the discontinuation of sterile male releases and nine months after the last wild-fly detection (Barclay et al., this volume; Vreysen, this volume).

#### *4.1.5. New World Screwworm in Libya*

The New World screwworm is native to the tropical and subtropical Americas, invading Florida in 1933 (Skoda et al. 2018). Its larvae produce myiasis by feeding on the living tissue of warm-blooded animals, including humans. Its damage to the livestock industry was the initial motivation for the development and successful application of the SIT in a 50-year area-wide campaign that started in Florida and culminated in the eradication of this pest from all of North and Central America (Klassen et al., this volume; Vargas-Terán et al., this volume).

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In the spring of 1988, the presence of the New World screwworm was detected in Libya. Shipments of live animals from Latin American countries were the likely source (Libya covered ca. half of its livestock needs with imports). The initial beachhead discovered extended already to more than 18 000 km$^2$, representing a major threat not only to Libya and North Africa, but to the whole Mediterranean region, as well as to wildlife throughout Africa (Vargas-Terán et al., this volume).

Supported financially and technically by a multi-institutional, multi-donor international effort, FAO took the lead in coordinating the emergency programme that not only focused on containment and eradication in Libya, but also on surveillance activities in the surrounding 7 first-line countries and 10 second-line countries. A Screwworm Emergency Centre for North Africa (SECNA) was established at FAO in Rome and the field programme in Tripoli, Libya. An intensive campaign informed and requested support from livestock owners and the general public in Libya and neighbouring countries. In addition, every 21 days more than 90 roving teams inspected the estimated 7 million head of livestock in Libya, treating wounds prophylactically. As a result of this major effort, together with the establishment of 13 quarantine stations to control livestock movement, containment was successful and no new foci developed elsewhere in Libya or the region. Nevertheless, by the time eradication activities were initiated, the New World screwworm had expanded to 25 000 km$^2$, and the severity of the infestation had increased significantly (FAO 1992).

Mating studies with wild Libyan screwworm flies were carried out to confirm sexual compatibility with the mass-reared screwworm flies produced in the mass-rearing facility in Mexico. Once it was confirmed, and the required infrastructure was established, weekly aerial shipments of sterile pupae from Mexico to Tripoli were initiated in December 1990. In total, until October 1991, 1300 million sterile pupae were transported, and sterile flies released over 41 000 km$^2$, with higher release densities over hot-spot areas. Releases were supported by surveillance, sentinel animals, wound treatment, and quarantine activities. The last myiasis case was detected in April 1991, although releases continued for another six months to assure the elimination of any remnants. Surveillance and quarantine inspection activities continued until June 1992, when eradication was declared (FAO 1992; Lindquist et al. 1993; Barclay et al., this volume; Vreysen, this volume).

Success was achieved earlier than foreseen, largely favoured by determined government support, effective management, and the decision to start the sterile fly releases in winter, when screwworm populations were naturally low. Nevertheless, there were some critical voices that claimed that the cold winter was responsible for screwworm eradication. However, as shown by Krafsur and Lindquist (1996), the screwworm population clearly did overwinter, and eradication would not have been achieved without SIT application. Originally estimated to cost USD 117.5 million, the actual cost of the eradication campaign was only ca. USD 80 million. An independent economic study commissioned by FAO estimated annual benefits of USD 300 million for the region, with a benefit/cost ratio of 50:1 (FAO 1992).

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There have also been multiple interceptions of New World screwworm-infested animals at ports of entry, and incipient outbreaks or reinvasions that have been eradicated, including in Aruba, Curaçao, Mexico, Florida Keys, etc., using the SIT (Skoda et al. 2018).

### *4.2. Successful Cases of SIT Preventive Releases in Areas of High Risk of Pest Establishment*

The above cases illustrate the effectiveness and environment-friendliness of SIT integration in achieving eradications of invasive pest populations -- simpler in the case of incipient and still-small incursions, and more costly and complex when large outbreaks are detected late or when the populations are already fully established. On the other hand, when repeated introductions occur into points of entry considered as high risk in view of historical evidence, and where the increasing flow of passengers and cargo is outdistancing inspection capacities, a preventive SIT approach is most cost-effective as shown by the following examples.

#### *4.2.1. Preventive Release Programmes against Mediterranean Fruit Fly in California and Florida*

California and Florida have been increasingly subject to incursions of the Mediterranean fruit fly, leading to repeated detections and outbreaks in urban areas, especially those with international airports, e.g. Los Angeles and San Diego in California, and Miami, Tampa, Ft. Lauderdale, and Palm Beach in Florida. The incursions have been linked to fruit smuggling, and the rapidly growing volume of shipments and air traffic. Liebhold et al. (2006) confirmed passenger baggage as a common pathway for entry of the Mediterranean fruit fly, and found a direct relationship between number of interceptions and travellers per year. They showed that, in spite of fines, dog brigades, and scanning devices or other equipment aiding the screening of baggage for quarantine material, this invasive pest arrives at a sustained level with fruit carried by passengers, explaining its repeated detection in urban areas but not in fruit-production areas.

Since the first Mediterranean fruit fly outbreak in 1975 in Los Angeles county, agriculture authorities in California have been responding to an ever-larger number of outbreaks, some triggering extensive and costly eradication programmes, initially largely based on aerial malathion bait sprays over urban areas that caused much public outrage (Scribner 1989; Mangan and Bouyer, this volume). Increasingly, the SIT was also included in a limited radius around outbreaks to eradicate them. Nevertheless, by 1993, this reactive localized strategy appeared to be no longer effective, since Mediterranean fruit fly infestations were being detected in 39 cities in five counties in the Los Angeles Basin, and there was no doubt that some population propagules had spread before achieving eradication (Dowell et al. 1999).

In response to an international advisory panel recommending an area-wide approach, a basin-wide sterile insect release programme was initiated in 1994 in 2358 $km^2$ that included all existing Mediterranean fruit fly infestations and areas in the Los Angeles Basin where Mediterranean fruit fly infestations had been eradicated in the

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previous 7 years. In addition to limited malathion bait sprays to hosts around fly finds, sterile flies were released basin-wide at a weekly rate of ca. 155 000 per km$^2$. This area-wide approach was very successful, with wild Mediterranean fruit fly captures decreasing from 400 in 1993, to 7 in 1994, and to no wild flies within the basin-wide area in 1995. It was also more cost-effective than the localized reactive approach, and avoided the expensive quarantine areas around each new incursion that are very disruptive to horticultural trade, both nationally and internationally.

In view of the success of this area-wide approach, and the continuous risk of Mediterranean fruit fly introductions, a permanent Mediterranean fruit fly Preventive Release Program (PRP) was initiated in 3470 km$^2$ that included the basin-wide programme area, and an additional 1112 km$^2$ in Riverside, San Bernardino, and Orange counties. Due to its preventive nature, the PRP reduced the weekly release rate of sterile flies by half to ca. 78 000 per km$^2$ (Dowell et al. 2000). The PRP has been in operation without interruption since 1996.

A similar PRP was established in Florida as a proactive approach to the heightened threat of new Mediterranean fruit fly introductions, due largely to increased international trade and travel and the high growth rate of urban areas. Since 1998, sterile Mediterranean fruit flies have been released weekly on a preventive basis in areas historically shown to be at high risk for new introductions (USDA 2014).

The fully operational fly emergence and release facilities have also enabled an effective response to detections of the Mediterranean fruit fly, and also some *Anastrepha* spp. fruit flies outside the PRP areas. This success has continued, and both PRPs are still fully operational but with some adjustments in the area and release densities. Therefore, the area-wide preventive SIT strategy has been a major success, substantially reducing the risk of Mediterranean fruit fly establishment in California and Florida. The PRPs provide protection to multi-billion-dollar (multi-thousand-million-dollar) industries, while emergency project costs have been reduced by 85%. Compared with pre-PRP years, outbreaks in California have been reduced by more than 98%. In PRP areas in Florida, no Mediterranean fruit fly outbreaks have occurred (USDA 2014). As a result, in spite of continuous incursions of this invasive pest, the continental USA is still internationally recognized as Mediterranean fruit fly-free. This has been disputed (Papadopoulos et al. 2013; Carey et al. 2017). However, as pointed out by McInnis et al. (2017) and Shelly et al. (2017), trading partners and foreign consumers have never detected larvae in Californian/Florida produce, and flies are not trapped in rural areas, where the vast majority of commercial horticultural production is located.

#### *4.2.2. Other Preventive SIT Programmes*

There have been other PRPs to protect free areas from pest invasion. One involved the release of sterile *Anastrepha ludens* (Loew) fruit flies near the Mexico border in southern California -- to prevent the entry of this pest from Baja California Norte (Klassen et al. 1994). This programme, in operation since 1964, was continued until the mid-1990s when it was no longer needed because Mexico eradicated *A. ludens* and *A. obliqua* (Macquart) from all of north-western Mexico (Reyes et al. 2000). A similar programme, also against *A. ludens*, has been in operation in the Rio Grande Valley in southern Texas (Klassen and Vreysen, this volume).

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As described above, the melon fly was eradicated from Okinawa. Nevertheless, there is evidence of isolated fly movement (probably wind-assisted) over considerable distances, based on captures in traps in some Japanese islands that are closer to Taiwan, but no infested fruits have been detected. Therefore, to prevent recolonization of the melon fly in Okinawa after eradication, sterile fly releases are carried out at lower densities in areas where the possibility of invasion is relatively high, e.g. near international airports, U.S. military bases, and residential areas (Kuba et al. 1996). A similar situation applies to the invasive solanum fruit fly after its eradication from Yonaguni Island, next to Taiwan, and which is therefore threatened by reinvasion.

### *4.3. SIT Releases to Contain Invasive Pest Populations to Protect Free Areas*

Sterile insects have also been used effectively to contain the spread of invasive pest populations into regions free of that pest, and where sterile insects are released along the front of the invasive pest population (see Fig. 1 in Hendrichs, Vreysen et al., this volume). Two large operational programmes stand out:

#### *4.3.1. Pink Bollworm Containment Programme in California*

The pink bollworm, originally native to Asia, has invaded most of the cotton-growing regions in the world. It first reached the cotton belt of the southern USA in 1917, and gradually advanced towards the south-western cotton region, where it became a major pest in the arid desert areas. It finally established in the cotton-growing Imperial and Palo Verde Valleys in southern California and north-western Mexico, and threatened to invade also the large San Joaquin Valley. In response, a sterile moth release containment programme became operational in 1968, funded largely by the cotton industry, with the objective of stopping the spread of the moth into this important valley. For more than three decades, sterile moths were released in 400 000 hectares as part of pink bollworm IPM; during this long period the pest was successfully excluded, thereby contributing to the survival of cotton production in the valley (Staten et al. 1993). Subsequent to the extensive planting of *Bt*-cotton, starting in the mid-2000s, this effective containment programme finally became unnecessary when the pink bollworm was gradually eradicated from all of south-western USA and north-western Mexico, largely through the integration of *Bt*-cotton and the SIT (Staten and Walters 2021; Simmons et al., this volume).

#### *4.3.2. Guatemala/Mexico/USA Moscamed Programme*

The other large programme, that has been applying the SIT effectively to contain the spread of an invasive insect pest, is the joint Mediterranean fruit fly programme between Guatemala, Mexico, and the United States (Enkerlin, this volume). This pest invaded Costa Rica in the 1950s, eventually spreading to neighbouring Central American countries, where it caused major damage to fruit and vegetable production, and resulted in the loss of important export markets, in particular the large USA market. Coming from El Salvador, it invaded and crossed Guatemala in only one year (1976), and in January 1977 was detected in Mexico close to the border with

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Guatemala. This threat to the horticultural industries of Mexico and the USA triggered the tri-national MOSCAMED Programme; while the first large mass-rearing facility was being built, a major aerial bait-spraying effort was made to contain the rapid advance of the pest (Schwarz et al. 1985). Even so, by early 1979, it had already spread along a 330-km coastal transect into the neighbouring State of Oaxaca. Once sterile male releases were added to the area-wide eradication campaign in 1979, the infested area in Mexico was gradually cleared of the pest (Hendrichs et al. 1983). Since 1984 to the present, this programme has been containing the fruit fly by releasing sterile males in a belt on both sides of the invasion front along the Guatemala-Mexico border, maintaining Mexico and the USA free of this pest, while also protecting Mediterranean fruit fly-free Belize and northern Guatemala (Enkerlin et al. 2015, 2017).

Another successful containment programme for the Mediterranean fruit fly is the one that was implemented along the Chile-Peru border from 1996 until the mid-2000s, after northern Chile was freed of this invasive pest; Peru's two southern provinces, Tacna and Moquegua, are now free of this pest (Enkerlin, this volume).

## 5. TRENDS, CONSTRAINTS, AND CONTINGENCY PLANNING FOR FUTURE SIT APPLICATIONS

The above cases illustrate different situations where the SIT has been effective in eradicating small or established invasive pest populations, in containing their spread, or in preventing their establishment in an environment-friendly way. There is great potential for integrating the SIT in similar situations against major invasive insect pests, particularly in situations where the implementation of "scorched earth" approaches is becoming increasingly difficult.

### *5.1. Social Issues and Trends*

A major trend probably favouring the expanded use of the SIT in invasive insect eradication programmes is public opinion in increasingly complex social landscapes (Epanchin-Niell et al. 2010). Establishing and maintaining public support is becoming a major challenge, especially in eradication campaigns in view of their "all or nothing" nature, and requiring the buy-in from major stakeholders (Perkins 1989; Myers et al. 1998)

Issues of free riders, public participation, and disagreements over the financing of public good, have in several cases severely hampered the positive outcome of area-wide programmes; this stresses the need for attention not only to ecological, environmental, and economic aspects, but also to social and management dimensions (Hendrichs et al. 2007). Therefore, under such circumstances, effective and professional management of all social issues related to eradication campaigns is becoming increasingly important (Owens 2017).

Among the consequences of the global phenomenon of rapid urbanization is a decreasing understanding and support for agriculture and related pest eradication or containment campaigns, especially if they involve the application of drastic control methods such as habitat destruction, area-wide removal of animal and plant hosts, and

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large-scale insecticide spraying. Campaigns that were possible in the past may no longer be possible today, and probably will not be acceptable to the public tomorrow. Even in the public-health sector, the human population no longer accepts unquestioningly area-wide action against invasive pests.

For some eradication programmes there have been public attempts to halt drastic actions, such as the mentioned Mediterranean fruit fly aerial spraying eradication campaigns in the Los Angeles Basin in the 1980s. Others were actually stopped by judicial action following public outrage, e.g. causing the failure of the citrus canker eradication programme in Florida between 1995 and 2006. More recently, efforts starting in late 2013 to stop the infection of olive groves in southern Italy by the bacterium *Xyllela fastidiosa* (vectored by the meadow spittlebug *Philaenus spumarius* (L.)) have been hampered repeatedly by local growers, environmentalists, and local authorities and prosecutors, allowing the spread of the disease northwards. Although the European Court of Justice finally officially backed the drastic eradication measures, including the removal of healthy trees within 100 m of a diseased tree, in this case it may be too late (Oggi 2016; Abbott 2017).

Such instances of public outrage are likely to increase because of the increasing frequency of invasive pests becoming established at ports of entry, such as near international airports, seaports, and elsewhere in urban and surrounding suburban areas (Hendrichs et al. 2007; Liebhold 2014). Under these situations, where the public can be non-supportive or even hostile, the integration of the SIT or mating disruption has considerable social advantages over aerial spraying and other drastic interventions, especially in urban/suburban settings, to avoid inconveniencing the public and raising public opposition (Dowell et al. 2000; Gamble et al. 2010). Nevertheless, also SIT-based area-wide campaigns should include a strong and well-designed public outreach component to anticipate any negative public reactions, and be proactive in mitigating those aspects that may cause outrage (Klassen 1989; Dyck, Regidor Fernández et al., this volume).

### *5.2. Constraints to SIT Applications against Invasive Pests*

For many pest species that have not yet arrived in regions or countries currently free of them, it is a matter of when, rather than if, they will invade. The exploding global travel and trade makes it increasingly likely that any species can reach anywhere in the world. This is compounded by a warming climate, allowing pest populations to survive and spread in previously inhospitable areas, for example the invasion of several disease-transmitting *Aedes* species into Europe (Cunze et al. 2016).

Expanding the use of the SIT to potential invasive pest insects is not straightforward. In some cases, the biology of the target non-native insect pest is not amenable to SIT application, or at least not practical economically (Lance and McInnis, this volume). Even when this is not the case, often the SIT package is only partially or not available for new invasive pests. Developing the missing technology in the short term, once an outbreak has occurred, is often complicated, although the described case of the painted apple moth in New Zealand is a successful example where the pest was suppressed and contained while the SIT was validated. Cases of taxonomically related pest species, for which the SIT is already being applied, are

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generally also feasible in view that the technology is easier to transfer and adapt, e.g. among the various tephritid fruit flies (IAEA 1999). On the other hand, the light brown apple moth (LBAM) *Epiphyas postvittana* (Walker) is an example of an attempt to develop the SIT as an eradication tool after detection occurred in California in 2007. The effort was eventually abandoned in 2008 because of public opposition to aerial spraying of mating disruption pheromone to suppress this pest (Bloem et al. 2014; Liebhold 2014), and the fact that it became widely established in the meantime. Analysing the origin of this environmental controversy, Lindeman (2013) concluded:

> CDFA [*California Department of Food and Agriculture*] lost the battle over LBAM aerial spraying largely because of a report and other supporting grey literature documents [*that are not available in the academic literature*] that expressed highly disputable facts, evidence, and conclusions.

Nevertheless, 10 years later, as a consequence of this case and the continued push from environmental advocates for overall tighter restrictions, the courts issued an injunction requiring the state of California to stop using 79 insecticides when combating non-indigenous pest outbreaks (McClurg 2018).

In view of the above, it would be very worthwhile for countries or regions to prepare themselves before the eventual expected invasions, at least for the potentially most-feared invasive pest species, both in terms of developing the surveillance capacity for early detection of incursions, as well as contingency plans and other tools to enable eliminating incipient outbreaks with the least social and environmental disruption. The Port Information Network database maintained by APHIS-USDA is a potentially valuable source of information on the most common invading pest incursions intercepted, and for understanding the pathways by which potential invaders arrive. There are also a number of invasive species lists and databases that compile information on invasive species (Lowe et al. 2004; NISIC/USDA 2017; Invasive Species Compendium 2018). Actual and potential worst arthropod invaders feature prominently on these lists, along with their risks of invasion, and potential damage and distribution; such lists help guide countries in preparing in advance for such events (Baker 2002; Klassen et al. 2002; NRC 2002; Worner 2002; Worner and Gevrey 2006; Wan et al. 2009; ISSG 2018).

When incursions occur, rather than waiting to pay for the accrued damages of an invasion, it is certainly much cheaper to have the technology ready for effective early detection and response. However, the full benefit might not always be realized when the investment occurs long before any impacts are experienced (Bradshaw et al. 2016). Therefore, it is often difficult to convince decision-makers to divert funds towards preparing for future pest problems that have not yet arrived.

### *5.3. Contingency Planning and Future SIT Applications*

The development of the SIT for the false codling moth *Thaumatotibia leucotreta* (Meyrick), a major potential invasive pest that is indigenous to sub-Saharan Africa, is a successful example of such forward-planning that now enables rapid SIT integration into area-wide eradication campaigns if this key pest were to invade other regions. It has a large host range and has already spread to several islands in the Indian Ocean and Atlantic. Larvae have been intercepted by APHIS-USDA from a wide variety of

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commercial hosts in shipments arriving in the USA from at least 16 different African countries (Carpenter et al. 2007). Since it became resistant to several insecticides, and a threat to South African exports, FAO/IAEA and USDA supported the South African citrus industry to develop the SIT as an additional method for effective false codling moth suppression, but also as an eradication method that can now be immediately used in future outbreaks of the false codling moth in the USA and elsewhere (Carpenter et al. 2007; Hofmeyr et al. 2015).

While the above false codling moth case resulted from a fortuitous alignment of interests that, in the future, would benefit the USA and other false codling moth-free countries, there has been very limited deliberate contingency planning and investment to develop environment-friendly tools to deal with future invasions of major insect pests. Among the exceptions, Australia and New Zealand stand out.

Australia has developed contingency plans to respond to a number of invasive pests, including the Old World screwworm *Chrysomya bezziana* (Villeneuve), a myiasis-causing fly in Africa, the Persian Gulf, India, and South-East Asia (Vargas-Terán et al., this volume). The Australian Veterinary Emergency Plan contains a strategy for Old World screwworm eradication should it gain a foothold in Australia (Anaman et al. 1994; Tweddle 2002; AHA 2017). The policy is to eradicate the screwworm in the shortest possible time, while limiting the economic impact. Therefore, Australia has made considerable effort to conduct research on the Old World screwworm and to develop the SIT against this livestock pest. Field trials in Papua New Guinea provided strong indications that the SIT would be effective in suppressing it, and later studies in Malaysia, which included the establishment of a 6-million-per-week pilot mass-rearing facility, validated the SIT for this screwworm species (Vargas-Terán et al., this volume).

New Zealand is probably the country most prepared in terms of major biosecurity threats. Among the most feared threats are the European grapevine moth *Lobesia botrana* (Denis and Schiffermüller), the Korean pumpkin fly *Zeugodacus depressus* Shiraki, the spotted-wing drosophila *Drosophila suzukii* (Matsumura), and the brown marmorated stink bug *Halyomorpha halys* Stål. If they became established, these species could wreak havoc in New Zealand orchards and vineyards. Therefore, Plant and Food Research scientists have been developing surveillance and eradication tools, including the SIT, for these potential invaders (Horner et al. 2016; Morton 2017; Welsh et al. 2017).

One of the limitations, in developing the SIT against a potential invasive pest that is still absent, is the need to establish partnerships with locations where the pest is present, e.g. the South Africa-USA collaboration on the false codling moth in South Africa, the Mexico-USA collaboration on the cactus moth in the USA, or the Australia-Malaysia collaboration on the Old World screwworm in South-East Asia. An alternative is to use model species, an approach being followed in New Zealand, where the southern green stink bug *Nezara viridula* (L.) is the model being researched in preparation for the brown marmorated stink bug. A further technical issue that needs to be managed, especially after successful eradications, is maintaining high-quality strains in mass-rearing facilities to be ready in case of new introductions (Kuba et al. 1996).

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Another option is to establish contractual arrangements to procure the sterile insects when mass-rearing capacity exists elsewhere. New Zealand is free of major fruit fly pests, but if the Mediterranean fruit fly or the Queensland fruit fly, both present in Australia, became established in New Zealand, they would create significant damage to crops and cause market access issues (Worner and Gevrey 2006; Horner et al. 2016). Therefore, New Zealand has contractual arrangements in place with mass-rearing facilities in Australia that produce sterile Mediterranean and Queensland fruit flies, providing the option of eradication by the SIT in case there are early detections of outbreaks in New Zealand.

California, which is free of pest fruit flies of the genus *Anastrepha*, has, in case of outbreaks, similar stand-by arrangements in place with Guatemala, Mexico and/or Texas for the provision of sterile flies of various *Anastrepha* species. Many incipient *Anastrepha* infestations have been eradicated using the SIT -- in Arizona (1963–1967), Florida (1972), and California (1983–1984), and various times since then, including Texas (Klassen et al. 1994). Thus, USDA has been supporting these mass-rearing facilities with the understanding that the USA will receive priority for sterile flies in case of outbreaks.

## 6. CONCLUSIONS

Given the current trends of globalization, the sustained incursion of non-native invasive insect pests will continue. Also, transboundary quarantine measures will not be fully effective, even with major improvements in exclusion efforts and technologies at ports of entry to increase interceptions without affecting travel and trade (Liebhold et al. 2006). Thus, reliable surveillance, effective action plans, and rapid outbreak-eradication capacities will increasingly play an indispensable role in excluding major non-indigenous pest species (Liebhold et al. 2016).

This entails investment in effective area-wide surveillance systems for major invasive pests, especially in high-risk areas, to increase the probability of detecting incipient populations before infestations reach an unmanageable size. This will be facilitated by the development of multiple-species traps to maximize the cost-effectiveness of surveillance systems for major invasive-pest threats (Brockerhoff et al. 2013). Early detection and rapid response to outbreaks of invasive pest populations significantly reduce the cost of eradication and the direct economic losses resulting from quarantine restrictions.

At the same time, the more established an invasive pest population is, the less likely eradication will be seen by decision-makers as a viable option (and the less likely that SIT may be used, unless it is combined with the application of effective population suppression measures). Also, the potential for controversy and non-target impacts is higher, and the investment, as well as social and political commitment required to eradicate it, are higher (Myers et al. 1998; Simberloff 2008; Pluess et al. 2012; Tobin et al. 2014; Liebhold et al. 2016; Suckling et al. 2016).

Implementing this exclusion strategy will also require better communication methodologies and more information provided to the public, as well as improved approaches to engage the community (Kolopack et al. 2015), and to include the main stakeholders in the decision-making process; social tolerability will increasingly

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define which eradication tools will be allowed (Gamble et al. 2010; Liebhold et al. 2016).

The eradication of invasive pest populations is feasible in many cases (Pluess et al. 2012; Tobin et al. 2014), but environmental constraints and public resistance to "scorched earth" eradication policies and treatments, commonly accepted in the past, has been increasing over the years, in conjunction with an urbanizing human population networked via social media. Thus, in future, only selective, economically justified, and socially palatable eradication measures, that allow achieving the objective of eradication with the least disruption, will be acceptable.

The SIT is a species-specific, environment-friendly, and inverse density-dependent method that is particularly suited to eliminate incipient invasive pest populations. It combines with Allee effects, representing considerable constraint to population persistence due to the difficulty of finding a receptive mate for sexual reproduction at low population densities (Gordillo 2015; Liebhold et al. 2016; Barclay, this volume). As the cases described illustrate, it has a very positive record of eliminating small or even large outbreaks of a number of invasive insect pests, in containing their spread, or in preventing their establishment.

There is great potential for integrating the SIT in similar situations against other major invasive insect pests. However, the SIT package is often only partially or not available when new invasive-pest outbreaks occur. Therefore, it is important to develop the SIT package for the worst potential non-indigenous pests that are amenable to SIT application (NRC 2002; ISSG 2018), although decision-makers are often reluctant to divert funds towards preparing for future pest problems that have not yet arrived. Nevertheless, benefit/cost analyses generally show that preparations for incursions that enable a rapid and effective response are much cheaper than having to run large and costly eradication campaigns with uncertain success, or letting a major pest become permanently established that then requires long-term control. According to Liebhold et al (2016), the eradication of many invasive pest populations would, in hindsight, have been economically beneficial; the magnitude of the potential impacts of the pest was often greatly underestimated (Bradshaw et al. 2016).

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# CHAPTER 6.3.

# PROCEDURES FOR DECLARING PEST FREE STATUS

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## SUMMARY

Procedures are presented for declaring an area to be "pest free" following an area-wide eradication programme against a population of an insect pest. These involve two probability models to deal with null trapping results, and a growth model to help verify that pests were no longer present when control actions were stopped. The two probability models are presented for a situation in which trapping for adult insects of the pest (and/or sampling for its immature stages) is ongoing, and for which the results are all negative. The models calculate the probability of such negative results if in fact insects were present. If this probability is sufficiently low, then the hypothesis that insects are present is rejected. The models depend on knowledge of the efficiency of the traps, and also the area of attractiveness of the traps. The possibility of a rebound of an incipient but non-detectable population that remains after control measures are discontinued is considered. Using a growth model, the rate of increase of an insect population that starts from one or two insects is examined. An example is given for tsetse flies — both means and confidence limits are calculated for a period of 24 reproductive periods after control has been terminated. If insects are disease vectors, it is also suggested that the progress of the disease be monitored to detect continuing transmission. This should be done in conjunction with a disease transmission model.

## 1. INTRODUCTION

Following an area-wide programme that has attempted to eradicate an insect population from a specific area, it is important to confirm the "pest free status". The criterion of programme success used is that the pest population has been eradicated from the target area (FAO 1996), a very different concept from that of the World Health Organization (WHO) regarding species eradication at a global level (WHO 2001).

There have been several successful insect pest eradication programmes, e.g. the New World screwworm *Cochliomyia hominivorax* (Coquerel) has been eradicated from North and Central America (Galvin and Wyss 1996; Wyss 2000a, b; Klassen et al., this volume; Vargas-Terán et al., this volume), and from Libya, using the sterile insect technique (SIT) together with wound treatments and quarantine measures (FAO 1992; Lindquist et al. 1992; Krafsur 1998; Wyss 2000b; Vargas-Terán et al., this volume). Many tephritid fruit fly populations around the world have been similarly eradicated by integrating the SIT with other methods (Sproule et al. 1992; MAG/SAG 1995; Kuba et al. 1996; Reyes F. et al. 2000; Villaseñor et al. 2000; Koyama et al. 2004; Enkerlin, this volume) or the male annihilation technique (MAT) for *Bactrocera* species that respond to the strong attractant methyl eugenol (Steiner et al. 1970; Koyama et al. 1984; Hancock et al. 2000; Seewooruthun et al. 2000; Suckling et al. 2016).

If continued attempts to trap adult insects or detect immature stages after the termination of control actions are unsuccessful, this could be taken as evidence that the population has been eliminated from the target area, but with the realization that null results do not necessarily imply non-existence (Mitchell 1980; Richards and Tarry 1992; Clift and Meats 1997; Barclay and Hargrove 2005; Hargrove 2005;

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Meats 2014). Low-density populations are often very difficult to detect. For example, even when trapping the mountain pine beetle *Dendroctonus ponderosae* Hopkins using pheromones that are known to be effective, if the species is endemic and at a very low density (e.g. one infested tree per 10 hectares), it may be almost impossible to trap beetles (Bartos and Schmitz 1998; L. Safranyik, personal communication).

Perkins (1989) listed seven scientific questions pertinent to eradication, including:

> What accommodations need to be made for the limitations in the detection methods of ultra-low populations?

In interpreting null trapping (or other sampling) results, some knowledge of the detectability of the species (when using specified traps), as well as a measure of the area of attraction surrounding traps, must be available (Shelly et al. 2010). Once these measures are known, probability estimates of non-existence can be formulated from null results. An added complication arises if detectability itself is density-dependent, or if the area of attraction depends on weather or other factors.

The Food and Agriculture Organization of the United Nations (FAO) recognizes three categories of pest status: presence, absence, and transience (FAO 1999). Absence is determined by one or more of the following factors: (1) the lack of any records of its presence, (2) the action of eradication, (3) a shift in a pest's range, or (4) the interpretation of positive records as being only temporary. The three stages in the establishment of a pest free area are: (1) systems to establish freedom from pests, (2) measures to maintain freedom, and (3) checks to verify that freedom has been maintained (FAO 1996). However, since these guidelines are very general (applying to all pests — plant, animal, and microbial), they do not include methods specifically applicable to insects.

At present, insect population eradication is usually declared after the pest has not been detected for a reasonable period of time, which includes a considerable safety margin. The usual procedure is to continue control measures for several generations after the last insect was captured, then monitor the area intensively for additional generations (section 2.2.), and finally, if no more insects are detected, declare success. The New World screwworm in Costa Rica was declared eradicated about 1 year after the last fly was found (Galvin and Wyss 1996; Wyss 2000b, 2001). In Libya, sterile releases were discontinued 6 months after the last wild fly was trapped, and eradication was formally declared 8 months later (FAO 1992; Lindquist et al. 1992; Wyss 2000b). The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) was considered eradicated in Chile after trapping (with a parapheromone and food attractants) for three generations without catching any wild flies (C. Flores and J. Gonzalez, personal communication). On the island of Kume, Japan, the melon fly *Zeugodacus cucurbitae* (Coquillett) was considered eradicated 6 months after the last capture, and following examination of 70 000 fruits (Iwahashi 1977). When the population of *Glossina austeni* Newstead was eradicated using an integrated approach on the island of Unguja, Zanzibar, sterile releases were maintained for six generations (after the last wild fly was captured) before eradication was deemed complete (Vreysen et al. 2000). The oriental fruit fly *Bactrocera (papayae) dorsalis* (Hendel) was introduced into Mauritius in 1996, and,

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immediately after detection, eradication activities were begun. The last fly was trapped in May 1997, and trapping continued for 2 years, after which time eradication was declared (Seewooruthun et al. 2000). Following detection of the last wild fly, and a 4-month effort using the MAT and SIT, the oriental fruit fly was declared eradicated from the Mariana Islands (Steiner et al. 1970). One year (about six generations) after the detection of the last wild fly, the oriental fruit fly was considered eradicated from the Okinawa Islands using the MAT (Koyama et al. 1984). Subsequent to seeing the last individual, and trapping for 190 days and examining over 300 000 fruits, this same pest was judged eradicated on Amami Island (Ushio et al. 1982). In Perth, Australia, a large infestation of the Queensland fruit fly *Bactrocera tryoni* (Froggatt), after integrating the SIT, was declared eradicated 12 months after the last wild fly was trapped (Sproule et al. 1992). Using the MAT in north Queensland, Australia, against the oriental fruit fly *B. dorsalis*, area-freedom was claimed 12 months after the capture of the last fly, and after another 12 months eradication was claimed (Hancock et al. 2000).

Under the auspices of the FAO and the International Atomic Energy Agency (IAEA), a meeting was held in Vienna, Austria, in August 2003, to review the procedures for declaring areas free of tsetse flies and the trypanosomosis problem. The results of that meeting are described in section 2.1.3.

## 2. ASSESSMENT METHODOLOGIES

Three methodologies, suitable for addressing the question of the non-existence of insects in a given area, are presented. These include probability models to assess the results of trapping, a model of a rebounding population following suppression, and the use of disease transmission information in the case of pest species that are disease vectors. These methodologies are illustrated for tsetse flies *Glossina* spp. (Barclay and Hargrove 2005; Hargrove 2005).

### *2.1. Probability Models*

#### *2.1.1. Developments in Japan*

Itô (1977) apparently made the first quantitative attempt to solve the problem of declaring a species to be absent from the area of interest or at minimal density, but since it was published in Japanese, it received little attention outside of Japan. Itô (1977) recommended the use of Wald sequential sampling to determine the minimum number of samples required (such as the number of fruits) in SIT projects for fruit flies. However, Kuno (1978) pointed out that the lower limit in sequential sampling need not be considered in eradication projects, so he recommended that the sample size ($n$) be based on either the binomial distribution or the Poisson distribution, giving:

$$n = \log(\beta) / \log(1\text{-}p) \quad (1)$$

for the binomial distribution, or

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$$n = \log(\beta) / p \quad (2)$$

for the Poisson distribution, where $n$ is the sample size (the number of examined sampling units), $\beta$ is the error rate that is permissible, and $p$ is the permissible proportion of infested sampling units. The symbol "$\beta$" is usually used for this probability instead of $\alpha$, since it is related to Type II error rate rather than Type I error rate. The above sample size regulates the risk in the sense that the actual error rate is always smaller than $\beta$ if the actual proportion of infested units is larger than $p$. Equations (1) and (2) are not effective if the total number of existing sampling units for survey is limited. Hence, in a later paper, Kuno (1991) recommended another equation:

$$n = N (1 - \beta^{1/Np}) \quad (3)$$

where $N$ is the total number of sampling units. Kuno noted that the length of the sequence of zero captures increases nearly inversely with the size of the assumed $p_0$; $p_0$ is assigned to be some very small probability of pest occurrence that is deemed acceptable. Thus, for very small values of $p_0$, the required length of the sequence becomes impossibly long. For example, if $N = 50\ 000$, and the required value of $p_0 = 0.0001$, then the length of the sampling sequence yielding zero captures would need to be 30 095, even though this may be achievable if routine trapping is being done.

Yamamura and Sugimoto (1995) summarized the relation between equations (1), (2), and (3) as follows: let $Y$ be the number of infested units in the sample, $P$ be the actual proportion of infested sampling units, $N$ be the total number of sampling units, and $n$ be the sample size. Then one of the criteria used in the Japanese quarantine procedure is that

$$Pr(Y = 0|P = pt) < \beta \text{ for all } p_t \text{ satisfying } p < p_t \leq 1.$$

The probability $Pr(Y = 0|\ P = p_t)$ is most precisely expressed by a hypergeometric distribution:

$$Pr(Y = 0|P = p_t) = (N - N p_t)!\ (N - n)!\ /\ N!\ (N - n - N p_t)! \quad (4)$$

The minimum number of samples, $n$, which satisfy the above condition for a given set of $p$ and $\beta$, can be given approximately by a simple formula (Japanese Industrial Standards Committee 1956):

$$n = (N - (Np - 1)/2)\ (1 - \beta^{1/Np}) \quad (5)$$

The $n$ calculated by the above formula is slightly larger than the exact $n$ for given $\beta$, $N$ and $p$. In that sense, the above approximation is a slightly conservative approximation. We can use the more conservative approximation given by equation (3) if $p$ is sufficiently small. If the sampling proportion $n/N$ is sufficiently small, we can use another conservative approximation given by equation (1) based on the binomial distribution. If both $p$ and $n/N$ are sufficiently small, we can use a much

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more conservative approximation given by equation (2) based on the Poisson distribution.

*2.1.2. Developments in Australia*

Clift and Meats (1997), using risk management software and trapping data up to May 1997, established criteria for 99% probability of localized eradication. Except for this last report, no probabilities were presented in any of the previous examples of eradication in Australia. Most authors simply did not provide the criteria on which eradication was judged, e.g. Schwarz et al. (1989).

An operational protocol, previously established in Australia (Clift and Meats 2004), specified that eradication could be claimed three generations (based on degree-day models) plus 28 days after the last fly was trapped. If one gets negative results, normally after three generations in some programmes (Tassan et al. 1983), one rejects the null hypothesis that flies are present. However, trapping records from a *B. dorsalis* campaign indicated that when trapping at only one trap per 1.5 $km^2$, 12 weeks of successive zeros could occur when flies were still present (Clift and Meats 1997; Clift et al. 1999). In addition, if there is a residual population and it is too small to be easily trapped or detected in fruit, then it will naturally increase when control stops, and so it becomes more detectable over time (Barclay and Hargrove 2005). In insects, such as tsetse, that have a slow rate of reproduction, this requires a long period, whereas for fruit flies, that have a much higher reproductive rate, any remnant population should soon become detectable (McInnis et al. 2017). The analytical methods of Meats and Clift (2005) represent a quantitative refinement of the ad hoc code of practice previously in use.

In countries such as Australia, Chile, Mexico, and the USA, cases of the eradication of spot infestations of pests (such as the Mediterranean fruit fly) in areas normally claiming area-freedom are much more common. Such eradications are virtually routine, and are not reported in the scientific literature (Suckling et al. 2016). Codes of practice for such procedures are also unpublished, and details differ according to both the country and pest involved. Meats et al. (2003) reviewed the codes of practice current in Australia for the Mediterranean and Queensland fruit flies, examined 25 years of data from spot eradications of each species, and calculated the radii of the areas of infestation where the probability of exceeding such radii was equal to probit 9 (containing 99.9968% of probability). If zones of area-freedom are to be established for other species, similar codes will be required. Obviously, it would be desirable if there were methods, with a pre-determined degree of probability, to calculate the radius of an affected area (where area-freedom would be suspended) and the length of the no-detection period needed to reinstate area-freedom within the previously calculated radius.

Clift and Meats (2004) and Meats and Clift (2005) assessed the probability of eradication of fruit flies in Australia by computing the probability that the fly population is below a certain density; this density can be adjusted to represent the critical density for population persistence, below which eradication would occur via the Allee effect (Allee 1931; Yamanaka and Liebhold 2009), whereby the rate of population growth declines or even becomes negative when the population declines below a given threshold (the Allee threshold). The number of traps and the length of

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trapping time can then be computed knowing the maximum acceptable density, somewhat similar to the approach of Kuno (1991). The method of Meats and Clift (2005) involved a probability model using the concept of catch per trap per week, called $c_{tw}$, where $t$ is the number of traps and $w$ is the number of weeks. They defined

$$m = c_{tw}\, tw. \tag{6}$$

The zero term of the Poisson distribution was then used to calculate the probability, $P_0$, of a zero catch with time as

$$P_0 = \exp(-m) \tag{7}$$

Then, to calculate the number of weeks with zero catches to achieve a critical value of $P_0$ (e.g. 0.01, 0.001, etc.), one forms

$$w = (-\ln P_0, crit)(c_{tw}\, t) \tag{8}$$

This allows the specification of a lower catch limit, $c_{tw}$, that signifies a pest density that is below the Allee threshold; then the population can be left to decline to zero by itself without any additional control being required.

In a comprehensive review of the potentials and limitations of trap arrays for fruit fly detection, Meats (2014) examined the efficiency of detection systems, required spacing of traps, surveillance for resurgence following eradication attempts, effects of weather and insect age on trappability, and expectation of success in eradication and the ability to declare eradication. The efficiency of existing programmes in Australia and California is compared with recommendations for trap spacing. In addition, considerations of generation time, as predicted by heat accumulation and its use in predicting phenological events, are reviewed.

### *2.1.3. Results of 2003 FAO/IAEA Meeting on Tsetse Flies*

The following probability models are based on trapping (or other method of sampling) with zero results, while assuming that insects are present. The models then give the probability of a zero catch for this assumption; if the probability is sufficiently low, one can reject the hypothesis that insects are present. For simplicity, all sampling systems are referred to below as “traps”. Techniques for declaring that eradication has occurred are nearly the same as those for a declaration that an infestation has not yet occurred (Yamamura and Katsumata 1999).

Two models are presented: (1) local sampling involving one trap or one group of traps (suitable for spot infestations), and (2) area-wide sampling (suitable for an area-wide eradication programme involving either an established pest or a large outbreak). Both models involve sampling a population that is close to elimination. Either approach can be used, and the results should be rather similar; when residual population sizes are very low, the models converge (Barclay and Hargrove 2005; Feldmann et al. 2018).

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For an insect to be caught in a trap on a given day, the following conditions must be met:

- A trap must be operative in the vicinity of the insect.
- The insect must be active.
- Given the above, the insect must succeed in finding the trap and be captured by it.

*Local Sampling with One Trap.* Regarding the probability of a zero catch in each of a number of traps, consider a single trap and the "circle" (area) of attraction around it, within which the probability of catching a given insect with a given trap during one day is $\sigma$, called the detectability; the probability of not catching a given insect is $1 - \sigma$. This assumes that detectability is constant within the circle; Meats (2014) has pointed out that attractiveness of baits falls off with distance, so the detectability might better be treated as a mean over the circle of attractiveness. If there are $k$ insects in the "circle", the mean number caught per activity period is $k\sigma$. If there are $k$ insects present, and if the insects are caught independent of each other, then the conditional probability of catching no insects during an activity period (or sampling period) is:

$$p(0|k) = (1 - \sigma)^k \tag{9}$$

The probability of both zero catch and a given number of insects being in the circle is $p(0 \cap k) = p(0|k)\, f(k)$, where the symbol $\cap$ refers to "and" or conjunction (Parzen 1960), and $f(k)$ is the probability of $k$ insects being present. The conditional probability of no catch, given that there is an undetermined positive number of insects present, is:

$$p(0|k>0) = \Sigma\, p(0|k)\, f(k) = \Sigma\, (1 - \sigma)^k\, f(k), \text{ where the sum is for } k \geq 1 \tag{10}$$

This equation could be used to construct probabilities of a zero catch, assuming that there are insects present near the trap. The problem arises in stipulating a distribution for $f(k)$, called the prior distribution on $k$.

Since the choice of a particular prior distribution is arbitrary unless there is some information about it, and since the probability given the presence of one insect has an easy closed form solution (i.e. $p(0|1) = 1 - \sigma$) and is more conservative, the latter probability is used in the development below (Barclay and Hargrove 2005).

The probability of a zero catch, given that there is one insect present, is $p(0|1) = 1 - \sigma$, so the probability of a succession of $n$ zero catches on $n$ independent sampling occasions is

$$P(0) = (1 - \sigma)^n \tag{11}$$

and this is true for each trap. For example, if the hypothesis (that there are pests present) is to be rejected at the $\beta = 0.01$ level, and if $\sigma$ was 0.1, then the number of trapping days, $n$, needs to be such that $(1 - 0.1)^n \leq 0.01$. This can be found using the

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equation: $n = \log(0.01) / \log(0.9) = -2.0 / -0.0458 = 43.7 \approx 44$. More generally, the equation is:

$$n = \log(\beta) / \log(1 - \sigma) \tag{12}$$

where $\beta$ is the chosen rejection level. The base of the logarithms is immaterial, as long as both logs are of the same base. Once the rejection level has been chosen, and the value of detectability, $\sigma$, is known, the required value of $n$ can be computed easily (however, the number of trapping days should be linked to the generation time under defined climatic conditions).

*SAMPLING FRACTION OF POPULATION.* Each trap has an area of attraction such that, within that small area, the probability of catching a given insect approximates the average detectability. If the number of traps is not sufficient to cover the whole area, and therefore the sum of the areas of attraction is less than the area to be evaluated for "pest free status" (called the "assessment area"), then one of two scenarios may occur. If the pests are sufficiently mobile so that they move in and out of areas of attraction in their normal daily or weekly movements, then the detectability is simply reduced, compared with the situation in which they are in the area of attraction all of the time. Alternatively, the traps could be moved from day to day or week to week, so as to cover the assessment area; then the detectability would similarly be reduced. Assuming that every insect spends roughly the same amount of time in areas of attraction to traps, then the detectability will be reduced by the sampling fraction. If the sum of the areas of attraction to traps is a fraction $f$ of the assessment area, then the average detectability will be $\sigma f$. In that case, the criterion becomes:

$$P(0) = (1 - \sigma f)^n < \beta \tag{13}$$

and solving for $n$:

$$n = \log(\beta) / \log(1 - \sigma f) \tag{14}$$

The size of the area of attraction will be crucial to the calculation of the sampling fraction, and this area may depend on weather, since odour plumes from lures in traps will vary in size with wind speed, and may also vary with topography, season, competing natural stimuli, and surrounding vegetation.

*Area-Wide Sampling.* Another way of looking at the problem, which leads to the same kind of result, is to consider area-wide or total population sampling, covering the whole assessment area. Having selected one or more sampling systems, the next problem is to decide on the required intensity of sampling, and for what period of time, before a series of zero catches can be interpreted as indicating eradication at some specified level of probability.

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The parameters are defined as follows:

$A$ Area sampled (km$^2$), assumed to be closed to immigration and emigration (either naturally or through the maintenance of a wide-enough barrier)

$k$ Total number of surviving pest insects, assumed to be randomly distributed in $A$

$\sigma$ Trap efficiency (detectability), i.e. the conditional probability that an insect is caught by a given trap, given that there is only one trap present in a 1 km$^2$ area containing the insect, and given that the insect is active

$s$ Number of traps present in all of $A$

$n$ Number of days that each trap is operated

If there are $s$ traps in the assessment area deployed for $n$ days, and if $s$ is sufficiently small that the traps act independently of each other, then the probability ($C'(k,s,\sigma,n)$) that none of the traps catches any of the $k$ surviving insects is:

$$C'(k,s,\sigma,t) = \exp ( - s\, n\, \sigma\, k / A) \tag{15}$$

assuming that the capture probability is identical for all insects, and is independent of time $t$ (Hargrove 2003). The result of interest is the function relating to the probability, $p(0)$, of observing a sequence of zero results, if in fact insects are in the assessment area:

$$p(0) = \exp (-sn\sigma\rho) \tag{16}$$

where $\rho = k/A$ is the population density, and other symbols are as defined above. The objective is to know when a series of zero catches is sufficiently long that the null hypothesis of the existence of pests at the assumed level can be rejected. For example, for the probability of a sequence of zero catches in the presence of insects to be below 0.01, then it is required that:

$$\exp (-sn\sigma\rho) < 0.01 \tag{17}$$

from which

$$-sn\sigma\rho < \ln (0.01) = - 4.605 \tag{18}$$

One can solve for one of the variables in terms of the others that are known. For example, if $t$ is determined, $\sigma$ is known, and $\rho$ can be surmised, then

$$s > 4.605 / n\sigma\rho \tag{19}$$

When this condition is met, the required probability has been achieved. If this calculation leads to an unreasonably large number of traps being required, then a different criterion may be considered. The value of $s$ obtained from the inequality above assumes a risk level of 0.01 in concluding that no insects are present, and this is shown in Fig. 1 for various values of the number of traps used ($s$) and number of insects present ($k$).

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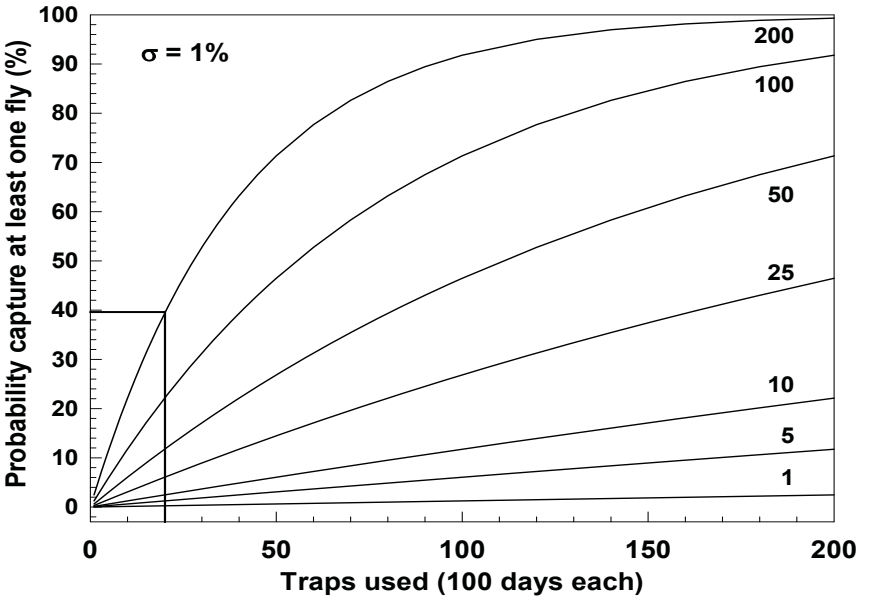



*Figure 1. Probability of catching a single tsetse fly in a small remnant population (numbers in body of figure) in an area of 8000 km$^2$ as a function of number of traps used and duration of trapping (when using a trap with efficiency of σ = 1%). (Figure from Barclay and Hargrove 2005, reproduced with permission.)*

It must be emphasized that the calculated number of traps ($s$) is independent of the size of the assessment area to be controlled (unless the area is very small). However, with a very large area, there is a greater probability that "pockets" of insects still exist (where the control treatments and/or released sterile males are locally overwhelmed), and these pockets must be identified and treated accordingly (Shiga 1991).

As a simple example of the application of the inequality (19) above, consider the insecticide aerial-spraying operation (sequential aerosol technique (SAT)) against tsetse flies in Botswana (Okavango Delta, about 16 000 km$^2$) (Allsopp and Phillemon-Motsu 2002; Tsetse News 2002), where apparently the last fly was caught on 30 August 2002, although very few traps had been installed in the Delta. Possibly the population of *Glossina morsitans centralis* Machado was actually eradicated in the SAT-treated area. However, suppose that 100 insects survived the sprays, what chance would there be of catching one of them? If the insects were distributed at random, 10 traps were deployed for about 3 months (90 days), $\sigma$ = 0.01, and using inequality (19), the probability of catching at least one insect is $C(k,s,n) \approx 0.06$. Thus, with this low level of trapping, there is a 94% chance that detection of the presence of insects will fail. To be 90% certain that at least one insect is detected (given that 100 insects were present), the application of inequality 19 suggests that more than 400 traps must be deployed for 3 months. To be 99% certain, this figure rises to more than 800 traps. Note that these figures assume a

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rather even distribution of traps. Deploying traps for a longer period, such as 1 year to include all seasons, may permit a reduction in the number of traps. Spreadsheets, that incorporate a variable number of trapping days, are available to calculate the minimum number of traps required. (It is important to be aware of the possible existence of insects in places that were avoided or ignored by field personnel, substantially reducing the chances of detecting a residual population.)

In inequality 19, $\rho$ must be surmised; the size of any residual population is not known. To some extent, this uncertainty is balanced by the fact that, if there is a residual population and it is too small to be easily trapped, naturally over time it will increase since it is no longer under control, and it will become more detectable; this is the subject of the next section.

### *2.2. Incipient Non-Detectable Populations*

If control actions have reduced the pest population until very few insects are present, so few that they cannot be detected by trapping, then when control is terminated natural reproduction will cause the population to increase if the population is not below the Allee threshold. How long will it take for this small population to increase to a detectable level? (It is assumed that surveillance trapping will continue or be increased (detection trapping/sampling) until there is a decision regarding the "pest free status" of the area.) The population can be modelled by a simple equation:

$$N_{t+1} = \lambda N_t \tag{20}$$

where $\lambda$ is the rate of increase each generation, and $N_t$ is the population size at generation $t$. Starting with a very small initial population of size $N_0$ (following termination of the eradication activity), the size of the population $t$ generations later would be $N_t = N_0\lambda^t$. When $t$ is large enough for the population to have become easily detectable, but continued trapping still yields no insects, then a declaration of "pest free status" can be made. In calculating this critical value of $t$, allowance must be made for dormant or non-growing periods when equation (20) above does not apply. In addition, the time covered by $t$ generations must be adjusted based on available pest-specific degree-day models to make allowance for slow-growing periods during unfavourable climatic conditions. Furthermore, before such a declaration could be made, the allowance of an adequate time-buffer is needed to permit sufficient time to elapse for the population to increase to perhaps 10 times the minimal detectable level. Since equation (20) is deterministic, and events in nature usually involve random elements, reasonable lower limits of $\lambda$ should be used, not mean values (often used in calculations of ordinary population growth). This is illustrated below by examples from fruit flies and tsetse flies (which have much lower rates of increase than fruit flies or most other insect pests).

#### *2.2.1. Observed Populations of* Bactrocera dorsalis *in an Eradication Context*

Clift and Meats (1997) described the trap catches obtained during a population resurgence of *B. dorsalis* part-way through an eradication programme in tropical northern Queensland, Australia. In discussing localized extinction and reinfestation

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of various areas within the pest-quarantine area, they noted intervals of up to 12 weeks between catches, and with the data available were unable to define localized extinction. After a declaration of eradication in 1999, subsequent data indicated that at least 16 weeks (favourable for the development of a fruit fly population) were needed to attain confidence that there were no flies in the area. Simulation data for 16 weeks were consistent with this result (Clift and Meats 2004). In practice, after 12 months of no catches under continued control, area-freedom was claimed, and after another 12 months with no catches and no control procedures applied, eradication was claimed. Throughout this interval, a 1 km grid of traps, with efficiency of at least 10%, was maintained over an 8000 $km^2$ area, and traps were checked at least weekly.

*2.2.2. Monte-Carlo Simulation of Population Growth of Tsetse Flies*

Females give live birth to a larva about every 9 days (Hargrove 1994), which then pupates, and about 30 days later an adult emerges (Phelps and Burrows 1969a, b). Assuming this rate of reproduction, and the least detectable population (one gravid female), a Monte-Carlo simulation was performed to compute the mean population numbers after each of 24 reproductive periods (i.e. a simulation length of about 1 year, with the first period including the pupal maturation and the rest following at about 2-week intervals), assuming that gender determination of larvae is a random phenomenon, and that at meiosis males and females are equally likely. At each reproductive period in this simulation, a decision was made (on the basis of a random number) for each adult gravid female (whether her offspring was to be male or female), and then the new numbers were tallied. This was repeated for 10 000 24-reproductive growth periods.

Mean values for the 10 000 simulation runs were calculated for each of the 24 reproductive periods. A cumulative frequency distribution of population sizes was computed for each reproductive period, and the lowest 1% of the 10 000 runs was noted. Population values bounding these proportions for the $24^{th}$ reproductive period are shown in Table 1 (the bound for the proportion 0.01 is the upper bound of the first percentile). Four mortality values for adult flies were used (daily mortality of 0, 0.5, 1, and 2 %). If these are accumulated over a 2-week period, they translate into 14-day survivorships of 1.00, 0.93, 0.87, and 0.75, respectively, and these were used in the Monte-Carlo simulation. Since tsetse has a reproductive rate of half a female every 2 weeks (or 10 days, etc.), the fertility rate for 2 weeks is 0.5. Hargrove (1988) suggested that a tsetse population could not sustain 4%-added daily mortality regardless of the amount of density-dependence in its natural mortality.

Table 1 shows that, with 1% daily mortality, the mean number of adult females (after 24 reproductive periods) resulting from one gravid female reached almost 711, and one would expect that most of them would still be within some local common area, if it were suitable. Since these population numbers could vary widely, effective lower limits of the population were established (for the numbers below which it is expected that the total female population would be in 1 out of 100 cases) (Table 1). At this point, the questions become, “What is the minimum detectable density of flies?” and “What is the maximal tolerable error level?” Once these questions are answered, an appropriate waiting period can be determined.

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The proportions of the 10 000 simulation runs that by chance ended in population extinction are shown in Table 2 (for the conditions used). Most extinctions occurred during the first five reproductive periods.

*Table 1. Number of tsetse flies (means and 1% confidence lower values) resulting from 10 000 simulated growing populations after 24 reproductive periods starting with one gravid female or with one male and one female*

| Initial population | | Survivorship per reproductive period | | | |
|---|---|---|---|---|---|
| | | 1.00 | 0.93 | 0.87 | 0.75 |
| One gravid female | Mean | 7480 | 2176 | 711 | 65 |
| | Below 1% | 830 | 1 | 1 | 1 |
| One male and one female | Mean | 11 211 | 3397 | 1157 | 112 |
| | Below 1% | 907 | 217 | 51 | 2 |

*Table 2. Proportions of 10 000 simulated growing tsetse populations that went extinct during 24 reproductive periods (table from Barclay and Hargrove 2005, reproduced with permission)*

| Initial population | Survivorship per reproductive period | | | |
|---|---|---|---|---|
| | 1.00 | 0.93 | 0.87 | 0.75 |
| One gravid female | 0.00 | 0.14 | 0.26 | 0.52 |
| One female, one male | 0.00 | 0.08 | 0.16 | 0.36 |
| Two gravid females | 0.00 | 0.01 | 0.04 | 0.18 |

### *2.3. Monitoring Vectored Infections*

If pests are difficult or impossible to detect at very low density, but nevertheless act as vectors of a disease, then, in addition to surveillance trapping, one supplementary method of detection is to use antigen tests and assess if the prevalence of vectored infections is decreasing (Delafosse et al. 1996). An epidemiological model using a zero-biting frequency could be applied to test the hypothesis that insects are absent (but bites from non-infected insects would not be monitored by this procedure (Dyck et al. 2000)). The models of Rogers (1988) or Baker (1992) could be used for tsetse, assuming that the insect density is zero. Infection rates in hosts should decrease with time. Also, if insects are truly absent, new host recruits should not be infected. A similar approach was used in the UK to decide if a warble fly (*Hypoderma* sp.) had been eradicated. In addition to trapping warble flies, blood sera from cattle were examined for antigens to the warble fly (Richards and Tarry 1992), and actually

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these blood antigens were detectable for several years after the last capture of a warble fly in a trap.

However, as this method has problems associated with it, it should usually be used only to provide additional information. The vector population may be too low for effective transmission, in which case the disease would decline even in the presence of a small vector population. On the other hand, diseases that do not kill the host may linger for a long period in the population after the vector is no longer present. Also, in antigen tests, false positives and false negatives are possible, and disease symptoms may be present without the real presence of the disease (Tazé and Gruvel 1978), giving the impression that eradication of the vector is not yet complete. Nevertheless, if the vector species is difficult or impossible to trap, this method may be the only one available.

### *2.4. Summary of Requirements for Models*

- Select sampling methods that will sample all individuals and stages of the population.
- Determine the detectability of each sampling method; to obtain good estimates, this is best done before the eradication programme begins.
- Determine the range of attraction for each trap and other sampling method used.
- Determine the fraction of the assessment area that is actually being sampled (product of the number of traps and the area of attraction of each trap, divided by the total assessment area).

## 3. ACTIVITIES BEFORE, DURING, AND AFTER ERADICATION PROCEDURES

### *3.1. Pre-Eradication Activities*

Before embarking on an eradication programme for any insect pest, a variety of actions must be taken and information collected. These include the acquisition of scientific background information, and also the creation of a plan for these activities. This information will be of value for monitoring and surveillance activities during, and subsequent to, the eradication programme. At least one, and preferably several, sampling techniques suitable for each of the pest species must be available (Itô et al., this volume; Vreysen, this volume). Costs and logistic difficulties associated with each trap and other sampling method should be known.

#### *3.1.1. Data Requirements and Complicating Factors*

*Choice of Risk Level.* It is impossible to conclude with complete certainty that a population has been eradicated. The best that can be achieved is to state that the probability, that observed zero or null trapping/sampling results are consistent with the presence of insects, is sufficiently low to reject the hypothesis of insects being

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present. For this hypothesis-testing approach, a level of probability (the $\beta$ or type II error level) must be specified; thus, when the probability of null results from trapping falls below this level, the hypothesis that insects are present will be rejected. The choice of $\beta$ is arbitrary, as it is in ordinary science where the rejection level, α, is usually 0.05. However, if human and animal lives, or a large investment in an eradication programme, are at stake, this level is probably too liberal. On the other hand, a lower level of $\beta$ means that more trapping is needed to satisfy the probability requirement. Thus, it is recommended that $\beta$ be 0.01, and this value is used below; Kuno (1991) also used this level. Kuno (1978) pointed out that null hypotheses are usually framed in terms of absence, rather than presence. Thus, he used a type II error with level $\beta$, the convention used by Kuno (1991) and Yamamura and Sugimoto (1995).

*Sampling Methods.* Different sampling methods, that sample different portions of a population, are often applied in area-wide integrated pest management (AW-IPM) programmes (Vreysen, this volume). Insects with free-living immature stages will usually require sampling methods different from those applicable to mature individuals, and hungry insects may be attracted to lures that are different from those that attract sexually active or ovipositing individuals (Jang et al. 1999). Traps that minimize the capture of sterile males, and instead capture mainly females, are a special advantage in the case of male-only sterile releases (Katsoyannos et al. 1999). In attempting to sample a sparse (or perhaps non-existent) population, careful planning is needed to ensure that all portions of the population receive attention. If there are portions of a population that cannot be trapped or sampled otherwise, then, before applying the probability models to them, sufficient time must be given for them to become responsive to a trap. Given the propensity of insects to congregate in certain areas, detection trapping needs to be done throughout the assessment area that will eventually be designated "pest free". Any information about the location of aggregated insects, times of greatest activity, and relative efficiency of various trap types and other sampling methods should be utilized to improve the chances of detecting insects that are at very low densities. In addition, to cover all life stages and physiological states, all effective trap types and other sampling methods should be utilized.

*Detectability.* Insect detectability (σ) can be estimated using a variety of methods (Thompson 2002); one method uses mark-recapture techniques (Shelly et al. 2010; Itô et al., this volume). Before an eradication programme commences, this should be done for each trap type used. Detectability can be estimated by the ratio of the number of marked individuals in the second sample (r) divided by the total number of marked individuals (m) released in the first sample:

$$\sigma = r / m \tag{21}$$

and $\sigma$ has an approximate variance given by

$$\mathrm{Var}(\sigma) = (n / mP)\,(1 - m/P)\,(P - n) / (P - 1) \tag{22}$$

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in which $P$ is either the known or estimated total population size (Thompson 2002). The standard error can be calculated from the variance. A further complication is that detectability may not be constant, but density-dependent. In the probabilistic approach outlined above, accurate measurement of detectability is of crucial importance; the way that detectability varies with density must be determined. If detectability declines at low density, then even estimating it presents a sampling problem since sparse captures will give a poor estimate of detectability. As an example of detectabilities that may be encountered in some pests, Table 3 shows the approximate estimated mean efficiencies for two tsetse species. The results in Table 3 underline the importance of selecting, for each pest species, the appropriate sampling systems, and of providing at least approximate estimates of the efficiency of each system that is used. If trap efficiency is unknown, it is necessary to conduct studies that estimate this parameter; only then can one use the technique to make defensible statements about the probability of an area being free of a pest species.

*Table 3. Estimates of probabilities (converted to percentages) of catching* Glossina morsitans morsitans *Westwood and* G. pallidipes *Austen using a variety of stationary and mobile baits (capture probabilities in this table for stationary baits apply to the probability for 1 day for flies in a 1 km² neighbourhood of the sampling device; for mobile baits the system may sample more than 1 km², but the time frame is the same (Vale (1974a, b) and Flint (1985) described the various sampling systems); table from Barclay and Hargrove 2005, reproduced with permission)*

| Baits | *Glossina m. morsitans* | | *Glossina pallidipes* | |
|---|---|---|---|---|
| | Males | Females | Males | Females |
| Mobile baits | | | | |
| Standard fly-round | 4.0 | 0.3 | 0.2 | c. 0.1 |
| Ox fly-round | 4.0 | 0.5 | 0.8 | 0.2 |
| Land Rover (electric net) | 12.3 | 2.4 | 1.5 | 0.7 |
| Decoy (electric net) | 5.6 | c. 0.1 | < 0.1 | < 0.1 |
| Trolley (electric net) | 9.5 | 2.1 | 3.9 | 1.4 |
| Stationary baits | | | | |
| Stationary ox | c. 0.1 | < 0.1 | 0.2 | 0.2 |
| Odour-baited F3 trap | c. 0.1 | c. 0.1 | 1.0 | 1.0 |
| Odour-baited electric net | c. 0.1 | c. 0.1 | 1.9 | 2.0 |

*Range of Trap Attraction.* To apply the concept of detectability, it is important to define the area in which a trap will attract insects. This area will differ for each trap type, and therefore each type must be estimated independently, and perhaps also in different seasons and weather conditions. If there are areas not covered by a trap,

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then the traps should be moved around so that the total area is susceptible to trapping.

*Problem of Immigration*. Unless formidable natural barriers to movement exist, most insects will eventually move into a favourable habitat. Therefore, eradication is possible only if such barriers exist or eradication programmes have long-term commitments to address whole populations or treat large eradication areas sequentially (Wyss 2000a; Hendrichs, Vreysen et al., this volume). Even with barriers, such as large lakes, oceans, mountain ranges, forests, large tracts of grassland, etc., invasions may still occur occasionally as a result of phoresy, strong winds, persistent flying, etc. If trapping is not carried out systematically and at sufficient intensity, it may be difficult to distinguish between a reinvasion and an incipient population that went undetected, unless population genetics methods are used (Krafsur and Ouma, this volume). Indeed, to verify the eradication of the oriental fruit fly and melon fly in the Mariana Islands, trapping continued for several years; several reintroductions were detected (Mitchell 1980). (In this case, another fly species that was also attracted to the traps verified the traps' attractiveness.)

*Unequal Propensity to be Trapped.* There is genetic variability in virtually all biological characteristics. Many insects exhibit variability in their attraction to pheromones and other natural attractants (Yaninek and Geraud 1989; Clearwater et al. 1991; Whittle et al. 1991). If eradication is attempted using only traps with odour attractants, there is a possibility that insects with a lower propensity to be trapped will eventually predominate (Barclay 1990, 1996; Shelly 1997). As the population declines, ultimately it could form a residual population that would not be detected by further trapping, and from which a rebound could occur. To avoid this problem, eradication should follow an integrated approach combining various control tactics, and assessment of the status of the population should deploy simultaneously several methods of sampling.

### *3.1.2. Characteristics of Targeted Pest Species*

- *Population density.* Population density is important in assessing both the control method and the progress towards eradication, and in determining the detectability of the trap types and possibly the effects of density on detectability.
- *Reproductive capacity.* In population growth and ease of control, the following parameters are important: fertility rate, generation length, number of reproductive periods per individual, number of generations per year, probability of finding a mate at low density (the Allee effect), and density-dependence of fertility.
- *Mortality.* Age-specific and stage-specific mortality, and density-dependence of mortality, are important in assessing both the method and difficulty of eradication, and also the speed of rebound from a small population after an attempted eradication or a pest reintroduction. Mortality is also important in assessing the probability of a small population becoming extinct by chance.

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- *Population movement.* Dispersal rate and distance, probability of exotic introductions, home range or territoriality, and density-dependence of movement are important in determining the size of sampling areas and the area to be covered with monitoring traps. Individuals may move from a suboptimal habitat into areas of known concentration that have been eradicated.
- *Periods of inactivity.* Certain portions of the population may be inactive and therefore cannot be trapped or otherwise sampled. Periods of inactivity are important in determining how long each trap type has effectively been trapping flies, and thus how many sampling periods may be counted in determining the probability that no insects are present.
- *Spatial distribution patterns.* Knowledge of spatial distribution is important in placing the control effort and simultaneous monitoring in the most effective positions. The distribution of the pest population within the targeted area, with particular regard to terrain, vegetation, and other features likely to affect distribution and density, should be ascertained. This information is important in case it is unnecessary or undesirable to uniformly apply control actions over the whole target area.

#### *3.1.3. Characteristics of Control Area*

- *Nature of terrain.* The topography, and availability and quality of roads, will determine the ease and cost of control and monitoring, and the logistics of trap location and visitation.
- *Boundaries, size of eradication areas, and barriers to reinvasion*. To prevent reinvasion, these must be known before eradication activities proceed.
- *Vegetation patterns.* These will affect the monitoring of insect density and distribution in suitable habitats.
- *Host availability.* This applies to host plant density for plant pests, or host animals for livestock or human pests.
- *Temperature and precipitation regimes.* Temperature will affect the speed of pupal development, influencing both the reproductive rate and the time required to ensure that no relict population exists after an eradication attempt, and precipitation regimes will affect the seasonal changes in vegetation patterns and also the availability of breeding habitats of insects such as mosquitoes.

### *3.2. Pest Control Phase: Suppression*

If the target insect is detected after eradication measures have stopped, delimitation trapping and more traps must be deployed immediately in the area where the insect was caught, and further control measures initiated. If, on continued monitoring, more insects are caught, control treatments should be continued (Mangan and Bouyer, this volume).

By collecting field data before and during control operations, the success of the control treatment can be assessed (Itô et al., this volume; Vreysen, this volume). As the density of the target population declines, the probability of a positive catch decreases. Simulation studies for tephritid fruit flies indicated that at very low

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population levels (capturing 0.001 flies per trap per week), consecutive zero catches for 16 weeks occurred in at least 5% of simulation runs (Clift et al. 1999).

However, given that the trap grid includes all the areas where the insects could be present, any relict population will increase and hence be detected eventually (McInnis et al. 2017). In the case of tephritid fruit flies, this occurs after three generations (Lance and Gates 1994). Trapping records from *B. dorsalis* showed that 12 weeks of successive zero catches occurred, even though wild insects were still present (Clift and Meats 1997; Clift et al. 1999). Often this represents a trade-off; it is not practical to have an optimal trapping grid of, for example, 400 m throughout the assessment area, but if the existing 1 km grid is maintained, some insects may go undetected until numbers build up to a detectable level.

### *3.3. Post-Eradication: Detection Trapping*

The intensity of surveillance or detection trapping should be highest after control ceases (Hendrichs, Vreysen et al., this volume; Vreysen, this volume). Immediately following an eradication attempt, surveillance traps must be increased throughout the target area. These surveillance traps should be chosen for their efficiency in detecting the target species at low populations (Lance and Gates 1994; Katsoyannos et al. 1999; Papadopoulos et al. 2001). The trapping time needs to be sufficient to conform to the probability model, and allow the eventual probability of occurrence of any insect in the trapping area to be lower than the chosen risk level, e.g. a probability of 0.01 of being wrong.

Vreysen and Van der Vloedt (1992) described a sensitive biological method to determine whether or not eradication has been achieved. This consists of the release of virgin sterile female flies (female sentinels), and their recapture and dissection, to determine if, while in the field, they became inseminated by wild males.

It should be recognized that remnant insects might still exist in areas where control actions were less effective, e.g. sterile insects that did not reach narrow canyons or insecticidal sprays that did not penetrate dense vegetation. If there is a way to identify such potential problem areas before eradication activities start, then particular attention must be given to these areas.

### *3.4. Evaluation Phases*

The first evaluation phase would normally be the calculation of the probability of insects being present given a sequence of null trapping results while control actions continue. The calculation can be done using any of the probability models, either on a trap-by-trap basis (most useful for spot infestations), in which all traps must satisfy the risk criterion, or on an area-wide basis (more useful for most programmes dealing with established pests), which gives one probability. When this probability becomes small enough, then the conclusion is reached that no insects are present at that trap site.

If, after terminating control actions, the number of insects remaining is extremely low, such as one insect in 10 or 100 $km^2$, the small number of traps indicated by the second probability model might not catch any of the remaining insects, simply

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because the insects never come into contact with the traps (they are too widely separated). In this case, either more traps must be deployed, or they should be moved around to ensure that for some time they are located in the vicinity of the insects. Alternatively, the SIT may be continued for some time as insurance, as in the eradication of *G. austeni* in Zanzibar (Vreysen et al. 2000).

The second phase involves stopping control actions but continuing detection trapping and waiting for an appropriate period (its length dictated by the population growth model (equation (20)) to assess the possibility of resurgence of a small remnant population. Generally, before declaring "pest free status", it would be advisable to wait long enough to allow the potential small population to grow to at least 10 times the minimum detectable level. If continued trapping proceeds without catching any insects, confidence in the eradication procedure will increase, either because elimination has succeeded, or an increase in the small remnant population would make detection easier.

In addition, there may be species for which, at present, no efficient trap exists. In this case, the trapping time would be unrealistically long or may even be incalculable, and there is little alternative except to wait (while still trapping) and see if a population rebound occurs during the minimal period. In such cases, a supportive technique proposed by Vreysen and Van der Vloedt (1992) is to release only virgin sterile females and, after recapture, assess if any of them had mated with wild males.

In both phases, it is advisable to monitor the progress of any diseases vectored by the insect pest, possibly using sentinel animals, trap trees, or other highly attractive features in highly suitable pest habitats. This is especially important in cases where detection trapping is very inefficient.

### *3.5. Criteria for Declaration of "Freedom from Pests"*

The criteria for making a declaration of eradication involve the above two phases of monitoring, and both should be satisfied before making a declaration. In the first phase, a series of zero results will have been obtained from surveillance traps (while still continuing control actions). From these results, the probability of such a sequence is calculated in accordance with one of the two probability models. If this probability is sufficiently low (below 0.01), then control activity can be stopped but surveillance trapping must continue. If the calculation results in an unreasonably large number of traps, then only the second phase of the criterion might be required.

The second phase involves calculating the minimal expected size of a population resulting from the rebound of a remnant population after a period of time. Calculating the population growth rate for the species is done using an appropriate population growth equation, such as the one in equation (20), and waiting until the expected population is at least 10 times the size of the minimally detectable population, assuming that a rebound is occurring. When both phases pass the numerical test, then eradication may be declared.

Throughout both phases, any diseases vectored by the pest should be monitored. By observing the presence or absence of new infections, especially in young hosts, the existence of continuing disease transmission can be ascertained (section 2.3.).

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As an illustration, the practical implementation of both phases has occurred in Australia, where experience in eradicating incursions of both endemic and exotic tephritid fruit flies has accumulated for over 50 years (Madge et al. 1997; Hancock et al. 2000). If a fruit fly species is introduced (usually in infested fruit) into an area that was free of that species, the young adults will disperse from the point of introduction, and could take from 1 or 2 days to several weeks to mature, mate, and infest more fruit. Dispersal can happen during and after the pre-maturation period. In a fly free area, an invading group of flies will disperse into a mate free void, so that only the few that stay around the origin will be at a sufficiently high density to encounter each other and breed (Meats 1998a, b). However, once mated, a female can disperse any distance that her lifetime permits, and spread the infestation as a new generation. Thus, we can expect that the occurrence of adults in a usually fly free zone would be clustered around the origin, and that the occurrence of larvae would be even more clustered. Transport of infested fruit is often the main problem.

In the case of the Mediterranean fruit fly, catching one or more male insects in a male-targeted trap indicates that supplementary traps (preferably baited with food lures attractive to females (Katsoyannos el al. 1999)) should be set up around it (CDFA 1999). In addition, a search for larvae is made within the array of supplementary traps. A catch of three male insects in the same or adjacent traps within 14 days, a catch of one female, or the detection of larvae in fruit, indicate the beginning of an outbreak and the need for the release of sterile insects within a suitable radius of probable movement within the time elapsed since detection. Also, the localized movement of soil or fruit leaving that area has to be prohibited, and a wider "suspension zone" imposed. A formula is then applied to establish criteria for the reinstatement of "area-freedom" status, involving a period free of both control measures and fly detection. This period is equivalent effectively to 12 weeks, or the length of one generation plus 28 days, whichever is longer (Tassan et al. 1983; Madge et al. 1997; Hancock et al. 2000).

### *3.6. Quarantine Issues*

For agricultural pests that are transported in produce, it is feasible to impose quarantine on fruits, vegetables, etc. entering an area where a pest has been eradicated. Such quarantine would not cause financial hardship to local growers who benefit from the absence of the pest, although the quarantine process itself might be expensive. For pests of veterinary importance, it is advisable to establish a programme of quarantine and testing of hosts that are imported from infested areas. For pests of medical importance, humans are often the host and quarantine is not feasible. Therefore, a medical history should be required of people travelling to areas in which a disease has been eradicated; luggage should also be inspected to prevent the unintentional introduction of exotic pests.

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# CHAPTER 7.1.

# IMPACT OF SCREWWORM ERADICATION PROGRAMMES USING THE STERILE INSECT TECHNIQUE

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## SUMMARY

The use of the sterile insect technique (SIT) in New World screwworm *Cochliomyia hominivorax* (Coquerel) eradication programmes has been successfully demonstrated. As a result of a 55-year area-wide campaign, suppression and eradication have been achieved in the USA, Mexico, Belize, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, Panama north of the Canal, some Caribbean Islands, and the outbreak in Libya, North Africa. The humans, livestock, and wildlife in these countries are now free of this dangerous pest. It has been estimated that the annual producer benefits are: USA – USD 896 million, Mexico – USD 329 million, and Central America – USD 88 million. In Libya, the estimated benefit/cost ratio was 5:1 in the infested zone, and 10:1 in the whole country; in addition, the pest never became established in the African and Mediterranean regions. If the New World screwworm were eradicated in South America, it has been estimated that each year about USD 3600 million would be saved. Small-scale field trials have confirmed that the SIT would also be effective for the area-wide control of the Old World screwworm *Chrysomya bezziana* (Villeneuve). Australia has developed extensive preparedness planning in case of potential screwworm outbreaks, which includes the application of the SIT to maintain its screwworm-free status.

## 1. INTRODUCTION

The application of the sterile insect technique (SIT), as part of an area-wide integrated pest management (AW-IPM) (Lindquist 2000) approach for the suppression and eradication of the New World screwworm C*ochliomyia hominivorax* (Coquerel), has been comprehensively demonstrated (Klassen et al., this volume). The similar biology of the New World screwworm and the Old World screwworm C*hrysomya bezziana* (Villeneuve) indicates that the SIT should also be effective against the Old World screwworm. Field trials in Papua New Guinea provided strong indications that the SIT would be effective in suppressing the Old World screwworm (Spradbery et al. 1989; Spradbery 1990), and later studies in Malaysia validated the SIT for this screwworm species (Mahon 2002).

Today, animal production is a high priority in world agriculture. There is an increasing demand for meat, dairy and egg production — major sources of animal protein for the world's growing population. To satisfy this demand, a diversity of livestock production systems is found in the different continents, including keeping cattle, buffaloes, sheep, goats, hogs, and poultry on traditional smallholder farms, and in extensive grazing or in more intensive systems, depending on the local circumstances.

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In developing countries, animal production makes a major contribution to local and national food supplies. This production provides food security, cash income to a large number of rural people, and benefits to the whole economy. Commercial livestock-keeping increases total farm produce and income, provides year-round employment, and reduces the investment risk of raising livestock. Income from livestock products provides funds to purchase additional means to improve crops, or for other farm investment. Livestock production enhances the economic viability and sustainability of the farming system (FAO 1992).

Predictions made by the International Food Policy Research Institute (IFPRI), International Livestock Research Institute (ILRI), and Food and Agriculture Organization of the United Nations (FAO) suggest that, between 1993 and 2020, total world meat consumption will double from 180 to 300 million tonnes, and milk production will increase from 400 to 650 million tonnes (Delgado et al. 1999). To satisfy this growing demand, the world must find mechanisms to develop greater production efficiencies without damaging the already stressed environment. Part of this productivity increase can be realized through improvements in animal health.

Animal diseases affect the livestock sector directly through mortality, reduced fertility, and loss of weight, and together with other factors have chronic debilitating effects on livestock and their production. This results in inefficient utilization of scarce resources. Both ectoparasitic and endoparasitic diseases are recognized as major factors limiting production. The cumulative effect of parasitic diseases is perhaps a greater cause of economic losses than that of any other disease.

Myiasis is caused by an infestation of a living vertebrate's tissue or fluids by larvae (maggots) of flies (Diptera). Even minor infestations cause annoyance to animals, disrupting normal habits including feeding and resting. In some situations there is loss of milk, meat or wool production, or in the value of hides.

There are at least 20 species of flies responsible for myiasis, feeding specifically on living animal tissues to complete the larval stage of the life cycle (James 1947). Two of the most important obligatory parasitic myiasis flies are the New World screwworm and the Old World screwworm.

The New World screwworm was first described in 1857, infesting humans on Devil's Island in French Guinea (Coquerel 1858). In the Western Hemisphere's tropical and subtropical regions, the New World screwworm is one of the most damaging insect parasites of livestock. It alone represents economic losses each year of hundreds of millions of dollars (USD) (section 2.3.). Losses result not only from direct reduction in productivity due to sickness and death, but also from the labour and insecticide costs incurred by continuously having to inspect and treat wounds. In endemic areas the annual cost of controlling New World screwworm myiases in domestic animals was estimated at USD 4.82–10.71 per head (Rawlins 1985). These flesh-eating larvae also represent a serious human-health problem (Reichard et al. 1992; Vargas-Terán 2002a; Wyss 2002a).

Gravid female flies are attracted to wounds, even those as small as a tick bite. Eggs are laid in, and around the border of, such wounds. After the eggs hatch, the larvae begin feeding on the live body tissue. As the maggots feed, they enlarge the wound, making it attractive for other female flies to oviposit and also susceptible to secondary infection. Without treatment, it is common for the animal to die.

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The New World screwworm has a high reproductive rate. Each female can lay several clutches of up to 400 eggs each. Under optimum conditions, a generation or life cycle can be as short as 3 weeks. Before suppression and eradication programmes commenced, the New World screwworm occurred naturally in subtropical and tropical North America, Central America, the Caribbean, and tropical and subtropical South America. Unintentionally, through animal movement, it spread to the USA, where it became established (FAO 1989); in the 1930s screwworms were present in the south-eastern United States (USDA/APHIS 2014).

The Old World screwworm causes myiases in Africa, Arabia, the Persian Gulf (Oman, Bahrain, Iraq, Iran), India, and South-East Asia (Spradbery 2002a). The life cycles of the Old World and New World screwworms are very similar, lasting about 21 days, and these species are a good example of co-evolution. However, the Old World screwworm is smaller than the New World screwworm, and the females are less fecund, producing egg clutches of 190–250 eggs.

The incidence and severity of the myiases depend on local conditions: livestock distribution and density, wildlife populations and their migratory habits, human population density, and the effectiveness of public health services. However, most important are the climatic conditions. Screwworm populations vary during the year, being most abundant in the hot and humid season. However, in the Middle East, the Old World screwworm is more abundant during the cooler months.

## 2. DIRECT AND INDIRECT BENEFITS OF NEW WORLD SCREWWORM ERADICATION

### *2.1. Benefits of Eradication in North America and North Africa*

The main direct beneficiaries of the elimination of the New World screwworm are livestock producers. However, direct and indirect benefits result for the community as a whole, through the increased availability of locally produced livestock and dairy products, reduced deficiencies caused by a shortage of meat and milk, and the increased availability of draught animals and manure. At a national level, economic benefits arise due to better-integrated agriculture and livestock production, and reduced dependency on food imports. There may also be public health benefits.

In North America, the economics of New World screwworm eradication programmes has been very positive, in spite of the high investment cost over the ca. 55 years of the programme (about USD 1300 million):

- USA: Cost to the United States Department of Agriculture (USDA) in 1958–1986 was about USD 650 million, in 2005 dollars (cost to producers and state governments not included) (Meyer 1994; J. H. Wyss, personal communication)
- Mexico: USD 413.5 million (FAO 2005)
- Central America: USD 268.4 million (Wyss 2002).

Estimates of annual producer benefits show the very large economic benefits that are accruing (Wyss 2000, 2002a), totalling USD 1300 million:

- USA: USD 896.1 million
- Mexico: USD 328.6 million
- Central America: USD 87.8 million.

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Initially, the Libyan campaign was estimated to cost about USD 117.5 million (supported by a multidonor fund), but it was completed at ca. USD 80 million. This fund was established by the governments of Australia, Austria, Belgium, Finland, France, Germany, Ireland, Italy, Luxembourg, Netherlands, Spain, Sweden, UK, and USA, and institutions such as the African Development Bank, European Economic Community, International Fund for Agricultural Development (IFAD), Islamic Development Bank, Organization of the Petroleum Exporting Countries (OPEC) Fund, and the World Wildlife Fund. An independent economic appraisal showed that the programme was a remarkably profitable investment, with benefit/cost ratios of 5:1 in the infested zone and 10:1 in the whole of Libya (Grindle 1991; FAO 1992; Vargas-Terán 2002b).

Screwworms still cause significant human morbidity and mortality in the tropical regions of South America and the Caribbean, as well as dramatic effects on mammalian wildlife. It is estimated that, throughout the Americas, about 330 million people reside in New World screwworm-endemic areas. Where the pest is still endemic (in the non-eradicated areas), in most countries the human disease has been brought under control through strict medical surveillance and treatment, but where surveillance is relaxed it threatens to develop into epidemic proportions. For example, before screwworm eradication in El Salvador, humans were found to be the third-most affected species (Reichard et al. 1992), and eradication relieved everyone of the personal risk of myiasis.

### *2.2. Importance of Livestock in South America*

Livestock production in South America is based predominantly on medium- and small-sized farms raising small numbers of a variety of animal species. These animals are used for family consumption, draught power, and some for sale. However, there is also an industrial commercial sector, which is totally market-oriented, and based primarily on cattle exports. Since the general economic situation is making remarkable improvements, this sector is growing very rapidly. Therefore, South American animal agriculture is developing in a complex and dynamic environment. Livestock and human populations are shown in Table 1.

### *2.3. Potential Economic Significance of Eradication in South America*

Using data collected through surveys, and from economic studies carried out in the Caribbean region during the 1980s, Rawlins (1985) estimated the annual cost of New World screwworm surveillance and medication in various countries at USD 4.82–10.71 per head. If an average of USD 7.76 per animal per year is taken as the theoretical cost, then the annual costs of the New World screwworm in South America may be in the order of USD 3600 million (Table 2). Wyss (2002a) estimated the potential annual producer benefits for New World screwworm eradication in South America at USD 2800 million (Fisher and Romero 2018).

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*Table 1. Number (x 1000) of animals and humans at risk of infestation by the New World screwworm (FAO 2014)*

| Country | Bovines | Equines | Suids | Ovines | Caprines | Total animals | Humans |
|---|---|---|---|---|---|---|---|
| Argentina | 51 646 | 3600 | 5100 | 14 700 | 4400 | 79 456 | 42 980 |
| Bolivia | 8865 | 490 | 2941 | 9499 | 2431 | 24 226 | 10 561 |
| Brazil | 212 343 | 5450 | 3792 | 17 614 | 8851 | 248 050 | 206 077 |
| Chile | 3000 | 310 | 2431 | 3300 | 450 | 9491 | 17 762 |
| Colombia | 24 205 | 821 | 5897 | 725 | 913 | 32 561 | 47 791 |
| Ecuador | 4604 | 282 | 1934 | 674 | 22 | 7516 | 15 902 |
| French Guiana (France) | 18 | - | 4 | 1 | 1 | 24 | 261 |
| Guyana | 113 | 2 | 12 | 132 | 82 | 341 | 763 |
| Paraguay | 14 465 | 300 | 1229 | 471 | 143 | 16 608 | 6552 |
| Peru | 5578 | 743 | 3232 | 12 415 | 1910 | 23 878 | 30 973 |
| Suriname | 36 | - | 36 | 5 | 4 | 81 | 538 |
| Uruguay | 11 600 | 408 | 280 | 8200 | 17 | 20 505 | 3419 |
| Venezuela | 16 816 | 522 | 3808 | 601 | 1432 | 23 179 | 30 693 |
| **Total** | **353 289** | **12 928** | **30 706** | **68 337** | **20 656** | **485 916** | **414 271** |

*Table 2. Estimated annual losses from New World screwworm in South America*

| Country | USD (million) |
|---|---|
| Brazil | 1885 |
| Argentina | 596 |
| Colombia | 250 |
| Bolivia | 184 |
| Peru | 181 |
| Venezuela | 176 |
| Uruguay | 155 |
| Paraguay | 126 |
| Ecuador | 57 |
| Suriname | 4 |
| French Guiana (France) | 1 |
| Guyana | 0.22 |
| Chile | 0 |

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## 3. NEW WORLD SCREWWORM PROGRAMMES

### *3.1. Successful SIT Eradication Programmes in North America*

The elimination of a residual indigenous screwworm population, after an intensive suppression programme, requires the area-wide application of the SIT. The SIT can most simply be described as a form of insect population control (Knipling 1985; Wyss 2002b; Klassen and Vreysen, this volume), supported by other disease-control activities including epidemiological surveillance, wound treatment, animal-movement control, and quarantine (Mangan and Bouyer, this volume). The eradication programmes, that over about 55 years implemented this integrated use of the SIT, have been phenomenally successful, as shown in Fig. 1 and Table 3 (Wyss 2000, 2002a; Klassen et al., this volume; Klassen and Vreysen, this volume).

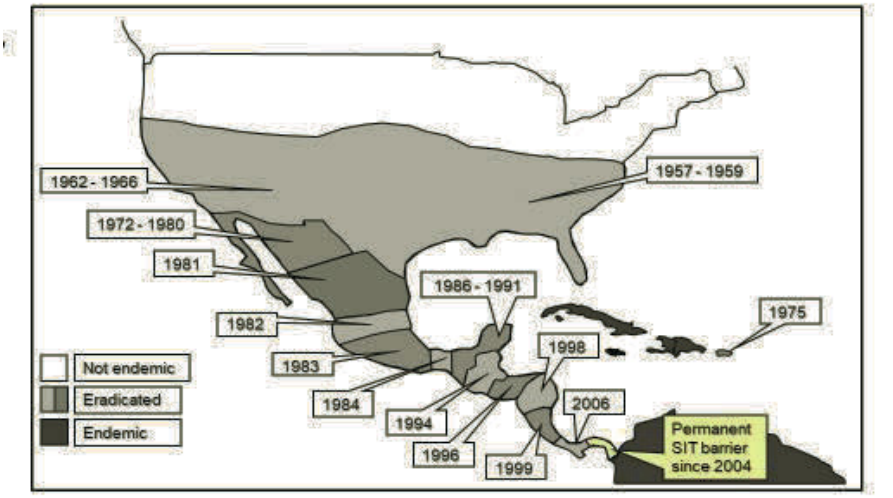



*Figure 1. Progressive shift over time of eradication zones in the New World screwworm AW-IPM programmes using the SIT from the southern USA to the countries of Central America. (Updated and modified map from Robinson 2002, reproduced with permission from Elsevier.)*

### *3.2. Naturally Free Areas in North America*

The countries and territories in the Caribbean, naturally free of the New World screwworm, are: Antigua and Barbuda, Bahamas, Barbados, Cayman Islands, Dominica, Grenada, Guadeloupe, Martinique, Montserrat, Netherlands Antilles (Bonaire), Saint Kitts and Nevis, Saint Lucia, Saint Vincent and Grenadines, and Turks and Caicos Islands.

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*Table 3. New World screwworm eradication programmes*[1]

| Country | Eradication period |
|---|---|
| USA south-east[2] | 1957–1959 |
| USA south-west[2] | 1960–1966 |
| Mexico[2] | 1972–1991 |
| Guatemala[2] | 1988–1994 |
| Belize[2] | 1988–1994 |
| El Salvador[2] | 1991–1995 |
| Honduras[2] | 1991–1995 |
| Nicaragua[2] | 1992–1999 |
| Costa Rica[2] | 1995–2000 |
| Panama[2] | 1997–2000 |
| Curaçao[3] | 1954 |
| Curaçao (reinfestation)[4] | 1976 |
| Puerto Rico[5] | 1975 |
| US Virgin Islands[5] | 1971–1972 |
| British Virgin Islands[5] | 1971–1972 |
| Aruba (outbreak)[6] | 2011 |
| USA – Florida (reinfestation)[7] | 2016–2017 |

[1]Information extracted from [2]Wyss 2000, [3]Knipling 1985, [4]Coppedge et al. 1980, [5]Williams et al. 1977, [6]Information provided by J. B. Welch ARS-USDA and F. Pinilla COPEG, and [7]Delgado et al. 2016; USDA/APHIS 2017; Skoda et al. 2018.

### *3.3. Remaining Endemic Areas in North America*

Screwworm myiases continue to be a serious animal and public health problem in Cuba, Dominican Republic, Jamaica, Haiti, and Trinidad and Tobago (Vargas-Terán 2002a; Klassen et al., this volume). These countries pose a risk of New World screwworm reintroduction to those countries already free of the pest. Therefore, the FAO and International Atomic Energy Agency (IAEA) have been providing technical assistance for New World screwworm suppression and eventual eradication.

- *Cuba (1995).* The presence of the New World screwworm was officially acknowledged in 1995. From 1995 to September 2003, 88 985 cases were reported. The animal species most affected were cattle, swine, sheep, goats, horses, dogs, and humans (FAO 1999, 2003). For the period 2004 to 2015, the Cuban Veterinary National Institute (CVNI) reported the number of New World screwworm cases and identification operations, totalling 167 141 cases (100 634 in 2004–2009 but only 66 507 in 2010–2015). Given that there is no national screwworm eradication programme, livestock producers may be becoming reluctant to report cases to authorities (L. Méndez-Mellor, CVNI, personal communication).

  The Cuban Government and FAO signed an agreement to establish a national suppression programme and design a project plan to implement screwworm

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eradication. The plan has two phases: (1) a pilot programme on Juventud Island, and (2) a full programme to cover the entire national territory. As a follow-up and part of the preparatory phase, the IAEA supported capacity building and an area-wide suppression trial on Juventud Island (García Rodríguez 2003; Garcia et al. 2007). The cost of New World screwworm eradication has been estimated at USD 62.5 million over 4 years. Recently, with the start of USA-Cuba communications, and in view of opening trade and the screwworm outbreak in Florida, there is a renewed interest in screwworm eradication in Cuba. The visit of the IAEA's Director General to Cuba in 2016 (Dixit 2016) was an indication of this interest.

- *Dominican Republic (1999).* Cases of the New World screwworm occur in all parts of the country, and without seasonal variation. Most neglected wounds and untreated navels of newborn animals soon become infested. Human cases are a common occurrence. In 1999, the governments of Jamaica, Haiti, and the Dominican Republic, and the FAO and IAEA, began regional technical assistance projects on capacity building and feasibility assessment of the suppression and possible eradication of the screwworm. As of February 2001, 1894 screwworm cases were diagnosed (FAO 2003). During the period 2004 to 2015 no major developments took place regarding the control of the New World screwworm (D. Vanderlinder, IICA, personal communication).
- *Haiti (1999).* The disease is endemic, with a high incidence throughout the country. It causes considerable losses in domestic livestock, and affects people of all ages. A Government of Haiti and FAO technical project reported 684 myiasis cases, of which 669 were positive for the New World screwworm; seven of those cases were in humans. When the project terminated in 2001, it was expected that a joint eradication programme with the Dominican Republic would be established (FAO 2003). Due to several factors, during 2004 to 2015 no progress was made on national or binational screwworm control (Max Millian, Chief, Veterinary Service, Haiti, personal communication).
- *Jamaica (1998).* Jamaica is one of the naturally screwworm-infested territories in the Caribbean. The high annual rainfall (over 1900 mm) and tropical climate sustain the very lush vegetation cover on the island, an ideal habitat for the New World screwworm. Consequently, the fly is widely distributed, regardless of season, altitude or ecological conditions. The island has about 400 000 cattle, 440 000 goats, and a large but unknown number of "stray" dogs. There are no wild animals, e.g. deer, rabbits, opossum, and peccaries, to support screwworm infestations. According to Snow et al. (1977), the New World screwworm is the second-most important arthropod pest of livestock, exceeded only by ticks. The annual economic losses inflicted by the screwworm on the Jamaican livestock sector, in terms of animal mortality and increased production costs, in 1998 amounted to USD 5.5–7.8 million (Vo 2000). Although all ways of keeping livestock are affected by the screwworm, its eradication from Jamaica would have the greatest implication for the many subsistence farmers who depend largely on small-animal holdings for their livelihood. The screwworm is also a severe human health problem, with 7 or 8 cases reported every month, and

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probably many more are unreported (M. J. B. Vreysen, personal communication).

In July 1998, the Government of Jamaica began a New World screwworm eradication programme, with assistance from the USDA and the cooperation of the IAEA and FAO (Robinson et al. 2000). The estimated cost was USD 9 million, and it was anticipated that 3 years would be required for completion (Vo 2000). From November 1998 to October 2004, on average 258 screwworm cases were reported every month. Weekly treatment of the island with sterile screwworm flies began in August 1999, with the release of about 16 million sterile flies per week. In mid-2002, in response to the prevailing high number of reported cases, a new strategy was adopted (M. J. B. Vreysen, personal communication). There were several logistical problems associated with the programme, so in spite of the new strategy, and the continuing commitment of the Government of Jamaica to eradicate the screwworm, little progress had been made by the end of 2004, and the programme was terminated (Grant et al. 2000; FAO 2003; Box 1 in Dyck, Reyes Flores et al., this volume). Vreysen et al. (2007) analysed the reasons for this failure and described the lessons learned.

- *Trinidad and Tobago.* The New World screwworm is endemic, and cases are found throughout the year. Wounds left untreated usually become infested. The proximity of the islands to Venezuela, and possible immigration of flies, could complicate screwworm suppression and eradication.

### *3.4. Potential for Eradication in Remaining Endemic Areas of the Americas*

#### *3.4.1. Threat of Reinvasion*

The Panama and USA governments have established a permanent biological barrier, in the Darien Gap in Panama, by releasing 15 million sterile New World screwworm flies per week; this barrier is maintained to protect the non-infested North and Central American countries. However, as long as Cuba, Dominican Republic, Haiti, Jamaica, and Trinidad and Tobago in the Caribbean, and a majority of South American countries, remain infested, they represent a high risk of reinvasion to the eradicated territories and the naturally screwworm-free countries in the Caribbean basin.

There are several examples of failure to prevent screwworm reinvasion in territories where it had been eradicated. In 1966, after eradication from the USA, a biological barrier was established along the Mexico–USA border. However, in 1972, a massive failure of the barrier occurred (due to favourable weather conditions for the insect, and intensive legal/illegal livestock trade between the two countries); 90 000 cases were detected in the USA. As a result, the Mexico-United States Commission (COMEXA) initiated eradication activities in Mexico, and by 1984, eradication had been achieved down to the Isthmus of Tehuantepec, the narrowest part of Mexico, where another barrier zone was established (COMEXA 2002). Then, in 1985, several outbreaks occurred in the central and northern territories of Mexico, in spite of the implementation and operation of a good quarantine network.

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The threat of reinvasion of the New World screwworm increases in proportion to the area eradicated, due to the risk posed by the international trade in animals, and the movement of pets and humans. Examples of screwworm outbreaks, both actual and potential, caused by such movements of infested animal/humans, are as follows: 1987 – dog from Honduras to the USA; 1988 – sheep from Latin America to Libya; 1989 – man from Panama to the USA; 1992 – woman from Brazil to New Zealand and Australia; 1994 – cattle from Central America to Mexico; 1998 – woman from Trinidad and Tobago to UK; and 2001–2002 in Chiapas, Mexico, due to the introduction of flies from Central America via a small aircraft. The cost of containment and eradication varies. The programme in Libya cost USD 80 million, Mexico's largest outbreak cost USD 8 million, and Aruba cost USD 200 000.

In 2016–2017 the screwworm was found infesting the wild deer population in the Florida Keys, probably due to reinvasion (Delgado et al. 2016). Sterile flies were released to re-eradicate the pest (USDA/APHIS 2017; Skoda et al. 2018).

The Mexico and United States governments, because of the cessation of its screwworm activities, in 2013 closed the sterile fly production facility of COMEXA in Tuxtla Gutiérrez, Mexico (SAGARPA 2013). Therefore, now the only existing screwworm production plant is in Panama, and belongs to the Panama and USA governments; it is operated by the Panama – United States Commission for the Eradication and Prevention of Screwworms (COPEG). Its weekly production of 15 million flies is used to maintain the Darien Gap sterile fly biological barrier to protect the screwworm-free countries in Central and North America. This facility is also the World Reference Laboratory for New World Screwworm Diagnosis of the World Organization for Animal Health (OIE). Presently, USDA-ARS is developing a genetically modified (GMO) male-only strain of screwworm to reduce potentially the costs of rearing, containment and eradication (COPEG 2015; Scott et al. 2017).

### *3.4.2. Caribbean*

The main objective of screwworm eradication in the Caribbean will be the promotion of sustainable agricultural development and food security, and simultaneously the protection of screwworm-free countries from reinvasion.

The Caribbean programme will demand the coordinated actions of all screwworm-infested and screwworm-free countries, as well as the institutions concerned with the suppression/eradication of the disease in the region. The experience and the resources accumulated by the Central and North American governments should be transferred to the infested countries in the Caribbean region.

The governments of the affected countries, with assistance from technical international agencies, will need to refine further national assessments of the current New World screwworm status, including geographical distribution, population genetics (Torres and Azeredo-Espin 2009), seasonal abundance, and economic impact, and to develop more economical methods to suppress the pest. Following these feasibility studies, a multidisciplinary mission should prepare a project proposal for the eradication of the New World screwworm from the Caribbean, to be submitted to potential donors for funding, or a strategic alliance be established with countries already screwworm-free in the region.

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Before launching such a regional programme, the following prerequisites should be resolved by the participating countries, donors, and stakeholders:

- The governments involved will be fully committed to the New World screwworm eradication programme, and there will be no change in their policy.
- Adequate funds will be available as required.
- Adequate numbers of sterile insects of the desired quality will be available from the Panamanian and possibly other insect production facilities to be built.
- All infested areas in the Caribbean will be progressively treated.
- Trinidad and Tobago should not be included in the Caribbean phase of the regional programme, due to its proximity to the endemic countries in South America and the associated real risk of reinvasion. However, once screwworm eradication has been achieved in the Caribbean, and suppression/eradication activities are underway in the coastal areas of Venezuela, it should be given priority consideration.
- All screwworm-free countries will maintain strong inspection and quarantine services to prevent the introduction of infested animals from endemic areas.
- Full support will be needed from international trade associations (North America Free Trade Agreement (NAFTA), Caribbean Community Regional Integration (CARICOM), Dominican Republic-Central America Free Trade Agreement (CAFTA-RD)) and global and international health organizations (FAO, OIE, WHO, IAEA, Inter-American Institute for Cooperation in Agriculture (IICA), and Regional International Organization for Animal and Plant Health (OIRSA)).

### *3.4.3. South America*

The New World screwworm is endemic throughout most of the South American continent (Vargas-Terán 2002a). All countries are infested except Chile, where the pest was last found in 1959 (although it is possible that Easter Island, a Chilean territory, remains infested). Although Chile shares borders with countries infested by the New World screwworm, it has been able to maintain its screwworm-free status as a result of strict controls imposed on the importation of animals and animal products. In South America, with the possible exception of Chile, there are no natural barriers known to prevent the spread of screwworms among countries.

Progress has been made in understanding the genetic diversity and population structure of the screwworm in the Amazon (Mastrangelo et al. 2014), while the presence or absence of screwworms at different altitudes in the Andes still remains to be determined. In addition to this groundwork, population genetic studies are also being carried out (Lessinger et al. 2000; Robinson et al. 2009; Fresia et al. 2014) to understand more about screwworm population variation in the region, and the possible existence of isolated populations or cryptic species. Unless barriers can be found, for eradication purposes all of South America must be considered as one region (Fresia et al. 2014). Once started, the programme would have to be progressive and continue until the whole continent (and thus the Southern Hemisphere) is completely free of the New World screwworm. To consider South America as a target area, considerable preparatory groundwork would be needed. The governments and livestock producers in each country involved must be

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convinced that eradication is technically, practically and economically justified. They must be ready to commit the resources and the energy to complete the task.

There will also be a need for mating compatibility studies among the different populations in the region (Mcdonagh et al. 2009; Robinson et al. 2009). Advances have been made on geographical information systems (GIS) and remote sensing applications (Bouyer et al., this volume) focused on improving the efficiency of the screwworm sterile fly barrier at the Darien Gap, and new fly strain collection locations have been identified to support the epidemiological case investigation (Phillips et al. 2004; Scott et al. 2017). Also, there is a better understanding of population genetics and distribution, and on how the biotic and abiotic factors influence community structure (Mastrangelo 2014), including screwworm historical movement among populations in endemic South American countries (Fresia 2014). These techniques will assist in identifying range shifts and expanded populations in the context of global environmental changes (Feldman and Ready 2014).

During 2007–2008, the governments of Brazil, Paraguay and Uruguay, with the support of the International Development Bank (BID) and COMEXA, carried out on the Brazil-Uruguay border a demonstration New World screwworm control programme to establish the basis for future programmes in the Southern Cone Common Market (MERCOSUR) countries (Muzio et al. 2009). In view of the advances in screwworm eradication in the Americas, and in response to the interest of South American countries, the FAO proposed in 2011 a "Road Map for the Suppression and Progressive Eradication of the New World Screwworm *C. hominivorax* in the Endemic Zones of the Western Hemisphere"; this would be a guide for regional efforts and avoid duplication of action among countries and international organizations (FAO 2011). In this context, the governments of Argentina, Brazil, Chile, Ecuador, Paraguay, Peru, Uruguay, and Panama requested technical assistance from the IAEA for screwworm control and prevention. The IAEA responded positively by approving in 2014 a regional project to "Support Capacity Building for Evaluation of Feasibility of a Progressive Control Programme for New World Screwworm" (W. R. Enkerlin, personal communication).

The following actions should be undertaken before the development and implementation of an eradication strategy:

- Conduct regional information campaign.
- Develop baseline data on New World screwworm case incidence in each country.
- Conduct studies on the economic impact of screwworms in each country.
- Develop an eradication strategy.
- Determine the cost of a regional screwworm eradication programme.
- Determine the benefit/cost ratio.
- Prepare an environmental-impact study on screwworm eradication in each country.
- Identify methods of financing its implementation (Vargas-Terán and Wyss 2000).

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### *3.5. Successful Eradication in Libya (1988–1992)*

The discovery of the New World screwworm in North Africa in 1988 posed an immediate threat to Libya, the continent of Africa, and the Mediterranean region. It rapidly became clear that the price to be paid for the persistent and inevitable spread of this pest from its beachhead in the Libyan Arab Jamahiriya would be very great indeed — in terms of suffering and loss in domestic animal populations. Also, the potential impact on wildlife in Africa was a grave concern to wildlife conservationists and enlightened people throughout the world (Van der Vloedt and Butt 1990; Woodford 1992).

Based on a review of Libyan livestock importations, the source of the New World screwworm outbreak was thought to be sheep (infested with New World screwworms) imported from South America. This was the first report of the New World screwworm occurring outside the Americas, and this incursion confirmed the New World screwworm as one of the most important transboundary animal-disease threats.

Immediately after official confirmation of the New World screwworm in Libya, a series of governmental and United Nations activities began. The FAO established the Screwworm Emergency Centre for North Africa (SECNA), based in the Animal Production and Health Division in Rome, to coordinate surveillance and control activities; its field programme was set up in Tripoli, Libya (Vargas-Terán 2002b). The FAO undertook the New World screwworm emergency programme in Libya on behalf of the countries threatened by the disease, and the 22 countries and agencies that provided the emergency funds (section 2.1.), including the technical support of the IAEA and other essential support provided by other United Nations agencies — IFAD and the United Nations Development Programme (UNDP).

To contain the outbreak, veterinary services in Libya provided 90 teams to undertake periodic inspections involving millions of animals in and around the infested area. Wounds were treated prophylactically, and the movement of livestock was controlled. Prevention programmes in all neighbouring North African countries concentrated on surveillance, public information, and control of animal movements.

Containment activities were successful. The infested area around Tripoli had expanded only from approximately 18 000 to 25 000 $km^2$ by the time field eradication activities commenced. As a consequence of the control of animal movement, no foci developed elsewhere in the region. However, the infestation had become more severe within the affected area, and more than 10 000 cases were recorded in the second half of 1990, compared with less than 2000 in the same period of 1989 (FAO 1991a, b).

From December 1990 to October 1991, 1300 million sterile New World screwworm pupae were transported from Mexico to Tripoli, and the emerging flies were aerially dispersed over an area of 41 000 $km^2$ around Tripoli (Lindquist et al. 1993). The last New World screwworm case was reported in April 1991 (Krafsur and Lindquist 1996). After 14 months without evidence of the parasite, and under continuous surveillance and quarantine inspection activities, Libya was declared officially screwworm-free on 22 June 1992 (FAO 1992; Vargas-Terán et al. 1994; Hendrichs, Enkerlin et al., this volume; Klassen et al., this volume).

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After eradication, the emphasis was on prevention. The FAO established the priority of improving surveillance technology and quarantine infrastructure to reduce the risk of screwworms spreading from endemic areas. This animal disease prevention approach is the background to the creation of FAO special programmes, such as the Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases (EMPRES) and the Regional Animal Disease Surveillance and Control Network for North Africa, the Middle East, and the Arab Peninsula (RADISCON).

## 4. POTENTIAL OLD WORLD SCREWWORM PROGRAMMES

The Old World screwworm is widely distributed, primarily in the Indian subcontinent and South-East Asia as far north as Taiwan and to Papua New Guinea in the south-east. It is present throughout mainland Papua New Guinea, except at altitudes of more than 2500 m above sea level (Norris and Murray 1964), and high population densities of this screwworm have been found in the coastal swamplands adjacent to Torres Strait (Spradbery and Tozer 2013). The fly is present in New Ireland and New Britain, but not further into the Pacific. This screwworm species is also found in the Indian subcontinent and tropical and subtropical sub-Saharan Africa, and also in several countries in the Middle East (Spradbery and El-Dessouky 1998; Spradbery 2002a; El-Azazy and Metenawy 2004). Kloft et al. (1981) reported repeated "transit infestations", via livestock vessels, of the Old World screwworm in the Persian Gulf. The Old World screwworm was discovered in Iraq for the first time during a 1995–96 survey of filth flies around Baghdad City (Abul-Hab and Kassal 1998), and by late 1996 a major outbreak was apparent. Seasonal changes in screwworm myiases in Iraq showed a peak in December-January, and were at their lowest in July-August (Siddig et al. 2005). In 1998 the Old World screwworm fly was discovered in Yemen for the first time (Robinson et al. 2009).

### *4.1. Feasibility of Using the SIT for Suppression or Eradication*

At a conceptual level, any suppression and eradication programmes in South-East Asia should first be initiated in the archipelagos of Indonesia and the Philippines, and other island nations such as Sri Lanka. Area-wide programmes could also be applied in peninsulas, such as peninsular Malaysia and southern Thailand, although a permanent buffer zone would be required at narrow interfaces between the eradicated and endemic zones, similar to the arrangement now in place for the New World screwworm in Panama. Treatment of islands should be extended progressively within a region before moving into the continental landmass.

On the Asian continent, the challenge would be much greater, similar to that of South America. Natural "ecological islands", bounded by deserts or high mountains unsuitable for screwworm survival, should be amenable to eradication programmes. However, more ecological and genetic studies on the screwworm are required before such a strategy could be implemented. There may also be a potential, at the edge of the pest's distribution, to reduce the range. This would be more feasible if the screwworm had already been eradicated in the "ecological islands" and peninsulas.

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If an eradication attempt were to be made on the continental landmass, it would be essential that a regional approach be adopted.

The screwworm outbreak in Iraq in the mid-1990s stimulated a combined response by the FAO, IAEA and the Arab Organisation for Agricultural Development (AOAD) with a view to containment and control in the region. Numerous initiatives were proposed (Hall 1997; Spradbery and El-Dessouky 1998), including a mobile mass-rearing facility on a dumb barge with a capacity for 50 million sterile flies per week that would be deployed initially in Muscat, Oman, and then be moved via the UAE towards Kuwait and Iraq as the programme progressed (Spradbery et al. 2002). The war in Iraq in 2003 and subsequent events put the Middle East screwworm control initiatives on indefinite hold.

Implementation of AW-IPM programmes using the SIT should target preferably the areas where the Old World screwworm is most damaging and vulnerable, and least able to migrate back to recolonize areas from which it had been eradicated.

### *4.2. Preparatory Activities*

In most areas where the Old World screwworm is endemic, there is a paucity of information about its population size, distribution, and possible seasonal variation. Anecdotal evidence suggests that the screwworm is a relatively minor problem under smallholder livestock production systems, but it becomes a serious production-limiting pest under more extensive grazing systems. Although farm records, where available, indicate that myiasis is a major animal-health problem under extensive grazing systems, screwworm suppression in large dairy herds is effective because the animals are observed closely every day, whereas herds of beef cattle and flocks of sheep require special mustering for inspection every few days, requiring a high labour cost that significantly reduces the efficiency of the production system.

Information about the incidence of Old World screwworm myiasis, and its significance for livestock production and public health, is limited. While this screwworm is reported widely, there are few reports of structured studies of the economic impact of the myiasis and of suppression measures; studies in Malaysia reported annual losses of USD 4.7 million (Feldmann and Slingenbergh 2002). If the screwworm became established in Australia, the estimated overall cost would be in excess of USD 794 million per annum (Kwabena Anaman, personal communication in Spradbery 2002b).

However, before an eradication programme is planned, an essential preliminary phase is the collection of comprehensive baseline data, including ecological and genetic studies to delineate the relevance of the problem (Hall et al. 2001), the infestation dynamics, and the economic, public health and environmental costs of the pest (Dyck, Reyes Flores et al., this volume). Priority areas include:

- Distribution and seasonal occurrence
- Population densities
- Incidence and severity of myiasis
- Economic impact studies
- Studies on screwworm migration behaviour

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- Modelling of climatic and other effects on populations
- Application of geographic information systems (GIS)
- Genetic diversity
- Risk analysis.

### *4.3. Other Important Considerations*

Countries proposing to undertake an AW-IPM programme that integrates the SIT must have a strong commitment to it. This commitment must come not only from the government, to provide resources and an operational framework, but also there must be very strong support from, and involvement of, the livestock industry and relevant private-sector groups. AW-IPM programmes require considerable input from local people to support surveillance and monitoring programmes, and to suppress outbreaks of screwworms (Dyck, Reyes Flores et al., this volume).

Clearly, the programme must be economically viable, with a favourable benefit/cost assessment, and have adequate funds. Benefits accrue from increased livestock production (better performance of animals and fewer deaths), and reduced production costs and pesticide usage.

The AW-IPM programme may also provide considerable benefits that are hard to quantify economically, e.g. environmental benefits from reduced chemical usage and impact on wildlife, and possibly public health benefits. The large mammal fauna of northern Africa and Australia are at risk from incursions of screwworms. Reductions in native fauna would impact tourism and ecosystems. In remote areas, or in areas with poor health services, there could be significant numbers of human cases. (In the poorer regions of Central America, screwworm strikes in humans resulted in up to 40% mortality.)

### *4.4. Australian Preparedness Planning for Potential Outbreaks*

#### *4.4.1. Threat of Screwworm Introductions into Australia*

Australia is fortunate that neither the New World screwworm nor the Old World screwworm has become established in the country, although large parts of its northern areas are environmentally suitable for these pests (Sutherst et al. 1989). Atzeni et al. (1994) predicted that about 2.3 million $km^2$ could be colonized within 4–5 years of an incursion. The development of GIS for managing and predicting invasive screwworm populations (Gilbert and Slingenbergh 2008; Feldmann and Ready 2014; Bouyer et al., this volume) has led to the application of GIS to a prospective Old World screwworm incursion of Australia (Welch et al. 2014). Additionally, spatial analysis has been used to determine targeted surveillance for detecting a screwworm fly incursion into Australia (Fruean and East 2014).

The introduction and establishment of the New World screwworm into Australia is considered unlikely, but not impossible. In 1992, an Australian tourist returning from South America accidentally brought, in a neck wound, live New World screwworm larvae into Australia (Searson et al. 1992). In 2012 a woman, who had visited the Amazon rainforest in Peru, returned to Australia with a screwworm

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myiasis in her scalp behind her ear, with 23 mature (up to 2 cm) live larvae of *C. hominivorax* (Lau et al. 2015). In both cases, the host patients lived on rural properties in south-eastern Australia, but the episodes occurred at a time of year when climatic conditions were unfavourable for the survival of the insect. These two case histories demonstrate the potential for inadvertent introduction into Australia via human transportation.

The Old World screwworm, prevalent in the neighbouring countries of Papua New Guinea and Indonesia, is a substantial threat to Australia. The export of live cattle from northern Australian ports to south-east Asian nations is an important export trade. In 1988 in Darwin harbour, several Old World screwworm flies were trapped by quarantine authorities aboard an empty livestock vessel that had just returned to Australia after delivering cattle to Brunei (Rajapaksa and Spradbery 1989). The Old World screwworm is also present near ports in the Middle East, such as Muscat in Oman, to which live sheep are exported from various Australian ports. Both of these situations provide an opportunity for the screwworm to enter Australia as pupae or adults in returning empty livestock-carrying vessels. While such vessels probably represent the most likely method for the pest to gain access to Australia, accidental transport on aircraft, active myiasis in humans or companion animals, are also distinct possibilities.

#### *4.4.2. Research on Screwworms*

In response to the threat of the Old World screwworm to the extensive pastoral cattle-producing areas in northern Australia, for many years Australia has conducted research on this pest. From 1973 to December 1991, the Commonwealth Scientific and Industrial Research Organization (CSIRO) studied the biology and ecology of the screwworm in Papua New Guinea. A study of dispersal by the screwworm fly demonstrated that adults could travel at least 100 km (Spradbery et al. 1995). In 1982, sterile chilled adult flies were dispersed from the air to evaluate their effectiveness in eradicating the Old World screwworm. In a small-scale field trial, it was found that up to 33% sterility could be induced in a wild population (Spradbery et al. 1989; Spradbery 1990). From 1995 to 2000, Australia and Malaysia undertook a collaborative myiasis control research project located at the Institut Haiwan, Kluang, Malaysia. The project assisted in suppression trials of the screwworm in Malaysia, and supported research that developed and evaluated improved Old World screwworm suppression and eradication techniques (Mahon et al. 2004). To confirm and provide confidence in the efficacy of the SIT, a demonstration to show that mass-reared and sterilized screwworms were fit and competitive in the field was implemented in 1986, with 62% sterility recorded (Mahon 2002, but see Spradbery 2006). Competitiveness of screwworms in the application of the SIT has been explored by Mahon (1996).

The sterile flies were produced successfully on a hydroponic diet. Most of the rearing methods for the Old World screwworm were based on techniques developed by the USDA for the New World screwworm (Wyss 2002b), but several innovations to mass-rearing larvae and harvesting chilled sterile flies for release were developed (Spradbery 1990). A small (6 million per week) pilot mass-rearing facility was built at the Institut Haiwan in Malaysia, where novel production-engineering methods

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were applied to rearing this species (Mahon and Ahmad 2000). Nevertheless, there is considerable scope for improving the efficiency of mass-rearing the Old World screwworm and of releasing sterile flies in the field.

*4.4.3. Impact of a Screwworm Introduction*

In 1990, to enhance the state of preparedness, Australia's long-term Old World screwworm preparedness was reviewed and a plan developed. Models of the impact of the Old World screwworm indicated that the cost of an invasion would be high. Anaman et al. (1993) estimated that the annual cost (at 1991 values) of an endemic establishment, to beef-cattle, sheep and dairy producers in an average climate year, would be approximately USD 200 million. In comparison with the cost to the community (several times the producer losses), these costs would be trivial (McKelvie et al. 1993). The models indicated that large areas of tropical and subtropical Australia are suitable for year-round survival of the Old World screwworm, with further southern extensions in summer that would recede in winter (Atzeni et al. 1994).

Extensive cattle-grazing is the dominant industry throughout much of the northern pastoral areas of Australia. Based on experiences in the USA, it is likely that extensive cattle production, as practised in northern Australia, would not be viable if the Old World screwworm became established. Failure of the livestock industries would, in turn, impact severely on the small- to medium-sized towns servicing the industries, and, as there are few or no viable alternative business opportunities, many communities could collapse. However, by undertaking an eradication programme, an 8:1 benefit/cost ratio would be achieved (Anaman et al. 1993).

Australia's native fauna is naive to this pest, and inevitably, should an incursion occur, there would be some impact on the fauna, although it is impossible to predict the extent of it. Records from the Zoo Negara in Kuala Lumpur, Malaysia, confirmed that a Red Kangaroo and an Agile Wallaby were infested by screwworm fly (Spradbery and Vanniasingham 1980). Human cases of screwworm myiasis would also occur.

*4.4.4. Australia's Contingency Plans in Case Screwworms are Introduced*

Australia has developed contingency plans to respond to a number of exotic diseases, including the Old World screwworm, involving collaboration between the commonwealth and state governments and the livestock industries. The Australian Veterinary Emergency Plan (AUSVETPLAN 2007) contains a strategy for the suppression and eradication of the Old World screwworm should it gain a foothold on the continent (Tweddle 2002). The policy is to eradicate the screwworm in the shortest possible time, while limiting the economic impact, using a combination of strategies including the following:

- Treatment of individual animals and groups to prevent or cure infestation, especially before movement.
- SIT to suppress and eradicate the fly.

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- Quarantine and movement controls in declared areas to prevent the movement of infested animals.
- Decontamination and disinfection of larvae-contaminated areas and things.
- Tracing and surveillance to determine the source and extent of the infestation, and provide proof of freedom from the disease.
- Zoning to define infected and disease-free areas.
- Public awareness campaign to encourage rapid reporting of suspected infestations, and to facilitate cooperation from industry and communities.

*4.4.5. Mass-Rearing Screwworms*

A fundamental plank in the eradication plan is to integrate the SIT, which at present is probably the only feasible method that can eradicate an incursion of the Old World screwworm (or New World screwworm) into Australia. It is envisaged that a facility producing 200–250 million sterile flies per week would be required, as it is difficult to predict how much a screwworm outbreak would spread before detection and containment. A facility with this capacity would be extremely expensive to construct, and there would be intense pressure to complete it as quickly as possible to prevent the screwworm from spreading and to minimize economic losses.

A design brief for a rearing facility (within Australia, if it were required), with a capacity to produce 250 million sterile screwworms per week, has been prepared (Phillimore 2002). Models have indicated that there is merit in constructing a facility, and then "mothballing it" until required. A multi-species sterile insect production facility was another attractive option evaluated by Anaman et al. (1993). In the multi-insect-facility concept, the facility would be used to produce sterile insects for the suppression or eradication of endemic pests, e.g. Queensland fruit fly *Bactrocera tryoni* (Froggatt) or Australian sheep blow fly *Lucilia cuprina* (Wiedemann) until an exotic pest incursion. After the exotic pest outbreak, the already-operational plant would fairly quickly be converted (in full or in part) to the production of sterile screwworms (or other exotic horticultural pests, e.g. the melon fly *Zeugodacus cucurbitae* (Coquillett)). Using such a multi-insect-facility, screwworm production would begin earlier in an outbreak, when the pest distribution was still restricted, and so a smaller capacity would be required.

A supplementary approach could be to maintain a culture of Old World screwworm in quarantine, e.g. Animal Health Laboratory, Geelong, Victoria, with regular infusions of wild material. A prefabricated and mothballed mass-rearing infrastructure that could be immediately deployed on a dumb barge near a future outbreak (see Spradbery et al. 2002), would provide an immediate response capability that is currently lacking. This initiative would also help maintain Australian-based expertise in culturing screwworm flies. Establishing a screwworm mass-rearing facility at an abandoned offshore detention centre on Manus Island in Papua New Guinea, for example, may be unrealistic.

*4.4.6. Geography and Genetic Variation of Screwworms*

The SIT could be ineffective if *Chrysomya bezziana* populations from different geographic locations proved to be a complex of sibling species (Strong and Mahon

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1991; Hall et al. 2014; Wardhana et al. 2014; Krafsur and Ouma, this volume). Studies were conducted to determine the genetic variation of *C. bezziana* samples from as many localities as possible within its geographic range, using a range of techniques including allozyme studies (Strong and Mahon 1991), a hybridization study in which flies from Papua New Guinea were crossed with material from South Africa, United Arab Emirates, Sultanate of Oman, Malaysia and Indonesia (Spradbery 1989), cytogenetic studies (Bedo et al. 1994), and biochemical profiles of cuticular hydrocarbons analysed by gas chromatography (Brown et al. 1998). Using morphological and molecular analyses of Old World screwworm fly material from 14 different countries, Hall et al. (2001, 2014) deduced from mitochondrial variation that there were two distinct geographical races, one from Asia and the Persian Gulf region, the other from sub-Saharan Africa. Specimens from Iraq and Iran probably originated from nearby Arab countries of the Gulf (Hall et al. 2009; Navidpour et al. 2009), while populations within the Gulf exhibited only a small degree of genetic diversity, suggesting a Gulf colony of screwworm could be used to implement the SIT in an integrated control programme in the Asia region (Hall et al. 2009; Ready et al. 2009; Ready et al. 2014). A phylogenetic study of the screwworm confirmed African and Asian lineages, with four daughter haplotypes in Asia (Wardhana et al. 2012b), and a morphological principal component analysis separated the Asian haplotypes from Oman, Central Indonesia, Sumatra, and Papua New Guinea (Wardhana et al. 2012a, 2014).

While differences among populations do occur, the studies on geographical variation of the Old World screwworm indicated that it is not essential that the colony used to breed sterile flies for the SIT is derived from the same location as the targeted Old World screwworm population (Hall et al. 2009).

#### *4.4.7. Screwworm Surveillance*

Other components of the strategy are designed to minimize losses and supplement the AW-IPM eradication programme. An early-warning system, part of the North Australian Quarantine Strategy (NAQS), has been established. It is based on enhanced quarantine surveillance, education, and a regular trapping regime. A programme to improve baits and traps for surveillance of the Old World screwworm fly, carried out jointly by Australia and Indonesia over many years, resulted in a new bucket-trap design (LuciTrap) and a newly formulated bait Bezzilure (Urech et al. 2012). The sensitivity of the new trapping system, when compared with inspections of cattle for myiasis, demonstrated that the traps were a very effective monitoring and surveillance tool (Urech et al. 2014; see also Al-Taweel et al. 2014). Also, it was found that the addition of a cattle-derived synthetic odour to the Bezzilure bait used in sticky traps enhanced its attractiveness to screwworm flies (Sulston et al. 2014). The detection of small numbers of screwworm flies in trap catches containing large numbers of secondary blowflies of similar appearance has been vastly improved using a highly sensitive, real-time polymerase chain reaction (PCR) test capable of detecting a single screwworm fly in a sample of 1000 non-target species or up to 95.5% probability of detecting one in a catch of 50 000 flies (Jarret et al. 2010; Morgan and Urech 2014).

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*4.4.8. Treating Animals*

Ivermectin and avermectin are effective systemic pesticides against the larval stages of the Old World screwworm (Spradbery et al. 1985), and would be used to treat infected animals and those wounded in the course of normal husbandry procedures. Ivermectin boluses prevent screwworm myiasis for 102 days (Wardhaugh et al. 2001). Ivermectin boluses, or another long-acting formulation such as insecticidal cattle ear tags (Tozer and Spradbery 2002), could limit the incidence and spread of the screwworm in extensive pastoral areas. This would reduce the population of screwworms, and perhaps thereby the incidence of myiasis, and certainly reduce dispersal. However, native and wild animals would be a problem since they cannot be treated effectively. The importance of insecticides, as an integral part of the application of the SIT, was emphasised by Wardhaugh and Mahon (2002). A review of chemicals that could be used to control the screwworm was undertaken by James et al. (2005). Subsequently, therapeutic and prophylactic studies in the laboratory and on animals were made in Indonesia to determine the efficacy of Australian-registered chemicals for potential use in screwworm fly control, with dicyclanil spray-on and ivermectin controlled-release capsule formulations giving longer protection than the currently recommended subcutaneous ivermectin (James et al. 2017).

Studies on vaccinations against screwworms showed that it is possible to induce dramatic immunological effects on feeding larvae, but a recombinant vaccine will prove elusive (Willadsen 2000; Willadsen and Partoutomo 2000).

*4.4.9. Public Awareness*

Public awareness and early reporting of a suspect myiasis are emphasized, both prior to, and in response to, an outbreak, especially in northern Australia. A video entitled "Recognizing exotic livestock disease number 7: screwworm fly" has been produced to train veterinarians and other health professionals. To encourage the submission of larvae from myiasis strikes, specimen collection kits have been supplied to producers (and health centres) in northern Australia. Spradbery (2002a) prepared a diagnostic manual, and people have been trained to identify the Old World screwworm and differentiate it from other species. A detailed diagnostic comparison of the two screwworm fly species has been published (Welch and Hall 2013). The "Old World Screwworm Fly Manual" published in 2002 is under revision with a new edition planned, possibly incorporating the latest diagnostic technologies from wing morphometrics to X-ray computerized tomography (CT) scanning (Beckett et al 2016; see also Animal Health Australia (AHA 2017)).

## 5. CONCLUSIONS

The myiasis caused by New and Old World screwworm flies, documented for its transboundary importance, is an animal and human disease that causes significant sanitary and economic damage when it enters a country previously free of the screwworms. Screwworm-free countries must be informed about its epidemiology and methods of suppressing it, and establish appropriate prevention measures to

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avoid introducing it. Nowadays, with international transport, the globalization of economies, and the rapid long-distance movement of animals and animal products, mobilization of pets and the increase of adventure tourism, the risk of transporting pests and diseases has greatly increased. As a result, stronger quarantine measures are being applied, with the potential of restricting free world trade. Some risks can be mitigated if the causal factor is removed. The elimination of the New World screwworm in North and Central America has removed an obstacle to animal movement within this zone.

Although in some areas the traditional methods of suppressing screwworms apparently give good results, the use of modern area-wide tactics must be considered together with the "One Health" (FAO, OIE, WHO) initiative approach to eliminate the disease where extensive grazing or more intensive livestock systems are predominant. In addition, other positive benefits will arise. As a direct result of the screwworm eradication programme in North and Central America, national animal-health organizations in the region are now working closely together. Also, within each country, the government animal-health sector has built stronger bridges to producers. Since the screwworm programme directly involved producers, and depended on them to assist in the eradication, they also became part of the programme and took pride in the results; this stimulated cooperation in other programmes.

The modern area-wide approach to eradicate screwworm populations has proved to be successful. However, it is essential that the governments of affected countries in Africa, Asia, South America (Fisher and Romero 2018), the Caribbean, and the Middle East give political support where the elimination of the disease would be feasible and cost-effective, and thus avoid continuous economic losses. As well, because of myiasis in humans, screwworm eradication would also improve human health.

## 6. ACKNOWLEDGEMENTS

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# CHAPTER 7.2.

# IMPACT OF FRUIT FLY CONTROL PROGRAMMES USING THE STERILE INSECT TECHNIQUE

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## SUMMARY

Measuring the impact of area-wide integrated pest management (AW-IPM) programmes, that use the sterile insect technique (SIT) to control fruit fly pests of economic significance, is complex. These programmes affect practically the whole horticultural food chain. In this chapter, the impact of the programmes is assessed by focusing only on the benefits generated to producers and traders of horticultural products, the direct beneficiaries. This is done first by describing the types of benefits accrued from these programmes, second by explaining some of the main factors that influence programme benefits, and finally by presenting several examples to illustrate how the SIT technology, when properly applied for eradication, containment, suppression, or prevention purposes, can generate substantial direct and indirect benefits to the horticulture industry.

## 1. INTRODUCTION

Recently, the FAO (Food and Agriculture Organization of the United Nations) estimated that, to meet the projected food demand by 2050, global agricultural production will have to increase by 60% (FAO 2016). Area-wide integrated pest management (AW-IPM) programmes, that use the sterile insect technique (SIT) to control insect pests, including fruit flies of economic significance, can substantially contribute to meeting this demand by enabling the production of more and better-quality horticultural products at a lower cost, increasing supply, diversifying markets, creating new jobs, and all in a more sustainable environment-friendly manner. Measuring the impact of such programmes integrating the SIT is a complex task.

This chapter will concentrate more on the direct benefits generated by such programmes to beneficiaries — the producers and traders of horticultural products, although benefits impact the whole food chain system from farmers to consumers, passing through all the intermediate links of packing, shipping, distributing to wholesale and retail markets, and selling. In addition, the benefits branch out to other related businesses, such as suppliers of farm inputs, packing and raw materials, and some other services.

In the past decades, several studies have prospectively assessed the economic feasibility of potential fruit fly programmes integrating the SIT. These studies have been the basis for deciding to invest or not to invest in such programmes (Reyes et al. 1991; IAEA 1995; Enkerlin and Mumford 1997; Larcher-Carvalho et al. 2001; Vo et al. 2002; Larcher-Carvalho and Mumford 2004; IAEA 2005; Mumford, this volume). However, more relevant to this chapter are similar retrospective studies to measure the economic returns and impact of ongoing programmes at different stages

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of implementation (USDA/APHIS 1993; Mumford et al. 2001; Kakazu 2002; Knight 2002; IICA 2009, 2010, 2013; Mumford, this volume).

This chapter assesses the impact of fruit fly programmes integrating the SIT. The most common direct and indirect benefits to the horticulture industry, especially to the producers and traders of horticultural commodities, are reviewed.

## 2. DIRECT AND INDIRECT BENEFITS

Inefficient pest control practices, or no control at all, result in direct and indirect losses from fruit flies that translate into direct and indirect benefits when using more effective alternative control methods and approaches such as the area-wide application of the SIT. For example, if direct fruit fly damage would normally cause a 25% loss in fruit yield, the area-wide application of an improved technology that reduces damage by 80% would lead to a direct benefit of a 20% increase in yield. Another example is the indirect damage from secondary pest outbreaks caused by killing natural enemies with regular insecticide "cover" sprays. If an effective and environment-friendly control technology is used, the amount of insecticide applied is reduced and more natural enemies survive to suppress the secondary pest populations; in this situation, a 10% indirect loss would potentially become a 10% indirect benefit.

The direct benefits commonly used to measure the impact of programmes integrating the SIT are:

- Increase in fruit yield and quality through reduced damage,
- Reduction in production costs through a more cost-effective control method.

The indirect benefits commonly used to measure the impact of programmes integrating the SIT are:

- Increase in fruit and vegetable export volumes, and market retention or diversification, through effective control of quarantine pests,
- Increase in export volumes through reduced rejections of commodities which do not comply with pest absence or the insecticide residue levels,
- Increase in fruit yield through reduced secondary pest outbreaks,
- Savings in medical costs, and occasionally deaths, through reduced exposure to insecticides, and also in legal costs arising from damage to private or public property as a result of insecticide misuse,
- Greater protection of beehives resulting in increased fruit yield through increased crop pollination,
- New jobs created in the horticulture industry and related businesses and services,
- Better human nutrition due to a per capita increase in fresh-fruit intake,
- Savings in environmental and public health costs through reduced insecticide residues in fruit, water reservoirs, and soil.

These indirect benefits are very difficult to assess and quantify, and in most cases have been accounted for only qualitatively (Pimentel et al. 1992).

The impact of programmes integrating the SIT has focused mostly on the direct benefits, but attempts have been made to quantify some indirect benefits using ad hoc methodologies (Enkerlin 1997). To partially quantify benefits to the

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environment from using methods such as the SIT, the monetary value of savings accrued from minimizing insecticide use has been estimated (Pimentel et al. 1992). For example, in 1997, it was estimated that some Middle East countries (Israel, Jordan, and Territories under the Jurisdiction of the Palestinian Authority) would over 6 years, if current control practices prevailed, spend USD 46.7 million for insecticides to suppress the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann). This compares with only USD 5.8 million that would be spent on insecticides over the same period if pest suppression with the SIT were adopted, a direct benefit of USD 40.9 million in 6 years related to reduced insecticide use, in addition to the indirect environmental benefits (Enkerlin and Mumford 1997).

Another example of an indirect benefit is the per capita increase in fruit production. A study (Vo et al. 2002), to assess the economic feasibility of fruit fly control in the Central American region using the SIT, indicated that the average per capita production of fruits was 17.2 kg/year, substantially lower than the world's average annual per capita production of 75 kg in 2016 (FAO 2016). Effective fruit fly control in the Central American region would reduce current fruit damage and increase the annual per capita production of fruits in the short term by 10.5% to almost 19 kg/year, equivalent to 48 600 tonnes valued at USD 25 million/year. On a country basis, the amount of fruit (from fruit fly hosts) produced per capita per year is as follows: Costa Rica — 85 kg, El Salvador — 12.2 kg, Guatemala — 18 kg, Honduras — 11.5 kg, Nicaragua — 24.7 kg, and Panama — 16 kg. (There are no data available for Belize, but being a Mediterranean fruit fly-free country, with a traditionally friendly market under the Commonwealth umbrella, the per capita production can be assumed to be well above the regional average.) Clearly in Costa Rica, where the fruit industry is well organized and fruit production has been modernized, the annual per capita fruit production is comparable with the highest values in the world. On the other hand, in the other countries in the region, especially El Salvador, Honduras, and Panama, the annual per capita fruit production is extremely low — due to a lack of appropriate infrastructure, deficient growers' organizational schemes, and insufficient government incentives for fruit production and commercialization. Effective fruit fly control would permit the development of fruit fly low-prevalence and fly-free areas, paving the way for private investment in fruit production and commercialization for both the domestic and export markets, contributing to increases in per capita production, preventing chronic disease, creating new jobs, and stimulating the economy (Vo et al. 2002).

In Jordan, the cost of poisonings arising from using organophosphate insecticides against fruit flies was assessed. It was estimated that USD 1.6 million/year are spent for treatment of moderate to severe poisonings, and for labour-days lost. Each year an average of 94 tonnes of insecticide, i.e. technical material, are applied to control the Mediterranean fruit fly. This represents 33.8% of the total insecticide use in Jordan. Thus, assuming that sprays against this fruit fly cause a corresponding one-third of poisoning cases, about USD 550 000 are spent each year to treat persons poisoned when spraying against the Mediterranean fruit fly. In the assessment of total benefits that would be produced should the SIT technology be adopted to suppress this pest in Jordan, this cost was included in the indirect benefits (IAEA 2001).

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Moreover, a study to measure the direct and indirect benefits of Mediterranean fruit fly control in the island of Madeira, Portugal (Dantas et al. 2004), was conducted using two valuation techniques. The first involves quantifying the costs incurred by society when dealing with the externalities caused by insecticides. In this case, only those costs that can be attributed specifically to the use of insecticides for Mediterranean fruit fly control in fruit crops are included. The second method, used for environmental valuation, is called "contingent valuation". This method is based on the choices people make when they are presented with a variety of goods and services; a "willingness to pay" (WTP) indicates a positive preference. Quantification of the indirect benefits accrued from using the SIT for Mediterranean fruit fly suppression in Madeira makes economic returns positive in two out of three control scenarios analysed (IAEA 2005).

The benefits generated by programmes that control fruit flies with an "integrated SIT approach" will be determined and shaped mainly by the characteristics of the pest problem, including pest status (presence or absence), level of pest risk, and access to the targeted market, as well as by programme strategic options (section 3.2.; Hendrichs, Vreysen et al., this volume). There are other factors that will influence the benefits: the type of organization (public, private or joint ventures) and organizational structure (centralized, decentralized or a combination) used to execute the programme, the level of participation of the main stakeholders, the readiness to take advantage of the improved phytosanitary condition, increased fruit production and quality including the necessary means for commercialization in national and international markets, global market supply-demand forces, and the strategic approach and tactics selected to achieve programme objectives (of particular relevance are the size of the intervention areas and the time period required to achieve the pest control goal).

A brief explanation of how some of these factors, in particular organizational structure, stakeholder participation, and programme strategic options, affect the benefits produced by programmes applying the SIT, and hence their impact, is presented in the following section.

## 3. FACTORS THAT AFFECT BENEFITS PRODUCED BY PROGRAMMES USING THE SIT

### *3.1. Organizational Structure and Stakeholder Participation*

To date, most fruit fly programmes integrating the SIT have been public projects, operated through a centralized federal government structure, with different degrees of participation from local governments, and with limited participation from the horticulture industry at the farm level. Often these have been prevention and emergency response programmes aimed at protecting the welfare of the horticulture industry, keeping out quarantine pests or eliminating outbreaks of recently introduced invasive pests. A typical example of such programmes is Chile's National Fruit Fly Programme aimed at maintaining fruit fly-free status through an effective early detection and emergency response system, operated by the

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Agriculture and Livestock Service of the federal government (Olalquiaga and Lobos 1993; section 4.2.).

Other types of programmes include those where strong alliances have been formed among the federal and local governments and the fruit industry, which share capital investment, operational costs, and responsibilities. These programmes aim to control established key fruit fly pests that, by directly affecting production and commercialization, limit the development of the horticulture industry. A typical example of such programmes is the National Fruit Fly Campaign of the Mexican government (SAGAR/IICA 2001; section 4.6.).

As the SIT technology becomes more cost-effective when compared with other more-conventional methods of fruit fly control, interest from the private sector in its application has slowly increased. Public-private organizational schemes, in which governments, the horticulture industry, and other private and/or civil organizations form alliances and partnerships for financing and operating area-wide programmes integrating the SIT, are becoming more common. In some cases, the participation of federal and local governments will be limited to a supporting role, e.g. creating the legal framework for smooth implementation of programme activities, and setting standard operating procedures (SOPs) in the form of phytosanitary norms for the production, shipment, importation, and release of sterile flies. Also, when required, governments must enforce quarantine regulations. An example of a scheme where the private sector has become involved in area-wide fruit fly control is the fruit fly suppression programme in the Hex River Valleys and other fruit producing areas in the Western Cape Province of South Africa, in operation since 1997 (Barnes et al. 2004; section 4.4.), and covering more than 22 000 ha of commercial deciduous fruit and table grapes (Venter et al. 2021).

As predicted earlier, and given prevailing diverse political and economic environments, programmes integrating the SIT will operate under different types of organizational structure. Countries such as Argentina, Australia, Brazil, and Morocco are operating programmes run by government-private sector alliances, and even some programmes or segments of programmes are operated fully by the private sector, for example in Israel and South Africa, but other countries, e.g. Belize, Chile, Croatia, Dominican Republic, Peru, Guatemala, Mexico (Moscamed), Spain, and the USA conduct mostly government-managed programmes. A key element for success in government or semi-government programmes is that the financial resources must be available in sufficient amount and with no delays according to an agreed technical and financial operational plan. Resources should be administered under an unbureaucratic flexible scheme where the budget adapts to the requirements and dynamics of the programme's operations, and not the programme to the budget (Enkerlin et al. 2017; Dyck, Reyes Flores et al., this volume).

Government-run programmes come at a low cost to the direct beneficiaries, since most of the required capital and operational inputs are fully or partially subsidized, and normally governments do not seek cost-recovery. If the programme is effectively executed, the beneficiaries start to accrue benefits or profits quickly. However, these programmes rely on political and economic stability, and under certain conditions the managerial and technical capabilities of governments are more exposed to failure. Since programmes integrating the SIT are financially demanding

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and management intensive, countries that decide to embark on such programmes must have a strong long-term political commitment and fulfil a minimum institutional capacity to manage them. Countries that begin a large-scale SIT governmental project, with little or no support and participation of the private sector, and no support from international organizations and other relevant stakeholders, are likely to fail in the endeavour. This situation contrasts with programmes that are established as strategic alliances between government and private sector, with the support of international organizations, and where the stakeholders are actively involved in programme execution or supervision. Under this scheme, with private assets and financial capital invested in the programme, there is little or no government subsidy. Direct beneficiaries and other stakeholders have to wait longer for payback of the initial investment, and for profits to start flowing. However, in the long term, since they are not so dependent on political stability and programme management is normally carried out more effectively, these programmes tend to be more sustainable.

### *3.2. Programme Strategic Options*

Based on the goals of the programme, and the status of the target pest in the area of interest, fruit fly programmes integrating the SIT can be grouped into four main strategic options, as follows: (1) eradication — eliminate from an area an established pest or an outbreak of an introduced pest to establish pest free areas, (2) suppression — reduce pest populations to establish low pest prevalence areas, (3) containment — apply phytosanitary and regulatory measures in and around an infested area to prevent the spread of the pest, and (4) prevention — apply phytosanitary and regulatory measures to prevent the introduction or reintroduction of a pest into a pest free area (FAO 2017; Hendrichs, Vreysen et al., this volume). Suppression, eradication, containment, and prevention programmes can provide the same types of direct and indirect benefits listed above (section 2.). However, in terms of required inputs, economic returns and subsequent impact, there are marked differences among these four types of programme.

In areas with a high risk of introductions, the most profitable strategy of using the SIT is prevention. It is always much more cost-effective to protect proactively a pest free area from the introduction and establishment of a pest than having to "live with" or eradicate it. However, when the target pest has already become established in an area, the options for using the SIT to control the pest are eradication, suppression, or containment.

Figure 1 shows a typical net-benefits trend for three control options — SIT suppression, SIT eradication, and insecticide-bait suppression. Net-benefits tend to be greater in an eradication programme than in a suppression programme. After eradication has been achieved, and the area has been certified fly-free, horticultural products can be exported to fly-free high-value markets without complying with expensive postharvest treatments and other quarantine regulations. Furthermore, the ongoing cost of maintaining an area fly-free (detection networks, quarantine regulations, and in some cases the release of sterile flies), although costly, would normally be lower than the cost of a permanent SIT suppression programme.

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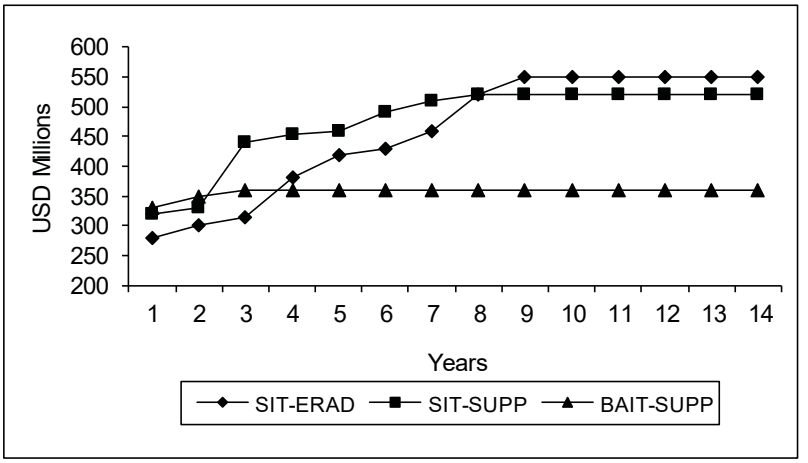



*Figure 1. Typical net-benefit trend for three control options: SIT eradication, SIT suppression, and insecticide-bait suppression. (Figure adapted from Enkerlin and Mumford 1997.)*

Once the eradication goal has been reached and benefits fully established, the combination of higher gross revenues at a lower cost makes the eradication approach more economical than the suppression approach (Fig. 1). However, an eradication strategy requires greater initial capital investment than a suppression strategy. In addition, after eradication has been achieved, the fly-free area will be exposed, depending on the situation, to a certain risk of reinfestation (quarantine operations are vulnerable and subject to some degree of failure). Recurrent pest outbreaks require costly emergency programmes to eradicate the reinfestations, and the enforcement of quarantine restrictions by trading partners can last for months or even years. This cost needs to be included when calculating the total cost of an eradication programme. There are situations where the risk of reinfestation is very high, and an eradication programme is not economically justifiable if there are recurrent outbreaks that disrupt agricultural trade; the stakeholders would be better off with suppression, which entails a lower risk. Alternatively, a preventive release programme can be cost-effective in situations where there is a high risk of reinfestation (Hendrichs, Vreysen et al., this volume; Mumford, this volume).

From technical and economic points of view, any one of the strategic approaches described above may, in a particular situation, be the best option. However, in some situations, the establishment and strict enforcement of quarantine barriers would be too disruptive to trade, excluding the eradication option. The choice depends on the conditions prevailing in the target area, such as the pest's status, the geography and ecology of the area, the available resources and overall plant protection and quarantine infrastructure, and the commitment and level of participation of stakeholders and their willingness to take risks. Therefore, it is critical that a decision to embark on one or the other approach is based on technically and economically sound analyses of the pest problem.

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## 4. BENEFITS ACCRUED FROM FRUIT FLY PROGRAMMES USING THE SIT

The SIT can be a cost-effective component of the AW-IPM of some of the most important fruit flies of economic significance, such as the Mediterranean fruit fly, oriental fruit fly *Bactrocera dorsalis* (Hendel), melon fly *Zeugodacus cucurbitae* (Coquillett), Mexican fruit fly *Anastrepha ludens* (Loew), West Indian fruit fly *Anastrepha obliqua* (Macquart), Queensland fruit fly *Bactrocera tryoni* (Froggatt), and Caribbean fruit fly *Anastrepha suspensa* (Loew) (Nigg et al. 2004).

In the past, at least 27 AW-IPM programmes in the world have integrated the SIT to control fruit flies. In Table 1, they are grouped by the strategic options pursued, i.e. eradication, suppression, containment, prevention, and combinations thereof (section 3.2.; Hendrichs, Vreysen et al., this volume). A few programmes have been selected to illustrate the benefits that have been produced.

Most of the data available on programme benefits and impact are based on quantifications of direct benefits, and in a few cases indirect benefits are also included. However, most of the indirect benefits are usually not accounted for, and therefore in most cases the overall benefits of these programmes are underestimated. Nevertheless, it is very evident that, when the technology is applied properly, the programmes generate substantial benefits to the horticulture industry and to society.

### *4.1. Fruit Fly Prevention Programme — California, USA (Box 1)*

#### *4.1.1. Problem Definition*

The Mediterranean fruit fly is a serious threat to California's economy. For the fruit industry alone, the annual value of susceptible crops in 2002 was about USD 4700 million (CASS 2002; USDA/NASS 2002, 2003a, b). The rate of introduction of this non-native pest, which can attack more than 250 different crops (Liquido et al. 2014), has been increasing with growing trade and tourism. Since 1975, more than USD 256 million (in state and federal funds) have been spent to eradicate small and large infestations, primarily in urban areas of the Los Angeles Basin and the San Francisco Bay area.

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*Table 1. Fruit fly AW-IPM programmes that integrated the SIT, grouped by strategic option*

| Strategic option | Programme |
|---|---|
| Prevention | Mediterranean Fruit Fly Preventive Release Programme (Los Angeles Basin, California, USA), 1996 – present |
| | Mediterranean Fruit Fly Preventive Release Programme (Tampa-Palm Beach-Miami, Florida, USA), 1998 – present |
| | Mexican Fruit Fly Preventive Release Programme (Rio Grande Valley, Texas, Mexico – US border), 1980s – present |
| | Mexican Fruit Fly Preventive Release Programme (Tijuana, Baja California, Mexico – US border), 1980s – present |
| | National Fruit Fly Control Programme (Chile), 1996 – present |
| Containment | Mediterranean Fruit Fly Programme "Programa Moscamed" (Guatemala, Mexico, USA), 1983 – present |
| | Binational Chile-Peru Programme for Mediterranean Fruit Fly Eradication, 1996 – present |
| | Queensland Fruit Fly SIT Control Programme (South-Eastern Australia), 1980s – present |
| Eradication | Mediterranean Fruit Fly Programme "Programa Moscamed" (Guatemala, Mexico, USA), 1978 – 1982 |
| | Melon Fly Eradication Programme (Okinawa, Japan), 1978 – 1993 |
| | Oriental Fruit Fly Eradication Programme (Guam), 1963 – 1964 |
| | National Mediterranean Fruit Fly Programme (Chile), 1990 – 1995 |
| | Carnarvon Medfly Eradication Project (Carnarvon, Australia), 2016 – present |
| | Mediterranean Fruit Fly SIT Control Programme (Southern Australia), 1980s – present |
| | Mexican Fruit Fly and West Indian Fruit Fly Eradication Programme (Northern Mexico), 1988 – present |
| | Mediterranean Fruit Fly Eradication Programme "PROCEM" (Mendoza – Patagonia, Argentina), 1992 – present |
| | Mediterranean Fruit Fly Eradication Programme (Altagracia, Dominican Republic), 2015 – 2017 |
| Suppression | Mediterranean Fruit Fly Suppression Programme (Arava, Israel), 1994 – present |
| | Mediterranean Fruit Fly Suppression Programme (South Africa), 1997 – present |
| | Oriental Fruit Fly Suppression in Pilot Areas (Thailand), 2000 – present |
| | Melon Fly, Oriental Fruit Fly, and Mediterranean Fruit Fly Area-Wide Integrated Pest Management Program (Hawaii), 1960s – present |
| | Mediterranean Fruit Fly Suppression Programme "Programa Madeira-Med" (Portugal), 1998 – 2008 |
| | *Bactrocera* spp. Pilot Suppression Programme (Guimaras Island, Philippines), 1996 – 1999 |
| | Mediterranean Fruit Fly Suppression Programme (Israel/Jordan), 1994 – present |
| | Mediterranean Fruit Fly SIT Control Programme (Peru), 1970s – present |
| | National Fruit Fly Campaign (Central and Southern Mexico), 1991 – present |
| | Mediterranean Fruit Fly Suppression (Tunisia), 2001 – 2007 |
| | Mediterranean Fruit Fly Suppression Programme (Valencia, Spain), 2005 – present |
| | Mediterranean Fruit Fly Suppression Programme (Neretva Valley, Croatia), 2009 -- present |
| | Mediterranean Fruit Fly Suppression Programme (Agadir, Morocco), 2010 -- present |

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*4.1.2. Major Achievements*

The Preventive Release Program (PRP) has resulted in a 96% reduction in the number of wild Mediterranean fruit flies caught in the treated Los Angeles basin, and more than a 96% reduction in the number of fly infestations. Before the change in 1994 to an area-wide approach (1987–1993), an average of seven or eight Mediterranean fruit fly outbreaks occurred every year, costing the state approximately USD 33 million per year (USDA/APHIS 1992). Since the implementation of the PRP in 1996, and after eliminating all outbreaks, the overall cost of the programme has been cut in half, and only a few small Mediterranean fruit fly infestations have occurred within the boundaries of the release area. These infestations remained confined and were quickly eliminated without applying aerial sprays of toxic bait and without trading partners establishing temporary quarantines for Californian exports.

*Box 1. Mediterranean Fruit Fly Preventive Release Programme (PRP) in the Los Angeles Basin, California Department of Food and Agriculture (CDFA 2002)*

After years of using the conventional reactive approach, i.e. detecting and eradicating Mediterranean fruit fly infestations at a local level, in 1994 the control strategy shifted to a proactive approach that emphasized area-wide measures over the whole Los Angeles Basin, including twice-weekly sterile-male releases. In view of its success, this release programme has continued preventively since 1996, and has become a major component of CDFA's comprehensive approach to prevent this pest from becoming established in California (Dowell et al. 2000). This programme is jointly coordinated and operated by county, state, and federal authorities.

*4.1.3. Estimated Benefits*

The impact of this programme has been measured in terms of the potential economic losses that the pest would inflict on the horticulture industry and home-garden fruit production if it became established in California. The parameters that have been used to measure the impact are the following: yield loss in agriculture and home-garden production, increased insecticide use (from 127 to 2270 tonnes/year of active ingredient), loss of export markets, and annual quarantine compliance costs (CDFA 2002). In 1991 it was estimated that a permanent Mediterranean fruit fly infestation could cost California's economy USD 1300–1900 million annually (yield loss, control costs, quarantine compliance costs, and loss of export markets, not including the social and environmental costs), with the loss of 14 000 or more jobs (Siebert and Pradham 1991). The current estimated benefit/cost ratio (BCR) of the PRP programme in California ranges from 87 to 187 (K. Hoffman, personal communication).

The insecticide savings (amount of active ingredient not used) have been very large. If the CDFA had continued to apply aerial toxic-bait sprays on the same 3800-$km^2$ area that was infested in 1994, some 458 tonnes of malathion would have been used (CDFA 2002). In contrast, since implementing the basin-wide sterile Mediterranean fruit fly releases, only a small amount of insecticide has occasionally been applied against pest incursions as part of the emergency reaction plan to some of the reduced number of Mediterranean fruit fly outbreaks that still occur periodically in California.

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### *4.2. Fruit Fly Prevention/Containment Programme — Chile* (Box 2)

#### *4.2.1. Problem Definition*

An important component of the Chilean economy is the production and export of fruits and vegetables, worth about USD 2000 million annually. As a prerequisite to importing these commodities, some of the main commercial partners, e.g. USA and Japan, require that produce be grown in certified fruit fly-free areas. Except for Arica province at the northern tip of the country, and occasional outbreaks in other provinces, Chile has historically been regarded as a fruit fly-free country. However, some countries used the presence of the Mediterranean fruit fly in northern Chile as a pretext to close their markets to Chilean horticultural products; as a result, since the 1980s, this stimulated significant eradication efforts in Arica province. Following various failed attempts to eradicate the Mediterranean fruit fly from northern Chile using baits sprays, in late 1990 the SIT was introduced. After 6 years of an intensive integrated area-wide programme, in 1995 the fly was eradicated in Arica, and Chile was declared a fruit fly-free country (MAG/SAG 1995).

#### *4.2.2. Major Achievements*

Since its inception in 1980, Chile's National Fruit Fly Programme has, through effective surveillance and eradication activities, successfully kept the country free of economic species of fruit flies. Chile's programme to remain fruit fly-free is one of the best in the world.

Strengthening binational cooperation with Argentina and Peru, and formalizing binational cooperation with Bolivia, are the cornerstones that sustain Chile's fruit fly-free status.

#### *4.2.3. Estimated Benefits*

Since Chile was declared a fruit fly-free country, fruit exports have grown to an annual 320 million boxes of fruits, mainly table grapes, apples, stone fruits, kiwis, and avocados, valued in 2016 at USD 4000 million (ASOEX 2017).

Each year the government of Chile spends on average USD 4 million to keep the country free of fruit flies. If multiple outbreaks occurred in Chile's Metropolitan Region, the industry would lose an estimated USD 78 million per year, just in market loss and compliance with US quarantine regulations. Chilean exports of fruits and vegetables are valued at approximately USD 4000 million per annum. If this figure is divided by the average annual cost of the national programme, a BCR of 1000:1 is obtained. This ratio would be even higher if other cost factors, e.g. control costs, yield losses, and general social and environmental costs, were included (Lindquist and Enkerlin 2000; R. Rodriguez, personal communication).

Chile's National Fruit Fly Programme (Box 2) has been the driving force behind the expansion of the fruit and vegetable export industry, one of the main contributors to the country's gross domestic product (GDP).

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*Box 2. National Fruit Fly Programme in Chile*

Chile's fruit fly-free status has allowed one of the most important export-oriented horticulture industries in the world to develop. To protect this valuable asset, in 1980 the Government of Chile, through the Servicio Agricola Ganadero de Chile (SAG) of the Ministry of Agriculture (MAG), created Chile's National Fruit Fly Programme — to prevent the introduction and establishment of any fruit fly species of economic importance, including the Mediterranean fruit fly and the economic species in the genera *Anastrepha* and *Bactrocera* (Olalquiaga and Lobos 1993).

The National Fruit Fly Programme in Chile operates through a centralized organizational structure of the Ministry of Agriculture. As part of a regional approach to the fruit fly problem, the Government of Chile has subscribed binational agreements with Argentina and Peru. Through these agreements the quarantine infrastructure and fruit fly control activities in these neighbouring countries have been strengthened, and thus the risk of introducing fruit fly pests from these countries has been reduced.

Chile has achieved its fly-free status by implementing two major strategic activities:

1. Effective national and international quarantine system (including interprovincial quarantine road stations and international quarantine at ports of entry), and an extensive and highly sensitive fruit fly-trapping network to detect fruit fly introductions at an early stage. Outbreaks of exotic fruit flies, mainly the Mediterranean fruit fly, have been eradicated through the effective execution of an emergency eradication plan based on detecting and eradicating infestations. A *B. dorsalis* outbreak on Easter Island was eradicated in 2011 at a cost of USD 100 000 (AGROMEAT 2017).
2. In Arica province, the ongoing Mediterranean fruit fly AW-IPM programme that integrates the SIT functions as a containment barrier to avoid the natural or artificial spread of fly populations into northern Chile, protecting the main fruit and vegetable production areas in the central and southern parts of the country.

For years, Chile has been subjected to increasing risks of pest introductions through more trade, tourism, and people coming from neighbouring countries. Consequently, there has been an increase in the rate of Mediterranean fruit fly detections and outbreaks. Nevertheless, the Government of Chile (through SAG) has strengthened its National Fruit Fly Programme by gradually incorporating state-of-the-art technology. This includes introducing an improved genetic sexing strain of the Mediterranean fruit fly into the mass-rearing facility in the Lluta Valley (Arica province), DNA-identification techniques to assess the geographical origin of introduced flies, updated fly-trapping systems, and X-ray machines at critical points of entry to facilitate detection and confiscation of fruit that are hosts of the Mediterranean fruit fly (Lindquist and Enkerlin 2000; Gonzalez and Troncoso 2007).

### *4.3. Fruit Fly Eradication Programme — Japan* (Box 3)

#### *4.3.1. Problem Definition*

In its current distribution range, the melon fly is the most destructive pest of cucurbit crops. It is found in Africa, India, South-East Asia, and islands in the Pacific (including Hawaii). In Japan, it was first discovered in 1919 in the Yaeyama Islands, and between 1919 and 1970 it invaded most island groups in the south of Japan. In Okinawa, this pest affected more than 40 important vegetables and fruits, inflicted substantial direct damage to fruits, and prevented the export of these infested commodities to fly-free areas.

Sugarcane was the most important cash crop in Okinawa, accounting for about 50% of cultivated land and 20% of all farm income. However, as a result of stagnant prices and productivity, as well as increased international competition, income from sugarcane production declined significantly. Okinawa's sugar industry survived only because of the government's price-support programme. As a result, farmers and the local government needed to diversify from monoculture sugarcane-centred agriculture into other cash crops, e.g. flowers, and tropical vegetables and fruits such

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as mango. The other promising agricultural strategy was to produce "healthy" food, and for Okinawa to become a brand name for "healthy longevity" because of its world-renowned "healthy island" image.

Due to this situation in Okinawa, in the early 1970s the eradication of the melon fly became the top priority project at local and national levels. The problem was urgent since the fly was rapidly spreading to other islands and, unless specific eradication measures were undertaken, might spread to mainland Japan.

#### *4.3.2. Major Achievements*

- First successful use of the SIT for melon fly eradication in island communities,
- Eradication was achieved with no harm to the health of the public or the environment,
- Lifting of internal quarantine regulations to allow transport to non-infested areas resulted in significant expansion of major horticultural products,
- Training of foreign technicians in the AW-IPM of the melon fly.

*Box 3. Melon Fly Eradication Programme in Okinawa, Japan (Kakazu 2002)*

Okinawa's prefectural government, with the support of the Japanese national government and active participation of staff of municipalities (mainly in field operations) and agricultural cooperatives, operated the programme. In 1972, an experimental melon fly eradication project using the male annihilation technique (MAT) and the SIT was conducted in Kume Island. Following successful eradication, in 1978 a project was initiated that gradually covered most archipelagos in the south of Japan; it was completed in 1993. In view of the geographic closeness of the southernmost islands to Taiwan, where the melon fly is present, in these islands the programme currently conducts a continuous detection, quarantine, and preventive release operation against re-establishment of the pest.

#### *4.3.3. Estimated Benefits*

A post-programme economic assessment, prepared by the Research Institute for Subtropics (Kakazu 2002) using the methodology described by Enkerlin and Mumford (1997), clearly shows the benefits of melon fly eradication. The estimated net-benefits are those arising from the commercial shipments of commodities that are hosts of the melon fly. Not included in the assessment were indirect benefits for the island economies, such as human health and environmental protection, insecticide-free farming, preservation of natural enemies, savings in fumigation and quarantine costs, and above all preventing the insect from spreading to the mainland of Japan. Furthermore, due to data constraints, the indirect social benefits were not estimated. The programme proved to be economically viable, even though only the increase in commodity shipments was considered in the benefit equation.

If the northward spread of the melon fly had not been stopped, the potential loss for farmers and the horticulture industry in general would have been very substantial, and the cost of an eradication programme would have risen enormously. As a result of the successful eradication programme, the production of Okinawa's high-valued niche products, such as bitter melon and mango, have risen sharply. Between 1990 and 2000, bitter melon production rose from 2720 to 6220 tonnes, a 2.3-fold increase. Similarly, over the same period, mango production increased from 278 to 1290 tonnes, a 4.6-fold increase; all together this is equivalent to USD 335

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million in 10 years. (These benefits are underestimated since the savings from fumigation and quarantine costs were not included in quantifying the benefits.) This figure should be compared with the much lower total programme cost, USD 172 million, during the 16-year eradication period (1978–1993).

In 1996, 3 years after achieving eradication in the last islands (Yaeyama Islands), the programme reached its break-even point and repaid the initial investment. Between 1997 and 2000, the programme cost was estimated to be USD 31 million, but gross revenues were USD 167 million (from the sale of commodities shipped to mainland Japan and other countries). If the total accumulated gross revenues are divided by the total accumulated costs during this time period, the BCR shows that 5.4 dollars were returned for each dollar invested. For a public investment project in Japan, it is a remarkable achievement that the programme was generating positive net-benefits only 3 years after eradication.

### *4.4. Fruit Fly Suppression Programme — South Africa* (Box 4)

#### *4.4.1. Problem Definition*

The Mediterranean fruit fly and the Natal fruit fly *Ceratitis rosa* Karsch occur in the Western Cape province, South Africa. The Hex River Valley is a major production area for table grapes; an average of 15.5 million cartons is exported annually. The dominant and economically important species is the Mediterranean fruit fly. It causes direct damage to fruit, requiring costly insecticide sprays, and infested fruit results in rejections of boxed table grapes by the phytosanitary inspectors of importing countries (Barnes and Eyles 2000; Barnes et al. 2015; Barnes 2016).

#### *4.4.2. Major Achievements*

Growers and scientists now understand the benefits of applying the concept of AW-IPM and the advantages to international trade of establishing low pest prevalence areas (FAO 2017). They also have effectively adopted the SIT technology and established the required infrastructure, which is now managed by the private sector. According to growers in the valley, integrating the SIT to suppress the Mediterranean fruit fly has been very successful. In the 3 years (1997–1999) before the release of sterile flies, fly mean population levels were 0.9–1 flies per trap per day, but in 3 years of the release period (2000–2002) the levels decreased to 0.1–0.4 flies per trap per day. From 1997 to 2002 insecticide use dramatically decreased (Barnes et al. 2004). These positive results have encouraged other associations of fruit growers outside the Hex River Valley to embark on SIT activities as well.

#### *4.4.3. Estimated Benefits*

By using environment-friendly technology, and reducing production costs and increasing revenues, the Mediterranean fruit fly suppression programme increased the profits of the table-grape industry in the Hex River Valley. By replacing insecticide applications with a combination of aerial and ground releases of sterile male flies at hot spots, the reduction in control costs was substantial, from USD

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350 000/year with chemical control to USD 130 000/year with the SIT. Rejections, due to fruit fly infestation, of exported cartons of table grapes from the valley were reduced by approximately 50%. In 2000, a reduction of 60% in rejection of cartons by phytosanitary inspectors of importing countries represented savings of USD 150 000. For the 2001/2002 season, the direct benefits totalled USD 370 000/year, at a cost of USD 130 000, which is equivalent to a BCR of 2.8:1.

The expected medium-term impacts of the area-wide project include:

- Expanding export markets by meeting sanitary and phytosanitary restrictions,
- Creating new jobs in agriculture (not only for large producers, but also emerging small producers) and related industries,
- Strengthening regulatory, research and development support of the fruit industry,
- Protecting the environment and the health of farm workers.

*Box 4. South Africa Mediterranean Fruit Fly Suppression Programme (IAEA 2002)*

In 1997, a pilot project to control fruit flies integrating the SIT was implemented in 10 000 hectares (100 $km^2$) in and around the Hex River Valley. The goal was to suppress, in a cost-effective and an environment-friendly manner, the Mediterranean fruit fly populations to below the economic threshold, and then create an internationally recognized low pest prevalence area (FAO 2017).

The organizational structure of this project is rather unique. It is a partnership between Infruitec/Nietvoorbij (a branch of the Agricultural Research Council (ARC), a parastatal body, with a mandate to conduct research, technology development and transfer) and the Hex River Valley Research Services Trust (which represents the deciduous fruit growers). Through an export carton levy, the growers raise funds to support programme operations (Barnes and Eyles 2000). The sterile male production, initially established by the ARC, is now managed by the private sector, and growers manage the fly release and other field operations (Venter et al. 2021).

### *4.5. Fruit Fly Containment Programme — Mexico and Guatemala* (Box 5)

#### *4.5.1. Problem Definition*

The Mediterranean fruit fly was introduced into Costa Rica in 1955 (Enkerlin et al. 1989). It spread across Central America, causing devastating effects on fruit production, and limiting the development and growth of the fruit industry (section 2). The pest became established in Guatemala in 1976, and in 1977 was detected in the border zone between Guatemala and Mexico. By 1979, the fly had spread to the Mexican states of Chiapas and Oaxaca, beyond the coffee belt along the South Sierra Madre Mountains, and threatened the states of Campeche, Tabasco, and Veracruz. If the pest had advanced beyond the Isthmus of Tehuantepec, 600 km into Mexican territory, the USA government threatened to close its border to imported Mexican fruits and vegetables, and fly eradication would have been practically impossible (Schwarz et al. 1989).

Gutierrez (1976) estimated that preventing the establishment of the Mediterranean fruit fly in Mexico translated into annual savings of at least USD 2000 million in direct damage (including yield loss and cost of insecticide treatment) and indirect damage (including loss of the price differential obtained from selling the produce in export markets). In addition, if the pest became established, thousands of jobs across the production chain would be lost, and substantial

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environmental costs would be generated by the tonnes of insecticides that would have to be sprayed to keep the pest under control.

*4.5.2. Major Achievements*

After 4 years of intensive eradication activities (1977–1982), the first programme objective was met — the Mediterranean fruit fly was eradicated from an infested area of 640 000 hectares (6400 $km^2$) in the state of Chiapas, Mexico, using an AW-IPM approach that included legal measures (e.g. quarantine regulations), various control methods (chemical, mechanical, and cultural), and the SIT (a mass-rearing facility, producing 500 million sterile flies per week, was completed in 1979). This was the first time that a tephritid fruit fly population was eradicated at a continental level, in a region of difficult topography, high ecological diversity, and using an environment-friendly technology (Hendrichs et al. 1983).

Since eradication was achieved in 1982, for 35 years (1982–2016) the programme successfully maintained a sterile fly containment barrier. This barrier has prevented the northward spread of the pest, protecting the horticulture industries in Mexico and the USA (worth thousands of millions of dollars) (Orozco et al. 1994; Villaseñor et al. 2000; Enkerlin et al. 2015, 2017). In addition, Belize and the area of Petén (northern part of Guatemala) have been kept free of the Mediterranean fruit fly. This fly-free status has been recognized officially by the Mexican and US governments, and benefits papaya fruit growers in Petén who can now export their produce to fruit fly-free countries. The value of papaya exports to the USA in 2013 was estimated at USD 13.6 million. Moreover, other Guatemalan fly-free and low prevalence areas have been certified — Quetzaltenango, where peaches are produced commercially, and Laguna de Retana, where tomatoes and peppers are produced and exported to the USA at an estimated value in 2014 of USD 30 million.

The programme has continued to develop and adapt technologies to large-scale conditions — new and more cost-effective mass-rearing, release and field technologies for Mediterranean fruit fly control, including the temperature-sensitive lethal (*tsl*) male-only fly strain (Cáceres et al. 2000, 2004; Franz et al., this volume), the more environment-safe insecticide spinosad (Rendón et al. 2000; USDA/APHIS/PPQ 2000), more sensitive female-biased trapping systems (Heath et al. 1995; IAEA 1999), more effective longer-lasting bait stations (Piñero et al. 2014), and more precise and intelligent chilled-adult release machines (Leal Mubarqui et al. 2014).

These technological advances, and continuing commitments from the governments of the three countries involved, have been the basis since 1999 for increased cost-effectiveness of the Mediterranean fruit fly eradication programme (Tween 2004). Moreover, it has created the basis for continuing eradication efforts in Guatemala, moving the leading edge of the infestation to the borders of El Salvador and Honduras, where the Central American isthmus starts and where the containment barrier would narrow to 50% of its current length (IICA 2013; Enkerlin et al. 2017; Rendón and Enkerlin 2021).

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*Box 5. Mediterranean Fruit Fly Containment Programme "Programa Moscamed"*

Due to the threat that this pest posed to horticulture industries in Guatemala, Mexico, and the USA, in 1976 and 1977 the governments of the three countries, with the support of several international agencies (including the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA)), subscribed cooperative agreements to create the "Moscamed Programme", a Mediterranean fruit fly programme using the SIT, with the following objectives:

- Eradicate the Mediterranean fruit fly in infested areas of the state of Chiapas, Mexico,
- Establish a containment barrier at the Mexico-Guatemala boarder, and continue eradication activities in Guatemala to create a fly-free buffer area between the containment barrier and the leading edge of the infestation in Guatemala,
- Eventually eradicate the Mediterranean fruit fly throughout Central America and Panama.

In 2015, the bilateral agreements were merged into a single Trinational Moscamed Agreement, which has strengthened the programme by facilitating harmonization of technical procedures and providing legal certainty.

### *4.5.3. Estimated Benefits*

The primary impact of the Moscamed Programme has been to protect the expanding horticulture industries of Mexico and the USA. As a result, and under the framework of the North American Free Trade Agreement (NAFTA), Mexico's gross revenues from horticultural products have tripled since 1994, to more than USD 9 billion per year (RIC 2017).

For Mexico, the net revenue (net present value) of the programme has been estimated at USD 39 300 million over 31 years (1978–2008), compared with the total cost of the programme in Mexico over the same period of time (estimated at USD 459 million), showing a remarkable economic return of USD 112 in crops and control costs saved for each dollar that was invested. The return is substantially higher for the USA, where the value of the fruits and vegetables that would be affected by the fly is several times higher than for Mexico. In addition, these returns are even underestimated, since no monetary value has been placed on the social and environmental savings obtained by preventing establishment of this pest.

The economic impact of the Moscamed Programme on the local economies of Chiapas (Mexico) and Guatemala has been substantial. The Moscamed Programme (which began operations in Chiapas) has permanently employed an average of 400 people (who sustain an estimated 2000 family members), apart from the hundreds that have been hired for temporary work in the factory and field operations. In 35 years, from the total programme budget, approximately USD 240 million have been spent in Chiapas, mostly for wages and the purchase of services, supplies, and equipment (Programa Moscamed 2002, 2015; IICA 2013). Moreover, maintaining the state of Chiapas free of the Mediterranean fruit fly has allowed the expansion of mango and papaya production areas and export volumes, valued at millions of US dollars per annum, and has created thousands of jobs in rural areas (IICA 2013).

Indirect benefits of the programme include:

- Strengthening the plant protection infrastructure in both Mexico and the USA, including the national and international quarantine, and national surveillance, systems — systems that protect the two countries from other exotic fruit fly pests, and also from other pests of quarantine importance that are intercepted through the same quarantine infrastructure and resources,

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- Recognition of the programme, by regional and international organizations, as an International Fruit Fly Training Centre — contributing greatly to technology transfer in more than 40 countries of Latin America and other parts of the world (including the Caribbean, Africa, and Asia) through the hands-on training of hundreds of professional scientists, and
- Transfer of the SIT technology, that has been improved and validated on a large scale, to other fruit fly AW-IPM programmes integrating the SIT in other parts of the world.

Keeping Mexico and the USA free of the Mediterranean fruit fly has not only protected the horticulture industries of these countries but, for Mexico, it has also created an opportunity to develop the industry into a multimillion dollar export-oriented enterprise (Economist 2004).

### *4.6. Fruit Fly Suppression/Eradication/Prevention Programme — Mexico* (Box 6)

#### *4.6.1. Problem Definition*

Mexico has more than 1 million hectares planted to fruit crops, with an estimated annual production value of more than USD 2500 million (SAGAR/IICA 2001). The fruit industry is significantly hindered by four fruit fly species: Mexican fruit fly, West Indian fruit fly, guava fruit fly *Anastrepha striata* Schiner, and sapote fruit fly *Anastrepha serpentina* (Wiedemann). The annual direct damage that these native fruit flies cause is more than USD 230 million (Reyes et al. 1991). This amount does not include the cost of insecticide applications, and the losses due to restrictions in fruit commercialization. These restrictions prevent the industry from benefiting from price differentials, and more importantly from market diversification, negatively affecting the general development of the industry. Some other losses are also not included, e.g. cost to human health from moderate and acute poisoning arising from applying insecticides, shortage in the supply of fruits, and negative impact on the environment.

#### *4.6.2. Major Achievements*

- Fruit flies of economic importance have been eradicated in more than 35 000 hectares of commercial plantations of citrus, mango, apple, and peach in north-west Mexico, completely freeing from fruit flies of economic importance the States of Chihuahua, Sonora, Baja California Norte, and Baja California Sur (SAGARPA 2001).
- In the north-east and central regions including the states of Nuevo Leon, Tamaulipas and San Luis Potosi, SIT suppression activities have reduced fruit fly populations to low-prevalence levels in parts of the more than 30 000 hectares of commercial citrus production (Orozco-Dávila et al. 2017).
- By creating federal legal instruments in support of the campaign, the construction of additional interstate quarantine checkpoints, and installation of X-ray equipment at specific ports of entry, have been possible. This has strengthened the international and national quarantine system, providing greater protection from exotic fruit flies and other pests of plants and animals.

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- Mobilization and organization of fruit growers at the local level into Plant Protection Committees that link the producer associations to the plant protection activities at the state and federal government levels.
- Through training courses and workshops, a work force of hundreds of professional scientists, specialized in the large-scale and area-wide operation of phytosanitary campaigns, has been deployed throughout the country.

*Box 6. Mexico National Campaign against* Anastrepha *Fruit Flies*

*Anastrepha* spp. fruit flies are major horticultural pests in Mexico. After a thorough economic feasibility study (Reyes et al. 1991), in which the returns of integrating the SIT into the control of fruit flies in major commercial fruit production areas were assessed, the Mexican federal government in 1992 approved the National Fruit Fly Campaign (Campaña Nacional Contra Moscas de la Fruta (CNCMF)), with the following objectives:

- To suppress, and in selected areas eradicate, fruit flies of economic importance in fruit production areas, using an AW-IPM approach including the SIT.
- To protect Mexico from the introduction and establishment of other economic fruit fly species that threaten the country.

The CNCMF is part of the Plant Protection General Directorate of the Ministry of Agriculture, Livestock and Rural Development (SAGARPA). The CNCMF operates through state governments and fruit-grower associations under compliance agreements subscribed to by the three parties (federal and state governments, and fruit industry).

The resources required to operate the programme are contributed in equal parts by the federal and state governments and the fruit industry, in financial and in-kind contributions. The federal government facilitates the legal instruments for smooth implementation of the programme, supplies the sterile flies, and operates international quarantine stations at ports of entry. The state governments, through their plant protection infrastructure, are responsible for distributing and releasing the sterile flies, and for conducting field activities outside the fruit production areas to assure area-wide control of the pests. Responsibilities also include operating quarantine interstate road stations. At the farm level, the programme is conducted by the fruit industry, through Plant Protection Committees, that link the producer associations with the state and federal governments. Activities of producers in orchards include trapping, applying bait sprays, and releasing sterile flies (Reyes et al. 2000).

### *4.6.3. Estimated Benefits*

In the first 4 years after 1997, when fruit fly eradication in north-west Mexico was officially declared, the direct benefits (reduced fruit fly damage and increased yield) amounted to USD 25 million. In addition, in the same time period, the benefits obtained from the price differential paid by export markets, and savings in postharvest treatments, totalled approximately USD 35 million. Thus, the total benefits in these fruit fly-free areas over 4 years amounted to USD 60 million, with a total cost of USD 4 million over the same time period, resulting in a BCR of 7.5 to 1 (SAGAR/IICA 2001).

A retrospective economic assessment was conducted to estimate the return on investment of the National Fruit Fly Campaign, specifically for mango and citrus, two of the mayor fruit crops produced in Mexico that are affected by fruit fly pests. Results indicated a BCR of 22 for mango and of 19 for citrus, clearly justifying the investment on fruit fly control done by the Mexican Federal Government and the fruit growers (IICA 2010).

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The eradication of fruit flies, and subsequent international recognition and maintenance of the fruit fly-free status, permitted an expansion of the area planted to fruit crops in the north-western states to 50 000 hectares. No doubt this has resulted in substantial economic and social benefits to that region of Mexico.

### *4.7. Other Successful Programmes*

Section 4 describes examples of programmes that have produced substantial economic returns after applying area-wide SIT against fruit fly pests; economic assessments or detailed quantification of costs and benefits are available for these programmes. However, there are other successful programmes for which detailed economic assessments have not yet been done, but that have also generated substantial benefits. One of these programmes is the SENASA Fruit Fly Control Programme in Peru, where in 2008 the eradication of the Mediterranean fruit fly and *Anastrepha* fruit flies from the regions of Tacna and Moquegua, integrating the SIT, was declared officially. This was achieved through a joint venture of the Peruvian State, producers, exporters, and regional and local authorities; financial support was also obtained from the Inter-American Development Bank, and technical and financial support from the FAO and IAEA. Programme achievements included: avoiding annual losses of more than USD 12 million in the production of fruits and vegetables, generating direct benefits to 17 876 producers in approximately 38 000 ha, avoiding annual use of over 600 000 litres of insecticides, helping to preserve the environment, and creating opportunities for the national and international trade of horticultural products within the framework of trade agreements with the USA (FAO/IAEA 2008).

Another successful programme is the Moscamed Programme in the Dominican Republic. A large Mediterranean fruit fly outbreak, covering an area of 2000 $km^2$, occurred in March 2015 in the eastern part of the country. In only 10 months, USD 40 million was lost because of quarantine restrictions on exports of host commodities including tomatoes, bell peppers, and avocados. Eradication was achieved after two years of intensive area-wide integrated pest management including pest monitoring, population suppression using ground and aerial bait sprays and bait stations, and eradication using the SIT. Eradication was declared officially by the Ministry of Agriculture in July 2017; net revenues from fruit and vegetable exports have since been re-established. Eradication of the Mediterranean fruit fly benefited the Dominican Republic, and also protected the horticultural industries in the Caribbean region and in neighbouring countries including the USA and Mexico (FAO/IAEA 2017; Gil 2017; Zavala-López et al. 2021).

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## 5. SUMMARY OF ECONOMIC RETURNS

The BCR achieved in some major fruit fly AW-IPM programmes integrating the SIT is high, ranging from 2.8 to 1000, clearly showing that the SIT technology, when properly integrated with other methods and applied on an area-wide basis, is feasible and economically viable (Table 2). Even when the types of benefits accrued from these programmes are similar, the returns on investment (BCR) vary widely, even among programmes with common fruit fly pest problems and similar strategic objectives. This is due to the different intrinsic characteristics of each programme, e.g. the magnitude of the pest damage, size and value of the crops being protected, targeted markets, commitment of the main stakeholders, resources available to execute the programme, and efficiency in programme management.

## 6. CONCLUSIONS

The high economic returns from some fruit fly AW-IPM programmes that integrate the SIT are possible primarily because of the environment-friendly and area-wide nature of the SIT technology. This technology allows cost-effective suppression, and in selected cases eradication, of insect pest populations, and also prevents the establishment of important fruit fly species in pest free areas through the use of pest risk-mitigation measures, such as SIT containment and prevention programmes. Such programmes protect, at a relatively low cost, high-value horticulture industries, such as those in Argentina, Australia, Chile, Mexico, and the USA. This is a major advantage of the SIT technology when compared with more conventional pest control methods such as insecticides (Enkerlin 2003). In contrast, the worldwide BCR of insecticides has been estimated at 4:1, if indirect costs are excluded, and only a 2:1 ratio if indirect environmental and public health costs are included (Pimentel 1991). As the use of the SIT technology for certain pests and crops expands, with increasing cost-effectiveness at economies of scale, it is expected that the release of sterile insects will gradually reduce the application of insecticides. The insecticide industry is gradually viewing the SIT as an opportunity for diversification rather than as a competing technology; this has already happened with other control methods that are biological in nature.

One of the unique features of the integrated application of the SIT is that, since its application is area-wide, the benefits spread beyond commercial fruit and vegetable producers to backyard gardens and subsistence farms in poor rural areas. In the future, the SIT will contribute even more to improved food security worldwide by increasing fruit and vegetable production in a cost-effective, environmentally clean, and sustainable manner.

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*Table 2. Estimated Costs, Benefits, and Benefit/Cost Ratios (BCR) for some Major Fruit Fly AW-IPM Programmes Integrating the SIT*

| Programme | Parameter used to measure benefits | Average annual cost[1] (USD million) | Average annual benefit (USD million) | Benefit/ cost ratio (BCR)[2] | References |
|---|---|---|---|---|---|
| Mediterranean Fruit Fly Containment Programme "Programa Moscamed" | Protection of horticulture industry in Mexico (includes only investment by Mexico and potential production losses and costs, and market loss) | 14.8 | 1660 | 112 | Gutierrez 1976; Reyes et al. 1991; IICA 2009, 2013 |
| Melon Fly Eradication Programme in Okinawa, Japan | Export of fruit commodities (mainly mango and bitter melon) after eradication was achieved | 7.7 | 41.7 | 5.4 | Kakazu 2002 |
| National Mediterranean Fruit Fly Control Programme in Chile | Export of horticultural products affected by fruit flies | 4 | 4000 | 1000 | MAG/SAG 1995; Lindquist and Enkerlin 2000; ASOEX 2017 |
| Hex Valley Mediterranean Fruit Fly Suppression Programme in South Africa | Savings in chemical sprays and table-grape rejections during the certification process | 0.13 | 0.37 | 2.8 | IAEA 2002 |
| Mexico National *Anastrepha* Fruit Fly Campaign | Savings in direct damage and value of fruit exports from the fly-free areas in north-west Mexico | 32 | 772 | 24 | IICA 2010 |
| Mediterranean Fruit Fly Preventive Release Programme in Los Angeles Basin, California, USA | Protection of California's horticulture industry from yield loss in agriculture and home-garden production, increased insecticide use, loss of export markets, and annual quarantine compliance cost | 15 | 1300–1900 | 87–127 | CDFA 2002 |
| Control of the Mediterranean Fruit Fly in Israel/Jordan | Export of vegetables from the Arava Valley | 0.8 | 8 | 10 | Cayol et al. 2004 |

[1]Includes fixed and variable operational costs, but not capital or financial costs.
[2]Assuming the whole benefit is lost in the event of fruit fly establishment and widespread infestation.

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# CHAPTER 7.3.

# IMPACT OF MOTH SUPPRESSION/ERADICATION PROGRAMMES USING THE STERILE INSECT TECHNIQUE OR INHERITED STERILITY

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## SUMMARY

More than 22 lepidopteran species have been investigated as candidates for control using the sterile insect technique (SIT) or inherited sterility (IS). However, to date only three programmes have been operationalized on a large-scale. The pink bollworm programme was successful at eradication across a broad swath of the cotton production area in south-western USA and northern Mexico by operating an area-wide control programme across this region using a combination of *Bt*-cotton, mating disruption, sanitation, and the SIT. The codling moth suppression programme in British Columbia, Canada, and the false codling moth in South Africa, have both been successful at effective suppression of the pest populations, reducing insecticide use, and improving interactions between growers and the general public. Other smaller-scale programmes against outbreaks of gypsy moth, cactus moth, and painted apple moth have also been successful, contributing to local eradications of invasive populations. New programmes are being investigated for managing a range of other target pests, including European grapevine moth in Chile, sugar cane borer in South Africa, tomato leafminer (for glasshouse populations in Europe), carob or date moth in North Africa, and naval orangeworm in California. Methods to further reduce the cost of lepidopteran programmes might include combining the SIT/IS with other environment-friendly pest control tactics such as mating disruption or the release of natural enemies, the development of genetic sexing strains, or the application of molecular technologies to develop genetic markers, genetic sexing, and genetic sterility. In the future, the greatest potential for impact of lepidopteran SIT/IS programmes may be in combating key invasive threats, with examples such as the eradication of an outbreak of the painted apple moth in New Zealand and the cactus moth in Mexico, or by adding an additional tool to pest control programmes where the use of insecticides may be limited by the development of resistance or the objection by residents in urban areas to ongoing treatments.

## 1. INTRODUCTION

Lepidopterans are among the most devastating agricultural and forest pests in the world. In the United States of America (USA) seven of the eight most serious insect pests of agricultural crops are lepidopterans (Peters 1987). According to a list of the 37 worst invasive insect pest threats to US agriculture and plant resources, 19 are lepidopteran species (ESA 2001). A review of global eradications revealed 144 eradication efforts against 28 species of Lepidoptera (Suckling et al. 2017). Control of these pests relies largely on insecticides, and the development of resistance is becoming a serious problem for many species, e.g. codling moth (Varela et al. 1993), diamondback moth (Tabashnick et al. 1990; Shelton et al. 1993), navel orangeworm (Demkovich et al. 2015), and pink bollworm (Bagla 2010; Tabashnik and Carrière 2010). In addition, the indiscriminate use of pesticides has had a significant negative

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impact on the environment. Of particular importance to agriculture is the destruction of crop pollinators and natural enemies that keep secondary pests in check (Edwards 2000; Zaller and Brühl 2019). Therefore, it is probably not surprising that more lepidopterans than any other group of insects have been investigated as potential candidates for integrated control using the sterile insect technique (SIT) or inherited sterility (IS) (a variation of the SIT that involves the release of partially sterile insects) (North 1975; LaChance 1985; Bloem and Carpenter 2001; Marec et al., this volume). At least 22 moth species have been investigated for their potential as SIT targets -- including those with completed radiation biology studies, with field tests, and with either pilot or operational programmes (Vreysen et al. 2016; Suckling et al. 2017).

In spite of the tremendous impact that area-wide control of key lepidopteran species could have, and that many species have been investigated in laboratory and field-cage studies for their suitability as candidates for SIT/IS programmes, field trials have been performed on only a limited number of species (Suckling et al. 2017). Of the 16 lepidopteran species that have been investigated in the field (Table 1), only three area-wide SIT/IS programmes have progressed to the operational stage. These are programmes against the pink bollworm in the southern USA and northern Mexico, the codling moth in British Columbia, Canada, and the false codling moth in South Africa. In these cases, partially sterile or sterile moths have been routinely released in the context of area-wide integrated pest management (AW-IPM) programmes that use a combination of pest control tactics.

*Table 1. Main moth pest species that have been investigated as SIT/IT candidates*

| Successful operational moth SIT/IS programmes | Past efforts to develop and apply moth SIT/IS | Ongoing methods development of moth SIT/IS |
|---|---|---|
| Pink bollworm *Pectinophora gossypiella* (Saunders) | Tobacco budworm *Heliothis virescens* (F.) | European grapevine moth *Lobesia botrana* (Denis and Schiffermüller) |
| Codling moth *Cydia pomonella* (L.) | Corn earworm *Helicoverpa zea* (Boddie) | African sugar cane borer[1] *Eldana saccharina* Walker |
| False codling moth *Thaumatotibia leucotreta* (Meyrick) | Light brown apple moth *Epiphyas postvittana* (Walker) | Navel orangeworm *Amyelois transitella* (Walker) |
| Painted apple moth *Teia anartoides* Walker | Oriental fruit moth *Grapholita molesta* (Busck) | Tomato leafminer[1] *Tuta absoluta* (Meyrick) |
| Cactus moth *Cactoblastis cactorum* (Berg) | European corn borer *Ostrinia nubilalis* (Hübner) | Carob/date moth *Ectomyelois ceratoniae* (Zeller) |
| Gypsy moth[2] *Lymantria dispar* (L.) | Asian corn borer *Ostrinia furnacalis* (Guenée) | |
| | Diamondback moth *Plutella xylostella* (L.) | |

[1] The SIT/IS has not been field-tested against these moth species.
[2] Even though successfully applied to eradicate gypsy moth outbreaks, the SIT/IS for this pest was discontinued eventually because more economic alternatives were developed (see section 3.1.).

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In addition, the release of partially sterilized male painted apple moths was successfully added to an eradication effort in Auckland, New Zealand, with aerial applications of *Bacillus thuringiensis v*ar. *kurstaki* (*Btk*) and an intensive trapping programme for this introduced pest, using virgin female moths (Suckling et al. 2007). The same approach was used to eradicate outbreaks of the invasive cactus moth in Mexico (Bello-Rivera et al. 2021).

Although studies on *G. molesta, O. nubilalis, O. furnacalis,* and *P. xylostella* generally reported positive results from releasing sterilized insects (Rosca and Barbulescu 1996; Apu 2002; Genchev 2002; Maung 2002; Wang et al. 2002; Yang et al. 2002), not enough detailed information concerning the size of treatment areas, release rates, release methods, and methods for evaluating efficacy of releases was provided. The programme against *L. dispar*, even though successfully applied to eradicate outbreaks, was eventually discontinued because more economic alternatives were developed. Also, programmes for *H. virescens* and *H. zea* were deemed uneconomic following a number of large-scale field studies.

## 2. SUCCESSFUL OPERATIONAL MOTH SIT/IS PROGRAMMES

The primary impact of successful lepidopteran SIT/IS programmes that has been reported is the degree of pest suppression or eradication, or the extent to which establishment of an invasive pest has been prevented in the treatment area. Quantification of other benefits, e.g. lower commodity-production costs, access to new markets, and fewer farm-worker health and safety problems or decreased ground-water contamination as a result of reduced insecticide use, has for the most part not taken place. Therefore, rather than limit this discussion to the little information available on accrued benefits from lepidopteran operational programmes, the major achievements of lepidopteran field programmes that have been undertaken are more broadly discussed to include their impact on the target pest population, the stakeholders involved, and the advancement of the SIT/IS as a tactic for lepidopteran control.

### *2.1. Pink Bollworm*

#### *2.1.1. Development of the Programme*

The pink bollworm *P. gossypiella* was first reported in North America from Mexico in 1911, probably entering on cotton seed shipped from Egypt (Noble 1969). The first reported infestation in the USA was in 1917 in Robertson County, Texas (Scholl 1919). By 1926, this highly invasive insect had spread from Texas through New Mexico and into eastern Arizona, and then quickly established itself as one of the major pests of cotton in the south-western USA and north-western Mexico (Burrows et al. 1984). It is considered to be among the most damaging cotton pests worldwide due to feeding while protected within the cotton boll, high reproductive capacity, high mobility, and frequent development of resistance (Henneberry 2007). Past management of the pink bollworm relied on the extensive use of broad-spectrum insecticides, and growers experienced significantly increased production costs and

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reduced yields (Watson and Fullerton 1969; Burrows et al. 1982, 1984). Ingram (1994) provided a worldwide perspective on the pest status and management of the pink bollworm, and Henneberry and Naranjo (1998) and Henneberry (2007) reviewed its status and the various integrated management approaches used for its control in the south-western USA.

Development of IPM strategies significantly improved the prospects for management of the pink bollworm in the south-western cotton belt which included the use of area-wide management with cultural controls, pheromone monitoring, coordinated insecticide applications, resistance monitoring, the widespread use of mating disruption, and the application of the SIT (Chu et al. 2006; Henneberry 2007; Lance et al. 2016). The work to integrate the use of the SIT into other AW-IPM tactics was based on research including mass-rearing and irradiation biology, extensive small-scale testing in field cages, several large-scale field trials, and demonstration projects (Staten et al. 1993; Henneberry 2007; Walters et al. 2009; Naranjo and Ellsworth 2010; Lance et al. 2016).

Stewart (1984) described the mass-rearing of the pink bollworm. Staten et al. (1993) and Walters et al. (2009) summarized the history and operational details of the programme. Miller et al. (2001) reviewed the efforts to enlarge the rearing facility in Phoenix, Arizona, developments like the twin-screw extruder technology to make high-volume and high-quality diet, and mechanization of the rearing process. These changes dramatically increased sterile-insect production capabilities at reduced costs, and opened up the possibility that sterile moths could be integrated into other AW-IPM programmes in the cotton belts of south-western USA and north-western Mexico.

Other developmental work included several field-cage studies and open-field trials to evaluate the potential of the SIT to control the pink bollworm. While the field-cage studies showed positive results, many of the early open-field trials failed or were only partially successful. These early failures were attributed to a lack of isolation from migrating moths, and the low competitiveness of mass-reared sterilized male moths that necessitated high (more than 60) sterile to wild overflooding ratios (Bartlett 1978). Henneberry and Keaveny (1985) provided detailed information on a large-scale SIT field trial in St. Croix, US Virgin Islands. Henneberry (1994) made a thorough review of all sterile-moth release trials for pink bollworm control, including the St. Croix project and the San Joaquin Valley programme.

### *2.1.2. Containment in the San Joaquin Valley*

The history of development of the SIT for the pink bollworm included a long-term area-wide preventive sterile-moth release programme in the San Joaquin Valley, which was the only cotton-growing area in the south-western USA not infested with the pink bollworm. The long-term prevention of pink bollworm establishment in that valley was attributed to an ongoing area-wide monitoring and SIT containment programme that had been in continuous operation since 1967 (Staten et al. 1993; Henneberry 1994; Walters et al. 2009). The objective of this programme was containment rather than suppression or eradication (Henneberry 1994; Hendrichs et al. 2007; Lance et al. 2016; Hendrichs, Vreysen et al., this volume). This was achieved by operating an area-wide monitoring and coordinated sanitation programme, and included the release of large numbers of sterile moths each year relative to the number

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of immigrating wild moths (Walters et al. 2009). The fact that the pink bollworm did not become established in the San Joaquin Valley, despite the annual immigration of moths from infested cotton-growing valleys to the south (Staten et al. 1993), and the demonstrated ability of the moth to successfully overwinter in the area (Henneberry and Keaveny 1985; Venette and Hutchison 1999), showed that the programme had been effective. The programme was financed by a self-imposed grower levy on cotton bale assessment, and USDA support for sterile moth release (Walters et al. 2009; CDFA 2019). Compared with cotton-production areas where the pink bollworm was established and not suppressed, growers saved an estimated USD 248–371/ha (CDFA 2019).

### *2.1.3. Area-Wide Integrated Pest Management*

The success of the San Joaquin Valley containment programme, coupled with the development of other biorational tools such as pheromone mating disruption and later *Bt*-cotton, created the opportunity to test the feasibility of using combinations of these tools for the integrated management of established pink bollworm populations on an area-wide basis. The first test was conducted in 1986–1989 involving 30 cotton fields in the Coachella Valley, California, using a high-rate pheromone disruption system and sterile insects. During the 4-year project, pink bollworm populations were maintained at low densities, and major reductions in conventional insecticide use were achieved (average of 7.3 insecticide applications per field per y decreased to 1.2) (Staten et al. 1993; Henneberry 1994).

A second area-wide pest suppression trial, combining the SIT with mating disruption, *Bt*-cotton, and cultural controls, was conducted in the Imperial Valley, California, from 1994 to 2000. Walters et al. (2000) reviewed the strategic objectives and results of this integrated management trial for the period 1994–1998. During 1994–1996, sterile moths were released on 6 d/wk in all fields at variable rates (70–560 moths per ha per d) calculated to deliver an overflooding ratio of at least 60:1 as measured by trap captures. Sterile moth releases were supplemented with mating disruption if a 60:1 sterile to wild moth ratio was not maintained. In 1997 the cotton planted in the Imperial Valley consisted of 81% *Bt*-cotton, 17.5% conventional or non-*Bt*-cotton protected with mating disruption, and 1.5% conventional untreated cotton as a refuge. Releases were reduced to 40 sterile moths per ha per d, 3 d/wk, throughout the Valley. The trial was expanded in 1998 to include the Blythe and Palo Verde Valleys, California, where there also was widespread use of *Bt*-cotton. The trial was terminated in 2000 after having achieved a high degree of suppression of established pink bollworm populations in all areas using essentially no insecticides.

### *2.1.4. Area-Wide Eradication*

Based on the success of these trials, a large area-wide eradication was launched to eradicate the pink bollworm from all cotton-producing areas of the USA and adjacent areas of northern Mexico (NCCA 2001, 2009; El-Lissy et al. 2002; Antilla and Liesner 2008; Tabashnik et al. 2010; Staten and Walters 2021).This large programme used a combination of tactics including mating disruption, regional widespread planting of genetically modified cotton expressing the *Bt* toxin, cultural control

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methods and the SIT (Grefenstette et al. 2009; Tabashnik et al. 2010; Liesner et al. 2014). Each of the programme areas used grower-planted *Bt*-cotton, and pheromone applications for mating disruption, for 1 or 2 y to lower the pest population levels. Then, in the following 2 or 3 y, sterile-insect releases were to be included to complete the eradication process. In 2001, the programme was initiated in Texas and Chihuahua, Mexico, and moved to New Mexico in 2002, south-western USA, and north-western Mexico; in 2006 the programme proceeded into Arizona and the lower desert production area in southern California, bringing all programme areas under full eradication (Antilla and Liesner 2008; Lance et al. 2016).

Sterile pink bollworms were released at variable rates of up to 250–600 moths per ha per d on conventional cotton, and at a lower rate of 36 moths per ha per d on *Bt*-cotton (Antilla and Liesner 2008; Grefenstette et al. 2009). The data before and after the eradication programme in Arizona show a steep decline in larval infestation of bolls and moth captures (Fig. 1) and pesticide applications (Fig. 2).

For the Arizona programme, the release of sterile moths over *Bt*-cotton pioneered a new use of the SIT, enabling the planting of 100% *Bt*-cotton without using the EPA-mandated *Bt*-resistance management strategy, i.e. planting a refuge of 10% conventional cotton. Using sterile insects in this manner works as a form of resistance management by reducing the probability that a moth with a rare resistant recessive allele would encounter another moth of the same genotype (Wu 2010).

This was a novel use of the SIT, enabling eradication programme officials to obtain an EPA (Environmental Protection Agency) section 24C local-use variance that permitted the planting of 100% *Bt*-cotton in all programme areas. The area-wide (near 100%) planting of *Bt*-cotton was credited as a major factor in the success of the programme (Henneberry 2007; Tabashnik et al. 2010; Liesner et al. 2014).

After 2009 no larvae were observed in the field, and after 2012 no wild moths had been caught in any of the programme areas (Blake 2014; Liesner et al. 2014; Lance et al. 2016). Since 2014, all programme areas were under a four-year "Confirmation of Eradication" designation where the area-wide cultural practice of planting nearly 100% *Bt*-cotton continued in most areas, but all other control activities, including sterile-insect release, ceased (Liesner et al. 2014). Area-wide monitoring continued with pheromone traps, a mandatory "plough-down" host-free period, and a small maintenance level of production of the pink bollworm to support a response with sterile insects in the event of pink bollworm captures (Liesner et al. 2014; Lance et al. 2016). After completion of the 2018 cotton-growing season, pink bollworm eradication was declared for all USA programme areas (Fitchette 2018; USDA 2018; Staten and Walters 2021).

Even though the eradication campaign is a success, the pink bollworm remains a threat for reinvasion and a significant worldwide pest. The pink bollworm is still the primary cotton pest in all major cotton-producing areas of the world, and resistance to *Btk* in transgenic cottons has been reported in India (Bagla 2010; Tabashnik and Carrière 2010). In North America, there are some parts of eastern New Mexico and the Southern Plains region of Texas not included in the eradication programme; in these areas pink bollworm populations have been historically low, and the status is uncertain (Pierce et al. 2013). Outbreaks in 2009–2011 were confined to a small number of fields planted to non-*Bt*-cotton (Pierce et al. 2013). In 2012, these

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populations were addressed by increased planting of *Bt*-cotton and releasing sterile insects; the area is considered eradicated (Pierce et al. 2013).

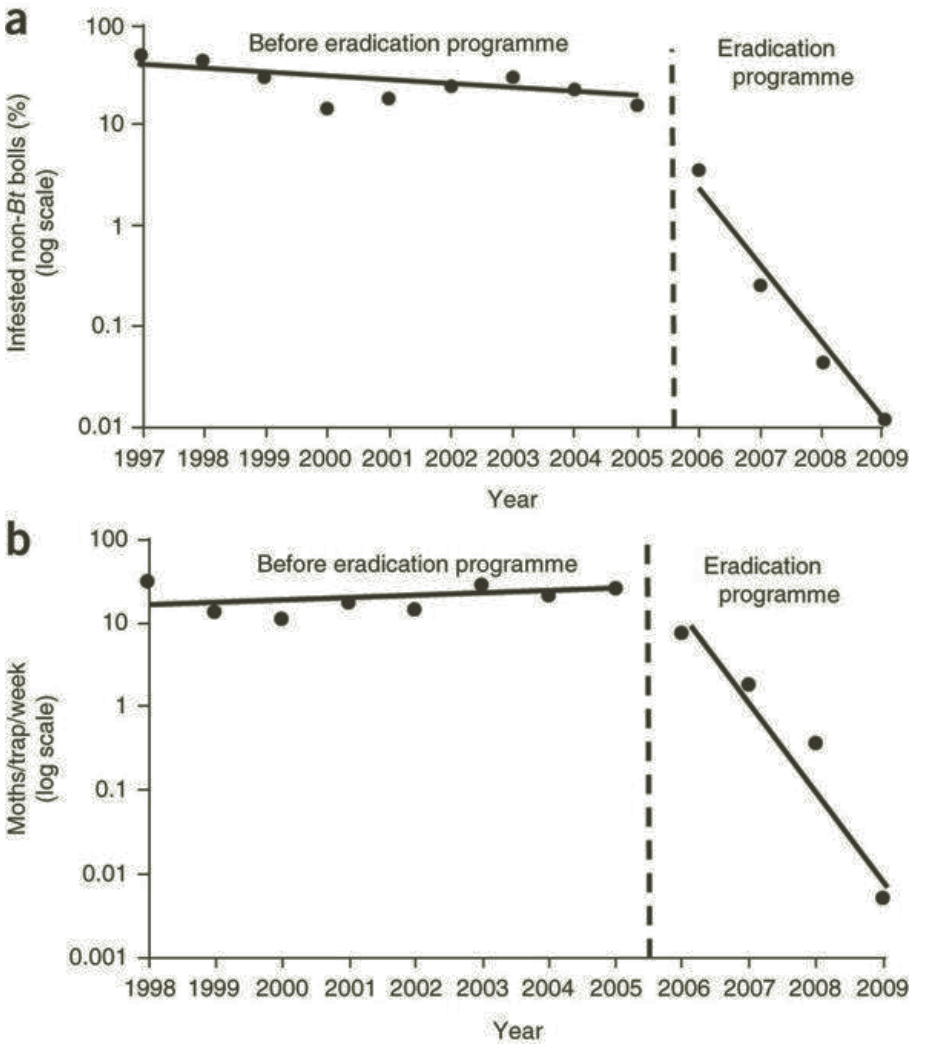



*Figure 1. a) Pink bollworm larval infestation of non-*Bt*-cotton bolls from 1997 to 2009 in Arizona, USA. b) Wild male pink bollworm moths trapped in* Bt*-cotton fields from 1998 to 2009. Analysis of covariance shows that the number of moths caught per trap per week (log transformed) was significantly affected by year, treatment (before versus during the eradication programme), and a year-by-treatment interaction ($P < 0.0001$ for each factor and their interaction, $r^2 = 0.95$). Linear regression shows that the slope, which indicates the change in moths trapped per year, was significantly negative from 2006 to 2009 (−1.0, $r^2 = 0.92$, $P = 0.04$), but did not differ significantly from 0 from 1998 to 2005 (0.017, $r^2 = 0.071$, $P = 0.52$). (Figure adapted from Tabashnik et al. 2010.)*

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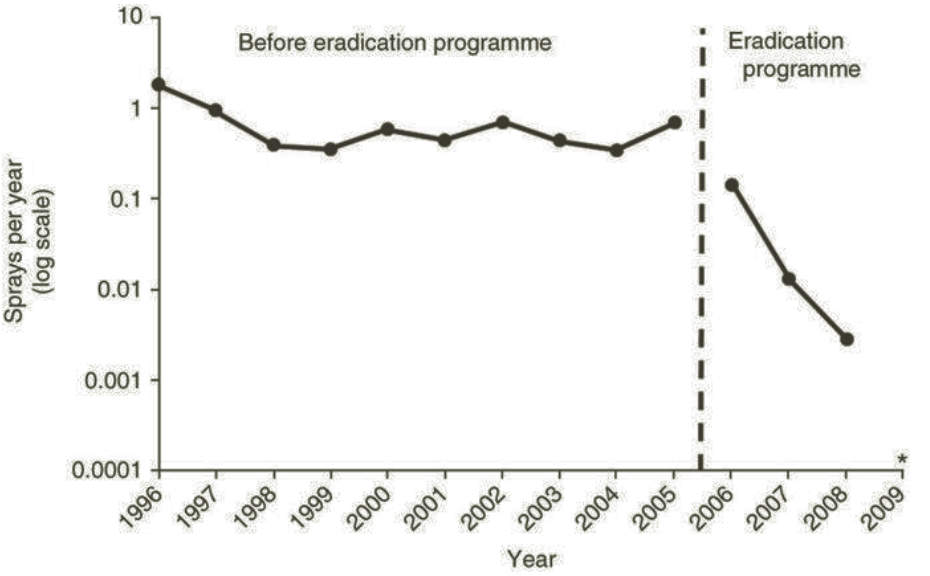



*Figure 2. Decline in insecticide applications to cotton in Arizona, USA, by comparing sprays per year before and after the initiation of the pink bollworm eradication programme. (Figure adapted from Tabashnik et al. 2010.)*

Other areas of concern include areas of central Mexico and the Caribbean where the pink bollworm remains a threat, and/or the status remains uncertain. Continued monitoring, mandatory "plough-down", and quarantine enforcement will be needed in US cotton-production areas to ensure that the pink bollworm does not reinvade.

The economic impact of the pink bollworm to US cotton producers was estimated at more than USD 32 million per year for the entire infested area -- from yield losses and control costs related to the pink bollworm (El-Lissy et al. 2002; Frisvold 2006; Antilla and Liesner 2008). The removal of the pink bollworm from cotton-production systems, and reductions in the need to treat with insecticides, has enabled the development of better IPM programmes for other key cotton pests, e.g. *Lygus hesperus* Knight and *Bemisia tabaci* (Gennadius). Also, overall, it has reduced costs and insecticide use to record low levels, making cotton-production systems in the western USA more economical and environmentally sustainable (Henneberry 2007; Naranjo and Ellsworth 2010).

### *2.2. Codling Moth*

The codling moth *C. pomonella* is the key pest of apples and pears in most regions of the world where pome fruit is grown (Vreysen et al. 2010). The larval stage burrows into the fruit, rendering it unmarketable and in breach of phytosanitary restrictions for trade. As a consequence, from the 1950s, organophosphate insecticides were applied to kill larvae as they emerge from eggs and before they can penetrate fruit. Control failures during the past 60 years, due to the development of insecticide resistance and

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concerns about the impact of insecticides on the environment, have led researchers in different parts of the world to make numerous attempts to use the SIT/IS against the codling moth.

The Agriculture and Agri-Food Research Centre in Summerland (in southern British Columbia, Canada) provided the extensive initial investigations (Proverbs 1962, 1969, 1974, 1982; Proverbs et al. 1973, 1982) that led to the implementation of an operational AW-IPM programme that routinely releases irradiated moths (Dyck et al. 1993; Bloem and Bloem 2000). After the ground-breaking work of Proverbs, notable examples include the work of scientists at the USDA/ARS laboratory in Yakima, Washington, USA (Hutt et al. 1972; Butt et al. 1973; White et al. 1976a, b; Hutt and White 1979), and the research in Switzerland that used diapaused $F_1$ sterile larvae released into small pome-fruit orchards (Charmillot et al. 1973, 1976a, b; Charmillot 1977).

Research targeted gamma radiation (Proverbs and Newton 1962a, b, c), an inexpensive, agar-free meridic diet (Brinton et al. 1969), design of a rotating oviposition cage (Proverbs and Logan 1970), and ground-release devices to distribute chilled moths in the orchard (McMechan and Proverbs 1972). These components are currently in use in the OKSIR Program (Dyck et al. 1993; Bloem and Bloem 2000; Bloem et al. 2007a; Dyck 2010; OKSIR 2019).

### *2.2.1. Okanagan-Kootenay Sterile Insect Release Program*

The Okanagan-Kootenay Sterile Insect Release (OKSIR) Program in Canada was launched in early 1992 (Dyck et al. 1993), more than a decade after Proverbs et al. (1982) had demonstrated in a 3-year (1976–1978) pilot project that local eradication of the codling moth was possible. Unfortunately, at that time, the cost of delivering this technology was about 2.4 times greater than the use of conventional insecticides (Proverbs et al. 1982). Between 1978 and 1992 several benefit/cost analyses were conducted at the request of growers to reassess the economics of the SIT (Holm 1985, 1986; Jeck and Hansen 1987). Following the more positive outcomes of these studies, an implementation plan was developed (DeBiasio 1988). A 2-year clean-up or sanitation phase (phase 1) was followed by 3 years of sterile moth releases (Phase 2) at an initial overflooding ratio of 40:1. Two zones, each containing about 4 000 ha, were treated sequentially, and urban trees were included in the programme. In addition, sterile codling moths were released along the USA-Canada border (phase 3 — containment) (Hendrichs et al. 2007; Hendrichs, Vreysen et al., this volume), which was (incorrectly) considered the only plausible route for reinfestation.

Construction of a mass-rearing facility at Osoyoos in the Okanagan Valley was completed in March 1993. Sterile moths were released into orchards for the first time in the spring of 1994. Unfortunately, clean-up activities during 1992 and 1993 were not entirely successful, and a higher-than-anticipated wild population resulted in poor overflooding ratios and poor control. In 1995, to help turn the programme around, reduce the wild population, and enable a 40:1 sterile:wild overflooding ratio to be achieved, growers received a one-time compliance grant of USD 115 per ha to support insecticide applications. Other strategic changes included tougher enforcement of codling moth control bylaws and an expanded communications campaign (Bloem and Bloem 2000; Bloem et al. 2007a).

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As a result of these strategic changes, the average wild codling moth captures in pheromone traps in the Zone 1 treatment area were reduced from 13 moths per trap per week during the first generation, and 2.5 during the second generation (in 1995), to 0.08 moths per trap per week during both first and second generations (in 2000). The amount of codling moth fruit damage at harvest was significantly reduced; in 1995 42% of orchards in the treatment area had no detectable level of codling moth damage at harvest.

The programme for commercial orchards in Zones 2 and 3 commenced with the sanitation phase using a combination of mating disruption and insecticide sprays. Releases of sterile moths in these zones began in 2002 (Bloem et al. 2007a). It was expected that by the end of 2005 all zones would have achieved minimal codling moth population levels similar to those achieved in Zone 1, but the reduction in wild moth trap catch and fruit damage did not occur as rapidly as observed in Zone 1 (Bloem et al. 2007a). The reasons cited were the larger urban areas in these zones with untreated backyard trees, and the use of mating disruption in these zones which may not have been as effective as insecticide treatments in Zone 1. Despite this initial slow start, since 1999 moth captures and fruit damage decreased in all programme areas to below the treatment action threshold of 2 moths per trap per week (Fig. 3). Since 2015, the economic damage threshold was met, with less than 10% of programme areas having >0.2% fruit damage (Nelson et al. 2021).

During the life of the programme, insecticide use was reduced by 96% of pre-OKSIR Program amounts (Fig. 4) (Nelson et al. 2021). Although other factors have contributed, the decreased reliance on insecticides for codling moth control has resulted in local packing houses encouraging their apple growers to consider switching to certified organic production or following new “Growing with Care” production practices where no insecticides are applied in orchards between blossom and fruit harvest.

Finally, the Canadian OKSIR Program is a model of the development of support from the general public to implement more environment-friendly AW-IPM programmes. From its inception, home and business owners were encouraged to recognize the value that apple growers brought to the community — in terms of quality of life and economic benefits through agriculture and tourism (Dendy et al. 2001). As a result, area residents took responsibility for helping growers to implement a mutually beneficial AW-IPM programme by paying a portion of the annual budget and actively participating in activities such as the removal of unmanaged host plants.

Despite ongoing success, the operation of a long-term sustainable pest-suppression programme is threatened by that same success; reduced pest pressure may result in decreased funding support because the pest is no longer perceived to be a problem. Ironically, the unique public-funding model of this programme may contribute to this perception. An additional economic threat is the low profitability of pome-fruit production and the consequent conversion of some orchards to higher-value wine grapes and sweet cherries; this has led to a decrease in pome-fruit production areas and a decrease in the need for sterile moths (Carpenter et al. 2014; Nelson et al. 2021).

To provide additional support to maintain a sustainable programme, OKSIR has been working with researchers from other regions to supply sterile or fertile codling moths to develop further area-wide management of the codling moth with pheromone

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mating disruption and sterile-insect releases (Carpenter et al. 2014; Horner et al. 2016; Nelson et al. 2021). Based on the successful pilot shipments from Canada to South Africa (Bloem et al. 2010), and using insects shipped "out-of-season" from Canada, since 2014 New Zealand researchers have been conducting a pilot programme in an isolated apple production area using mating disruption and regular sterile-moth releases (Horner et al. 2016).

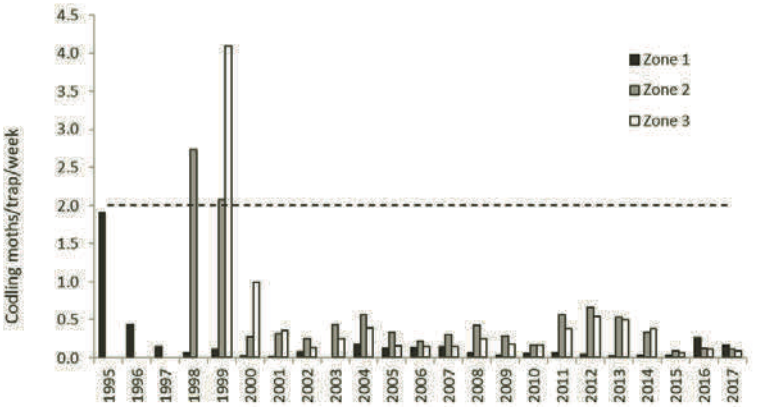



*Figure 3. Mean number of wild codling moths captured per trap per week for each zone managed by the OKSIR programme in Zone 1 (from 1995), in Zone 2 (from 1998), and in Zone 3 (from 1999). The dashed line indicates the recommended threshold (two codling moths per trap per week for two consecutive weeks) at which insecticide controls supplementary to the SIT would be required. (Figure from Nelson et al. 2021.)*

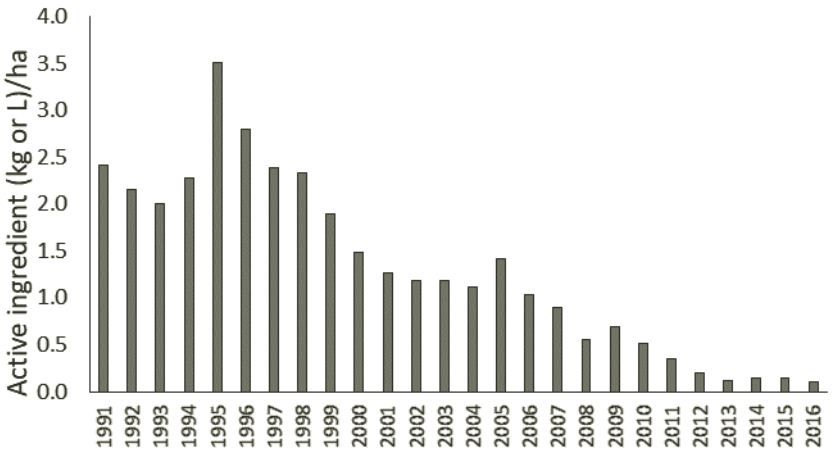



*Figure 4. Estimated insecticide active ingredient (kg or L) applied per ha per year for all zones managed by the SIR programme based on the estimated proportion of sales for the 15 products registered for use against the codling moth (note: some of these insecticides are also applied for other pests and/or crops). The estimates of active ingredients are divided by the area (ha) of planted pome fruit in the programme area to account for changes in sales due to the amount of pome fruit under cultivation. (Figure from Nelson et al. 2021.)*

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### *2.3. False Codling Moth*

The false codling moth *T. leucotreta* is an indigenous pest in sub-Saharan Africa, and also in Atlantic Ocean islands off the African coast, islands in the Indian Ocean, and Israel (Newton 1998; Malan et al. 2018). It is a significant pest of several important crops, with citrus as one of its main hosts (Newton 1989; Hofmeyr et al. 2015; Malan et al. 2018). The larvae feed internally, causing fruit lesions (Malan et al. 2018). Until about 1969, it was unknown in the fruit-growing regions of the Western Cape province, South Africa, but by the end of the 1970s it had spread throughout all the important ones (Hofmeyr et al. 2015). The false codling moth can have 5–6 generations per year.

This moth is not present in North America, Europe and Asia -- this has phytosanitary implications for fruit exports to those regions (Hofmeyr et al. 2015; Malan et al. 2018; Boersma 2021). The South African citrus industry developed integrated pest management tactics to suppress the false codling moth, including sanitation, mating disruption, and chemical, microbial and augmentative biological controls (Newton 1998; Hofmeyr et al. 2015; Malan et al. 2018; Boersma 2021). However, the pest management programme was not adequate for effective moth control, insecticide resistance developed, and export markets increased in importance. Therefore, the citrus industry developed a more comprehensive area-wide control programme with the SIT as an additional method to suppress false codling moth populations (Bloem et al. 2003; Carpenter et al. 2007; Stotter et al. 2014; Hofmeyr et al. 2015; Boersma 2021).

A comprehensive research programme was organized by the industry, with support from the FAO/IAEA and USDA, to investigate the potential for applying the SIT against the false codling moth. There were several well-organized phases -- determine the radiation biology, evaluate mass-rearing technology, and conduct small-scale field-cage tests and subsequently a pilot project. The radiation biology experiments demonstrated that female moths were completely sterile at 200 Gy, but male moths were partially sterile with residual fertility of 5% at 350 Gy when crossed to fertile females (Bloem et al. 2003; Hofmeyr et al. 2015). Evaluating the fertility of the $F_1$ progeny showed that treatments of 150 Gy to male moths resulted in 100% sterility of their $F_1$ progeny, and demonstrated that an effective dose for an IS programme would be between 150–200 Gy. The field-cage test found that a dose of 150 Gy was effective for a mixed-sex release at a ratio of 10:1 sterile to fertile, and resulted in the highest percentage of uninfested fruit (Hofmeyr et al. 2005).

Based on these results, the pilot phase began with a treatment to 35 ha of citrus with a season-long release of moths treated at 150 Gy for a period of 29 weeks; the goal was to maintain an overflooding ratio of at least 10 to 1 over the course of the season. This trial resulted in a 95–97% reduction in fruit loss compared with the control plots (Fig. 5) (Hofmeyr et al. 2015).

Given the positive results of the first three phases of testing, and in support of a new operational SIT programme, the South African citrus industry constructed a mass-rearing facility (1900 $m^2$); the goal was to produce 21 million moths per week (Hofmeyr et al. 2015). The facility incorporated many new improvements to the rearing system and infrastructure, including a new mass-rearing diet, improved oviposition cages, a new moth-collection system, an irradiation source, and new

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release systems using ground-based and aerial release vehicles. Operational releases started in the 2007–2008 citrus-production season (Hofmeyr and Pretorius 2010; Hofmeyr et al. 2015).

During this period there was significant programme success, but also there were obstacles to overcome -- leading to a change to an extruded-diet production, a change in the release systems (using helicopters for sterile-moth release), and increasing the irradiation dose to 200 Gy (Xsit 2018, 2019). Over the next decade, the operational programme steadily continued to expand, reducing wild moth populations and fruit damage to low levels -- 95% or more below pre-programme levels, and reducing export-fruit damage-rejection levels (Fig. 6) (Hofmeyr et al. 2015; IAEA 2016; Boersma 2021).

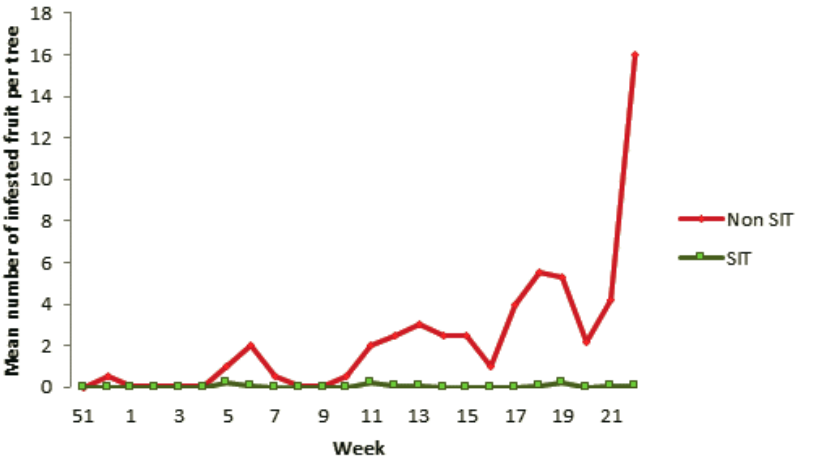



*Figure 5. Fruit drop due to* T. leucotreta *infestation in non-SIT and SIT-treated citrus orchards (35 ha) as part of an SIT pilot project conducted in the Citrusdal region during the 2005–2006 season. (Figure from Boersma 2021.)*

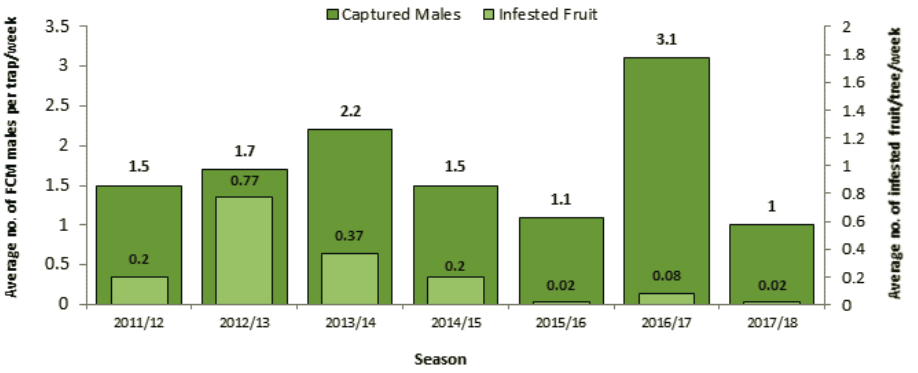



*Figure 6. Reduction in the number of wild* T. leucotreta *males and infested fruit in the Sundays River Valley, Eastern Cape Province, South Africa, as a result of sterile insect releases (data obtained from Xsit). (Figure from Boersma 2021.)*

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The programme has gained additional participation, increasing operations to encompass nearly 20 000 ha of the programme's three main operational treatment areas in citrus of the Western Cape and the Eastern Cape provinces (Boersma 2021). The programme attributes its success to focussing on a single crop industry, with broad support from growers to focus on the demands of the export market, integrating the SIT with other area-wide control tactics, and making the necessary investments in research and methods development to improve rearing, handling, and release procedures to maintain high moth quality and competitiveness (Boersma 2021).

### *2.4. Cactus Moth*

The cactus moth *C. cactorum* is famous as a very successful biological control agent of invasive cacti (*Opuntia* spp.) in Australia, South Africa, and other regions, but it has proved to be a "two-edged sword" (Suckling and Sforza 2014). Following its accidental introduction into Florida, and subsequent spread along the coast of the Gulf of Mexico, and subsequent detections in Mexico (Fig. 7), the threat posed to native *Opuntia* ecosystems and agricultural production led to the launch of a binational emergency response against this invasive pest (Bloem et al. 2007b; Hernández et al. 2007; Bello-Rivera et al. 2021).




*Figure 7. Location of Isla Contoy and Isla Mujeres near Cancun, Quintana Roo, in the Caribbean Sea, where infestations of* Cactoblastis cactorum *occurred but were later eradicated. (Map from Bello-Rivera et al. 2021.)*

To address this threat, an area-wide control programme, with containment and eradication as the main goals, was implemented (Bloem et al. 2007b; Hernández et al. 2007; Bello-Rivera et al. 2021). This was a challenge because few control or surveillance tools were available (Stiling 2002; Bloem et al. 2005). The main methods

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of cactus moth control consisted of insecticide treatments and hand-removal of infested cladodes and egg sticks. No pheromone was available for use in a detection system or for mating disruption (Bloem et al. 2003). Manual removal methods are effective but labour intensive (Hight et al. 2005), and insecticide treatments cannot be applied over large natural areas (Leibee and Osborne 2001; Zimmermann et al. 2004).

To address the limited number of tools available, research was initiated to improve monitoring methods, and to develop the SIT for eradication, barrier establishment, and as a resource to increase hosts for natural-enemy establishment (Carpenter et al. 2001a, b; Bloem et al. 2003; Hight et al. 2005; Heath et al. 2006). An artificial diet and mass-rearing system were developed (Carpenter and Hight 2012). A dose of 200 Gy was selected for implementation of an IS release programme where females were 100% sterile and male moths had residual fertility of between 40–50% (Carpenter et al. 2001; Hight et al. 2005).

The operational programme used surveillance, removal of host plants, sanitation efforts, and releases of sterile moths to contain and reduce cactus moth populations along the US Gulf Coast, and to eradicate this invasive pest in Isla Mujeres and Isla Contoy, preventing an invasion into the mainland of Mexico (Fig. 8) (Carpenter et al. 2008; NAPPO 2009; Hight and Carpenter 2016; Bello-Rivera et al. 2021).

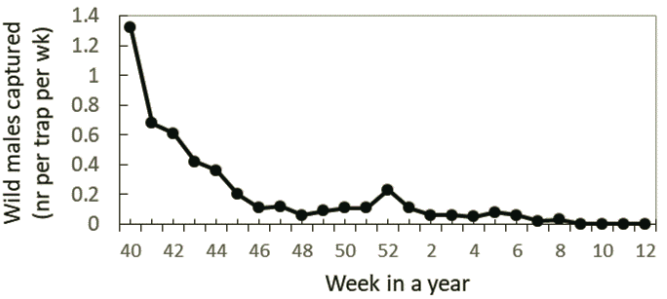



*Figure 8. Wild* C. cactorum *male moths caught per week in pheromone-baited traps from October 2006 to March 2007 in Isla Mujeres, Mexico (66 traps were deployed, except 115 in the last month). (Figure modified from Bello-Rivera et al. 2021.)*

### *2.5. Painted Apple Moth*

The painted apple moth *T. anartoides* is an Australian tussock moth species with flightless females (Fig. 9). There was concern that larvae would feed on many different plants of importance to horticulture in New Zealand, e.g. apple, forestry, e.g. plantation pine, and native ecosystems (Stephens et al. 2007). In fact, it has since been shown that the potential host range was wider in male than in female moths (Suckling et al. 2014); this has not been recorded previously in insects. This invasive pest was estimated to have a potential total cost to New Zealand of USD 52–203 million.

An eradication programme was approved, with a budget of up to USD 52 million (including communications and human-health monitoring costs). Operations began in January 2002, initially applying aerial sprays of *Btk*. In February 2003, releases of partially sterile males irradiated as pupae at 100 Gy (Suckling et al. 2002; Wee et al. 2005) were initiated at three sites with known or suspected painted apple moth

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breeding populations. Regular quality assessments of males were made in a wind tunnel (Fig. 9). By May 2003, 45 000 males had been released. In virgin female-baited traps, recapture ratios of sterile to wild males averaged ca. 100:1.

Outbreaks of this invasive pest in Auckland, New Zealand, were eradicated in 2007 (Suckling et al. 2007).

Earlier modelling of the impact of the aerial *Btk* spray programme on the insect pest population suggested that a protracted programme would be needed to achieve eradication using this tactic alone. The addition of IS to eradication efforts was welcomed by public factions that opposed the spray applications. However, an assessment of the full benefit of the addition of IS to the successful eradication of the painted apple moth is not possible because of confounding among several techniques used together. Nevertheless, because reared virgin females were being used a lot as lures in a trapping grid across Auckland, the additional cost of rearing, sterilizing, and releasing males was rather minor (less than USD 145 000 per year). All indications from the IS programme were positive, including a highly favourable benefit/cost analysis (Brockerhoff et al. 2010) and little public resistance (Gamble et al. 2010).



*Figure 9. Male painted apple moth attracted to a caged calling virgin-female moth -- part of a quality assurance bioassay conducted weekly on a subsample of irradiated male moths released during the successful eradication in urban Auckland. (Photo from D. M. Suckling.)*

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## 3. PAST EFFORTS TO DEVELOP AND APPLY MOTH SIT/IS

### *3.1. Gypsy Moth*

The gypsy moth *L. dispar* was accidentally introduced into the USA in 1869 near Boston, Massachusetts, from where it has been gradually expanding its distribution. The area infested by the gypsy moth in North America is confined to the eastern USA (behind an advancing front slowly moving in a south-westerly direction) and the eastern provinces of Canada (Sharov et al. 2002a; Liebhold et al. 2021). It remains an important forest defoliator that periodically builds to outbreak levels resulting in serious economic, environmental, and public nuisance problems (Liebhold et al. 2000; Thorpe et al. 2006). Since 1924 more than 32.8 million ha of US forests have been defoliated by the gypsy moth (USDA Forest Service 2001).

The potential of using the SIT to contain and manage gypsy moth leading-edge populations, and to eliminate isolated outbreak areas resulting from the accidental transportation of egg masses and other life stages through commerce and recreation, led to the initiation in 1957 of radiation biology studies (Godwin et al. 1964). Based on several criteria, the gypsy moth appeared to be well suited for population management with the SIT — females do not fly, males may mate several times, and females typically mate only once, producing an egg mass in the fall from which larvae hatch the following spring (Reardon and Mastro 1993).

Mastro et al. (1981) and Reardon and Mastro (1993) provided good reviews of the considerable amount of research that was conducted throughout the 1960s, 1970s, and 1980s. This research defined the sterility effects of various doses of radiation when applied to different gypsy moth developmental stages, assessed and developed methods to optimize competitiveness of sterile gypsy moths, and quantified the impact of releasing sterile and partially sterile insects. Three different release strategies were investigated (Reardon and Mastro 1993): (1) field-placement (from the ground) of male pupae (sexed visually by size and form) treated with at least 150 Gy that emerged as fully sterile adults, (2) deployment (from the ground) of substerilized male pupae treated with 100 Gy, and (3) broadcast release (from the ground or air) of diapausing $F_1$ sterile eggs produced from untreated females mated with males treated with 100 Gy. Schwalbe et al. (1991) discussed the gypsy moth problem in North America and the rationale for focusing on the use of $F_1$ sterile eggs to eradicate isolated infestations. Unfortunately, the programmatic details of the various field trials that were conducted are lacking, with the exception of a few key pilot tests (Berrien County, Michigan, 1980–1982, using fully sterile male pupae; Horry County, South Carolina, 1982, using partially sterile male pupae; and Bellingham, Washington, 1984–1985, using sterile $F_1$ egg masses) (Mastro and Schwalbe 1988; Mastro et al. 1989; Schwalbe et al. 1991; Reardon and Mastro 1993).

What little additional information exists on gypsy moth SIT is contained principally in a series of unpublished annual progress reports by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) Laboratory, at Buzzards Bay, Massachusetts. However, in a report to the USDA, Agricultural Research Service (ARS), National Technical Advisory Board, LaChance (1976) provided some information on early gypsy moth field tests.

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Small SIT field trials to suppress the gypsy moth also were conducted in Yugoslavia in 1969–1971 (Maksimović 1971, 1972, 1974). Even though it was concluded that the release of sterile males reduced wild population levels, an assessment of the actual impact of these releases is difficult given the experimental design that was used (LaChance 1976).

*3.1.1. Release of Irradiated Pupae and $F_1$ Sterile Egg Masses*

In general, the release of irradiated pupae was effective in eradicating isolated infestations and in suppressing low-density populations along the leading edges of spreading infestations. However, despite the technique's effectiveness, several operational difficulties were identified, and the technique was judged to be impractical for large-scale use (Mastro et al. 1989). Some of the problems were: (1) pupae were fragile and required special care in packaging and shipping (Reardon and Mastro 1993), (2) pupae had to be released in emergence cages that were expensive to deploy and maintain (Reardon and Mastro 1993), (3) pupae and newly emerged adults were sometimes subject to intense predation, (4) overcrowding in emergence cages resulted in poor-quality adults (LaChance 1976), and (5) since released males lived only 2 or 3 d, frequent releases were necessary to maintain high ratios of released to wild insects (Schwalbe et al. 1991; Reardon and Mastro 1993). In addition, since male flight activity lasts only about 4 wk, a mass-rearing facility would be underutilized for most of the year (except potentially for producing the gypsy moth nuclear polyhedrosis virus) (Mastro 1993; Reardon and Mastro 1993).

As a result of the difficulties in releasing pupae, research efforts were refocused on the release of $F_1$ sterile egg masses. Male pupae were irradiated with 100 Gy, and then these males were mated to fertile females; the $F_1$ egg masses were stored in diapause until needed for release the following spring. In this way, in theory, some $F_1$ sterile larvae would emerge and develop in the field in synchrony with the wild population. This technique had the advantages that the $F_1$ sterile egg masses were easier than pupae to handle and ship, could easily be produced and stockpiled in diapause throughout the year using a relatively small rearing facility, and required only a single release per season (Schwalbe et al. 1991; Reardon and Mastro 1993). In spite of these advantages, the additional population suppression that IS offers (suppression by the release of partially sterile males followed by the field production of their $F_1$ sterile progeny) could not be taken advantage of when this approach was used. Also, some larvae (the feeding stage) resulting from the released $F_1$ eggs might cause some plant damage (although it was never actually observed) (Mastro et al. 1989; Mastro 1993).

Although the early use of gypsy moth $F_1$ sterile egg masses was successful in eradicating an isolated population in Bellingham, Washington (Mastro and Schwalbe 1988; Mastro et al. 1989; Schwalbe et al. 1991; Reardon and Mastro 1993), similar results were not consistently obtained in subsequent trials against isolated outbreak populations, and the technique proved inadequate for managing gypsy moth populations along invading fronts of established infestations (Reardon and Mastro 1993). Apparently, the low efficacy and inconsistent results of releasing $F_1$ sterile egg masses were due to the fact that the resulting sterile adults that developed in the field were less competitive than the wild population, and were actually less competitive than laboratory-reared males irradiated and released as pupae. Part of the poor

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competitiveness was due to developmental asynchrony with wild moths, which was likely exacerbated by variably delayed development that was subsequently shown to be caused by low bioavailability of iron in the diet of the maternal parent (Keena et al. 1998). Nevertheless, between 1988 and 1992, eight isolated populations of the gypsy moth were treated with $F_1$ sterile egg masses with apparently "generally positive results" (Reardon and Mastro 1993). Between 1980 and 1992, infestations covering a total of 2385 ha were eradicated using sterile gypsy moth releases of all types (USDA Forest Service 2001). However, after nearly 35 years of study and application, it was determined that the SIT/IS was not sufficiently cost-effective relative to other less-expensive options. After 1993, this technology was no longer employed against the gypsy moth.

Current programmes for gypsy moth suppression rely on the use of *Btk*, a gypsy moth nuclear polyhedrosis virus, and the synthetic sex pheromone disparlure (Sharov et al. 2002b; Lance et al. 2016). In recent years, sterile gypsy moths have been used only for such purposes as calibrating trapping grids to confirm that phenological models are giving reasonably accurate estimates of the timing of egg hatch, and determining parasitoid host preferences. Nevertheless, the investigations associated with the development of the SIT for this pest led to a better understanding of its biology and behaviour that is being utilized in ongoing management activities (Mastro et al. 1989).

In Europe, more recent investigations have been conducted on the release of $F_1$ sterile egg masses in advance of imminent gypsy moth outbreaks; the objective is to enable natural enemy populations to build-up early, and thus reduce the magnitude of an outbreak (Zúbrik and Novotný 2009).

### *3.2. Tobacco Budworm*

The tobacco budworm *H. virescens* is a key pest of cotton and tobacco, and has been developing resistance to most insecticides used for its control (Harris et al. 1972; Elzen et al. 1992). In an attempt to develop an alternative control strategy for the tobacco budworm, Laster (1972) discovered hybrid sterility in $F_1$ male moths when male *H. virescens* were hybridized with female *Heliothis subflexa* (Guenée). The $F_1$ (hybrid) females produced from this cross, when backcrossed to *H. virescens* males, produced sterile male and fertile female progeny. This male sterility persisted in all subsequent backcross generations. Genetic studies of tobacco budworm backcross sterility discovered abnormalities in the sperm of hybrid and backcross males (Richard et al. 1975; Goodpasture et al. 1980). LaChance (1985) and Laster et al. (1988) reviewed in detail the different biological mechanisms responsible for sterility in the hybrid, including the early backcross and subsequent backcross generations. The potential use of backcross sterility was examined using population models (Makela and Huettel 1979; Levins and Parker 1983) that predicted a decline in a natural population of tobacco budworm following the release of backcross insects. Following encouraging results from many studies of host-plant preference, mating preference, and mating competitiveness (Laster et al. 1988), a pilot release programme was planned for the island of St. Croix, US Virgin Islands.

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The objectives of the pilot programme on St. Croix during 1977–1981 were to introduce a measurable amount of backcross sterility into the natural population of tobacco budworm, and to evaluate the population suppression and the level of backcross sterility in subsequent generations. This project was a cooperative effort between the USDA and the Mississippi Agricultural and Forestry Experiment Station. During 1979 and 1980, four separate releases were made (Proshold et al. 1983). Male sterility in tobacco budworm wild populations continued to increase as long as backcross insects continued to be released. During the last 6 weeks of 1981, 94% of wild tobacco budworm males were sterile (Proshold 1983). However, as the backcross frequency declined following the last release, tobacco budworm populations returned to pre-release levels. Proshold and Smith (1990) were not able to detect the backcross phenotype five years after the last release, presumably because of genetic drift and selection.

Following the pilot release programme on St. Croix, in 1991–1993 a pilot test was conducted in the central delta of Mississippi, USA, to study the effects of released backcross insects on natural tobacco budworm populations in a typical agricultural production area (where it overwinters and from where it annually undergoes long-distance dispersal to northern states and Canada (Laster et al. 1993, 1996; Hardee and Laster 1996). Backcrossed moths were released by placing pupae in emergence boxes at the test location, a 16.7-$km^2$ area in Washington and Sunflower Counties, Mississippi, in 1992, and in Bolivar County, Mississippi, in 1993. Control areas of the same size were used for each year. In 1992, the backcross to budworm overflooding ratio achieved was 3:1. After releases had ceased, this ratio declined to 1.3:1 during June, and to 1:2.3 during July. In 1993 the backcross:budworm ratio in the same area was 1:2.2 (29.9% sterility) for the overwintering generation. Releases in Bolivar County during 1993 achieved a backcross:budworm ratio of 2.6:1. After releases had ceased, this ratio declined in June to 1:1.6, in July to 1:3.6, and in August to 1:4, producing in 1994 a 12.1% sterility carry over. Hardee and Laster (1996) concluded that backcross release results were favourable. However, considering the survival and migration potential of the tobacco budworm, higher overflooding ratios of released to wild insects would be needed on an area-wide basis in overwintering areas of the budworm to achieve more sustainable results.

### *3.3. Corn Earworm*

The corn earworm *H. zea* is a major pest of maize, cotton, and many other field crops in the Western Hemisphere. Due to the importance of this pest, a method to mass-rear the corn earworm was developed (Burton 1969), and several attempts to eradicate this pest using the SIT in St. Croix, US Virgin Islands, were made (Snow et al. 1971; Laster et al. 1988). The first eradication trials were conducted for 3 months in 1968, and 6 months in 1969, with a second campaign being conducted from 1972 to early 1974.

Many problems were encountered during the 1968 trial, including an unexpected increase in the area planted to maize, inconsistent releases of irradiated insects, and poor and inconsistent insect production resulting from disease contaminants in the laboratory colony. Shipping and disease problems were reduced in 1969, but still

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caused periodic slumps in the supply of sterile corn earworms. Nevertheless, when there was no slump in the insect supply, releases of corn earworm males (treated with 320 Gy) resulted in sterile to wild overflooding ratios of 10:1–15:1. As a result of the 1969 programme, there was a reduction in the field in the number of fertile corn earworm eggs, rather than an increase in the number of sterile eggs. It was concluded that this reduction in oviposition was caused by a high incidence (50%) of locking (failure of mating pairs to disengage upon completion of copulation) between released and wild adults (Snow et al. 1971; Laster et al. 1988).

For the 1972–1974 eradication campaign, changes were made in the rearing system to improve the insect quality and reliability of supply of insects for release. In general, only males were released, and the radiation dose used to sterilize males was reduced from 320 to 225 Gy (Hamm et al. 1971; Young et al. 1976). However, several times during the course of the campaign, changes were made in the radiation dose actually used, and in the sex (males alone or mixed sex) of the insects actually released. Local eradication of the corn earworm population was not achieved during either campaign, but much knowledge and experience were gained concerning the operation of an area-wide programme against a lepidopteran pest. Laster et al. (1988) concluded that improved rearing techniques and more competitive insects were critical, and suggested that using a lower radiation dose would improve the efficacy of future AW-IPM programmes against the corn earworm.

To assess the influence of released males treated with a substerilizing dose of radiation (100 Gy), and to measure the level of IS induced in wild populations of the corn earworm, Carpenter and Gross (1993) conducted a pilot test in small mountain valleys in western North Carolina, USA, from 1986–1990. They found that the number of wild males captured per ha was positively correlated with the distance from the release site of the substerilized moths. Analyses of seasonal population levels of wild corn earworms, estimated from mark-recapture data, indicated that seasonal increases of wild males were significantly delayed or reduced (or both) in mountain valleys where substerile males had been released. The incidence of corn earworm larvae with chromosome aberrations (indicating they were progeny of irradiated, released males) collected from the test sites during the growing seasons demonstrated that substerile males were competitive with wild males in mating with wild females, and were successful in producing sterile $F_1$ progeny that further reduced the wild population. These significant reductions (73.5%) in populations of the corn earworm resulted even though the average overflooding ratio of irradiated to wild males (5.3:1) was low compared with that of other programmes that release sterile insects.

The use of the SIT has not been implemented into operational programmes for the control of either *H. virescens* or *H. zea*. Although hybrid backcross sterility and $F_1$ sterility suppressed pest populations in the field, the cost of rearing the insects, the highly mobile nature of these species, and in particular the development and adoption of effective *Bt* transgenic crop varieties, make it economically impractical at the present time to use the SIT to control these pests. However, preventive releases of sterile moths at overwintering sites, when wild populations are concentrated and are present in naturally low populations, would be an opportunity to apply IS to minimize the moth populations migrating north in the spring (Hardee et al. 1999).

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### *3.4. Light Brown Apple Moth*

The light brown apple moth *E. postvittana,* a native to Australia, was first detected in California in 2007 (Brown et al. 2010). As a pest, the light brown apple moth is best known from tree fruits (e.g. apples, pears, citrus, peaches, nectarines, and apricots), vines, berry fruit, and to a lesser extent from forestry, and vegetable and flower crops (Wearing et al. 1991). The suspected-host list is estimated at about 500 species (Brockerhoff et al. 2010; Suckling and Brockerhoff 2010). The potential for harm from this pest triggered a major response from the USDA and the State of California agricultural authorities (Suckling and Brockerhoff 2010). Infested areas in the core California coastal areas were quarantined, and regulatory and control measures were put in place (including a large-scale effort to apply aerial pheromones in an attempt at eradication). As the programme progressed, with both public opposition leading to some challenges to the State's authority to implement a pheromone-based control programme (Lance et al. 2016), and the realization that more control tools were needed to contain this pest, a research and pilot project to develop the SIT was launched. It developed mass-rearing technology, examined both pupal (Soopaya et al. 2011) and adult irradiation biology (Jang et al. 2012), and conducted field trials in New Zealand and California (Suckling et al. 2011; USDA/APHIS 2011; Stringer et al. 2013). Modelling the overflooding ratios showed that, at 300 Gy, the population extinction was 95% probable when the ratio of released to wild males in monitoring traps exceeds 6.4 (Kean et al. 2011). Higher overflooding rates would achieve eradication more rapidly. There was an advantage to using IS over full sterility early in such a programme (due to the superior fitness of males at lower doses) (Kean et al. 2011; Suckling et al. 2011). A male-biased sex ratio was shown (Soopaya et al. 2011), adding to the multiplier effect of matings by sterile $F_1$ offspring.

In 2011, a pilot SIT project was initiated in California; it included sterility testing of adult moths, confirming the dose of 300 Gy for full sterility (shown by Soopaya et al. 2011). The mass-rearing and adult-collection systems were adapted from methods used in the pink bollworm and codling moth, and a sterile-insect field release trial followed in a 2.6-km² residential area in a coastal city in southern California. Twice-weekly releases of sterile moths (60 000–70 000 sterile moths per week) were made by hand on a grid system for 11 weeks, with a total release of 650 000 sterile moths. Due to the high population of wild moths in the area, satisfactory overflooding ratios were not achieved. However, moth quality appeared to be high, with moth recaptures of about 0.1% in the widely spread trap grid, with dispersal distances of up to 0.5 km, and field longevity of released adult moths observed for greater than 2 wk after release. At the end of 2011, the project was stopped -- due to financial constraints, and the evolution of the project away from a control programme towards a regulatory programme designed to limit further spread out of the quarantine areas.

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## 4. ONGOING METHODS DEVELOPMENT OF MOTH SIT/IS

### *4.1. European Grapevine Moth*

Historically the European grapevine moth *L. botrana* has been a pest of the Mediterranean regions of Europe, North Africa, and Asia. Recently, it was introduced into the Americas region, with first detections in Chile in 2008, California, USA, in 2009, and Argentina in 2010 (Varela et al. 2010; Gilligan et al. 2011; Ioriatti et al. 2011, 2012). A successful eradication campaign was completed in the USA in 2016 (Schartel et al. 2019; Simmons et al. 2021), but there is increasing pest pressure in the South America region. Infestations in urban areas are difficult to treat, and market-access problems affect the trade of other hosts, e.g. blueberries. Increasing problems for grape production in the Mediterranean regions are related to the development of insecticide resistance, warmer growing seasons with climate change, and the high cost of mating-disruption treatments (Martin-Vertedor et al. 2010; Ioriatti et al. 2011; Gutierrez et al. 2018). These factors have increased interest in the development of the sterile insect technique for *L. botrana* (Mansour 2014; Saour 2014, 2016).

Saour (2014) found complete female sterility when irradiating adults at 150 Gy, but a much higher dose of 400 Gy was needed to achieve near 100% sterility in males. A dose of 150 Gy was recommended for use in an IS strategy, a trade-off between high field competitiveness, as measured by flight ability, and higher residual male fertility (Saour 2016). (Higher irradiation doses significantly impacted the flight ability of male moths compared with a dose of 150 Gy.) At 150 Gy, the frequency of $F_1$ male progeny increased, and these had high sterility levels (Saour 2014).

In Chile, *L. botrana* was first detected in Santiago in urban grapes, and is now present in grape production areas in the central region of the country (Ioriatti et al. 2012; SAG 2019), and has also caused problems for the export of the large and valuable blueberry (*Vaccinium* spp.) crop. In urban areas (that are near grape and fruit production areas) many Chileans grow wine grapes in their gardens; this is a significant problem for the control programme because urban treatments can be very expensive and logistically challenging (residents can refuse treatments). The government's national control programme strategy for urban areas includes the use of mating disruption. A pilot project to test releases of sterile moths and parasitoids has been initiated (SAG 2018, 2019; H. Donoso, personal communication).

For the development and evaluation of the SIT as a control tool, the Chilean government and the fruit industry have formed a partnership to develop *L. botrana* mass-rearing systems, determine the radiation biology, and in 2016 began field-cage and pilot-scale field releases (H. Donoso and S. Izquierdo, personal communication). These trials were designed to measure the field-performance traits of irradiated males (e.g. dispersal, longevity, and mating with wild moths), determine release rates and frequency, and identify any needed changes in the rearing and handling of moths to improve performance.

The programme developed a sterile-insect laboratory in Arica with a capacity to produce >100 000 mass-reared moths per week to support a larger programme; currently it is producing 50 000–75 000 per week. This project has access to the gamma irradiator (Bakri et al., this volume) used for Chile's Mediterranean fruit fly

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programme. The work in Arica has focused on optimizing the adult diet, developing quality-control procedures, and developing methods to collect and irradiate adult moths. The programme is also working with its fruit-industry partner Fundación para el Desarrollo Frutícola (FDF) to develop mass-rearing procedures, verify previous radiation-biology studies, and begin pilot-scale releases of sterile moths. Production capacity at the FDF mass-rearing laboratory in Santiago exceeds 100 000 pupae per week. The FDF programme is focused on methods of pupal collection and irradiation. Even though it is challenging to collect and irradiate pupae for large-scale moth SIT programmes, there are advantages to using pupae, e.g. a decrease in the impact on moth quality compared with handling and storage of adults. Early results from the programme suggest that it is having success – good insect dispersal performance and recapture rates. However, while a system of using irradiated pupae may be suitable for a small-scale programme targeting residential areas (Sucking et al. 2007) and selected vineyards (Stringer et al. 2013), a larger production effort for the area-wide release of sterile moths over a region will likely require the collection and irradiation of adult moths.

In 2018, the programme initiated several larger pilot-release evaluations in residential areas measuring 12.5 to 25 ha, and for a longer period of time releasing up to 16 700 sterile moths per week (FAO/IAEA 2018; SAG 2018; H. Donoso and S. Izquierdo, personal communication). This programme was expanded in 2019 to release 50 000 moths per week for a season-long project in a 25-ha residential area next to commercial vineyard and agricultural production areas. The goals of this larger phase of testing are to evaluate dispersal, field longevity, overflooding ratios, and suppression of wild populations, and also to develop the operational procedures needed to conduct an SIT programme. While it remains to be seen whether this pilot project will succeed and lead to operational releases, the first results are promising. The Chilean programme is making substantial progress, and may be the only solution available to reduce the extent of *L. botrana* movement from residential into production areas.

### *4.2. African Sugar Cane Borer*

The African sugar cane borer *E. saccharina* is a native pest in several regions of Africa. It infests sugar cane, maize, and sorghum, as well as other grain crops of the grass family, and several native grasses and sedges (Walton and Conlong 2016a, b). The larvae's boring activity causes crop loss and reduced yields in sugar cane (Conlong 1994). After its spread into South Africa, the sugar cane industry launched a large effort to understand its biology, use of other hosts, methods of biological control, and prospects for area-wide management using various habitat management strategies. Efforts to determine if the SIT could be incorporated into the current area-wide programme were launched with work on mass-rearing, radiation biology, mating competitiveness, and modelling (Barnes et al. 2015; Mudavanhu et al. 2016; Walton and Conlong 2016a, b; Conlong and Rutherford 2017).

Work by Walton and Conlong (2016b) showed complete sterility of females at 200 Gy and residual male fertility of 20%, and suggested the potential of an IS approach for this species using 200 Gy. Mudavanhu et al. (2016) found that reared males,

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irradiated at a dose of 200 Gy, were as competitive as wild fertile males, and have a high mating compatibility with wild females, suggesting that the effects of colonization, mass-rearing, and irradiation would not greatly affect the competitiveness of mass-reared irradiated moths.

### *4.3. Navel Orangeworm*

The navel orangeworm *A. transitella* is the key pest of California tree nuts: almonds, pistachios, and walnuts. It causes feeding damage on kernels that can also lead to the introduction of aflatoxin producing the *Aspergilli* fungus. The insect has a wide host range that includes other tree and fruit crops such as oranges, figs, pomegranates, pecans, and ornamental species (Bentley et al. 2016; Ferguson and Haviland 2016). These crops are planted on more than 768 900 ha, and valued at more than USD 6200 million per year. For all of these crops, tolerance to pest damage by the navel orangeworm is extremely low, especially for pistachios (because of the difficulty of sorting out damaged nuts while still in the shell). Contamination by aflatoxin can impact quality and marketability. Levels of navel orangeworm damage are directly correlated with those of aflatoxin contamination. Standards of aflatoxin and insect damage for export markets are very demanding; this is a prominent concern (Bentley et al. 2016; Ferguson and Haviland 2016).

In view of concerns about the long-term sustainability of the current navel orangeworm management methods, there is interest in developing the SIT as an additional tool for navel orangeworm control; also, there is support to develop an area-wide control programme (Northcutt 2015). Work on radiation biology by USDA scientists in California (Light et al. 2015), and strong support shown by the tree-nut industry, has led to a pilot project to develop mass-rearing and to field-test sterile navel orangeworms (Wilson and Burks 2019). The USDA-APHIS-PPQ pink bollworm sterile-insect mass-rearing facility in Phoenix, Arizona, may be used to rear navel orangeworms (Blake 2015). An earlier demonstration project developed and evaluated navel orangeworm area-wide control measures (mating disruption, sanitation, and coordinated applications of insecticides), and showed that navel orangeworm control could be improved by using area-wide tactics. Since the use of the SIT is complementary with area-wide control, development of the SIT as a new tool would be especially useful for the long-term sustainability of navel orangeworm management in California.

Development of mass-rearing (focusing on evaluations of diet, rearing trays, and adult collection at the USDA-APHIS-PPQ facility) shows that there is a potential to produce several million navel orangeworm per week. That this was accomplished (without making significant changes to a system optimized for the pink bollworm) demonstrates that much higher numbers of navel orangeworm could be produced if the entire facility was committed to rear navel orangeworm on a much larger scale. Current production levels are being maintained to support the pilot-project field testing needed to develop an SIT system. Field trials were initiated in 2018, and are ongoing in 2019.

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### *4.4. Tomato Leafminer*

The tomato leafminer *T. absoluta* is an emerging pest of solanaceous crops from the neotropics that has expanded into North America, Europe, the Middle East, Africa, and parts of South-East Asia (Biondi et al. 2018; Biondi and Desneux 2019; Han et al. 2019). Internal feeding damage to fruit, leaves, and stems may cause losses as high as 100%; such damage increases the need for insecticide applications, but they disrupt the integrated management programmes of other tomato pests. Given its rapid range expansion and adaptability to diverse climatic conditions, this pest is predicted to have a high impact on tomato growers throughout these regions (Biondi et al. 2018).

Current control practices often rely on insecticide applications, but more sustainable control practices are needed (there are several reports in several regions of the development of insecticide resistance) (Biondi and Desneux 2019; Guedes et al. 2019; Silva et al. 2019). Furthermore, augmentative biological control using Neotropical parasitoids may not be available in other regions due to increased biosecurity that is increasingly excluding non-native natural enemies. Thus, there is interest in developing the SIT for the tomato leafminer for use in greenhouses (as an alternate tool to insecticide treatments) which would be compatible with biological control-based tactics (FAO/IAEA 2016, 2019).

Recent work includes completion of radiation-biology studies, field-cage testing showing pest suppression on infested tomatoes, and incorporation of predator releases with sterile-moth releases in glasshouse tomato production (Cagnotti et al. 2016; Kuyulu and Hanife 2016; Vreysen et al. 2016; FAO/IAEA 2019). Radiation-biology data for *T. absoluta* suggest that doses of 200–250 Gy could be used to induce IS in males (Cagnotti et al. 2016; Carabajal Paladino et al. 2016).

### *4.5. Carob/Date Moth*

The carob or date moth *E. ceratoniae* is a major pest of dates in North Africa. The use of *Btk*, parasitoids, and postharvest fumigation does not provide sufficient pest control to enable the industry to reap the potential economic benefits from increased exports of dates.

Laboratory and field studies have been carried out in Tunisia and Algeria, with a view to integrating the SIT into AW-IPM programmes for date and pomegranate plantations. A mass-rearing system was established, and some small-scale releases of partially sterile moths were made (Dhouibi et al. 2000; Mediouni and Dhouibi 2007; Chakroun et al. 2017).

## 5. IMPACT, CHALLENGES, AND FUTURE DIRECTIONS

The long list of pestiferous lepidopterans (with increasing development of resistance to insecticides, and their tremendous impact on agriculture and forestry) necessitates an ongoing search for more efficient, economical, and environment-friendly ways of dealing with these pests. The SIT/IS is a species-specific technique that is highly compatible to integration with other pest management tactics, including the use of ground or aerial spraying of selective or biorational insecticides, mass-trapping,

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cultural controls, sanitation, host removal, host-plant resistance, and biological control (Carpenter et al. 2007; Vreysen et al. 2016; Suckling et al. 2017; Mangan and Bouyer, this volume). It is likely to be especially effective when combined with other inversely density-dependent tactics such as mating disruption (Suckling et al. 2012).

### *5.1. Effectiveness and Impact*

The overall effectiveness of the SIT/IS has been demonstrated to reduce lepidopteran pest populations in several operational programmes, resulting in the successful eradication of the pink bollworm in four states in the south-western cotton belt in the USA and in northern Mexico, and the eradication of outbreaks of the gypsy moth in the USA, painted apple moth in New Zealand, and cactus moth in Mexico. The application of the SIT/IS continues (in suppression programmes) to manage moth pests in the OKSIR programme against the codling moth in Canada, and now New Zealand, by reducing fruit-damage levels and reliance on insecticides. Within a comparatively short timespan of about 15 years, a programme targeting the false codling moth was developed in South Africa; it is effectively expanding the SIT-based area-wide control to additional citrus-production areas to reduce fruit-damage levels below demanding export thresholds.

At the same time, efforts started in recent years are continuing to develop the SIT against several emerging pests: the programme in Chile on European grapevine moth has moved into pilot-phase testing, with interest from several other countries; progress has been made towards the SIT for the African sugar cane borer in South Africa for a potential addition to a programme of comprehensive AW-IPM; and radiation-biology studies, and the development of mass-rearing systems, to develop the SIT are targeting the navel orangeworm. Also, research is underway to enhance the potential for the SIT to manage the carob moth in date production for export from North Africa, as well as the tomato leafminer in greenhouses (an emerging pest of solanaceous crops that has expanded into new regions).

### *5.2. Challenges*

Despite these successes, there remain serious challenges to be met to make the SIT/IS for Lepidoptera more cost-effective relative to other pest-management tactics (Simmons et al. 2010; Vreysen et al. 2016; Suckling et al. 2017). Development of lepidopteran mass-rearing systems are complex endeavours, cost-effective meridic diets are difficult to develop, insect developmental times are long, larvae are often cannibalistic, sanitation measures must be stringent to prevent pathogen contamination (fungi, viruses, microsporidia) (Abd-Alla et al., this volume), and the insects, especially adult moths, are fragile.

Unlike in dipteran SIT programmes, methods to collect and irradiate the more robust pupae have not been widely developed for operational programmes. Using pupae in moth SIT programmes could reduce costs related to handling, storage, irradiation, and transport. However, the collection, handling, and irradiation procedures for current operational programmes are based on adult moths, which require optimized protocols to maintain high quality and low mortality rates of sterile

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insects for release. This is in part because, for many moth-rearing systems, larvae pupate within the diet, and are difficult to separate out easily. Also, compared with dipteran mass-rearing systems, collections of pupae from lepidopteran rearing systems are of a less uniform physiological age, ranging from young pupae several days from emergence to pharate pupae (G. S. Simmons, personal observations). There are significant reductions in moth quality and emergence rates when irradiating pupae that are less developed than the pharate state. While there are some examples of small-scale programmes based on pupal irradiation and releases, there are significant obstacles for a larger programme to adopt these methods.

Another challenge, unlike dipteran SIT programmes (e.g. tephritid fruit flies and the New World screwworm), is that until recently relatively little attention had been given to development of procedures to maintain quality, fitness, and competitiveness traits in mass-rearing colonies of Lepidoptera (Simmons et al. 2010). While there have been no documented failures of Lepidoptera SIT programmes based on colony strain or mass-reared quality defects, in the past ten years there has been significant emphasis devoted to the importance of maintaining high quality in Lepidoptera to obtain mass-reared insects that are highly competitive (Simmons et al. 2010; Vreysen et al. 2016). This includes the idea of building rearing systems that can resist the negative effects of genetic drift and natural selection that can either result in the loss of important genetic variability associated with high performance in the field or selection for high reproduction and fitness in the rearing system that comes at the expense of high field performance (Woodworth et al. 2002; Simmons et al. 2010).

Important components to maintain in a mass-rearing system are the capacity to fly (e.g. using moths from an adult collection system in oviposition cages because they have to fly to reproduce) and the capacity for male moths to respond to a sex pheromone (Simmons et al. 2010). There is a general idea in many SIT programmes that high performance of an SIT release strain can be maintained by adding to or replacing on a regular basis the colony strain with new wild individuals. As Woodworth et al. (2002) and others show there is very strong selection for performance in captive populations, and the "wild" traits introduced with the field-collected individuals are quickly lost in a rearing system containing "captive" selected individuals. This process can occur in just a few generations, which will easily swamp the effect of new beneficial traits introduced into a mass-rearing colony, and will not improve SIT performance unless specific rearing procedures are in place to maintain competitive characteristics in the colony (Nunney 2001; Simmons et al. 2010; Hendrichs and Robinson, this volume).

Rearing large numbers of lepidopterans produces potent allergens due to the presence of scales and setae (Davis and Jenkins 1995; Parker, Mamai et al., this volume), requiring specialized equipment and air-filtration systems to reduce/mitigate allergic reactions suffered by workers. The fact that only a limited number of SIT/IS programmes for Lepidoptera have become operational has unfortunately also meant that they have not been able to take advantage of shared learning experiences in the way that the many fruit fly programmes have, and the beneficial impacts of such programmes are less well documented and accepted.

Methods to reduce the cost of lepidopteran programmes might also include combining the SIT/IS with other suppression tactics such as the release of natural

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enemies. In this instance, the cost of rearing might be reduced by using the same facility to rear both insect species, while the efficiency and effectiveness of a combined programme can help meet objectives in a more timely fashion through synergistic action of both tactics. Especially in the case of implementation of an IS strategy, the residual fertility in the parental release generation can generate some field reproduction to produce a completely sterile $F_1$ generation that can provide a significant additional resource for the build-up of natural enemy populations (Barclay 1987; Knipling 1992; Carpenter 1993; Vreysen et al. 2016; Mangan and Bouyer, this volume).

As it has for dipteran programmes, the development of genetic sexing strains (Marec et al. 2005) would greatly reduce the costs of rearing and release in lepidopteran programmes. Marec et al. (2005) demonstrated genetic sexing in the Mediterranean flour moth *Ephestia kuehniella* Zeller, using a mutant-male strain that is trans-heterozygous for two lethal genes (Marec et al., this volume). However, there is not universal agreement that having the ability to develop single-sex male release strains is necessary for increased effectiveness of the SIT in Lepidoptera; a large number of female sterile moths released in the field can distract (through sex pheromone release) fertile wild male moths, thereby assisting with mating disruption (Stringer et al. 2013).

### *5.3. Future Directions*

The application of molecular technologies might be used to develop genetic sterility, sexing systems, and reliable genetic markers for the released moths (Peloquin et al. 2000; Miller et al. 2001; Marec et al. 2005, 2007; Simmons et al. 2007; Simmons et al. 2011; Morrison et al. 2012; Bolton et al. 2019; Häcker et al., this volume). Since about 2007 there has been significant progress to develop and evaluate transgenic technology for two of the most difficult moth pests, the pink bollworm and the diamondback moth. In the pink bollworm, there were two efforts to introduce a sterile release moth into an operational programme. Genetically marked strains with fluorescent proteins could provide more reliable monitoring methods for standard sterile release programmes; they could also provide a heritable marker enabling implementation of IS systems. Field production of highly sterile $F_1$ progeny (Simmons et al. 2007, 2011), and production of a genetically sterile strain, could avoid radiation treatments and enable the production of male-only release strains (Simmons et al. 2007; Morrison et al. 2012; Jin et al. 2013). Work on the diamondback moth has progressed to the stage of field-testing genetically sterile or self-limiting strains to determine their field performance and competitiveness compared with wild strains (Bolton et al. 2019). It remains to be seen whether these technologies will be applied in new or existing operational programmes, but with further development these technologies could enable the operation of more cost-effective programmes.

The SIT/IS has special attributes that make it a desirable and unique insect pest management tool. However, because this technique requires a large start-up investment and a management-intensive support system, candidate pest species to be controlled with the SIT/IS should be selected carefully. A decision process to evaluate the suitability of the SIT/IS for controlling a pest lepidopteran should consider many

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factors including: (1) key pest status, (2) economic importance, (3) mass-rearing costs and technical feasibility, (4) favourable radiation biology, (5) migration ability, (6) potential for an area-wide approach to minimize the treatment area becoming reinfested, (7) host specificity, (8) availability of monitoring tools, (9) stakeholder and customer support, and (10) the availability and effectiveness of other compatible control options for the target pest (Simmons et al. 2010; Vreysen et al. 2010, 2016; Suckling et al. 2017).

In addition to selecting carefully the most appropriate candidate species to be controlled with the SIT/IS, the strategic objective and type of programme should also be evaluated (Hendrichs, Vreysen et al., this volume). Since prevention is far more cost-effective and environmentally desirable than long-term measures that would be required once a non-native invasive species has become established, future considerations for selecting target lepidopterans should emphasize key invasive threats, similar to the SIT preparedness model that was developed for use in Australia for the Old World screwworm *Chrysomya bezziana* (Villeneuve) (IAEA 1998; Tweddle 2002; Hendrichs, Enkerlin et al., this volume; Vargas-Terán et al., this volume). For example, the false codling moth, the leek moth *Acrolepiopsis assectella* (Zeller), the European grapevine moth, and the Central American potato moth *Tecia solanivora* (Povolny) are all serious invasive threats to the USA and other countries such as Australia and New Zealand (Suckling 2003). The proactive development of the SIT/IS technologies offshore at the point of origin offers the following advantages:

- Capitalization on contributions by foreign counterparts due to their interest in controlling the same pest,
- Reduction in pest pressure at the point of origin (offshore risk mitigation) and thereby the risk of the pest being accidentally introduced elsewhere, and
- Availability of the SIT/IS for invasive moth pests, allowing significant reduction in response time to implement an SIT/IS eradication programme should such pests become established in a new location while pest populations are still restricted geographically and at low densities.

Rapid responses to incursions rely on the ability of scientists to make use of existing knowledge, and quickly provide decision-makers with ready-response capabilities (Bloem et al. 2014). Market drivers for reducing insecticide use for IPM also provide grounds for optimism for the future of SIT/IS implementation, particularly against high-value pests of export horticulture, where the absence of insecticide residues on fruit increasingly creates market value (Walker et al. 2017). These technologies are versatile, and can be used for pest management as well as for eradication goals.

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# CHAPTER 7.4.

# IMPACT OF TSETSE FLY ERADICATION PROGRAMMES USING THE STERILE INSECT TECHNIQUE

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## SUMMARY

Hunger and poverty persist in rural sub-Saharan Africa. Many affected communities could produce enough food for themselves, and even for sale, if they had the basics — livestock and crops. In most of these communities, the presence of tsetse flies and the disease they vector, trypanosomosis, prevents optimal productive livestock-keeping and mixed farming, resulting in inadequate local food production. Since a vast majority of the rural communities depends on agriculture, the removal of a key development problem like tsetse and trypanosomosis (T and T) would permit increased local agricultural production, socio-economic and market development, and alleviate hunger and poverty. A sustained alleviation, if possible a complete lasting removal of the T and T problem, is therefore considered a prerequisite to rural self-sufficient agriculture, in which productive livestock can provide milk, meat, draught power to cultivate the land, and eventually generate higher income and market opportunities. Hence the removal of such a key problem would catalyse overall development in rural areas. However, the poverty and food security status of communities in Africa is rather heterogeneous, and reflects the impact of various constraining factors, including T and T, on the current agricultural production process and human well-being, as well as on the overall development potential. Correspondingly, the benefits to sustainable agriculture and rural development (SARD), resulting from an elimination of the T and T problem, will also vary from area to area. In view of the substantial funding required over the next decades to address this key problem, and the need for early "success stories" that show tangible benefits, it is important that the initial T and T control areas are carefully selected according to technical feasibility, and to the predicted potential of increasing agricultural productivity in the context of SARD. Trypanosomosis is a major, but technically solvable, development problem, and the effectiveness of the sterile insect technique (SIT), as a component of area-wide integrated pest management (AW-IPM) programmes, to create tsetse-free zones, has been demonstrated in Unguja Island, Zanzibar, and other locations. This chapter (1) outlines the causal relationship between the T and T problem and food insecurity, malnutrition, poverty, and related disease and development constraints, (2) describes the impact of the problem on African rural communities and the overall economy, and (3) indicates the benefits of a reduced T and T burden, or even of its zonal elimination from selected priority areas in support of sustainable rural development.

## 1. INTRODUCTION

Tsetse flies are the cyclical vectors of trypanosomosis, a disease occurring mostly in rural areas of sub-Saharan Africa where it gravely affects agro-pastoral activities. The fly infests an estimated area of 8.7 million km² where it transmits human African trypanosomosis (HAT) or "sleeping sickness" in humans and African animal trypanosomosis (AAT) or "nagana" in livestock. While human suffering and deaths from sleeping sickness have been declining and are confined to certain areas, livestock production in rural areas continues to be severely constrained by nagana. The resulting

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food insecurity and the limitations on health, food production, and even survival, imposed by these vectors and transmitted diseases, make the tsetse and trypanosomosis (T and T) constraint one of the most underestimated and severe problems for sub-Saharan rural communities.

In 1997, sleeping sickness was a potential threat to 60 million people living in the infected areas, and up to 350 000 people were assumed to be infected. However, the number of reported cases decreased from >25 000 in 2000 to 7106 cases in 2012, and the areas at high or very high risk were reduced by 60% (Simarro et al. 2015; Franco et al. 2017). In 2017, less than 1600 new cases of sleeping sickness were reported to the WHO, which represents the lowest number of sleeping sickness cases ever recorded (WHO 2017; Jose Ramon Franco, personal communication). However, there appears to be a general consensus that active and passive surveillance of the human population alone will not be enough to reduce further the number of reported cases. Therefore, vector control needs to be embraced as a requirement to keep reducing the incidence of sleeping sickness (recommendation of the third WHO stakeholders meeting (Geneva, April 2018) on *gambiense* human African trypanosomiasis (g-HAT) elimination).

Considering the overall impact (large direct and indirect losses), and the causal relationship among poverty, food insecurity, and the tsetse-related rural development constraints, it is inevitable that, in the long term, the suppression of vector populations, and better yet the creation of tsetse-free zones, are both economical and moral imperatives. If the T and T constraint were removed in an area, thereby making it feasible for rural people in subtropical and tropical Africa to produce more food than for their subsistence through mixed livestock-crop farms, they will obtain better nutrition and higher incomes, and the poorest region of the world will experience improved economic and social development.

Although some livestock producers apply methods of suppressing tsetse flies and trypanosomosis with reasonable success, the benefits are partial and usually last only a short time (Vreysen 2006). The rural poor cannot generally afford and sustain enduring T- and T-control efforts. Therefore, it appears more appropriate to identify those T- and T-infested areas that have a high rural-development potential and contain isolated or at least geographically confined target tsetse fly populations, and then implement programmes aiming at removing vector populations in a phased, integrated, and sustainable manner by applying — where feasible and justifiable — the sterile insect technique (SIT) as part of an area-wide integrated pest management (AW-IPM) programme (Feldmann 2004; Vreysen et al. 2013a; Feldmann et al. 2018). The SIT lends itself to be the final component of an integrated programme for the creation of sustainable tsetse-free zones in some, e.g. humid riparian forests and moist savannah areas in West Africa, but not all, e.g. open savannah woodland in East Africa, ecosystems. The potential to eliminate the disease in a sustainable manner may open a new dimension of benefits, derived from introducing upgraded livestock breeds and cross-breds, resulting in more productive agricultural farming practices (Bouyer et al. 2014).

Although important for economic development and conservation of the environment (Ford 1973; Reichard 2002; Nagel and Peveling, this volume), the potential for changes in land use resulting from tsetse control is not discussed here.

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## 2. POVERTY AND HUNGER

The International Fund for Agricultural Development (IFAD) stated in its 2016 rural development report that, although rural poverty declined in all regions, the Asia and the Pacific region, Latin America and Near East, North Africa, Europe, and Central Asia cut by half their rates of extreme poverty, whereas extreme poverty fell by only 10 per cent over the 1990–2010 period for eastern, southern, western, and central Africa (IFAD 2016). The annual growth of the gross domestic product (GDP) per capita in sub-Saharan Africa was reduced from 3.6% in 2000 to 1.2% in 2016 (WB 2016).

Reducing poverty in poor countries, especially in sub-Saharan Africa, is now the primary objective of development programmes. In 1990, 35% of the world's population lived in extreme poverty (USD <1.9 per day), but by 2013 this had declined to 10.7%. However, half of the extreme poor live in sub-Saharan Africa. During this same time period the number of poor in the region fell by only 4 million; in 2013 there were 389 million people living on less than USD 1.90 a day, more people than in all the other regions combined (WB 2018).

An initiative by the World Bank (WB) and the International Monetary Fund (IMF) to help the Least Developed Countries (LDC) (34 of 49 are in Africa), and especially the Heavily Indebted Poor Countries (HIPC) (34 of 42 are in Africa), to obtain debt relief, led to these countries preparing Poverty Reduction Strategy Papers (PRSPs) (UN 2000b, 2001). Significant debt relief was granted, which would, it was hoped, enhance food security and agriculture (FAO 2001a; IMF 2018). FAO has provided recent estimates; the number of chronically undernourished people in the world stands at 815 million. Most of the hungry live in low-income and lower middle-income countries, many of which have yet to make the necessary headway towards the structural transformation of their economies (FAO 2017). Although the prevalence of undernourishment in sub-Saharan Africa has decreased from 33.3% in 1991 to 18.6% in 2015, it is still higher when compared with the other regions, i.e. 16.3% in South Asia, 10.2% in East Asia and the Pacific, and 7.6% in Latin America (Roser and Ritchie 2018).

Sub-Saharan Africa remains the poorest region of the world. In 2010, average real per capita income was USD 688 compared with USD 1717 in the rest of the developing world. Over the past 30 years, GDP growth per capita in sub-Saharan Africa has averaged 0.16% per year (Chauvin et al. 2012). The World Bank estimates that the share of Africans who are poor fell from 56% in 1990 to 43% in 2012. Although the poverty rate may have declined, many more people are poor because of population growth, i.e. 330 million poor in 2012, up from about 280 million in 1990. Poverty reduction has been slowest in fragile countries, and rural areas remain much poorer, although the urban-rural gap has narrowed (Beegle et al. 2016). Poverty rates have declined only slightly in most sub-Saharan African countries, and have actually increased in Kenya and Zambia (FAO 2017).

In 2013, 6.3 million African children under the age of five died, i.e. almost 17 000 every day, and the risk of a child dying before completing five years of age is highest in Africa (90 per 1000 live births), about 7 times higher than in Europe (12 per 1000 live births). Approximately 3.1 million children die from hunger each year. In 2011 poor nutrition caused nearly half (45%) of deaths in children under five. Children who

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are poorly nourished suffer up to 160 days of illness each year, and undernutrition magnifies the effect of every disease, including measles and malaria (Hunger Notes 2018).

Boosting poor-country agriculture is critical to reducing hunger (Economist 2002), and the sustainable removal of this key development problem appears to be a prerequisite for tackling poverty and food insecurity.

> A sharper focus on hunger and agricultural development is needed within the broader objective of poverty reduction (FAO 2001b).

Hunger leads to reduced productivity, to environmental degradation, and to conflict at national and international levels (FAO 2002a; Sachs 2004; Diamond 2005). It also increases sickness, which reduces productivity, quantifiable through the Disability-Adjusted Life Year (DALY) Index (Murray 1994; Murray and Lopez 1994). Every year, across the developing world, 66 million primary school-age children (with 23 million in Africa alone) attend classes hungry, which greatly impacts their ability to learn (Hunger Notes 2018)

At the 1996 World Food Summit, a goal was set to eradicate hunger in all countries, and to halve the number of chronically undernourished people in the world (from about 840 to 420 million) by the year 2015 (FAO 1996). The target, later called one of the United Nations' Millennium Development Goals (MDG), was to achieve sustainable food production and security for all. According to the Food and Agriculture Organization of the United Nations (FAO), in 2015, sub-Saharan Africa was the region furthest from reaching the MDGs, with the actual number of hungry people increasing rather than decreasing (FAO 2017).

As part of a new sustainable development agenda, in September 2015 countries adopted a new set of goals to end poverty, protect the planet, and ensure prosperity for all. These 17 global Sustainable Development Goals (SDGs) were set by the United Nations with specific targets to be achieved over the next 15 years. The SDGs cover a broad range of social and economic development issues, including poverty, hunger, health, education, climate change, etc. Although the SDGs replaced the MDGs, unlike the MDGs, they do not distinguish between "developed" and "developing" nations, and apply to all countries (UN 2015).

Programmes that feed the hungry with imported food, as important as they are at critical times, provide only temporary relief, and, if wrongly implemented, they can have a devastating effect on rural people whose livelihoods depend on the production and sale of staple crops (Clark 2001). The role of agriculture, in generating food supplies and incomes necessary for development and access to food, is paramount in developing countries, especially the Low-Income Food-Deficit Countries (LIFDCs) (FAO 2001d).

Agriculture employs on average 65% of Africa's labour force, but it accounts for only about 32% of gross domestic product, reflecting the relatively low productivity of the sector (Chauvin et al. 2012). Success in raising small-farmer productivity will lead to improvements in household food security, level of nutrition, and income. Efforts to eradicate hunger need to focus on empowering families to achieve inclusive food security, encouraging a maximum of self-reliance (FAO 2001c).

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> As incomes grow and diets diversify during structural transformation, the demand for food generally shifts from basic staples to horticultural and livestock products. This leads to a shift in the overall structure of agricultural production. But evidence from Africa suggests that while such a shift is occurring in some countries, it is not yet the norm across the continent. Agriculture shows healthy growth in terms of both output and productivity, but it is not diversifying its commodity mix much. The picture that emerges is of an expanding agricultural sector, but one with weak fundamentals that are preventing a broad-based reduction in poverty and inequality (IFAD 2016).

## 3. LIVESTOCK AND CROP PRODUCTION

A major barrier to significantly improving agriculture in sub-Saharan Africa is the lack of productive livestock. Productive livestock are the key to agricultural improvement because they: (1) provide food (milk and meat), (2) aid in crop production, especially of staples and cash crops, through draught power to cultivate the land for crops and manure for fertilizer, and they can live off crop residues as well as grazing, (3) provide manure for fuel, hides for leather and power for transport, (4) act as a form of savings, a "walking bank", and thereby reduce risk, and (5) provide a vital, and often the only, source of income for the poorest and most marginal of the rural poor, such as pastoralists and those living on marginal land, sharecroppers and widows (Delgado et al. 1999). Livestock, for example a few cattle and goats, owned by each rural family are the means to food production on a continuing basis.

An estimated one-third of all children in developing countries, and perhaps a higher share of pregnant or lactating women, suffer from mild to moderate protein-energy malnutrition (FAO 2001d). People in developing countries typically consume annually only one-fifth to one-sixth the milk, and one-third to one-fourth the meat, of those in industrial countries (FAO 2000). In 1995–1997, people in developing countries obtained an average of only 11% of their calories, and 22% of their protein, from animal food products, compared with more than twice these figures (25% and 49%, respectively) in developed countries (FAO 2001d).

> These low consumption levels in developing countries indicate the potential for growth in the consumption of animal food products without adverse consequences on human health. Increased consumption, of even a relatively small additional amount of meat and milk, would supply the necessary protein and micronutrients, as well as needed additional calories, especially for children (FAO 2001d).

Farming families with small holdings must be enabled, through mixed farming, to increase agricultural productivity for their own immediate benefit.

> The integration of livestock and crop operations is the main avenue for sustainable intensification of agriculture in many regions of the developing world (FAO 2000). Zimbabwean smallholders, who combine livestock and crop production, have incomes twice as high as those with only crops (IFAD 2001).

Traditional agriculture in Africa is based on crops grown on land worked with a hand-hoe. According to the FAO, in sub-Saharan Africa, 89% of all primary cultivation is carried out by hand (Budd 1999). However, the hand-hoe is not enough to produce sufficient crops to feed rural Africa! Animal draught power enables a family to cultivate at least twice as much land as can be done by hand (2 hectares with a team of oxen compared with 1 hectare with a hoe) (Swallow 1999). Animal traction

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has been shown to generate higher returns on land (25–45%) and labour (140%) than the hoe, partly from the advantage of using manure as a fertilizer (Swallow 1999).

If the land is not suitable for crops, then livestock can still be grazed on it. In the case of natural disasters, such as drought and floods, livestock provide longer-lasting food security, and more risk-free agriculture, than crops. Even landless people can own cattle or small livestock, which graze on communal-pasture land, or are kept indoors with feed brought to them (zero grazing).

## 4. TSETSE AND TRYPANOSOMOSIS (T AND T) PROBLEM

### *4.1. Human African Trypanosomosis (HAT) (Sleeping Sickness)*

Tsetse-transmitted human African trypanosomosis, sleeping sickness, has been a major burden on African people in rural areas between northern Angola (Independent Online 2004) and southern Sudan. If left untreated, the disease is inevitably fatal; it has been estimated that, at the beginning of the last century, 50 000–100 000 people died every year from the disease (Shaw 2004). Although the number of persons dying from the disease drastically decreased between 1900 and 1960, since many African countries obtained their independence in the early 1960's the number of people succumbing to the disease increased again until the end of the last century. However, since then, enormous progress has been made in reducing the disease prevalence in Africa, from many thousand new HAT cases in 2000 to 977 cases in 2018 (Fig. 1).

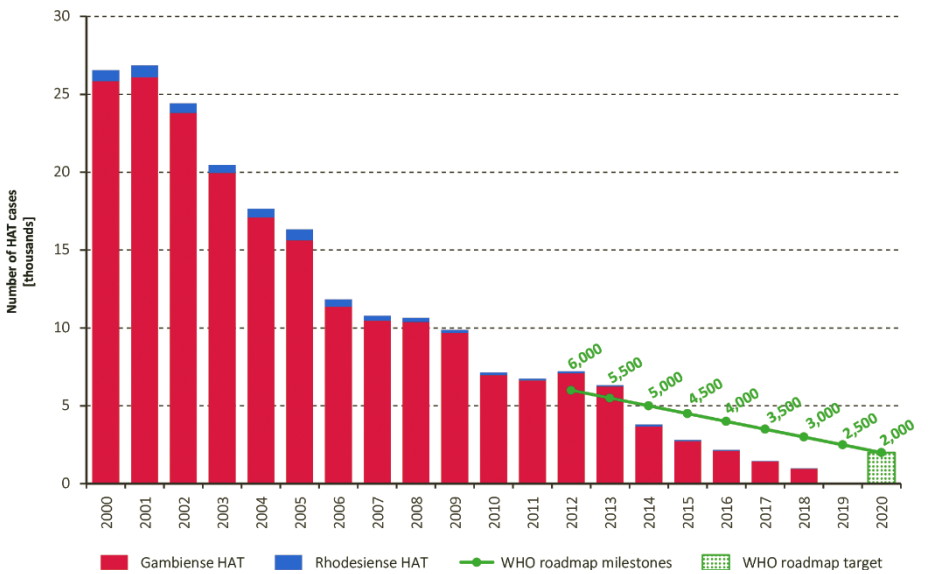



*Figure 1. Total number of reported cases of HAT* (gambiense *and* rhodesiense) *per year (2000–2018). The green line and the green bar show the milestones and target set in the WHO Roadmap for HAT elimination. (Reproduced from Franco et al. 2020.)*

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### *4.2. African Animal Trypanosomosis (AAT) (Nagana)*

#### *4.2.1. Problem of African Animal Trypanosomosis*

Unlike HAT, the disease continues to be especially devastating to livestock, with 45–50 million animals at risk in T- and T-affected countries (Kamuanga 2003; Shaw 2004); in these countries, the vast majority of the animals are crowded into the few tsetse-free areas. In some areas, there is evidence that the problem is exacerbated by an expansion in the distribution of tsetse flies (Leak and Mulatu 1993; Stevenson 1998), even reaching, as in Ethiopia, higher altitudes than was previously recorded (Vreysen et al. 1999).

As stated in ILRI (1999),

> Trypanosomosis is probably the single greatest health constraint to increased livestock productivity in sub-Saharan Africa,

and it significantly impairs agricultural development (Swallow 1999). When not lethal, trypanosomosis in livestock leads to a chronic debilitating condition that reduces fertility (calving rates), weight gain, meat and milk offtake by at least 50% (USD 2750 million per year), and the work efficiency of oxen used to cultivate the land (Budd 1999; Swallow 1999; DFID 2001; Shaw 2004). However, if left untreated, it is often fatal (especially for calves), with at least 3 million cattle and other domestic livestock dying each year (Hursey and Slingenbergh 1995). The problem is getting worse rather than better.

Besides the direct impact of the disease on livestock, there are also indirect negative effects. It discourages using more-productive exotic and cross-bred cattle, depresses the growth and affects the distribution of livestock populations, reduces the potential opportunities for livestock and crop production (mixed farming) through less draught power to cultivate land and less manure to fertilize (in an environment-friendly way) soils for better crop production, and affects human settlements (people tend to avoid areas with tsetse flies) (Shaw 2004).

T and T are enormous barriers to raising productive cattle in most of the humid and sub-humid zones of Africa (FAO 2000), especially exotic breeds and cross-bred cattle, which are much better milk and meat producers than local breeds but more susceptible to the disease. At present, cattle (especially cross-breds) can only be raised relatively satisfactorily outside (or around the periphery) of the tsetse zone, or by giving regular treatments of expensive trypanocidal drugs, in addition to other health care. Therapeutic chemical treatments are expensive and must be administered regularly. It has been estimated that as many as 35 million doses of trypanocides are used annually in sub-Saharan Africa alone (Holmes 2013), which represents a figure suitable to treat only about one-third of the cattle at risk (Swallow 2000). Inclusion of trypanocides sold informally in the African market may substantially increase the total number of doses sold annually, which may be as high as 70 million doses (Giordani et al. 2016). This practice elicits drug resistance in the trypanosomes, in combination with the sale of fake trypanocides drugs (Melaku and Birasa 2013). Consequently, treatments are no longer effective, and the future of cattle production in these areas may become problematic.

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The use of trypanotolerant cattle in West Africa shows some promise as a way of coping with the disease threat, but these animals are rather small (DFID 2001) and thus are less suitable as draught animals. Shaw et al. (2004) compared trypanotolerant, Zebu and cross-bred (trypanotolerant x Zebu) cattle in herd models. Benefits with and without T and T, expressed per head of cattle over a 20-year period, were highest with cross-bred cattle.

Current methods of controlling tsetse, and the disease in humans and livestock, are by no means perfect. The current situation with respect to AAT is untenable, with risk to the environment arising from high pressure on natural resources in uninfested areas where livestock are being concentrated. The problem remains a major barrier to meeting the basic livelihood needs of the rural poor.

### *4.2.2. Benefits of Controlling African Animal Trypanosomosis*

Everyone agrees that AAT

> . . . is sufficiently important for virtually any intervention to be beneficial (Shaw 2003).

*Livestock Production.* The benefits of T and T control are reduced disease prevalence or even disease elimination, reduced mortality rates and treatment expenses, and improved health and agricultural productivity. These benefits can be estimated using a dynamic herd model, which includes animal traction among the herd outputs. The reduced livestock production cost (38% and 25% per tonne of milk and meat, respectively) would increase the amount of milk and meat supplied by farmers, and lower the price for consumers. For areas freed of tsetse, milk and meat production would almost double, increasing by 83% and 97%, respectively. Benefits from traction power and manure production have been estimated to be, on average, 34% of the total value of livestock production (Kristjanson et al. 1999). The monetary values of losses and benefits are summarized in Box 1.

The 36 countries (10 million km², population 260 million) that have some level of tsetse infestation have about 172 million cattle, but only 45 million of them are kept in tsetse-infested areas, while the remainder are forced to crowd around the periphery, leading in some instances to land degradation (Swallow 1999). The overcrowding of cattle and people in tsetse-free areas is a problem for both good land-use practices, in terms of sustainable utilization of natural resources, and long-term community development activities.

It is expected that enhanced trypanosomosis control will allow the replacement of low-productive cattle by high-productive cross-breeds that can be kept at a lower average density per unit of land (with more rural families owning cattle) which will in many instances bring relief to the often fragile ecosystems in sub-Saharan Africa (Vreysen et al. 2021).

Therefore, the creation of sustainable T- and T-free zones, and the resulting rural development and the opportunities for profitable investment into productive agricultural and livestock systems, are not expected only to be major contributions in the fight against food insecurity and poverty, but also to reduce the environmental risks of a potentially large increase in the number of cattle.

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*Box 1. Monetary Values — Estimates of Losses from the T and T Problem, and Potential Benefits of Controlling African Animal Trypanosomosis*

Hursey and Slingenbergh (1995) valued the direct annual losses in cattle production due to AAT at USD 1000–1200 million.

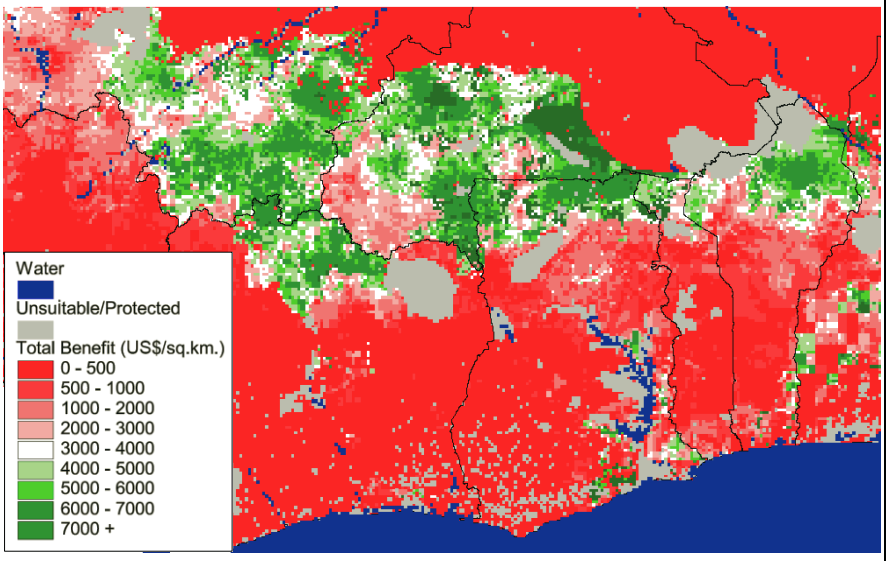
Shaw et al. (2004) created maps that show the estimated potential benefits from T and T control in Benin, Burkina Faso, Ghana, Mali, and Togo. The research modelled cattle density, animal draught work, rate of herd growth, and rate of cattle spread. If the disease were removed, the total benefits after 20 years (in the form of a present value discounted over 20 years) ranged from less than USD 500 to well over 5000 per km² (Fig. below). Benefits included milk, meat, draught power, the extra cattle present, and the spread of cattle. The estimated benefits from the removal of the tsetse fly from the Niayes were much higher, amounting to about USD 3250 per $km^2$ per year, mainly by replacing indigenous breeds with cross-breeds or exotic breeds (Bouyer et al. 2014; section 6.2.).

The potential benefit of enhanced trypanosomosis control in livestock in Africa is USD 700 million per year, arising from increased milk and meat productivity (Kristjanson et al. 1999). If one includes other potential benefits that would accrue from trypanosomosis elimination, such as animal traction and manure,

> …the total cost of the disease is likely to be well in excess of USD 1338 million per year (Kristjanson et al. 1999).

In 10 fully infested countries alone, the impact of T and T on the agricultural GDP was estimated to amount to 10% below a theoretical T- and T-free GDP level (Swallow 1999), which corresponds to USD 1000 million in monetary terms.

Estimates of the overall annual lost potential in livestock and crop production range from USD 1950 to 4500–4750 million (Budd 1999; DFID 2001; Shaw 2004; Gooding and Krafsur 2005).




*Total calculated potential benefit (USD per km²), after 20 years, of removing trypanosomosis (benefit range USD 0–500 [dark red] to 7000+ [dark green] per km²). (Modified from Shaw et al. (2004) and W. Wint for the countries Benin, Burkina Faso, Ghana, Mali, and Togo; reproduced with permission.)*

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*Crop Production and Mixed Farming.* In sub-Saharan Africa, livestock and crops are usually separated due to T and T, but there is a huge potential benefit if livestock and crop production can be integrated (Box 1).

In total, about 8.2 million $km^2$ in Africa are classed as cultivable land (but half of it is marginal) (Harrison 1996). Up to 6 million $km^2$, especially in Central and southern Africa, are not cultivated for various reasons (Ford 1973). The best potential for fodder production lies in the humid zones, 18% of Africa's land area, but only 6% of the livestock are found there (Harrison 1996). In some cases, tsetse flies deny access to fertile, arable land, and their removal makes it accessible to livestock-agricultural production.

> African animal trypanosomosis constrains agricultural production in the areas of Africa that hold the continent's greatest potential for expanded agricultural production (Swallow 1999).

### *4.2.3. Causal Relationship between T and T, Hunger, and Poverty*

Evidence for the causal relationship between the T and T problem and the prevalence of hunger and poverty is presented in this section. It cannot simply be a coincidence that most of the 37 tsetse-infested countries of Africa are also poor, debt-ridden, and underdeveloped. A comparison between the tsetse-infested areas and the 34 Heavily Indebted Poor Countries in Africa (WB 2001) (32 of which have tsetse flies), reveals a striking overlap (Fig. 2); it is logical to conclude that the T and T problem is a major contributor to hunger and poverty.

Of course there are other problems creating food insecurity, such as limited water and fertilizer supply, severe human diseases, lack of credit, poor governance, civil unrest and wars, as well as other livestock diseases, and these are also important, and some are interconnected and interdependent. Solutions to these problems must be found as well. However, to achieve rather quickly the objective of reducing hunger and poverty in such a needy region as sub-Saharan Africa, it is vital first to adopt the solution that will bring about the biggest change in the fundamental problem, and for which strategies and technology packages already exist and need to be integrated into the overall context of sustainable agriculture and rural development (SARD). It is a matter of giving a high priority to a problem that is fundamental, and to a solution that will have a great impact in the near future. This approach is part of the comprehensive policy of the Programme Against African Trypanosomiasis (PAAT), an international alliance comprising the FAO, African Union/Inter African Bureau for Animal Resources (AU/IBAR), International Atomic Energy Agency (IAEA), and WHO. The removal of T and T is expected to have a catalytic effect on overall rural development initiatives aimed at food security and poverty reduction.

### *4.2.4. Why is the T and T Problem so Hard to Solve?*

Why has the barrier of T and T remained so strong, and prevented a breakthrough in livestock and crop production? The T and T problem is a rural problem, and most political leaders, policy makers, and planners are probably already overwhelmed by various needs and deficiencies requiring immediate attention, ranging from water supply, infrastructure issues like roads and schools, to medical and other services.

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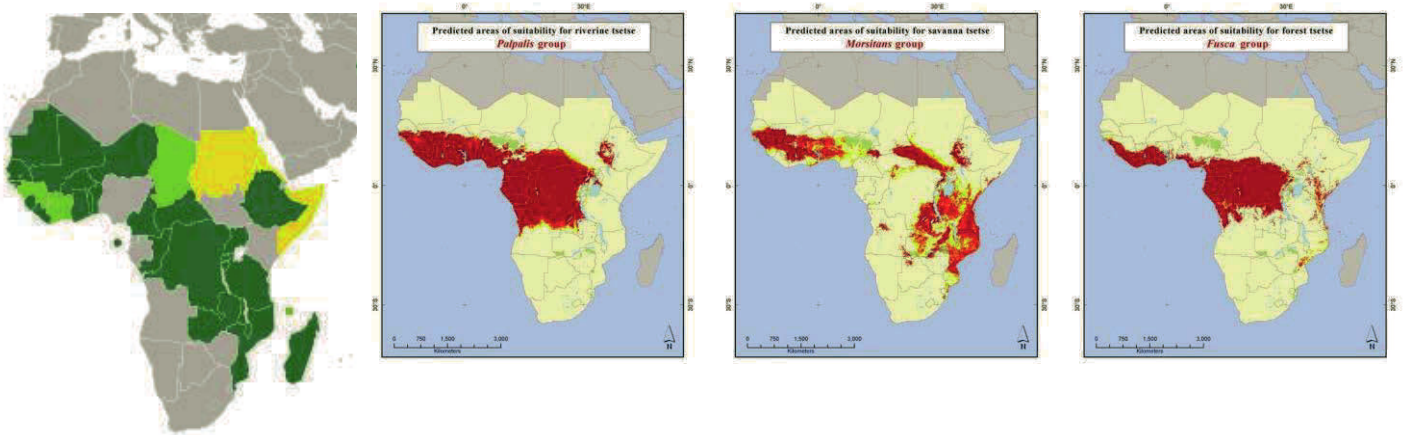



*Figure 2. Left map: Heavily Indebted Poor Countries (dark green – countries qualifying for full HIPC relief, light green – countries qualifying for partial HIPC relief, yellow – countries eligible for HIPC relief but have not met the conditions) (map by Christallkeks (2016) - Own work, CC BY-SA 4.0); Right maps: Predicted areas of suitability (yellow – 10–40%, orange – 40–70%, red – 70–95%, brown – >95%) for the* palpalis *(riverine),* morsitans *(savannah), and* fusca *(forest) groups of tsetse flies (FAO/DFID, reproduced with permission).*

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Actions against a problem like T and T are expected to result in benefits only in the medium to long term, and thus may receive a low or inadequate level of attention and political support. The attention of donors and the media tends to focus on acute, novel, or emerging problems such as the Ebola (WHO 2016) and Zika (CDC 2016) crises of 2015–2016, and not on chronic problems such as T and T. This is probably a contributing factor why the HAT and AAT are considered to be "neglected tropical diseases". Since several efforts in the past to control the T and T problem did not lead to a sustainable improvement, it is difficult now to generate the necessary awareness and commitment among decision-makers.

Also, several influential scientists maintain the view that tsetse flies and the disease trypanosomosis cannot be eradicated, and that there is no alternative except to "live with the problem" at the lowest possible level of disease transmission. Many people have spoken out against "eradication" of tsetse populations, and many are pessimistic, believing that the "curse of tsetse" must simply be endured. This prevailing fatalistic attitude has led to a submissive acceptance of the seemingly inevitable need to suffer hunger and poverty, with no hope of change. This attitude and approach to the T and T problem has prevented farmers, governments, and donors from trying to create, and consistently and gradually expand in a sustainable manner, zones that are free of the problem.

In past years, several community-based programmes to reduce fly populations were initiated enthusiastically, and relatively successfully suppressed tsetse populations and consequently the disease risk. However, enthusiasm waned, suppression was neglected, and the flies and disease returned (Jordan 1995; Barrett and Okali 1998). Unfortunately, even during phases of good tsetse suppression, the flies were still efficient vectors, and therefore continued to discourage the introduction of productive livestock breeds. This was recently demonstrated again in the Mouhoun area of Burkina Faso where, despite a reduction in apparent density of the tsetse fly population from >10 flies/trap/day to 0.43 flies/trap/day, there was no impact on the prevalence of AAT (Percoma et al. 2018).

#### *4.2.5. T and T, and Sustainable Agriculture and Rural Development (SARD)*

Large-scale AW-IPM programmes against T and T should focus on priority development areas in affected countries. The selection of such priority areas in the context of SARD, and in view of the need for substantial funding over the next decades, should focus on areas where the required investments are expected to result in maximum benefits. FAO provided some criteria and guidelines for the selection of initial priority intervention areas (FAO 2002b; Cecchi et al. 2014, 2015; Diall et al. 2017.

To generate impact and benefits for the affected communities, it is essential that control measures against the T and T problem be firmly embedded in overall development efforts towards SARD. A programme called Farming in Tsetse Controlled Areas (FITCA), which was completed in 2004, aimed to improve the welfare of people through sustainable rural development, and to increase livestock productivity by improving animal health through community-based T and T suppression. FITCA had anticipated that the generation of integrated crop/livestock production systems would increase food production (Daily Nation 2003;

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AU/IBAR/FITCA 2004). However, the last 20 years have shown that sustaining such community-reliant methods of T and T suppression beyond the phase of funded institutional assistance has been challenging if not impossible (Vreysen 2001, 2006). It has been demonstrated numerous times that, after the successful introduction of community-based tsetse suppression and a discontinuation of donor support, very few farmers pursue the work and pay for the traps, insecticides, etc. Whenever an approach, that necessitates continued action, is not sustained, no breakthrough is possible. Under such scenarios, sub-Saharan Africa will likely remain a "green desert", preventing sustainable self-sufficiency in food for the rural poor.

#### *4.2.6. PATTEC*

The African Heads of State and Government, at the 36th Ordinary Session of the Organization of African Unity (OAU) (now called African Union (AU)) summit meeting in Lomé, Togo, in July 2000, recognized the seriousness of the T and T problem and made an historic decision (OAU 2000). The year 2001 was declared as the "Year of the Control of Tsetse Fly" to mark the beginning of renewed efforts to suppress tsetse flies and trypanosomosis. This new effort, initiated by the OAU, is called the Pan-African Tsetse and Trypanosomosis Eradication Campaign (PATTEC) (PATTEC 2000; Pesticide Outlook 2002). The PATTEC Plan of Action to be implemented was approved at OAU's Lusaka meeting in 2001 (OAU 2001). This decision was received with great enthusiasm by the T- and T-affected countries; they anticipated that this new African-owned and African-led initiative would result in a useful contribution to reducing rural hunger and poverty. Despite the initial euphoria and high expectations, the reality after almost 20 years is more sobering and, with a few exceptions, little overall progress can be reported.

## 5. TSETSE- AND TRYPANOSOMOSIS-FREE ZONES

### *5.1. Concept*

To implement this approach of creating and progressively expanding T- and T-free zones in areas with great potential for livestock-agricultural development, properly developed plans and programme documents are needed. As the problem is complex, a major effort is required that necessitates full ownership, and a high, consistent commitment by the affected countries. Long-term partnerships with local, national, and international stakeholders, non-governmental organizations (NGOs), and the private sector will be instrumental in the gradual creation of zones free of the most important tsetse species in priority areas for rural development. The very large benefits of complete disease removal will only be realized if the disease threat is virtually non-existent, which would encourage poor producers to take the step of investing in productive cattle.

Whereas suppression of tsetse fly populations, to temporarily alleviate the burden of the disease, will remain important in many areas of sub-Saharan Africa, in certain ecological settings the creation and progressive expansion of sustainable T- and T-free

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zones (Feldmann and Jannin 2001; UN 2001) will provide substantial benefits for the rural poor, enabling them to produce enough food (Feldmann 2004).

Given the current technologies available, it is not possible to apply this concept to the whole of tsetse-infested areas of sub-Saharan Africa. In addition, the use of the SIT against tsetse populations would only be justifiable in those ecological zones where the SIT would bring a comparative advantage. Successful examples, where the SIT was not used, include: 1) the combined use of insecticide applications by air and ground teams and habitat destruction, which resulted in long-lasting relief from the disease in northern Nigeria (Spielberger et al. 1977), and 2) the integrated use of the sequential aerosol technique (SAT) with trapping in the border areas, which resulted in the creation of a sustainable zone free of *Glossina morsitans centralis* Machado in the Okavango Delta of Botswana (Allsopp and Phillemon-Motsu 2002; Kgori et al. 2006). Both programmes followed, intentionally or not, the principles of AW-IPM, and capitalized on favourable agro-ecological or other relevant trends or developments.

### *5.1.1. African Animal Trypanosomosis*

In animal trypanosomosis, tsetse suppression has an important role to play in immediate (but with long-term perspectives) problem alleviation in priority areas, and as a forerunner of creating tsetse-free zones (PAAT 2000).

The concept of creating and progressively expanding T- and T-free zones following AW-IPM approaches will require substantial funding over many decades (Vreysen et al. 2007). Initial resources must be focussed on a few selected priority areas — with strong demographic pressures, and high agricultural, livestock, and overall development potential — and must achieve good progress with early successes. An advantage that should be utilized is the fragmented distribution of some tsetse fly species, with discrete populations in isolated or well-confined habitats. In that respect, Bouyer et al. (2014) identified natural barriers that isolate populations of *Glossina palpalis gambiensis* Vanderplank in West Africa using a statistical model that assessed the genetic distance between 37 populations. Potentially isolated clusters of *G. p. gambiensis* habitat were identified based on a species distribution model and ranked according to their predicted genetic distance to the main tsetse population (Bouyer et al. 2014; Feldmann and Ready 2014; Vreysen, this volume).

The initially targeted areas will be those with such isolated or confined fly populations. Progressively, over time, the size of infested areas will be reduced. The transboundary nature of fly infestations requires a regional approach, based on the AW-IPM concept, to reduce substantially the risk of fly reinvasion.

The international T and T community has agreed that efforts to create and subsequently expand T- and T-free zones should be initiated in areas with a high potential for agricultural development, e.g. Southern Rift Valley in Ethiopia and the Niayes area in Senegal (Swallow 1999; Feldmann and Jannin 2001; FAO 2002b; PAAT 2002; Alemu et al. 2007; Zerihun 2017; Vreysen et al. 2021). Whereas the Moist Savannah Zone in West Africa has great potential for agricultural development, and the watersheds of the main river basins were initially considered as effective barriers to migration (Hendrickx et al. 2004), new data have indicated the potential of flies to cross these barriers, demonstrating the need for artificial barriers (Vreysen et

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al. 2013b). In addition, the use of the SAT against *Glossina tachinoides* Westwood and *G. p. gambiensis* was shown to have its limitations in the Moist Savanna Zone of Ghana, West Africa. The SAT operations failed to achieve eradication in an area of ≈18 000 km$^2$, and both target species were still detected in the sprayed blocks, albeit at low densities (Adam et al. 2013). In view of the extreme low fly population densities obtained, the project in Ghana would most likely have been successful in creating a tsetse-free zone had the spraying operation been followed by the release of an adequate number of high-quality sterile males.

There may be opportunities to embark on projects for creating T- and T-free areas in several other countries, but further evaluations are needed, e.g. Chad (Mahamat et al. 2017), South Africa (Kappmeier et al. 2007), Kenya, Uganda, and the United Republic of Tanzania (IAEA 2001). In some of these areas, particularly where other vector suppression techniques face technical limitations, or for environmental or other reasons can only be applied as a temporary measure, the SIT may play a role.

To minimize ecological disturbances, and ensure environmentally appropriate utilization of natural resources, components of the environment, and land-use practices, must be monitored before and after removal of the vector population (Feldmann and Hendrichs 2001). Even though there is no factual basis for the fear that tsetse eradication will lead to ecological imbalance (AU 2002; Nagel and Peveling, this volume), it is wise to monitor continuously several components of the biological and physical environments to permit, at an early stage, the identification and correction of undesirable developments in the field.

#### *5.1.2. Human African Trypanosomosis*

In addition to efforts to create T- and T-free zones in selected areas that have great potential for agricultural development, T and T suppression needs to be pursued in the remaining sleeping sickness foci (Fig. 3). The neglected tropical disease roadmap of the WHO calls for the elimination of *gambiense* HAT (g-HAT) as a public health problem by 2020, and complete elimination of transmission by 2030 (Franco et al. 2017). In 2017, there were 1370 centres that were equipped to screen for *gambiense* HAT, and more than 600 centres were equipped to provide treatment. As a result, the number of cases reported in 2017 was low (<1600). Even though 80% of all the new cases were reported from the Democratic Republic of the Congo, they were still distributed over 17 countries (J. R. Franco, WHO, personal communication). In view of the ever-decreasing number of new *gambiense* HAT cases detected each year, there is evidence that vector control will gain in importance in the overall human sleeping sickness elimination approach (Mahamat et al. 2017).

### *5.2. Potential Role of the SIT*

The SIT has been demonstrated to be effective and efficient against tsetse flies under certain ecological conditions when integrated in a phased conditional manner with other suitable tsetse suppression methods (Cuisance et al. 1986; Clair et al. 1990; Oladunmade et al. 1990; Vreysen et al. 2000, 2021). The scientific principles of the technology against tsetse flies have been well studied in Africa, and have now been

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proven (Offori 1993; Feldmann and Hendrichs 2001; Feldmann and Jannin 2001; Feldmann 2004; Vreysen 2006; Vreysen et al. 2021). When applied on an AW basis, the integrated approach benefits all segments of rural society, including poor farmers, because, instead of protecting selected animals or farms, entire economically important populations of tsetse flies are targeted (Klassen and Vreysen, this volume). Furthermore, because these programmes aim at a public good, local people would usually not be charged for the SIT component. This approach involves, in a first phase, the area-wide application of control tactics suited for tsetse suppression (e.g. insecticide impregnated traps/targets, pour-on for livestock, stationary bait technology, and the SAT) (Mangan and Bouyer, this volume), which, in certain ecological settings, are followed by a second phase – the release of sterile male insects to remove the remaining relic populations.

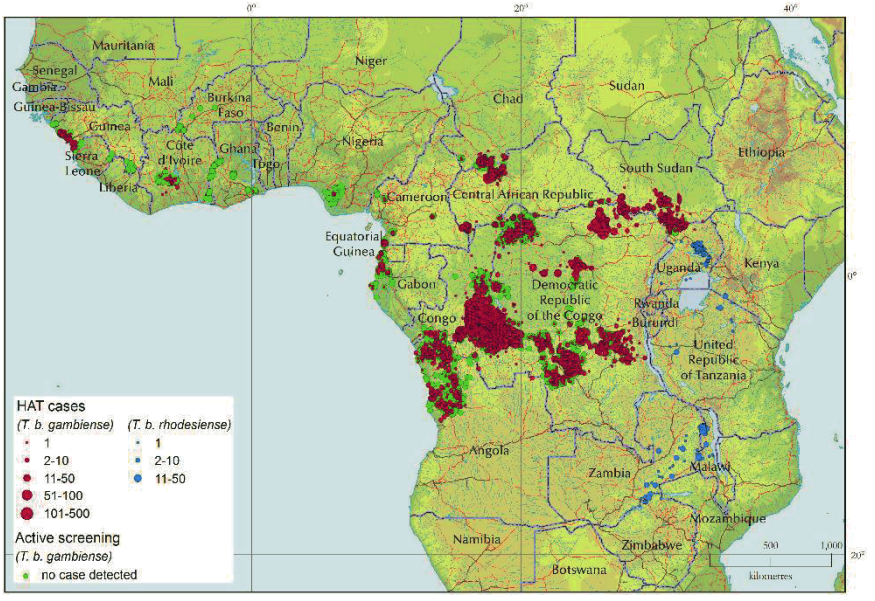



*Figure 3. Remaining foci of* gambiense *(red circles) and* rhodesiense *(blue circles) HAT in sub-Saharan Africa for the period 2010–2014. Green circles: active screening in which no HAT case was detected. (From Franco et al. 2017.)*

The SIT is one of the few control tactics that acts in an inverse density-dependent manner, and is therefore most efficient at low pest densities (Knipling 1979). Therefore, the SIT would best be applied when the pest density is naturally low or due to prior suppression. When the SIT is integrated as a final component into an area-wide pest management system, the treatment area can ultimately become a tsetse-free zone (Vreysen et al. 2000, 2021).

The insecticide-based and other suppression techniques, e.g. trapping, most often used in such integrated area-wide campaigns are environmentally acceptable, provided they are applied for a short time (Nagel and Peveling, this volume). The insecticide is applied in a localized way – on cloth targets, cloth traps, netting, or

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livestock, and is therefore not dissipated in the environment. Even after the application of the SAT (repeated application of ultra-low-volume formulations of non-persistent insecticides, usually from aircraft) used to eradicate *G. m. centralis* from the Okavango Delta in Botswana (Kgori et al. 2006), Perkins and Ramberg (2004) showed that terrestrial and the vast majority of aquatic non-target invertebrates recovered within 1 year to the pre-spray composition and abundance. After the temporary use of such techniques to suppress efficiently the vector population to a very low level, the application of the SIT to remove the relic tsetse population involves using the only vector-control method that has no known side-effects on non-target organisms (Müller and Nagel 1994; Feldmann and Hendrichs 2001).

The weekly cost of tsetse SIT per km², i.e. USD 2.7–9.4, is in the range of, or lower than, the required expenditure (weekly, per $km^2$) in AW-IPM programmes integrating the SIT against other insect pests of agricultural and veterinary importance, e.g. codling moth *Cydia pomonella* (L.) (USD 100), Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (USD 80), and the New World screwworm *Cochliomyia hominivorax* (Coquerel) (USD 3.5) (Hendrichs, Vreysen et al., this volume). Therefore, in those ecological settings where the SIT brings a comparative advantage, integrating the SIT into area-wide programmes, and sustainably removing a target tsetse fly population, will be cost effective. If programmes to create and expand T- and T-free zones are focused, and relatively small incremental steps are taken following the phased conditional approach (Vreysen et al. 2021), financing field projects in African countries should be feasible. Budd (1999) stated that an

> . . . economic analysis indicates that the cost of controlling trypanosomosis through controlling the tsetse fly populations will be covered several times by the benefits of tsetse-free status.

## 6. BENEFITS FROM TSETSE ERADICATION

### *6.1. Benefits from Eradicating* Glossina austeni *in Unguja Island, Zanzibar, Tanzania*

In 1994–1997 it was demonstrated that, after pest suppression with insecticides, the SIT completely eradicated the *Glossina austeni* Newstead population in Unguja Island of Zanzibar (Vreysen et al. 2000); subsequently, trypanosomosis in local cattle could no longer be found (Vreysen et al. 2014). This successful AW-IPM programme created significant opportunities to improve livestock and crop farming, although Unguja Island is not the best example of the agricultural benefits that would accrue from eliminating the T and T constraint compared with the African mainland. Unguja Island has poor soils, and hence the contribution of agriculture to the GDP on Unguja has been rather low and not consistent, i.e. it declined from 38% in 1997 to 24.7% in 2007, but increased again to 30.8% in 2013 (Mwaseba et al. 2015). According to a government census in 2007/2008, crop production dominated agricultural activity, and engaged 86 509 households (66%); this compares with 43 844 households (33%) that were engaged in both crops and livestock, and 1840 households (1%) that were engaged in livestock only. According to the socio-economic survey of 2014 (Mwaseba et al. 2015), 57.4% and 27% of respondents listed farming and livestock

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keeping, respectively, as their major occupation. This is a significant reduction when compared with earlier studies that indicated 88 or 89% of the population was engaged in farming (Tambi et al. 1999; Mdoe 2003).

Socio-economic studies were carried out in Unguja Island in 1997, 2002, and 2014 (Tambi et al. 1999; Mdoe 2003; Mwaseba et al. 2015) using collected local data, interviews with farmers, and relevant socio-economic parameters. The data illustrate the range of agricultural and socio-economic benefits that can accrue after removing the T and T constraint. (Sleeping sickness did not occur in Unguja, so no direct human health benefits were assessed.) It is important to recognize that the benefits began accruing as soon as the tsetse fly population and disease transmission were reduced, in some areas starting in 1985, using insecticides, and continued until, and especially after, the target fly population collapsed in 1996 as a result of applying the SIT. For the past 20 years or more, the island of Unguja has remained free of *G. austeni* and AAT (Saleh et al. 1999; Vreysen and Dixit 2016).

The following summarizes some of the benefits, but also some of the constraints experienced after the removal of the T and T problem. An analysis is made as to why agricultural development on Unguja Island has not expanded to its full potential.

### *6.1.1. Benefits to Livestock Farmers*

Although the contribution of the livestock sub-sector to the agricultural GDP increased from 12% in 1986 to 34% in 1997, in the following 15-year period (1999–2014) the percentage of the population engaged in global farming decreased, suggesting a significant shift to better paid employment opportunities other than farming. This is reflected in a reduction of the population engaged in farming as a principal and secondary occupation, i.e. from 88–89% in 1999–2002 to 57% in 2014 (as principal occupation), and from 40.7% in 2002 to 25% in 2014 (as secondary occupation).

*Livestock Keeping*

- Whereas the proportion of households raising improved cattle increased from 2% in 1985 to 18% in 1999 and to 24% in 2002, this percentage declined to 13% in 2014.
- Generally, there has been improvement in agriculture as evidenced by increases in crop and livestock numbers. Whereas from 1985 to 1999 the number of indigenous cattle increased by a factor of 1.5 and that of cross-bred cattle increased 7 fold, between 2009 and 2014 the number of improved cattle breeds increased by only 4%. However, during the same 5-year period, there was a significant increase in the numbers of improved chickens (82%), sheep and goats (69%), and donkeys (62%), and there was a decline in indigenous cattle (-21%), local chickens (-22%), and indigenous sheep and goats (-15%).
- Even though 38% of the farmers preferred local breeds of cattle, the majority (61%) preferred improved breeds, primarily because of their high productivity. Although the 2014 survey showed that farmers keep more local cattle breeds than improved ones, about 96% of those who preferred improved breeds said that they produced more milk than the indigenous ones. This is slightly higher than the 94%

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reported in a similar study by Mdoe (2003). In 2014, a shortage of land and feeds was often mentioned as a constraint on increasing the number of livestock kept, especially dairy animals.

- Only about half (48.2%) of livestock keepers were using zero grazing in dairy production, down from 91.6% in 2002 (Mdoe 2003). The limited expansion and development of the dairy sector is largely explained by the constraints that the sector continues to face: low adoption of rearing improved cattle because of the high cost of improved heifers, low financial ability among poor farmers identified, and tick-borne diseases (especially East Coast Fever). There was limited use of by-products and supplements, perhaps because tethering rather than zero grazing is common in the area; as a result, farmers used grasses and legumes to feed their animals. These constraints have not been addressed properly, and as a result the potential of the dairy sector in Unguja has not been realized.
- The higher increase of improved breeds/cross-breeds of goats when compared with cattle may be explained by pressure on the land due to, among others, population growth, and therefore a limited availability of grazing land. In this context, it is easier to get enough feed for dairy goats than to meet the nutritional needs of dairy cattle.
- The 2014 study showed that among farmers who keep cattle, about 67% used local bulls for breeding, but about 20% used improved bulls, and only about 14% used artificial insemination. This is in contrast to 2002, when 72% of dairy farmers used artificial insemination while 68% of them used improved bulls [assuming that some farmers used both methods]. This might be related to the fact that famers find keeping local cattle cheaper in terms of operational costs, and the fact that exotic breeds are easily attacked by diseases. The absence of artificial insemination projects might also have contributed to this situation.
- There was an increase in meat production. The proportion of domestic versus imported cattle slaughtered for meat doubled (29 to 66%) between 1978–1985 and 1986–1995, indicating an increase in slaughter cattle obtained domestically. Between 1999 and 2001, the production of beef increased by 7%.

*Milk Production*

- Average milk production increased for both indigenous and improved cattle – for indigenous cattle from 2.4 to 2.5 to 4.6 litres per cow per day in 1997, 2002, and 2014, respectively, and for improved cattle from 8.2 to 8.3 to 9.7 litres per cow per day in 1997, 2002, and 2014, respectively.
- There was an increase in the number of farmers selling milk, and the quantity of milk being sold. Milk processing is not well developed in Zanzibar; only 1% of dairy-cattle keepers participated in some form of milk processing in 2014. Most of the milk is marketed in its raw form, and therefore this has not improved since 2002. However, producers have little difficulty in finding buyers – street vendors and hawkers provide a market outlet for about 73% of the milk produced. About 12% of the milk produced is consumed by local households, 4.4% is sold through collection centres, and 3.9% is sold to hotels and restaurants. An increase in milk

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production and consumption probably contributes to an improvement in the nutritional status of rural households.

*Livestock-Crop Integration*

- Crop productivity in mixed-farming systems increased, resulting from a greater capability to use animal power (stronger and healthier animals) for ploughing and transporting farm products. The use of animal traction for land preparation is, in general, rather low, but it increased from 3% in 1997 to 5% in 2002 and to 10% in 2014. In 2002 about 21% and 18% of farmers used animals to transport their own and commercial products, respectively. By 2014, the percentage of farmers using animals for their own transport increased further to about 64% of sample households, but decreased to 10% for those who used them for commercial transport.
- Livestock-crop integration increased because more farmers used manure for crop production. About 51% of farmers in 1999, and 54% in 2002, grew crops on fields fertilized with animal manure. From 1999 to 2002, the increase in productivity of cassava, rice, maize, coconut, and vegetables may be attributed to the use of improved seed varieties and manure for crop production. In 2014 similar figures were found – 58% of respondents used manure for crop production. In addition, more farmers are using crop by-products to feed their animals. In 1999 only 13% of farmers were feeding crop by-products to livestock, but in 2002 this figure had increased to 22%. Also, farmers are increasingly feeding improved fodder to their cattle. Some 92% of dairy farmers raising improved breeds zero-grazed their cattle. The 2014 study found a limited use of by-products and supplements. Most farmers were using grasses (62%) and legumes (50%) to feed their animals (probably because tethering rather than zero grazing is common in the area).
- In the 2014 survey, 68% of respondents indicated that cultivated land has remained the same, whereas 25% and 8% of the farmers reported an increase and decrease in cultivated land, respectively. This compares well with the data from the 2002 study where 52%, 35%, and 7% cultivated the same land, increased or decreased the land cultivated, respectively. These findings suggest that farmers have not been able to expand the area under cultivation.

*Household Income.* Household income increased over time – in 1999 the average household income per month was estimated at USD 51.5, in 2003 USD 53.5, and in 2014 USD 116. A strong correlation was observed between household income and milk yields, milk sales, and use of manure and animal power for cultivation and transport. However, given the reduction in recent years in the percentage of families engaged in farming, the increase in household income might also be attributed to additional family income coming from employment outside of the agricultural sector, e.g. the growing tourism industry. Nevertheless, the economic benefits to those who continue to keep livestock are higher now than they were before the eradication of T and T.

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*6.1.2. Red Colobus Monkey*

The number of Zanzibar red colobus monkeys *Pilicolobus kirkii*, an endangered and protected species found primarily in forests (also former major habitat of *G. austeni)*, increased from 1000–1500 in 1991 to more than 2500 in 1999 (Masoud et al. 2003). In the period 2013–2014, a systematic assessment of the demography and distribution of the red colobus monkey indicated a total population of 5862 individuals in Unguja, of which 2907 were present in the Jozani-Chwaka Bay National Park (formerly the Jozani Forest Reserve) (Davenport et al. 2017). Therefore, subsequent to eradicating *G. austeni* in Zanzibar, there was no decline in the monkey population. This contradicts the opinion of several conservationists that eradication of tsetse flies would lead inevitably to habitat destruction followed by a reduction in wildlife.

*6.1.3. Challenges – Constraints*

Much of Unguja Island is coral rag, and consists of low-fertile soils that are not really suited for permanent agriculture (Hettige 1990). This in itself poses a constraint on the development of more sustainable and efficient agriculture systems in general, and livestock systems in particular. There are several reasons why agricultural development on Unguja Island, after the sustainable removal of the tsetse fly and AAT, has not been realized to its full potential. The following provides a non-exhaustive analysis:

- Before the eradication of *G. austeni* in Unguja Island in 1997, AAT was the major constraint to the development of more effective livestock systems and the intensification of the dairy sector. Clearly, eradication of the disease offered real opportunities for the intensification of livestock production in Unguja. These opportunities were most evident immediately after the eradication programme, with a rapid increase in the number of improved cattle, more use of improved seeds that resulted in increased use of crop by-products as animal feed, use of manure for crop production, and use of animal power for ploughing and transport activities. However, the numbers of pure breed and cross-breed cattle increased only slightly between 2009 and 2014, although the numbers of improved sheep and goats were more impressive. These data suggest that, although intensification is on-going, it has in the last decade not evolved at the same rapid pace as in the 1999–2003 period.
- Improvement of the livestock sector requires the availability of improved cattle breeds. The 2014 study showed clearly that only 46% of respondents indicated that improved livestock breeds were readily available. In addition, some farmers, despite changes and development of the dairy industry in Unguja, still preferred to keep local cattle breeds largely because it is easier to raise them (76%), and they are more resistant to other diseases (21%). Other constraints that can partially explain the low adoption of improved dairy cattle are the high cost of improved dairy heifers, the low financial ability of resource-poor farmers, limited access to credit facilities, poor quality of cattle feed that consists mainly of local grasses with low use of concentrates and mineral supplements, high costs of veterinary drugs for disease treatment compared with the milk and meat prices (animal drugs and feeds increased by 92–110% in the period 2005–2008 compared with a 43% increase in food commodities), and the absence of a good milk-marketing system.

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- The creation of a tsetse- and AAT-free Unguja removed a significant constraint to agricultural development on the island. However, livestock keepers still had to cope with other debilitating animal diseases such as the tick-borne disease East Coast Fever. Although the disease prevalence declined from 84% in 2002 to 56.9% in 2014, it is still very important. Other diseases that cannot be neglected are lumpy-skin disease and helminthiasis.
- The importance of market functions in terms of economic development, and the stimulation of agricultural production and development, should not be underestimated. The 2014 study found that the marketing system on Unguja Island tends to be highly controlled by a network of intermediaries, resulting in a situation that does not give farmers equitable benefits for their commodities but allocates high margins to the middleman (Mwaseba et al. 2015).

### 6.2. *Benefits from Eradicating* Glossina palpalis gambiensis *in the Niayes of Senegal*

In the Niayes of Senegal, an area located north-east of the capital Dakar, three main cattle farming systems can be identified – a traditional system that uses trypanotolerant cattle, and two systems that use more productive cattle breeds for milk and meat production. Before the project started, herd size was 45% lower, and annual cattle sales >3 times higher, in the improved farming systems as compared with the traditional farming system (USD 290 per head versus USD 86 per head, respectively) (Bouyer et al. 2014). In the tsetse-infested areas only a small proportion (6%) of the farmers kept improved breeds, whereas almost half (43%) of the farmers living in the tsetse-free areas owned more productive cattle (Bouyer et al. 2014).

An ex-ante socio-economic study that assessed the potential benefits of the sustainable removal of *G. p. gambiensis*, the only tsetse species present, considered two possible scenarios with respect to potential increases in cattle sales to be expected after eradication. The first, a conservative scenario, assumed an annual replacement rate of 2% of traditional by improved systems, and the second assumed a higher replacement rate of 10% five years after the removal of the tsetse fly population. The final annual increase in cattle sales was estimated at USD 3250/km$^2$ versus a total cost of the eradication campaign of USD 7430/km$^2$ (Box 1). In spite of this relatively high cost, the benefit/cost analysis indicated that the project was highly cost-effective, with internal rates of return (IRR) of 9.8 and 19.1%, and payback periods of 18 and 13 years, for the two scenarios, respectively (Bouyer et al. 2014; Bouyer and Vreysen 2018). The replacement of traditional local cattle with more productive exotic animals is already apparent in the areas that have been cleared from tsetse (Block 1) or are close to eradication (Block 2) (Paquette 2019; Vreysen et al. 2021)

### 6.3. *Prediction for the Ethiopian Southern Rift Valley*

The anticipated benefits of removing the T and T problem in the Ethiopian Southern Rift Valley are substantial (Alemu et al. 2007; Zerihun 2017). At the request of the Ethiopian government, Knight (2001) conducted a preliminary benefit/cost analysis,

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pointing out that there are significant benefits to be gained from sustainably removing tsetse flies by involving the SIT as part of an AW-IPM strategy. For investments made to create a tsetse-free zone in the Southern Rift Valley, the break-even point should be reached in year 5 or 6, and the net present value (NPV) over 12 years is estimated at between USD 36.6 and 52.7 million. This represents an internal rate of return of between 33 and 43%.

### *6.4. Benefits from a Planned Programme to Eradicate* Glossina austeni *and* Glossina brevipalpis *in KwaZulu Natal, South Africa*

The province of KwaZulu Natal in South Africa harbours two species of tsetse flies, *Glossina austeni* and *Glossina brevipalpis* Newstead. These populations were responsible for a major outbreak of nagana in 1990, prompting the government to assess potential benefits from a sustained removal of these populations (Kappmeier et al. 1998)

Kappmeier Green et al. (2007) developed an AW-IPM strategy to remove sustainably the two tsetse populations. It included two cycles of the sequential aerosol technique (SAT) followed by the release of sterile males. A benefit/cost analysis indicated that such an approach would reach a break-even point after 8 years, with a cumulative total net benefit (net present value, taking into account a discount rate of 8%) of USD 51 million, and an overall benefit/cost ratio of 3.4, over a 15-year period. These figures do not take into account additional benefits, such as improved agricultural productivity because of healthier draft animals. Starting in year 9, the project would reach the maintenance phase, and benefits would be fully established; the annual benefit/cost ratio would fluctuate between USD 90 and 493 for each dollar invested (year 9 to year 15). Moreover, the project would have an internal rate of return of 23%, meaning that the discount rate could be almost three times higher than the estimated value of 8% and the project would still break even in a 15-year time frame (Kappmeier Green et al. 2007; Bouyer and Vreysen 2018).

## 7. CONCLUSIONS

Keeping livestock, and especially operating a mixed farm, is fundamental to the rural poor being able to produce their own food. There are many problems of food security that, even though they limit the availability of adequate food supplies, can be solved, step-by-step. However, if approached from the viewpoint of conventional pest suppression, the T and T problem remains an insurmountable barrier to productive agriculture. A partial or unsustainable solution is really no solution. Without breaking this barrier to enable the introduction of higher productive agriculture and livestock systems, and without the resulting development and profitable investment opportunities, there is no hope that the hungry in sub-Saharan Africa will become food self-sufficient within a reasonable time period. The poor farmers of Africa, because of where they live, are helpless in the face of a problem that, even though they may be able to alleviate it temporarily, on their own they cannot remove it in a sustainable manner.

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# CHAPTER 7.5.

# POTENTIAL IMPACT OF INTEGRATING THE STERILE INSECT TECHNIQUE INTO THE FIGHT AGAINST DISEASE-TRANSMITTING MOSQUITOES

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## SUMMARY

More than three thousand million people live with the risk of malaria. Due to the widespread resistance of mosquitoes to insecticides and of parasites to chemotherapies, previous gains made in disease reduction are being reversed. In addition to this perennial threat, there is now a rapid invasion of *Aedes* mosquitoes across the globe and the associated spread of the arboviruses (arthropod-borne viruses) they carry. One half of the world's population is now at risk of dengue, and chikungunya (having emerged from Africa) is an increasing public-health problem in Asia and the Americas. The economic and social costs of these diseases is so great that, in some areas, they have slowed the development of nations. Current vector-control methods are inadequate (especially against container-breeding species) because they are losing their effectiveness, the global burden of mosquito-borne diseases is increasing, and no specific drugs or effective global vaccines are available to treat or prevent the diseases. Therefore, there is a need for additional suppression methods to be applied as part of Integrated Vector Management (IVM). Since the early 2000s, there has been a renewed interest in applying the sterile insect technique (SIT) against mosquito vectors of disease. The explosive outbreaks of the Zika virus (and associated birth defects) across the tropics increased the urgency. The recent availability of technology to rear and release the sterilized males of many mosquito species on a large scale has increased the expectation that the SIT could help reduce the suffering caused by mosquito-borne diseases. Much progress has been made in developing the SIT technology for mosquitoes, based on historic SIT efforts and the experiences gained in the successful large-scale application of the technique against agricultural pest species. The SIT is a suitable technology for suppressing mosquitoes because: (1) they can be mass-reared in a laboratory, (2) natural sexual dimorphism in many species aids sex separation, and (3) females become refractory after mating. There has been a perception that mosquitoes are more vulnerable than many pest species to damage during handling, sterilization, and release. However, technological and methodological improvements can cope with this lower robustness, and indeed take advantage of their smaller size and weight. Nevertheless, the need for perfect sex separation for male-only release to preclude any biting and disease transmission by released females, remains a technical bottleneck to scaling the SIT beyond small-scale pilot trials. As a remedy for this, genetic sexing strains are being developed. However, until they are available, combining the SIT with cytoplasmic incompatibility conferred by *Wolbachia* infection (incompatible insect technique (IIT)) has been proposed as an advantageous strategy. The advantage of including the IIT is that *Wolbachia* infection may prevent potential disease transmission by any released females, whereas sterilization guarantees that such females cannot reproduce, avoiding the loss of the cytoplasmic incompatibility due to *Wolbachia* establishment in the target population. Another advantage of simultaneous IIT use is that it enables the radiation dose to be minimized. Other challenges remain, particularly in release technology and quality control. Nevertheless, in recent years, pilot trials have been conducted or have been initiated, e.g. China, Germany, Greece, Italy, Mauritius, Mexico, Singapore, and Thailand, achieving encouraging results in suppressing adult populations of *Aedes* species. Area-wide releases, focused on urban and suburban settings, appear particularly promising in terms of sustainable and cost-effective IVM of *Aedes* vectors (eventually provided commercially by the private sector) because they can protect many people concentrated in relatively small areas. In the case of *Anopheles* vectors, the SIT may become a useful complementary tool, especially against outdoor-biting species which are not well-controlled by mosquito nets.


## 1. INTRODUCTION

Mosquito-transmitted dengue is now the world's most common mosquito-borne viral disease; over the last 50 years the incidence has grown more than 30-fold (Bhatt et al. 2013). Dengue viruses are estimated to infect about 400 million people per year, and over half of the world's population is at risk of the disease. Moreover, chikungunya virus emerged from Africa in the mid-2000s, and spread across Asia and the Americas in 2013 (Mayer et al. 2017). Due to the increasing spread of invasive mosquito species (Kraemer et al. 2015), these arboviruses were already becoming a major international public-health concern, even before Zika virus outbreaks occurred in the South Pacific in 2013 and in the Americas in 2015 (Mayer et al. 2017). Epidemics of the Zika virus in the Americas were associated with cases of microcephaly and other congenital

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abnormalities. On 1 February 2016 the World Health Organization (WHO) declared a public-health emergency of international concern. To date there are no drugs or effective global vaccines available to treat or prevent Zika; this situation led to calls for an urgent response against its *Aedes* vectors. Even though one of the best vaccines ever developed exists for yellow fever (another arbovirus), it is re-emerging in some countries (including Angola, Brazil, China, Democratic Republic of Congo, and Kenya (Zwizwai 2017)).

Subsequent to a major expansion of its geographical range, *Aedes aegypti* (L.) is the most important vector of arboviruses worldwide, particularly in tropical and subtropical urban settings (Mayer et al. 2017). *Aedes albopictus* (Skuse) is also a major public-health concern because it is a very good vector of several arboviruses including dengue, chikungunya, and Zika (Mitchell 1995). The epidemiological risks associated with *Ae. albopictus* are increasingly great in Europe, where significant disease outbreaks are now occurring (Gossner et al. 2018). While arboviruses are increasing in impact and notoriety, 3.2 thousand million people remain at risk of malaria, transmitted by Anopheline mosquitoes; in 2016 alone, an estimated 216 million new cases of malaria and 445 000 deaths occurred (WHO 2017b).

### *1.1. Burden of Disease*

Seven of the major vector-borne diseases (malaria, lymphatic filariasis, leishmaniasis, dengue, Japanese encephalitis, yellow fever, and Chagas' disease) share a largely overlapping pattern of global distribution; many parts of the world are at risk of up to six of these (Fig. 1) (Golding et al. 2015). Controlling the mosquito vectors would simultaneously combat several co-localized diseases, improving the benefit/cost ratio and sustainability of control efforts, particularly Integrated Vector Management (IVM) programmes, which could include a sterile insect technique (SIT) component. Alongside the human suffering (morbidity and mortality) caused directly by mosquito-borne diseases, they also exert a high financial burden -- direct costs of treatments and prevention activities, and indirect costs through loss of human productivity and subsequent impact on a region's economy. The concentration of malaria cases in Africa means that malaria in particular constitutes a major obstacle to sustainable development and poverty eradication on the continent.

The cost of treating cases of disease can vary greatly depending on the economic and social setting. The direct and indirect costs may fall on the state, on the patients and their families, or on both. In Ghana, for example, the treatment cost for each malaria episode varies from USD 5.70 to 48.73, depending on the severity of disease; the household is responsible for 55% of the treatment cost. Indirect costs, such as a funeral, can in some African countries be equivalent to one year's total household income (Sicuri et al. 2013). The Philippines carries the fourth-highest burden of dengue cases in South-East Asia; an analysis by Edillo et al. (2015) estimated that over 840 000 clinically diagnosed cases led to direct medical costs of USD 345 million (USD 3.26 per capita). Thirty-five percent of cases were treated as outpatients, representing 10% of indirect costs, compared with the 65% of patients who were hospitalized and constituted 90% of direct costs.

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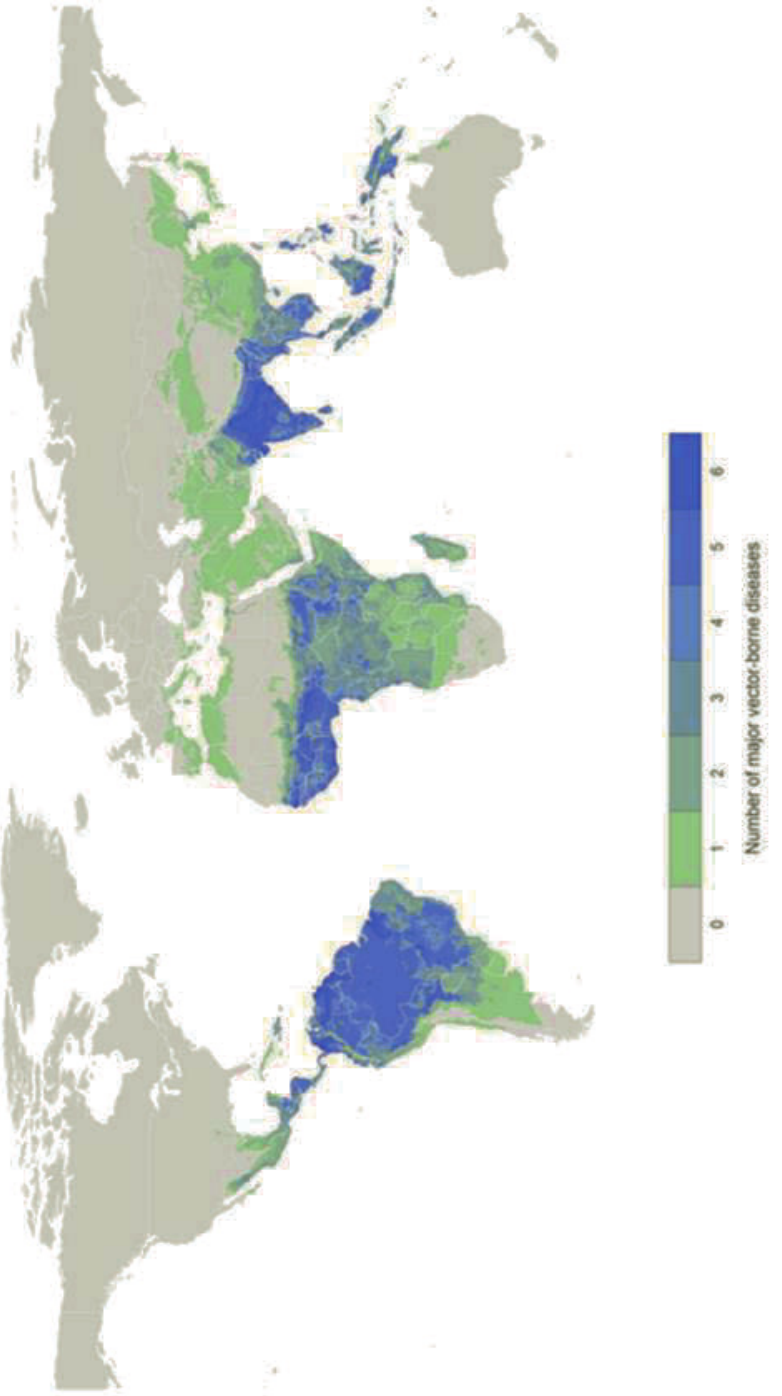



*Figure 1. Combined global distribution of seven major vector-borne diseases for which integration of vector control programmes may be beneficial: malaria, lymphatic filariasis, leishmaniasis, dengue, Japanese encephalitis, yellow fever, and Chagas' disease. Colours indicate the number of vector-borne diseases that pose a risk at each 5 x 5 km grid cell. (Map from Golding et al. 2015, reproduced with permission.)*

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The cost of fighting dengue in 18 countries at purchasing power parity (PPP) was calculated at about USD 3.3 thousand million in 2015; using standardized PPP costs permits comparing socio-economic impacts in the different countries (Oliveira et al. 2019).

The economic burden of vector-control costs, in an attempt to reduce or prevent cases of disease, needs to be factored into estimates of the cost of disease to a country or region. To the USD 102.2 million direct cost of treating the almost 40 000 cases of dengue reported annually in Malaysia from 2007 to 2012, another USD 73.5 million (0.03% of Gross Domestic Product (GDP)) need to be added for the National Dengue Vector Control Programme, mostly for adulticidal fogging, increasing the cost by 72% (Raviwharmman Packierisamy et al. 2015).

Vector-control costs can apply even where a disease is not yet a problem or has previously been brought under control, e.g. countries (such as Mauritius) which are at risk from importation of arboviruses due to their high inflow of international visitors (Beesoon et al. 2008; Ramchurn et al. 2009) or those countries at risk of invasion by *Aedes* vectors. A balance must be made between potential disease costs and preventive vector control efforts. As the Zika virus was spreading through the Americas, the US president requested USD 1.8 billion from Congress in February 2016 to combat Zika in Costa Rica and Brazil, expenditure which was found to be justified in a study by Alfaro-Murillo et al. (2016). The judgement was based on estimated probabilities of microcephaly in babies born to infected mothers and direct medical costs, which both vary by country, and the loss of Disability-Adjusted Life Years (DALYs) per case of microcephaly (29.95 DALYs) and of Guillain-Barré syndrome (1.25 DALYs).

The wider benefits of disease reduction or eradication may be significant. A study conducted in Ghana into the economic-burden impact of malaria mentions that a 1% increase in malaria morbidity reduces economic growth by 0.41% (Asante and Asenso-Okyere 2003). Due to malaria, each business in the nation lost on average about a month's productivity per year; this corresponds to a drastic decrease in average income. A dynamic macrosimulation model to estimate the effects of eradicating malaria shows that higher economic development can be achieved in the long-term (within 30 to 50 years) from malaria eradication (Ashraf et al. 2009).

While the burden of agricultural pest insects is usually felt by individual growers and sector cooperatives, the burden of vector-borne diseases is felt by the whole population; therefore, the management of such diseases is commonly the responsibility of local organizations (including non-governmental mosquito control districts (Foley IV et al. 2021)) or supplemented by governments as part of their social policy, where it is a constitutional requirement to provide health care. Tang et al. (2004) developed a framework outlining for the USA the ten coordination and regulatory roles that government may play in health-care quality (which could be applied in any country):

> (1) purchase health care, (2) provide health care, (3) ensure access to quality care for vulnerable populations, (4) regulate health care markets, (5) support acquisition of new knowledge, (6) develop and evaluate health technologies and practices, (7) monitor health care quality, (8) inform health care decision-makers, (9) develop the health care workforce, and (10) convene stakeholders from across the health care system.

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Each role proposed above would have an impact on the control of neglected diseases. In facing the continuous increase in the number of cases and disease expansion to new areas, item six highlights how critical it is to develop an effective contribution to reducing the number of transmitted cases over time -- by incorporating new strategies and control methods for vector-borne disease control (Araújo et al. 2015; Bourtzis et al. 2016).

Various countries have developed their own guidelines and protocols to deal with vector-borne diseases (based on guidelines developed by the WHO (WHO 2009, 2012)) in which they formulate evidence-based strategies and policies. For example, the Brazilian health ministry developed the National Dengue Control Plan (PNCD), operating since 2002. It comprises a great range of activities and instructions in an effort to reduce the number of dengue cases all over the country. This plan evolved after the first attempt to eradicate the mosquito in the 1970s -- aggregating and developing strategies based on different approaches including epidemiological and entomological surveillance, frequent house inspections, and insecticide application (Braga and Valle 2007). Until now, the PNCD's objectives have not been fully achieved, and the fact that dengue epidemics can still be expected every year illustrates the need for a full review and evaluation of the strategies and control methods applied. There are local problems related to achieving full implementation of the plan (a result of insufficient support from stakeholders), in addition to growing resistance to the commonly used temephos larvicide and deltamethrin adulticide (Valle et al. 2019), and the general challenge of eradicating populations of container-breeding *Aedes* vectors. Brazil alone always represents more than 95% of the number of dengue cases in Latin America (Pessanha et al. 2009; Salles et al. 2018). Until effective universal vaccines, and safe, effective, and inexpensive drugs, are developed and become available, control of the mosquito vectors of disease is likely to be the most effective method of reducing cases of disease and controlling their spread.

### *1.2. Need for Better Vector Control*

Currently most mosquito control strategies rely primarily on the use of insecticides, but with the increasing spread and significance of resistance in vectors of malaria (Ranson and Lissenden 2016) and arboviruses (Ranson et al. 2010; Moyes et al. 2017) there is a need for sustainable tools that enhance the arsenal against key vectors, particularly in the face of public concern about the human health and environmental impact of widespread insecticide use. Although great gains have been made in reducing the burden of malaria (e.g. there were 20 million fewer malaria cases in 2017 than in 2010 (WHO 2018)), these gains have been achieved through applying artemisinin combination therapies (ACTs) and especially insecticide-treated bednets (ITNs). The 2018 World Malaria Report suggested that these gains are being reversed; no significant progress was made in reducing cases between 2015 and 2017 -- due in large part to drug and insecticide resistance becoming increasingly established and widespread (WHO 2018).

In its global vector control response 2017–2030, the WHO pointed out the urgent need for the development and integration of innovative mosquito control methods, including the SIT, particularly against *Aedes* vectors (WHO 2017a). A major

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advantage of suppressing a mosquito vector population with such an integrated approach is that it can address several diseases at once; different diseases, such as arboviruses, are often transmitted by the same *Aedes* vectors, whereas other approaches, e.g. vaccination, need to be developed for each new emerging disease.

The behavioural ecology of anthropophilic mosquitoes, and in particular their use of disseminated microhabitats as oviposition sites, challenges the integrated control of these insects in many countries and climatic conditions, and prohibits a satisfactory level of population reduction (Reiter 2016). Moreover, the application of many insecticides is more and more restricted worldwide; this reduces the available vector control options, particularly in the face of spreading resistance against all but the newest classes of insecticides in vectors of both malaria (Sokhna et al. 2013) and arboviruses (Ranson et al. 2010; Grigoraki et al. 2017; Pichler et al. 2018). In addition, insecticide-treated bednets are not effective in combating *Aedes* vectors (which are active during the day).

Therefore, new techniques to control mosquitoes are under development and being evaluated in the field, including genetic control strategies targeting the reproductive capacity of disease-transmitting mosquitoes (McGraw and O'Neill 2013; Lees et al. 2015; Bourtzis et al. 2016; Flores and O'Neill 2018). Amongst these, the SIT and the incompatible insect technique (IIT) show great promise (Oliva et al. 2014).

### *1.3. Integration of the SIT Offers the Potential for Sustainable Mosquito Suppression*

Pilot trials against mosquitoes started in about 1960 in the USA, with the goal of assessing the potential of the SIT to reduce populations of *Ae. aegypti* and *Anopheles quadrimaculatus* Say, but in both cases there was no evident population suppression in the target areas after 43–48 weeks of releases of sterile males (irradiated with gamma rays) (Morlan et al. 1962; Weidhaas et al. 1962). Many other pilot projects took place with various mosquito species in various countries using different rearing protocols, irradiation sources, and methods of sterilization (chemosterilization, translocations, inversions, and cytoplasmic incompatibility (CI)) (Benedict and Robinson 2003; Dame et al. 2009; Klassen et al., this volume). Some were able to demonstrate suppression and even elimination/eradication of the target population, e.g. elimination of *Anopheles albimanus* Wiedemann in El Salvador (Breeland et al. 1974; Dame et al. 1974).

Interest in applying the SIT against mosquitoes had waned since these intial trials in the 1960s and 1970s. Rather than technical failure, this was due largely to political instability affecting their implementation and insufficient practical governmental support (Klassen et al., this volume). Moreover, in view of the availability of new insecticides, mosquito control has relied heavily on the use of a limited number of insecticides. Recently, there has been a resurgence of interest due to: (1) increased pressure exerted by emerging arboviruses and the spread of resistant malaria, (2) the loss of current methods of control because of malaria resistance to drugs and resistance to insecticides in both *Anopheles* and *Aedes*, and (3) the availability of molecular techniques that have enabled the development of improved strains for the SIT (Bourtzis and Hendrichs 2014; Bourtzis and Tu 2018; FAO/IAEA 2018c; Lutrat et al. 2019) and made alternative versions of genetic control possible (Alphey 2014).

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Releasing insects sterilized by ionizing radiation has been a very important method for the area-wide suppression, containment, and even eradication of major insect pest populations (Klassen et al., this volume). Given the experiences obtained in those programmes applying the SIT, there is reason to believe that it could also be applied -- in combination with other control methods as part of an area-wide integrated pest management (AW-IPM) approach -- to suppress disease vectors below the threshold required for disease transmission. Building on previous experiences in the 1960s and 1970s to develop the SIT for disease vectors, many parameters have been investigated to better understand, enable and optimize the application of the SIT against mosquitoes, including mass-rearing procedures, sterilization methods, transport and release methods, and trapping systems (Pepin et al. 2013; Puggioli et al. 2013; Balestrino et al. 2014a, b; Carvalho et al. 2014; Codeço et al. 2015; Lees et al. 2015; Eiras et al. 2018; Bakri et al., this volume; Dowell et al., this volume; Parker, Mamai et al., this volume; Parker, Vreysen et al., this volume; Vreysen, this volume). Part of the development of the SIT against mosquitoes has also been based on the experiences of successful programmes against other insects, e.g. those against *Ceratitis capitata* (Wiedemann) in the USA, Guatemala, Mexico, and Chile, and *Cochliomyia hominivorax* (Coquerel) in Central and North America, as well as Libya (Enkerlin, this volume; Klassen et al., this volume; Vargas-Terán et al., this volume). However, more research and testing need to be done before "mosquito SIT" reaches the same level of development as these programmes (Krafsur 1998; Vargas et al. 2008; Enkerlin et al. 2015).

Several features of mosquito biology make mosquitoes suitable targets for the SIT (Beier et al. 2014). Most of the key mosquito disease vectors can be colonized and reared in the laboratory, adapting to feeding on artificial larval diets and taking blood meals through artificial membranes. Therefore, the production of sufficient numbers to achieve an overflooding release ratio is feasible, even if reducing natural population densities using other complementary suppression techniques will often be necessary against mosquitoes, particularly the reduction of larval populations and habitats (Mangan and Bouyer, this volume).

Sexual dimorphism, particularly in *Aedes* species, can be exploited to help remove females prior to sterilization and release of males, which is an essential requirement for applying the SIT against mosquitoes -- females are the disease vectors, and releasing even small numbers that may transmit disease is problematic for the public and regulators, even if not for technical efficacy.

In comparison with fruit and tsetse flies, mosquitoes are smaller and lighter, making the challenges of handling, transport, and release more tractable (especially to take advantage of new technologies such as automated release from unmanned aerial vehicles (UAVs) (Dowell et al., this volume)). However, their small size also confers some fragility; this must be taken into account to prevent a reduction in survival or quality of released males. Finally, female mosquitoes are mostly refractory to remating -- not an absolute requirement of the SIT but beneficial in reducing the numbers for, and frequency of, release required to induce sterility in the female population (Lance and McInnis, this volume; Whitten and Mahon, this volume).

The container-breeding species, such as *Ae. aegypti* and *Ae. albopictus*, are particularly challenging to control because their life cycle relies on oviposition in

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small, and often temporary, sources of water. To mitigate the risks of a whole egg batch being lost due to a small body of water drying out before the offspring can emerge and escape, and also predation or competition for space or nutrition, an Aedine female will lay her egg batch, up to 100 eggs per gonotrophic cycle, in multiple sites. Many of those sites (such as discarded drink cans or tree holes) are very small and difficult to target with chemical control, and many are difficult to remove or treat (such as water butts used to collect rain water in areas of unreliable piped-water supply or buckets in fishing communities), and may be located in urban sites which are not amenable for control, e.g. abandoned lots or balconies of apartments in high-rise buildings. This cryptic behaviour also means that females often rest in locations which are not reached by insecticide applications (Dzul-Manzanilla et al. 2017). The advantage of sterile males is that they will locate mates, which then will lay non-viable eggs, and negate the need to find and treat these hard-to-reach oviposition sites. Increasing global trade and urbanization, as well as reliance on disposable containers without sufficient waste disposal infrastructure in many areas affected by mosquito-borne disease, help explain the rapid growth in distribution of *Aedes* vectors. In this context, the reliance on the dispersal of sterile males rather than human operators is an important advantage of control efforts that integrate mosquito release over other methods; a similar advantage applies to autodissemination stations for juvenile hormones.

In the case of *Anopheles* females, these are targeted by conventional vector-control methods while they are trying to take a blood meal through an insecticide-treated bednet or resting on a wall treated with indoor residual spray (IRS), but those that remain in untreated houses or communities will be unaffected. Moreover, some exophilous species (such as *Anopheles arabiensis* Patton) that bite mainly outside will not be impacted by these methods. One major benefit of SIT application against *Anopheles* mosquitoes, in particular *An. arabiensis* which is the sole vector of malaria in most of its area of distribution, is that human operators do not need to find and treat these sites or target every mosquito, but instead male mosquitoes are released to seek out and sterilize females, whose eggs (wherever she lays them) will then be sterile.

## 2. DEVELOPMENT OF THE SIT TECHNOLOGY AGAINST MOSQUITOES

Increasing interest in applying the SIT, and other control methods relying on the large-scale rearing and release of insects, has led to a rapid improvement in the available technology and methodology to mass-rear, sterilize, assess the quality of, and release and conduct surveillance on, *Aedes* and *Anopheles* mosquitoes (Benedict et al. 2009a; Lees et al. 2015). A significant requirement of applying the SIT against mosquito vectors of disease is the need for male-only release, not only to maximize the efficacy of releases and the efficiency of rearing efforts, as in other insects (Franz et al., this volume; Häcker et al., this volume), but also for the public perception and regulatory challenges surrounding the release of even a small number of potentially disease-transmitting females. Pilot trials of the SIT and associated techniques have been conducted or initiated in recent years in a number of settings, against both genera of mosquitoes, and evidence of the potential for the SIT to suppress mosquito populations is being produced.

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### *2.1. Rearing and Sterilizing Males*

#### *2.1.1. Colonization and Mass-Rearing*

The need for effective colonization and rearing to maintain essential qualities in mosquitoes released for vector control have been reviewed by Benedict et al. (2009b). Colonization and establishment of a new mosquito colony is a painstaking process (FAO/IAEA 2017a, 2018b). Blood-fed females, or immature developmental stages, are collected from a field site where the species of interest is likely to dominate, and individual families are reared while morphological and/or molecular analysis is used to confirm the species. Conspecific families can then be pooled to establish a colony which is often small, and must go through a bottleneck as it becomes adapted to artificial rearing conditions, particularly feeding from an artificial membrane instead of a natural host. Once a colony is established and stabilized, laboratory-adapted mosquitoes are relatively amenable to large-scale rearing, though for a release programme to be efficient each element of the rearing process must be optimized (Parker, Mamai et al., this volume).

Key to affordable production of high-quality adults is the selection of a larval diet which provides all the nutrients needed by developing larvae to grow and establish the nutritional reserves they will require as adults for foraging and mating. Ideally, these should consist of locally available ingredients, be reliably available and of a consistent quality, and even if inexpensive yet still effective in producing high-quality adults (Khan et al. 2013 give *Anopheles stephensi* Liston as an example). A well-proven diet (consisting of tuna meal, bovine-liver powder, and vitamin mix) is effective for rearing *An. arabiensis* (Damiens et al. 2012), *Ae. albopictus* (Puggioli et al. 2013), and *Anopheles gambiae* Giles (Yahouédo et al. 2014). The addition of Brewer's yeast increases the protein content; it is particularly helpful for improving sexual dimorphism in *Aedes* rearing (Balestrino et al. 2014a). Given the cost and difficulty in obtaining bovine-liver powder, alternative diets have been validated (e.g. Bimbilé Somda et al. 2017), and other cheaper proteins, e.g. insect proteins, are also being developed (Bimbilé Somda et al. 2019).

Since they have an aquatic larval stage, rearing mosquitoes is more labour-intensive than other insect species targeted by the SIT. In addition, the pupal stage, lasting only 24–48 h, must be collected and transferred to a cage before adult emergence. Therefore, some level of automation and large-scale equipment for larvae and adults are required to prevent labour costs from becoming prohibitive. A tray-and-rack system (consisting of 50 trays stacked in one rack for easy filling using piped water, seeded with first-instar larvae, reared to pupation, then tilted to recover pupae for transfer to adult cages) has been validated to rear up to 200 000 *An. arabiensis* larvae (Balestrino et al. 2012) and about 900 000 *Ae. albopictus* larvae (Balestrino et al. 2014b). Accurate quantification of eggs or larvae used to seed a rearing tray is critical because density-dependent competition acts at the larval stage (affecting the speed and synchronicity of development, the size and nutritional status, and hence the performance of the resulting adults). Trays can be filled in a standardized way using a larval counter (Mamai et al. 2019). Once the trays have been tilted, *Anopheles* pupae can be separated from remaining larvae (on the basis of their different buoyancy) using a cold-water vortex system (Balestrino et al. 2011), and *Aedes* pupae are

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separated using the Fay-Morlan separator (Focks 1980). Pupae are quantified volumetrically, and then the required number is transferred to adult emergence cages. In China and Singapore, ongoing programmes recover 70–80% of male pupae in only one tilting event, and sex separation is very efficient (only 0.3% female contamination). An automatic sex sorter based on a robotized Fay-Morlan separator has been developed by the Wolbaki company, with the same efficiency and a throughput of up to 150 000 pupae per hour (Xi, Z., personal communication).

Adult cages, inspired by those used for mass-rearing fruit flies, have been validated for up to 25 000 *Ae. albopictus* (Balestrino et al. 2014a; FAO/IAEA 2017b) or 15 000 *An. arabiensis* (FAO/IAEA 2017a) pupae. Adults must be blood-fed so that females can develop eggs. Adults are blood-fed using a Hemotek membrane feeder modified for mass-rearing (Damiens et al. 2013). *Anopheles* eggs are collected in water on the bottom of the oviposition cage which is then flushed out (Maïga et al. 2016); *Aedes* eggs are laid on wet filter paper which can then be removed and dried for storage (Zheng et al. 2015). Eggs can be quantified at this stage (Zheng et al. 2015; Maïga et al. 2016) before they are stored (*Aedes* only) or hatched. Mass-rearing using this system does not appear to impact male quality significantly (Soma et al. 2017) as judged in a laboratory setting. A new cheaper mass-rearing cage for *Aedes* has also been validated recently; it costs 90% less than the former IAEA reference cage and can be produced locally in any country (Maïga et al. 2019). Alternative designs for mass-rearing cages are also proving to be effective (Zhang et al. 2018).

It is essential to separate males (destined for release) from females (potential disease vectors). A perfect method for sexing mosquitoes on a mass-scale has not yet been developed (Gilles et al. 2014), although promising methods are being evaluated (section 4.1.). Nevertheless, currently *Aedes* can be separated on the basis of sexual dimorphism (differential speed of development and pupal size) (Fay and Morlan 1959; Focks 1980). In addition, the mass-removal of female *Anopheles* can be achieved by spiking blood meals with toxicants such as ivermectin (Yamada et al. 2013).

### *2.1.2. Irradiation*

There is a perception by some that mosquitoes are more susceptible to somatic damage caused by irradiation (with the resulting reduction in performance) than other species targeted by the SIT (Bakri et al., this volume). There are reports from historic mosquito SIT projects that failures were due to poor male performance (Dame et al. 2009). Nevertheless, it is possible to select a suitable dose to induce sufficient sterility without inflicting unacceptable somatic damage, although rearing is key to ensuring a consistent high-quality output of sterile males for release (Parker, Vreysen et al., this volume). A key observation during historic mosquito SIT releases (Darrow 1968) -- that field-collected *Culex tarsalis* Coquillett males subsequently irradiated as pupae were competitive following re-release, unlike mass-reared and irradiated males -- points to the mass-rearing and handling process, and not irradiation, as the more important factor impacting male-mating success.

At the present time, sterile males for release are obtained by irradiating pupae -- they are more easily handled and less damaged by radiation than adults (Helinski et al. 2006, 2009). However, strong density-dependent efficiency related to water content and anoxia suggests a method for irradiating chilled compacted adults (that is

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presently under development) will provide better results (H. Yamada, personal communication). On the other hand, pupae can be irradiated at a high density in a small volume of water, minimizing the volume to be irradiated and thereby increasing the uniformity of dose. Protocols and dose-response relationships have been established (to achieve an optimal balance between a high level of sterility and a low impact on male performance) for *Ae. albopictus* (Balestrino et al. 2010), *An. stephensi* Liston (Akram and Aslamkhan 1975), *Anopheles pharoensis* Theobald (Wakid et al. 1976), and *An. arabiensis* (Helinski et al. 2006). Even though gamma irradiators are commonly available, X-ray irradiation offers a more convenient and less costly alternative, with lower security requirements (Ndo et al. 2014; Yamada et al. 2014; Bakri et al., this volume).

### *2.1.3. Quality Management and Monitoring*

Quality management and monitoring of sterile males are critical for the SIT -- to ensure good mating performance and that released males are competitive (Parker, Vreysen et al., this volume; Vreysen, this volume). Quality-control monitoring of rearing conditions is also essential to assure sexual dimorphism in *Aedes*, synchronous pupal production, consistent productivity and quality, and a reliable number of adults for release (Parker, Mamai et al., this volume; Parker, Vreysen et al., this volume). Regular measurement of important life history traits and mating competitiveness is critical in a colony reared for release. Rearing should be as standardized as possible to ensure consistent quality of material for release, and predictable synchronized development. In *Aedes* colonies it is important to maximize sexual dimorphism (key to sex-separation). Parameters such as water temperature and larval density must be optimized to consistently produce high-quality adults (Mamai et al. 2018).

A useful method for assessing if rearing conditions are optimal for a given species in a given setting was described by Valerio et al. (2016). To quantify and help minimize the impact of mass-rearing, irradiation, and handling on sterile male mosquitoes, several methods to judge the consistency, quality, and predicted performance following release have been suggested. The relative impact of different handling treatments, and combinations of different parameters, could be estimated by comparing the longevity of exposed males (Chung et al. 2018; Culbert et al. 2018b). Longevity is an important parameter of released males, also sometimes used as a proxy measure of quality.

Balestrino et al. (2017) proposed for *Aedes* pupae a system of quality control based on flight performance (an improvement on the conventional flight cylinder), and a further development has been designed (Fig. 2), to measure the flight ability of adult mosquitoes; it may be the most practical and robust of the tests for proxies of success. This parameter has been validated as a predictor of survival (a product of being able to forage for food and evade predators) and mating capacity in *Ae. aegypti* (Culbert et al. 2018a). However, it may be necessary to assess more than one quality indicator to fully understand the impact of colonization, mass-rearing, and manipulations on the overall quality of sterile males (Poda et al. 2018).

Semi-field experiments have demonstrated the ability of sterile male mosquitoes to attract and compete for mates (e.g. Howell and Knols 2009; Bellini et al. 2013a; Madakacherry et al. 2014; Yamada et al. 2014). *An. arabiensis* adults transported by

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air survived and competed for matings to an acceptable degree in field cages near the intended release site in Sudan (Helinski et al. 2008). Although it is difficult to judge the effect of irradiation and other manipulations in an open field setting, this is a valuable step in evaluation. Field observations of sterile *An. arabiensis* males participating in swarms within 2 h of release (Ageep et al. 2014) is a very encouraging measure of the potential success of applying the SIT to control these mosquitoes.

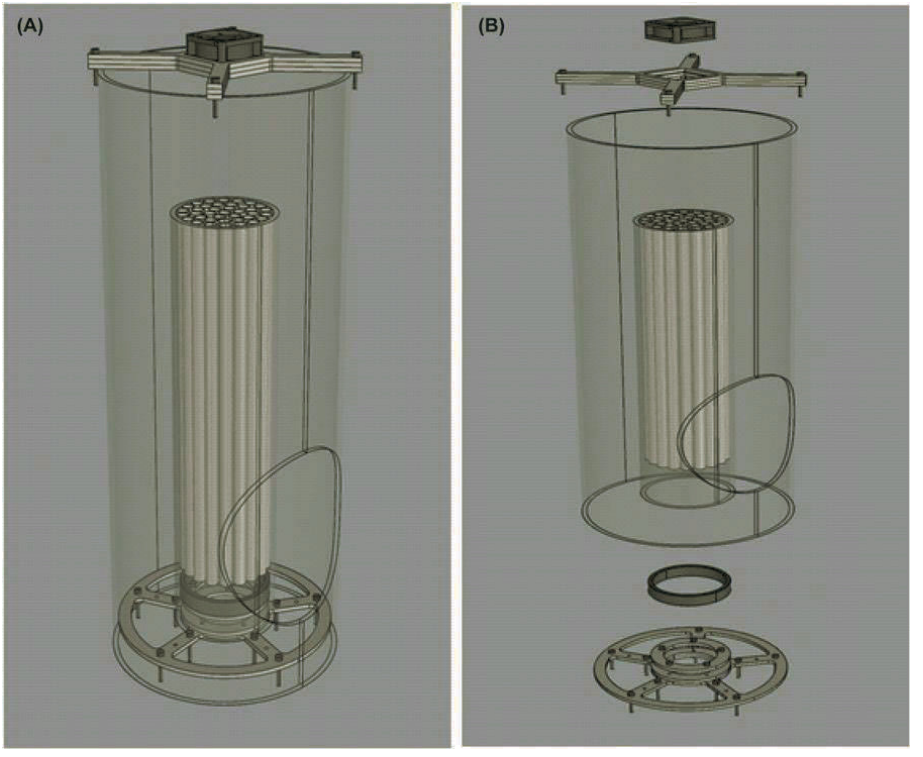



*Figure 2. A: Flight test device; B: Device showing components. (Drawings from Culbert et al. 2018a, reproduced with permission.)*

### *2.2. Release and Monitoring*

Until now the pilot releases of sterile male mosquitoes have been small enough to permit manual release from small plastic containers. Male pupae emerge directly into these containers; they are transported to the field and opened by hand at set ground release points to release the males. This method of release has been used in all of the SIT field pilots until now, as well as in the release of 10 000 transgenic *Ae. aegypti* males per week in Juazeiro, Bahia, Brazil as part of a RIDL (Release of Insects carrying a Dominant Lethal) suppression trial (Carvalho et al. 2015; Häcker et al., this volume). However, the large-scale releases required for operational application of the SIT will necessitate more sophisticated methods of handling, transport, and release,

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i.e. methods that are less labour-intensive and have the capacity to be standardized between sites. The most likely scenario is that adult males will be chilled, loaded into a release device with some level of compaction, and released either aerially or from a motorized vehicle (FAO/IAEA 2014; Chung et al. 2018). In some situations, existing infrastructure may be used, e.g. designing a release device and strategy that utilizes the coverage of large cities by bus routes, or shipping sterile mosquitoes from rearing facility to release site by commercial courier (Mains et al. 2019). Release by UAVs will probably be used to reach areas that are not reliably accessible by road, such as dense favelas or isolated rural hotspots, and importantly to increase the speed of release and uniformity of coverage while reducing costs (Benavente-Sánchez et al. 2021; Dowell et al., this volume).

It is important that release procedures do not unduly damage the males or impact their subsequent performance and survival. Laboratory studies show that mosquitoes are damaged to some degree by chilling, packing, compaction, and release into simulated field conditions (Chung et al. 2018; Culbert et al. 2018a, b). However, some degree of chilling improves survival during compaction (Chung et al. 2018), and parameters such as the chilling temperature can be optimized to minimize the impact. Also, conditions can be optimized by ensuring sufficient ventilation during chilling and transport. For example, the best chilling-temperature range for the transport of compacted *An. arabiensis* adults is 8–11°C (Culbert et al. 2017). Aedine mosquitoes appear to be more robust than Anophelines, and the time of release is likely to be more critical in the latter case.

For an SIT project to be effective, sufficient sterile males must be released in relation to the target population. In an efficient programme, males should be directed to the target area at a fine scale; this requires an understanding of the behaviour, size and spatial distribution of the population before intervention (Lees et al. 2014). Such data are also crucial in monitoring the progress, and demonstrating the success, of the project as it progresses (Vreysen, this volume). During the planning and development phase of an SIT project, it is important to trap male mosquitoes (during mark-release-recapture experiments) to make assessments about the survival of released males, distance dispersed, and level of mating competitiveness. Ongoing monitoring of the fate of released males, and the size of the target population, is necessary as an SIT programme progresses. The benefits of detailed population data are increased by making releases assisted by automated geographic information systems (GIS) (Bouyer et al., this volume). Software is available which allows releases to be tailored according to real-time surveillance data for maximum efficacy.

Since female mosquitoes are the vectors of disease, most existing traps have been designed to collect females for surveillance, and in some cases for control. It is necessary to produce male-specific tools, or to adapt the timing, location or baiting of traps to target males specifically. For *Aedes* surveillance, males and females can be collected indoors using aspirators. The BG-Sentinel trap is effective for collecting males, but most of the effective traps attract only gravid females. Thus, there is an especially urgent need for effective methods to survey Anopheline mosquitoes (Batista et al. 2019; van de Straat et al. 2019). The CDC light trap often catches few *Anopheles* mosquitoes, and other tools such as the Suna trap, sticky resting boxes, baited traps, and human-landing catches require better standardization. Swarm capture

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is a powerful approach but requires great expertise, and this technique is not possible in all locations (Bimbilé Somda et al. 2018). Better understanding of the cues which attract male mosquitoes is leading to traps with improved efficiency, e.g. the Sound-GAT (Johnson et al. 2018). In Singapore, mosquito surveillance is achieved using a network or more than 30 000 GAT traps monitored weekly (N. Lee Ching, personal communication). Emerging technology for automated passive mosquito surveillance shows great promise; in theory, a network of intelligent traps could automatically identify in real time the species, sex, size, and marker status of insects entering or passing close by (FAO/IAEA 2019a).

## 3. PILOT TRIALS AGAINST MOSQUITO VECTORS

In this new era of interest in applying the SIT against mosquito vectors, until now the pilot trials have been on a small scale, usually focussed on isolated villages or islands. It is perhaps no coincidence that the first projects to reach pilot-scale demonstrations of the SIT are to control Aedine mosquitoes, given their relative amenability to mass-rearing and sex separation. These small-scale trials are sufficient to demonstrate an impact on some entomological indicators, usually the number of eggs collected per ovitrap, egg-hatch rate, and adult-catch rate. These indicators suggest success, but for the technique to be taken up by abatement districts, governments or charities for implementation on an operational scale, evidence of epidemiological impact of the SIT (reduction of disease incidence) as part of integrated management schemes will be required, involving much larger area-wide trials. Transmission of disease by mosquitoes occurs not just in the home, but also in schools and workplaces, and therefore, unless a community is very isolated with little human movement outside a given area, it will be a challenge to demonstrate disruption of transmission or case reduction. Moreover, in some circumstances, female *Aedes* appear to disperse much farther than males (up to 800 m) (Honório et al. 2003); this will make demonstration of efficacy by current randomized cluster trials very challenging. Current equipment and techniques, in particular sex separation and release methods, will need to be improved dramatically, particularly against Anophelines, before such large-scale trials are possible. Through small-scale pilot trials, many lessons are being learned, which will inform the development of larger projects, e.g. the significance of *Ae. albopictus* immigration from neighbouring vegetation into urban or semi-urban areas targeted with the SIT, and the requirement for reduction of target populations using conventional methods or IVM prior to, and in conjunction with, applying the SIT.

### *3.1.* Aedes albopictus *in Italy*

Feasibility studies on using the SIT to target invasive *Ae. albopictus* mosquito populations were started in northern Italy in 2000 (Bellini et al. 2007). The studies included several releases of irradiated males; promising levels of induced sterility were achieved. Where release ratios were high enough, population reduction occurred (Bellini et al. 2013b).

Between 2008 and 2012, six pilot studies of sterile *Ae. albopictus* male releases were made to test the efficacy of the SIT approach to suppress mosquito populations.

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The release sites were selected as being representative of urban conditions, small enough (10–17 ha) to achieve the required release ratio given the level of sterile-male output that was possible in the Bologna mosquito production unit, and well-isolated from other urban areas. The dynamics of the mosquito populations were monitored weekly in the release and control areas, using standard ovitraps. Eggs collected from the traps were counted under a stereomicroscope and hatched using standard procedures to assess fertility and, conversely, induced sterility (Bellini et al. 2007). Sterile males, released at the rate of 900–1500 males/ha/week, induced sterility levels between 15 and 70% of the background fertility of the local population. Where induced sterility reached 70%, a similar reduction was found in egg numbers collected by ovitraps. Therefore, inducing sterility levels of >80% in the native *Ae. albopictus* females for an entire season was expected to be sufficient to suppress effectively the mosquito population.

A recent meta-analysis of these trials (R. Bellini, personal communication) found that the released males demonstrated a mean competitiveness value of 0.188 (SD±0.33). This competitiveness (measured as the Capacity to Induce Sterility or CIS index) was highly variable between pilot trials despite very similar environmental conditions being experienced among the sites and similar sterile-male release methods being used in all studies. A strong temporal variability was observed, with lower values found at the beginning and at the end of the summer season (when the wild population density is usually lower and therefore the male sterile:wild ratio is higher) (Albieri et al. 2010; Carrieri et al. 2011). The strong negative correlation between the sterile:wild ratios and the competitiveness values demonstrated in this study was also observed in previous trials conducted under semi-field and field conditions using irradiated and transgenic sterile males (Harris et al. 2011; Damiens et al. 2016). In practical terms, an optimal ratio must be used in SIT operations to maximize cost-effectiveness; increasing the release ratio will not result in a proportional increase in induced sterility.

### *3.2.* Aedes albopictus *in Mauritius*

As part of its Operational Plan for Prevention and Control of Chikungunya and Dengue, the Ministry of Health and Quality of Life in Mauritius is evaluating integrating the SIT to control *Ae. albopictus* populations; the objectives are to prevent outbreaks and the re-establishment of arboviruses in an island benefitting from large numbers of international visitors (Beesoon et al. 2008; Ramchurn et al. 2009). Several characteristics of Mauritius make it a very suitable country in which to test the feasibility of applying the SIT. Identifying potentially suitable sites for pilot-trial releases, and corresponding control areas, is straightforward due to the largely agricultural nature of the island of Mauritius. Small discrete villages exist, often located on the coast or surrounded by sugar cane fields providing geographical isolation. In some villages *Ae. albopictus* is the only Aedine mosquito present or at least the dominant species. Two suitable villages were selected for the first pilot trial: Pointe des Lascars (0.3 $km^2$, consisting of 203 houses and 800 inhabitants) and Panchvati (0.03 $km^2$, consisting of 67 houses and 270 inhabitants) (Iyaloo et al. 2014). The villages, located 1.6 km from each other, are well-matched in terms of the human

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inhabitants, natural geography, infrastructure, and mosquito populations. Routine monitoring and mark-release-recapture experiments showed that the majority of ovitraps are positive for eggs throughout the year, with peaks in December-March and troughs in July-September (Iyaloo et al. 2019).

To determine survival and dispersal of sterile males in this ecological setting, three mark-release-recapture (MRR) experiments (Itô et al., this volume; Vreysen, this volume) were performed in Pointe des Lascars (Iyaloo et al. 2019). A release rate of 6000 males/ha was applied during the winter season, and at least twice that number in the summer. When assessed in a laboratory, marking males (with a fluorescent dust) did not affect their performance (Dowell et al., this volume). After applying a dose of 40 Gy with a gamma irradiator, tests showed that: (1) dispersal was not affected by irradiation, and (2) irradiated males survived up to 12 days; on average, unirradiated males survived 4 days longer (Bakri et al., this volume).

In parallel with baseline mosquito surveillance (using ovitraps and BG-Sentinel traps), a colony of mosquitoes from the pilot villages was established in a climate-controlled insectary at the Vector Biology and Control Division. For larval diets, two animal feeds were shown to be suitable and cost-effective (USD 1/kg); they are manufactured locally (important for an island nation) (Iyaloo and Facknath 2017). Mosquitoes were reared using the tray and rack system described by Balestrino et al. (2014b), and large commercially available adult cages. Sex separation of release cohorts, done on the basis of pupal size (using graded sieves), achieved a maximum of 4% female contamination. Adult females were further removed from the release cages with an aspirator. Batches of 2000 male pupae were irradiated at 30–40 hours old, as was done for the MRR trials, and allowed to emerge into small adult cages containing a sugar meal. Adults were transported to the field in the small cages for release 3 days after emergence.

Prior to the pilot trial, staff of the Ministry of Health and Quality of Life went from house to house (in the release and control sites) inviting people to attend sensitization meetings, held in the village hall, during which the project was explained, and also a cage of male mosquitoes was used to demonstrate that they do not bite. During the trial, field officers working in the pilot sites were mobilized to talk to the public at least once per week to hear and address any concerns (D. P. Ilyaloo, personal communication).

In the first mosquito SIT trial in Mauritius, IVM was applied during the first two months of the project, applying larval-source reduction, weekly *Bacillus thuringiensis israelensis* (*Bti*) applications, and biweekly pyrethroid fogging alongside sterile-male releases (Mangan and Bouyer, this volume). Each week, for 9 months, 60 000 sterile males were released, equally distributed among 10 release sites in Panchvati for the first 6 months and 20 sites for the remainder of the trial period. Ovitrap surveillance within (and in a 150-m radius around) Panchvati and within Pointe des Lascars, and biweekly 24-h collections using 8 BG-Sentinel traps in both villages, showed a significant decrease in oviposition and the adult population as a result of the IVM treatments. This was reversed in the control village when vector treatments were halted, but not in the village where sterile-male releases alone continued. Egg fertility remained stable throughout the trial period in Pointe des Lascars, but was significantly lower in Panchvati during the period of releases (except for a period in the immediate

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aftermath of tropical cyclone Berguitta). During the period of sterile-male releases, induced sterility reached more than 30%, the number of eggs collected per ovitrap dropped by more than 50%, and adult catch was less than one half that in the control village (D. P. Ilyaloo, personal communication). Ovitraps in the area surrounding Panchvati tended to collect more eggs than within the village, suggesting that immigration of fertile females into the treatment area was acting against the population suppression efforts.

As in the Italian SIT pilot studies, releases were more effective the lower the initial population density at the target site; low densities enabled a higher release ratio given the rearing resources available, and possibly also a greater competitive advantage for the sterile insects. Permitting sterile males to mature, and take a sugar meal before release, improved mating competitiveness. In the ecological setting of Mauritius, initiating the targeted release of sterile males in the winter season is likely to be most effective in reducing the peak summer adult population, but sustained releases over several years would be required to sustainably reduce, or even locally eliminate, populations due to the stockpile of eggs of the wild population; these eggs are laid each season and hatch when the rains arrive.

### *3.3. Other Pilot Trials against Mosquitoes*

Many other pilot trials are ongoing or have been completed in various countries, and many more are in the planning stages (Table 1), indicating the current high level of interest and ongoing activities to develop and integrate the SIT and/or IIT, as well as transgenic approaches, against disease-transmitting mosquitoes.

## 4. LOOKING TO THE FUTURE

The major elements required to apply the SIT against major mosquito vectors of disease are in place. The remaining challenges, that need to be overcome before it can be applied operationally on a large scale, are just technical improvements and upscaling, particularly in terms of sex separation, methods of releasing males, and accurate monitoring of male populations.

The technology for mass-rearing mosquitoes is well advanced (section 2.1.1.; Parker, Mamai et al., this volume): mass-production of larvae in racks of large trays (Balestrino et al. 2014a), separation of larvae from pupae (Balestrino et al. 2011), and housing and feeding of adults for egg production and storage (Balestrino et al. 2014b; Maïga et al. 2017). Methodological improvements are ongoing to increase efficiency and decrease costs (directly and through automation to reduce the labour required). For example, the reuse or recycling of larval-rearing water (Mamai et al. 2017) is a significant factor in the feasibility of mass-rearing mosquitoes in water-limited environments. The costs of mass-rearing equipment are being reduced (Maïga et al. 2019) and adult and larval diets improved (Bimbilé Somda et al. 2017, 2019). Currently, two issues are still being addressed (to enable mass-deployment) -- the need for a sex-sorting system (section 4.1.) that is efficient on a large scale (particularly for Anophelines), and the development of technologies to release mosquitoes aerially using UAVs (Dowell et al., this volume).

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*Table 1. SIT, IIT, and transgenic pilot trials against mosquitoes*

| Type of approach used | Mosquito species | Location | Trial staus | Reference |
|---|---|---|---|---|
| SIT pilots[1] | *Aedes aegypti* | Mexico | Concluded | FAO/IAEA 2018c |
| | *Aedes albopictus* | Italy, Mauritius | Concluded | Bellini 2013b; D. P. Iyaloo, personal communication |
| | | La Réunion (France), Germany, Greece, Italy, Montenegro, Spain | Ongoing | TIS 2019; R. Bellini, personal communication; I. Pla Mora, personal communication |
| SIT/IIT pilots | *Aedes aegypti* | Thailand | Concluded | Kittayapong et al. 2018; Kittayapong 2021 |
| | | Singapore | Ongoing | FAO/IAEA 2018c |
| | *Aedes albopictus* | China | Concluded | Jozuka 2016; Zheng et al. 2019; Baton et al. 2021 |
| IIT pilots for suppression – releasing only *Wolbachia*-infected males | *Aedes aegypti* | Debug-Verily in Australia and USA | Not clear | Haridy 2017; Debug-Verily 2019 |
| | | Singapore (Project *Wolbachia*) | Ongoing | Co 2019; NEA 2019a, b; Liew et al. 2021 |
| | *Aedes albopictus* | Florida (USA) | Concluded | Mains et al. 2016, 2019 |
| | | MosquitoMate in USA | Ongoing | MosquitoMate 2019 |
| | *Aedes polynesiensis* | Raiatea (French Polynesia) | Concluded | O'Connor et al. 2012 |
| | | Tetiaroa (French Polynesia) | Concluded | Bown 2019; Strugarek et al. 2019 |
| IIT pilots for population replacement -- releasing also *Wolbachia*-infected females | *Aedes aegypti* | World Mosquito Programme -- Australia, Brazil, Colombia, India, Indonesia, Mexico, Pacific Islands, Sri Lanka, Vietnam | Ongoing | Flores and O'Neill 2018; WMP 2019 |
| Transgenic mosquito pilots | *Aedes aegypti* | Oxitec -- Brazil, Cayman Islands, Panama | Concluded | Harris et al. 2011; Carvalho et al. 2015; Gorman et al. 2015 |
| | | Oxitec – Brazil, Panama, USA | Ongoing | Oxitec 2019 |

[1]In addition, pilot SIT projects will begin in 2019 against *Ae. aegypti* in Brazil, Cuba, Indonesia, Malaysia, the Philippines, and the USA (Florida), and against *An. arabiensis* in South Africa.

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The process of handling and transporting sterile males from a mass-rearing facility to release sites could be streamlined and standardized so as to minimize the impact on male survival and performance, and to enable upscaling the release technology. For example, handling could be minimized by loading sterilized male pupae into emergence cages; after emergence the adults can be fed a sugar meal and then chilled and concentrated into transport cassettes (with the emergence cages removed) which are loaded into ground- or aerial-release vehicles.

Aerial release of mosquitoes will enable the SIT to be applied in a wider range of circumstances, including situations where access by road is difficult or the labour costs of the fine-scale release of sterile males are prohibitive. A releasing system on a UAV was tested successfully in Brazil (FAO/IAEA 2016a, 2018a). The survival of aerially released *Ae. aegypti* males was not significantly different from that of males released on the ground, suggesting that the impact of the aerial release system on insect quality was minimal. Moreover, at a sterile:wild ratio of only 3:1, the induced egg sterility reached 50% in the release area; this is very promising (J. Bouyer, unpublished data). However, further fine-tuning of the technology and approach, and the likely involvement of the private sector, will be needed to produce reliable industrial versions of the release system, and enable it to be used, eventually also commercially, in operational vector-control programmes (Dowell et al., this volume).

The priority for the operational development of the SIT against mosquitoes is to upscale field trials, demonstrate impact on mosquito populations, and ultimately prevent cases of disease. The FAO/IAEA and WHO are presently collaborating to develop common operational guidance on applying the SIT to control mosquito-borne diseases, and to offer technical guidance to countries planning to integrate the SIT into their integrated management strategies. This guidance will cover all practical aspects relating to the trial and early application of the technology, e.g. production, transport, and release of sterile males, associated quality-control parameters, clearly defined entomological and epidemiological indicators of efficacy in large-scale entomological trials, and frameworks to assess risk, safety implications, and cost-effectiveness. Guidance will follow a phase-conditional approach, and will include strategies for community and media engagement, disease surveillance under large-scale deployment, monitoring, and evaluation of success, and a description of legal frameworks for registration and regulation related to operational SIT programmes (FAO/IAEA 2019b; Bouyer et al. 2020; WHO/IAEA 2020).

### *4.1. Need for Scalable and Effective Sex Separation*

Although perfect sex separation may not be an absolute requirement for the efficacy of the SIT, in the case of mosquitoes (where females are the vectors of disease) the tolerance for even low-level female contamination in the release population is very low (section 2.1.1.). Options for improved sex-sorting that are currently available include using classical genetics to produce a genetic sexing strain (GSS), such as the dieldrin-sexing strain in *An. arabiensis* (Yamada et al. 2012). This system was very effective in sorting males (resistant to rearing solutions containing 2 to 4 ppm dieldrin, unlike females) but presented two drawbacks: a reduced egg fertility and thus low strain productivity, and the presence of dieldrin residues on the released males and the

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associated risk of bioaccumulation in the environment (Yamada et al. 2015). Another option is to exploit the fact that only female mosquitoes feed on blood, i.e. add a toxicant to the blood meal (Yamada et al. 2013); though effective on a small scale, this may not be feasible for mass-rearing, particularly where a 100% male-release population is absolutely required.

Significant effort is being expended to develop new methods of sex separation (Papathanos et al. 2009, 2018; Bourtzis and Tu 2018). Sorting systems have been developed in *Aedes* species based on their sexual dimorphism, attempting to improve on the Fay-Morlan separator (Fay and Morlan 1959; Focks 1980) for larger-scale rearing. They enable mature pupae to be separated automatically on the basis of sex (Fig. 3) (Araújo et al. 2018; Bellini et al. 2018; Zacarés et al. 2018); at small-scale rearing up to 99% of males are recovered, and contamination by females is lower than 0.1%. An automated sieving system has been developed to separate *Ae. aegypti* pupae on the basis of smaller (male) pupae being able to pass up through openings in a submerged surface while larger (female) pupae are trapped below (Justia Patents 2017; Debug-Verily 2019). Another automatic pupae sex-separator, that can sort up to 150 000 pupae per hour with a female contamination rate below 0.3%, has been developed for *Ae. albopictus* by Wolbaki Biotech in China (Baton et al. 2021).

The size difference between *Anopheles* male and female pupae is less pronounced, and they are less robust and more easily damaged, making such sorting impossible (Mashatola et al. 2018). However, a similar system is under development to sort mature pupae of a sexing strain expressing sexual dimorphism in eye colour (K. Bourtzis, personal communication), which may be more amenable for *Anopheles* sex sorting, and the two systems may be combined to further improve the purity of the male-only release population. Nevertheless, these sexing systems are based on the sorting of pupae, making it necessary to produce female larvae; if females could be removed earlier in development, production costs could potentially be reduced by one half. Also, these methods are labour-intensive; it is very challenging to remove every female without losing a large proportion of males, further escalating the rearing costs.

Mechanical systems are unlikely ever to separate the sexes with 100% efficiency, and certainly not on a large scale; a few females will always be released. There is then a danger of "population replacement" when using only the IIT, but when the IIT and radiation-sterilization are combined, this cannot happen – any released females are sterile (and cannot transmit disease because of the *Wolbachia* infection) (section 4.2.).

A more efficient method of separation will be required before the SIT can be applied to mosquitoes on an operational scale, most likely relying on a GSS. A GSS in *An. albimanus* was produced in the 1970s for the SIT project in El Salvador but was lost after the trial was terminated (Dame et al. 2009). Producing GSSs by classical genetics is lengthy and labour-intensive because it relies on mutagenizing and screening large numbers of individual families (due to the low probability of mutagenesis producing the desired linkage event) (Lebon et al. 2018; Ndo et al. 2018). In *Aedes,* species sex is determined by a male-determining factor located on a small, non-recombining M locus on chromosome 1 and not on a heteromorphic sex chromosome (Hall et al. 2015), making it more unlikely and therefore more laborious to achieve translocation of the selectable marker to the sex-determining chromosome (Papathanos et al. 2018).

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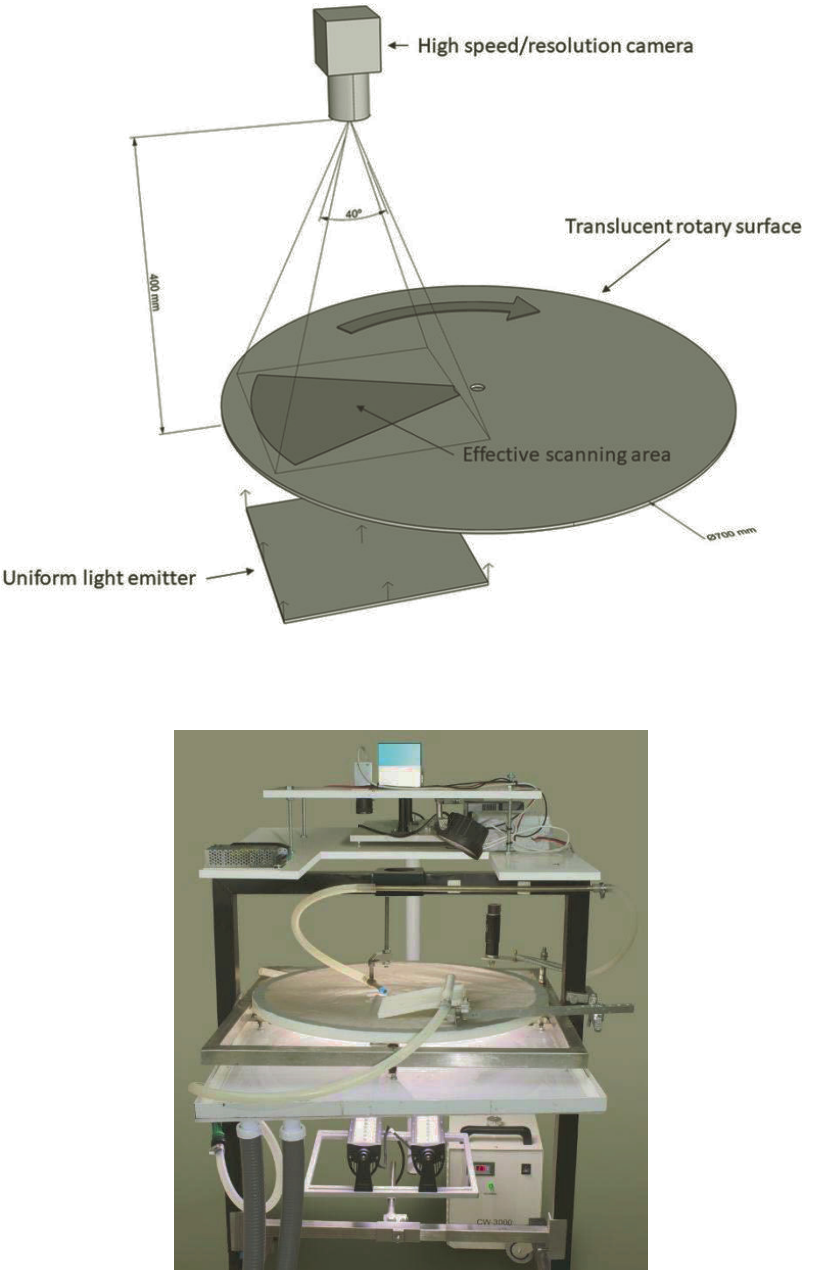



*Figure 3. Upper drawing: Camera set-up for sorting male and female* Aedes *pupae based on size dimorphism. (Drawing from Zacarés et al. 2018, reproduced with permission.) Lower photo: Prototype laser sex-sorting machine (Bourtzis 2019).*

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However, a promising approach, using modern molecular techniques, is to identify *temperature-sensitive lethal* (*tsl)* genes, and to link them to sex-specific genes to enable the removal of females as first-instar larvae or even as embryos through classical genetics (as is done for *C. capitata* (Franz et al., this volume)). Modern molecular tools that can be used to produce transgenic as well as non-transgenic sexing strains have been widely reviewed (Catteruccia et al. 2009; Bernardini et al. 2018; Häcker and Schetelig 2018; Lutrat et al. 2019; Häcker et al., this volume).

An alternative approach could be to feed double-stranded RNAs (dsRNAs) to mosquito larvae (Whyard et al. 2015). By targeting both the testis genes and a female sex determination gene (*doublesex*) to induce RNA interference (RNAi), female development can be successfully inhibited (Häcker et al., this volume). To be effective, the dsRNA must be available constantly during relevant developmental stages to guarantee the silencing effect, though the cost of producing these dsRNAs may be reduced through the culture of transformed bacteria or yeast.

Flow cytometry machines (COPAS®, Union Biometrica) could be used for high-throughput separation of males from females based on the sex-specific expression of a fluorescence marker, e.g. sperm-specific expression in the testes (Marois et al. 2012). This approach is still being upscaled for mass-rearing, but relies on the use of transgenic strains, whose "open release" is limited or prohibited in some countries. Irradiating these transgenic strains to sterilize them before release would enable them to benefit from a non-GMO status in European countries, where only fertile material is considered as an organism. Under the Nagoya protocol (CBD 2014), even the release of sterile GMOs is tightly controlled, though their release is authorized in Europe (Lutrat et al. 2019). Using approaches such as CRISPR/Cas engineering, targeted mutagenesis through gene editing could be used to create GSSs which do not contain exogenous DNA and thus are not classed as transgenics, facilitating their acceptance for release (Kandul et al. 2019; Häcker et al., this volume).

### *4.2. Combined SIT/IIT Approach to Control* Aedes

In parallel with the increased interest in applying the SIT against mosquito vectors of disease, there has also been much interest in developing and applying the incompatible insect technique (IIT), and exploring its potential through *Wolbachia*-based approaches to enhance the SIT (Zabalou et al. 2004; Moretti et al. 2018; Baton et al. 2021). The IIT relies on cytoplasmic incompatibility (CI) between released males (which carry a *Wolbachia* infection) and wild females (with no infection, or a different and incompatible infection), thus resulting in sterile matings. Moreover, introduced *Wolbachia* infection makes females refractory to arboviral transmission. Starting in 2011 in Australia, field trials releasing *Wolbachia*-infected *Ae. aegypti* females and males have been successful at population replacement (Hoffmann et al. 2011). However, such *self-sustaining* releases (Alphey 2014) have the intent to establish permanently the *Wolbachia* strain in the target population, a process (that unlike the SIT) is irreversible and leaves an "ecological footprint". On the other hand, releasing only *Wolbachia*-infected males is *self-limiting,* and results in population suppression similar to the SIT, without any permanent changes in the target population (Bourtzis et

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al. 2014, 2016); it is essential that only males are released, otherwise population replacement can take place.

Until effective genetic sexing strains become available for large-scale SIT application, combining the SIT with the IIT provides important benefits for an operational scale (Arunachalam and Curtis 1985; Bourtzis and Robinson 2006; Brelsfoard et al. 2009). A strain of *Ae. albopictus,* carrying three strains of *Wolbachia* (*w*AlbA, *w*AlbB, and *w*PiP), has been shown to express strong cytoplasmic incompatibility and good mating competitiveness in semi-field tests after irradiation at 28 Gy (a fully sterilizing dose for females) (Zhang et al. 2016). Thus, due to the sterility given to males by cytoplasmic incompatibility, a lower irradiation dose can be used to sterilize females, minimizing the impact on male performance (Zhang et al. 2015a, b). At the same time, including the IIT precludes potential disease transmission by any inadvertently released females (which make up at least 1% of the release population in upscaled SIT releases using current sexing systems). On the other hand, simultaneous sterilization guarantees that such inadvertently released females cannot reproduce; this will prevent *Wolbachia* from becoming established in the target population (resulting in the loss of cytoplasmic incompatibility and creating resistance to the IIT approach) (Lees et al. 2015; Bourtzis et al. 2016; NEA 2018). However, in Europe, this approach is at present limited by the absence of regulation.

An early pilot trial of the combined IIT and SIT approach against *Ae. aegypti*, conducted in a village in Chachoengsao Province, eastern Thailand, showed a significant reduction in hatch rate, and a lower total adult catch, during 6 months of weekly releases compared with the control area (Kittayapong et al. 2018; Kittayapong 2021). Released males were infected with *Wolbachia* collected from a local *Ae. albopictus* strain, irradiated as pupae with 70 Gy using gamma rays, and confirmed to be sterile by crossing a sample of each release cohort with non-irradiated *Wolbachia*-infected females to score fertility. In a small-cage laboratory test, released males were shown to be equally competitive to non-irradiated *Wolbachia*-infected males. A total of 10 000–25 000 one-day-old males was released (100–200 per household per week) for 6 months from delivery containers in which pupae had emerged and been provided with a sugar feed. Local support was garnered in the pilot site through strong community engagement and public awareness activities, even involving householders in the releases (Dyck, Regidor Fernández et al., this volume). Adult abundance was monitored monthly using sticky traps, and collections were made with portable vacuum aspirators. Ovitraps were collected weekly to monitor hatch rate (Vreysen, this volume).

### *4.3. New Opportunities in the Integrated Management of Mosquito-Borne Diseases Offered by the SIT*

The SIT technology is ready to be applied in pilot trials and small-scale population suppression programmes for integrated mosquito control (FAO/IAEA 2015; Lees et al. 2015). In the efforts to control malaria, the SIT may, under specific conditions (particularly against exophilic species such as *An. arabiensis* where conventional control by insecticide-treated nets and indoor residual sprays is less effective), become a powerful adjunct to other technologies. This would be in accordance with the World

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Health Organization's Roll Back Malaria strategy (Nabarro 1999), and more recently the WHO's Global Vector Control Response 2017–2030 (WHO 2017a), which promote integrated vector management rather than reliance on any single approach to control malaria. Given the advanced state of development of the SIT against *Ae. aegypti*, and in response to the widespread epidemic of the Zika disease in 2015–2016, FAO/IAEA proposed the technology as part of an integrated Zika management strategy (FAO/IAEA 2016b).

Advances in molecular biology and biotechnology have provided several potential genetic methods to manage mosquito populations, offering different opportunities and challenges relative to the SIT (Catteruccia et al. 2009; Alphey 2014; Bouyer and Marois 2018; Flores and O'Neill 2018; Häcker et al., this volume). Also, it has been proposed that the SIT can be boosted by treating the males with biocides, like juvenile hormone analogues or specific biopesticides like densovirus, before release (Bouyer and Lefrançois 2014; Bouyer et al. 2016). During mating, or even mating attempts, sterile males could transmit these biocides to females, which would in turn transfer them to oviposition sites. As an example, treating sterile male *Ae. albopictus* with pyriproxyfen may enable the number of males released into a given area to be reduced by more than ten-fold, and achieve a more reliable and sustainable impact on dengue transmission (Pleydell and Bouyer 2019). The principle has been tested with success on a very small scale against *Ae. aegypti* in Kentucky (Mains et al. 2015), and further trials are planned in Spain and France.

The fact that the SIT is self-limiting, unlike population replacement based on *Wolbachia* or gene drive of transgenic traits, is one of its advantages. However, this is also a drawback -- releases must be sustained over time, involving permanent costs to prevent disease transmission in a given area. Other advantages of the SIT are that it is species-specific, and has no regulatory requirements (unlike most other proposed genetic control methods that require approval for releases) (Reeves et al. 2012; Hendrichs and Robinson, this volume). Also, it has a positive public perception and no restrictions on intellectual property rights -- each country is able to use its own local mosquito strains to apply the technology.

Finally, random mutations, chromosome breakages, and gross gonad damage caused by radiation eliminates the risk of resistance development – unlike insecticides and potentially other genetic control methods (Alphey et al. 2011; Eckermann et al. 2014; Handler 2016; The Economist 2017; Häcker et al., this volume; Hendrichs and Robinson, this volume; Whitten and Mahon, this volume). Contrary to a wrong perception that this sterilization process is necessarily associated with a loss of competitiveness of the sterile males, field trials of alternative technologies, especially RIDL, demonstrated that competitiveness of transgenic males can be much lower than what has been observed with irradiated males (Benedict and Robinson 2003; Facchinelli et al. 2013). This is related primarily to the fact that in mosquitoes the SIT is based on local strains that are well-adapted to local environments, whereas transgenic strains have often been colonized for decades.

In other insects, e.g. tsetse flies, it has been demonstrated that a loss of competitiveness is usually the result of a combination of factors such as mass-rearing, handling, chilling, and transport rather than irradiation itself (Diallo et al. 2018). This will affect equally all genetic control methods based on the mass-release of males.

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Optimizing the rearing conditions is critical to minimize the impact of laboratory adaptation and other manipulations on adult male quality (Bargielowski et al. 2011).

An understanding of the reasons for a negative correlation between competitiveness and the sterile:wild male ratio (seen in the SIT trials in Italy) will be instrumental for planning future mosquito control programmes that have an SIT component. One of the major hypotheses for this phenomenon is that a proportion of the female population might be protected from sterile males regardless of the release ratio because they are located in cryptic habitats, and it is difficult for the released sterile males to access these. This illustrates the importance of released sterile males matching the behaviour of wild males by, for example, aggregating in the same micro-habitats (Vreysen at al. 2011). Also, there probably is an optimum ratio of sterile to wild males beyond which no increase in the ratio results in a higher induced sterility. It is important to identify this optimal ratio because accurate estimates of sterile-male release densities would maximize the cost-efficiency of SIT projects.

Another reason might be immigration of fertile females into the target area from neighbouring sites where sterile males are not released (the females have a much higher dispersal capacity than males) (Honório et al. 2003). Therefore, a tailored distribution of sterile males in the target area and surroundings is needed so as to achieve an adequate overflooding ratio even where wild populations are at high densities, especially when releasing into urban settings characterized by many favourable cryptic micro-habitats. Hot spots with a high mosquito population density should be identified and targeted appropriately, although this is challenging with existing monitoring tools and is not currently achievable on a large scale.

The SIT is not a stand-alone technology, and needs to be applied in combination with other pest control methods as part of an area-wide integrated pest management (AW-IPM) approach (Hendrichs, Vreysen et al., this volume; Klassen and Vreysen, this volume; Mangan and Bouyer, this volume). In particular, population reduction achieved through public participation in larval-site reduction will usually be a prerequisite, and will require that there is a strong stakeholder engagement in all projects (Dyck, Regidor Fernández et al., this volume).

## 5. CONCLUSIONS

At present, many SIT pilot trials against mosquitoes are ongoing or in preparation (Table 1). These field trials will create important knowledge needed for future upscaling of the technology. The most illustrative recent example is the SIT/IIT pilot trial in China that successfully suppressed, and nearly eliminated, two field populations of *Ae. albopictus* over a two-year period. Millions of sterile males were released, with prior pupal irradiation. Community support for the SIT/IIT approach strongly increased following mosquito releases, as nuisance-biting decreased. This successful field trial has fully demonstrated the feasibility of area-wide application of SIT/IIT for mosquito vector control (Zheng et al. 2019).

Area-wide releases, focused on urban/suburban settings and touristic sites, appear particularly promising in terms of sustainable and cost-effective IVM with an SIT component (eventually provided commercially by the private sector), as they can protect many people concentrated in relatively small areas.

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The potential impact of the successful control of mosquito vectors, through the combined use of the SIT and other control methods, can be estimated based on previous successful programmes. When the Panama Canal was constructed, mosquito control during a period of 5 y had a significant economic and social impact on the region – eliminating yellow fever and drastically reducing malaria transmission, enabling the Panama Canal to be completed, and promoting the development of the entire area (RES 2014). In Brazil in the 1930s and 1940s, similar benefits were seen when a broad range of strategies was used to eradicate the invasive *An. gambiae* (Hendrichs, Enkerlin et al., this volume). In each of these cases, the community saw these programmes as drastic, due to, for example, the use of military force to achieve the goals, but the resulting impact on morbidity and development overcame the negative reactions (Severo 1956; Ockenhouse et al. 2005; Griffing et al. 2015).

Between 1990 and 2010, the global burden from neglected tropical diseases declined by 27%; however, this reduction occurred mostly in upper-middle-income countries. If the latest WHO targets are met between 2010 and 2020, a 55% reduction in the global burden in each country would be achieved, with an even greater reduction in low- and lower-middle-income countries (Stolk et al. 2016). It is expected that including the SIT among methods used to control mosquitoes will contribute to achieving the WHO goal, and result in increased human well-being, reduced deaths from vector-borne diseases, and increased development in deeply affected areas.

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# CHAPTER 8.1.

# PROSPECTS FOR THE FUTURE DEVELOPMENT AND APPLICATION OF THE STERILE INSECT TECHNIQUE

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## SUMMARY

Science-based modern agriculture and international trade in agricultural commodities have achieved that, even though the world population has doubled in the last 40 years, the absolute number of people in poverty and hunger has been falling steadily. The major challenge in the immediate future is to consolidate these positive gains, while simultaneously expanding sustainability through environment-friendly agricultural and public-health practices. Within this context, the sterile insect technique (SIT), as part of area-wide integrated pest management (AW-IPM) programmes, will continue to gain momentum for application against certain key insect pests. This is in response to the demands for cleaner food and a better environment, the need to facilitate increasing international trade by overcoming pest related trade barriers to the movement of agricultural commodities, and the imperative of dealing with the increasing invasion of invasive pests and disease vectors. As the use of the technology increases, changes will continue to be made to improve the overall efficiency of the technique for those species where the SIT is already being used, and to expand the use of the technique to new key species. Modern biotechnology and paratransgenesis will increasingly also contribute to improving SIT efficiency in terms of strain marking, genetic sexing, molecular sterilization and disease refractoriness. However, whereas the shipment and use of *Wolbachia*-infected male insects as part of combined SIT/IIT (Incompatible Insect Technique) strategies for disease vectors is making considerable progress, public opposition and regulatory hurdles have largely limited to a few countries the release of transgenic insects. There appears to be much promise in improving sterile male performance by exposing male insects to hormonal, nutritional, microbial, and semiochemical supplements. Furthermore, the management of mother colonies will be significantly improved to reduce the effects of colonization and to slow down mass-rearing effects on key behavioural parameters that often result in rapid colony deterioration. Progress will also need to be made in the cost-effectiveness of all components of SIT implementation, from cage design to facility design, and from programme planning to evaluation. The trend of increasingly using sterile insects for routine pest suppression rather than eradication, particularly in commercially important commodities, will favour the involvement of the private sector and hence accelerate these improvements. Commercial producers of beneficial insects will probably be the natural investors, in view of the complementarities with sterile insects, experience in managing living organisms, and understanding the biological control market. As programme implementation is logistically complex, management will remain the key issue determining the success or failure of any area-wide approach to insect control. Thus, in spite of the many successes achieved and to be expected, in many least-developed countries the SIT may be a technology that is "ahead of its time" and beyond the animal and public health, as well plant protection infrastructures. Failures in SIT application, mostly confined to such countries, have not been due to science but to the poor management of large-scale operations. Increased involvement of the private sector in such countries probably would assure effective implementation.

## 1. INTRODUCTION

It is likely that the use of sterile insects in area-wide integrated pest management (AW-IPM) programmes will continue growing as increasing constraints are placed on the use of chemical pesticides responding to the public's growing demands for a cleaner environment and residue-free food. Global food chains increasingly link agricultural production with powerful supermarket chains that compete to offer their customers higher quality food in terms of maximum residue levels of insecticides. Even without these constraints, resistance problems and the cost of development and

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regulatory approval of new insecticides will continue to increase. Added to this, more species-specific methods to control key pests, such as mating disruption or sterile insects, will play a more important role to complement the increasing use of biological control agents, which are often disrupted by insecticide applications. Moreover, as a result of the global spread of mosquitoes and other vectors, vector-borne and zoonotic diseases are on the rise (Higgs 2018). In the absence of vaccines and efficient, safe and inexpensive drugs to combat the emerging diseases they transmit, interest in the genetic control of public health pests is undergoing a revival (Lees et al., this volume). Furthermore, the international standards that regulate global agricultural trade, including measures for pest management where these affect trade (under the World Trade Organization's (WTO) Agreement on Sanitary and Phytosanitary Standards (SPS), are having a major impact on agricultural production and trade, and this is creating an environment in which area-wide approaches to the management of insect pests have a comparative advantage (Devorshak 2007; Klassen and Vreysen, this volume).

With this expansion in the use of the technology, the basic components of the sterile insect technique (SIT) — mass-rearing and release of sterile insects — are hardly likely to change. However, changes will continue to be made to improve the overall effectiveness and efficiency of the technique for those species where the SIT is already being used, and research needs will be addressed to expand the use of the technique for new key pest species. For some potential candidate species, technical bottlenecks have already been identified that prevent the development of the technology, e.g. artificial rearing systems or obligate diapause, which often can be addressed by a concerted research effort, whereas other pest species, because of their biology, are not amenable to SIT application (Lance and McInnis, this volume).

Expanding globalization will inevitably lead to a further rise in the invasion of non-native or invasive insect pests into new areas, where they increasingly survive in previously inhospitable areas due to a changing climate. The concept of using the SIT against such pest incursions to preclude the establishment of such invasive populations has proven effective (Hendrichs, Enkerlin et al., this volume) and will probably gain increasing support. This can take the preventive approach of the California model in which sterile Mediterranean fruit flies *Ceratitis capitata* (Wiedemann) are continuously released in areas of high risk of establishment (due to the regular incursions into the region) (Dowell et al. 2000; Enkerlin, this volume). Alternatively, it can take the form of the Australian approach where the technology, expertise, and contingency plans are developed to be able to respond should an introduction of the Old World screwworm *Chrysomya bezziana* (Villeneuve) occur (Tweddle and Mahon 2000). The trend to counter the impact of increasing globalization, by moving plant protection "offshore" for risk mitigation of non-native pest incursions, will open up new opportunities to integrate the SIT into the process of creating pest free or low prevalence areas under a systems approach (FAO 2016a, b, 2017a), from which agricultural products can be safely exported.

Currently there is no interest from pesticide companies to become involved with the SIT technology, probably because it is not impacting on their market to any significant extent. Even with continued expansion, the SIT will remain a niche market. Commercial producers of pollinators and other beneficial insects will probably be the

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natural investors, in view of their experience in managing living organisms and their understanding of the biological control market. Such companies aim to offer a complete "biological package", i.e. the SIT can be used to manage some of the major key pests, while the reduced insecticide applications allow a significant expansion of the sales of parasitoids and predators to control secondary pests. The use of sterile insects for routine pest suppression rather than eradication, thus creating a sustainable demand analogous to the current use of augmentative biological control, will favour the involvement of the private sector (Hendrichs et al. 1995). However, for commercial companies to become more involved in the SIT, some issues including intellectual property rights (IPR) and regulation may have to be addressed (Barnes 2007; Bassi et al. 2007) (although see section 2.6.).

Programmes with the strategic goal of eradication can suffer from the price of success, e.g. maintaining staff and infrastructure in the face of a successful but declining programme. In such cases it is important to have a long-term vision, developing alternative uses of the rearing facility and programmes for other relevant pests. Converting the pink bollworm *Pectinophora gossypiella* (Saunders) mass-rearing facility to navel orangeworm *Amyelois transitella* (Walker) production, and from melon fly *Zeugodacus* (formerly *Bactrocera*) *cucurbitae* (Coquillett) to sweet potato weevils, are examples in the USA and Japan, respectively.

For future expansion of the SIT, a major constraint that will always need to be addressed is the significant upfront cost of constructing a facility. Of course, industrial plants producing and formulating insecticides represent larger investments, and compared with the costs of insecticide development, the costs of SIT development are minimal. Nevertheless, raising funds is not easy, and so far most rearing facilities have been constructed with public funds, and the beneficiaries often pay, partially or fully, for the sterile insects and/or the field operations. (The costs to beneficiaries usually do not include costs for constructing the facility; this is in contrast to the costs of insecticide where the development costs are recovered in the pricing policy of the company). In some situations, true commercialization may be a way out of the dilemma (section 2.1.).

Another constraint that must be overcome at the start of an area-wide programme is to involve, and get the commitment of, all or at least a majority of stakeholders in the programme (Loosjes 2000). Again, this free-rider problem is not faced in a local pesticide situation; an individual farmer can make his/her own decision, and be approached on an individual basis by chemical companies.

Quality control protocols, both for the product, i.e. the sterile insect, and the process, i.e. the procedures used to produce, sterilize, and distribute the sterile insect, will need to be further improved, and in some way standardized (Parker, Vreysen et al., this volume). This has already been done for sterile fruit flies, where an internationally agreed set of protocols is used to monitor the quality of the sterile insect (FAO/IAEA/USDA 2019). It is reasonably straightforward to develop the protocols and to update them regularly. This is a pre-requisite for commercialization. Producers and users require harmonized protocols to measure sterile insect quality, and the increasing transboundary shipment and trade in sterile insects will gradually ensure that all programmes implement them (Enkerlin and Quinlan 2004; Dowell et al., this volume).

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Management remains the key issue determining the success or failure of any area-wide approach to insect control (Dyck, Reyes Flores et al., this volume). Since the SIT is logistically complex and management intensive, its implementation requires flexible procedures and non-bureaucratic management structures. In general, the scientists who have been closely involved with developing the technology should not be responsible for programme implementation, as other skills are needed. Also, research activities should not be part of an operational programme; instead, a separate unit (but associated with the programme) is needed for problem-solving and continuous fine-tuning of procedures; technology can always be improved to increase cost-effectiveness. The "secret" is to find a balance between sterile insect quality and operational stability, predictability, and innovation (Rull et al. 2012).

## 2. UTILIZATION OF THE SIT TECHNOLOGY

### *2.1. Commercialization*

Almost all of the field programmes integrating the sit described in this book have been carried out by public-funded organizations, with or without some financial support from the direct beneficiaries. Although area-wide programmes often address a public good, in the long run full reliance on governments may not be sustainable. Furthermore, continued expansion of the technique will be facilitated when commercial enterprises become involved. Even if public funds are used initially to establish a programme, governments, to increase implementation efficiency, subcontract private companies to provide services or operate the whole programme or parts thereof. However, thus far commercialization of the sit has been a difficult concept to promote, for the following reasons: (1) there are no equivalent models through which commercial confidence can be generated, with the possible exception of the growing biological control industry (Steinberg and Cayol 2009), (2) currently there are only some international agreements or regulations for the production and transboundary shipment of sterile insects (but see section 2.6.), (3) only a very few detailed business plans for the production and use of sterile insects have been developed (FAO/IAEA 2008), (4) the initial capital cost of constructing a mass-rearing facility is rather high (although this can be mitigated by following a gradual modular approach, where new rearing modules are added only with expanding sterile insect demand (Tween 1987)), (5) some public-funded programmes have been providing sterile insects at subsidized prices, thus undercutting competition from private sector companies that have to sell at real prices that include the cost of capital (Barnes 2007; Bassi et al. 2007), and (6) the area-wide nature of sit programmes requires at least some degree of organization and coordinated participation of stakeholders. In spite of this list of constraints to the commercialization of sterile insect production, rather paradoxically there is currently no shortage of customers who would purchase sterile insects if they were available. While the use of sterile insects only for eradication of pest populations is normally not an attractive proposition for commercialization because these are time-limited programmes, the permanent use of sterile insects for suppression, containment and prevention programmes (Hendrichs, Vreysen et al., this volume) provides some continuity in the

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need for sterile insects, and has already resulted in some private investment (Steinberg and Cayol 2009; Boersma 2021; Venter et al. 2021).

Commercialization of the SIT can involve the delivery of a complete package, or more likely be partitioned into several different components, depending on the type of programme. A commercial company could be responsible for rearing, shipping, emerging, and releasing sterile insects, and charge customers (who would only manage the field monitoring) according to the number of insects released or the area treated. This would be suitable where there are large agricultural areas growing mainly one crop, or where governments contract such turnkey operations to deal with pests of animal or public health importance. In an alternative strategy, a grower cooperative could buy sterile insects from a supplier, manage the monitoring and fly emergence, and subcontract the release activities; the Mediterranean fruit fly programme in Patagonia, Argentina, already follows such an approach. On the other hand, in The Netherlands, onion growers individually contract with a commercial producer of sterile onion maggots for the combined service of monitoring and suppression to guarantee maintaining the pest in their onion fields below a pre-agreed threshold level (Loosjes 2000; Fournier 2014; Everaarts 2016).

Another strategy that lends itself to commercialization is the production of eggs in a large facility for satellite facilities (maybe even in another country) which then only rear, sterilize, and release the insects. This removes the necessity for every programme to manage mother colonies and to maintain a large egg production colony, an expensive and highly skilled operation, especially if genetic sexing strains are being used (Caceres et al. 2004; sections 3.1.1. and 3.1.2.).

### *2.2. Non-Native Pest Incursions*

Globalization, increased movement of agricultural products, and changes in climatic conditions will all lead to increases in the spread of pests and in their ability to establish in new locations. This increasing problem of non-native or invasive species incursions and outbreaks is causing much concern; often no remedial actions can be taken when such a pest enters a new area, or no environment-friendly eradication tools are available that are acceptable to the public (Hendrichs, Enkerlin et al., this volume). A good example of how such problems can be addressed is the accidental introduction into Libya in the 1990s of the New World screwworm *Cochliomyia hominivorax* (Coquerel). In the case of this incursion, fortunately a solution was at hand; the technology was already available for this pest, and sterile pupae could be brought from Mexico. For the eradication in 1990–1991, 40 million irradiated pupae pre-packed in boxes for release were air-freighted from Mexico each week in aircraft (especially outfitted for long-distance transport of chicks). The insects were released in Libya, and the large outbreak was successfully eradicated (FAO 1992; Lindquist et al. 1992). Of course, the major drawback to this approach is that a monitoring and rearing/release system must be in place, and the procedures must be implemented rather quickly.

The Australian government has taken this proactive approach and developed offshore a mass-rearing system for the Old World screwworm; should there be an incursion of this devastating pest into the country, the needed expertise and experience

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are available (Mahon and Ahmad 2000; Vargas-Terán et al., this volume). The recurrent incursions (through cargo ships) of the Asian gypsy moth *Lymantria dispar* (L.) into British Columbia, Canada, and north-western USA, but also as far as New Zealand, are also potential targets for integrating the SIT with repeated aerial *Bt* sprays, increasing the effectiveness of ongoing efforts to eradicate such outbreaks (Suckling 2003). New Zealand also has contractual arrangements in place with mass-rearing facilities in Australia in the case of early detections of incursions of Mediterranean and Queensland fruit flies *Bactrocera tryoni* (Froggatt) (Horner et al. 2016).

Countries or regions may decide to identify their most important non-native insect threats, and then in advance develop the required pest-specific technology and expertise, as well as the general legal and physical infrastructure, that provide some degree of remedial action (Suckling 2003; Horner et al. 2016). This could include the SIT; it has a unique ability to eradicate incipient outbreaks of non-native insects in an environment-friendly way and with minimum public resistance (Hendrichs, Enkerlin et al., this volume). For example, one of the most feared non-native pests is the false codling moth *Thaumatotibia leucotreta* (Meyrick), still largely confined to the southern part of the African continent. To prepare for any future outbreak, the US government has supported in South Africa the development of the SIT against this major polyphagous pest (Carpenter et al. 2007; Boersma 2021).

### *2.3. SIT Application to Environmental Problems*

Non-native insects, as well as causing problems for humans, their livestock and crops, can have a devastating impact on the environment. A textbook example of successful classical biological control is the deliberate introduction of the cactus moth *Cactoblastis cactorum* (Berg) to Australia to control invasive *Opuntia* cacti. However, after its accidental arrival in North America (where *Opuntia* cacti are endemic), *C. cactorum* has become an invasive pest. It has spread from the Caribbean to the south-eastern USA, attacking endangered cacti. If this pest reaches the American southwest and Mexico, it is expected that it will have a devastating effect on whole ecosystems based on *Opuntia* cacti. In response, radiation biology, mass-rearing, and pheromone studies have been carried out on *C. cactorum.* The aim is to apply SIT/inherited sterility to contain this environmental pest, in its westward spread along the Gulf of Mexico, before it reaches the south-western USA (Zimmermann et al. 2004; Bloem et al. 2007).

Since the SIT has virtually no environmental impact, it is an ideal tool for use in protected areas such as natural parks and biological reserves where other kinds of control are prohibited. One example is the eradication of an outbreak of the cactus moth on the island of Contoy, Mexico, which is a natural park where the mechanical removal of host cacti was not possible (Bello-Rivera et al. 2021). Other examples are some national parks in Africa where conventional tsetse control is no longer acceptable, but sterile insects can be released without any impact on biodiversity (Nagel and Peveling, this volume). Similarly, the SIT plays a special role in the large organic coffee production areas of Central America where suppressing the Mediterranean fruit fly using conventional bait sprays is prohibited.

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SIT/inherited sterility also has considerable potential to assess under natural conditions the environmental impact of introducing non-native herbivores as biological control agents of invasive weeds (Hendrichs et al. 2009). There is increased awareness of possible risks of non-target effects (Follett and Duan 2000). To obtain importation and release permits for such species, increasingly stringent host specificity testing is required. Tests carried out under unnatural quarantine conditions often overestimate the host range, leading to rejection of effective candidates. Releasing sterile herbivores can be another risk-management tool for host-range assessment under natural conditions. There can be no leakage of genetic material into the gene pool because there is no native population present, and in the case of inherited sterility, released females are fully sterile. The survival of $F_1$ larvae under various abiotic and biotic conditions can be assessed, and if non-target species are attacked, releases can be suspended with no risk of permanent establishment.

This concept is being developed in Florida for an herbivore of the Brazilian peppertree *Schinus terebinthifolius* Raddi (Anacardiaceae), a major invasive non-native that is altering native plant communities in many subtropical regions. The South American leaf-rolling moth *Episimus utilis* Zimmerman (Tortricidae) has been imported into Florida for quarantine evaluation, and radiation biology studies have already been conducted to determine the dose that results in full female sterility (Moeri et al. 2009).

### *2.4. New Target Species*

The biological criteria, to be considered before embarking on an AW-IPM programme integrating the SIT for a particular species, are described by Lance and McInnis (this volume), but the external conditions under which a pest population can become a candidate for the SIT are changing continually. For example, problems in the citrus trade between Spain and the USA led to the incorporation of the SIT into the area-wide control of the Mediterranean fruit fly in Valencia's large citrus-growing areas (Juan-Blasco et al. 2014), which until then had been based largely on insecticide bait sprays. The same has been occurring in Morocco's main citrus exporting region in Agadir (Qadda et al. 2017). Therefore, increasing concerns about biosecurity, food safety, and residues will also impact positively on the economics and desirability of using sterile insects.

The rapid adoption and expansion in the area planted to crops expressing *Bt* toxins had been expected to reduce the demand in some candidate species for SIT integration. For example, the use of *Bt*-cotton would remove insect pests of this crop from consideration as potential SIT targets. Nevertheless, even though transgenic cotton is now planted annually on many million ha, there is increasing resistance developing in some of the targeted moth pests because the area-wide compliance of farmers in following the refuge strategy has not always been adequate (Tabashnik et al. 2010). The systematic maintenance of refuges, by planting non-*Bt* plants on parts of their fields, is supposed to delay development of pest resistance by producing susceptible insects to mate with resistant insects. Another disadvantage of this strategy, besides the potential for resistance development, is that populations of the pest need to be maintained so that refuges can fulfil their role (Fig. 1) (Wu 2010).

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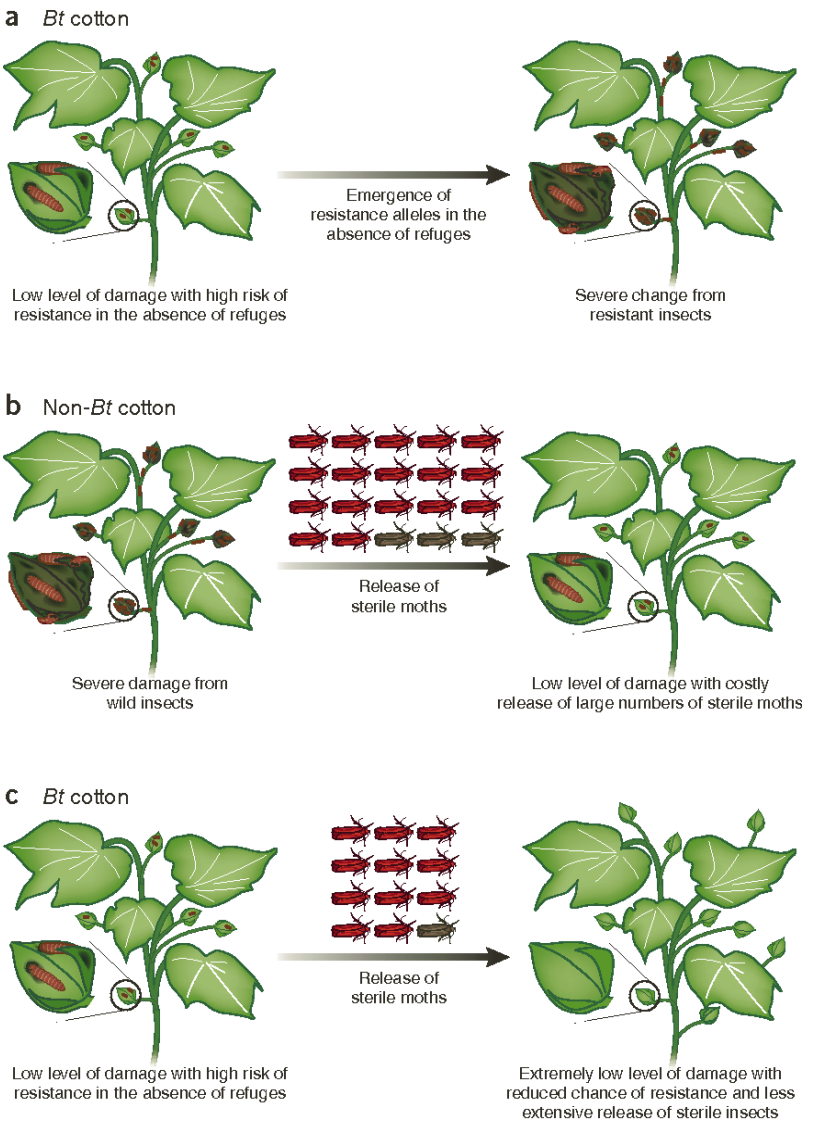



*Figure 1. Use of the SIT together with transgenic cotton expressing the* Bt *transgene suppresses the growth of the pink bollworm population and facilitates management of resistance to* Bt *toxin. The pink bollworm feeds only on cotton bolls and does not damage other tissues. (a) Sustainable use of* Bt*-cotton to control pink bollworm populations is threatened by the emergence of resistance. (b) Although costly, repeated release of sterile pink bollworm moths (red) in vast excess to the number of wild moths (brown) can suppress the growth of pink bollworm populations. (c) Combined use of* Bt*-cotton and the SIT ensures that the release of fewer sterile moths can suppress the growth of pink bollworm populations while preventing the emergence of resistance to* Bt *toxin. (Figure from Wu 2010, reproduced with permission.)*

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An alternative strategy in such situations where refuges may be insufficient to mitigate permanently the development of resistance, and taking advantage of the *Bt*-suppressed pest populations, is to combine the use of the *Bt* crop with the simultaneous release of sterile insects so that they mate with, and suppress the reproduction of, the resistant insects (Fig. 1) (Tabashnik et al. 2010; Wu 2010).

This synergistic approach was successfully applied against the low pink bollworm populations (resulting from the extensive growing of *Bt*-cotton) and motivated the upgrading of the existing containment programme to an eradication programme (Henneberry 2007). The application of the complementary SIT, mating disruption, and transgenic technologies, requiring a less extensive release of sterile moths, succeeded in eradicating all populations of this pest from the south-western USA and northern Mexico, while also eliminating insecticide sprays against this key invasive pest (Staten and Walters 2021; Simmons et al., this volume). Therefore, while area-wide application of the SIT can help delay the establishment of resistance in a pest population exposed to *Bt* crops, and allows growers to avoid planting non-*Bt* refuges and the related damage, the area-wide expression of the *Bt* toxin without refuges suppresses drastically the pest population, thereby increasing efficacy and economic feasibility of SIT application (Wu 2010).

Pest lepidopterans constitute a major threat to many agricultural crops, resulting in the use of large amounts of insecticide. Currently there are already several operational programmes being implemented against this group of pests (Simmons et al., this volume), supported by much research that has been conducted in this area (IAEA 2002; Marec et al. 2005; Kean et al. 2007; Vreysen et al. 2010, 2016; Marec et al., this volume). Thus, the number of lepidopteran species, for which SIT technology will be developed, will probably increase, but this will be only gradual since its application is usually more expensive compared with dipteran species, transgenic crops primarily target moth pests, and mating disruption is most effective in lepidopteran pests (Cardé 2021).

Insect vectors of malaria and other human diseases continue to exact a huge toll in human lives and livelihoods, and in the 1970s many SIT pilot projects against vectors were implemented with varying degrees of success, including a very successful field trial in El Salvador (Weidhaas et al. 1974; Klassen et al., this volume). Since that time, many technologies related to SIT development and implementation have changed dramatically, and the use of this technology against important vectors is being re-evaluated. This trend will be accelerating because of the increasing burden of mosquito-borne diseases and the global spread of emerging diseases such as chikungunya, dengue, yellow fever and Zika, growing public awareness and concerns about the impact of chemical control on human health and the environment, the increasing incidences of resistance to insecticides, and recognition by the WHO (World Health Organization) and other major stakeholders of the need for more sustainable, effective and biologically based methods that are complementary to current control (WHO 2017).

The technical problems in suppressing mosquito populations using the release of sterile males are quite formidable, but significant progress is being made in developing the SIT and the combined SIT/IIT (Incompatible Insect Technique) (Zabalou et al. 2004) strategies for important vectors such as *Aedes aegypti* (L.), *Ae.*

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*albopictus* (Skuse), and *Anopheles arabiensis* Patton (Bourtzis and Robinson 2006; Beier et al. 2014; Lees et al. 2015; Bourtzis et al. 2016; Zheng et al. 2019). The problems in developing and implementing the SIT are significantly smaller for *Aedes* than *Anopheles* populations, and a number of mosquito pilot programmes that are ongoing or in preparation are focusing mainly on suppressing *Aedes* populations in urban areas (Bellini et al. 2010; Lees et al., this volume).

### *2.5. Integration with Other Measures*

The SIT is always applied in combination with other methods of pest management; in eradication programmes it is often the final component of an integrated sequential approach (Mangan and Bouyer, this volume). Knipling (1992) suggested that the combinatorial approach involving the simultaneous release of natural enemies together with sterile insects may be extremely efficient in population suppression and eradication. Theoretical modelling (Barclay, this volume) has confirmed the effectiveness of this integration. While sterile insects have their impact on receptive adult females, parasitoids target the immature stages, and pathogens and predators all life stages of the pest. Although there has been limited experimental work to assess the veracity of this prediction, an expansion of this approach can be foreseen.

In Lepidoptera, where the release of partially sterile insects offers greater suppressive potential than the release of fully sterile insects, the synergism of combined augmentation of natural enemies and inherited sterility has been repeatedly demonstrated, with released biological control agents reproducing/feeding/infecting on sterile or substerile offspring (Mannion et al. 1995; Carpenter et al. 1996; Hamm and Carpenter 1997; Bloem et al. 1998; Seth and Barik 2009).

Seeding substerile or sterile supplemental hosts or prey in the field to increase the initial survival of inoculatively released biological control agents, or to increase the early build-up of native biological control agents in advance of pest population build-up, also has potential (Fatima et al. 2009). This approach is currently providing effective management of several species of sugar cane borers in a 40 000-ha area of sugar cane in Pakistan.

Integration of augmentative releases of natural enemies and sterile insects can also increasingly be expected in protected or greenhouse crops, where insecticide use must be avoided so as not to interfere with the release of pollinators and biocontrol agents controlling other pests (Kaspi and Parella 2006, 2008; Steinberg and Cayol 2009; Robinson 2018). Already a number of SIT studies are ongoing to address *Drosophila suzukii* (Matsumura) (Lanouette et al. 2017; Nikolouli et al. 2018) and other greenhouse pest problems, such as whiteflies (Calvitti et al. 2000), leafminers (Cagnotti et al. 2012; Walker 2012; Sultan et al. 2017), and the pepper weevil *Anthonomus eugenii* Cano (Robinson 2018), for which other biological control solutions are not available or sufficient as part of a complete biologically based control package. Also, in terms of integration of mass-production processes, biological control and the SIT can be complementary, reinforcing the applicability and cost-effectiveness of these augmentative technologies (Steinberg and Cayol 2009).

Today the control of mosquitoes relies heavily on the use of *Bti* for the suppression of immatures, and insecticide-treated bednets that target female mosquitoes during

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their host-seeking behaviour. These technologies are very compatible with the release of sterile male mosquitoes; they do not seek the host for feeding but rather target the mate-seeking behaviour of female mosquitoes. Similarly, success in New World screwworm eradication programmes requires the simultaneous treatment of wounds with insecticides, which are directed at the immature stages, and the systematic release of sterile insects, which are directed at the adult stage.

There will be increased efforts to achieve field sterilization or inoculation through deploying pathogen- or chemosterilant-baited traps or autodissemination devices (Charmillot et al. 2002; Navarro-Llopis et al. 2011, 2015; Maniania and Ekesi 2013; Bouyer and Lefrançois 2014; Toledo et al. 2017). Such approaches attempt to achieve autosterilization and dissemination through slow-acting compounds (such as insect growth regulators, chitin synthesis inhibitors and pathogens) that are transferred by contact or the ingestion of a bait and spread in the target population through intraspecific interactions. In the case of chemosterilants, this effect can be especially complementary to the release of sterile insects because, unlike wild insects, they are not affected. Nevertheless, the potential for large-scale application is limited to special areas and situations; high-density deployment of such devices is not practical for area-wide implementation, and the environmental impacts on non-target species must be thoroughly assessed.

### *2.6. Regulatory Issues*

Future expansion of the SIT will be facilitated by a regulatory framework to support the commercial production, trade, shipment, and release of sterile insects. Historically, since they are not self-replicating, sterile insects were not considered to be biological control agents, and therefore not included in the regulatory framework provided by the International Plant Protection Convention (IPPC) in the form of International Standard for Phytosanitary Measures (ISPM) Number 3 "Code of Conduct for the Import and Release of Exotic Biological Control Agents" (FAO 1996), which specifically dealt with the import and release of biological control agents. Notwithstanding this lack of a regulatory framework, over the last 60 years there have been significant transboundary shipments of sterile insects, totalling over a trillion (million million) insects (Enkerlin and Quinlan 2004; FAO/IAEA 2018a). This significant international transport of live sterile insect pests testifies to the inherent safety of radiation-induced sterility and its general acceptance by the international plant protection community.

The increasing interest shown by some commercial companies in producing sterile insects emphasized the importance of having a regulatory framework. In response, in 2005, ISPM Number 3 was revised by representatives of national and regional plant protection organizations, resulting in the "Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and Other Beneficial Organisms" (FAO 2017b). In this revised ISPM 3, the definition of biological control agents has been broadened, sterile insects have been specifically included as "beneficials", and the SIT is now officially accepted internationally as a type of biological control. In addition, the terms "sterile insect" and "sterile insect technique" have been defined for inclusion in the ISPM Glossary of Phytosanitary Terms (FAO 2017c; Klassen and Vreysen, this volume). Thus, the application of sterile insects as part of the integrated

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management of plant pests is now recognized by the IPPC, and this should facilitate their transboundary shipment and use, especially in terms of their commercial use. Since this framework only covers those cases where plant pests are involved, the use of the SIT against insects of veterinary or public health importance, that are vectors of disease, will require other regulatory action, possibly through the Office Internationale des Épizooties (OIE) and the WHO. While the shipment and use of sterile and/or *Wolbachia*-infected male mosquitoes as part of the SIT, IIT (EPA 2017), or combined SIT/IIT strategies (Bourtzis et al. 2016; Zheng et al. 2019; Lees et al., this volume) for such vectors are recently making considerable progress, the lack of regulation has limited (largely to a few countries) the transport and release of transgenic male mosquitoes and other insects (Carvalho et al. 2015; Miller and Cohrssen 2018).

### *2.7. Resistance to the SIT*

The increased use of sterile insects to suppress pest populations may bring with it concerns about the development of resistance (Itô et al. 2003; Lance and McInnis, this volume; Whitten and Mahon, this volume). As suppression programmes by definition are long-term, this will provide opportunity for natural selection to select individuals that can, in some way, differentiate between a released sterile male and a fertile wild male. This may be especially relevant where an island population is targeted on an area-wide basis because it represents an isolated genetic environment (Itô et al., this volume). The danger for resistance to occur is probably more remote when a population is targeted for eradication, although the development of resistance in suppression programmes will be modulated by the occurrence of immigration and refugia from where "susceptible" individuals re-enter the target population.

For resistance to develop and be selected, three conditions must be fulfilled: (1) a recognizable difference or differences between a sterile male and a wild male that a wild female can recognize, (2) a fitness cost to the wild female if she mates with a sterile male, and (3) a genetic basis for the recognition of the sterile male by the wild female. It is not inconceivable that, for certain species, all these conditions could be met. For condition (1), routine application of quality management systems should identify any significant anomalies (Cáceres et al. 2007), allowing correction through the introduction of a new strain and better mother-colony management; for (2), as the fitness of a wild female mating with a sterile male is zero, this condition will always be met in a programme integrating the SIT, and for (3), for most species this is largely unknown. The development of resistance in a target population can be dealt with by re-colonizing a population from the target field site and replacing the original colony. This becomes slightly more problematic when specialized strains, such as genetic sexing strains, are used for release (Robinson et al. 1999). Field cage tests under semi-natural conditions, that continuously monitor the effectiveness of sterile males when competing with wild males to mate with wild females, become a very critical component of all programmes (Hendrichs et al. 2002).

In those programmes where sterile females are also released, tests need to evaluate the ability of wild males to discriminate between sterile females and wild females. If wild males mate only with wild females, but sterile males cannot distinguish and mate with both types of females, modelling indicates that a doubling of the sterile insect

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release rate is required to overcome this wild male discrimination against sterile females (Vreysen et al. 2006).

## 3. TECHNICAL IMPROVEMENTS

### *3.1. Improving Insect Quality*

A critical factor determining the success or failure of a programme that includes an SIT component is the ability of the released sterile males to effectively inseminate wild females with sperm that is competitive with normal sperm (although in some species aspermic sterile males can also inhibit female remating at the same rate as normal matings involving sperm transfer (Itô et al. 1993)). This ability is generally termed "competitiveness", and is determined by many factors related both to the treatment of a particular cohort of released insects and the developmental history of the mass-reared strain used to produce the cohort for release. Both sets of factors can impact negatively on the competitive ability of released insects, and efforts will continue to be made to minimize these negative effects. As the "production philosophy" still tends to dominate decision-making, even though theoretical models continue to stress the importance of insect quality over the number of insects released (Itô et al., this volume), more efforts to educate decision-makers and managers are needed. A major component of any quality improvement strategy is to have in place a series of quality control protocols that can be used as a baseline to evaluate the effect of any experimental changes on insect quality (Cáceres et al. 2007; FAO/IAEA/USDA 2019; Parker, Vreysen et al., this volume).

Since quality refers only to a field parameter, then it would seem appropriate to develop field-based protocols for its assessment, unless laboratory-based protocols can be identified that correlate directly with field competitiveness (Boller and Chambers 1977). In the past, probably too much attention was focussed on laboratory-based protocols that had very little predictive value in terms of field performance. Quality is relative; it is dependent on the space and complexity of the testing arena. In a restricted space, for example in a small laboratory cage with a reduced distance between individuals, sterile males are often more effective than wild ones, but in larger cages the effectiveness of the same sterile males decreases significantly, and in field cages with vegetation it decreases even further (Soemori et al. 1980; Miyatake and Haraguchi 1996). It is also of little value to develop quality control parameters using only mass-reared insects, especially for species with a mating system in which the females choose among mating partners (Lance and McInnis, this volume). Therefore, the quality of mass-reared insects must increasingly be measured directly under semi-natural or natural conditions and in competition with wild insects (FAO/IAEA/USDA 2019; Parker, Vreysen et al., this volume).

In fruit flies, a standard field-cage system, including host plants, has been developed, where mass-reared sterile insects have to compete with wild insects (Cáceres et al. 2007; FAO/IAEA/USDA 2019). A similar system has also been developed for tsetse flies (Mutika et al. 2001). This type of approach needs to be expanded and refined to include semi-field cage systems that contain a cycling wild population, and in which the competitiveness of sterile insects can be maintained and

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assessed. This has already been done for the Mediterranean fruit fly (McInnis et al. 2002) and for mosquitoes (Knols et al. 2002).

The key field parameter to assess the quality of mass-reared sterile males is the amount of sterility induced in wild females; improved protocols need to be developed that provide information on this parameter. In screwworms (collection of egg masses), tsetse flies (aberrations in the reproductive system of dissected wild females), and moths (mating tables), the opportunity to monitor the behaviour and fertility of a field population under challenge from sterile insect releases is a tremendous advantage to routine programme evaluation (Vreysen, this volume). In fruit flies, on the other hand, this is not practical, although molecular tools now allow identifying the origin of sperm in captured wild females as an indicator of the degree of induced sterility in the target population (San Andrés et al. 2007; Juan-Blasco et al. 2013, 2014). In mosquitoes, sugar-feeding of males before release with a rhodamine dye can be used to stain their sperm and seminal fluid that can then be detected in the wild females of the target population (Johnson et al. 2017).

To a limited extent, compensation for low insect quality is possible by releasing a larger number of sterile insects. However, there is a limit to this in terms of the overall economy of a programme, and there is a biological limit in that, below a certain quality value, increasing the number of released insects will simply not be effective (Barclay, this volume; Itô et al., this volume). In future, much more attention should be paid to sterile insect quality as opposed to quantity, irrespective of the cost of producing a specific insect.

The introduction of a colony mass-rearing protocol based on the filter rearing system, currently used to maintain stability in genetic sexing strains (Parker, Mamai et al., this volume), can also make a major contribution to the quality of mass-reared insects (section 3.1.2.).

### *3.1.1. Colony Initiation and Maintenance*

There are virtually no procedures or protocols on how to establish an initial colony of a species for future mass-rearing (McDonald 1976). Questions about the number of insects that should be sampled, where they should be sampled, and when the sampling should be done, remain largely unanswered. Even if guidance could be given on the above points, there is still the well-known phenomenon of a "bottleneck" which occurs during the first one or two generations of laboratory rearing, and which can negate the effects of any science-based approach to field sampling (Cayol 2000a; Rull and Barreda-Landa 2007; Hoffmann and Ross 2018; Parker, Mamai et al., this volume).

The answer to the question, "How many insects should be sampled?' is generally, "As many as possible". Of course this is not very satisfactory, and the number of insects used is usually influenced by the logistics involved in collecting insects in the field, coupled with the known size of the bottleneck that occurs during initial colonization. If the aim of the initial sampling of the field population is to sample the available genetic variability, then, based on population genetic studies of natural populations, samples of 20–100 insects generally reveal most of the genetic variability present in a field population, indicating that a sample in the hundreds is probably sufficient for most situations, provided that most individuals reproduce in the

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laboratory. Then the major task becomes the preservation of that variability during initial colonization and subsequent mass-rearing in the laboratory (Hoffmann and Ross 2018).

From where in its distribution should the field population be sampled? Preferably this should be from the target population, although again logistics tends to determine the specific location of sampling sites and hence the parts of the insect distribution that are sampled. A related question to this concerns the strengths and weaknesses of using a single mass-reared strain for all regions where a particular species is a pest. This is especially important for species that are distributed worldwide, e.g. the codling moth *Cydia pomonella* (L.) and the Mediterranean fruit fly, and even for other species showing very discontinuous distributions. To a point, this can be tested by conducting mating compatibility studies, using the target populations from the different regions and the mass-reared strain (Cayol 2000b; Taret et al. 2010). Even if tests reveal no mating problems between the two populations, still programme managers often request that a particular genetic background be backcrossed into the strain to be used, despite the absence of any evidence that this is beneficial. In biological control circles, there has been much discussion on whether to sample marginal or central populations, but some of the hypotheses proposed to answer this question are, in practice, very difficult to test (Mackauer 1976).

The underlying assumption made during this discussion is that the level of genetic variability, per se, in the mass-reared colony is correlated with the overall quality of an insect once sterilized and released in the field, and hence is important to maintain. Krafsur and Ouma (this volume) challenge this assumption, and suggest that inbreeding, i.e. reduction of overall variability during colonization and mass-rearing, may not always affect competitiveness in the field. At the moment, probably there are insufficient data to make a general conclusion regarding the correlation of genetic variability in the mass-reared colony with competitiveness of the insects in the field. As suggested by Krafsur and Ouma, in the future, quality control procedures should be expanded to include routine measurements of genetic diversity in the colony over time, coupled with effective tests to monitor the competitiveness of the insect in the field. In the colonization strategy for the New World screwworm, procedures have been developed that involve the establishment of isofemale lines, followed by their evaluation and subsequent hybridization to establish a strain for mass-rearing (Mangan 1992).

The decision-making procedure, as to when a strain needs to be replaced, has to be improved. Since strain domestication or deterioration is normally correlated with the number of generations under selective mass-rearing conditions, at present programme managers usually establish a time-based replacement schedule. Better tools need to be developed to monitor the quality of released sterile insects in the field. A science-based and data-driven approach to strain initiation and replacement is needed (but see next section). Also, mother-colony maintenance procedures that slow strain deterioration should be developed, thus extending the life of a strain under mass-rearing. These will probably include a combination of the following actions:

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- Maintaining founder/back-up strains under cryopreservation to guard against genetic drift and laboratory adaptation (Leopold et al. 2001; Leopold 2007),
- Holding mother colonies under relaxed, preferably semi-natural, rearing conditions that introduce some variation and complexity, and from which the rearing colonies are regularly derived (see filter rearing system below),
- Making simple modifications to the rearing processes and holding conditions of the mass-reared colony that reduce the selective pressures that result in directional changes in some of the key behavioural processes (Miyatake and Haraguchi 1996; Liedo et al. 2007), and
- Implementing an "active" quality control programme that establishes heritabilities and genetic correlations between desirable traits such as mating behaviour and traits that can be counter-selected during routine mass-rearing (Miyatake and Yamagishi 1993; Miyatake et al. 1997; McInnis et al. 2002; Sánchez-Rosario et al. 2017).

*3.1.2. Strain Replacement Procedures and the Filter Rearing System (FRS)*

For all insect species that are mass-reared, the artificial environment of a facility presents the insect with a tremendous challenge, i.e. to adapt to the artificial conditions, both biotic and abiotic. Such conditions impose new selective pressures that are not present in nature (for example, high densities), while selective pressures that are strong in nature are weakened or absent (for example, predation) (Hoffmann and Ross 2018). While holding large colony sizes and refreshing them with new wild insects can help minimize genetic drift, they will generally not ameliorate adaptation (Schneider 2009). Therefore, successful colonization will inevitably result in the often rapid selection for adapted genotypes, and this can impact negatively on the competitiveness of a sterile insect once it is released in the field (Miyatake and Haraguchi 1996; Briceño and Eberhard 2002; Hoffmann and Ross 2018).

Most mass-rearing protocols follow the principle of large cycling colonies, whereby a proportion of the production is used for sterilization and release, while the remainder is returned to maintain the production colony. In this system, over time there is an inevitable accumulation of highly selected genotypes, such as early reproduction, faster development, and simplified courtship behaviour (Briceño and Eberhard 2002), some of which can significantly compromise the quality of the insects released in the field. This fact has prompted most operational programmes to replace their strains regularly, and to establish improved quality control procedures that monitor the overall competitiveness of the mass-reared strain. Strain replacement is a major logistical exercise, and invariably the new strain requires considerable time before it is as productive as the old strain. It is not clear how much of the overall quality of a strain is lost during this adaptation process, and how often a strain needs to be changed. Rull and Barreda-Landa (2007) found that new colonies established by exclusively crossing wild males and laboratory females enabled the restoration of male mating competitiveness without compromising mass-rearing production. Nevertheless, improved methodologies to monitor strain quality are needed.

As discussed by Franz et al. (this volume) and Parker, Mamai et al. (this volume), the use of genetic sexing strains in Mediterranean fruit fly programmes incorporating the SIT required the development of a filter rearing system (FRS) to maintain the

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integrity of the sexing strain (Fisher and Caceres 2000; Caceres et al. 2004). The FRS relies on the maintenance of a mother colony that each generation is checked for unwanted individuals (which are then removed). Eggs from this colony are harvested as required, and following 3–4 generations of mass-rearing amplification the males are sterilized and released. In the FRS, no insects that have been through mass-rearing are returned to the mother colony, and therefore there is no accumulation of highly selected genotypes in the colony.

The FRS can, of course, be used for purposes other than maintenance of the integrity of genetic sexing strains, and can make a major contribution to the overall competitiveness of mass-reared strains. The mother colony can be kept under more natural environmental conditions, at reduced adult and larval densities, and with reduced selection pressure for genotypes adapted to mass-rearing conditions. In addition, a more natural environment, preferably under greenhouse conditions with hosts and natural light, could also help address the major problem of loss of irritability and predator-evasion behaviour in mass-reared insects, requiring significantly higher overflooding ratios as a result of the high mortality suffered by released insects due to predation (Hendrichs et al. 2007).

A major advantage of developing a mass-rearing system based on the FRS is that strain replacement becomes a much simpler procedure, and can be done without major disruption of the production process. The size of the population in the initial small mother colony will, of course, depend on the production level required in the facility, but it will probably not exceed several thousand individuals. Therefore, a new strain can be introduced into the FRS in a sequential manner during one generation, and there will be little interruption in production. This procedure has been used to introduce new Mediterranean fruit fly genetic sexing strains into production facilities (Caceres et al. 2004).

### *3.1.3. Larval Diets*

All insects that are mass-reared are maintained on an artificial diet. For most species, the larval and adult stages need to be fed. One exception is tsetse flies where larval development takes place in the female, and only adults need to feed. The larval diet of many species is an expensive component of mass-rearing costs, and presents logistical problems if the diet includes components that are poorly defined, requiring bioassays before undertaking large bulk purchases to verify their quality. From the perspective of quality control, defined diets would be preferable, but very few are available, and anyway these would be too costly for mass-rearing purposes. Nevertheless, one can foresee the commercial availability of pre-mixed diets (Thomas et al. 2018).

Disposal of large quantities of spent larval diet can also be a concern, although some facilities sell it as livestock feed, after sterilizing with a steam extruder or other method to kill any live insects remaining in the diet. Since the spent larval diet can still be rich in organic matter, recycling of some components has been considered (the larvae do not actually exploit part of the nutritional value of the diet), but the problem of metabolite accumulation in the diet has yet to be overcome, and thus far no effective practical procedure for any larval diet has been identified.

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A challenge for the future of larval diets is to include micro-organisms that naturally contribute nutrients (Kyritsis et al. 2017; Stathopoulou et al. 2021; Augustinos et al., this volume). Inoculation of diets with such probiotic symbionts would not only reduce the need for diet preservatives, but also for the protein supplements, normally the costliest ingredient that larvae often only partially use (Chan Jr. et al. 2000). Whether such probiotic bacteria can be mass-produced cost-effectively for the larval diet, compared with protein supplements, is being investigated (Stathopoulou et al. 2021).

Books by Schneider (2009) and Cohen (2015) review and highlight many critical issues related to insect diet development and rearing. More research on ways to artificially rear several important pest species otherwise amenable to SIT application is required. The lack of cost-effective mass-rearing procedures for these species has been the major obstacle to the implementation of area-wide control programmes.

### *3.1.4. Post-Factory Sterile Male Performance*

By applying nutritional, probiotic, hormonal or semiochemical supplements or other bioactive materials to post-teneral sterile males before their release, there is a great potential to improve their subsequent performance or impact in the field (Pereira et al. 2013). Until recently, research on the critical period between the arrival of sterile pupae at emergence facilities and the release of adults in the field has been neglected. Current and future research could result in significant breakthroughs, and it may be possible to recover some of the quality deterioration that has occurred due to colonization, mass-rearing, and irradiation (FAO/IAEA 2017a; Pereira et al., this volume). Thus far, operational programmes have only gradually been adopting the findings already made on this subject.

Juvenile hormones (JH) are known to regulate the development of reproductive capacity and sexual signalling in Diptera and other insect orders. Hormonal treatments in the form of JH mimics such as methoprene, applied to emerging sterile males, have been shown to advance significantly sexual maturation. For example, in *Anastrepha* spp. fruit flies, treated males mate 5–7 days earlier than untreated males (Teal et al. 2000). Also, in *Bactrocera* spp., early sexual maturation is promoted by methoprene- or raspberry ketone-treatment of immature sterile males (Akter et al. 2017; Akter and Taylor 2018; Adnan et al. 2019). This acceleration of reproductive development is crucial for improved SIT application in insects with a long imaginal pre-maturation period, where normally a majority of released sterile males are lost to predation and other causes before they reach sexual maturity in the field (Hendrichs et al. 1993).

Nutritional supplements are equally critical for sexual development and signalling in anautogenous species, in which the imaginal stage has to forage for protein sources to be able to reach sexual maturity. Therefore, adding protein to the diet fed to sterile males prior to release often significantly increases sexual performance (Shelly et al. 2002a; Yuval et al. 2002; Gavriel et al. 2011), although the form of delivery and the ratio of protein to sugar requires fine-tuning for each species. Often in such insects the adults are adapted to ingesting bacteria as protein sources, generally a scarce resource under natural conditions (Lauzon et al. 2004). In tephritid flies this adaptation is reflected in mouthparts that allow the ingestion of only liquids and suspended particles less than 0.5 μm in size, such as bacteria of the Enterobacteriaceae

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(Vijaysegaran et al. 1997; Coronado-Gonzales et al. 2008; Augustinos et al., this volume). Nevertheless, the importance of bacteria in the behavioural ecology of some target insects, for example their role in nitrogen fixation and by making other nutrients available through enzymatic degradation, is still not fully understood (Behar et al. 2005). Current mass-rearing practices may even be promoting non-beneficial or harmful bacteria. Thus, the inoculation of post-teneral diets with probiotic diets, i.e. a diet that contains beneficial gut micro-organisms, is an area with much potential to improve sterile male performance (Niyazi et al. 2004; Akami et al. 2019; Stathopoulou et al. 2021; Augustinos et al., this volume).

Combining hormone exposure and protein-enriched or probiotic adult diets results in synergistic interactions, thus increasing sterile male competitiveness many-fold when compared with either only protein or only hormone treatments (Teal et al. 2007; Haq and Hendrichs 2013; Haq et al. 2014). Such hormonal and nutritional therapies are affordable, easily incorporated into protocols followed at fly emergence and release facilities, and earlier release reduces costs associated with holding maturing sterile males (Pereira et al., this volume).

### *3.1.5. Sterile Males as Carriers of Bioactive Materials*

There is also great potential to use semiochemical supplements or treatments to boost post-factory sterile male performance. Some species that are attracted to natural attractants sequester these as pheromone precursors into their pheromonal systems, and subsequently release them during courtship. For example, sterile males of *Bactrocera* spp. exposed to such components before release can significantly improve their mating competitiveness (Haq et al. 2018), and, in addition, such components can act as very potent allomones to deter predators (Tan 2000). Furthermore, pre-release exposure to such attractants can significantly reduce sterile male response to male-annihilation baits in the field (Shelly 1994). On the other hand, non-exposed wild males would still be attracted and killed by male annihilation technique (MAT) baits, thus potentially allowing a "male replacement" strategy, consisting of the simultaneous application of male annihilation and sterile male release. Modelling such a concurrent synergistic SIT/MAT implementation confirmed that it is an extremely powerful control strategy, enabling the sterile fly release rate to be reduced to only ca. 5% of that when the SIT is applied alone (Barclay et al. 2014).

The mating competitiveness of wild or mass-reared Mediterranean fruit fly males is similarly enhanced considerably by exposure of pre-release sterile males to ginger root oil and citrus peel oils (Papadopoulos et al. 2001; Shelly et al. 2002b; Kouloussis et al. 2013, 2017). This has great potential in increasing the cost-effectiveness of deploying sterile males, and even exposure to vapour has this effect on emerging males, thus enabling the development of an "aromatherapy" that has facilitated application in fly emergence and release facilities (Shelly et al. 2004a, b; Pereira et al., this volume). Another advantage of aromatherapy is that wild females mated initially with wild males are more likely to remate with ginger root oil-treated sterile males, and furthermore, initial matings with ginger root oil-treated sterile males reduce the likelihood of wild female remating with wild males (Shelly et al. 2004a, b). This is important since, in fruit flies, normally the final male partner fertilizes the majority of the eggs in multiple-mated females.

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The identification of the stimulatory cuticular hydrocarbons in New World screwworm female flies, which elicit mating responses in screwworm males, opens the possibility of applying them to sterile females (Carlson et al. 2005). Commonly, after rearing a newly colonized screwworm strain for a few generations under mass-production conditions, screwworm females experience the loss of these cuticular pheromones, and only sterile males accept them as partners; wild males consistently reject them and mate only with wild females (Vreysen et al. 2006). Similar pheromonal compounds may exist in the ecological spheres of other pest species, and still await discovery for post-factory application to boost sterile insect performance.

Finally, there is a great potential to use sterile males or sterile females as carriers of various other bioactive materials (Knipling 1979). One possibility is autodissemination, in which sterile insects would be inoculated with electrostatically charged powder formulated with slow-acting insecticides or entomopathogens (Vickers et al. 2004), which would be spread throughout the pest population through intraspecific interactions (Howse et al. 2007; Bouyer and Lefrançois 2014). Large-scale field application against Mediterranean fruit flies showed that sterile males used as vectors or disseminators of *Beauveria bassiana* Vuillemin can result in effective area-wide suppression, with at least 57% of the wild fly population becoming infected in the treatment area, resulting in total population reduction of over 90%, compared with 0% infection in the non-treated area (Flores et al. 2013; Toledo et al. 2017). The additional cost when used in combination with the SIT appears low when compared with the benefit obtained from the complementary effect of using both techniques. Such a combined approach can obtain high levels of population suppression prior to the release of sterile insects, thus achieving favourable sterile-to-fertile ratios and sterility induction in the wild population (FAO/IAEA 2019).

Another possibility is autoconfusion, a type of mating disruption particularly suited for moth pests, in which released sterile males would carry on their bodies pheromone particles that attract wild males. This would transfer particles to them, and subsequently these males would contaminate other males, resulting in increased mating disruption (Knipling 1979; Howse et al. 2007). Since the few released males that would eventually encounter pheromone-calling females are sterile, autoconfusion and the SIT are clearly compatible. This approach may be more cost-effective for area-wide application than conventional mating disruption or even the establishment of a reduced number of dispensers in the field where wild males would get contaminated. A further possibility is simultaneous mating disruption of a moth pest and releases of another sterilized pest that is much cheaper to mass-rear (Suckling et al. 2011). The moth pheromone-inoculated sterile males, e.g. Mediterranean fruit flies, would significantly increase the mating disruption of the target moth pest population due to a higher overflooding ratio, and simultaneously suppress the local Mediterranean fruit fly population.

There are probably many other possibilities of applying bioactive materials to emerging sterile males, but so far these are mostly theoretical, and much research is required to explore the various possibilities and to assure the environment-friendliness of such approaches.

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*3.1.6. Hybrid Vigour*

Hybrid vigour relies on the crossing of two inbred strains to produce an $F_1$ generation that exhibits increased fitness. It is a general phenomenon, used widely in plant breeding to improve the fitness of plants in the field. To be applied to the SIT, it would require that two inbred strains be reared in a facility, and a simple way exists to select males and females from both strains for directed mating to produce $F_1$ insects for sterilization and release. At present, in most species, it is not possible to do this automatically. However, it can be tested in the Mediterranean fruit fly, where genetic sexing strains are being mass-produced. In facilities using these strains, two separate sexing strains, both based on pupal colour and temperature sensitivity, could be maintained in separate production modules. In the generation before release, male pupae (brown) from one strain could be placed with female pupae (white) from the second strain, and vice versa, in adult cages and $F_1$ eggs collected. These could then be heat-treated, and the surviving males reared, sterilized, and released. This procedure could even be carried out without the need for a pupal colour sorter since there is a difference in the developmental time of white and brown pupae in the genetic sexing strains (Franz et al., this volume). Some protocols based on this principle have been evaluated (Mangan 1992; Zapater et al. 1996; McInnis et al. 2002; Gilchrist and Meats 2014; Ekechukwu et al. 2015; Sánchez-Rosario et al. 2017).

*3.1.7. Radiation Dose*

Radiation is one of the many contributing factors to reduced competitiveness of sterile insects; the higher the radiation dose, the more the competitiveness of the insect can be compromised. In most programmes, the radiation dose used produces almost full sterility (as measured by percentage egg hatch from a mating involving either an irradiated male or female insect). Even though this parameter is convenient, it is probably not the only one to use when deciding on the appropriate dose. Measuring egg hatch in the laboratory does not take into account factors such as male competitiveness and sperm competitiveness that, in the end, will combine to determine the hatchability of eggs produced by females of the wild population. These last two parameters will be the major factors in inducing sterility in the wild insects, and they must be taken into account when deciding on the radiation dose to be applied. If the dose is reduced below that which gives full egg sterility, a more competitive insect is released, which in the end induces more sterility in the wild population (Toledo et al. 2004; Rull et al. 2007; Yamada et al. 2014; Marec et al., this volume). Thus, an optimum radiation dose based on induced sterility should be identified for each particular species and strain (Rull et al. 2012). However, better decision-making tools are needed to be able to identify the optimum radiation dose (Parker and Mehta 2007). Of course, the rate of increase of the target population must be considered; it determines the effectiveness of various combinations of male sterility and release ratios in reducing the target population (Klassen and Creech 1973).

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### *3.2. Improving Technology*

As well as improving the quality of sterile insects, progress will need to be made in all components of SIT implementation, from cage and facility design, to mass-production and other processes, and to programme planning and evaluation. Improved and more reliable air-handling systems in mass-rearing facilities can have a major impact on the efficiency and cost of rearing. Several facilities now have centralized systems where environmental conditions throughout the facility can be monitored and adjusted as necessary. This centralized system brings with it some disadvantages; other facilities have been built using a modular design (Tween 1987). Facility design, equipment needs, cost, and space allocation for the different production processes are key components. Interactive spreadsheets are now available to integrate the relevant data for various production volumes. This facilitates the planning, financing, construction, and equipping of rearing facilities that produce different numbers of sterile males (Cáceres et al. 2012), as well as managing operations and product quality based on the timely analysis of information (FAO/IAEA 2018b).

#### *3.2.1. Shelf-Life and Shipment*

In many areas of the world, pest problems are seasonal, and thus operational programmes need to release sterile insects only during specific times of the year. Nevertheless, insects can be produced year-round in a rearing facility; it makes economic sense to keep a facility productive for most of the year. In species that have a facultative diapause, this behavioural trait can be used to stockpile insects during the time when they are not required for release; this appears to have been achieved for the commercial production of the onion maggot. This trait can also be exploited when shipping sterile insects (Bloem et al. 1997). However, many tropical and subtropical pest species have no diapause, and these insects cannot be stored, although in some insects eggs can be stored indefinitely in a dry state, e.g. eggs of *Aedes* spp. Mosquitoes enter into dormancy after a drying process (Zheng et al. 2015). However, for temperate-climate species that have an obligate diapause, this prevents the development of mass-rearing technology and thus limits the application of the SIT. Research on developing strains of these species that do not enter diapause, or in which diapause termination can be induced, is therefore needed so as to overcome this barrier (Colinet et al. 2015; Chen et al. 2016; FAO/IAEA 2017b).

Sterile insects are often transported over long distances and across international borders (Dyck, Reyes Flores et al., this volume). This is usually done when the insect is in the late pupal stage, and some form of cooling and/or anoxia is used to prevent adult emergence during transit. This procedure can reduce insect quality, particularly if the transport period exceeds 48 hours. Pupae are usually shipped in cardboard containers containing cool packs to maintain a low temperature, and then the packing materials are discarded, sometimes creating a disposal problem. It would be desirable if durable equipment were designed that would maintain the correct temperature and atmospheric conditions, and increase the safety of insect shipments (Dowell et al., this volume). There are constraints in transporting biological material, both within and between some countries, and anything that can be done to overcome such constraints or improve the biosafety of shipments is valuable.

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There is now another way to ship insects. Fertile eggs are being shipped from egg production facilities to larval rearing facilities that only rear, sterilize, and release sterile adults. The egg reception facilities do not need to maintain mother and large adult colonies for egg production, and this greatly simplifies their operational protocols. It has been demonstrated that Mediterranean fruit fly eggs can be shipped over long distances for extended periods of time without losing their viability and affecting the quality of the insects that are subsequently mass-reared. This concept permits the egg production facility to concentrate its efforts on the maintenance of a mother colony and a large colony for production purposes (section 3.1.2.). In principle, one large central facility could provide eggs to several satellite insect rearing and sterilizing facilities. This concept has already been put into practice in the Moscamed Programme in Guatemala and Mexico, where ca. 100 million heat-treated eggs from a genetic sexing strain maintained at the mass-rearing facility in Guatemala are shipped daily to the male-rearing facility in Tapachula, Mexico (Tween 2004).

### *3.2.2. Rearing Systems*

The hardware used to produce sterile insects is continually being modified and improved. In countries with high labour costs and good maintenance practices, partial to full automation of mass-rearing systems will become common. Even in less developed countries, some degree of automation for key processes will be required to provide improved consistency in sterile insect quality (IAEA 2003). There are two key biological components, i.e. egg production and larval rearing, where new design components would have a major impact on mass-rearing efficiency. At present, the cages used for maintaining production colonies often allow females to produce only a portion of their full egg potential. This is caused mainly by males harassing the ovipositing females (cages have a high insect density) and a shortening of the female adult lifespan. New cage designs that address these issues will have an immediate positive impact on rearing efficiency and insect quality (Liedo et al. 2007).

Larvae are usually reared on an artificial diet held in a container. When the larval period is complete, larvae leave the diet or are separated from it and allowed to pupate. This process can be costly, wasteful, and in some cases unpredictable. In some species, the larvae pupate in the diet, and special procedures are required to obtain adult insects, i.e. by positive phototaxis (Dowell et al., this volume). The development of a defined liquid diet held in a type of fermenter, in which the larvae grow to maturation before being separated from the diet, would be a major change in rearing philosophy. Unless unexpected problems in rearing systems arise, the status quo tends to be maintained. Facility managers must ensure that innovations and new concepts are continually evaluated (Parker, Mamai et al., this volume).

### *3.2.3. Sterilization*

Insects are usually sterilized by ionizing radiation produced from $^{60}Co$ or $^{137}Cs$ sources. These irradiators are effective, but have some disadvantages. They require a considerable amount of regulatory support. The radioactive source declines with time, and thus radiation times get longer; eventually the source has to be recharged, a

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complicated and expensive procedure. It is becoming increasingly difficult to arrange for the international transport of this type of source to some parts of the world.

At present, exposure to ionizing radiation is the best way to sterilize insects. However, in view of the logistical difficulties associated with shipping and using radioactive sources, other options are being explored (Bakri et al., this volume). Electron beam technology to generate electrons, or photons such as X-rays, is an option. These systems are being encouraged because they do not involve any radioactive materials, only require access to electricity and cooling water, and can be switched on and off as needed (IAEA 2012). They require no complicated regulatory framework, and avoid the complex and costly shipment of radioactive sources across international borders. On the other hand, at present their availability is limited, they are relatively expensive to purchase and operate, and require substantial maintenance expertise. Nevertheless, increasingly, information on the development and use of alternative equipment to sterilize insects is becoming available (Mehta and Parker 2011; IAEA 2017).

Sterility can also be generated biologically through cytoplasmic incompatibility (CI), when different insect populations of the same species are mated that carry endosymbionts of the genus *Wolbachia* (Townson 2002; Robinson, this volume). *Wolbachia*-induced CI will be used increasingly as a tool for vector population suppression in a self-limiting way (IIT), in combination with radiation to avoid the release of any fertile females, as well as to avoid the transmission of animal, human or plant diseases (Bourtzis and Robinson 2006; Bourtzis et al. 2016; Lees et al., this volume), or as a self-sustaining drive system to deliberately replace a target population (Alphey 2014; Hoffmann et al. 2014). The development of molecular methods (Häcker et al., this volume) of sterilizing insects is discussed in section 4.3.

### *3.2.4. Release Technology*

The area-wide approach to pest management requires that sterile insects be distributed as adults over large areas; this is usually done by aircraft, guided by the Global Positioning System (GPS) and Geographic Information Systems (GIS) technologies that have significantly improved the precision of sterile insect distribution (Bouyer et al., this volume).

Most programmes release chilled adult sterile insects into the wild by means of small fixed-wing aircraft. While this is the fastest method of release, providing uniform distribution over target areas, for some situations it is not the most appropriate or cost-effective release platform. Depending on the target insect and the scale of the programme, there are alternative types of aerial vehicles that should be assessed further since they have the potential for significant cost savings (Tan and Tan 2013). In recent years, technological developments related to Remotely Piloted Aircraft Systems (RPAS) have created opportunities for their use in releasing sterile insects under specific conditions and regulatory frameworks (Benavente-Sánchez et al. 2021). Their disadvantages, such as reduced payload and flight duration, are counterbalanced by advantages such as accuracy, increased safety, and cost-efficiency for small- and medium-scale operations, with the largest cost saving represented by eliminating the need for a pilot and related wages and fuel consumption. However, mandatory special operational permits are required from regulatory agencies, in

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particular for operations beyond the visual line of sight (Benavente-Sánchez et al. 2021; Dowell et al., this volume). Cheaper alternatives to currently used aircraft will enable widening applicability of the SIT to smaller-scale programmes, for example mosquito suppression operations that are focused largely on urban and suburban areas.

The current practice is to release sterile insects via an auger or conveyor belt from a chilled container in the aircraft, removing the need to hold and release insects in bulky biodegradable containers, saving airplane space and thus greatly reducing flying time and hence costs. This procedure requires that adult insects, after emergence from pupae, be collected and placed in the chilled container. The logistics of this procedure for very large numbers of insects are daunting, given that insect quality must be maintained at as high a level as possible. The technology to hold insects at a low temperature for several hours in an aircraft, and to achieve the required distribution over a release area, has greatly improved (Leal Mubarqui et al. 2014). Nevertheless, there is still a major concern about the negative impact on insect quality from the stress of low temperature and high insect density in the release machine (Andress et al. 2012, 2013; Shelly et al. 2013). The low temperature, when combined with a high humidity, results in water condensation that makes the insects wet. In the dense holding columns such wet insects become stuck together, exiting the release machine in clumps instead of a continuous stream. Also, because the moving augers of the mechanical delivery system can damage the insects, conveyor belts will be increasingly preferred (Leal Mubarqui et al. 2014).

One indication of the future is new systems that are based on cryogenics (such as liquid $CO_2$ to make dry ice pellets) as the cooling component. Also, passive chilling systems like those based on the use of phase-change materials can maintain the required cool-temperature range in the insect-holding container at a low payload cost (Tween and Rendón 2007; Benavente-Sánchez et al. 2021). They simplify maintenance, eliminate the high electric load on the aircraft, and allow greater control over temperature and humidity, thus minimizing damage to the sterile insects. The cryogenic system is in use in Guatemala, can deliver up to 50 million sterile *C. capitata* males per flight, and enables the simultaneous release of several species of sterile insects and parasitoids (Tween and Rendón 2007). Computer software linked to a satellite-guided aerial navigation system is programmed to deliver an adjustable number of sterile insects (as needed in each release block), and to turn off the release machine when outside the target blocks. The performances of the pilot, aircraft, and machine are recorded, and can be analysed after each flight (Dowell et al., this volume).

### *3.2.5. Field Monitoring*

The widespread use of GPS/GIS technologies has dramatically increased the accuracy and ease with which insect populations can be monitored before, during, and after the implementation of a programme (Bouyer et al., this volume). This increase in precision is enabling much better use of programme resources, and rapid decisions to modify programme activities are now possible. The use of bar-coded traps, and the ability to enter field data directly into hand-held computers for rapid downloading at the field centre, will increasingly make a major contribution to the accuracy and accessibility of data, and enable managers to monitor the efficiency of trapping

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personnel. New technologies are also allowing the development of automated insect monitoring systems to provide real-time alerts of the presence and location of a pest, as conventional monitoring systems can result in delays in the information reaching decision-makers, potentially risking outbreaks and the loss of markets. Such technologies will increasingly be deployed in countries with high labour costs because manual checking of thousands of traps is labour intensive and also costly in terms of vehicles and logistics (Potamitis et al. 2017; Schellhorn and Jones 2021).

Field monitoring also requires effective methods to attract and trap insects of both sexes, and in many species there is active research on trapping methods. Data from traps are used to calculate the ratio of released to wild insects, and to directly monitor the relative size of the wild population (Vreysen, this volume). Traditionally, since females are responsible for population growth and hence damage levels, most emphasis has been placed on developing efficient female traps. However, when sterile insects are released, it is important to monitor males in the field. Nevertheless, when all-male releases of Mediterranean fruit flies are made, traps biased to preferentially collect females are useful to minimize the recapture of sterile males, and to facilitate discrimination between wild and recaptured sterile flies (Epsky et al. 2014).

During monitoring it is useful that released insects be easily and unequivocally differentiated from wild insects trapped in the field. (However, it is acknowledged that the New World screwworm eradication programme in Central America was implemented without marking the released sterile insects.) To accomplish this, usually larvae or pupae destined for release as sterile insects are marked with a dye (Dowell et al., this volume; Parker, Mamai et al., this volume; Vreysen, this volume). When trapped insects are brought in from the field, their origin is determined by examining them for the presence of the dye. This process is very labour intensive, expensive, and subject to significant errors of interpretation. Hagler and Jackson (2001) and Johnson et al. (2017) have described several new technologies that may be improvements on these dyes. Morphological markers have often been suggested as possible ways to monitor insects in the field, but usually they are associated with reduced competitiveness and therefore cannot be used effectively in the field. In the Mediterranean fruit fly, a phenotypic mutation has been isolated; it may be useful in the field since it does not appear to be associated with reduced competitiveness both during mass-rearing and in field-cage competition tests (Niyazi et al. 2005). Molecular methods offer a solution to marking for special situations, e.g. to identify the genetic origin of the sperm in spermatheca as an indicator of the degree of induced sterility in the target population (San Andrés et al. 2007; Juan-Blasco et al. 2013, 2014; Smidler et al. 2018; Häcker et al., this volume), but generally they are not practical for routine screening of large numbers of trapped insects. As indicated below (section 4.2.), markers involving the use of transgenic insects would involve regulatory problems.

### *3.2.6. Suppression*

Before releasing sterile insects, it is essential to suppress the field pest population so as to facilitate achieving favourable sterile to wild insect overflooding ratios. Since the SIT must be carried out on an area-wide basis, suppression techniques are included in this same requirement (Mangan and Bouyer, this volume). The area-wide approach will often require that pest populations have to be suppressed in inaccessible areas and

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also in areas where there is no human population. Although sterile insects can be successfully distributed by aircraft, the same is not always possible with a suppression "technology", e.g. in many countries, it is not permitted to conduct aerial spraying with traditional insecticides, especially over organic crops or sensitive areas. This puts severe restrictions on the type of suppression technology that is appropriate for area-wide application.

Another difficulty involves suppression activities in urban areas, where many vector breeding sites or host plants may be located. The human populations in these areas need to be persuaded that what can be a very intrusive intervention will eventually benefit the health or economy of their community, but better and more selective suppression methods are needed. There is considerable interest in developing, for use in such sensitive areas, bait stations that both attract and kill insect populations (Piñero et al. 2014). Mass-trapping in urban areas significantly reduced *Ae. aegypti* density and disease prevalence in mosquitoes (Barrera 2017). Also, autodissemination stations from which adult mosquitoes transfer juvenile hormone analogue or insect growth regulators between resting and oviposition sites have been shown to be effective (Devine et al. 2009). The method has since been validated against various *Aedes* species, but is quite expensive because high densities of deployed dissemination stations are necessary (given their low attractiveness) to achieve a good suppression level.

Additional suppression technologies that can be applied by aircraft are highly desirable, and this has led to the identification of a new insecticide-bait formulation that is quite selective for fruit fly suppression. Spinosad is an insecticide developed from the bacterium *Saccharopolyspora spinosa* Mertz and Yao, and it has been incorporated into a protein bait spray that has been organically certified (Peck and McQuate 2000; USDA/APHIS/PPQ 2000; Mangan and Bouyer, this volume; Nagel and Peveling, this volume). However, this insecticide is not immune to the resistance problem; strains resistant to spinosad were rapidly selected in the house fly *Musca domestica* L. (Shono and Scott 2003).

Mating disruption of lepidopteran pests, and male annihilation for *Bactrocera* spp. fruit flies, are other effective and more compatible suppression methods that can also be delivered from the air (Vargas et al. 2014; Cardé 2021; Staten and Walters 2021). Normally, the MAT is applied before sterile males are released; however, the simultaneous implementation of the SIT/MAT is highly synergistic when sterile males have been exposed to the parapheromone methyl eugenol before release (Barclay et al. 2014).

Effective tsetse fly suppression is now possible using the sequential aerosol technique (SAT), where extremely low amounts of pyrethroid insecticide are delivered by low-flying aircraft at certain times when climatic conditions are optimal (Allsopp and Phillemon-Motsu 2002). Effective suppression technology, and an "environment" in which it can be used, will always be an absolutely essential component of AW-IPM programmes integrating the SIT.

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## 4. TRANSGENESIS AND GENE EDITING

During the last 30 years, modern biotechnology has developed molecular technologies that allowed first to routinely introduce foreign genes (transgenesis) into the germ line of many pest species (Hoy 1992; Robinson and Franz 2000; Robinson et al. 2004), and more recently site-specific genome editing techniques such as the CRISPR/Cas system (clustered regularly interspaced short palindromic repeats) that permit the precise editing of genomes at pre-defined positions (Meccariello et al. 2017; Nielsen 2021). There are also RNA interference approaches under development that do not change genomic information, but instead influence gene expression (Baum and Roberts 2014).

All these modern molecular technologies have significantly increased, and will continue to increase, the potential for understanding and modifying the genetic information in support of SIT application (Häcker et al., this volume). They can be used for self-sustaining approaches that aim at population replacement strategies, e.g. using gene drives or *Wolbachia*, or for self-limiting approaches, such as the SIT, that require continuous releases but aim at leaving no ecological footprint. These molecular technologies may benefit operational programmes in the four areas outlined below (Handler 2002; Häcker et al., this volume).

There has been much investment and speculation in this area, but until now very few transgenic or gene-edited strains of pest insects have been produced and field tested in pilot projects. Their wider adoption has been slow because of the low public acceptance of such approaches (NAS 2002) and the regulatory requirements and approvals that are needed in most countries for their application (Reeves et al. 2012; EFSA 2013); however, paradoxically, the release of sterile transgenic insects is probably one of the lowest risk strategies for transgenic organisms (Hoy 1995, 2000; FAO/IAEA 2006).

### *4.1. Genetic Sexing*

As described by Franz et al. (this volume), the SIT can often be made much more effective if only males are mass-reared and released (Rendón et al. 2004; Lance and McInnis, this volume). However, in mosquitoes and other vectors, where the females transmit disease, developing effective genetic sexing systems is essential (Lebon et al. 2018; Ndo et al. 2018). Traditionally, genetic sexing strains have been based on classical Mendelian genetics, and use a combination of selectable markers and male-linked translocations to achieve sex-linkage. These strains have disadvantages — the mass-reared colony is only 50% fertile, females in the colony are homozygous for the marker, and they show reduced viability. Most importantly, the systems are not transferable to other species. Considering that the Mendelian sexing systems can take many years to develop for a single species, this is probably the major disadvantage.

Genetic sexing using molecular approaches, although they have to overcome similar problems such as strain stability and competitiveness, are expected to be more generic, and thus transferable between different pest species. They can either be targeted towards killing females or transforming putative female zygotes into males, and both systems require conditionality to ensure maintenance of colonies. As in

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conventional sexing strains, if females are targeted for killing, then lethality should be induced at an early stage, ideally in the egg, as very large numbers of zygotes can be treated together. This requires that early-acting sex-specific promoters are identified and placed under conditional control, as has been achieved for *Anastrepha suspensa* (Loew) (Schetelig and Handler 2012), and then transferred to *C. capitata* (Ogaugwu et al. 2013), *Lucilia cuprina* (Wiedemann) (Yan and Scott 2015), *Anastrepha ludens* (Loew) (Schetelig et al. 2016), and *C. hominivorax* (Concha et al. 2016). Also, much progress has been made recently in mosquitoes (Bourtzis and Tu 2018).

Transforming females into males has the advantage of doubling the male output, and in the future will increasingly be pursued. It requires a detailed knowledge of sex determination in the species under study (Meccariello et al. 2019). While the sex-determining factor appears to be highly conserved in Diptera, Lepidoptera, and Hymenoptera, its sequence is not, and there is considerable variation in the functional relationships (Shearman 2002; Verhulst et al. 2010). Nevertheless, sex reversion via a transient RNAi effect has already been performed successfully in *C. capitata* (Pane et al. 2002), *Bactrocera oleae* (Rossi) (Lagos et al. 2007), and *L. cuprina* (Concha and Scott 2009), resulting in fertile fully transformed XX males. The same sex transformation effects have been demonstrated in *C. capitata* following transgenesis (Saccone et al. 2005). Furthermore, a CRISPR/Cas-based X shredder, that shreds the X chromosome in sperm during spermatogenesis, or the targeted disruption of the expression of female fertility genes (resulting in sex ratio distortion), have been developed for the *Anopheles gambiae* complex (Galizi et al. 2016; Kyrou et al. 2018; Fasulo et al. 2021).

### *4.2. Marking*

As indicated above, sterile insects for release are usually marked with fluorescent powders or other dyes (Dowell et al., this volume; Parker, Mamai et al., this volume; Vreysen, this volume); however, they are not reliable, and daily exposure during mass-rearing may be harmful to workers. Molecular techniques enable marking with fluorescent proteins. Using such genetic markers for released insects requires that the marker be dominant, and that it can be monitored even in dead adults, as insects are usually dead when removed from traps. Transgenic strains carrying fluorescent markers have already been developed in a wide variety of insect pests (Häcker et al., this volume), and their stability in dead specimens after trapping has been confirmed by fluorescence microscopy and PCR (Meza et al. 2011; Simmons et al. 2011). However, the fitness cost to the insect for the production of this exogenous protein is unknown. Also, no data are available on the effect of this marker on the behaviour of the insect in the field, or indeed on the response of conspecifics or predators to fluorescent insects. Assessing this is especially relevant since insects use the UV spectrum for vision.

Strains with fluorescent sperm can also be used to monitor the mating success of the released males by screening the spermathecae of wild females (Juan-Blasco et al. 2013). Furthermore, sex-specific expression of fluorescent markers can allow automated sexing, as shown for immature stages or adults of *Ae. aegypti* (Smith et al.

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2007), *Anopheles stephensi* Liston (Catteruccia et al. 2005), or *An. gambiae* (Marois et al. 2012) using the Complex Object Parametric Analyser and Sorter (COPAS).

### *4.3. Sterilization*

The use of ionizing radiation has proven to be an extremely effective way to sterilize insects for release in the field (Bakri et al., this volume). To reduce somatic damage, the procedure is done at as late a developmental stage as possible. This physical process is a fail-safe procedure when the correct protocols are followed. It is also not subject to the development of resistance, can be used on any strain, and does not interfere with the mass-rearing process. As with all the procedures to which released insects have to be subjected, radiation does have some negative effect on the competitiveness of treated insects. However, the detrimental effects of radiation have sometimes been exaggerated. The overall competitiveness of a released insect is determined by a whole combination of different factors related to factory adaptation, selection, mass-rearing, transport, and handling and release procedures, etc. Itô et al. (this volume) strongly make the case that reductions in competitiveness in the field are much more likely to be due to colonization and long-term mass-rearing effects, as well as post-factory handling, than to radiation.

Reproductive sterilization can also be achieved through genetic approaches, eliminating the need for irradiation. Any system to induce sterility in the field through dominant lethality must be conditional in some way so that efficient mass-rearing can be carried out. This conditionality can be achieved by using transcriptional activation or suppression systems based on the presence or absence of antibiotics in the larval diet (Heinrich and Scott 2000; Thomas et al. 2000). The permissive condition in the facility will require the presence of an antibiotic or its analogue during the mass-rearing of the colony, although not the male production for release. Following release of the transgenic males, the female progeny of a wild female mated with such a male would die in the absence of the antibiotic.

Horn and Wimmer (2003) engineered reproductive sterility in a *Drosophila* strain by transferring embryonically lethal transgenes, so that both male and female progeny die as embryos. Subsequently, this sterilization system was adapted and transferred to tephritid pests such as *C. capitata, A. ludens*, and *A. suspensa* (Schetelig et al. 2009, 2016; Schetelig and Handler 2012). Alternatively, transgenic strains developed based on the RIDL system (Release of Insects carrying a Dominant Lethal) (Thomas et al. 2000), are late-acting conditional lethality systems that kill progeny in late larval or pupal stages. They have been designed for *C. capitata* and *Ae. aegypti* (Gong et al. 2005; Phuc et al. 2007; Harris et al. 2012). However, some of these strains will require further refinement because they are not completely sterile in matings with wild females, and thus their genome may become incorporated into the target population (Evans et al. 2019). The lethality in the late larval stage is another disadvantage, resulting in damage from crop pests. Nevertheless, for *Ae. aegypti,* open-field trials with transgenic mosquitoes, carried out in Grand Cayman (Harris et al. 2012) and Brazil (Carvalho et al. 2015), achieved over 80% suppression of local populations. Open-field trials are also planned for a transgenic sexing strain of the New World screwworm in Panama (Concha et al. 2016).

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A specific concern regarding these types of systems (that require the addition of a bioactive compound to very large volumes of larval diet) is the disposal of the diet, e.g. the Mediterranean fruit fly facility in Guatemala produces about 25 tons of diet per day. This is a real issue since mass-rearing facilities often sell spent larval diet as cattle feed. In addition, maintaining the appropriate concentration throughout the diet will not be easy. A third concern is the effect of the diet on the level of antibiotic. As the diet is a microcosm of bacterial and fungal growth, it will not be easy to standardize the exposure of the larvae to the antibiotic. It will also be important to choose the appropriate type of dominant lethality with which to kill the progeny in the field. Any genes involved in cell death mechanisms, or which show general cell toxicity, would probably not be suitable and/or would raise environmental concerns. Since effective sterilization of wild females remains the key to a successful programme that includes the release of sterile insects, there is no room for error in the sterilization procedure, and any proposed biological system must be extremely robust, controllable, and accurate.

There has been much argument about the relative merits of using either biological or physical methods to induce lethality in field populations (Alphey and Andreasen 2002). While it may appear that the molecular approach has some potential advantages, it is important to distinguish the characteristics of these two methods in producing sterility in a field population. Both approaches, to be effective, must act in a dominant manner and induce lethality in eggs produced by wild females following mating with released males. The key difference, and the one that will finally determine whether the molecular approach has any practical value, is that molecular sterility is based on a single dominant factor. Any variation in the expression of the sterility factor following interaction with the large and heterogeneous genome in the field population will very quickly lead to selection for non-sensitive females and loss of sterility induction. This is a major weakness of any molecular approach; there is no practical way to evaluate the possibility that this might happen without compromising the eventual use of the technology. One solution that has been proposed is to introduce several constructs into the same individual, but the fitness costs of such multiple insertions still has to be determined.

In contrast, sterility produced by radiation is based on the action of an almost infinite number of dominant lethal mutations, and every released male carries a different set of sterilizing factors (Robinson 2002); thus, it is very difficult for a field population to develop any sort of resistance (Whitten and Mahon, this volume). Radiation-induced sterility has built-in redundancy, and in spite of some disadvantages it remains the only proven and environment-friendly technique for the introduction of sterility into field populations of pest insects.

### *4.4. Paratransgenesis*

A fourth area in which transgenic technology may benefit future programmes that include the release of sterile insects is manipulating their symbiotic organisms (Miller 2010; Augustinos et al., this volume; Häcker et al., this volume). Such mutualists are often required by some insect vectors for the transmission of important animal, human or plant diseases. Paratransgenesis of symbionts offers major possibilities to develop

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vector strains that are refractory to transmission by introducing into the symbionts genes coding for proteins that prevent the vector from transmitting the pathogen. One example is *Rhodnius prolixus* Stål, an important insect vector of Chagas disease caused by *Trypanosoma cruzi*. *R. prolixus* is associated with the symbiont *Rhodococcus rhodnii,* which was modified to express proteins that are toxic to, or block the transmission of, *T. cruzi* (Beard et al. 2001). Another example is tsetse fly species, where the paratransgenesis has been developed to impede trypanosome transmission by blood-feeding sterile males (Abd-Alla et al. 2013; Demirbas-Uzel et al. 2018; De Vooght et al. 2018; Kariithi et al. 2018). Similar developments are in progress for several mosquito species using fungi, viruses, or bacteria (Wilke and Marrelli 2015).

An approach that is showing great potential is to harness, for paratransgenesis purposes, the *Wolbachia*-induced cytoplasmic incompatibility phenomenon that can be used to induce sterilization to suppress or modify natural populations (Zabalou et al. 2004; Bourtzis et al. 2016). Infection of *Ae. aegypti* by different *Wolbachia* strains can lead to several or different outcomes such as a shortened lifespan, resulting in: (1) a reduction in the female potential to transmit pathogens that must infect adult mosquitoes to complete their development cycle, (2) a limited susceptibility to infection with the dengue or chikungunya virus or the *Plasmodium* parasite, or (3) depending on the strain of *Wolbachia*, an induction of cytoplasmic incompatibility, apparently with no significant fitness cost and high horizontal transmission (Wilke and Marrelli 2015; Flores and O'Neill 2018). However, each specific vector situation endeavouring to use *Wolbachia* for paratransgenesis and cytoplasmic incompatibility will need to be assessed carefully (Hughes et al. 2014); also, there are cases where *Wolbachia* enhanced disease infection (Dodson et al. 2014).

Since 2011, the World Mosquito Program has been releasing successfully *Wolbachia*-infected *Ae. aegypti* males and females for population replacement in various pilot locations in Australia, and also more recently in Colombia and Indonesia (Flores and O'Neill 2018). Releases (for population suppression) of only male *Wolbachia*-infected *Ae. aegypti* are ongoing or planned in Brazil, China, Singapore, and the USA (Callaway 2016; Mains et al. 2016; Waltz 2017; Häcker et al., this volume; Lees et al., this volume).

### *4.5. Technical and Regulatory Constraints*

Any transgenic strain proposed for sterile insect release will have to meet stringent criteria regarding the expression of the specific trait that it carries and the overall quality of the strain itself. These are not inconsiderable technical constraints, as has been demonstrated for the introduction of sexing strains based on classical approaches (Franz et al., this volume). The impact of large-scale rearing under extremely stressful conditions, and the number of individuals reared (often exceeding millions/week), can uncover significant genetic and biological events that can never be induced and studied under typical laboratory conditions, and for which the consequences are at present unknown. It is safe to say that extensive evaluations of transgenic strains are required before they can be used operationally in the field.

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Major constraints in using transgenic strains for area-wide field release, even when sterilized, are the often considerable opposition from the public and special interest groups such as organic farmers (Reeves and Phillipson 2017), as well as the regulatory aspect (Lehane and Aksoy 2012). Whereas there are no real regulatory constraints for strains based on classical approaches (section 2.6.), for transgenic strains there are a number of regulatory approvals required (Reeves et al. 2012) (that represent major hurdles to be overcome in a majority of countries) before transgenic insects can be released. Furthermore, regulatory frameworks for transgenic insects are not yet harmonized nationally and internationally (NAPPO 2007; EFSA 2013), usually requiring lengthy case-by-case evaluations.

An elegant way to overcome such constraints has been proposed in Lepidoptera, where females are the heterogametic sex. Transformation to develop sexing strains using dominant conditional lethals, only expressed in females, would result in only colony females carrying the transgenic constructs, with the resulting sterile males being non-transgenic (Marec et al. 2005). Alternatively, besides classical genetic approaches, other rapidly evolving non-transgenic technologies can be pursued for self-limiting strategies, such as CRISPR/Cas and RNAi approaches (Beetham 2018), although in Europe strains resulting from genome editing are being subject to the same stringent regulations as transgenic organisms. Fortunately, in many other countries, including Japan and the USA, such editing that results in small insertions or deletions will not be treated as transgenic organisms (Nature 2018), permitting, for example, the removal of genes required for sex determination to create non-transgenic sexing strains (Meccariello et al. 2019). RNAi methods also offer a way to work without transgenic organisms, as long as the dsRNA is not conditionally or constitutively expressed in the insect, and if it does not persist in the environment (Häcker et al., this volume).

On the other hand, there is the great potential in the future of using CRISPR/Cas gene drives for self-replicating purposes, to spread rapidly beneficial traits through interbreeding pest and vector populations, thereby eliminating the need for continuous mass-rearing and release. However, even though such approaches hold great promise for high impact and low cost, they have raised a number of societal concerns and regulatory demands that will necessitate careful consideration (James et al. 2018; Nielsen 2021). There are also technical issues that will first need to be overcome, such as the emergence of resistance and other evolutionary processes, before the application of such a promising approach can become reality (Champer et al. 2017).

## 5. CONCLUSIONS

Global trends towards a cleaner agriculture and less aggressive pest control will increase the demand for more target-specific, biologically based, and sustainable suppression methods such as the release of sterile insects. Globalization is resulting in more incursions and outbreaks of invasive pests, requiring effective and environment-friendly eradication tools such as the SIT. Furthermore, international trade is creating an environment in which area-wide approaches to the management of insect pests have a comparative advantage. Increasingly, overcoming technical and scientific constraints, and introducing new technological innovations such as refractory vectors,

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diapause termination, sterile-insect stockpiling, and post-factory performance-boosting treatments for sterile males will enable the SIT to be used against new target pests, and improve cost-effectiveness against current targets.

Nevertheless, these innovations and improvements are no guarantee for the successful implementation of the SIT as part of AW-IPM programmes. These programmes are management-intensive compared with most other control operations. Close coordination among many simultaneously occurring activities is required; their execution requires precision in time and space. Since the area-wide approach also depends on the cooperation and participation of all stakeholders in the target area, excellent management of communications with all stakeholders is indispensable, paying close attention to political, socio-economic, and environmental sensitivities. Therefore, effective programme implementation depends on the establishment of an efficient management structure that is semi-independent of normal government bureaucracy and corruption; mosquito abatement districts are good examples of effective and largely autonomous self-governing programmes (Foley IV et al. 2021). A common denominator of failed SIT projects has been the lack of such dynamic management structures and dedicated leadership to respond flexibly to changing situations. Equally important is transparency, external technical advice, and an effective oversight structure to maximize limited resources and to attract stakeholder funding (Dyck, Reyes Flores et al., this volume).

A major unexploited opportunity is the integration of sterile insects and augmentative biological control. As long as key pests are treated with insecticides, natural and augmentative biocontrol is disrupted, and hence there is only a limited potential for growth in the application of pollinators or natural enemies to deal with secondary pests. On the other hand, when a key pest is managed using biologically based tools (e.g. host resistance, mating disruption, sterile insects, etc.), natural and mass-reared biological control agents can complement these methods, and also play an important role in controlling less important pests. Thus, the existing augmentative biocontrol industry (ANBP 2018; IBMA 2018) and the SIT appear to be natural allies, and the expected gradual progress in commercializing the SIT will probably advance within this context. The private-sector biocontrol industry already has the technical "know how" to manage the mass-rearing, quality control, handling, and shipping of beneficials, and therefore could make significant contributions that improve and advance the SIT technology. Adding sterile insects to their products can provide a complete "biological package". A pioneer in this approach is BioBee in Israel, which is expanding from greenhouse to field pest management (Bassi et al. 2007; Steinberg and Cayol 2009).

Also, there is much unexploited potential in applying sterility to natural enemy production and facilitation. This involves using sterile insects not to transfer sterile sperm but as sterile hosts/prey/vectors for parasitoids/predators/pathogens to facilitate the use, and enhance the efficacy, of biological control agents (Hendrichs et al. 2009). At present, considerable research is being conducted to exploit the many potential possibilities and applications to facilitate natural enemy production, handling, and shipment, and also to provide sterile hosts for the establishment and maintenance over critical periods, or early season build-up (or in border or trap crops), of natural enemy populations in the field.

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# Sterile Insect Technique

The sterile insect technique (SIT) is an environment-friendly method of pest control that integrates well into area-wide integrated pest management (AW-IPM) programmes. This book takes a generic, thematic, comprehensive, and global approach in describing the principles and practice of the SIT. The strengths and weaknesses, and successes and failures, of the SIT are evaluated openly and fairly from a scientific perspective. The SIT is applicable to some major pests of plant-, animal-, and human-health importance, and criteria are provided to guide in the selection of pests appropriate for the SIT.

In the second edition, all aspects of the SIT have been updated and the content considerably expanded. A great variety of subjects is covered, from the history of the SIT to improved prospects for its future application. The major chapters discuss the principles and technical components of applying sterile insects. The four main strategic options in using the SIT suppression, containment, prevention, and eradication with examples of each option are described in detail. Other chapters deal with supportive technologies, economic, environmental, and management considerations, and the socio-economic impact of AW-IPM programmes that integrate the SIT. In addition, this second edition includes six new chapters covering the latest developments in the technology: managing pathogens in insect mass-rearing, using symbionts and modern molecular technologies in support of the SIT, applying post-factory nutritional, hormonal, and semiochemical treatments, applying the SIT to eradicate outbreaks of invasive pests, and using the SIT against mosquito vectors of disease.

This book will be useful reading for students in animal-, human-, and plant-health courses. The in-depth reviews of all aspects of the SIT and its integration into AW-IPM programmes, complete with extensive lists of scientific references, will be of great value to researchers, teachers, animal-, human-, and plant-health practitioners, and policy makers.



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