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Tactical Sciences for Biosecurity in Animal and Plant Systems

Kitty F. Cardwell and Keith L. Bailey

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The goal of biosecurity is to minimize the risk of introduction and transmission of infectious diseases to people, animals, and plants. This is achieved by accurately identifying pathogens and instituting appropriate methods to prevent their introduction, reemergence, and/or spread. However, disease is dynamic, and biosecurity needs to continually change to keep pace as pathogens evolve. As described in this chapter, a basic understanding of evolution is central in considering how genetic changes and their associated phenotypes can alter the disease presentation of pathogens. In addition, evolution leaves a trail of genetic information that can be leveraged to inform biosecurity because the spatiotemporal patterns of these past changes provide clues as to how the pathogen might be spreading. This chapter aims to provide insights into how various genetic alterations occur, the background on how these are informative for biosecurity, and illustrations of applications to real-world examples. Evolution underlies the abilities of pathogens to adapt, emerge, and to cause epidemics.

Humans have always played an important role in dispersing plants, animals, and other organisms—either intentionally or inadvertently. Over the last several decades, rapid developments in infrastructure and transportation have led to dramatic increases in trade, travel, and mass migration; this in turn has accelerated the human-mediated spread of organisms across the globe. In their new environments, introduced species may thrive and cause severe economic and ecological impacts. Mitigating the entry, establishment, and spread of exotic pests and pathogens is crucial for protecting agriculture, ecosystems, and people. To do this, it is important to understand the pathways by which invasive species spread, assess the associated risks, and develop effective mitigation measures. This chapter describes the role of risk analysis for understanding human-mediated pathways of pest introduction and spread and provides case studies from both the plant and animal health arenas.

Biosecurity, in the context of agriculture and natural ecosystems, refers to a strategic framework of policies and procedures intended to prevent the introduction or release of biological agents and materials that have the potential to threaten or compromise the agricultural sector in the form of invasive species, exotic pathogens, and foreign pests. Exchange of plants, animals, and agricultural products along with wood packaging material and dunnage that are transported through commerce and trade can lead to accidental introductions of foreign pathogens and pests unless sound biosecurity protocols are implemented to ensure the quality and safety of imported commodities at the local, transboundary, and global levels. Principal stakeholders at risk are those with interests in food, feed, fiber, oil, ornamental, and industrial crops; commercial forestry, natural ecosystems, and parks; and the livestock, poultry, aquaculture, fisheries, and apiculture industries.

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Surveillance is one of the core activities of national organizations responsible for human, animal, or plant health, with the goal of demonstrating the absence of infection or infestation, determining the presence or distribution of infection or infestation, and/or detecting as early as possible exotic or emerging pests and pathogens that may be harmful to agriculture and the environment. Surveillance is a tool to establish absence of the pest or pathogen, monitor trends, facilitate the mitigation and control of infection or infestation, provide data for use in risk analysis, substantiate the rationale for sanitary measures, and provide assurances to trading partners, producers, and the public. The type of surveillance applied depends on the objectives of the surveillance, the available data sources, resources, and the outputs needed to support decision-making.

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Surveillance is the collection, analysis, and dissemination of information to support prevention and mitigation of pest and pathogen impacts across natural and managed health systems. Surveillance provides an informational foundation for the risks posed by the organism, current status of the outbreak, directing limited resources, and effectiveness of management actions within the context of a response. Each response may have a series of management goals to accomplish over time and the information needs to support each goal will vary. Surveillance must be appropriately designed to align with the response goal and be well supported by risk assessment information on the biology of the invasive pest/pathogen, biology of the host or host system, pathways of introduction and spread, types and magnitude of impact, etc. This chapter proposes a generalized framework as a starting place for designing surveillance schemes using core design factors and how to effectively narrow parameterization of these factors within the context of a response goal.

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Diagnosis of disease is a process of hypothesis, investigation, and synthesis. Regardless of whether a human, animal, or plant is afflicted, the process of diagnosis is strikingly similar. Positioned on the biosecurity continuum between surveillance and response, early and accurate diagnosis is critical to effective mitigation and management of disease. Infectious diseases have the potential to spread among animal or plant populations, jump species barriers, and result in epidemics and global pandemics. Additionally, zoonotic infectious agents can also significantly impact human health on a mass scale. It is critical that infectious diseases be identified and detected in a timely fashion to prevent spread. This chapter will delve into the resources and supporting activities for that process, demonstrated via case studies from animal and plant systems, illuminating similarities and differences in the diagnostic process tools that can be mobilized and enhanced for biosecurity.

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This chapter focuses on emergency response following an initial detection of an invasive plant pest or foreign animal disease (FAD) as well as the regulatory authority utilized to initiate a response. Many emergency responses will be associated with crops and livestock on farms and ranches while others may involve nurseries, forests, wildlife, and exotic animals in various urban and rural locations. The incident command system framework is typically utilized to organize response efforts. Standard response preparedness and mobilization will be discussed with a consideration of the multitude of internal and external influences that can impact the strategy and tactics used during an outbreak. Sources of emergency funding and the critical need to manage public perception and information are also explained.

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The policy and resource infrastructure required to manage agricultural and environmental pest and pathogen incursions evolve and strengthen over time. Animal and plant health responses involve the highly coordinated efforts of various entities. Governments partner with state and territory officials, subject matter experts, and representatives of the commodity(ies) that are/may be impacted by the invasion. Short-, intermediate-, and long-term animal and plant health incident management tactics may change over time depending upon multiple conditions and externalities that will be described in this chapter. Results to response may range from fully successful eradication to learning to live with the pest and deregulation. Although the scope, timeframe, and consequences of events can vary, actions taken in response to the identification of an exotic plant or animal pest, disease, or condition are designed to minimize economic and environmental impacts, ensure trade and food security, assure business continuity, and avoid social unrest.

The secure and continuous production of agricultural commodities and food security are key to U.S. national security. The introduction of foreign-origin or emerging animal, plant, and human diseases by intentional acts of espionage, terrorism, biological warfare, or criminal activity can lead to severe consequences for domestic and international agricultural markets, the economic security of the agricultural community, food safety and food security, and the credibility of responsible state and federal agencies. Early public, animal, plant health, law enforcement, and intelligence assessments and investigations of suspected or confirmed intentional threats are critical additions to existing interagency prevention, response, and management protocols. Forensic microbiology, a multidisciplinary science, is essential to the nation's readiness for responding to a potentially criminal, intentional, or otherwise nefarious incident in the agricultural sector (plant or animal), and of eventual supporting attribution and the prosecution of the perpetrators.

Advances in technologies, increased globalization, impacts of changes in climate and land use on food production practices, and the expanding world population will continue to exert significant pressures on global biosecurity systems. The world must be prepared to face novel biosecurity threats, whether a consequence of natural pest and pathogen emergence or an intentional or unintentional release into a community. It is imperative that public and private sectors develop comprehensive and innovative strategies to mitigate these ever-evolving threats rapidly and effectively. This chapter reviews several opportunities that currently exist in global biosecurity of animal and plant systems with the hope that it will provide researchers, health experts, educators, and first responders with the awareness and impetus to adopt biosecurity tactics that enhance preparedness, reduce risk, and prevent catastrophic outcomes.

Foreword

The term and concept of “biosecurity” came into vogue in the 1990s, even though safeguarding systems to prevent the entry of harmful or invasive pests, diseases, and organisms into a country or territory were in place long before then. Biosecurity and safeguarding systems generally refer to official regulatory activities, including border inspections and controls, surveillance and detection activities, and outbreak response plans and programs.

New international trade agreements, a shift toward market-oriented economic policies, the advent of offshore production and supply chains, and the rapid expansion in travel and commerce in the 1990s helped bring biosecurity to the forefront. As the movement of people and goods accelerated, so did concerns about the spread of pests and diseases through new and expanding pathways. Around the same time, the term “globalization” emerged in the popular lexicon, reflecting these rapid changes and the opportunities and anxieties associated with them.

Trade will continue to be an essential element in countries’ economic development and growth. Policy makers in most countries also view commercial and cultural openness as necessary to enable the exchange and flow of competitively priced consumer goods and improve their access to foreign services, tourism dollars, foreign sources of investment, the latest science and technology, and foreign development assistance. However, governments also clearly recognize and agree that they must supervise and regulate these new active commercial pathways and global interactions to filter out and prevent the inflow of pests, diseases, and other potential health threats.

Several key treaties today reflect international consensus on the enduring right and responsibilities of governments to safeguard their nation’s agricultural, livestock, and natural resource base. These include the World Trade Organization (WTO) Agreement on Sanitary and Phytosanitary Measures (SPS Agreement), International Plant Protection Organization (IPPC), World Organization for Animal Health (OIE,) and Convention on Biological Diversity (CBD). These international agreements share a core tenant that countries are fully entitled and, in fact, obligated to protect the health of their people, food production, and their resource base from potentially foreign invasive organisms.

However, these agreements further require that health related measures taken to protect a country’s resources must be scientifically justified and only used to the extent necessary to manage a given risk. These trade and biosecurity related treaties give science a leading role in the assessment of biological risks and design and implementation of biosecurity measures, programs, and policies, especially in the context of international trade. In addition to contributing technical tools and approaches for ensuring a safely regulated trading system, biosecurity-related sciences help ensure that government regulatory programs are targeted on legitimate risk concerns and that society’s limited resources are used in an efficacious, prioritized, and cost-effective fashion to manage those verified threats.

Today, the concern for maintaining effective biosecurity and safeguarding systems is growing. Millions of sea containers, which can serve as a pathway for hitchhiking pests and contaminants, speed around the world. Climate change is impacting pest biology and the ability of pathogens to spread into, survive and cause havoc in new habitats. E-commerce has grown rapidly, creating new opportunities for importing harmful materials that evade normal commercial and inspection channels.

Biosecurity officials, industry, civil society, and international organizations will continue to rely on science for innovation and new technologies and ideas—e.g., surveillance systems, diagnostic tools, alternative pest treatment options, risk modeling and forecasting techniques, and pest response and control strategies. "Tactical Sciences for Biosecurity of Animal and Plant Systems" is as an important contribution by scientists and animal and plant health professionals to advance our thinking about the changes and challenges we face in keeping our nations healthy and safe and offer insights and ideas to help inform biosecurity policy decisions and risk management strategies moving forward.

Note: The views expressed in this prefatory essay do not necessarily represent the views of USDA.

John K. Greifer
International Phytosanitary Standards (IPS), United States Department of Agriculture, Animal Plant Health Inspection Service (USDA, APHIS), Plant Protection and Quarantine, USA

Preface

In today's interconnected world, a disease threat anywhere is a disease threat everywhere. U.S. Centers for Disease Control and Prevention (CDC)

The ease and frequency of international travel and commerce help drive the world's economy. This phenomenon is often referred to as globalization, which encompasses notions of world-wide commerce, market expansions, global distribution systems, integrated currencies, integrated communication, and extensive travel and transportation systems. Upsides of globalization have been in the reduction of poverty and increased cultural awareness. One downside, however, is that these activities also have potential to endanger the health and well-being of global communities through the accidental introduction of biohazards, i.e. diseases, pests, and invasive species (Work, et al., 2005). In order to successfully defend against the unrelenting threat of exotic pests and diseases, all countries have a shared responsibility to adopt strategies that rapidly detect and mitigate global disease threats, while recognizing that national and global health are inseparable. The underpinning of a national or international health strategy is an effective biosecurity system.

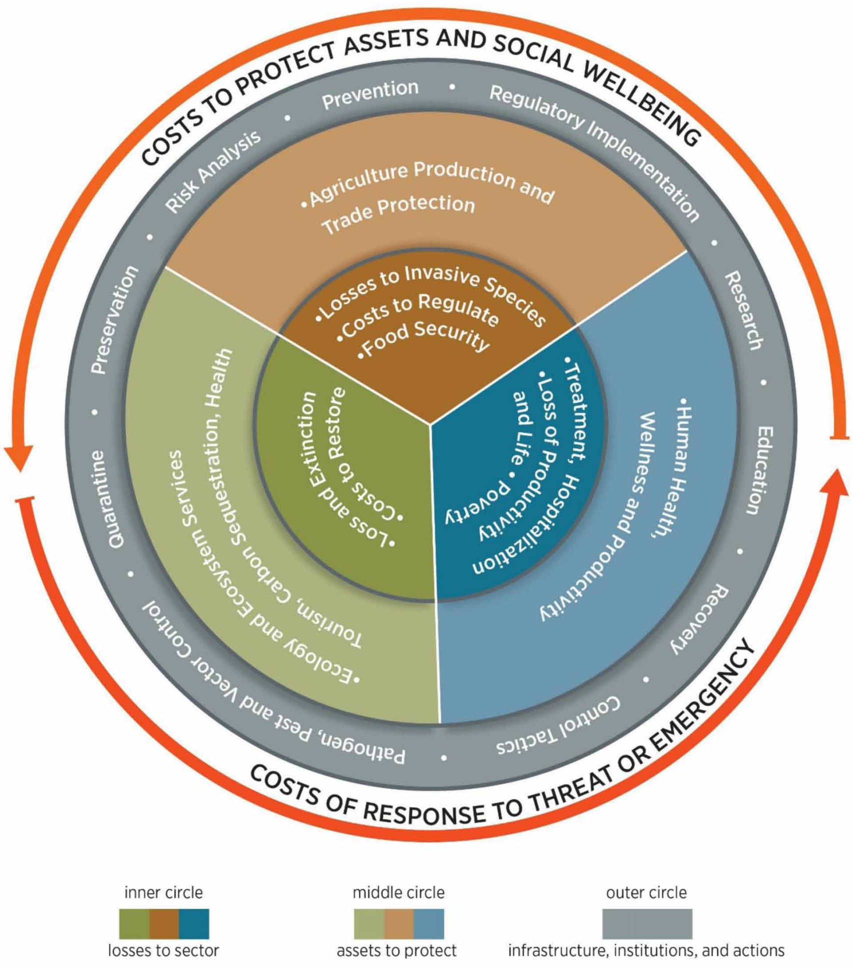
An effective biosecurity system should be regarded as an essential national asset as it safeguards the health and preservation of people, animal and plant production systems, and environments. As such, it is imperative that governments, agencies and non-governmental entities have a clear understanding of the interrelationships between science, research, technology, public health measures, pest and disease threats, production systems and their vulnerabilities, diagnostic capabilities, ecosystems, and global trade and travel. Understanding this vast body of knowledge serves as the basis of tactical sciences that are deployed to mitigate risks. Tactical sciences are the principles and activities that are directly applied to support strategic actions and policy decisions. Tactical sciences in biosecurity span many technical applications and disciplines in clinical and diagnostic laboratories, mathematical modeling for epidemiology and risk analysis, engineering for monitoring and data systems, chemistry for surveillance, therapeutic treatments and laboratory reagents; and social sciences for communications, coordination, and integrated actions.

This book is designed to illustrate the scientific principles behind the tactics that are applied to achieve biosecurity and highlight the interoperability of these tactics in animal and plant systems. The intent is to focus on *what* is done, *not who* does it. That is because at every scale and around the world, individuals do not stop pest and pathogen introductions, systems of tactics do. Conversely, institutions do not stop pest and pathogen introductions, but groups of people wielding knowledge and science-driven tactics do. Nevertheless, in this introduction we will provide a brief overview of the kinds of institutional infrastructure that exist at different scales.

WHY ANIMAL AND PLANT BIOSECURITY MATTERS

Not all introductions of non-indigenous plants and animals are bad, as many are intentionally introduced for food security and nutritional benefits such as crop and livestock imports, and social and environmental benefits by importation of exotic plants for gardens and pets. Indeed, global agricultural production has benefited from transfer of plants and animal species from the original habitats to new locations around the world. For example, the center of origin of corn (maize) was Central America, but it is now produced globally. Potatoes originated in the Andes of South America, but they have been intentionally spread around the world. Swine were domesticated in Asia and moved to Europe around 7,500 years ago. During most of this movement, the microflora and fauna that lived in close association with these exotic plants and animals were moved with them, including pathogens. Some of the affiliated biota were neutral to the new environment, however, many times, non-indigenous species have caused serious damage and economic losses to agriculture, forests, native environments, and society in general.

Figure 1. Costs to prevent and/or react to invasive species (Adapted from Bradshaw, et al., 2016)



In the U.S. alone, the annual cost is around $120 billion from the cumulative burden of introduction of approximately 50,000 species of exotic plant and animal pathogens, arthropods, weeds, mollusks, and snakes, etc. (Pimentel, et al., 2005). It has been estimated that invasive alien species cost Chinese food production systems an equivalent of 1.4% of the annual GDP (Xu, 2006). Globally, exotic arthropod pest and disease outbreaks cause losses in total agricultural output, costs of infrastructure to try to prevent, manage and control invasive species, and losses to societies from disrupted landscape, environmental degradation and declining natural habitats. Pimentel, et al. (2005) estimates that 42% of all endangered species are endangered because of the presence of an invasive species. Work et al. (2005) concluded that U.S. inspectors charged with intercepting invasive organisms coming in through trade can only examine up to 2% of cargo arriving at maritime ports, airports, and border crossings. Globally, inspection services face increasing volumes of trade and import quantities, with finite resources, (Heikkila, 2008); therefore, how to sustainably and effectively protect borders presents economic and technical questions (Figure 1).

These factors drive biosecurity actions from global to farm scale to reduce the social, environmental, and agricultural impacts of invasive species. Biosecurity is a public and private enterprise established with strategic policies, regulatory frameworks and tactical, science-driven actions at multiple scales that help ensure the health and well-being of human, animal, and plant populations. Important indicators of an effective biosecurity system include economic sustainability and food security.

An effective biosecurity system prevents the unwanted entrance or escape of pathogens or pests from a specified area with a specific boundary. To most people, the term ‘border’ indicates a geopolitical boundary. However, it is important to recognize that the border construct may be applied at different scales such as global or outlining continents, countries, regions, states, provinces, counties, cities, towns, farms, barns, or individual animal pens. Within each border, environmental features may favor the persistence or establishment of pathogens or pests, as well as influence a corresponding disease outbreak. Regardless of the scale, the chapters in this book address specific pre-border activities and post-border tactics and the science that informs the tactics used in biosecurity.

BASIC TACTICS: BIOSAFETY, BIOCONTAINMENT, AND BIOSECURITY

Biosafety is the prevention of loss of containment or escape of a potentially harmful organism, a biohazard. Laboratory clinicians and first responders in a field can be at risk of exposure to a potentially hazardous biological agent that could cause human illness. The environment and agriculture are potentially vulnerable to the introduction of a harmful pest or pathogen, for example when the organism is exotic and moving into a population without innate immunity. Tactics in biosafety are those activities that help prevent human, agricultural, and environmental exposure to an organism through protection of the person or containment of the organism to avoid release to the environment.

Protection of humans from biohazards and containment procedures are categorized according to risk. The lowest level of biosafety precaution to protect humans may be as simple as wearing a mask and frequent hand washing. As risk increases, more protective measures are required, including personnel protective equipment (PPE) ranging from gloves and face shields to positive pressure biosafety suits (Figure 2). When working with a potentially hazardous organism, risks might include inhalation, accidental ingestions, percutaneous punctures when using needles or scalpels, or bites and scratches if handling animals. Therefore, management of biosafety risk is generally based on tested procedures and

Figure 2. A CDC scientist dons pressurized PPE before entering a high-risk biocontainment lab
Source: Wikipedia

guidelines provided by global, national, and institutional biosafety organizations (e.g. World Health, Centers for Disease Control (CDC), Institutional Biosafety Committees, etc.).

Biocontainment is an aspect of Biosafety in which the physical containment of pathogenic agents (generally bacteria, viruses and fungi) or dangerous agents such as toxins, by isolation in environmentally and biologically secure cabinets or rooms, to prevent accidental infection of workers or release into the surrounding community and environment. In facilities that study or treat agricultural pathogens, biocontainment is the sum of practices to prevent unintended infection of plants or animals by release of a high-consequence pathogen.

Biosecurity in the context of Biosafety is a combination of physical features that eliminate access to high-consequence pathogens except for trained and known individuals. Physical features such as locks, entrance codes, alarms and cameras are often combined to assure that only very specific personnel can enter the space. Additionally, in very high biosecurity risk, individuals who work in that space must undergo a security clearance. In the U.S., research on and handling of high-consequence biohazardous agents can come with a legal liability to the individual and the institution should a biosecurity lapse and/or inadvertent release happen (U.S. Code of Federal Regulations, Title 7 CFR Part 331 and Title 9 CFR Part 121).

Biosecurity in the context of protection of plants and animals also has to do with hazard or risk assessments, barriers and borders, containment, individual and environmental systems protections, and preparedness to manage a situation should a biosecurity breach or invasion occurs. Biosecurity is a system of practices designed to reduce the risk of accidentally introducing a disease or pest into an operation or an area. Biosecurity occurs at different scales, ranging from individual laboratories, small- to large-scale animal and plant production operations, food processing centers, state and national parks, regional and national safe-guards and, finally, to global infrastructure and practices. At all levels, tactics are implemented to maintain profitability, protect the environment, assure safe and beneficial trade, and promote food security, societal well-being, and human health.

Motivations for Biosecurity

Safeguarding Production Systems

In many countries around the world, agriculture represents a significant share of their overall economy. Globally, an estimated 884 million people (27% of the overall workforce) were employed in the agriculture sector in 2019 (FAO World Food and Agriculture Statistical Yearbook, 2020). The percentage of people relying on agriculture for employment is highest in less developed regions of the world. For example, an estimated 70% of jobs in Africa is related to agriculture (AGRA, 2017). In 2019, agriculture and related industries contributed slightly more than US$1.1 trillion to the U.S. gross domestic product, a 5.2% share. The same year, agriculture and food sectors accounted for 22.2 million jobs in the U.S., with 2.6 million of the jobs being performed on the farm (USDA ERS, 2021).

Economies whose backbone is based on agricultural systems, particularly in less developed regions of the world, may lack resources for an effective biosecurity system to mitigate the risks of pest or pathogen invasions. However, affluent countries have the resources to effectively mitigate disease threats, including those with potential to create economic hardships, food insecurity, and civil unrest.

In 2014-2015, the U.S. experienced an outbreak of highly pathogenic avian influenza (HPAI) in domestic poultry and wild birds. HPAI is a contagious viral respiratory disease of birds; however, some variants also have potential to cause fatal human disease. In total, there were 211 detections on commercial poultry facilities and 21 detections on backyard premises, resulting in the death and/or depopulation of approximately 43 million chickens and 7.4 million turkeys (USDA VS, 2016). This outbreak represented the single largest and most expensive HPAI outbreak in U.S. history, with approximately US$850 million spent on response activities and indemnity payments. An additional US$100 million was spent on further preparedness activities, including biosecurity enhancement and education. The HPAI outbreak was successfully ended in late 2015 due to an aggressive, coordinated, and effective campaign.

Safe and Beneficial Trade

Free trade enhances societal well-being in multiple ways. Greater varieties of goods and services are now available than any time in human history. Lowered prices and enhanced availability of products are key advantages to consumers. Distribution of products around the world has lifted all global economies and opportunities.

Despite global biosecurity infrastructure and investment, serious and damaging pest and pathogen outbreaks are regular global occurrences. Commerce and travel have provided pathways for movement that overwhelm the biosecurity systems of countries around the world, reducing food crop and animal yields, contributing to food insecurity, damaging the environment, and harming economies.

Ensuring Global Health

From a global health perspective, effective biosecurity systems must continually evolve to combat new, emerging, and evolving biological threats such as insect pests and disease vectors, plant and animal pathogens, and invasive plants and animals. Managing biosecurity risks is inherently complex and costly. Fortunately, many of the tactics employed in combating pests and diseases of animal and plant systems are based on the same scientific principles, and this book highlights potential synergies. From a practical point, the implementation of effective biosecurity systems will allow countries to offset serious societal challenges such as food insecurity, health care inequities, and climate-related crises.

The SARS-COV2 pandemic of 2019 has reinforced the importance of science-based approaches to biosecurity and the reliance on input from trusted health and disease experts. It has also highlighted the critical need for formalized training in global biosecurity to ensure a continued pool of highly qualified first responders. To this end, the number of courses offered on college and university campuses pertaining to global biosecurity is expected to expand greatly to meet the increasing need for workforce development. Biosecurity courses would provide important training for Public Health majors, as well as undergraduates pursuing healthcare occupations. Institutions of higher education currently offering specialized training programs in Biosecurity include Kansas State University, Oklahoma State University, Penn State University, Saint Louis University and Stanford University.

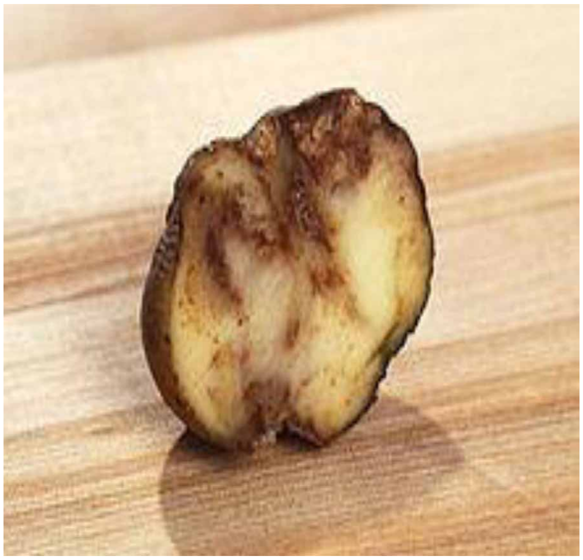
Food Security

The global distribution of foods has resulted in decreasing global hunger and increases in child survivorship (von Grebmer, et al., 2020). However, periodic international food price spikes, food price volatility, and a general trend upwards of food costs globally are comprehensively documented (Tadesse, et al., 2014). Nominal price spikes of up to 50% globally occurred in 2007-2008 and 2011-2012. Although there appear to have been numerous drivers, one certainty is that low-income people around the world were the most impacted. Poor people in poor countries spend as much as 50% of their income on food. Even in the U.S., people in the bottom income quintile spent 36% of their income on food in 2019 (USDA ERS, 2019). Households that spend 25-50% of their income on food become food insecure when there are price increases. The global spread of Covid-19 has had a significant impact on food security, in many countries reversing recent trends (von Grebmer, et al., 2020), making food availability and distribution even more critical.

There are historical cases where enough food was available, but due to speculation and market perturbation, price spikes made it unaffordable resulting in famine. Several notable famines have been caused either directly or indirectly by crop disease.

The Irish potato famine or the Great Hunger (1845-1852) resulted in the death of 1 million Irish people and the migration of at least another million forced to leave for lack of food. An additional 100,000 people perished from hunger throughout Europe, leading to widespread civil unrest. This famine was caused directly by a fungal plant pathogen, *Phytophthora infestans* that destroyed the primary staple food of the Irish, potatoes (Figure 3). This pathogen had moved with potatoes from the center of origin in the Andes and had been noticed and written about in Europe as early as the early to mid-1800s (Berkley, 1948). Warm wet conditions commenced an epidemic of the fungus in the potato crop that rendered the tuber inedible. Starvation was the direct result of lack of this staple food.

Figure 3. Potato affected by a plant disease (Source: Encyclopedia Britannica, 26 Oct. 2017, https://www.britannica.com/science/late-blight. Accessed 7 January 2022.)



A famine in Bengal (1943) resulted in over 3 million deaths. It started with an epidemic of brown spot disease of rice, caused by another fungus, *Cochlibolus miyabeanus*, that significantly reduced yields across the Indian sub-continent. The famine, however, was caused by policy failure, not lack of food *per se* (Sen, 1977). First the government denied that there was a problem, then attempted to hold down the price of rice paddy, but instead created a black market and encouraged hording, leading to hyperinflation for the price of rice. Enough rice was available, but for millions of Bengalis it was inaccessible because they could not afford it (Sen, 1977). Today, it is less likely that fungal crop diseases will result in famine because of fungicides and better crop protection infrastructure globally. Nevertheless, when a crop disease epidemic occurs, it brings a tremendous economic burden to producers and governments to control and

Figure 4. Mother with her starving children

manage the situation. [Figure 4 famous photo published in *The Statesman* on 22 August 1943 showing famine conditions in Calcutta, Bengal province, India. Copyright was held by *The Statesman* newspaper of Kolkata, India, however the image is now in the public domain (photographs are protected for only 60 years from the date of publication.)]

Fortunately, most countries do not rely solely on a single food source and are able to access interlinked markets to ensure food resilience. In 2018, an outbreak of African swine fever (ASF) virus emerged in China, the world's largest pork producing and consuming country (Zhou et al., 2018). ASF has since spread to provinces throughout China and currently represents a global disease threat to the swine industry. In 2018-2019, nearly half of China's pig population died or were culled because of this highly contagious hemorrhagic viral disease. Fortunately, China increased pork importations from other countries and many residents were able to identify alternative protein sources. Based on a partial equilibrium model, an 80% reduction in pork production in China resulted in a 0.78% decline in household welfare and a 1.8% declined in household income (Mason-D'Croz, et al., 2020). Additionally, the estimated overall effect of China's decreased pork production on global food security was small due to global food trade, stocks of frozen meat stores, and consumers able to utilize other protein sources (Mason-D'Croz, et al., 2020).

Environmental Protection

Imagine if all the trees in your town died suddenly. Imagine that all the birds in an area are killed in a short time. Imagine there is an invasion of ants, so ferocious and omnivorous that they can kill a newborn calf before it can stand. These are what are called landscape-scale events, in which large, detrimental changes have occurred. These three disastrous scenarios have already happened due to movement of biohazards around the world. The magnificent American Chestnut, a major hardwood forest tree, was eliminated from the U.S. landscape by an accidentally introduced fungus, *Cryphonectria parasitica* (Rankin, 1912). The Brown Treesnake arrived in Guam, presumably in shipping containers, and quickly invaded the island territory, and wiped out the majority of native bird species. *Solenopsis invicta*, known in the United States as the red imported fire ant (RIFA) is an invasive pest in many areas of the world, including the United States, Australia, China and Taiwan (Ascunce et al., 2011). The RIFA was believed to have been accidentally introduced to these countries via shipping crates. An estimated US$5 billion is spent annually in the U.S. on medical treatment, damage, and control in RIFA-infested areas. Furthermore, the ants cause approximately $750 million in damage annually to agricultural assets, including veterinary bills and livestock and crop losses. Many native animals and beneficial insects in the U.S. have become extinct due to the establishment of fire ants (Wojcik, et al., 2001).

The final example of environmental destruction due to an invasive species is feral swine in North and South America. Pigs were introduced into the Americas in the 1500s by early explorers and settlers. In the U.S., the environmental impact of invasive feral swine has been augmented by the more recent introduction of Eurasian and Russian wild boar for sport hunting. Feral swine currently reside in approximately 35 states in the U.S., and their population is estimated at more than 6 million (USDA APHIS, 2020). Feral swine are rapidly expanding in number and geographic range due to their high fecundity. Feral swine herds damage domestic vegetative crops, compete with other wildlife for wild plants, and kill and eat other native species of animals. In 2007, the economic cost attributed to feral swine in the U.S. was US$1.5 billion (Pimentel, 2007). In addition to directly damaging ecosystems, feral swine are reservoirs for more than 30 viral, bacterial, and parasitic diseases of humans and other livestock (Williams and Barker, 2001). Two of the infectious diseases harbored by feral swine (i.e., pseudorabies virus and brucellosis) have been targets of eradication from U.S. domestic swine herds for decades.

These were just 4 cases of the kinds of environmental harm that has come from invasive species. There is now heightened awareness of the need for biosecurity tactics to control biohazards in shipping containers, wood packing materials, and imported plants and animals. Nevertheless, the pressure on natural systems is relentless, driven by globalization of human activity. The global annual cost of managing invasive species are estimated to be around US$1.4 trillion, but that cipher is very likely an underestimation and will continue to increase (Pimentel, et al., 2001; Bradshaw, et al., 2016; Daigne, et al., 2021).

INFRASTRUCTURE FOR BIOSECURITY

All Pests and Pathogens Are Local Somewhere, but They Are Exotic Somewhere Else

When pests and pathogens move or are moved into new areas, there may be very little preparedness and at-risk populations have little or no resistance or immunity. Often, outbreaks seem to ‘come out of nowhere’, unanticipated, a surprise. These are reminders that nature can find a way around the biosecurity systems that humans put in place, often with the help of humans.

Governments typically implement the highest levels of animal and plant biosecurity in accordance with global entities such as the World Organisation for Animal Health (OIE), Food and Agriculture Organization of the United Nations (FAO), and International Plant Protection Convention (IPPC).

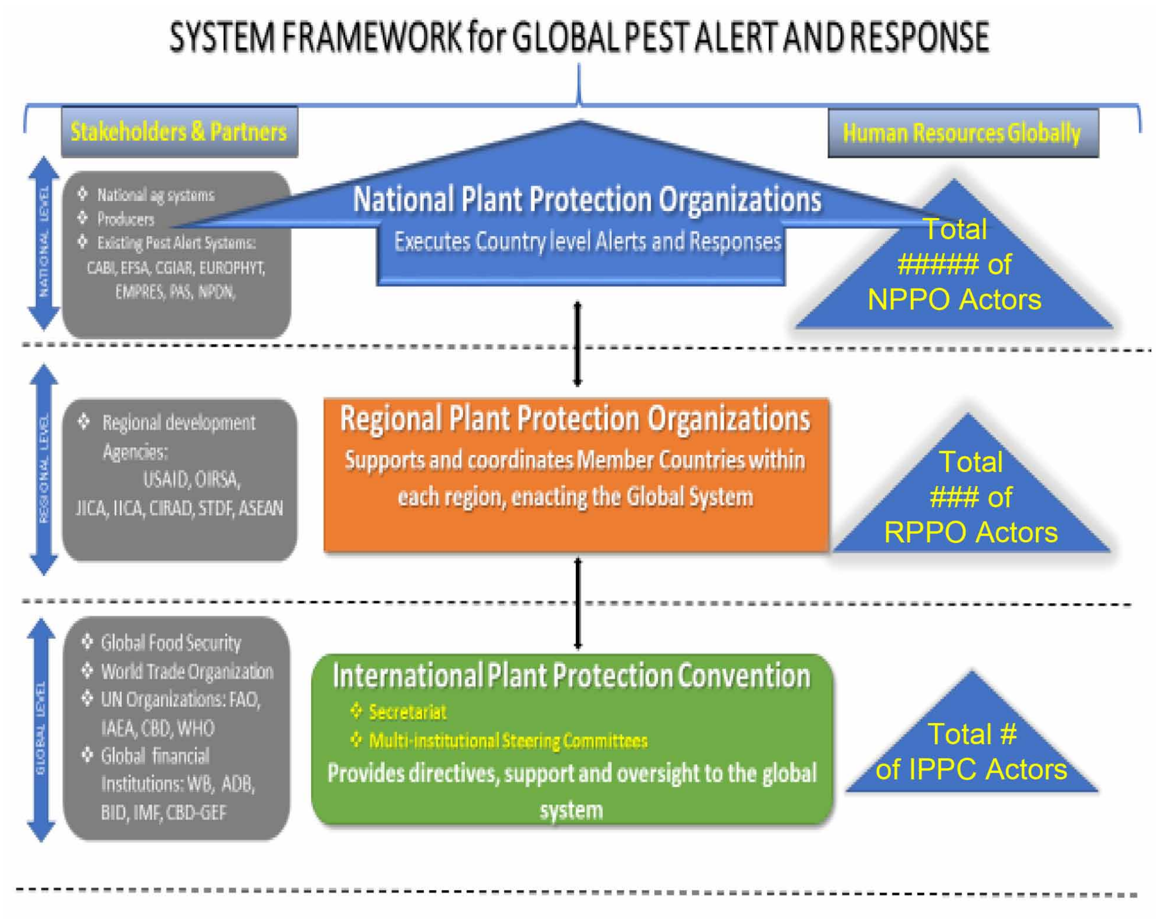
The OIE was established in 1924 due to a recognized need to combat animal diseases in a coordinated manner on a global scale. As of 2018, the OIE was comprised of 182 member states (Home - OIE - World Organisation for Animal Health), each represented by delegates who meet annually to discuss sustainable animal and human health initiatives. The OIE utilizes members and specialists to develop international standards and strategies that provide a global framework for:

- Controlling animal diseases and zoonoses
- Ensuring food safety and animal welfare
- Investigating antimicrobial resistance
- Promoting veterinary services
- Contributing to a sustainable world

IPPC development agenda (2020-2030) is to ‘Facilitate safe trade development and economic growth. Protect forests and the environment. Enhance global food security and increase sustainable agricultural production.’ The IPPC, which has 183 signatories, is governed by the Commission on Phytosanitary Measures (CPM); the CPM establishes and reviews international standards on how plants and plant products are handled during their transport (https://www.ippc.int/en/). The IPPC is the global framework for all formal activities related to plant protection and trade (Figure 5). Tactical activities overseen by the IPPC include:

- International Standards and Phytosanitary Measures – international consensus on tactics including diagnostic protocols, therapeutic treatments, wood pallet sanitation (ISPM-15) https://www.ippc.int/en/.
- Maintaining oversight of National Reporting Obligations among member countries
- Implementation and capacity development

Figure 5. Global infrastructure for plant protection



- Pest outbreak alert and response systems
- Oversight and coordination of the Regional and National Plant Protection Organizations (RPPOs and NPPOs)

Regional Plant Protection Organizations (RPPOs) function as a coordinating body for the National plant protection organizations (NPPOs) in their region. There are currently 10 RPPOs who are charged with cooperating in information collection and sharing. NPPOs are the governmental plant protection officers of each country. The NPPOs have the responsibility to implement tactics:

- Issuance of Phytosanitary Certificates
- Reporting the occurrence, outbreak and spread of pests
- Inspecting consignments of plant and plant products and other regulated items
- Managing treatments related to plant health (either disinfections or disinfestations) of consignments
- Surveillance and maintenance of pest free areas and areas of low pest prevalence
- Conducting pest risk analyses
- Ensuring the maintenance of phytosanitary security of consignments after certification.
- Staff training and development.
- Reporting any changes in its organizational structure, regulations and other phytosanitary issues. (IICA, 2021)

Each country and region have specific pests and pathogens of concern, indicated by risk and pathway analyses, for which customs and border protection processes and tactics are developed. There are many interceptions every year. For example, on a typical day in fiscal year 2020, the U.S. Customs and Border Protection discovered 250 pests at U.S. ports of entry and quarantined 3,091 suspicious materials such as plants, meats, animal by-products and soil (US CBP Snapshot, 2020).

Within countries, various networks have been established to facilitate the rapid detection of pests and pathogens that represent significant biosecurity risks, as well as a coordinated national response to these threats. For example, the U.S. developed the National Animal Health Laboratory Network (NAHLN) and the National Plant Diagnostic Network (NPDN) to protect animal and plant systems. These networks represent strong collaborations between diagnostic laboratories, regulators, and public health partners.

The NAHLN was established in 2002 and is administered by two agencies in the Unites States Department of Agriculture (USDA); specifically, the Animal and Plant Health Inspection Service (APHIS) and the National Institute of Food and Agriculture (NIFA). The NAHLN represents a partnership of more than 60 federal, state and university-associated animal health laboratories in the U.S., with most laboratories also members of the American Association of Veterinary Laboratory Diagnosticians (AAVLD). Member laboratories of the NAHLN utilize harmonized diagnostic testing protocols that are developed by federal laboratories and are subject to strict quality assurance and quality control measures. The member laboratories are capable of rapidly testing for high-consequence animal pathogens throughout all regions of the U.S. and have the in-house resources to accommodate surge capacity testing. Additionally, early in the SARS-CoV-2 outbreak, the member laboratories were able to quickly provide high-throughput testing as a public health service, with at least one veterinary diagnostic laboratory testing more than 1 million human specimens by polymerase chain reaction (PCR) assay for SARS-CoV-2 in a 4-month period.

The USDA Plant Protection and Quarantine (PPQ) is the regulatory body that oversees biosecurity tactics for plant imports and exports from the U.S. It is the National Plant Protection Organization under the IPPC (Figure 5). Much of the work and efforts conducted by APHIS PPQ are discussed throughout this book. From overseas monitoring, inspection, detection to response, all are coordinated by this organization.

Nevertheless, the U.S. has over 1 billion acres of farm, forage, and forest lands. When all efforts to exclude an organism are unsuccessful, an outbreak of a new invasive pest or pathogen happens. There are about 15 emergency pest outbreaks per year (pers comm, APHIS). To improve the ability to quickly detect new outbreaks, the National Plant Diagnostic Network was developed and has become a critical component of the U.S. Biosecurity infrastructure (Stack, et al., 2014). The NPDN is a distributed, coordinated system of plant diagnostic laboratories at Universities and State Departments of agriculture in every State and Territory in the country. NPDN diagnosticians are trained to provide timely and secure communications to the State and National regulatory officers when they have a presumptive diagnosis of a new, potentially invasive, and/or select agent pathogen, often providing the critical first detection in the country. By facilitating early detection and providing triage and surge support during plant disease and pest outbreaks, NPDN has become an important partner to the federal and state agencies, meanwhile providing a critical service to their local agricultural and environmental enterprises (Stack et all, 2014).

It is important to note that both the NPDN and NAHLN laboratories have been rigorously trained in appropriate communication protocols. The detection and identification of a new pest or pathogen in managed (agricultural and horticultural production) or natural (forest, prairie) plant or animal systems can have significant ramifications (Stack, et al., 2014). Consequences of an unconfirmed media report or journal publication announcing a new pest or pathogen outbreak can be significant. Large scale response and mitigation measures may be deployed. Trade partners may block trade of that commodity from the entire country, farms may fail, and commodities futures react negatively. Thus, the partnership between the regulatory agencies and the non-regulatory laboratories needs to be well coordinated, with system fail-safes in place.

It is also of utmost importance that all networked laboratories use the same best practices and standard operating procedures and laboratory personnel are proficiency tested to minimize the chance for false diagnostic outcomes. The consequence of a false positive diagnosis is that inaccurate information is provided to a producer or a responsible authority setting off ineffective and costly response operations. The consequence of a false negative diagnosis is that inaccurate information leads to potential establishment and or spread of the pathogen or pest and delays mitigating response. The networked laboratories understand the serious nature of the part they play in national biosecurity, otherwise the system would not work.

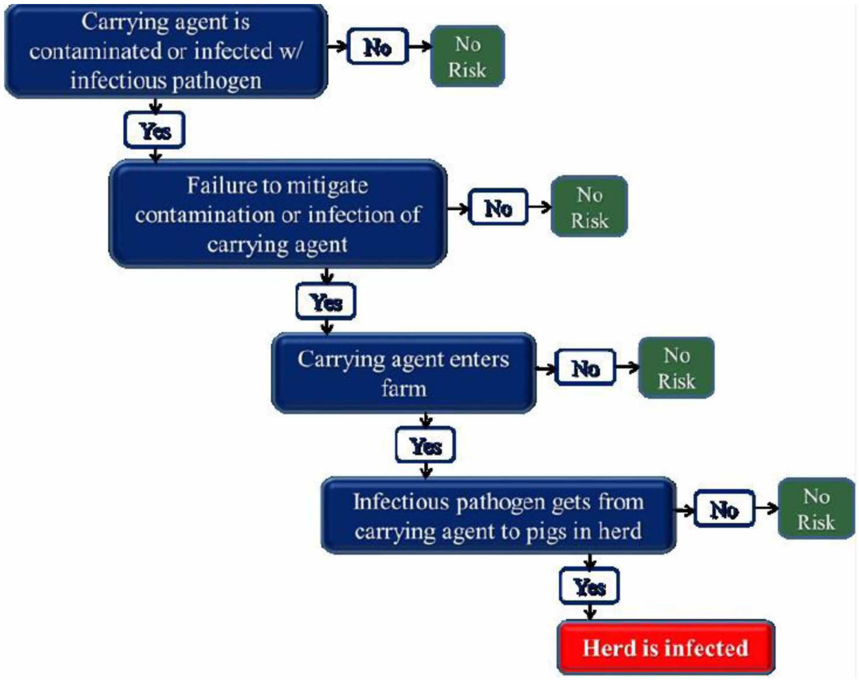
Biosecurity at the Farm Level

In addition to global and national biosecurity initiatives, arguably the highest levels of biosecurity occur within the private sector. Commercial animal and plant industries serve as critical control points for animal and plant diseases, and as such, these entities commit significant financial resources towards biosecurity each year. Excellent resources are available that outline biosecurity practices in animal and plant production systems (Gullino, et al., 2008; Gordh and McKirdy, eds. 2014; Dewulf and Van Immerseel, 2018), so most farm-specific tactics are not addressed in this book.

An example of animal production-specific tactics includes the bio-exclusion practices on commercial swine farms outlined in Figure 6 (Holtkamp, et al., 2020). In this example, pathogens may be introduced into the herd during routine operational practices (e.g. pigs entering or leaving the farm, vehicles delivering semen or feed, farm staff, infectious organisms in the air and water, etc.), with each event constituting one or more risks. Farm biosecurity efforts are primarily concentrated on reducing the likelihood of pathogens entering the farm and mitigation strategies for pathogens that reach the farm (Dewulf and Van Immerseel, 2018).

There are also biosecurity tactics for crop production farms, grasslands, greenhouses, public gardens and natural habitats such as parks and wilderness areas. An excellent guide to developing an enterprise-level biosecurity plan can be found at the Canadian Food and Inspection Service website (CFIA, 2021). They promote creating a biosecurity plan to reduce pest or disease introduction and spread within and between production areas, and to minimize impacts of pests already present. The tactics are essentially

Figure 6. Biosecurity failures that facilitate the entry of a pathogen into a swine herd



the same at any scale: understand the risks, identify potential entry pathways and control points, and implement biosafety and biocontainment practices. At a farm level, these can include strategies such as regular monitoring and not moving equipment from infected to non-infected fields. In high-value commercial greenhouses, people may be required to wear PPE. Whatever the size and scope of the operation, understanding and implementing biosecurity principles just makes sense and will save money. The tactics that we have discussed at the farm level are voluntary, and every operation must decide what is in their best interest. But, awareness of biosecurity principles will certainly improve chances to avoid pest and disease related losses.

Global and Social Implications of Biosecurity

There are similarities and differences between countries and continents where biosecurity is concerned. Most countries have bought into and become signatories to international trade treaties (Bhagwati, 2002). However, what is considered a quarantine pest may be very different from one location to another. Additionally, socio-cultural differences can play an outsized role in how different countries react and respond to biosecurity strictures. For example, places that rely heavily on ecotourism may naturally place more emphasis on protection of environmental services, even as higher tourism puts the ecology at risk. Locations that do not produce a specific commodity may have no rules about biohazards to that crop, while those that depend on producing the commodity will be strict. Where food security is an issue, policies and procedures will lean towards conserving health and well-being at home, while other places may be more focused on economic growth. Some cultures are naturally conservative in approach, where others strive for innovation and change.

Therefore, biosecurity regulations and practices can create tensions between countries, neighbors, people, and nature. There is a normative tension between country regulatory frameworks and the regulated industries therein. Although the tactics and policy implementation are designed to protect the regulated industry, the individual industry/company bears the cost of compliance.

At the airport, foods, spices, and animals are regularly confiscated and destroyed, to the dismay of the passenger who wanted those things. At the port, customs exams can be a significant source of frustration and confusion when shippers are moving goods into a country. When a shipment is put on a custom hold for inspection and examination, it derails timelines for the shipping company and costs money for the shipper(s). Shipments may be selected at random for inspection, or there may be algorithms evaluating risks associated each shipment. Criteria may include past history and trust of the shipping company, whether goods were consolidated at various locations, provenance of the goods, documentation and bill of lading, etc. An intensive customs exam could result in delays of weeks to a month. The tension can go both ways. Companies can and do take governments to court for losses, putting the regulatory officer and system in the position of needing to defend their actions.

Another social dimension of biosecurity is the interface with the public during a biosecurity operation, particularly in response to an emergency or high consequence outbreak of a pest or pathogen. Tactics such as stop-movement, depopulation, and area-wide spraying programs create optics that can lead to public concerns (Figure 7). Communication by authorities must be clear and calming, even more so when the public is being asked to participate in an operation, such as the removal of a backyard tree or flock of birds.

Figure 7. Destruction of cattle and sheep during 2001 Foot and Mouth Disease epidemic in England as reported in BBC News, January 14, 2002

There are offsets between biosecurity regulations and societal well-being. Trade and global movement of products creates economic opportunities, jobs, tourism, increased opportunities and purchasing options. The balance between too much regulation and not enough is a constant flux. Many biosecurity regulations are guidelines and voluntary because they are not enforceable but rely upon the good will and societal altruism for the greater good.

The biggest, most ignored tension by far, is that between humans and the natural world. What can we as humans do to protect (or at least not destroy) the natural world around us? Everything we do, from farming to manufacture, from tourism to game hunting is a direct interface with nature. In many ways, the biosecurity regulations we put on ourselves now, may determine how well we survive as a species.

Uncertainty and Risk

What do we not know and what is the impact of what we do not know? Similarly, what is the impact of randomness (i.e. variability) on a biosecurity system?

Biosecurity is primarily about managing risk, defined as a continuum of possible events or future changes in state. Risk is the combination of likelihood of an event happening and the economic consequences, or the net cost of the event (Heikkila, 2009). Throughout this book you will see risk analysis and management described with respect to the tactical decisions made along the continuum of biosecurity implementation.

The difficulty with analyzing risk is that there are always unknowns, and uncertainty itself must be managed. Uncertainty in biosecurity is found in natural and biological processes, as well as in how humans act and react. Biological uncertainties revolve around whether a biohazard could survive transport, find a susceptible host, find the environment conducive to survival, adapt to the new environment, and spread outward from the invaded area. It is also uncertain whether the invader would be/could be found early enough for successful interdiction and control. Uncertainty concerning humans has to do with how humans react and make decisions, as well as the societal and institutional ability and will.

Risk and uncertainty have economic implications. Resources must be allocated to minimize the negative effects of risk, balanced with the costs required to reduce the risk, while maximizing benefit. Failure to address uncertainty during risk assessments may lead policy makers to rely on their implicit beliefs, prior experiences, or inaccurate perception of the probabilities of various outcomes (Kahneman, 1982 and Koch, 2015). If uncertainty and potential negative consequences are both very high, the policy decision may be to avoid the risk altogether by, for example, not allowing trade of the risky good. When uncertainty is lower and risk analysis indicates that the risk can be managed, tactics such as surveillance or offshore mitigation may be used. Often, the perceived benefit outweighs the potential risk to the extent that the decision is to live with the risk and be prepared in the case that it materializes. Another risk management tactic is to plan for it by diversifying or creating insurance options.

Biosecurity to protect agricultural production, ecosystems, public health, and wellbeing is a series of decisions that have biological and anthropogenic uncertainty and economic costs. The basic question is, ‘what should we do about this risk and will the benefits of what we do be worth the cost of doing it?’

OVERVIEW OF THE BOOK

The foundational tactics, concepts, strategies, and tools outlined in this book can be applied at scale to any biosecurity system and thereby enhance preparedness, reduce risk, and prevent catastrophic outcomes. Each chapter contains Case Studies that are designed to provide real-life examples of how animal and plant threats were detected and managed.

Economic considerations represent a major driver in policies designed to safeguard animal and plant systems. However, the benefit-cost tradeoffs of biosecurity policy are difficult to accurately predict in the rapidly evolving global agricultural marketplace. Chapter 1 outlines an economic framework to assess policies pertaining to invasive pests and pathogens. Simultaneously considering economic and biologic outcomes provides the ability to estimate the economic outcomes *prior to* implementation, allows for comparisons across alternative strategies, and potentially improves the efficiency of management choices.

To reduce the risk of introduction and transmission of infectious disease to animals and plants, biosecurity seeks to understand the mechanisms and patterns of pathogen emergence and implement strategies to effectively manage disease. Chapter 2 describes how evolution underpins pathogen dynamics and its impact on biosecurity tactics. Pathogen populations change as pressures are exerted on them from the environment, the host, or human practices. The foundational approaches discussed in this chapter form the scientific basis for effective animal and plant biosecurity management strategies, while recognizing that the applied approaches used in detecting and characterizing pathogen populations need to be continually evaluated and adapted as pathogen populations change.

Humans have always played an important role in dispersing plants, animals, and other organisms – either intentionally or inadvertently. Over the last several decades, the expansion of infrastructure and transportation has led to dramatic increases in trade, travel, and mass migration. Mitigating the entry, establishment, and spread of exotic pests and pathogens is crucial for protecting agriculture, ecosystems, and people. However, a thorough understanding of the pathways by which invasive species spread and their associated risks is required for a successful biosecurity system. Chapter 3 describes the role of risk analysis for understanding human-mediated pathways and develops a risk analysis framework to guide decision making.

Border biosecurity has a critical role in reducing risk to domestic agricultural resources, native flora and fauna, and the environment. Transboundary biosecurity measures focus on all sources of movement of plants, animals, germplasm (including pollen), and their associated products. Successful mitigation strategies are directed at critical points along the production and distribution chain. Chapter 4 provides a practical overview of management and control practices to safeguard agricultural biosecurity against human-mediated threats, emphasizing validated control measures, emerging technologies, inherent challenges, and potential biosecurity gaps related to activities leading up to (i.e. pre-border) and at the point of entry (i.e. border).

Introduced pests and pathogens require effective biosecurity strategies to ensure their rapid detection, identification, and prevention of spread. The goal of early detection is to find and identify pests and pathogens before they become established and cause widespread damage and economic harm to the agricultural, landscape, and environmental sectors. The focus of an early detection surveillance program is to determine if a pest or pathogen is present or absent in order to facilitate market access, trade, and the movement of crops, forest products, animals, animal products, and other commodities and goods within a country or internationally. Chapter 5 describes the various components, topics, and issues that are part of an early detection surveillance program.

After initial detection, the dynamics of an outbreak evolve based on the nuances of the system where the hazard is found, the type of hazard introduced, the risks presented by the hazard, and interactions between host, hazard, humans, and the environment across space and time. As the situation evolves, the objectives of a surveillance scheme should also shift according to feasible management options available to address the hazard and eliminate (or reduce) the risk. Chapter 6 provides information on how a surveillance scheme must deliver "fit to purpose" information to coincide with the management goal. The chapter introduces foundational concepts for designing surveillance schemes and the key elements for consideration to accommodate the management goal. It provides a generalized framework for surveillance design for a subset of common goals across a wide range of applications including plant and animal diseases and incursions of invasive species and pests.

At its most basic level, diagnostics is the process of figuring out what is causing a problem so that a solution can be prescribed. Diagnosis of disease is a process of hypothesis, investigation, and synthesis that encompasses hard science skills of infesting and testing hypotheses, as well as soft skills of conversation and imagination. Positioned on the continuum between surveillance and response, early and accurate diagnosis is critical to effective mitigation and management of invasive pests and pathogens. Chapter 7 focuses on the diagnosis of a causal agent once an outbreak has been detected. The chapter outlines current diagnostic techniques and technologies to confirm the presence of a pest or pathogen in a system, as well as describes novel and emerging diagnostic methodologies.

Emergency response to animal and plant disease outbreaks encompasses much more than eliminating an infectious agent or pest. Responses are rapidly changing and complex, often involving multiple organizations. Decisive actions must be taken to contain, control, mitigate, or eradicate invasive pests and infectious agents. Emergency preparedness well in advance of a disease incursion supports detection and diagnosis, mobilization of emergency personnel, treatments, and recovery. The effective response to animal or plant disease requires extensive cooperation and coordination between regulators, private industry, and many segments of society. Chapter 8 describes the organizational structure and coordination during the initial emergency response to a threat and outlines the Incident Command System as an effective biosecurity tool for response coordination and initial management of invasive pests or pathogens.

Following the rapid, coordinated response to an animal or plant emergency, multiple entities work together to effectively manage the threat. Short-, intermediate- and long-term animal and plant health incident management tactics may change over time depending upon multiple conditions and externalities that will be described in Chapter 9. Results to response may range from fully successful eradication to learning to live with the pest and deregulation. Although the scope, time-frame and consequences of events can vary, actions taken in response to the identification of an exotic plant or animal pest, disease, or condition are designed to minimize economic and environmental impacts, ensure trade and food security, assure continuity of operations, and avoid social upheaval.

The secure and continuous production of agricultural commodities is a key component of an effective biosecurity program. The introduction of foreign-origin or emerging animal, plant, and human diseases can occur by natural incursion, accidental introduction, or by intentional acts of terrorism, biological warfare, or criminal activity. These threat agents, which are mostly biological, can lead to severe consequences for domestic and international agricultural markets, the economic security of the agricultural community, food safety and food security, and the credibility of responsible state and federal agencies. Chapter 10 describes the microbial forensic investigation of disease outbreaks in agricultural settings, with particular emphasis on the biological characterization and potential attribution of the invasive pest or pathogen. The chapter also discusses the investigative tools and approaches to exclude the intentional release of a high-consequence animal or plant pathogen into a system.

Future advances in technologies, increased globalization, impacts of changes in climate and land use on food production practices, and the expanding world population will continue to exert significant pressures on global biosecurity systems. The world must be prepared to face novel biosecurity threats, whether a consequence of natural pest and pathogen emergence or an intentional or unintentional release into a community. It is imperative that public and private sectors develop comprehensive and innovative strategies to mitigate these ever-evolving threats rapidly and effectively. Chapter 11 reviews several opportunities that currently exist in global biosecurity of animal and plant systems with the hope that it will provide researchers, health experts, educators and first responders with the awareness and impetus to adopt biosecurity tactics that enhance preparedness, reduce risk, and prevent catastrophic outcomes.

GAPS THIS BOOK FILLS

Several books and publications outline detailed components of an effective biosecurity system (Gordh & McKirdy, 2005; DeWulf & Immerseel, 2018); however, the value of this book is that it highlights the interoperability of biosecurity tactics employed in animal and plant systems. Science courses in agricultural colleges are typically a collection of exposures to different classes of pathogens and arthropods, scientific principles, and academic thinking. Environmental, plant and animal sciences are often taught in separate colleges, with little opportunity for crosstalk. In some countries, higher degrees are conferred to people trained to be generalists, such as Ingieur Agronome titles in Europe and Latin America. In a few cases, University degrees are designed to be practical such as the Doctorate of Plant Medicine at the University of Florida.

The SARS-CoV-2 pandemic demonstrated the need for critical infrastructure, trained biosecurity responders and a comprehensive battery of biosecurity tactics. Moving forward, many colleges and universities are well positioned to offer formal, comprehensive and integrated biosecurity courses that train our next generation of biosecurity responders, as well as help educate researchers and scientists.

This book is intended to provide readers with an overview of the scientific principles and activities that are directly applied to support strategic actions and policy decisions employed in an effective biosecurity system. To do so, it is important to understand the motivations for biosecurity measures which include economic considerations and impacts on global health, well-being, and environmental protection. The book also outlines processes that facilitate pathogen emergence and provides biosecurity tactics that range from assessing and managing biosecurity risks to early detection and diagnosis of threats to the emergency response and management of invasive pests and pathogens. Additionally, one chapter pertains to the tactical sciences of microbial forensics and potential attribution of an intentional effort to introduce and release a biohazard.

Biosecurity is a continuum of efforts comprised of multiple tactics designed from understanding of biological and social science principles. The chapters are arranged to highlight biosecurity tactics to prevent, intercept, and stop movement of damaging organisms into and out of a country; and when those efforts fail the tactics turn to detection and diagnosis, mitigation, control, and management. There are elements of risk analysis and surveillance tactics in several chapters, depending upon whether the pathogen or pest is still outside a border, or whether it has gotten inside the border and response is needed. Each chapter is authored by expert practitioners who dedicate their lives to biosecurity efforts. The chapters of this book provide glossaries that define specific technical terms and concepts as well as case studies designed to assist the reader in understanding the real-life application of tactical sciences of biosecurity in animal and plant systems.

DISCLAIMER

The views expressed in this book do not necessarily represent the views of any Governmental Agency.

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When we embarked upon the task of assembling this book, we were innocently unaware of the thousands upon thousands of person-hours that would be invested. For the dedication and passion of the assembled co-authors, we are immensely grateful. Subject matter experts and university and government scientists voluntarily spent countless hours on videoconference team meetings and writing and rewriting sections of text. We hope you have pride in the output of your labors and the breadth and rigor of the entire exercise. Our deepest gratitude is also extended to many external reviewers who provided insightful and constructive comments that helped strengthen each chapter.

Initially, our idea was that this book would be a generic treatment of biosecurity tactical sciences, generally applicable to anywhere. In effect, however, the book explores most deeply the biosecurity mechanisms primarily in the U.S. It is our hope that there will be content of interest to readers around the world. We have already begun using these materials for graduate level coursework, helping students see where their discipline focus fits into the larger continuum of biosecurity.

Finally, the term ‘Tactical Sciences’ arose from a brainstorming team, Gary Sherman and Kitty Cardwell, at the USDA National Institute of Food and Agriculture (NIFA), with the mission of unifying under one title the disparate granting opportunities for applied programs such as the National Animal Health Laboratory Network (NAHLN), National Plant Diagnostic Network (NPDN), National Clean Plant Network (NCPN), Extension Disaster Education Network (EDEN), integrated pest management (IPM) centers, pesticide applicator training, National Veterinary Stockpile, Veterinary Medicine Loan Repayment Plan, etc. These programs are more infrastructural and applied than the usual competitive grant research programs and it is important to call them out for the important contribution they make. All of these require a highly skilled and educated workforce, and they are the infrastructural network of practitioners at academic institutions who support the biosecurity efforts of the United States. The team struggled with what to call such a diverse set of programs. Finally, one bright morning, Gary Sherman, DVM, Virginia Tech (ret), walked in and said, “I’ve got it! These are all tactical sciences.” Thanks to Gary for providing clarity and the perfect phrase!

Chapter 1
Introduction to the Economics of Animal and Plant Biosecurity

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ABSTRACT

The economics of plant and animal health protection influence country policies through rapidly evolving benefit-cost tradeoffs that are difficult to forecast. Increased threat of infestation by invasive species following novel trade pathways is one recent trend, being counteracted by advances in data analytics to target interventions on higher risk pathways. The availability of increasingly large, complicated datasets generated from daily enforcement of regulations are available to safeguarding analysts. These data resources used to monitor and evaluate pathways are increasingly available electronically with shorter time lags. But the efficacy of increased analytic capabilities requires a clear objective of what is optimal. Economic frameworks can help focus the analytics. For example, increased protection that costs more than the benefit generated is not efficient. Economic theory provides a systematic method with which to develop policy or to assess existing programs. This chapter provides basic economic concepts and examples relevant to biosecurity safeguarding.

INTRODUCTION TO THE ECONOMICS OF PLANT AND ANIMAL BIOSECURITY

International export markets for U.S. domestic products, and U.S. markets for international products, are pathways for the spread of agricultural and environmental pests and diseases. International shipping and air travel, as well as trade in non-agricultural products, even hurricanes and storms can lead to pest and disease spread. These factors increase the likelihood of novel introductions and re-introduction of previously controlled pests and diseases.

The economics of plant and animal health protection in the U.S. influence policy through a labyrinth of benefit-cost tradeoffs that are difficult to predict and rapidly evolving. Two opposing forces are gaining

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strength in this landscape: increases in the likelihood of infestations by invasive species following novel pathways; versus analysts' ability to use data analytics to target interventions on higher risk pathways to lower the risk of infestations taking place.

Historically, plant and animal protection has operated in a command-and-control environment (see glossary) which was inherently reacting to an introduction. Ideally, an agricultural safeguarding continuum requires a proactive rather than reactive response to agricultural pest and disease threats, and more simultaneous than sequential consideration of economic implications of different approaches to dealing with threats. There was a time when we could address the cost of pest and disease exclusion, followed by an economic analysis of what dangers were avoided and at what cost. Increased threats, increased mobilization, and limited economic resources to address them has led to a proactive consideration of the value of exclusion, relative to the risk of introduction and establishment for various agricultural commodities.

Large datasets are generated from daily enforcement of animal and plant protection regulations to safeguard from pests and pathogens associated with the different arrival pathways for various plant and animal commodities. Risk-based sampling of cargo and conveyances for pests and pathogens is based on interpretation of that data. At the same time, data resources to monitor and evaluate plant and animal pests and diseases are increasingly available electronically with shorter time lags. Analytical capability that can be applied to this data is growing in both rigor and ease of implementation including statistical and geographic information system (GIS) map-layering capability. Data mining, simulation, and optimization algorithms have gained sophistication and can be used to predict future events or discover patterns that would be otherwise undetectable.

The news media regularly reports the environmental, social and agricultural costs of introduced pests and diseases, and have even created "glamor pests" that catch the public's imagination like the "murder hornet" or Asian giant hornet (AGH), one of the world's largest hornets and one that is extremely aggressive towards honeybees, threatening their pollinator functions (Kumar, 2020). This celebrity is likely to influence the economic tradeoffs associated with different exclusion, treatment, and AGH control strategies. The prioritization and timing of AGH actions are likely to be influenced towards proactive strategies and away from reactive ones. The result is more resources are likely to be allocated to develop an early warning system, rather than a more standard detect and react approach.

These opposing forces of expanding exposure and increasing analytical capability leads to some important agricultural safeguarding questions. Can practitioners predict the next costly invasive pest? Can they forecast how it might get here, or how they could stop it before it ever reaches our shores? Can policymakers develop a best practices approach for managing a domestic outbreak before it happens? This chapter discusses the economics of these opposing forces. It relates those economic principles to specific, recent examples of plant and animal pests and diseases impacting U.S. agriculture, and how enhanced analytical capability has helped control or eradicate problems for individual agricultural products.

The Animal and Plant Health Inspection Service (APHIS) agency of the U.S. Dept. of Agriculture strongly supports the publication of a chapter that describes the rapidly evolving landscape of plant and animal health safeguarding and protection as it supports the basic mission of the agency to protect and promote U.S. agricultural health (APHIS, 2021).

A Framework for Economic Analysis of Plant and Animal Biosecurity: Along the Safeguarding Continuum

Non-native species can have unintended consequences long after the initial introduction of the species. The questions surrounding the economic impact of invasive species or the efficacy of a plant and animal protection policy are not new, nor are the discussions of how best to measure impacts or assess efficacy of policies.

Post-implementation assessments often focus on the market costs and benefits of the plan in order to ascertain if a policy has been successful. That is, do the benefits outweigh the costs? Without considering all impacts, market and non-market (e.g., environmental and/or ecological impacts), the assessment of a specific policy may under or overestimate its impact. In other words, more data improves the accuracy of economic models.

The dynamic nature of many invasive species, where the population grows or declines over time, creates consequences that are difficult to assess. Different actions in early time periods can result in different outcomes in later time periods. In some cases, border detection may be reliable and cost effective but when detection is difficult, deterrence may be more effective. This can result in varying cost and benefit paths over time. An ex-post assessment of a policy can provide an assessment of the chosen policy, but an ex ante assessment of potential policies may provide insight into the optimal policy (Heikkila, 2011). Agricultural pests may be the first to come to mind, but these principles apply equally well to non-native animal species like common starlings, Asian carp, snakehead fish, and zebra mussels (USFS, 2015) and/or plants such as cheatgrass or kudzu (USFS, 2021).

Policies to curb the introduction of non-native species into the system, take into consideration the cost of an optimal level of detection (see glossary) compared to the benefits of reduced potential harm from the species. Thus, in either detection or deterrence, a policy that is developed without an economic assessment *prior* to implementation may be sub-optimal, as a different set of actions, a different time path of actions, or a different level of intensity of chosen actions may result in an improved outcome. An economic framework can provide an assessment tool that allows comparisons across potential plans, or can assess the efficacy of a single plan prior to implementation, rather than as a post-analysis of the efficacy of dollars spent. Planning based on *"What is the optimal management or detection plan?"* may provide improved outcomes (Heikkila, 2011; Kim, et al., 2012).

This chapter develops the economic framework with which invasive species policies can be assessed. Economics is sometimes equated to calculating costs and benefits of an outcome, or discounting future cash flows to today's dollars. These are both techniques used in economics (Lewis and Tietenberg, 2020; Schmid, 1989). Economics is broader and provides a methodological approach to estimate the outcome of policies or choices as a planning tool. Simultaneously considering economic and biologic outcomes provides an ability to estimate the economic outcomes *prior* to implementation.

A priori comparisons across alternative strategies can improve the efficacy of management choices. Outcomes will vary predictably as characteristics of the system vary. For example, countries, companies, and individuals may make strategic choices that benefit them individually but may not be in the best interests of multiple actors at a larger scale. From an economic perspective, what incentives or regulations are best to incentivize individual actions to be aligned with a broader societal good? Another consideration may be the impact of spatial and temporal changes in an efficient outcome. How does the choice of managing an invasive species today impact its spread tomorrow? Economics, including market analysis using the laws of supply and demand, principles of international trade, strategic behavior, and timing uncertainty provide frameworks to incorporate these types of questions into an ex-ante, assessment of a proposed management policy.

The following pages provide a background of relevant economic concepts and theories with which to assess threats. Relevant case studies are provided to illustrate the use of economics in the specific case. Starting with the relatively simple framework of supply and demand and market equilibrium, the chapter progresses through case studies, and focuses on additional concepts that are applicable to each one. In many cases, the new concepts are additional factors added to those already discussed in prior cases. The goal is to provide an overview of not only where economics fits into biosecurity tactics, but also to showcase how using bio-economic modeling can improve management decisions and outcomes in the future.

Market Equilibrium

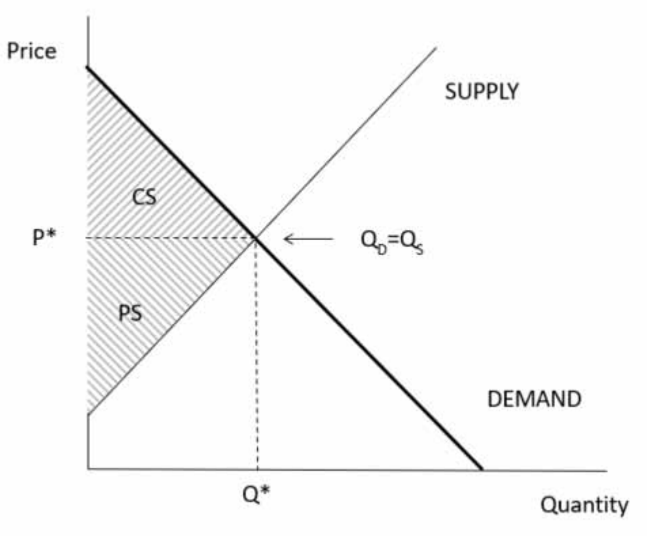
A market equilibrium is simply defined as the condition where a price is struck such that the quantity of a good demanded is exactly equal to the quantity of the good supplied (Black et al., 2009). In a perfectly competitive market, characterized as a price taking market, where no buyer or seller has market power, this is defined by the intersection of the supply curve and the demand curve. A demand curve represents the relationship between quantity demanded (Q_D) by consumers and price (P), holding all other factors constant. The price is the maximum price consumers will pay for the good and so represents the incremental, or marginal benefit (MB) of consuming each unit of the good.

For most goods, this is an inverse relationship meaning at a higher price for the good a consumer won't buy as much of the good, as they would at a lower price because the consumer receives less benefit from consuming additional units of a good. This means the demand curve is normally downward sloping and is referred to as the "law of demand" (Marshall, 1892). For example, someone whose favorite food is a hamburger will gain a level of satisfaction from consuming a hamburger. If that person eats a second hamburger, their satisfaction may not be as great as the first (they're already full, they could have eaten something else). And, if they eat a third, fourth and fifth hamburger, their satisfaction continues to decline.

The supply curve represents the relationship between the quantities firms are willing to supply (Q_S) at each price, P. This is the minimum price the firm would accept and is, in a perfectly competitive market, equal to the incremental or marginal cost (MC) to supply each additional unit of a good. As firms are able to cover higher costs, at higher prices, the supply curve is upward sloping. The equilibrium condition for a perfectly competitive market is depicted in figure 1. Notice that at the equilibrium price (P*), $Q_D=Q_S$, so price is a function of Q, denoted P(Q). Note, in a perfectly competitive market, all units of the good are sold at the equilibrium price, resulting in benefits in excess of price to consumers and profits to the firm for each unit sold up the equilibrium quantity.

A perfectly competitive market is also considered the first best outcome as it maximizes social welfare (Heikkila, 2011). Social welfare is the sum of the benefits and profits described above, or consumer surplus (CS) and producer surplus (PS). That is, Welfare = CS+PS. Given the characteristics of demand and supply, represented by the demand and supply curves, there is not a single price that would result in a larger welfare measure.

Figure 1. Market equilibrium



Not all markets fit the perfectly competitive outcome described above, nor are markets static, with unchanging conditions. Indeed, most are not. This is certainly true in the case where invasive species are concerned. However, the equilibrium condition provides a starting point with which to incorporate various market changes, failures, biologic considerations, or institutional characteristics that move us away from the static, perfectly competitive market.

CASE STUDY: MARKET EQUILIBRIUM AND A SHIFT IN THE MARKET - PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV)

Livestock diseases often have major economic impacts. Tonsor and Schulz (2020) cite the potential impacts as significant threats at both national and international levels. An outbreak can result in increased morbidity and/or mortality, reduce productivity and decreased total production, resulting in an impact on equilibrium market price. Depending on the disease, the duration and impact can be short or long-lived. If short-lived the most likely impact would be on supply, as depicted in figure 2. A decrease in supply results in a shift to the left of the supply curve and an increase in price and a decrease in the quantity sold.

Consider the case of Porcine Epidemic Diarrhea Virus (PEDV), which manifested itself in the U.S. in 2013. PEDV is a coronavirus, with a high mortality rate, especially among pre-weaned pigs. Shulz and Tonsur (2015) estimated a 3.03% decrease in saved pigs per litter from September 2013 through August 2014. They report that during 2014, the price of pork increased over 10%, while per capita consumption fell less than 1%, which is consistent with a shift down in supply in Figure 2. The impact on the market can be seen in figure 3 (ERS, 2014), which presents U.S. hog prices and pork production from the first quarter 2011 through second quarter 2014. While there is a seasonal nature to pork production, the distinct impact of PEDV, is the decline in production between fourth quarter 2013 and second quarter 2014 and the steep increase in prices, consistent with a leftward shift in supply.

Figure 2. Shift in supply

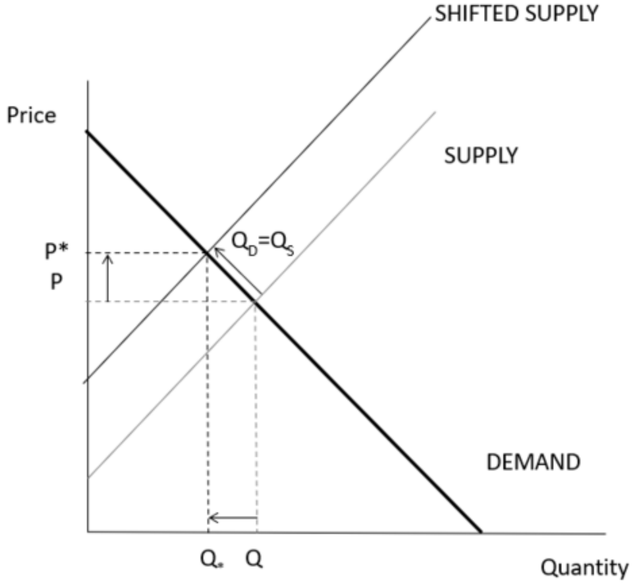
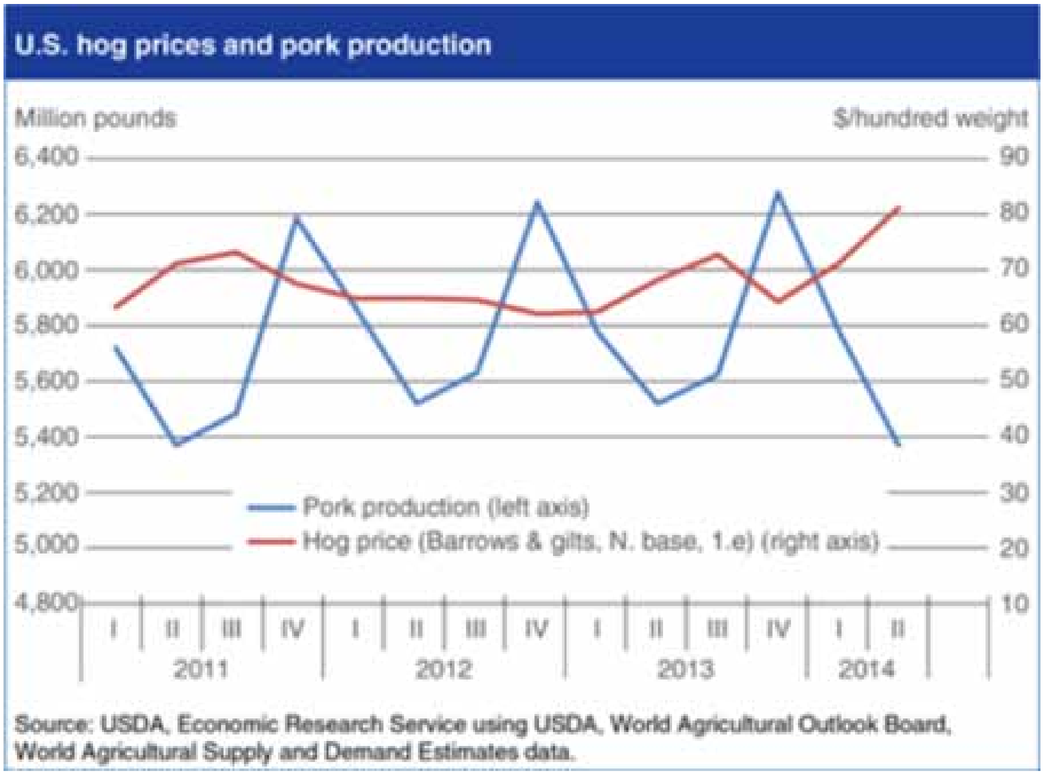


Figure 3. Production and prices



Source: USDA, Economic Research Service using USDA, World Agricultural Outlook Board, World Agricultural Supply and Demand Estimates data.

CASE STUDY: MARKET EQUILIBRIUM AND SUPPLY AND DEMAND ANALYSIS IN USDA APHIS' SPOTTED LANTERNFLY (SLF) PROGRAM

The spotted lanternfly (SLF) was detected in Pennsylvania in 2014. Affecting ornamental, fruit and woody trees, it is both a "hitchhiker" and short distance flyer moving from one location to another with the availability of host plant material like grapevines, maples, black walnut, and its preferred Tree of Heaven. The response by the USDA's Animal and Plant Health Inspection Service (APHIS) and its SLF Program is, from an economic perspective, one of basic "supply" and "demand" and "equilibrium."

The questions addressed by supply and demand analysis can be framed in terms of how much any responding governmental biosecurity agency, such as APHIS, and/or external and private sources of funding can afford for the program. The analyses determine the supply of program activities and balances that against demands and needs of the stakeholders. It is where supply and demand meet that the greatest return on that investment is likely to be found (Wakie, et al., 2020).

Thus, the question could be articulated as: what is the economic value of any phytosanitary measures given available resources? Or what is the value of making changes in an approach while maintaining level budgets? Such questions establish the scope of the economic evaluation without including an assumption of additional financial resources to address the problem.

With respect to the Spotted Lantern Fly (SLF), such basic questions can be addressed with monitoring, evaluation and quarantine data collected by APHIS Field Operations staff as well as program evaluations established by a team of analysts that develop models of potential SLF spread and compares that to estimates of treatment effectiveness.

A scenario approach is a useful way to add economic value analysis into alternative program evaluations by comparing treatment cost effectiveness to potential SLF spread. The idea is to simply collect all the costs of different treatment options and compare them to the effectiveness of the scenario in changing the dynamics of the spread of the SLF population. Three scenarios are considered here: status quo, control the spread, or eradication.

The Australian government (Agriculture Victoria, 2021) has implemented a process for systematizing the variables considered in an analysis like this called the analytical hierarchy process (AHP). This process (Saaty, 1995) has been applied to numerous public decisions with multiple stakeholders and the usual budget constraints.

1. **Status Quo** – over five years of experience and the data to support it indicate that current programs have reduced the rate of spread of SLF. However, they have not controlled the spread; only slowed the advance. From an epicenter detected in and around the city of Reading, Pennsylvania (PA), USA, in 2014, by 2019 most of the area infected by SLF in PA is in a rough circle centered on the town of Reading that covers 44,876 square kilometers (Pennsylvania Department of Agriculture, 2021a). The estimated average annual rate of spread in Pennsylvania from 2015-2019 is 27.2 km/year in all directions from the perimeter of a circular infected area in 2015. This led APHIS to create a 24,066-square kilometer surveillance band on the outside of the infected area, that extends outward 27.2 km from the perimeter of the core infested area. This surveillance band is taken to represent the area of potential spread of SLF for 2020. Basically, this tactic involves applying insecticides within the perimeter and monitoring outside the perimeter.

The cost of implementing this scenario was an approximately $30 million annual budget for SLF control for 2019 in Pennsylvania. That budget was associated with a calculated rate of spread as well as a calculated area protected as a result of monitoring and spray activities and the budgetary allocation needed for the control actually achieved. That is a key metric: how much area is being protected by containing and slowing the spread of SLF under current hand-sprayer treatment practices? This decision about control technology is the focus of the economic analysis of the program to control SLF.

Looking to the future and adjusting tactics within a fixed budget, may consider other technologies for controlling SLF spread. Area-wide aerial spraying from fixed aircraft is considered impractical because of potential drift of the chemical spray into population centers, such as housing, retail, and farming centers. Helicopter application of chemicals have the benefit of the downdraft produced by helicopter rotors that force the spray chemicals down into a smaller area, and into the branches and through the leaves of targeted trees. High-capacity sprayers mounted on pickup trucks were finally chosen for future applications beyond the status quo period for their combination of increased chemical application over hand sprayers used in previous years, with reduced risk of pesticide drift by wind disbursement into densely populated areas.

It is worth noting that new satellite infestations of SLF in Winchester, Virginia, and Western Pennsylvania appear to be related to the SLF's ability to hitchhike on rail cars when they are parked near the host trees of SLF. SLF has two sets of wings but hops more than flies between hosts; and appears to be able to hop on a stationary rail car. Rail cars themselves may offer a convenient form of transportation for SLFs when the rail cars stop and wait, before moving again, sometimes long distances. The economics of controlling the spread to satellite locations using rail lines has been treated as a high priority for future years, and more economically important than spread within an established territory just discussed. Within a fixed budget scenario, tradeoffs would be needed.

However, partnering with rail company executives, APHIS has recommended to adjust the time of trash tree removals along rail lines to more closely match the lifecycle stages of SLF. Thus, host trees like the Tree-of-Heaven (Pennsylvania Department of Agriculture, 2021b), along rail lines are removed before adults can feed on them. Removals have traditionally been scheduled and paid for by the rail company without considering SLF control implications, so change of timing will not necessarily add to the tactical costs of SLF control. This Status Quo scenario then considers the costs and benefits of SLF control measures taken through 2020, and changes to the program that are likely in 2021/22.

2. **"Control the Spread" focusing on high-risk commodities** – Long-term spread analysis from two different sources give fairly consistent results. Even though somewhat different data sources and analysis assumptions were used in these studies they are in 77% agreement (Gaydos, 2021). When these studies disagree, the disagreements were most often in low probability areas for SLF spread. The combined spread analysis from these studies indicates that despite Status Quo slowing the spread of SLF, agricultural commodities with high values will be impacted by SLF infestation in the next 10-15 years. The approach envisioned under this second scenario is to use economic and treatment efficacy analysis to prioritize future SLF program funding to avoid impacts on major commodities for as long as possible using an Analytical Hierarchy Process (AHP) (Agriculture Victoria, 2021).

The first step when using AHP is to define the alternatives to use in the evaluation. These alternatives could be the individual commodities or growing areas that need protection from SLF infestations.

Alternatives are used to evaluate the potential efficacy of possible solutions. The spread rate and direction have been considered with SLF spread modeling using geographical information systems (GIS) maps. Likely habitat and weather characteristics are used with suitability models to produce maps that show potentially impacted areas. Another approach is to use previous experience with insects that are believed to have similar spread characteristics to SLF like the brown marmorated stinkbug. The stinkbug spread rapidly through the East and Midwest of the U.S., before becoming established on the West coast. (Penca and Hodges, 2019). This approach to spread modeling has been known as informing analysis by analogy.

The second AHP step is to model characteristics of the problem. These characteristics are usually a related set of sub-problems. The AHP method therefore relies on breaking the problem into a hierarchy of smaller problems. For instance, there are often common elements to different locations that can simplify the problem analysis. Certain commodities are grown in different regions with some similar characteristics such as climate, topography, and soil type, in addition to the commodity itself as a likely host for SLF.

The way cost effectiveness and spread rate were evaluated under Status Quo, are also factors in the third AHP step. Under this "Control the Spread" scenario using AHP the focus is on how to cost effectively avoid the spread of SLF to certain growing areas and commodities. Here the emphasis is on control. It may be cost-effective to allow more of the SLF spread to occur into areas where higher value commodities are not grown, so more monetary and human resources can be devoted to controlling the spread of SLF from reaching higher value commodities.

The fourth step when using AHP is to establish priority activities using pairwise comparisons of the combined first three AHP characteristics. Each commodity may have several bundles of characteristics that might include the specific regions where high value crops like grapes, almonds, apples, walnuts, cherries, hops, peaches, plums and apricots are grown. These crops, produced in different regions are likely to have somewhat different growing area characteristics, that will influence the SLF spread rate in that area where the crop is grown, and the effectiveness of different treatments.

A recent geospatial analytical project that uses maps to forecast SLF spread, found that 50% of the U.S. crop of grapes, almonds, peaches, apricots and walnuts will be at medium or higher risk of SLF infestation by 2035. The crops with the highest production value at stake will be grapes, almonds and apples. These production values for crops potentially impacted by SLF can be used to weight different treatment and control strategies that would allocate different resources to protecting different crops. This should give an internally consistent strategy for controlling SLF based on crop value protected that can allow for the volume of rail traffic and rail node density, along with other crop and region characteristics.

3. **Eradication** – The third and final scenario considered is listed third because without economic considerations, it might be the default scenario for pest and disease treatment options. The problem with taking eradication as the highest priority without considering economic factors, is that eradication may not be attainable (Jones et al., 2021). The second "control" strategy may help prioritize the use of available funds, and eradication can remain an option if sufficient funding is available. The benefit of considering eradication as a scenario is that it can lead to future cost savings if SLF can be eliminated, and future program costs avoided. Costs to avoid reintroduction of SLF are however likely to remain a continuing expense, even after successful eradication. The cost of SLF eradication may be extrapolated from previous levels of control spending and success rates experienced. Eradication costs are simply the sum of all the anticipated costs to wipe out SLF in the United States; and keep it from coming back. The most useful approach to this scenario has been developed in Australia to eliminate entire populations of fruit flies from the continent and

is known as area wide management programs (Hort Innovation, 2018; Florec, 2010; Victorian Government, 2010)

In addition to potential future cost savings of avoided pest population control when carrying out pest eradication, there may also be trade-related benefits of eradication. Agricultural commodity exporters may face Sanitary and Phytosanitary (SPS) measures imposed by trading partners. The World Trade Organization recognizes the right of country members to protect themselves from the risks posed by exotic pests and diseases through the application of SPS (WTO, 2021). One possible response from exporting countries facing these SPS trade barriers is to obtain pest-free area (PFA) certification. PFA certification is certified eradication of a pest in an officially monitored and maintained commodity growing area that is free of a specific pest.

The welfare impacts of PFAs accrue to producers that avoid SPS restrictions, by investing in the costs of surveillance, monitoring and eradication protocols necessary to maintain area freedom certification. These efforts are supported by government organizations and/or producer interest groups supported by farmers and stakeholders. Growers may face reduced pest control costs and expensive post-harvest treatments such as fumigation or chilling prior to the supply of produce to export and domestic markets to offset PFA costs. Producers, participants in the supply chain, and consumers also benefit from increased crop supplies due to reduced pest damage and improved plant health. To weigh the costs and benefits of avoiding SPS trade barriers in this way, an analysis would need to compare production outcomes for a country or a region over a planning horizon with and without PFA certification.

Several other factors related to those already discussed are that fruit marketed from a PFA without post-harvest treatment, may be higher quality and command a market premium on both domestic and export markets. Another consideration is that a PFA may attract investment and establishment of new production capacity in the controlled area; or the reverse if a PFA is allowed to expire.

CASE STUDY: THE SOYBEAN RUST COORDINATED FRAMEWORK - INFORMATION FOR IMPROVED DECISIONMAKING IN FARMERS' RESPONSE TO SOYBEAN RUST

Soybean rust (SBR) caused by a fungus, *Phakopsora pachyrhizi*, has been a recurrent problem for soybean producers around the world [REF]. Until 2004, SBR reduced yields and raised production costs for soybean growers in every major production region of the world except the United States.

SBR, considered a dangerous threat to soybean production in the U.S., was first detected in the Southern U.S. in fall 2004, late enough in the season that it did not pose a threat to that year's soybean crop. After overwintering in the South, SBR posed a new, uncertain, and potentially large threat at the beginning of the 2005 U.S. soybean season. Fields infected with SBR were anticipated to see markedly reduced soybean yields if not treated with fungicides. Although SBR has the potential to cause significant yield losses, these can be almost entirely mitigated with application of fungicides, but at considerable cost (Roberts, et al., 2009).

Significant media attention heightened concerns that a major outbreak of the disease could occur in 2005. In response to urgent requests by the American Soybean Association and United Soybean Board, by May of 2005, USDA had initiated the Soybean Rust Coordinated Framework to track laboratory verified detections in the U.S. soybean crop and forecast its spread (Personal Communication, Kitty Cardwell, USDA NIFA).

With sufficient notice of an imminent SBR threat, farmers could treat their fields with preventative fungicides, if they were in a high-risk pathway. Another approach to the threat was to carefully monitor fields and immediately treat with curative fungicides once the disease was detected. Because curative fungicides must be applied immediately after first infection, this approach also benefits from timely information on the spread of SBR by allowing farmers to limit scouting to times when infection risks are highest. In addition to fungicides being costly, the efficacy of both preventative and curative fungicides is sensitive to the timing of application, which means that better information about the likelihood of infection helps farmers improve management decisions and increase profits.

Information was (and still is) collected and analyzed within the framework and communicated to the public via a website called the Pest Information Platform for Extension and Education (https://ipmpipe.org/) (Isard, 2005). The public website included a regularly updated map showing where field and test-plot monitoring by trained extension agents in 30 states had detected or not detected evidence of SBR; national and local commentary discussing the incidence and likely spread of SBR; and management strategies, often delineated by county. The framework also used a chat-based system to facilitate communication between the many experts, comprised from government and nongovernment agencies and universities, who monitor for SBR in soybean fields and sentinel plots strategically located throughout the country. USDA built and tested the new SBR-information infrastructure before SBR had caused any significant U.S. crop losses.

The website pictured below, was updated almost daily during the growing season, and was viewed about 4.9 million times in 2005. Approximately 4,500 users of USDA's SBR Internet website signed up for email alerts when new information, such as a new incidence of SBR in the U.S., was posted. This was the broadest USDA delivery over the Internet of information to provide plant pest or disease forecasts to farmers and other stakeholders up to that time.

By the end of the 2005 growing season, the rust had not spread north out of the deep south. This fact was possibly explained by the active scouting and fungicide spraying in southern states, reducing the potential amount of rust spores that could spread north. The main body of the soybean growing region of the U.S. and Canada had been entirely spared.

Estimating the Value of Soybean Rust Forecasts

How valuable was the information provided by the framework? This question became particularly salient in light of the modest outbreak of SBR during the 2005 season. Given the expense of developing the website and its underlying infrastructure, some have questioned whether the framework was a worthwhile endeavor.

Farmers' decisions are fundamentally different with and without information about the SBR threat. Without the coordinated framework, some farmers might have simply managed their crops as if there were no SBR, saving time and expense. With no website information but awareness of the potential problem, farmers must decide whether to spray or not without knowing if their fields could become infected with SBR. In this instance, farmers will sometimes spray when not needed and sometimes not spray when a spray is needed. Information about natural events will seldom be perfect, but it might be useful to think first about decision outcomes with a perfect forecast.

With perfect information, farmers can always make the correct decision whether to spray a preventative fungicide and will earn higher profits than with less information. The Economic Research Service (ERS) of the USDA conducted an analysis of the value of SBR information addressed several intermediate scenarios that allowed for less than perfect information and a wait-and-see (monitor-and-cure) treatment option. This richer analysis with less than perfect information about the SBR threat and another curative option in addition to prevention, it is possible that they could have fared as well as or better than they actually did in 2005.

Even with this richer set of options and payoffs for soybean producers, the scenarios had to acknowledge the widespread perception at the beginning of the soybean growing season in 2005, that SBR posed a threat throughout all growing regions (of unknown magnitude). This reality is difficult to capture because it is not clear how farmers might have prepared for that threat in the absence of the framework, which provided real-time information about local conditions and detections. It could not have been known in advance that optimal conditions for infection ultimately would not arise in most growing areas. Indeed, without the framework, individual farmers in some areas may have incurred even greater expenses in monitoring their own fields and perhaps spraying fungicides wastefully for a threat that did not exist. Without the framework, some farmers may have forgone planting soybeans entirely and planted a less profitable alternative crop.

ERS' analysis assessed the framework's value by estimating farmers' expected profits with and without the information from the framework from the beginning of the growing season. Making this calculation involved quantifying farmers' expectations about the likelihood of SBR at the beginning of the season—that is, how likely they perceived the SBR threat to be. It also involved evaluating what farmers' decisions and profit outcomes would have been without the framework.

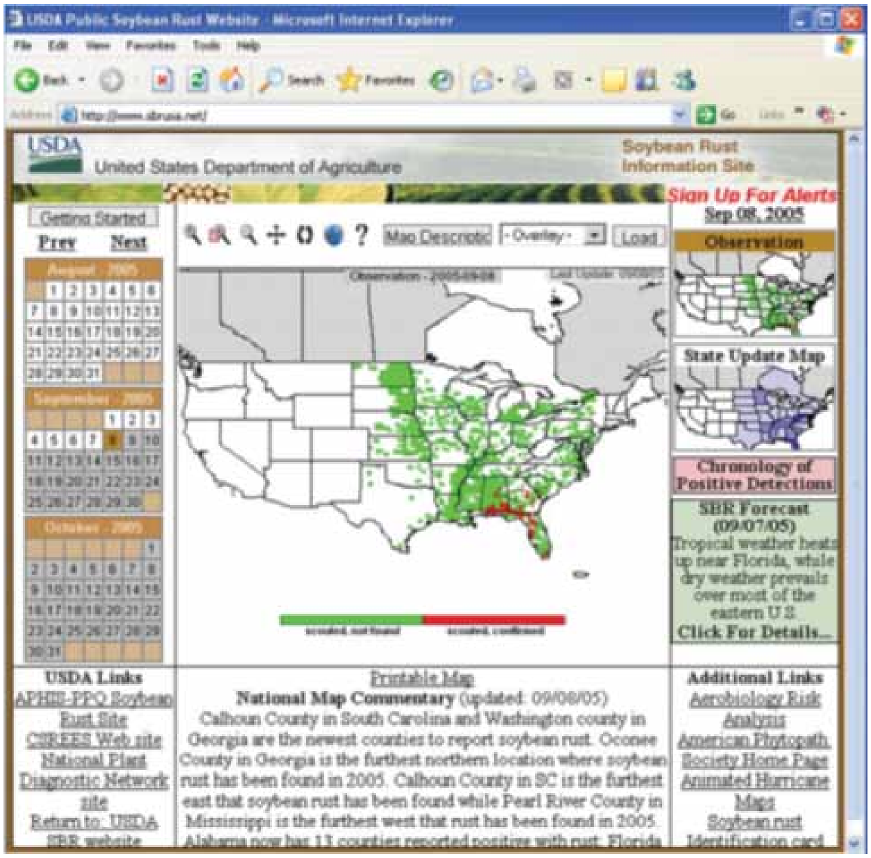
Across all scenarios and forecast accuracies considered, the ERS analysis found that the value of information from the framework ranged between \$11 million and \$299 million in fungicides not sprayed, or about \$0.16 to \$4.12 per acre (Roberts, et al., 2006). This value is made up of a combination of reduced expected costs and higher expected yields. This range of possible information values is small relative to total U.S. soybean production and sales (about \$16.1 billion, or \$214 per acre), but quite large relative to the cost of establishing the framework and protecting that large soybean crop. The framework's total development cost in 2005 was \$2.6 million making the benefits of the framework exceeded its costs by several orders of magnitude.

From an economic perspective, the SBR initiative is assessed from a public good or positive externality perspective (see glossary for definition). A public good is one that is both nonexcludable (the good is freely available to everyone) and nonrival (consumption by one person does not prevent consumption by another person). These goods are typically underprovided by private markets. Though there are relatively few true public goods, there are many goods that have some characteristics of public goods and may similarly be underprovided (positive externalities) or overprovided (negative externalities) relative to socially optimal outcomes.

A public good is thus one that cannot efficiently be provided by a private market - in this case, information about the SBR would not have been considered a valuable commodity for a private, profit motivated firm. This does not equate to lack of value, but rather, the nature of the good (information), which provides a positive externality to society. A positive externality is a benefit that accrues to someone outside of the private transaction. Conversely, a negative externality is a cost (see glossary for more detailed definition) that accrues to someone outside of the private transaction. This results in a deviation between the private market outcome and the societal outcome.

Graphically, the optimal societal outcome would be the equivalent of shifting the demand curve in figure 1 to the right. This would depict the difference between the optimal private market and optimal societal outcome. Conversely, if a negative externality is present the private market would produce too much at too low of price. Again, focusing on figure 1, the impact of a negative could be depicted by shifting the supply curve to the left, illustrating the impact on society. Expanding on the frame from the perfectly competitive outcome in figure 1. Figure 4 incorporates the impact of the externality by showing the spatial distribution of SBR.

Figure 4. Map for soybean rust coordinated framework



SBR Forecast
(09/07/05)
Tropical weather heats up near Florida, while dry weather prevails over most of the eastern U.S.
Click For Details...

National Map Commentary (updated: 09/08/05)
Calhoun County in South Carolina and Washington county in Georgia are the newest counties to report soybean rust. Oconee County in Georgia is the furthest northern location where soybean rust has been found in 2005. Calhoun County in SC is the furthest east that soybean rust has been found while Pearl River County in Mississippi is the furthest west that rust has been found in 2005. Alabama now has 13 counties reported positive with rust. Florida

For the SBR example, the downward sloping Marginal Benefit (MB) curve is equivalent to the demand for information, while the upward sloping Marginal Private Cost (MPC) curve is the supply of information that would be provided by the private market. As in many other cases of information supply, the supply of information on SBR has positive externalities, which means purely private provision would yield too little supply and too high a price. The ERS analysis concluded that the public information about SBR was still quite valuable even without a large outbreak, because it helped farmers make better decisions in managing their operations (Roberts and Schimmelpfennig, 2006).

Information is an unusual kind of economic good. It is not bought and sold in stores like apples, cars, or electronics devices, mainly because people can easily share or replicate information. As a result, markets do not always create and disseminate information as efficiently as other kinds of goods and services because it is hard for businesses to control access and charge all users. Without fair and reasonable methods to charge for the information, it might not be provided at all. Sometimes state, local and federal governments can step in and provide information, like hurricane or crop disease forecasts, that private markets do not provide.

Governmental agencies propose, approve, and implement regulations that create incentives for individuals and businesses to provide information they otherwise may not. For example, the Food and Drug Administration (FDA) requires “Nutrition Facts” labels on food products, which helps consumers make more informed dietary choices (FDA, 2018). In another program, the FDA monitors maximum pesticide residue tolerances established by the Environmental Protection Agency (EPA) (FDA, 2020).

Companies that produce and sell pesticides MUST provide explicit scientific evidence that when used as directed, the pesticide will not leave an intolerable amount of residue in food. These examples are a few of the many ways that government agencies can influence the creation and dissemination of useful information that might not otherwise be available without regulations on the production sector. Nevertheless, there has to be a balance between the social benefit of the information, the cost of delivering the information, and the profitability of the regulated industry

Also, information is not normally traded in competitive markets like consumer electronics. The value is difficult to determine because it involves examining decisions farmers might have made without the information and what the consequences of those decisions might have been. ERS estimated the value of public information from USDA’s SBR initiative by comparing farmers’ expected profits, as viewed pre-season, with and without the information. This value reflects the degree to which information allows farmers to adjust their decisions to suit the particular situation they might face. Estimating this value involves quantifying how large the perception of the threat by soybean farmers would have been without real-time detections and forecasts. It also involves evaluating what farmers’ decisions and profit outcomes would have been without the framework.

SOME CONCLUDING, CLARIFYING COMMENTS FOR THE SBR CASE STUDY

Information from the SBR framework was particularly valuable for SBR management, mainly because the incidence tracking, and movement forecasts aided farmers in their decisions whether to apply costly fungicides or not. Because of the high cost of monitoring SBR and applying fungicides, farmers would have wanted to apply preventative or curative treatments only if an SBR threat were likely. Without a forecast, they would have been more likely to spray when it was unnecessary and not spray when it was necessary. The lesson learned from SBR is that the more information influences decisions, the greater its value. This is true regardless of whether the information takes the form of hurricane forecasts, food nutrition labels, crop production forecasts, Internet searches, or SBR forecasts. Forecasters are using recent advances in machine learning as a tool for forecasting pest arrivals (Kumar, 2020).

Strategic Economic Behavior and a Negative Externality

An invasive species is described by Hanley and Roberts (2019) as "those introduced into a novel environment with negative environmental, economic, or social impacts." These impacts result in a negative externality, which is defined as a cost that accrues to individuals outside of the market transaction. Sudden Oak Death is a tree disease caused by the fungus-like plant pathogen *Phytophthora ramorum*. The disease was first recognized in the mid-1990s in Marin, California, and only kills certain oak species like coast live oak, *Quercus agrifolia*, and an oak relative, tanoak, *Notholithocarpus densiflorus* (USDA, 2021). Some groups highly value these tree species, and to others the disease creates space for their preferred trees. These external costs may give rise to potential strategic behavior to take advantage of those costs, or strategic behavior may result in external costs imposed on others.

The economic principles and cases to this point are based on optimal choices and outcomes where no individual can influence the outcome. That is, we assume a perfectly competitive market where no individual consumer or producer can impact the market, the good is identical, and consumers and producers have the same information. In reality, when these assumptions are not met, we move away from a perfectly competitive market to one where an individual, or group of individuals, can impact the outcome, because of the market structure, as well as market specialization. At the extreme, we could have a single supplier or consumer, resulting in a monopoly or a monopsony. This results in that single individual choosing the optimal outcome for themself and because of their greater market power, the market is impacted.

Examples of market power can be seen in various plant or animal commodities. For example, in poultry operations, broiler operation size has increased substantially, providing more power to individual producers, while cow-calf operations are more dispersed (MacDonald and McBride 2009). In the former, large-scale operations can provide the opportunity for individual producers to impact the market - especially, if they can act together. In the case of the more dispersed cow-calf operations, these operators have less impact on the market outcome. In addition to the number of producers, the structure of the industry is important.

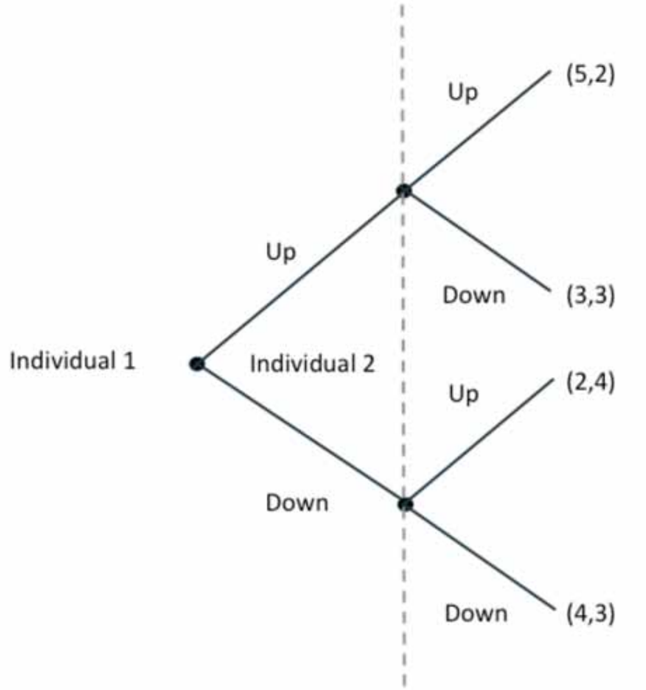
More applicable to many issues with invasive species, is the situation where there may be a few decision makers whose collective actions impact the market. The outcome for an individual depends on not only their choice, but the choices made by others as well. This allows for individuals to make strategic choices based on what they believe others will do to optimize their personal "payoff" given the externalities.

We can use results from a field of economics called Game Theory to model these strategic choices and interactions. The structure of the "game" depends on the characteristics of the game. For example, are decisions made simultaneously or sequentially? Is the game a series of repeated rounds or is it a one-time occurrence? If the game is repeated, does one individual have the opportunity to retaliate against the other, depending on the choice made in the prior round? Do individuals have prior knowledge of when the game ends?

A simple game is depicted in figure 5. This is a one-period game, where the decision makers make their choices sequentially. Decision maker 1 moves first and chooses either Up (U) or Down (D). After decision maker 1 makes their move, decision maker 2 makes their choice and can respond by either choosing U or D. The payoff to each individual depends on the sequence chosen. For example, if both choose U, then the payoff to decision maker 1 is 5 and the payoff to decision maker 2 is 2.

The optimal choice for each will be the move that results in their best payoff, given the choice of the other decision maker. Because the game is defined by sequential moves, 1 has an advantage and can consider 2’s choice given 1’s first choice. In this case assume that a large number is more desirable. If 1 chooses Up, then 2’s best response is Down, resulting in payoffs of 3 and 3 for 1 and 2, respectively. If 1 chooses Down, then 2’s optimal response is Up, resulting in payoffs of 2 and 4. Based on this, 1 chooses Up because a payoff of 3 is preferred to a payoff of 2. The outcome for the game would be Up/Down. This is a Nash Equilibrium, where 1 would not change their choice, if 2 didn’t, and 2 wouldn’t change their choice if 1 didn’t. That is, 1’s choice is optimal given 2’s choice and 2’s choice is optimal, given 1’s choice.

Figure 5. Game tree and payoffs



Most games are more complicated than the one described above and certainly strategic behavior in invasive species is more complicated, but the concepts can be applied to a number of examples from management of rangeland by multiple individual ranchers to companies involved in trade and imports of agricultural commodities.

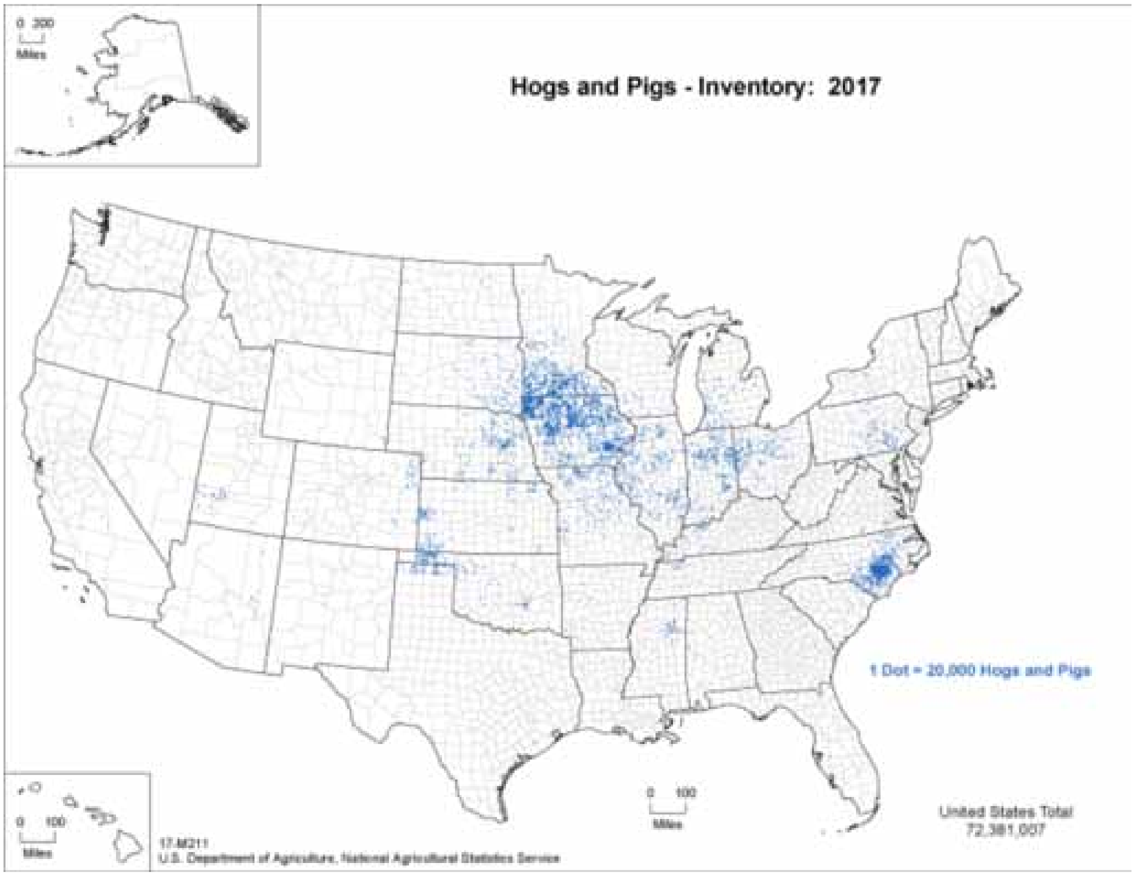
Game theory can be used to better understand the success of measures to reduce the spread of high-consequence foreign animal diseases or pathogens in the U.S. livestock industry (Ordeshook, 1988). The primary reason that strategic behavior is necessary for considering this question is that surveys show that if one of these diseases was introduced into the U.S., the swine, beef cattle, and dairy industries would not be likely to respond equally in the first year of introduction (Wu, et al., 2018). A factor identified in the study to influence adoption of control measures for high consequence pathogens in livestock is whether either the producer or a neighbor had ever personally experienced a high consequence foreign disease on their operation.

Other strategic factors identified that influence behavior include a producer's view on their own likelihood of experiencing a high consequence novel disease given their current situation, and a producer's view of the effectiveness of control measures in reducing high consequence disease risks. Availability of educational materials to explain high consequence disease risks and the benefits of risk mitigating biosecurity measures were found to be the least important factors influencing adoption and implementation of measures.

After combining these strategic factors with other characteristics of each livestock industry, the swine industry is likely to see the highest and the beef cattle industry the lowest biosecurity measure adoption in the first year of a large, high consequence disease outbreak. Risk reduction is shown to have a small positive effect on biosecurity adoption, and a firm's own risk reduction should be considered as well as their closest neighbor's risk reduction; and costs have a small negative effect on biosecurity adoption.

A key strategic reason influencing these partial adoption results may be tied to the level of vertical integration between sectors within the industry from production to processing to marketing. The swine industry is characterized by vertical integration with producers, either contract or independent, selling to packing companies, where the 10 largest companies account for almost 90% of pork (Dunn, 2017). The concentration of producers in specific areas of the U.S., as shown in figure 6 (USDA, 2017) has further enforced the vertical integration in the industry. This has resulted in uniformity across the industry and

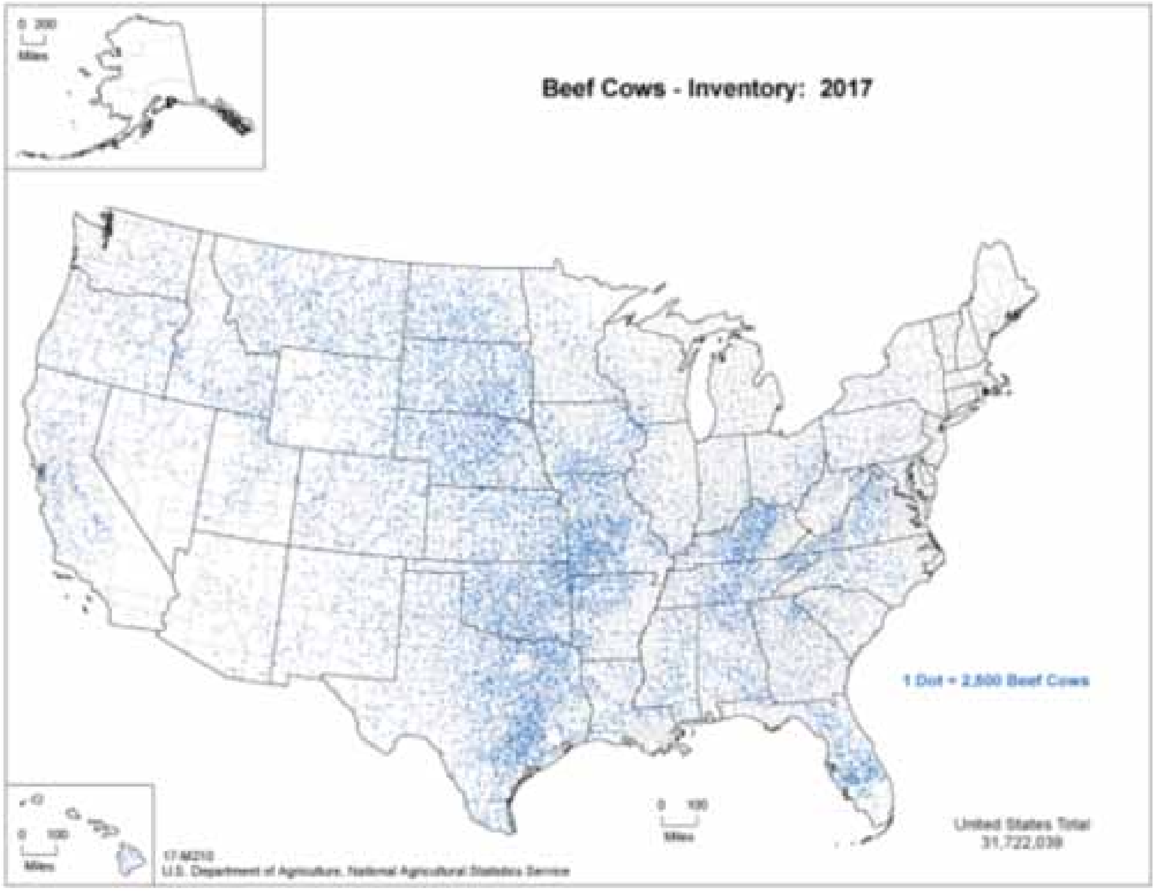
Figure 6. Hog and pig inventory



provides the packing companies with market power, resulting in contract producers adhering to biosecurity standards as a requirement of contract. Mitchell et al. (2019) find that contract producers have a statistically higher adoption rate for specific biosecurity practices. Thus, the biosecurity investment likely brings benefits primarily to downstream sectors in the supply chain and the contract producers may bear more of the costs.

The beef industry has a relatively low level of integration (compared to the swine industry) within in the industry, as shown in figure 7 (USDA, 2017). This can be attributed not only to a relatively large number of cow-calf producers with relatively small herd sizes (MacDonald and McBride, 2009), but also with the dispersion of the industry across the U.S. This reduces the potential for vertical integration and the potential influence of packing companies specific to biosecurity risks. This results in an industry where biosecurity adoption may be driven more by individual risk perception, rather than adoption influenced by other activities within a vertically integrated industry.

Figure 7. Beef cow inventory 2017



An application of economic principles of strategic behavior to animal and plant biosecurity is an example of how principles of game theory can help ensure that cost-effective approaches are used to support safe trade for U.S. agricultural producers of food and fiber, and to safeguard U.S. producers from pests and diseases that might enter the U.S. from abroad. The scope of this analysis includes all offshore, domestic, transportation, and other pathways where biosecurity requires safeguarding plants and livestock from pests and disease.

For plant pest and disease problem economic modeling, a potentially large factor that can impact pathways related to international trade, is ‘gaming’ the system or strategically manipulating the trade system. This can happen for a number of reasons, and these motivations for trade flows are seldom modeled but may present significant challenges for maintaining biosecurity.

Adding Strategic Behavior to Trade and Regulation Biosecurity Considerations

While economics provides optimal theoretical models of response (where the response could be from any individual or organization that operates within an economy), in real life, there are instances where a response is not consistent with the predicted optimal. There is a substantial literature focusing on the how choices are made strategically and are influenced by external factors that result in choices that deviate from the optimal.

Broadly speaking how information is presented is one category where choices can be impacted. Included in this framing, anchoring, bracketing, as well the amount of information provided. Barney and Tekeila (2020) raise a concern about the difficulties of presenting the ecological impacts of an invasive species when there may be both positive and negative economic impacts that can vary spatially. An example they cite is yellow star thistle, which has a positive impact on honey production, but a negative impact on rangeland health. Parsing through the pros and cons of the impacts may result in too much information, while simplifying may result in framing effects (included in glossary).

Other factors that can impact choices include uncertainty and updating, learning by doing, risk preferences, timing of a choice, as well as social norms, or fairness. Johnson et al. (2011) conducted a survey of ranchers in sagebrush steppe rangeland. The focus of the survey was on the management of an alien grass, Medusahead, which negatively impacts livestock forage production and has negative ecological impacts as well. They found participation in management was impacted by experience. Ranchers were more likely to participate in management if they had been negatively impacted by the grass.

This provides an example of uncertainty and a Bayesian update, where new knowledge is incorporated into the rancher’s choice, reducing the uncertainty. Dorfman (1997) provides a useful survey of the field of Bayesian economics and a guide to using an information updating framework for statistical econometric analysis and an approach to decision-making when prior information is available. Further, in the rancher’s survey, respondents indicated that the mode of information delivery was important, with most ranchers preferring information be provided in a pamphlet or face-to-face from a specialist. Web-based or videos were less desirable, suggesting the potential for knowledge delivery is also a potential external factor of behavior.

Finally, social norms have been found to be significant in the management of invasive species. Thacher et al. (2010), as well as Johnson et al. (2011) found survey respondents were more likely to manage an invasive species when more of neighboring ranchers were also managing the species. The former study focused on yellow star thistle in New Mexico (NM) and the latter on Medusahead, as previously discussed.

Trade Pathways, Regulations, and Strategic Behavior by Traders

Some of the most important economic factors influencing the biosecurity of international trade pathways, are different types of conveyances that can transport pests and disease agents along with international trade goods. The section discusses recent research articles that identify trade-related factors influencing the likelihood of invasive species and disease introductions. International trade has come under careful consideration recently as trade pathways have become increasingly numerous and complex as traders have worked to exploit possible gains from trade posited in influential work by Frankel and Romer (1999), Fisher (1993), and Samuelson (1939; 1962).

Early et al. (2016) find national capacities to prevent and manage species invasions differ greatly. They point out while only one-sixth of the global land surface is highly vulnerable to invasions, substantial portions of these areas occur in developing economies, which also may be biodiversity hotspots. Important for the U.S. and the mission of the Plant, Protection and Quarantine and Veterinary Services divisions of the APHIS of the USDA, is that for high-income countries, the dominant invasion vectors are trade in plants and pets. Research on externalities imposed by networks of trade activities provides helpful insights from economic analysis on international plant and animal biosecurity.

Balcana et al. (2009) propose human disease mobility network models and find that for realistic computational modeling of infectious diseases, it is important to consider both short-range commuting flows and long-range airline traffic in shaping spatiotemporal patterns of global epidemic transmission. These findings point to the importance for plant and animal protection considering both contiguous country trade flows often by rail and trucking, along with longer distance international trade of produce and products carried by air and on shipping.

Some of the economic factors that influence international trade flows beside commodity supply and demand, and tariffs and quotas in individual countries, are food safety and quality issues that might be subject to country-specific regulations. Differences in these regulatory requirements between countries can lead to tensions that can result in retaliatory regulations that restrict trade flows (Josling, et al., 2004).

Incorporating Time into the Economic Trade Model

In many cases, the cost, or security risk of a detrimental invasive plant and/or animal species, depends on the population of the invasive species, or level of infestation, which can change over time. While species dependent, often a logistic growth function is used to describe population growth. Lotka (1925) described biologic growth with the differential equation

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K}\right),$$

where N is population, r is the growth rate and K is carrying capacity or the occupied area, so (N/K) is the density of the species (Bacaër, 2011). The economic implications of logistic growth are substantial, as an environment may be able to absorb all or some of the negative impacts of an invasive species as long as the population at a point in time, is relatively small.

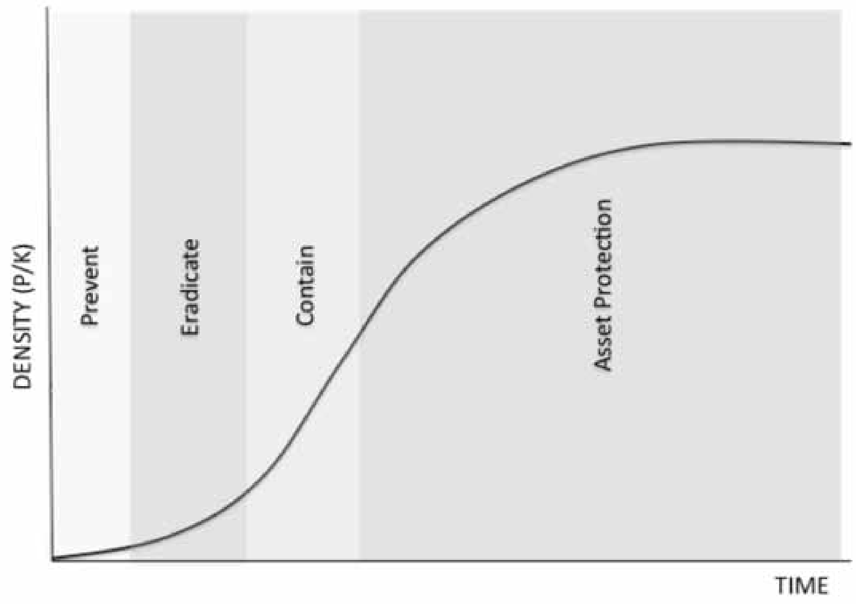
Note that this changes the impact of the invasive species on the simple, static result that the impact on production of the invasive species would now be $Q(N(t))$. This helps emphasize the importance of timing in economic management strategies, as well as that the optimal effort may change, depending on the population. This is because both the societal impact and the cost of the management strategy can change with population.

Foot-and-mouth disease (FMD) is a serious transboundary animal disease threat recognized as a priority problem in both Europe and the U.S. The disease could severely disrupt regional and international trade in livestock products on both continents. The U.S. has developed a Secure Beef Supply (SBS) Plan, which includes a list of enhanced biosecurity practices that aim to prevent FMD transmission and facilitate continuity of business during an outbreak. Critically important to the success of the plan is producers' willingness to be early adopters of the enhanced biosecurity practices included in the SBS plan. Even with willingness to adopt, the question remains of perceptions for U.S. cattle producers of feasibility-of-adoption and this likely also impacts the timing of adoption.

Recent research by Pudenz et al. (in review) shows early adoption of the thirteen U.S. SBS enhanced biosecurity practices is generally low. Even three strongly-recommended pre-outbreak practices had low potential adoption rates — having a biosecurity manager, having a written operation-specific enhanced biosecurity plan, and having a line of separation between different cattle operations. Adoption of pre-outbreak practices is likely to be low because the benefits of adopting the practices depend on a low probability, uncertain event.

Further delaying the timing of producer response to FMD is that producers who have not adopted the pre-outbreak practices are more likely to have lower feasibility ratings for the remaining enhanced biosecurity practices, suggesting that lack of adoption of the strongly recommended practices delays adoption of remaining biosecurity measures during an FMD outbreak. The reverse is also true and the adoption of the strongly recommended pre-outbreak practices helps facilitate a quicker and more effective U.S. cattle industry response to an FMD outbreak in the U.S.

Figure 8. Stylized invasion curve



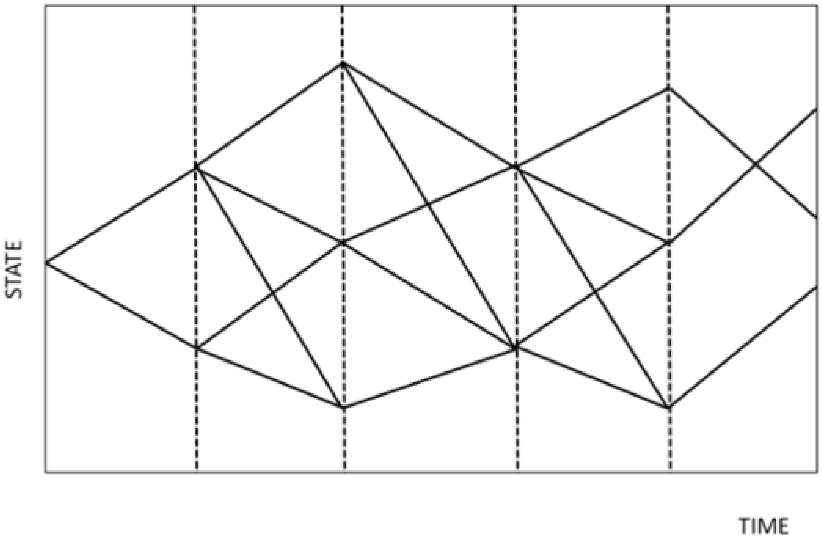
The basic relationship between the economic returns of management and the population can be depicted with the "invasion curve," in figure 8 (adapted from Fleming et al., 2017). As the density increases, the management objective and optimal response will change. Prior to invasion, preventing the species establishing itself in a region through education may be optimal. After introduction, at low densities, the optimal response may be eradication, followed by containment. At high densities, economic management may turn to protection of assets. The growth, chosen action, impact on growth, and timing of optimal action is often species specific.

Within a simple framework, economic management actions in a single period would have contemporaneous benefits (i.e., reduced security risks) and an optimal outcome would be similar to that depicted in figure 2, where the societal costs are incorporated into the market outcome. A complexity is that the cost structure may change as the level of invasion changes. If we assume economic management costs are related to the population, that is C(P), then as the population and density changes, so may the incremental cost of reducing the population.

The Economics of Dynamic Biosecurity

In a more realistic economic framework, the potential complexity of the bio-economics of invasive species maybe somewhat obscured by the invasion curve, as management actions taken today may impact the potential future states of the world. This means there is not a one-to-one mapping of benefits and costs in a single period to the current period, but instead, the benefits and costs are more appropriately considered over a period of time or management horizon. As the states of the invasion in each period are determined based on the management actions in the prior period. Figure 9 (based on Chang, 1992) illustrates this concept. Assume that we start at the left most point. This is an initial state of the infestation of an invasive species and the end result of the choices made over periods are found on the right-hand

Figure 9. Choice dependent paths over time



axis. The final "state-of-the-invasion" depends on the choices made over time. In some cases, taking one path precludes alternatives over time and dictates the end result.

The outcome depends on the choices made and where we end up depends not only the biology of the species, but also the economics and management alternatives. Dynamic optimization, or economic optimal control theory allows us to model systems where the future state of the system is impacted by today's choices. And today's choices are impacted by the objective. For example, an objective may be to maximize societal welfare over the management horizon and at the end of the economic management horizon, the level of infestation of an invasive specifies must be at or below an acceptable threshold. This now combines the economics with the biologic growth of a species.

CASE STUDY: WESTERN RANGELAND AS AN EXAMPLE OF A DYNAMIC ECONOMIC BIOSECURITY PROBLEM

Many invasive species do not become an economic problem until they reach a critical level. Focusing on the western U.S., there are a number of examples where a single invasive weed lies on a different point on the invasion curve, depending on the location. An example is Yellow Star Thistle (*Centaurea solstitialis*). Yellow Star Thistle (YST), a native of the Mediterranean region, was introduced to the U.S. in the mid 1800's - most likely intermixed with imported alfalfa seeds. It is now present in 41 states of the lower 48 states (Randall et al., 2017). It is classified as a noxious weed in 11 U.S. states, where up to 20 million acres are affected, and 2 Canadian provinces (USDA, 2019). The potential impact is not only on rangeland, but also on the animals that graze on infested lands (Tsynkevych, 2018).

YST's impact is mainly felt in a number of western states. Positive impacts from YST include late season pollen that can increase an economically valuable commodity to beekeepers. Immature YST plants also have value as forage. The negative externalities of YST are mainly focused on pasture, rangeland, and livestock. Overall forage value declines and YST can crowd out native plants.

Amongst the states most impacted is California (CA), with over 14 million acres impacted (Pitcairn et al., 2006) and exponential growth (USDA, 2019). In 2006, the economic impact on CA from YST was estimated to be between 6% and 7% of the total annual value of harvested pastureland in the state, which negatively impacts livestock (Eagle et al., 2007). The impact on livestock can be through reduced forage, as in the case of cattle, or through potential toxicity, as is the case for horses. While it varies, depending upon the time of year, rangeland infested with YST has less crude protein and digestible nutrients than un-infested rangeland, resulting in weight loss for cattle (USDA, 2019). Long-term ingestion of YST by horses is linked to a neurologic disease (nigropallidal encephalomalaci), resulting in an on-set of systems similar to Parkinson's and, ultimately, death (Chang et al., 2012).

Eagle et al. (2007) conducted a survey of CA ranchers, with the objective of analyzing the economic impact of YST rangeland, and on ranch revenues. The survey targeted ranchers in three important ranching and agricultural counties but also included responses from across the state. The results from this study include an estimated impact of 15.3% reduction in forage yield from YST on native land and a 12.8% reduction yield on non-native improved pasture lands. The direct impact of these reductions included selling animals, as well as purchasing additional forage. As the forage loss increased, the probability of these actions increased as well. In addition, the majority of ranchers reported actively trying to control YST through chemical applications, mowing, timed grazing, cultivation, prescribed burns, or biological control.

Of note, some survey respondents indicated that there was also a non-forage, or “non-market” value of the land to them. Eagle et al. estimate the impact of reduced forage in 2003 to be between $5.7 and $9.9 million, with out-of-pocket expenditures for YST control between $4.9 and $13.9 million. In total, the impact on ranchers was estimated to be between $10.6 and $23.8 million. While not at the level of CA, Oregon, Idaho, and Washington also have substantial acres impacted (USDA, 2019). As you move eastward, the level of impact lessens. This illustrates two economic aspects.

The first is the variation we might expect as to where different states would fall on an invasion curve. CA falls furthest with management plans that fall somewhere between containment and asset protection portion on the invasion curve. States like New Mexico (NM), Colorado (CO), or Montana (MT) and Canadian provinces would fall towards the left on a curve.

Because of the variation in infestation levels, the optimal management choices across the states could vary dramatically. Historically, management strategies for YST include chemical, mechanical, cultural, and biologic control (USDA, 2019). A difficulty in the management of YST is the cost of management relative to the annual value of the forage, as is indicated in Eagle et al. (2007).

Both CO and MT have ongoing eradication programs in order to prevent YST from establishing in the states. CO (Colorado Department of Agriculture, 2015) provides an educational pamphlet describing YST and an integrated management plan that focuses on cultural control by establishing competing native species and mechanical (hand) removal. Biologic control is explicitly not included as part of the management plan.

CA has promoted integrated management plans that include mechanical, cultural, biologic, and chemical control (DiTomaso et al., 2006). The optimal plan is site specific, depending on the level of infestation. However, the success of these plans may be questionable. The USDA (2019) has proposed the introduction of a weevil, Ceratapion basicorne (Illiger) (Coleoptera: Apionidae) to combat YST in CA. An argument for the introduction of the weevil is given as “*Conventional control strategies have been inadequate because of the size of the infestation, economic and environmental costs of herbicides, and the relatively low monetary return from grazing and recreational land use*” (USDA 2019, p. 2).

The second economic aspect illustrated by YST is the economic dynamics of the problem, which follows from the above quote. The management actions described above can impact the level of infestation of YST through impacting either the population itself (e.g., mechanical removal) or through the growth rate (e.g., chemical treatment). This results in the cost being borne today for an expected future benefit (reduced expected future economic impacts of YST). At low levels of infestation, where eradication (prior to economic impact) may be optimal, the current cost can outweigh the current benefit. For individual ranchers, investment in management of YST may be low on their economic priority list when faced with budgetary constraints as investments with more immediate returns are preferred (Thacher et al., 2010).

The spread of YST across the landscape adds an additional complexity to economic management choices. Similar to the single period game introduced earlier, the spread of YST from one plot of land to another requires management that is either coordinated, or at least takes into account the actions of others, as those actions can impact the spread. And, specific to individual action, Thacher et al. (2010) found the individual ranchers in NM would be more likely to manage YST on their land, if their neighbors were as well.

The case of YST draws together several aspects of the need for economics to be considered in management plans. Outcomes in CA and knowledge gained in the last three decades, suggests coordinated management may be more effective. And states starting early with eradication plans rather than containment may reduce the long-term harm from YST. While the cost of eradication may exceed the current benefits, the future benefits need to be considered. The development of an ex-ante management plan that considers the biologic and economic dynamics, the behavioral, the game theoretic aspects, as well as the basic costs and benefits, may provide more effective, longer-term solutions.

INCORPORATING RISK INTO ECONOMIC ASSESSMENTS

Park and Shapira (2017) defined risk as "*the situation under which the decision outcomes and their probabilities of occurrences are known to the decision-maker*," and they define uncertainty as "*the situation under which such information is not available to the decision-maker*." Throughout this chapter we've considered outcomes under certainty and yet, biosecurity is fraught with risk, as there are multiple factors that can impact the efficacy of any choice and, consequently, the resulting outcome. Incorporation of risk into economic assessment will depend on the assessment, but as a starting point, if the future outcomes are known as are the probabilities of occurrence, then the expected value can be incorporated into an analysis.

Consider the case where there are two future states of the world A and B. State A occurs with probability p and state B occurs with probability $(1-p)$. If the value of A is \$X and the value of B is \$Y, then the expected value (EV) is $EV=p\$X+(1-p)\$Y$. For example, if a management plan had a 60% probability of being successful and a net benefit of \$1 million and a 40% probability of no success, with cost of \$500,000, the expected value of the project would be \$400,000 (EV= (.6*\$1,000,000) +(.4*(-\$500,000)) =\$600,000-\$200,000). This type of analysis can provide more accurate evaluation the cost of outcomes, provided the information is available with which to develop the analysis.

POLITICAL CONSIDERATIONS FOR ECONOMIC BIOSECURITY CHOICES

The above discussion and examples are presented from a policy perspective and incorporating economics into assessing biosecurity policy. This does not consider, however, political aspects of biosecurity choices, which can compound the complexity of decisions and available paths. There are a number of factors that may be of importance, some of which are discussed below.

As shown in the above examples, biosecurity policies may be dynamic in nature and an optimal policy may best be developed over a period of time. In the case of YST, an optimal policy may have required action prior to noticeable, economic impacts and policies could focus on the impact of animals, e.g., re-sizing herds, or purchasing additional forage, or on management or eradication of YST In the U.S., as in many countries around the world, budget requests are for the fiscal year and agencies cannot expend funds that have not been authorized and appropriated. Policies in place may be impacted by a change of the party in power, as well as by external events impacting budgets.

Elected officials may have as their relevant time horizon, their election cycle, while optimal management policies may be substantially different. Policy developed on an election cycle time period, rather than on sound scientific inquiry may result in short term impacts but may lack meaningful long-term outcomes. This is not a new phenomenon and was termed the "political business cycle." Choices made concerning biosecurity may also be a part of a larger political action where biosecurity is either a small part of the objective or, in some cases, a part of the constraint. These issues are compounded on a global scale (Norton, 2020)

For example, in the U.S., the political objective of smaller federal budgets and smaller government, results in moving obligations, decisions and choices to a state, local or individual level. State requirements for balanced annual budgets and an objective of invasive species management may be at odds with smaller state budgets and multi-year management plans (Funk et al., 2012).

At an international level, trade liberalization policies may be counter-productive to reducing the introduction of alien species; Levine and D'Antonio (2003) find a relationship between trade and accumulated invasive species. Seebens et al. (2017) suggest an exponential increase in first records of invasive species over the last 200 years with more that 37% of all first recorded species reported between 1970 and 2014, suggesting an inconsistency between trade and invasive species policy.

Regardless of the reason, the politics of biosecurity may impact the policies of biosecurity and the management choices made. Incorporating political choices into economic assessment provides an additional layer of complexity to wickedly difficult problems that may best be solved through the simultaneous consideration of science, economics, and politics.

DISCLAIMER

The views expressed in this chapter are those of the authors and should not be attributed to APHIS or the U.S. Department of Agriculture.

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KEY TERMS AND DEFINITIONS

Command-and-Control Environment: A type of regulation that sets the outcome and mandates the technology that must be used.

Framing Effects: The presentation of options with a positive or a negative connotation. The context within which problems and solutions are presented often impact how positive or negative they are perceived to be.

Marginal Benefit: The incremental benefit, or the benefit of one additional unit of a good or service.

Market: A mechanism that allows buyers and sellers to strike an agreement on price and quantity traded.

Market Power: The ability by participant, or group of participants to influence the outcome of a market.

Market Structure: The characteristics of a specific market that impact how prices are determined and if any participants in the market have market power.

Monopoly: A market structure where one producer or firm produces and supplies all the product to a market.

Monopsony: A market structure where a single consumer is the only buyer of the good in a market.

Nash Equilibrium: A stable state of interactions between participants where the outcome for each participant depends on their choice as well as others' choices and no individual will be better off switching their choice if no one else switches.

Negative Externality: A cost that accrues to a third party outside of a private market transaction.

Optimal Level of Detection: For budgetary reasons, there will likely be a low level of detection that is considered acceptable. An optimal level of detection balances the costs of additional detections against the damage caused by the remaining pests or pathogens.

Perfectly Competitive or a Price Taking Market: A market where consumers have no ability to influence prices they pay, and suppliers have no ability to influence the prices they receive. Prices are determined in the market and are taken as given by consumers and suppliers.

Pest and Disease Exclusion: Actions and procedures used to intercept pests and diseases before they become established in a country or jurisdiction.

Positive Externality: A benefit that accrues to a third party outside of a private market transaction.

Vertical Integration: An arrangement a single company operates or controls multiple activities within the supply chain.

Chapter 2

Genetic Processes Facilitating Pathogen Emergence

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ABSTRACT

The goal of biosecurity is to minimize the risk of introduction and transmission of infectious diseases to people, animals, and plants. This is achieved by accurately identifying pathogens and instituting appropriate methods to prevent their introduction, reemergence, and/or spread. However, disease is dynamic, and biosecurity needs to continually change to keep pace as pathogens evolve. As described in this chapter, a basic understanding of evolution is central in considering how genetic changes and their associated phenotypes can alter the disease presentation of pathogens. In addition, evolution leaves a trail of genetic information that can be leveraged to inform biosecurity because the spatiotemporal patterns of these past changes provide clues as to how the pathogen might be spreading. This chapter aims to provide insights into how various genetic alterations occur, the background on how these are informative for biosecurity, and illustrations of applications to real-world examples. Evolution underlies the abilities of pathogens to adapt, emerge, and to cause epidemics.

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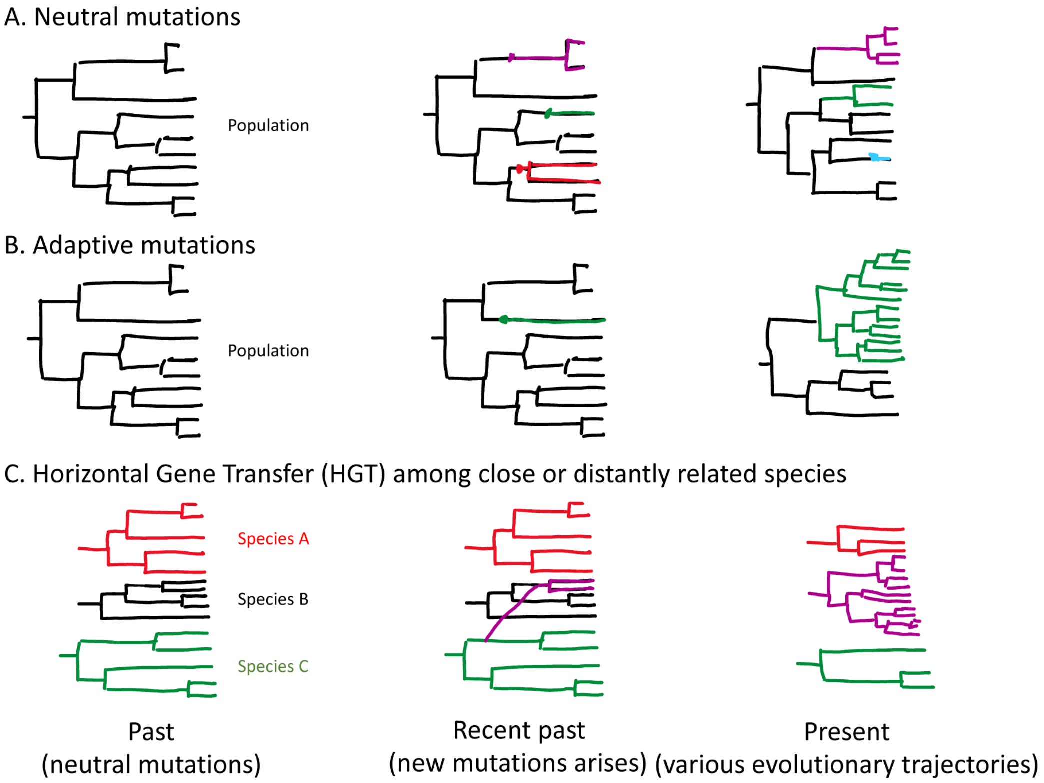
INTRODUCTION

The goal of biosecurity is to minimize the risk of introduction and transmission of infectious diseases to people, animals, and plants. This is achieved by accurately identifying pathogens and instituting appropriate methods to prevent their introduction, reemergence, and/or spread. However, disease is dynamic, and biosecurity needs to continually change to keep pace as pathogens evolve. "Nothing in biology makes sense except in the light of evolution," is an oft-repeated quote from the eminent American geneticist Theodosius Dobzhansky (Dobzhansky, 1973). This sentiment holds true for biosecurity, as pathogens are constantly emerging and evolving. As described in this chapter, a basic understanding of evolution is central in considering how genetic changes and their associated phenotypes can alter the disease presentation of pathogens. In addition, evolution leaves a trail of genetic information that can be leveraged to inform biosecurity because the spatiotemporal patterns of these past changes provide clues as to how the pathogen might be spreading (Koblentz, 2010). This chapter aims to provide insights into how various genetic alterations occur, the background on how these are informative for biosecurity, and illustrations of applications to real-world examples.

Evolution underlies the abilities of pathogens to adapt, emerge, and to cause epidemics. Evolution occurs when genomes and their corresponding phenotypes change over time and when these changes become established in a **population** (for words in **bold**, see glossary for definitions). Genetic information is encoded in one of two classes of nucleic acids, DNA or RNA, and must be replicated to create new progeny. During replication, inheritable changes, e.g. **mutation**s, can be passed on to subsequent generations. Thus, mutations accumulate in populations over time creating alleles, namely variations of the same gene in the population. Mutations occur within an individual, but evolution occurs in a population of individuals with varying mutations. Many mutations are neutral, meaning that they have no effect on the resulting RNA or protein products and have no measurable effect on the **fitness** of an individual (Figure 1A). Others lead to truncations or loss of function in proteins that can make individuals that inherit the mutations less fit. However, a few of these mutations might confer a fitness advantage by, for instance, changing the activity of a protein to work in new ways. Individuals that inherit the beneficial mutations gain an advantage that may allow them to eventually outnumber other individuals in the population that do not possess the advantageous phenotype (Figure 1B). This is the process of natural selection where individuals with favorable genes and alleles increase in abundance in a population. Natural selection occurs in all populations of organisms, including viral, prokaryotic, and eukaryotic pathogens of animals and plants. The genomes of some viral pathogens consist only of RNA, but the principles of selection and evolution are the same.

The genomes of pathogens change for a variety of reasons, including simple errors during replication. Mutations, when they occur in regions that code for functional products like proteins, can be *silent*, which is when the change results in encoding for the identical amino acid, or they can be a *missense* mutation, where the changed sequence then codes for a different amino acid (Figure 2). Missense mutations are a major mechanism for functional changes and evolution. For example, a bacterium might be able to feed on a substrate that previously could not be utilized as a food source. Or if the mutation changes the amino acids on the external domain of a surface antigen to a different amino acid, would not be recognized by antibodies targeting the original antigenic **epitope**. In this case, the missense mutation would be under ***positive*** **selection**, since any pathogen that possesses the surface amino acid change would be able to escape the host immune response and outgrow others without such a change. Last are nonsense mutations where a sequence that once coded for an amino acid leads to a stop, thereby prematurely truncating the

Figure 1. Pathogens evolve in many ways. Here, we illustrate three common scenarios including neutral mutations, adaptive mutations, and horizontal gene transfer. A. Most mutations (red, green, purple, cyan) do not affect fitness of an individual. These mutations are called neutral mutations and can be very useful in tracing migration of a pathogen genotype/lineage. B. Rarely, novel mutations can be beneficial (green). Beneficial mutations are under selection and become abundant in a population and displace other older lineages. In A and B, inheritance follows a vertical pattern, from parent to offspring C. Horizontal gene transfer (HGT) can move genes or a combination of genes (e.g., plasmids in bacteria shown in purple) among different species (red, black, and green). In this example Species B obtains a plasmid transferred horizontally from species C that provides novel capabilities and results in strong selection for presence of the plasmid in the purple sub population. The purple subpopulation of the black species with the HGT is fitter and displaces the black population by natural selection.

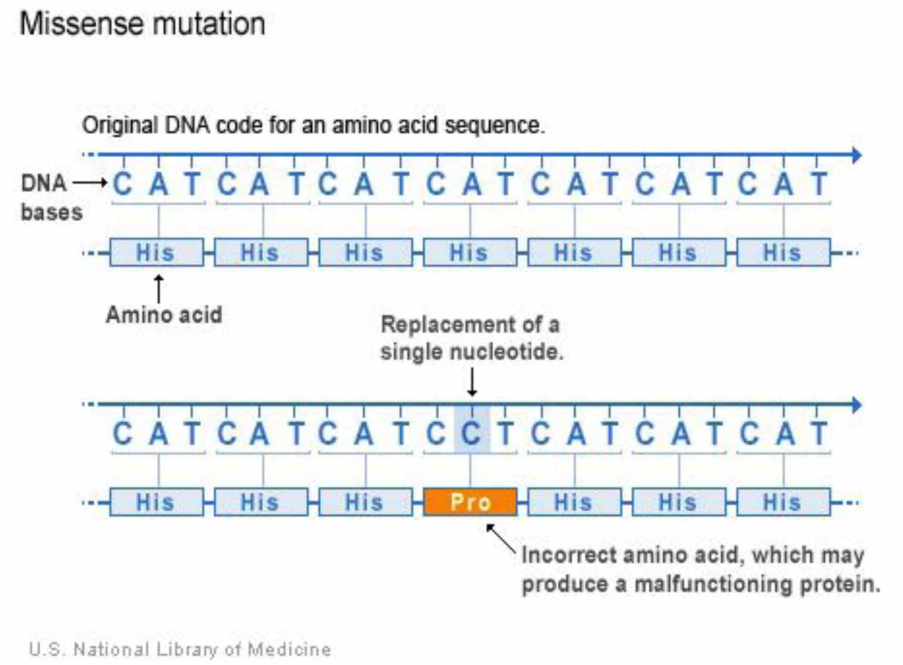


protein. These types of mutations can significantly compromise the function of the protein depending on the location of the premature stop codon.

Horizontal gene transfer (HGT), which is sometimes referred to as lateral gene transfer, is another process that fuels the evolution of pathogens (Figure 1C). In this process, fragments of DNA are exchanged in a pattern that deviates from the traditional vertical parent to offspring path. On the one hand, the transferred regions can result in subtle changes such that recombination can occur and displace the original gene sequence, leading to allelic variation, similar to mutation. On the other hand, HGT can introduce few to many new genes in a single step that provides the recipient with novel functions, such as virulence, symbiosis, and new metabolic potential that allows organisms to adopt new lifestyles and

Figure 2. Example of a nonsense or missense mutation that creates a stop codon which disables translation of the full protein sequences and thus can disable gene function commonly occurring in natural populations of pathogens

(Source: U.S. National Library of Medicine; https://ghr.nlm.nih.gov/primer/mutationsanddisorders/possiblemutations)



invade new environments. Thus, HGT is an extremely powerful evolutionary process because DNA can be exchanged between genotypes within the same species, strains of different species and even between organisms of different kingdoms. Such an exchange of genetic material can provide a fitness advantage more quickly than the gradual accumulation of point mutations.

HGT represents a major concern for biosecurity. Clinical and agricultural environments promote the intense mixing of diverse pathogens and provide increased opportunities for HGT to occur. Studies have shown that virulence genes of bacteria can be transferred between species within a genus and antibiotic resistance genes can be spread between species across different genera (Conlan et al., 2014; Weisberg et al., 2020). The potential to constantly generate a diverse pool of pathogens means that treatments in clinics and management strategies in agricultural settings will select for pathogens with new combinations of genes, some of which may overcome the treatments (Mutreja et al., 2011).

Host-pathogen interactions also have major impacts on pathogen evolution that influences biosecurity. Hosts can include plants, animal, insect, or any other vector. Pathogens and their hosts are constantly coevolving in an evolutionary arms-race. Host immune systems are strong selective pressures that shape the evolution of pathogens (and their hosts). In terms of biosecurity, the hosts of concern here are primarily vertebrate animals and plants. Though there are fundamental differences in the mechanisms by which their respective immune systems work, there are important similarities in how they affect the evolution of pathogens.

Animal immunity consists of two systems that cooperate to differentiate between self and non-self. The innate, or early, immune system consists of a collection of non-self-recognizing molecules, called pattern recognition receptors, that are encoded by genes present in the genome and "fixed" in each individual (Buchmann, 2014). Pattern recognition receptors detect conserved motifs of potential pathogens and activate cells to help clear the body of these potential pathogens. However, pathogens have evolved diverse mechanisms to avoid, co-opt, or even incite the innate immune response to promote their infection (Beachboard & Horner, 2016; Finlay & McFadden, 2006; Reddick & Alto, 2014). The second system is the adaptive immune system, which consists of antibody (B-cell) and cell-mediated (T-cell) immunity (Boehm & Swann, 2014). A key feature of this system is that recognition is not from a fixed set of receptors; B- and T-cell populations are diversified throughout the life of the individual. Antigens derived from non-self-entities are presented and can lead to the expansions of subsets of B- and T-cells that recognize them. This activates a clearance mechanism and results in a so-called memory that protects the animal from subsequent infections by the same genotype of pathogen. Vaccination relies on this principle by priming the immune response of the host for recognition of the pathogen. Because microbes have large population sizes and short lifecycles, they can evolve more rapidly than their hosts, and evade detection by host memory and thus require a new immune response from the host. For certain pathogens, annual vaccinations are thus required, as is illustrated by the influenza virus (van de Sandt et al., 2012).

In contrast to animals, plants do not have circulating cells and do not clear the plant body of potential pathogens. Instead, plants dramatically slow the growth of pathogens and limit damage. Their immunity consists of two systems, both of which are forms of innate immunity (Jones & Dangl, 2006). Pattern-Triggered Immunity (PTI) is the most analogous to animal innate immunity in that PTI relies on pattern recognition receptors that recognize conserved motifs of potential pathogens. Activation of PTI leads to a variety of responses that collectively limit growth of the pathogen. As is the case of animal pathogens, plant pathogens have evolved mechanisms to overcome PTI. One of the more intensively studied mechanisms is the type III secretion system of bacteria. This system injects effector proteins into plant cells to interfere with PTI (Büttner, 2016). The second form of plant immunity is Effector-Triggered Immunity (ETI). As the name implies, ETI directly recognizes effector molecules of pathogens or indirectly recognizes the action of the effector molecule. This form of immunity is mediated by plant resistance or "*R*" genes, which are commonly used in breeding programs to develop resistant cultivars of plants. ETI confers upon plants the ability to mount an immune response that is faster and stronger than PTI. In addition, ETI is often associated with a localized cell death called the hypersensitive response (HR), which is a form of programmed cell death of host cells.

Regardless of differences between animal and plant immune systems, they are both strong forces that shape pathogen populations. Lineages that escape detection by the host immune response are concerns for biosecurity for three main reasons. First, diseases that were once thought to be controlled by resistant host populations may reemerge. Second, these lineages are new genetic variants which may require new tools for detection. Third, new genetic variants may jump to new host species or evolve greater virulence towards their original hosts.

The mode of reproduction also heavily influences the evolution of pathogens. For example, pathogens can exist as both clonal or sexual populations. Clonal pathogens asexually reproduce without recombination and therefore, with the exception of point mutations, which accumulate slowly, transmit the same genotype (e.g., the overall combination of genetic material) across generations. In contrast, sexual recombination shuffles genetic material and each progeny of sexual reproduction has a unique combination of the genetic material derived originally from parents. Between eukaryotic pathogens varying in these modes of reproduction, those that can recombine sexually are thought to have higher evolutionary potential and have a higher risk for developing a more aggressive pathogen (McDonald & Linde, 2002).

A case in point is the potato late blight pathogen *Phytophthora infestans*. This pathogen exists as a clonal population in most of North America, Europe, Africa, Asia and South America. However, in central Mexico this pathogen reproduces sexually and is predicted to be the source of new **clonal lineages** that emerged, migrated to the USA, and displaced older lineages (Fry et al., 1993, 2015; Goss et al., 2014).

Methods Used to Characterize Pathogen Emergence and Evolution

A cornerstone of biosecurity efforts is the ability to accurately identify a pathogen. Traditionally, this has involved culturing and using morphological traits and biochemical tests to identify the organism. Advances in molecular technologies over the last few decades have dramatically changed the methods used for pathogen identification. Both DNA- and RNA-based methods have greatly enhanced the specificity, sensitivity and speed with which this can be accomplished. Advances in nucleic acid sequencing technology now allow for the rapid identification of specific mutations (Martin et al., 2000; Miles et al., 2021). Combining the record of genetic mutations with sophisticated analytical tools on populations then allows inferences on the patterns and processes giving rise to those observed today. Understanding patterns and processes shaping pathogen populations is foundational to biosecurity. Such knowledge can be used to inform strategies aimed at controlling pathogens and restricting their transmission (Deng et al., 2016).

Traditional and contemporary methods used to detect pathogens are extremely diverse, but they are all founded on similar principles. They identify genotypes and/or **phenotype**s that discriminate a pathogen from a non-pathogen or one lineage from another. The polymerase chain reaction (PCR) is a fundamental method. PCR can be used to quickly amplify short regions from the genome of a pathogen so that it can be readily detected even without having to culture the pathogen. PCR can be a quick method if a unique and specific nucleic acid sequence of the organism is known and diagnostic of its identity as a pathogen (Martin et al., 2000). Antigen or antibody-based methods are another form of diagnostic tests that rely on unique phenotypes. The *Streptococcus* test in the form of a dipstick is one such example. The test uses an antibody that specifically recognizes an antigen on the surface of group A *Streptococcus* bacteria and a positive result then allows health care providers to prescribe the appropriate antibiotic against these bacteria.

Some PCR-based methods rely on detecting allelic variants of genes that can be used to infer, but not directly discriminate, pathogenic genotypes. In these methods, PCR is used to amplify a region of the genome that is common among microbes. The product is subsequently sequenced to determine the DNA sequence. If we take the example in figure 1A, sequencing PCR products could be used to identify strains with neutral mutations that distinguish between purple, green, and red lineages. The advantage of this approach is that the neutral mutation needs to only correlate with pathogenicity and the causative genes do not need to be known. However, this approach is susceptible to technical artifacts and the possibility that other unrelated lineages have experienced identical mutations. One way of mitigating these problems is to analyze multiple genes or interrogate multiple regions of the genome.

Significant advances in technologies, dramatic drops in cost, and development of computational tools have made whole genome sequencing (WGS) a powerful method for informing biosecurity (Cooke et al., 2012). Whole genome sequencing captures most or all of the genetic information to discriminate pathogens or lineages from each other, track their movement, and inform on management strategies. Analyses can be carried out to infer vertical (Fig. 1A and 1B) and horizontal (Fig. 1C) evolutionary processes and thus to draw well-supported conclusions on disease transmission. Because mutations accumulate over time and separate into lineages, analyses can be used to identify clusters of pathogens that have similar or identical genome sequences to inform on recent transmissions and enable a rapid response to slow disease spread (Jackson et al., 2016). WGS can simultaneously be used to identify genes, such as those that confer resistance to antibiotics, that provide valuable information on effective treatments.

Capitalizing on the power of massively parallel sequencing methodologies and instruments initially developed for WGS, a recent variation is metabarcoding and metagenomics. Instead of sequencing the complete genome of an organism, sequencing of shorter genetic markers that can be used to identify organisms are used. In this way, the genetic markers function like molecular barcodes and can not only identify more than one organism in a single sample, but the relative abundance of each organism's barcode is a reflection of the abundance of that organism in the sample. Metabarcodes and metagenomics have been particularly useful in the study of microbiomes in the soil and in applications where changes in the microbial community structure are important.

Use of whole genome sequencing for biosecurity is not without its challenges. First and foremost, despite the large toolbox of analytical methods, analyses of genome sequences still require a sufficient infrastructure (e.g., computational cloud to maintain databases, computational power, and algorithms to process the data) and expertise in genomics and bioinformatics in working with large datasets. Second, except for viruses and bacteria with small genomes, whole genome sequencing does not typically yield a complete genome sequence and instead yields fragmented sequences that require additional investments of time and resources to finish, if necessary. Third, WGS is not yet a cost-effective approach for the many pathogens with large genomes. Fourth, to fully capitalize on the power of whole genome sequencing, the biosecurity community needs to build databases for each pathogen of interest with known and well characterized population samples. General databases are currently available, but the appropriate data need to be extracted and robustly classified for each taxon of pathogen to be useable in biosecurity. A considerable investment of resources for each pathogen of interest is required.

When sufficient resources are available, use of whole genome sequencing is demonstrably powerful as evidenced by its use in the global tracking of genetic variants during the coronavirus pandemic. Likewise, a network of laboratories was established to use and share whole genome sequencing data to track and manage disease caused by foodborne pathogens, such as *Salmonella* and *Listeria* (Gwinn et al., 2017; Timme et al., 2019). There are three important features for how this network integrated whole genome sequencing with diagnostics. First, data are centralized, using data archives such as GenBank to store sequence data. Second, method standardization includes a substantial data reduction step to allow those with less expertise in computational biology to draw meaningful conclusions. Third, when warranted, more intensive methods that rely on whole genome sequencing are then used to identify links between small numbers of identical or very closely related **strains**. As stated previously, this allows for a more rapid response to slow the spread.

In addition, HGT represents another major concern for biosecurity that can be addressed with WGS. HGT can impact the interpretation of results. Results from more traditional methods that use marker genes common to pathogenic taxa for detection can be misleading because related, but non-pathogenic lineages that lack virulence plasmids, will also be detected. Results from the detection of virulence genes is a more direct method to detect pathogenic lineages, but if such genes are transferred horizontally, exclusive reliance on their detection will fail to reveal the emergence of novel lineages or divergence of pathogens and potentially result in an incorrect diagnosis. Whole genome sequencing, in contrast, provides genetic data to identify pathogens and differentiate vertical from horizontal inheritance (Weisberg et al., 2020). Hence, this method can provide high resolution information to more accurately model how pathogen populations evolved. In essence, WGS can provide the sequence data for genetic elements acquired by HGT such as virulence plasmids.

Select Examples of Genetic Analyses in Animal and Plant Epidemics

Use of Neutral Mutations in Tracking Pathogens

Mutations gradually accumulating in a pathogen leave an evolutionary history in the genome. They can be used to reconstruct the genetic history of a pathogen. Neutral mutations can inform us on how pathogens emerge and spread. The concept of tracking neutral mutations is illustrated by work documenting the emergence of the sudden oak death pathogen, *Phytophthora ramorum*, on the US West coast (Grünwald et al., 2012). This pathogen exists as four distinct clonal lineages, that is, genetic subpopulations that do not share alleles because they do not reproduce sexually. Each of the four clonal lineages have accumulated many mutations that are unique to each lineage. Furthermore, these neutral mutations were used to infer how these **clone**s have moved around the world. By tracing mutations in space and time, scientists could infer that populations were moved via nursery plant shipments form the West coast to the East coast (Goss et al., 2009). This information allows federal and state agencies to prioritize eradication strategies. Recent work documented a new introduction of the pathogen into southwest Oregon forests (Peterson, Navarro, LeBodus and Grunwald, unpublished). This new introduction is of concern because lineages are of opposite sex and could potentially reproduce sexually. Sexual reproduction could result in creation of new genotypes of the pathogen that might become more aggressive and cause more severe epidemics due to recombination of genes and **natural selection**. This finding was insightful to biosecurity and the Oregon Department of Forestry has made eradication of this new introduction a top priority.

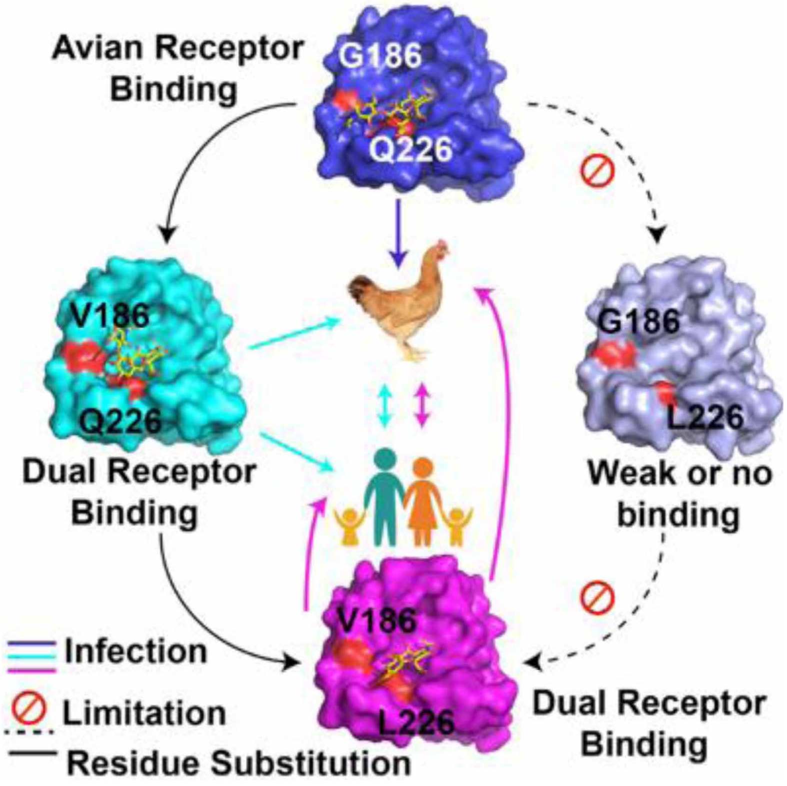
Selection for Adaptive Mutations in Pathogen Populations

Emergence of antimicrobial resistance (AMR) is one of the most serious medical problems of modern times. It provides a classic example of mutation, HGT, and selection leading to novel pathogen adaptations that jeopardize public health (Baker et al., 2018). Bacteria have relatively short generation times, and have the potential to rapidly diversify populations via mutation and HGT. Thus, whenever they are confronted with antimicrobials intended to control them, strong selection will act to enhance the fitness of individual bacterial cells that are resistant.

One excellent example is provided by the seventh cholera pandemic (Mutreja et al., 2011). There have been several pandemic waves of human cholera, caused by *Vibrio cholera*. Between the first and second waves of the seventh pandemic, estimated to be in the late 1970s, a lineage of *V. cholerae* was predicted to have horizontally acquired a genetic element that conferred antibiotic resistance. This was approximately 15 years after antibiotic use started in clinics to treat disease, suggesting that management practices were a strong selective pressure on the evolution of antimicrobial resistant *V. cholera*. Another important example was reported in *Pseudomonas aeruginosa,* an opportunistic pathogen of cystic fibrosis and immunocompromised patients (Hocquet et al., 2012). In this case, frequent use of a preventative antibiotic, one that has no efficacy against *P. aeruginosa,* is associated with genomic changes that confer resistance to another antibiotic that is used to control *P. aeruginosa.* Hence, the use of one antibiotic drove evolutionary changes that resulted in resistance to another.

The next two examples include the host immune system and its **coevolution** with pathogens. The first example is of an adaptive mutation allowing for a jump from avian to human hosts of viruses. Influenza A viruses have genomes that are composed of eight single-stranded RNA molecules. RNA replication has a higher error rate than DNA replication. Most daughter RNA molecules will result in progeny viruses that do not function as well as the parental virus. However, with the large population sizes of progeny, there is a chance that some mutations will confer an evolutionary advantage. An avian influenza virus, adapted to the high body temperature of avian hosts, might have a mutation that affects its ability to replicate its genome more efficiently at the lower mammalian body temperature and thus adapt to become more pathogenic on mammalian hosts. A different way in which an avian influenza virus can become adapted to mammalian hosts is by mutation that affects the hemagglutinin protein (Figure 3). The hemagglutinin protein is found on the influenza virus particle and functions to bind to the host cell surface receptor (Xu et al., 2019). But the avian and mammalian cell surface receptors have a number of differences, and mutations in the influenza hemagglutinin protein can lead to improvements in the affinity of the variant to bind to mammalian receptors. A mutation affecting the SARS-CoV-2 spike protein has emerged and has had similar consequences (Korber et al., 2020). In this case the mutation does not increase the pathogenicity of the virus or alter host specificity, but enables the virus to be more transmissible (Long et al., 2020). Similar fitness-enhancing mutations have been identified in West Nile virus and SARS-CoV-2.

Figure 3. Substitutions of two amino acids in the influenza virus hemagglutinin protein alters the virus binding to cell surface receptors resulting in changes in host specificity
Source: Xu et al., 2019



A final example of adaptive mutations impacting biosecurity efforts includes the coevolutionary arms race between plant pathogens and their hosts. An analogous process to the avian influenza virus-animal host recognition occurs among interactions between plants and pathogens. Many plant pathogens deploy collections of effectors to dampen the Pattern-Triggered Immunity (PTI) arm of the immune system, but effectors are at risk of being detected by the Effector-Triggered Immunity (ETI) arm of the plant immune system. Collections of effector genes in the pathogen are highly modular and robust (Cunnac et al., 2011). These features allow pathogens to lose and gain effector genes with little to no effect on the ability of the pathogen to cause disease. The collection of effectors is necessary, but few individual effectors are essential. Hence, analogous to how host pathogens evade the animal adaptive immune system, plant pathogens can diversify their collections of effector genes to evade ETI. Likewise, as in adaptive immunity where animal systems are continually diversifying B- and T-cells in response to changing pathogen populations, plants diversify their sets of *R* genes (Baumgarten et al., 2003). But whereas adaptive immunity diversifies over the life of the animal, ETI diversifies in plant species over evolutionary time.

Elegant work on evolutionary changes in populations of the tomato pathogen *Pseudomonas syringae,* showed that mutations have occurred within genes that encode patterns perceived by PTI as well as effectors perceived by ETI (Cai et al., 2011; Clarke et al., 2013). Both of these changes are associated with changes in pathogen populations. The flagellum is an important and conserved apparatus that confers motility to *P. syringae.* Small fragments, or epitopes, of the flagellum are also PAMPs recognized by the PTI immune system. The epitope from a more recently derived variant of flagella is associated with a weaker immune response than the ancestral epitope. Strains of *P. syringae* encoding the new epitope of flagella also have mutations that yield a truncated effector. Consistent with expectations, strains with functional, but non-mutant variants elicited responses indicative of ETI. Hence, both arms of the plant immune response are inferred to have driven selection for pathogen genotypes that can evade resistance and persist as pathogens.

Innovation by Horizontal Gene Transfer (HGT)

HGT is another evolutionary mechanism whereby pathogens emerge. Several groups of human pathogens require virulence plasmids, acquisitions of which have been identified as key in transitioning lineages to become pathogenic. The *Yersinia* genus consists mostly of environmental species of bacteria. Three, including the causative agent of the “Black Death” plague, can cause disease in humans. Original hypotheses predicted that the three are derived from a common ancestor. However, findings from whole genome sequencing concluded that ancestors of the three pathogenic lineages evolved independently, each catalyzed by the horizontal acquisition of a virulence plasmid (Reuter et al., 2014). An important function encoded on the plasmid is the type III secretion system. Like plant pathogens, this apparatus allows human and animal pathogens to shape host cells to be more accommodating to infection. Pathogenic *Shigella* species evolved in a similar fashion (The et al., 2016). Horizontal acquisition of a virulence plasmid, also encoding a type III secretion system, was critical to the emergence of pathogenic *Escherichia coli.* Detailed analyses of genome sequences suggested that these so-called foothold moments may not have yielded strains optimized for virulence. Several additional horizontal acquisitions of pathogenesis-associated genomic regions, called pathogenicity islands, as well as other genomic changes occurred subsequently.

One interesting form of HGT is observed among the chestnut blight pathogen *Cryphonectria parasitica.* In European forests, this pathogen is found to harbor mycoviruses that can lower the fitness of the pathogen in chestnut trees. These mycoviruses can also readily be transferred horizontally among individuals (Cortesi et al., 2001). However, this transfer of the virus is only possible among strains of the fungal pathogen that are vegetatively compatible, meaning that the individuals can exchange genetic material asexually. Incompatible strains cannot merge their fungal bodies to exchange materials.

Agrobacterium is a genus of plant pathogenic bacteria and HGT has two roles in the diseases they cause. *Agrobacterium* are the causative agents of both crown gall and hairy root diseases, which require tumor-inducing (Ti) or root-inducing (Ri) plasmids (Nester, 2014). The two diseases are associated with growth deformations of plants and are major economic problems in several sectors of agriculture. Ti and Ri plasmids provide *Agrobacterium* the very unusual capacity to transfer genes into plants, which is a form of inter-kingdom HGT (Gordon & Christie, 2014). The portion of the plasmid that is transferred into plants is called the transfer DNA or T-DNA. The T-DNA integrates into the genome of the host and also carries plant growth promoting hormones that cause unregulated proliferation of host tissues. The T-DNA also carries genes necessary for the biosynthesis of novel metabolites that *Agrobacterium* can in turn live off. Hence, pathogenic *Agrobacterium* are bacteria that genetically transform plants to establish a novel niche, rich in nutrients from which the bacteria can gain a fitness advantage.

Disease by *Agrobacterium* involves a second form of horizontal gene transfer and that is the transfer of Ti and Ri plasmids among strains within the genus (Gordon & Christie, 2014). As described earlier in this chapter, HGT is a powerful process allowing acquisition of novel traits. The metabolites produced by transformed host cells have dual roles (Oger & Farrand, 2002). These metabolites are not only nutrients for the pathogen, but they are also signals that upregulate copy numbers of Ti and Ri plasmids and induce expression of genes that mediate conjugation (e.g., the transfer of plasmids between bacterial cells). Work has shown that divergent lineages of *Agrobacterium,* which are separated by millions of years, have exchanged plasmids and diversified the number of species that are capable of causing disease (Weisberg et al., 2020). Importantly, use of whole genome sequencing has uncovered plasmid molecules identical in sequences present in genetically distinct strains isolated from diseased plants distributed across the world (Weisberg et al., 2020). This work shows that some plasmids, upon HGT into another species, are sufficient to confer novel abilities to extant strains.

OPPORTUNITIES AND FUTURE DIRECTIONS

To reduce the risk of introduction and transmission of infectious disease to animals and plants, biosecurity seeks to understand the mechanisms and patterns of pathogen emergence and implement strategies to effectively manage disease. As described in this chapter, evolution underpins not only pathogen dynamics, but also the methods used in biosecurity. A key concept here is that via mutation and/or HGT, pathogen populations diversify. Pressures, whether they are from the environment, the host, or from human practices, select for individuals with the fittest combination of genes. This will result in a change in the population. The foundational approaches discussed in this chapter form the scientific basis for effective crop and animal biosecurity management strategies. However, many of the applied approaches used in characterizing pathogen populations, such as methods that detect marker genes or use of products to control pathogens, need to be continually evaluated and adapted as pathogen populations change.

Analyses of whole genome sequences are important in changing our understanding of pathogen evolution. In the near future, whole genome sequencing will routinely be applied to characterize pathogen populations in real-time for not only human but also animal and plant health. This coming era will provide unprecedented capabilities to infer the genetic changes in the populations that give rise to epidemics. However, this era will require development of generalizable computational platforms for rapid analysis of whole genome data. In addition, curated databases with known diversity for each pathogen group need to be developed.

Case Study: The Sudden Oak Death Emergence

Sudden oak death (SOD) is caused by the invasive plant pathogen *Phytophthora ramorum*. This pathogen was first detected in California causing SOD in the mid-1990s. The pathogen has since spread into Oregon forests. The pathogen infects tree stems of certain oaks or tanoaks and eventually kills the trees (Figure 4).

Figure 4. Detection of the first tanoaks killed by sudden oak death in Curry County, Oregon, 2001
Source: Photo courtesy of Mike McWilliams, Oregon Department of Forestry (https://pnwhandbooks.org/plantdisease/host-disease/oak-quercus-spp-sudden-oak-death

Unfortunately, the pathogen has a wide host range infecting several ornamental plants grown in nurseries such as rhododendron, viburnum or pieris. The pathogen can be moved with movement of life plants across vast geographic ranges depending on shipping patterns.

To date four distinct variants, typically referred to as clonal lineages, are distinguished. These variants are distinguished using DNA nucleotide polymorphisms or **SNPS** and are found to cluster together (e.g., being genetically most similar to each other) in phylogenetic trees (Figure 5).

Analyzing genetic variation found in the pathogen populations was used in a range of studies to document repeated migrations in Europe and North America. These efforts have identified at least 5 global migrations of the SOD pathogen (Grünwald et al., 2012, 2019; Van Poucke et al., 2012). More recently, genetic analysis identified three distinct introductions into SW Oregon forests, two of both belonging to the NA1 variant and one of the EU1 (Figure 6).

Figure 5. Two phylogenetic trees using highly variable simple sequence repeats (A) or mitochondrial DNA polymorphisms (B) show presence of three genetic variants named NA1, EU1 and NA2
Source: Grünwald et al., 2009

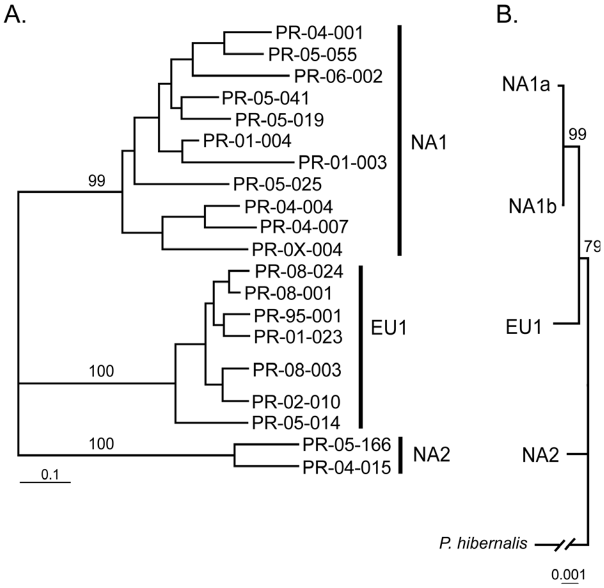
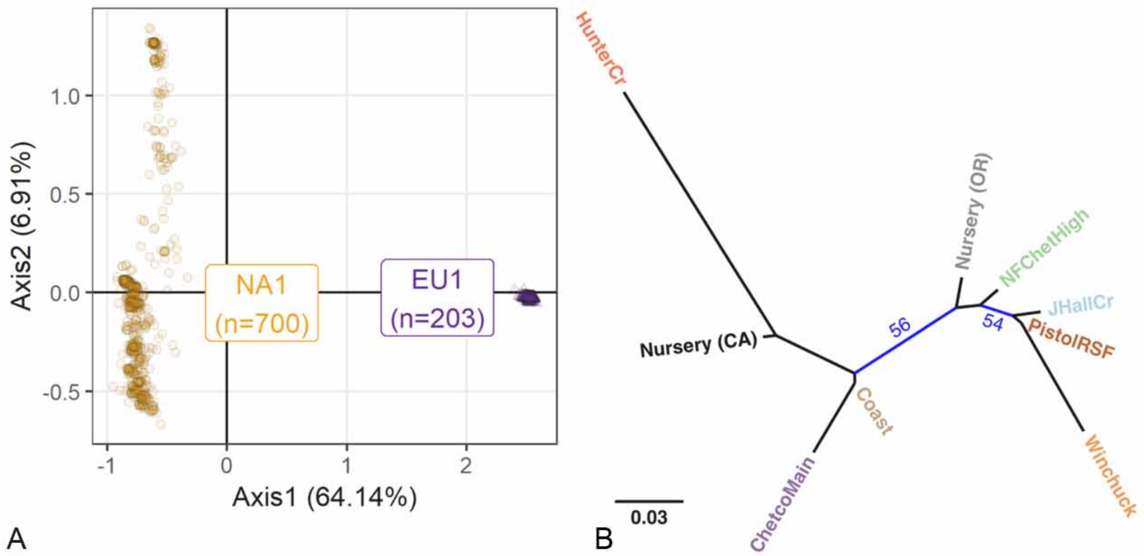


Figure 6. A: Distinct clustering and geographic distribution shows evidence for two NA1 and one EU1 introductions of the SOD pathogen into SW Oregon forests (Carleson et al., 2021). Shown is a cluster for the population in the Hunter Creek watershed (top left) that is unrelated to the other populations found in Oregon Forests watersheds labelled Chetco Main, Coast, North Fork of Chetco, Pistol River and Winchuck. B: Populations sampled in commercial nurseries growing woody ornamentals such as rhododendron are shown in black (California) and grey (Oregon). The Hunter Creek population clusters of NA1 (yellow clusters top left and bottom left) and one cluster of EU1 (purple, right) strains most closely to the CA nursery population.



More recent genetic analysis has shown evidence supporting a third introduction of the EU1 variant into SW Oregon forests (Figure 6A) (Grünwald et al., 2016).

CASE STUDY: APPLICATION OF MOLECULAR BIOLOGY TO AVIAN INFLUENZA BIOSECURITY

Avian influenza virus (AIV) is a virus with eight single-stranded RNA segments. One of the RNA segments encodes the hemagglutinin (H) protein and a second segment encodes for the neuraminidase (N) protein. Wild birds carry a wide variety of AIV, and the viruses are grouped into 16 H and 9 N subtypes according to serological relatedness. Each strain is named after an H & N combination, such as H1N1 or H5N1. Be aware that the H & N designation is only the beginning of characterizing a particular strain of AIV, as there are, for example, many different H5N1 viruses and each strain can behave very differently.

Molecular Testing for rapid threat assessment. AIV strains carrying the H5 and H7 subtypes are the ones that are most often associated with disease outbreaks in poultry. In the laboratory, PCR tests designed to detect the H5 or H7 subtypes can detect the presence of these RNA segments in about two hours. Knowing that the AIV subtype is, or is not, one of the two dangerous subtypes will give farm operators and resource managers critical information needed for appropriate response.

Nucleotide sequencing for strain characterization. Avian influenza viruses can be characterized into low pathogenic avian influenza (LPAI) or highly pathogenic avian influenza (HPAI) groups. LPAI viruses typically cause infections in the respiratory or gastrointestinal tract in domestic poultry (Figure 7). One of the reasons for this tissue tropism is that the hemagglutinin protein is made as a precursor that must be cleaved by a host protease for the protein to function. In the LPAI viruses, the amino acid sequence at the protease cleavage site within the hemagglutinin protein is recognized by a protease that is found only in the respiratory and gastrointestinal tract, and hence these two organs are the only ones infected by LPAI viruses. On the other hand, HPAI virus infections are associated with systemic infection in domestic poultry. This systemic infection is one factor in the virulence of these viruses. The hemagglutinin RNA segment in HPAI viruses contains mutations that change the encoded amino acid sequence at the protease cleavage site into one that can be recognized by a host protease that is widely distributed in the body (Figure 7). In the laboratory, nucleotide sequencing of the protease cleavage site region of the hemagglutinin RNA segment can be done in a few hours. In practice, this is often done overnight following the detection of H5/H7 AIV. Deducing the predicted amino acid sequence from the nucleotide sequencing on the next morning allows determination of whether the virus is the LPAI or HPAI kind, and provides responders with a key piece of information on the pathogen they are facing. Depending on policy, identification of an HPAI virus might trigger an immediate culling of a flock, while LPAI-infected flocks might be allowed to remain and retested until the flock is negative.

Phylogenetic analysis provides clues as to pattern or history of transmission. The genetic sequence can also be used in analyses that can provide additional information such as the pattern and timing of spread, because the genetic mutations accumulate as the virus is passed from host to host. Phylogenetic information can greatly enhance traditional epidemiological data. For example, during the 2014-2015 HPAI H5N2/H5N8 outbreak in the United States, epidemiological data suggests a clear difference in the location and timing of events in the Pacific Flyway versus that in the Midwest (Figure 8A), but the genetic analysis of the viruses provides further insight that the Midwest outbreaks were associated with only a few introductions, and that those introductions were then associated with large secondary, and

Figure 7. Schematic diagram of the sites of avian influenza virus infection in the domestic chicken (blue stars). The hemagglutinin protein is synthesized as a precursor protein (HA0) containing an amino acid sequence that is recognized by host proteases (region in black). Protease cleavage results in two proteins HA1, and HA2. LPAI, low pathogenic avian influenza. HPAI, highly pathogenic avian influenza.

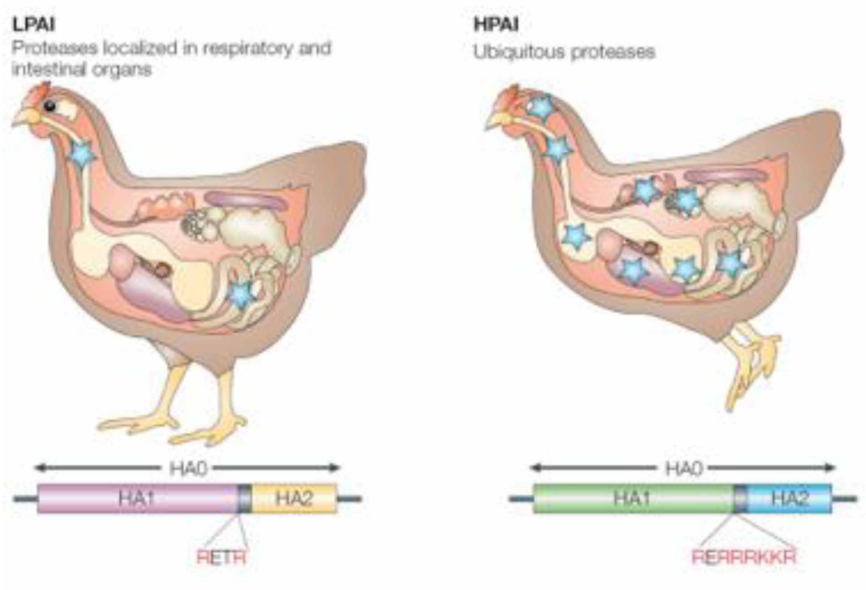
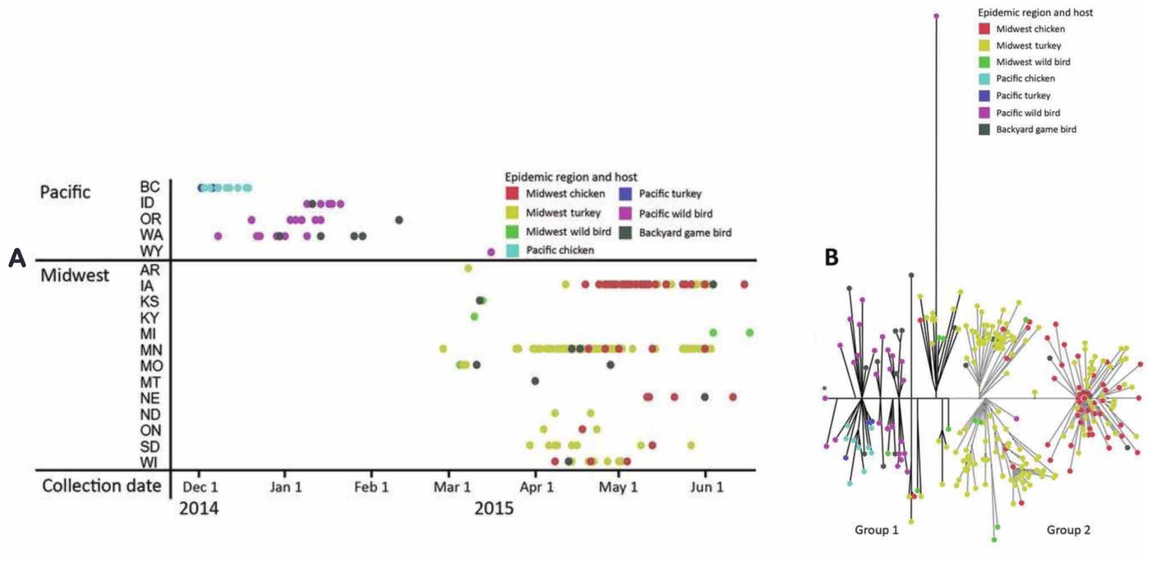


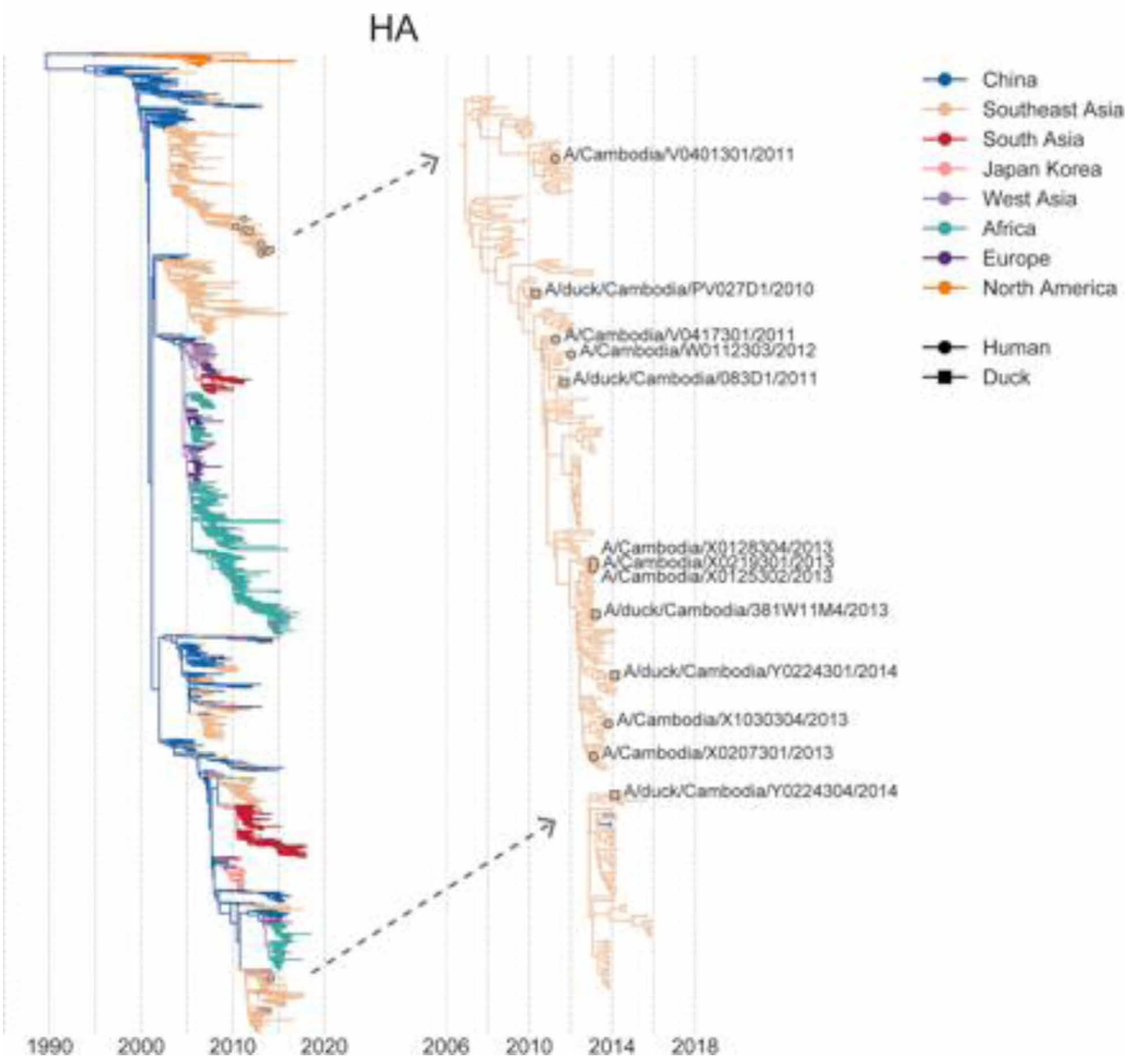
Figure 8. **(A)** *Pattern of the HPAI H5N2/H5N8 outbreaks in the United States in 2014-2015. Individual outbreaks are separated by states (US) and provinces (Canada) and plotted over time. The geographic region (Pacific flyway or Midwest) as well as different avian categories are coded in different colors to show the changes in the pattern and nature of the transmission during this event. (***B***) A Network Analysis of the HPAI viruses isolated from the different avian categories in the 2014-2105 outbreak. Clustering indicates relative genetic relatedness and possible transmission chains. The virus located on a very long branch represents the last known descendant of the 2014-2015 virus*



in some cases, tertiary, spreads (Figure 8B). Understanding such patterns of spread in a timely manner would provide farm operators and response agencies with critical information to target strategies to interrupt these transmission chains.

As demonstrated by the SARS-CoV-2 pandemic, rapid sequencing of viruses during outbreaks can provide insight on how the virus is changing and the ways an epidemic might be changing. This strategy has also been used in animal diseases. Figure 9 is a phylogenetic tree of HPAI H5N1 viruses. Each branch of the tree is a virus and is color coded with respect to the region in the world where it was isolated. In the light tan color, for example, are viruses that were isolated from Southeast Asia. Since 1996, this

Figure 9. Global phylogenetic tree of the HPAI H5N1 hemagglutinin (HA) gene. The tree is color coded to indicate the originating region. Arrows indicate enlarged regions. Some viruses from Cambodia are highlighted with symbols indicating the viral host and the full name of the virus, including the sample collection year is presented



region has suffered from multiple HPAI H5N1 introductions, some of which were limited in scale, as indicated by the small number of branches with short temporal duration. Other introductions, such as the region of the tree indicated by the upper arrow and expanded on the right-hand panel, resulted in extensive spread and decade-long transmission. The lower arrow highlights a different region of the tree, also from Southeast Asia. From the year of isolation, it is clear that multiple strains were circulating within the same year and in several instances the same strain was introduced more than once. This kind of information should prompt investigations to determine if the same introductory routes are used repeatedly, or if there are as yet unidentified routes of introduction that need to be discovered and halted.

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KEY TERMS AND DEFINITIONS

Alleles: Alleles are different variants of the same gene.

Clonal lineage or clone: An asexually reproducing subpopulation. This can be recognized by genotyping individuals and finding them to cluster together (e.g., being genetically most similar to each other). Genetic markers are found to be linked across the genome in contrast to sexual populations were markers are unlinked. Furthermore, genetic variation within a clone is low relative to a sexual population.

Coevolution: Evolution resulting from two species affecting each other's evolution. Plant or animal hosts and their pathogens are continually adapting and counter-adapting to each other. This conflict results in the escalation of attack and defense mechanisms in what is often referred to as a coevolutionary arms race.

Epitope: The part of an antigen (protein) that is recognized by the immune system.

Fitness: Fitness refers to the relative degree by which an organism can reproduce and transmit its genes to the next generation.

Genotype: A unique combination of genetic markers. Genetic markers can include stretches of DNA sequence or a combination of SNPs.

Horizontal Gene Transfer: Movement of DNA between unrelated organisms other than by the transmission of DNA from parent to offspring.

Lineage: An evolutionary clade of individuals that are descended from the same common ancestor. Typically, this is inferred by graphing trees and obtaining statistical support that all individuals should be placed in this clade by genetic similarity and are not assigned to any other clade.

Missense Mutation: A mutation in which a single base pair substitution alters the genetic code such that an amino acid is different form the usual amino acid at that position in the petide.

Mutation: A change in the genetic code of an organism. Mutations can occur anywhere, but in sexually reproducing organisms, only those mutations that occur in the germ cells can be inherited by the progeny.

Natural Selection: The differential reproduction and survival of individuals due to inheritable, genetic changes that affect fitness.

Negative Selection: During negative selection an allele becomes less frequent in a population.

Neutral Mutation: A mutation that does not change reproduction or survival of an organism.

Phenotype: An expressed trait of an individual organism, such as resistance to antibiotics.

Plasmid: Plasmids are extra-chromosomal DNA molecules that typically replicate independent of cell replication. Plasmids often carry genes that are adaptive to new environments. Plasmids are not essential for cell viability.

Population: A group of individuals of the same species that coexist and share ancestry.

Positive Selection: During positive selection an allele becomes more common in a **population**.

SNP: Single nucleotide polymorphism, that is a change in a single nucleotide in a DNA sequence.

Strain: A genetic variant of an organism. For bacteria, a strain is derived from a cell isolated and purified away from other cells.

Chapter 3

Risk Analysis for Human-Mediated Movement of Pests and Pathogens

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ABSTRACT

Humans have always played an important role in dispersing plants, animals, and other organisms—either intentionally or inadvertently. Over the last several decades, rapid developments in infrastructure and transportation have led to dramatic increases in trade, travel, and mass migration; this in turn has accelerated the human-mediated spread of organisms across the globe. In their new environments, introduced species may thrive and cause severe economic and ecological impacts. Mitigating the entry, establishment, and spread of exotic pests and pathogens is crucial for protecting agriculture, ecosystems, and people. To do this, it is important to understand the pathways by which invasive species spread, assess the associated risks, and develop effective mitigation measures. This chapter describes the role of risk analysis for understanding human-mediated pathways of pest introduction and spread and provides case studies from both the plant and animal health arenas.

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INTRODUCTION

Humans have always played an important role in dispersing plants, animals, and other organisms—either intentionally or inadvertently. Over the last several decades, rapid developments in infrastructure and transportation have led to dramatic increases in trade, travel, and mass migration; this in turn has accelerated the human-mediated spread of exotic organisms across the globe (Hulme 2009).

In most instances, new species arrivals do not lead to the establishment of permanent populations because conditions (e.g. climate, availability of hosts or mates) are not favorable for establishment. However, under suitable conditions, introduced species may thrive and in some cases, can cause severe economic and ecological impacts in their new locations. For example, the Asian longhorned beetle, *Anoplophora glabripennis*, and the emerald ash borer, *Agrilus planipennis,* are tree pests that were introduced from Asia to the United States, presumably through wood packaging materials used in trade (Haack, Herard et al., 2010). By killing their host trees, both species have severely impacted forests and urban landscapes in the United States, causing billions of dollars in economic losses (Herms and McCullough 2014).

Citrus greening or Huanglongbing (HLB), is a bacterial disease introduced from Asia that infects citrus trees, shortening their lifespan and reducing fruit yield and quality. The first U.S. detection of citrus greening was in Florida in 2005; today, an estimated 90% of citrus acreage and 80% of citrus trees in Florida are affected by the disease (Singerman and Useche 2017). Although no one knows how citrus greening arrived in the United States, illegally imported plant material is believed to be the most likely pathway (USDA 2020). The bacterium is transmitted from tree to tree by an insect vector, the Asian citrus psyllid. Infected psyllids can be transported over large distances by prevailing winds or in shipments of citrus nursery stock or fruit (Stelinsky 2012). Over the past 5 years, citrus greening has caused economic damages of over $1 billion annually, with nearly 5,000 jobs lost per year (Li, Wu et al., 2020). Since the detection of the disease in 2005, Florida has suffered a 30% reduction in grove-bearing area and a 74% decline in production (Court 2017).

The yellow star thistle, *Centaurea solstitialis,* is a weed that was accidentally introduced to the United States in the 1800s, probably through importation of contaminated alfalfa seed. The thistle is toxic to horses, often killing them (Plant Conservation Alliance's Alien Plant Working Group 2005). Since its introduction, the thistle has spread over at least 15 million acres in 17 states, reducing biodiversity and rangeland values, and causing significant economic impacts (Juliá, Holland et al., 2007); (Eagle, Eiswerth et al., 2007).

Exotic Newcastle disease, one of the world's most important poultry diseases, was introduced to the United States in infected live pet birds or game fowl on three separate occasions, and the U.S. Federal Government spent hundreds of millions of dollars to eradicate the resulting outbreaks (ARS, 2016). The movement of asymptomatic, infected birds and contact with smuggled birds at live-bird markets (USDA 2013) contributed to the silent spread of the disease. The pathogen can also be spread via contaminated clothing or equipment.

Foot-and-mouth disease is a contagious disease of cattle, swine, sheep, goats, deer, and other cloven-hoofed animals. The primary means of introductions is through imports of livestock, as well as human contact with infected animals and eating infected meat. Foot-and-mouth disease is among the most economically devastating livestock diseases in the world because of its rapid spread, the number of species affected, and the difficulty of controlling outbreaks. The United States experienced nine outbreaks of foot-and-mouth disease between 1905 and 1929. In 2001, an outbreak in Great Britain resulted in the slaughter of more than 6 million animals, costing 20 billion dollars (American Veterinary Medical Association 2007).

Exotic pests and diseases represent biological hazards, and mitigating their entry, establishment, and spread is crucial for protecting agriculture, livestock, ecosystems, and people. To do this, we must understand the pathways by which biological hazards may enter a biological system and spread, assess the associated risks, and develop effective mitigation measures. This chapter describes the role of risk analysis for understanding human-mediated pathways and provides case studies from both the plant and animal health arena.

WHAT IS A PATHWAY?

The International Plant Protection Convention defines a pathway as “any means that allows the entry or spread of a pest” (IPPC 2012). Similarly, in the 2016 U.S. Executive Order 13751, a pathway is defined as “the mechanisms and processes by which non-native species are moved, intentionally or unintentionally, into a new ecosystem” (Executive Office of the President 2016). Because this chapter covers plant pests, as well as animal diseases, we will refer to them collectively as “biological hazards” in the remainder of this chapter.

A multitude of pathways are known to have facilitated the spread of biological hazards (USDA-APHHIS-PPQ 2009) (Meurisse, Rassati et al., 2019) (Webber 2010) (Smith, Baker et al., 2007). In the trade of agricultural commodities, biological hazards can be carried in the commodities themselves but also on or in containers, conveyances, and packaging materials. International travelers may intentionally or unintentionally carry these organisms in their luggage or on their shoes or clothing. Animals -both livestock and wildlife -can spread pathogens, either as infected hosts or mediated by vectors. In addition, the spread of biological hazards can be aided by wind, water, or the movement of infested soil.

A pathway is comprised of the entire chain of events and conditions that may lead to introduction. For example, if an agricultural pest is introduced on an imported plant commodity, the pathway would include:

- Commodity production in the exporting country (location, level of pest surveillance, management practices)
- Harvest and packaging (*e.g.* time of harvest, inspection and culling practices, washing, disinfecting, waxing, post-harvest treatments)
- Shipping to importing country (duration and conditions of transport)
- Arrival at port of entry (inspection practices, ease of detection, import regulations)
- Retail (duration and conditions of transport and storage)
- Use and/or disposal (*e.g.* is commodity consumed or planted? discarded in landfill or compost pile?)

Because a biological hazard may “enter” or “exit” the pathway at any point, a pathway analysis considers how the events and conditions of each section of the pathway affect the likelihood that a biological hazard will enter the pathway and remain in it.

RISK ANALYSIS FRAMEWORK FOR BIOLOGICAL HAZARDS

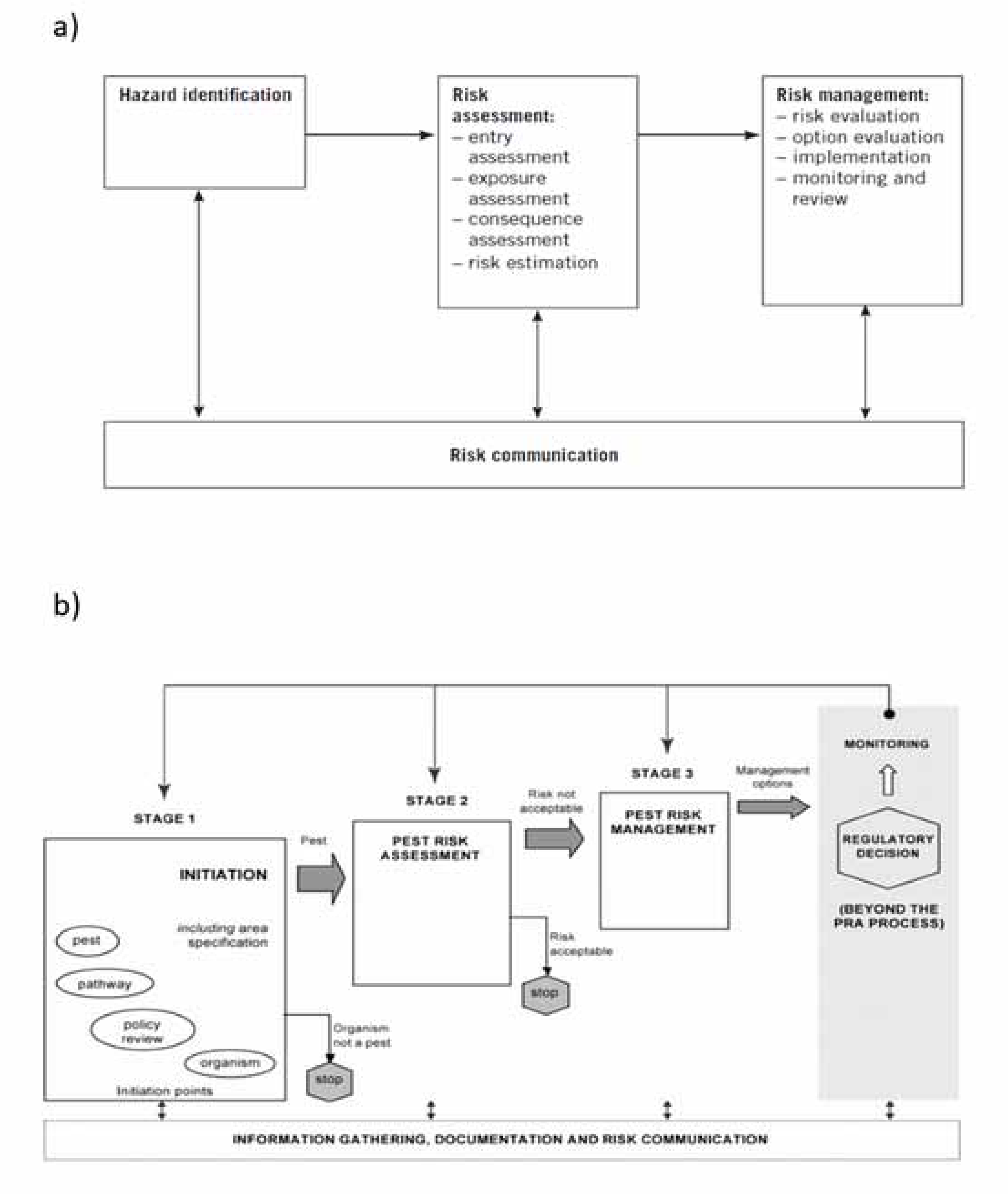
Risk analysis is a generalizable framework used across many disciplines to guide decision making. It has been codified by international plant health (IPPC 2007) and animal health (OIE 2019) organizations and is therefore a recognized standard that can be used to evaluate human-assisted pathways of introduction for biological hazards to agricultural and natural ecosystems. Risk analysis is the process of determining the magnitude of risk and whether and how that risk should be mitigated. While plant and animal health communities use slightly different risk analysis frameworks (Figure 1a-b), the general concept is the same and includes:

- **Biological hazard identification**, also known as threat characterization. This step describes the biological hazard, i.e. the pest(s) or pathogen(s) of concern. In the plant health arena, biological hazard identification is part of the initiation process and often takes the form of a pest list, which in many cases includes hundreds of pests and/or pathogens. In animal health, biological hazard identification usually focuses on a single pathogen.
- **Assessment of risk associated with each hazard.** The concept of risk combines the potential impact caused by a hazard and the likelihood that this impact will occur. In plant health, the process of risk assessment evaluates the likelihood of introduction (entry and establishment) of a biological hazard, as well as the magnitude of its expected impact if introduced.
- **Risk management** is the process of identifying measures to mitigate the identified risk(s) by reducing either the likelihood of introduction or the expected impact at specific points along the pathway.
- **Risk communication** is the process of explaining the logic and the results of the risk analysis to the appropriate audience (often decision-makers and stakeholders).

It is worth noting that while these processes appear sequential, in practical application, they often occur simultaneously or cyclically. Risk analysis terminology varies slightly between plant (IPPC 2007) and animal health (OIE 2019) systems. For example, in the plant health framework, the biological hazard identification step is considered to be part of risk assessment, while the animal health community considers risk assessment as separate from hazard identification. In plant health risk assessment, likelihood of establishment refers to the ability of a newly arrived pest to form lasting, reproducing populations. In animal health risk assessment, the term exposure is used instead. Exposure is the post-entry likelihood of a given pathogen infecting a susceptible animal population and maintaining onward transmission.

There is no single method to conduct a risk assessment. The methodology used may involve qualitative or quantitative data analysis, simulation, geospatial modeling, or any combination of these approaches. Both likelihood and magnitude of impact may be expressed qualitatively (*e.g.* high, medium, low) or quantitatively, depending on the application and available data. In any risk analysis, there will be unknowns (uncertainties), meaning that assumptions will have to be made. As in any science-based work, it is essential to define terms, methods, criteria, and assumptions and to document all data sources used in analyses.

Figure 1. Risk analysis frameworks of a) the World Organization of Animal Health (OIE, 2019) and b) the International Plant Protection Convention. Both frameworks include identifying the biological hazard, assessing the risk of those hazards, evaluating risk management options, and communicating. However, in the IPPC framework hazard identification takes place in the initiation stage, rather than being its own step. Further, risk management in the IPPC framework only includes evaluating risk management options. Determining, implementing, and monitoring risk management options are considered to be outside the Pest Risk Analysis (PRA) process, whereas in the animal health area they are part of the framework.
Source: IPPC, 2007



RISK ASSESSMENT FOR HUMAN-MEDIATED PATHWAYS OF INTRODUCTION

The term "pathway analysis" is generally used to refer to any analysis that focuses on how a biological hazard may enter a country or spread to new areas or populations. Pathway analyses are a type of risk assessment and can be hazard-centric or pathway-centric. The goal of a hazard-centric pathway analysis

Table 1. Potential factors to consider in pathways analysis

Factor	*How it affects risk*
ENTRY-SPECIFIC FACTORS	
Likelihood of hazard presence in the pathway	The actual quantity of pests or pathogens being moved along a specific pathway is usually unknown but can sometimes be estimated based on factors such as: prevalence of the pest/pathogen in the area of origin; association with the commodity; immunization, control, and sanitation practices; time of harvest; post-harvest processing and inspection practices.
Volume of shipments	The larger the volume of imported goods, the larger the number of pest/pathogen propagules associated with the import, and thus the higher the likelihood of establishment.
Frequency of shipments	The number of shipments per time can impact risk because each shipment represents an opportunity for some event to occur that favors biological hazard introduction, e.g. contact opportunity to transfer the biological hazard to the pathway.
Survival during transport	The conditions during transport can impact a pest's or pathogen's survival or rate of spread. For example, high or low temperatures may kill pests or pathogens; long transport times may lead to starvation of pests; or an infectious disease may spread within a shipment of live animals.
Likelihood of detection	Pests or pathogens may be detected during port-of-entry inspections or at other points along the pathway, thus reducing the risk of entry. The likelihood of detection is determined by the conspicuousness of the biological hazard (or its symptoms); the inspection methods and tools used; and the frequency, intensity, and thoroughness of inspection.
Effectiveness of mitigation measures	Manufacturing processes, regulatory or trade restrictions, pre-export health inspections/certification, inspection, quality control, sanitary measures, etc that reduce the likelihood of a biological hazard to enter a new region.
ESTABLISHMENT-SPECIFIC FACTORS	
Timing of entry	Timing of entry affects whether hosts are available during certain parts of the year (such as post-harvest crop areas), or if temperatures are suitable for survival (cold temperatures reduce insect vector activity and pathogen transmission).
Likelihood of transfer to recipient ecosystem	After entry, how likely is it that the biological hazard will come into contact with a suitable host or environment?
Likelihood of an environment or host organism	The likelihood that a pest/pathogen will encounter conditions suitable for establishment depends on numerous factors, chief among which are climatic suitability, host availability, and propagule pressure/dose response. Pests/pathogens entering in/on live hosts generally have a higher chance of establishment than those entering in commodities for consumptions or on non-living carriers.
Effectiveness of mitigation measures	Measures aimed at reducing the incidence of biological hazards (e.g. vaccination, biosecurity protocols, sanitation, phytosanitary treatments) will reduce their likelihood of introduction.
Presence of reservoirs	Alternative populations (such as vectors or wildlife) may help carry and transmit disease to managed populations.
SPREAD-SPECIFIC FACTORS	
Population growth and stability	The population growth rate influences a pest/pathogen's ability to spread across an area.
Speed of response	A fast response (e.g. quarantines, stop-movements, biosecurity protocols) can help slow the spread and reduce the impact pest/pathogen. Among other things, the speed of response can be influenced by how easily or how quickly a biological hazard can be detected.

adapted from (Hulme, 2009)

is to identify and assess all possible pathways by which a particular biological hazard may be introduced, or to analyze the likelihood that the biological hazard will move by a specific pathway. Conversely, the goal of a pathway-centric analysis is to identify all biological hazards that may be associated with that pathway and assess the associated risks.

Regardless of the approach, a crucial step of any pathway analysis is to define, or "map," the pathway(s) because a detailed description of the pathway provides the necessary foundation and transparency for the analysis. Defining the pathway involves specifying all events and conditions that are relevant for the analysis. This requires finding the right balance between being too detailed and too general. In some cases, only part of a pathway (e.g. transportation) may be considered. The choice of how much detail to include depends on the purpose of the analysis, the amount of data available, the desired precision of the results, and the time available to conduct the analysis.

After defining the pathway, the likelihood of hazard presence at each step (node) of the pathway is assessed by evaluating the interaction of pathway conditions and biology/epidemiology. The use of scenario trees or event diagrams can help visualize the thought process.

While each pathway is unique and must be treated as such, a pathway analysis commonly involves information on origin, destination, volume, and level of contamination, as well as other factors (Table 1), many of which are interrelated. A pathway analysis would generally accomplish one or more of the following objectives:

1. Identify and describe plausible mechanisms for the biological hazard to enter a new geographic region.
2. Post-entry, identify and describe plausible mechanisms for the biological hazard to come into contact with a susceptible host population or host system.
3. Identify vulnerable points in the pathway where a large transference of risk could be mitigated.
4. Identify uncertainties (i.e. aspects of the pathway, the hazard, or the interaction between two that are not sufficiently understood)
5. Final risk evaluation (estimation of likelihood of risk of entry and spread)

RISK MANAGEMENT AND BIOSECURITY TACTICS ACROSS THE "SAFEGUARDING CONTINUUM"

Consistent with the concept of a pathway as a chain of events and conditions that lead to the introduction and spread of a pest, the "safeguarding continuum" refers to a system of safeguards to prevent the introduction of biological hazards into and their spread within a country by reducing risk at multiple points along the pathway (USDA APHIS 2020). Geographically, the safeguarding continuum begins in the country where the pathway originates, continues to the borders of the receiving country, and then extends across that country's territory. Functionally, the safeguarding concept can be divided into four main types of activities (USDA, 2017):

- Preparedness (activities carried out before a biological hazard enters the country, aimed at speeding up the response);
- Prevention/Pest exclusion (activities carried out to prevent the entry of a biological hazard);
- Response (activities carried out after a biological hazard enters the country, aimed at preventing establishment, limiting spread, and/or minimizing impact); and

- Recovery (activities carried out after a response).

Examples of **preparedness** activities include:

- Establishing and continuously improving a comprehensive safeguarding system. Such a system may include early-warning and biological hazard prioritization mechanisms; surveillance programs; proactive development of response plans; risk analysis capability; established roles and responsibilities, as well as communication protocols for emergency response situations.
- Identifying and securing resources necessary for biological hazard management, emergency response, and coordination.
- Annually conducting appropriate training to ensure and maintain rapid, consistent, and effective biological hazard management, emergency response, and coordination.
- Identifying and prioritizing knowledge, skill, and method needs and closing existing gaps before a biological hazard is introduced.

Examples of **prevention** activities include:

- Offshore programs ensuring foreign commodities are free of biological hazards before being exported to the United States.
- Import regulations, based on risk assessment, that prescribe specific mitigation practices to reduce the likelihood of introducing biological hazards.
- Agriculture quarantine inspections at ports of entry.

Response activities are carried out immediately after a new biological hazard introduction is discovered. Examples of response activities include:

- Prompt development of a response plan that accounts for the specific circumstances and conditions surrounding the new introduction.
- Rapid detection and delimiting surveys to gain a full understanding of the extent of the biological hazard presence.
- Mobilization of emergency personnel and resources using the Incident Command System under a unified command structure.
- Obtaining emergency funding and enacting an emergency regulatory framework (e.g. USDA, 2017), while maintaining compliance with environmental and other applicable laws.
- Implementing communication and data management plans.

Recovery elements of the safeguarding system provide stability and protection to a pest response, with the goal of returning to normalcy, and include activities such as:

- Demobilization of emergency response personnel and resources.
- Critical evaluation of the response program.
- Process improvements.

The previous sections explained how pathway analysis combines the idea of pathways of biological hazard introduction with the general concept of risk analysis. We also explained how the "safeguarding continuum" can be used to reduce the likelihood of introduction, limit the spread, and minimize the impact of a biological hazard. In the following, we present two case studies that demonstrate the practical aspects of these concepts.

PLANT HEALTH CASE STUDY: LIKELIHOOD OF INTRODUCING PINE WOOD NEMATODE FROM THE UNITED STATES INTO EUROPE IN WOODEN AMMUNITION BOXES

The United States Department of Agriculture (USDA), at the request of the U.S. Department of Defense (US DoD), carried out a project that exemplifies a successful application of pathway analysis concepts.

The DoD keeps stockpiles of ammunition, packed in wooden boxes, in depots throughout the United States. There is an ongoing need to move ammunition to Europe. The European Union (Meurisse, Rassati et al.) requires all wood packing material to be heat-treated or fumigated in compliance with the International Standard for Phytosanitary Measures No. 15 (ISPM15) of the International Plant Protection Convention (IPPC). However, because many of the DoD ammunition boxes have been in storage since before ISPM No. 15 went into effect, they do not meet the EU requirements. Treating these boxes prior to shipping is logistically difficult and expensive. Because the boxes contain explosive ammunition, it is also dangerous.

The purpose of the EU-required treatment is to prevent the introduction of wood pests, and especially the pinewood nematode (PWN), *Bursaphelenchus xylophilus*. Therefore, the USDA pathway analysis aimed to determine the likelihood that PWN would be introduced into the EU in the DoD ammunition boxes that do not currently meet EU requirements.

The pathway analysis considered the entire life cycle of the DoD ammunition boxes, from sourcing of the wood to disposal of the boxes. Likelihood of pest presence and survival were evaluated based on the following factors: number of boxes exported; origin and quality of the wood, including presence of bark; treatments and inspections applied to the wood; time in storage; storage and transport conditions; and the level of inventory control exercised by the DoD. In addition, the USDA conducted a pest survey to determine the actual pest incidence in the ammunition boxes.

The analysis concluded that it was extremely unlikely for pinewood nematode to be introduced into the EU in the ammunition boxes (Figure 2), for the following reasons:

The boards from which the boxes are made are kiln dried at or above 70°C (Boone, Kozlik et al., 1998, Bergman 2010), which has been shown to kill PWN in wood (Tomminen and Nuorteva 1992). In addition, these temperatures have been shown to kill the blue stain fungi (USDA-Forest Service 2005) PWN needs as a food source in the absence of living plant cells (Evans, McNamara et al., 1996, Sousa, Naves et al., 2011).

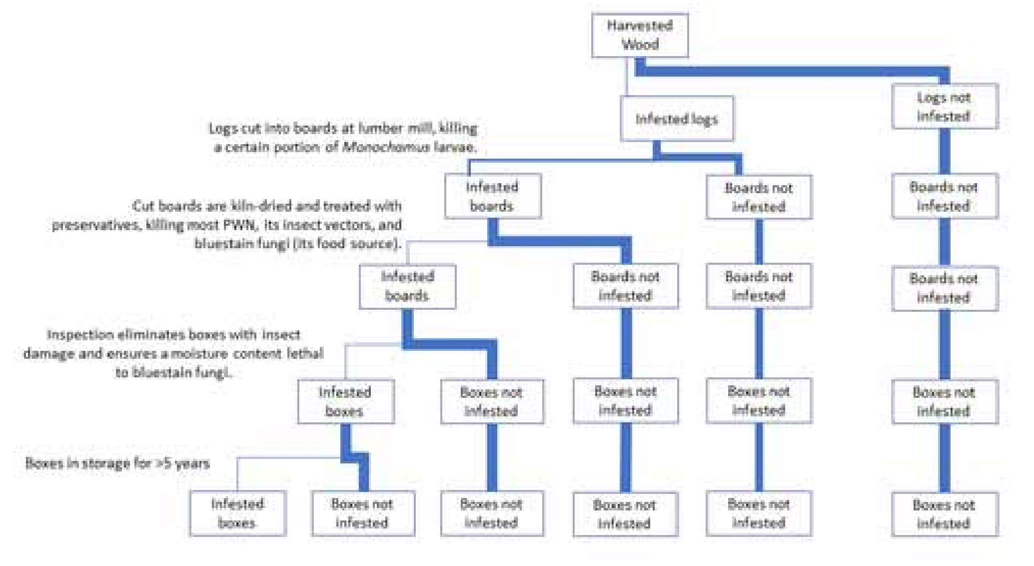
After the boards are made into boxes, they are subject to strict DoD requirements ((US DoD 2012)): they must be dried to a moisture level of 9-18 percent, and be free from rot and insect damage. Quality control inspections are carried out to ensure these requirements are met. Studies show that blue stain fungi do not grow in or infect wood with a moisture content below 20 percent (Miller 1980), which means that pinewood nematodes would be without a food source. Also, Dwinell (2004) found that pinewood nematode populations in air-dried lumber declined when the wood moisture content fell below a certain point.

Storage conditions further reduce the likelihood of PWN being in the ammunition boxes. The softwood ammunition boxes in question have been in storage for at least five years. Sousa et al., (2011) found that PWN populations in non-kiln treated boards made from infested trees largely disappeared by week 40, as the wood dried out. Sousa et al., (2011) also found that transfer of PWN from infested wood to suitable recipient wood is only possible for a period of less than 40 weeks after production of the boards.

Consistent with the above conclusions, the USDA survey of ammunition boxes found no pinewood nematodes, other wood pests, or blue-stain fungi in any of the 630 boxes sampled. Furthermore, the survey found the moisture content to be at or below 20 percent in 96 percent of the softwood ammunition boxes.

Ammunition boxes are typically shipped to Europe by sea. While exposure to the environment may occur as boxes are loaded into a shipping container, boxes do not touch the soil, so infestation by pinewood nematode during the shipping process can be ruled out.

Figure 2. Scenario tree depicting the pathway of introduction of Pine Wood Nematode and its vector Monochamus spp., from tree harvest to arrival at a European port of entry



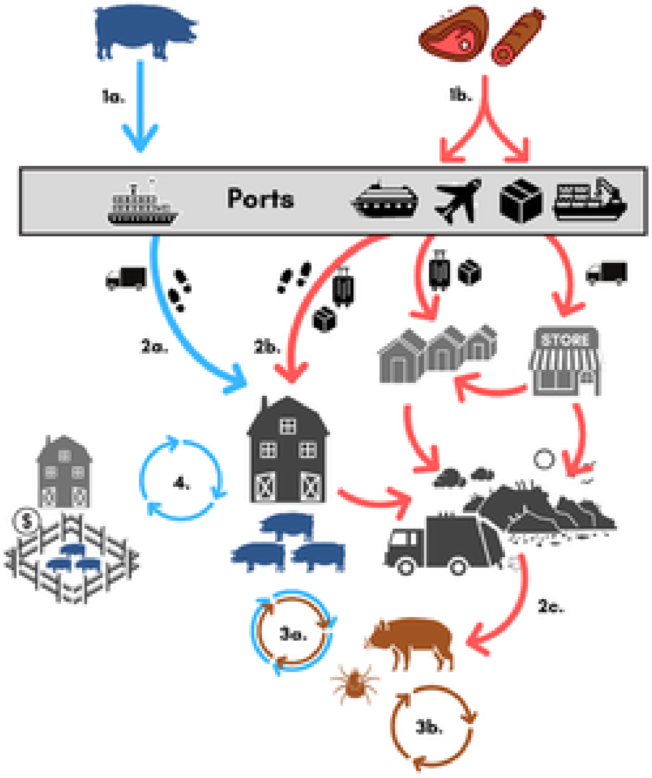
Upon arrival in Europe, boxes remain inside the shipping container. Boxes are shipped to the Ammunition Center Europe if ammunition is for military exercises, or to a demilitarization contractor if ammunition is to be destroyed. According to DoD requirements, ammunition used in training exercises must be stored under cover, away from soil, and away from trees. Empty boxes are stored in closed, clean buildings. Disposal of ammunition boxes in the EU follows strict host nation and local laws; disposal methods (e.g. incineration or recycling) do not allow the spread of pinewood nematode and generally lead to the destruction of any pests.

Based on its pathway analysis, the USDA successfully argued that the processes, requirements and conditions already applied to or in place for the DoD softwood ammunition boxes can be regarded as at least equivalent to the EU-requirements, reducing the phytosanitary risk these boxes pose to the EU to a negligible level. Convinced by the analysis, the EU exempted the DoD ammunition boxes from the treatment requirements. This means savings of hundreds of millions of dollars for the DoD, as well as the avoidance of negative environmental impacts caused by unnecessary phytosanitary treatments.

ANIMAL HEALTH CASE STUDY: LIKELIHOOD OF INTRODUCING AFRICAN SWINE FEVER TO THE UNITED STATES

African swine fever (ASF) is a hemorrhagic disease that has recently emerged as a significant threat to global domestic swine production. The United States is the world's third largest producer and consumer of pork (ERS 2020), reporting a hog population of 77.5 million in 2020 (USDA 2020). While the United States is not currently affected by African swine fever, the estimated potential impacts of over US$4.25

Figure 3. Human-mediated pathways affecting the entry (1a-1b), exposure (2a-2c), establishment (3a-3b) and spread (4) of African swine fever virus. Arrow colors indicate grouping by live animal, animal product, or wild pigs/vectors. Pathway segments are defined as: 1a. Entry – Live Animal, 1b. Entry – Animal Products, 2a. Exposure – Direct, 2b. Exposure – Swill/backyard feeding, 2c. Exposure – Landfill Scavenging, 3a. Establishment – between Commercial and Wild Pigs, 3b. Establishment – within Wild Pigs or Vectors, 4. Spread – Market sales or farm-to-farm. Blue arrows indicate pathways involving live commercial swine, red arrows involving swine products, and brown arrows involve vector/wild pig pathways



billion to the US economy (Rendleman and Spinelli 1999) have triggered the development of several studies of transboundary introduction risks (Brown and Bevins 2018; Brown, Miller et al., 2020; Corso 1997; Jurado et al 2019). This case study addresses the scenario of analyzing multiple pathways for entry and spread of ASF virus (ASFv) to the United States and summarizes the safeguarding activities reported by the USDA in response to this threat.

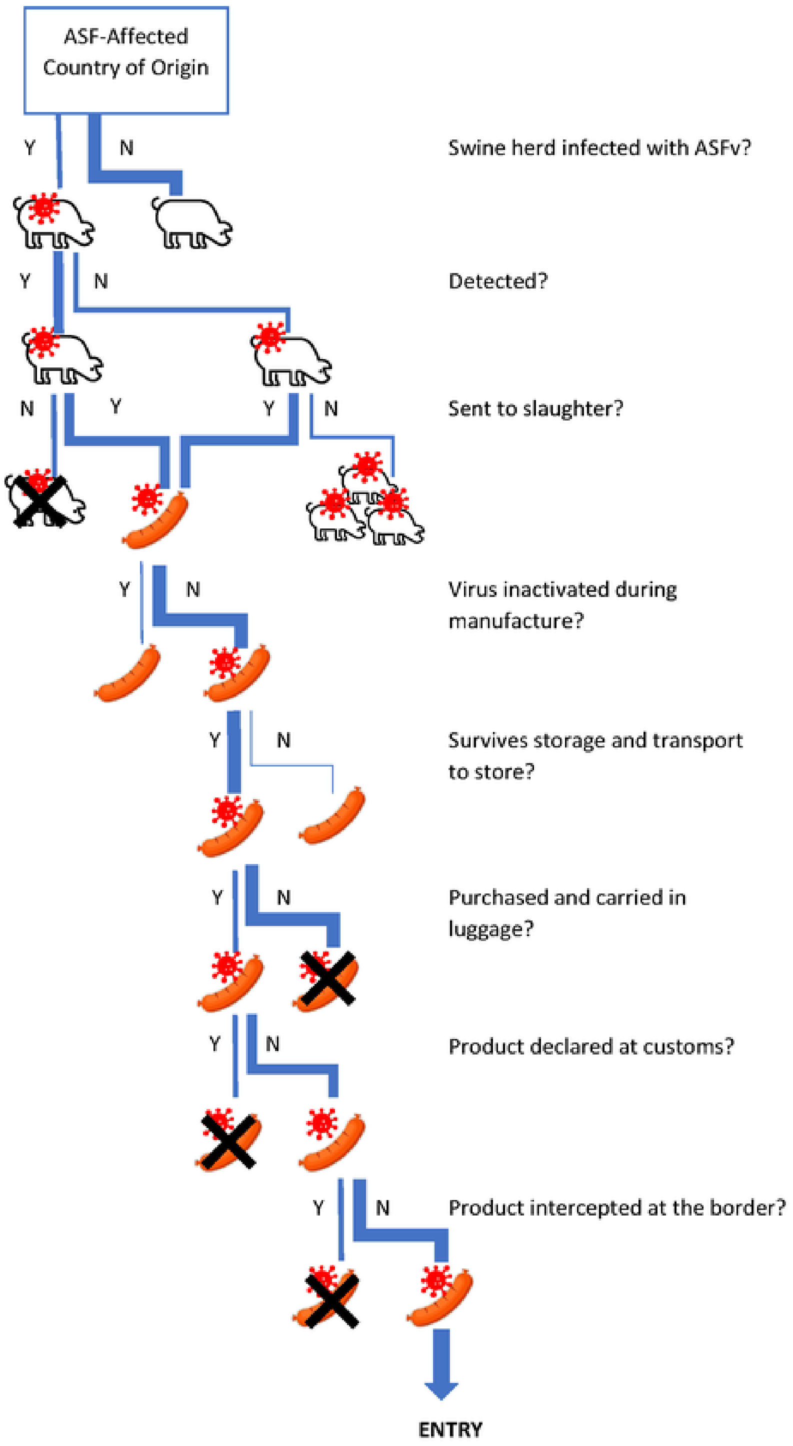
Following the spread of ASFv into China in 2018, the USDA evaluated fourteen potential pathways with regard to their likelihood of introducing ASFv to the U.S. (USDA 2019). Based on the results of these evaluations and for brevity of the case study, we focus on two pathways which can be facilitated by humans, introduction of ASFv via live animal imports and smuggling of animal products. Ranking the risk of other pathways, such as import of wildlife trophies, can be found in the report by USDA (2019). In this case study, we extend the analysis beyond entry to illustrate how humans also create pathways of in-country spread through vectors and reservoir populations (Figure 3). The nature of this analysis is much higher level and more complex than the example given for pine wood nematode, because multiple pathways are being evaluated. Therefore, the depiction of pathways in Figure 3 is more generalized than process-oriented but serves its purpose to visualize pathways and pathway interactions.

Likelihood of Entry

The United States imports live swine only from Canada, but this import pathway (Figure 3: 1a) constitutes 13% of the global trade volume for live pigs (FAO 2019). In order to reduce the likelihood of ASFv entering with import of live pigs, the USDA requires valid health certificates stating that the animals have been resident in Canada for at least 60 days and have not originated from any country affected by ASF (USDA 2020). The animals must also have undergone veterinary inspection 14 days prior to export, certifying that the animals are free of disease. The extensive inspection, testing, and health certification of live pigs entering the U.S. makes entry unlikely, thus mitigating downstream risks of transmission and disease. While smuggling live pigs into the U.S. is possible, the lack of interception data or qualitative reports suggests that this is unlikely (i.e. a negligible risk).

In contrast, the illegal entry of pork products (Figure 4) in passenger baggage, cruise ships, mail, and ship/plane garbage (Figure 3: 1b) is more likely to lead to the entry of ASF due to the volume of these products and the difficulty to entirely mitigate entry risks for this pathway (Corso 1997; Jurado et al 2019; USDA 2019). The entry of pork products from ASF-affected countries is prohibited, but most illegal pork is unknowingly being transported into the U.S. by citizens and tourists unfamiliar with federal regulations. While the U.S. monitors and inspects these pathways, the variety of entry points and large volume make it difficult to fully mitigate. The illegal entry of insufficiently heat-treated pork products has been identified by most risk and pathway assessments as the cause of previous global outbreaks of ASFv (Penrith and Vosloo 2009, Beltran-Alcrudo, Falco et al., 2019). If we narrow the scope to just air passenger baggage and evaluate its potential for entry of ASFv, we can use a scenario tree to evaluate the likelihood of entry (Figure 4). In the early stages of an outbreak, prior to detection, or in situations with poor biosecurity, infectious animals may be sold for slaughter before exhibiting visible signs of disease. In some countries, resources to rapidly control the outbreak may be insufficient and infected animals may be sent to slaughter to limit economic impacts. ASFv is incredibly stable in meat tissues, and manufacturing processes may not be sufficient to completely inactive the virus in pork products. Substantial volumes of contaminated or potentially contaminated pork products are transported illegally in airline passenger baggage (Hong et al., 2005; Wooldridge et al., 2006; Chaber et al., 2010; Falk et al., 2013;

Figure 4. Scenario tree depicting the entry of pork products from ASF-affected countries in the air/ cruise passenger baggage pathway



Beutlich et al., 2015; Melo et al., 2018). On average, the United States Customs and Border Protection intercepts 8,000 pork products annually from air passenger baggage and mail (Jurado, Paternoster et al., 2019). Interception records show that the majority of interceptions are not declared. It is estimated that 10-50% percent of such products are estimated to have been intercepted (Corso 1997), the remainder enters the U.S. undetected (Jurado, Paternoster et al., 2019). While not all pork products entering the U.S. illegally are contaminated with ASF virus, there have been several reports globally of such products testing positive for ASF virus in other international airports (Farmer 2019, Hodal 2019, MAFF 2020).

Likelihood of Exposure

After entry, the virus must come into contact with and infect commercial swine herds to be of consequence. Swine may be exposed via direct contact with infected animals (Olesen 2017; Sanchez-Vizcaino 2010), ingestion of food contaminated with infected animal tissues, secretions, or excretions, or contact with contaminated objects. ASFv transmission occurs most easily via direct contact or inhalation of aerosolized virus from infectious pigs (Figure 3: 2a); transmission via ingestion of contaminated pork products is less likely to occur (Figure 3: 2b). However, even a low probability of transmission through ingestion of contaminated pork products may occur given the very high volume of entry. A pathway of great concern is the feeding of contaminated pork products to susceptible pigs, particularly backyard swine operations. The Swine Health Protection Act prohibits the feeding of raw or undercooked meat products to swine. Garbage feeding operations in the U.S. require a permit to operate and comply with State and Federal requirements to ensure infectious organisms are killed. However, backyard garbage feeding does not require a permit and may lead to exposure of domestic swine (DEFRA 2019) if the garbage is not sufficiently heat treated prior to feeding.

Swine populations may also be exposed to ASFv through landfills, where consumers or commercial stores may discard infectious imported pork products. The United States has a large wild pig (frequently referred to as feral swine) population that is known to forage in landfills (Lewis, Farnsworth et al., 2017, Lewis, Corn et al., 2019) where they may come into contact with infectious food products (Fig 3 2c). They may then transmit ASFv to commercial swine populations if biosecurity is insufficient to prevent contact between pigs or their potentially infectious tissues/secretions/excretions.

Likelihood of Establishment

In countries where wild pigs have been involved in ASFv transmission, the disease has persisted in wild and feral populations, causing continued exposure to domestic swine (Figure 3: 3a; Miller and Pepin 2019, Pepin, Golnar et al., 2020). Persistence of ASFv through direct transmission alone appears to require relatively high wild pig densities (>4 pigs/km^2) (Pepin, Golnar et al., 2020). However, when carcass-based transmission occurs simultaneously with direct transmission, establishment can occur at densities as low as 1 pig /km^2. Pepin, Golnar et al., (2020) inferred that as much as 66% of transmission can be carcass-based. In Europe, this has been recognized as an important pathway for transmission (Wooldridge, Hartnett et al., 2006) and some countries actively remove wild pig carcasses from the landscape. In North America, wild pig densities have been estimated to average 1.9 pigs/km^2 and reach up to 16 pigs/km^2 (Lewis, Farnsworth et al., 2017). This suggests that introduction of ASFv into North American wild pig populations may result in persistence (Figure 3: 3b) if allowed to establish.

Ticks can also play a role in transmission among pigs and the potential for establishment of ASF. Studies in Europe indicate limited evidence of tick-mediated ASFv spread among wild pigs (Frant, Woźniakowski et al., 2017). However, in North America the risks of transmission via ticks is less clear and may contribute to spread and establishment of ASFv in wild pigs (Figure 3: 3b). Five species of *Ornithodoros* ticks, the genus of soft-bodied ticks associated with ASFv transmission, are found in the North America: *O. coriaceus, Ornithodoros hermsi*, and *O. parkeri* occur in central and western North America; *O. turicata* and *O. talaje* are found in arid regions of southwestern US and Mexico (Butler and Gibbs 1984, Brown and Bevins 2018). Laboratory studies have demonstrated that *O. coriaceus, O. parkeri*, and *O. turicata* can become infected with ASFv, and *O. coriaceus* was able to transmit the virus (Kleiboeker and Scoles 2001). All of these species are present in areas with wild pig populations (Miller and Pepin 2019). *O. turicata* is most likely to cause transmission, as most of its distribution range has high wild pig densities (Miller and Pepin 2019). The presence of *O. turicata* in regions with large wild pig populations may have the potential for maintenance of ASFv by this tick in the event ASFv is introduced into wild pigs (Pérez, Showler et al., 2015). While possible, there remain significant scientific gaps concerning the suitability of wild pigs as maintenance hosts for these tick species.

Likelihood of Spread

After initial infection in a pig, there are many paths by which humans can move ASFv between premises. Animal(s) that are infected but not showing clinical signs may be moved off a premise (Figure 3: 4) to new locations, inadvertently spreading infection. The likelihood of ASFv spread is not equivalent for all swine movements. For example, animals commingling at markets may lead to multiple premises infected (Ferdousi, Moon et al., 2019) while movements in terminal channels (i.e. directly to slaughter) may not lead to any new pig to pig infections. In addition, trucks used to transport swine may spread ASFv if not properly sanitized between shipments. Much of the commercial swine industry is vertically integrated, meaning animals are moved at each stage of production to specialized operations within a supply chain. This industry structure helps limit disease spread to the next phase of production in the chain or to facilities outside of the supply chain. With proper biosecurity in place, disease shouldn't spread backwards in the chain, i.e. from grower/finisher back to the nursery.

The underlying structure in the industry drives the direction of swine movements (Gorsich, Miller et al., 2019), but estimating how that may ultimately drive disease spread through the swine industry is difficult. Testing scenarios in disease simulation models can be a useful method to evaluate the likelihood of disease spread through complex spatial structures in landscapes and evaluate potential effectiveness of mitigations options (Tsao, Sellman et al., 2020). For example, USDA combined limited real-time electronic data on swine movements (simulating the movement of swine between premises) with disease simulation models (Lindstrom, Grear et al., 2013).

Safeguarding Tactics: Preparedness and Prevention

Since the outbreak of ASF in China in 2019, USDA has been developing new pathways analyses to update safeguarding activities to prevent the entry of ASF. In preparation for a potential response, USDA updated its ASFv preparedness and response plan and executed a national functional exercise in 2019 with several pork producer states (USDA 2020). An ASF "red book" or response plan has been developed which outlines specific activities which would be activated in a response to an ASF incursion (USDA

2020). In order to prepare for the large numbers of samples expected in an outbreak, USDA expanded the number of diagnostic laboratories in the National Animal Health Laboratory network approved for testing ASF (Farmer 2020). Finally, USDA implemented an active surveillance plan for ASF in addition to its existing passive surveillance data streams (USDA 2019).

With the recent confirmation of an ASF detection in the country of Dominican Republic in July 2021, USDA again used pathways analyses to further increase safeguards. In particular, a new Federal Order was established that halts movement of swine, germplasm, products, and by-products through U.S. territories to the mainland (USDA 2021). Information from pathways analyses are being evaluated for potential use in targeting surveillance activities in the protection zone.

By leveraging risk analysis methodologies, USDA has developed a science-based preparedness strategy in response to the threat of ASF incursion. The continuing global spread of ASF, combined with pathways studies (only some of which are summarized in this case study) indicates that an incursion of ASF in the United States is plausible. Regulatory controls, inspection, and biosecurity measures have been put in place and recently strengthened but cannot eliminate all risks. USDA continues to evaluate strategies for mitigating pathways of concern and leverage internal and external partnerships to accomplish the mission of safeguarding U.S. agriculture.

OPPORTUNITIES AND FUTURE DIRECTIONS

Data on human movement, particularly origin-destination data, is key to understanding pathways for spread. The continual integration of technology into daily life presents new opportunities for linking human activity to the introduction of pests and pathogens, similar to how social media has already been leveraged as an investigative tool for criminal activity (Abdalla and Yayilgan, 2014). Countries could benefit from drafting internationally accepted data collection standards around human movement datasets for the purposes of modeling pest and disease spread.

Estimating the expected impact of introduced pests or pathogens is an important component of risk analysis. However, shared standards for how to assess economic impacts do not yet exist. Scientific studies do not report pest or pathogen damage in a consistent manner, making it difficult to assemble generalized impact estimates from the scientific literature. The international community could benefit from developing a standard for reporting pest and pathogen impacts. The creation of a clearinghouse for reported economic impact data could also be useful.

Advances in artificial intelligence (AI) provide exciting opportunities for improving pathway analysis. One application of AI is *machine learning*, which are mathematical models or algorithms that "learn" or improve by adapting tunable parameters to observed data and provide a powerful means of prediction (Mitchell 1997). The objective of machine learning is to find generalizable, predictive patterns through analysis of often large quantities of data enabling the prediction of future out comes or classify new observational data (Bzdok, Altman et al., 2018). Machine learning techniques are currently being applied to a large diversity of problems such as predicting whether or not a specific email is spam (e.g., Guzella and Caminhas 2009), predicting the selling price of a house (e.g., Park and Bae 2015), identifying a person or animals in digital photos (e.g., Bartlett, Littewort et al., 2004), making product recommendations (e.g., Park and Chang 2009), or finding gene expression profiles that are similar (e.g., Jin, Xu et al., 2006). Application in the biosecurity field is nascent, but there are opportunities to apply these techniques, to help analysts identify which pests/pathogens are most likely to establish, where and when they are most likely to establish, which pathways they are most likely to arrive on, or which critical point(s) on the pathway are most likely to fail.

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KEY TERMS AND DEFINITIONS

Biological Hazard: A biological agent (such as a pest or pathogen) with the potential to cause an adverse effect in natural or agricultural ecosystems.

Entry: Movement of a pest or pathogen into an area where it is not yet present.

Establishment: Persistence of a pest or pathogen within an area after entry.

Exotic: Not known to be present in an area.

Exposure: An animal pathogen infecting a susceptible animal population and maintaining onward transmission after entry to a new area.

Infection: The invasion and multiplication of a pathogen inside an animal or plant.

Introduction: For plant pests, the entry and subsequent establishment in a new area; or for animal pathogens, the entry of the pathogen and subsequent infection of susceptible hosts.

Pathway: Any means that allows entry or spread of pathogen or pest in a new area.

Phytosanitary: Relating to the health of plants, especially with respect to the requirements of international trade.

Reservoir: A living organism or inanimate matter (e.g., soil) in which an infectious agent normally lives and multiplies and from which it can be transmitted.

Transmission: The passing of a pathogen from one host to another.

Chapter 4

Control Practices for Safeguarding Agricultural and Environmental Biosecurity Before Entry Points

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ABSTRACT

Biosecurity, in the context of agriculture and natural ecosystems, refers to a strategic framework of policies and procedures intended to prevent the introduction or release of biological agents and materials that have the potential to threaten or compromise the agricultural sector in the form of invasive species, exotic pathogens, and foreign pests. Exchange of plants, animals, and agricultural products along with wood packaging material and dunnage that are transported through commerce and trade can lead to accidental introductions of foreign pathogens and pests unless sound biosecurity protocols are implemented to ensure the quality and safety of imported commodities at the local, transboundary, and global levels. Principal stakeholders at risk are those with interests in food, feed, fiber, oil, ornamental, and industrial crops; commercial forestry, natural ecosystems, and parks; and the livestock, poultry, aquaculture, fisheries, and apiculture industries.

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INTRODUCTION

Biosecurity, in the context of agriculture and natural ecosystems, refers to a strategic framework of policies and procedures intended to prevent the introduction or release of biological agents and materials that have the potential to threaten or compromise the agricultural sector in the form of invasive species, exotic pathogens, and foreign pests. Exchange of plants, animals, and agricultural products along with wood packaging material and dunnage that are transported through commerce and trade can lead to accidental introductions of foreign pathogens and pests unless sound biosecurity protocols are implemented to ensure the quality and safety of imported commodities at the local, transboundary, and global levels. Principal stakeholders at risk are those with interests in food, feed, fiber, oil, ornamental, and industrial crops; commercial forestry, natural ecosystems and parks; and the livestock, poultry, aquaculture, fisheries, and apiculture industries.

Primary factors contributing to the vulnerability of various sectors include: 1) wide variations in the types, sizes, and management practices of production systems around the world; 2) challenges in establishing and maintaining effective monitoring, surveillance, and control programs to identify and successfully manage current and evolving biosecurity threats; 3) the growing interdependence of large-scale agricultural businesses, combined with an increasing reliance on vertical integration; and 4) administrative and political barriers that impede timely communication and sharing of critical information between various national oversight systems.

A nation's economy can be easily sabotaged by the accidental or intentional release and dissemination of biological agents that have the capacity to cause widespread harm and destruction to people, animals, plants, and/or the environment. Risks are managed through early detection and rapid control measures that are designed to prevent unauthorized introduction of invasive species, pests, and foreign diseases with the potential to compromise a country or region's agricultural assets. Basic control techniques focus on preventing introductions; identifying threats in a timely manner; and managing consequences to minimize their impact. The main objective is to reduce opportunities for any species, pests, or pathogens with elevated biosecurity concerns to spread or become established.

Foreign pathogens and pest species can be introduced as contaminants of agricultural products and other goods that are legally exchanged between countries (Capinha et al., 2015; Seebens et al., 2017; Tabak et al., 2017). However, they can also be indirectly transported over long distances through natural currents of air and water; routine movements of migratory wildlife and birds; and by lapses in sanitation or disinfection procedures during the shipping process. Inanimate objects such as shipping vessels, ballast water, sea containers, machinery carts, wooden transport crates, packing materials, soil, and other contaminated products can conceal non-indigenous plants, pollen, animals, insects, and their associated microbial flora while in transit, allowing their accidental escape or release during the shipping process (McNeill et al., 2011; Ruiz et al., 1997).

Humans can also accelerate the movement of foreign species, pests, and pathogens through negligent acts that reflect an inadequate understanding of sound biosecurity principles or poor judgment. These can include careless handling or intentional release of nonindigenous plants, animals, and contaminated agricultural commodities into new ecosystems compatible with their survival. Proliferation of feral swine throughout the western hemisphere is a classic example of unforeseen consequences resulting from the casual introduction of a new species, without proactive consideration of the potential risks (West et al., 2009). Wild pigs contribute to the spread of various diseases that are endemic to many swine-producing regions, such as leptospirosis, brucellosis, tuberculosis, and porcine epidemic diarrhea virus (PEDV), and their presence significantly impedes control efforts directed at other high consequence pathogens, such as Foot and Mouth Disease (FMD) and African Swine Fever (ASF), when disease-free areas are threatened by introduction of these agents (Brown et al., 2021).

Exotic plants, animals, pathogens and pests that become established following unplanned or unauthorized introductions are referred to as invasive species, defined by U.S. Presidential Executive Order 13112 as species that are "non-native (or alien) to the ecosystem under consideration" and "likely to cause economic or environmental harm or harm to human health" (Exec. Order No. 13,112, 1999). Following the introduction of invasive species, ecosystems can be permanently altered or degraded as native species are forced to compete for limited resources. Recent publications have estimated costs of biological invasions to be between $22.1 to 26.8 billion (U.S. dollars) each year (Diagne et al., 2021; Moodley et al., 2021), with the most recent data projecting costs to be around $162.7 billion (U.S. dollars) in 2017 (Diagne et al., 2021).

Covert introduction of foreign pathogens and pests can also occur through malicious acts of bioterrorism deliberately targeting a country's vulnerable agricultural assets as well as through smuggling and illegal sales of prohibited biological materials and wildlife (Hitchens & Blakeslee, 2020; Wyatt, 2016). To date, there has been one suspected agro-bioterrorism incident linked to a plant pathogen which caused significant economic losses at a cacao plantation in Brazil and may have been intentionally released (Caldas & Perz, 2013), and numerous allegations associated with the questionable emergence of animal pests that have also never been fully substantiated (Keremidis et al., 2013).

By comparison, smuggling appears to be a more likely and common route for foreign pests and pathogens to be introduced. Contraband that is contaminated or infested poses serious threats to agriculture, and rigorous countermeasures are being developed to deter these criminal activities (Casagrande, 2000). The now well-established Varroa bee mite population in New Zealand originated from queen bees that were illegally imported (Stevenson et al., 2005; Wyatt, 2016). Private citizens also released Rabbit Hemorrhagic Disease Virus in New Zealand without authorization in 1997, after the government specifically decided against introducing it to control the rapidly expanding wild rabbit population (Abrantes et al., 2012). Many authorities suspect the Asian citrus psyllid (*Diaphorina citri* Kuwayama), which is implicated in the transmission of pathogens responsible for citrus greening disease (huanglongbing), entered the U.S. through contaminated ornamentals and infested citrus concealed in cargo that originated in Asia (Halbert & Manjunath, 2004). Prohibited meat and meat products contaminated with FMD or ASF virus are a constant threat to countries free of these agents, such as New Zealand and the U.S. (Pharo, 2002). The ASF virus has been documented to survive in dry-cured pork products for up to 83 days (Petrini et al., 2019), which means the virus can easily survive transport in contaminated meats and become a source of infection if these commodities are fed to, or come into direct contact with, susceptible swine populations during that timeframe.

Transboundary biosecurity focuses on all sources and movements of plants, animals, germplasm (including pollen), and their associated products. Restrictions are placed on the entry of these goods until they are confirmed free of actionable pathogens and pests through comprehensive testing, a satisfactory quarantine period, and/or effective treatment programs. Trade partners jointly establish intergovernmental standards, which define specific criteria needed to verify the disease and/or pest-free status of exchanged commodities and products. In some cases, common management practices that are intended to prevent pathogen or pest transmission may have unexpected consequences that complicate screening efforts. For example, subclinical infections can sometimes be masked by vaccination, use of prophylactic antimicrobials, and/or routine disinfection procedures, making timely and accurate detection more challenging (Jaemwimol et al., 2019; Richter et al., 2020; Tizard, 2021). A complete record of treatments and other mitigations performed should accompany commodities and products that are exchanged as part of the verification process.

Successful mitigation strategies are directed at critical points along the production and distribution chain. The emphasis is on preventing or minimizing the impact and global spread of human-mediated threats to agriculture and the environment. Proactive biosecurity programs incorporate sound management practices, including: 1) monitoring the transport and movement of people, plants, animals, and related products across, and within, national borders; 2) verifying the biosecurity status and quality of all imported goods and products; 3) adhering to established biorisk management strategies focused on eliminating or controlling significant pests and pathogens (i.e., quarantine, minimum holding times, diagnostic testing, vaccination, and/or prophylactic treatment programs); and 4) maintaining a clean and secure environment that promotes product integrity throughout the trade cycle. The most effective oversight systems generally require an integrated approach, with tiered actions implemented at the international, national, state, regional, and local farm levels.

This chapter provides a practical overview of management and control practices to safeguard agricultural biosecurity against human-mediated threats, emphasizing validated control measures, emerging technologies, inherent challenges, and potential biosecurity gaps related to activities leading up to (i.e., pre-border) and at the point of entry (i.e., border). For purposes of discussion, a border is defined as the official boundary of a distinct geographical or political jurisdiction and serves as a critical control point for regulating or limiting the movement of people, animals, plants, and other goods into, and out of, a designated area or region. National borders may correspond to natural geographic features, such as mountain ranges or water boundaries that are easily recognized and defined, or they may be legally established through agreements negotiated between political or social entities that exercise dominion over a specified territory or region. Some ports of entry are located at international airports and seaports that are internal to a country's peripheral boundaries. In the U.S., borders can also refer to the boundaries between or within states. In some cases, states have the option to regulate commercial activities with other states by restricting the movement of certain products and goods that have the potential to compromise agricultural assets and industries (CDFA, 2021). The attempt is to provide a perspective of biosecurity at international borders. However, emphasis is given to established practices in nations that are at the forefront of these global efforts, including New Zealand, Australia, the U.S., and Canada.

PRE-BORDER ACTIVITIES

Pre-border control activities are strategically designed to minimize opportunities for potential threats to be introduced beyond or across the border. These actions require an ongoing analysis of potential risks (FAO, 2007; Leung et al., 2014) and establishment of international partnerships that contribute resources, intelligence, and policy frameworks to meet common objectives (Jeggo, 2012).

Control Systems

Active pre-border control systems are a major component of any successful threat reduction program and provide the first line of defense against the introduction of excluded products, pests and pathogens. Frequent assessment of potential risks is a prerequisite for all border transactions and helps to guide these initiatives. The process starts by establishing a clear understanding of the biosecurity status of neighboring countries and trading partners. Building alliances through trust lays the foundation for successful trans-border risk management and control efforts. Maintaining effective communication networks ensures prompt and accurate reporting of biosecurity lapses and other unusual occurrences between partners. Breakdowns within the information exchange system leads to lost opportunities and can have negative consequences on resources, trading, and other commercial activities. Control options to maintain and protect vulnerable crops and animal populations are often limited or completely ineffective after pathogens or invasive species have been released and become established in the environment (see Table 1).

Table 1. Notable foreign animal and plant disease outbreaks

Year	Disease	Country	Circumstances
1845	Potato late blight	Ireland	Strain introduction led to the Great Irish Potato Famine (Kentaro et al., 2013)
1933	Johne's Disease	Iceland	Infected sheep imported from Europe (Sigurdsson, 1954)
1966-67	Foot and Mouth Disease	United Kingdom	Contaminated lamb/sheep meat imported from Argentina (Wright et al., 2013)
1980s	Bovine Spongiform Encephalopathy	United Kingdom	Initially found in the United Kingdom, with spread to 24 countries through infected cattle and contaminated meat/bone meal (Smith & Bradley, 2003)
1990s	Varroa Bee Mite	New Zealand	Unidentified source (Iwasaki et al., 2015)
1997	Classical Swine Fever	Netherlands	Pigs transported in a vehicle contaminated with CSF in Germany (Elbers et al., 1999)
2001	Foot and Mouth Disease	United Kingdom	Pigs fed unprocessed waste that contained contaminated meat products (Davies, 2002)
2011	Maize Lethal Necrosis	East Africa	Disease due to combination of two viruses (Maize Chlorotic Mottle and any Potyviridae cereal virus) (Mahuku et al., 2015)
2013	Olive Quick Decline	Italy	Emergence of a new *Xyllela fastidiosa* pauca disease via an insect vector from Central America (Martelli et al., 2016)
2013	Porcine Epidemic Diarrhea Virus	United States	Suspected entry through contaminated animal feed components (Scott et al., 2016)
2016	Wheat Blast	Bangladesh	Arrived in food aid traced to South America (Callaway, 2016)
2017	*Mycoplasma bovis*	New Zealand	Unidentified source (New Zealand Ministry for Primary Industries, 2020e)
2019	Tomato Brown Rugose virus	United States	First described in Jordan and Israel, arrived in U.S. in 2019 (Ling et al., 2019)

Basic components of a sound bioexclusion program include: 1) tactical use of inherent geographical features that act as physical barriers to deter the migration and transport of pathogens and pests, 2) unified regulatory initiatives such as internationally established legislation (policies, regulations, and laws), and 3) commercial controls (negotiated treaty and trade agreements) that restrict unauthorized movement of people and goods.

Landscape characteristics, such as naturally occurring water systems and rough terrain, can significantly impede natural transmission of pathogens and pests between distinct geographic regions (Bessell et al., 2008; Smith et al., 2002). Prominent topographical features help to minimize casual movement of animals, reduce dispersal of biological materials through wind and water transmission, and create seasonal variations that are not conducive to the spread or establishment of nonindigenous species. Unfortunately, reliance on defined ecological niches to suppress species translocation and dissemination has become increasingly threatened by ecological and social changes. Persistent social and economic pressures have contributed to shifts in climate, habitat, and biodiversity that challenge the integrity of physical and environmental obstacles, which have historically served as the first line of defense in protecting the health and security of agricultural products and natural ecosystems (Ogden et al., 2019).

Regulatory barriers include permit requirements and other trade restrictions on agricultural goods. Conditions for international commerce are defined through legal frameworks that establish clear biosecurity standards and other controls needed for the safe exchange of products and treatment of packaging material. The presence or absence of specific diseases and pests within a particular jurisdiction generally define limitations on the movement of products and goods originating from that location. Jurisdictions can be defined as a distinct legal entity (country, zone, county, province, or state), a group of entities (countries), or combined parts of various separate entities (region). Program success largely depends on the willingness of various partners to uniformly commit to these agreements as a condition of trade. (APHIS, 2021d).

The World Organization for Animal Health, Office International des Épizooties (OIE), is the global body responsible for collecting, analyzing, and disseminating information on the distribution and occurrence of animal diseases. These data are used to develop and continuously update international biosecurity guidelines focused on baseline health, diagnostic testing, and vaccination practices required for the safe trade of animals and their products, as documented in the OIE's Terrestrial Animal Health Code (OIE, 2021a). Under the Sanitary and Phytosanitary Agreement, the World Trade Organization uses the OIE Code to establish scientifically based regulatory standards that govern the health and movement of animals and their products in international commerce. Participating countries are guided by common core principles, emphasizing a shared commitment to work cohesively toward the eradication of important transboundary animal diseases that pose risks to trading partners.

Commonly used tactics include pre-border vaccination programs or treatment of animal-based products to prevent the introduction of dangerous and costly diseases. Immunization programs can be strategically designed to focus on diseases of national interest and emerging pathogens that pose imminent threats to vulnerable native populations (i.e., humans, susceptible livestock, companion animals, etc.). International and state-sponsored vaccine banks help to ensure high-quality vaccines manufactured in accordance with uniform intergovernmental standards are procured and can be delivered in a timely manner (OIE, 2021b). The United States Department of Agriculture (USDA) administers the National Vaccine Stockpile (APHIS, 2021a) and National Animal Vaccine and Veterinary Countermeasures Bank (NAVVCB) for livestock in the U.S. (Agriculture Improvement Act, 2018). These programs stockpile approved vaccines, diagnostic kits, and other veterinary countermeasures that can be rapidly deployed to prevent high-consequence animal diseases from entering and spreading in the U.S., including Highly Pathogenic Avian Influenza, FMD, Exotic Newcastle Disease, and Classical Swine Fever (CSF). The U.S. also partners with Canada and Mexico to maintain a shared FMD vaccine repository through the North American FMD Vaccine Bank (NAFMDVB) (Beckham et al., 2018).

Vaccination of livestock for FMD varies, depending on the disease status of a country. Typically, disease-free countries do not pre-emptively vaccinate for this agent. However, vaccination programs, in combination with other sanitary and quarantine measures, have evolved as effective tools to protect animals in countries where the disease is endemic. Immunized populations can serve as barriers to limit spread within an affected area and to control disease incursion into bordering countries during outbreaks. Vaccines have even been used to overcome commercial barriers and support the safe and fair exchange of animals and animal products between countries of different zoosanitary status. Under the OIE guidelines, participating countries can continue international trade with reliable assurance that potential biosecurity risks are being effectively managed through these import restrictions (OIE, 2021a).

Immunization of susceptible non-target species, combined with enhanced control measures, provides an additional barrier against the casual spread of foreign pathogens and pests across physical borders in defense of animal and public health. These programs have been used to successfully control the incidence and spread of wildlife diseases that present significant risks to humans and domesticated species. One of the better-known examples is the oral rabies vaccine, which is distributed as a palatable bait and has been highly successful in eliminating rabies in coyotes throughout most of the U.S., the gray fox in Texas, and the red fox in parts of Europe (Rupprecht et al., 2004). Similar efforts to tackle other diseases transmitted from wildlife to domestic livestock are in progress, including bovine tuberculosis caused by *Mycobacterium bovis* and spread by brushtail possums in New Zealand (Rouco et al., 2018), Eurasian badgers in the United Kingdom, wild boars in Spain, and white-tailed deer in the U.S. Early program results are promising and have laid the groundwork for future investigations on optimal bait formulation and distribution techniques for various species of interest, as well as developing diagnostic tests capable of distinguishing infected animals from those that are vaccinated (Balseiro et al., 2020).

Targeting pests that serve as vectors in animal disease transmission is another effective strategy that minimizes the spread of foreign pathogens across physical borders. Basic control methods often require treatment of animal hosts while pests are feeding or the physical environment where animals are kept. Insecticides can be applied topically to animals, administered systemically, or by combining both methods. A sound husbandry program is the foundation for a comprehensive control program, so housing areas should be thoroughly cleaned and disinfected before premises are treated to eliminate conditions favorable to insect life stages completed in the environment. Immature stages that are less mobile are more vulnerable to environmental treatment, so formal control programs targeting these developmental phases are often more effective and practical.

USDA leads efforts to incorporate OIE principles into national animal health standards for the U.S. Importation of animals from restricted countries may be completely banned or only allowed under specific conditions (i.e., confirmation of health status through veterinary inspection, diagnostic testing, and sometimes treatment or immunization) to minimize accidental introduction of costly and dangerous pests and diseases (see Table 2). A pre- and/or post-transport quarantine period may be required when reliable diagnostics are not available or a disease or pest has a lengthy incubation period that delays detection. Products of animal origin must also be screened for potential pathogens and pests. In some cases, animal products may need to be altered or treated with heat, desiccation, irradiation, or some other means prior to importation.

New Zealand has legislated and adopted a National Animal Identification and Tracing (NAIT) system, requiring all cattle and deer to be registered and permanently identified with official ear tags. Individuals responsible for these animals must maintain a complete record of their movement, from birth until final disposition. The system ensures rapid contact tracing and a coordinated response to animal disease outbreaks, food safety concerns, and other quality assurance issues that affect animal health and welfare (New Zealand Ministry for Primary Industries, 2019).

As part of its mission, the Food and Agriculture Organization of the United Nations (FAO) has an important role in setting international health standards for plants which parallels the OIE's contributions

Table 2. Past impact of foreign animal and plant diseases on global economics and production

Disease	Site	Year	Economic Consequences
Foot and Mouth Disease	Taiwan	1997	• $6 billion due to slaughter of over 4 million pigs (or 40% of the national inventory) • $1.6 billion due to Japan's ban on pork from Taiwan (Polyak, 2004)
Classical Swine Fever	Netherlands	1997	• Slaughter of 700,000 pigs across 429 infected farms • Pre-emptive depopulation of 11 million pigs across 1300 additional farms (Elbers et al., 1999)
Foot and Mouth Disease	United Kingdom	2001	Total $11.9-$18.4 billion loss due to: • slaughter of 7 million animals • $4.8 billion loss to agriculture, the food industry, and the public sector • $4.2-$4.9 billion in lost tourism • $2.9-$3.4 billion in indirect losses (Polyak, 2004)
Asian Soybean Rust	United States	2004	• $640 million – 1.3 billion in first year alone (Livingston et al., 2004)
Citrus greening	United States	2005	• $1 billion/year from 2015-2019 (Li et al., 2020b)
Porcine Epidemic Diarrhea Virus	United States	2013	• 10% reduction in 2013-2014 pig crop (7 million pigs lost) • $481-$969 million annual losses (Schultz & Tonsor, 2015)
Tomato Brown Rugose Virus	Israel, Jordan	2015	• $262 million/year impact (Klap et al., 2020)

to animal health. FAO administers the International Plant Protection Convention (IPPC), an intergovernmental treaty signed by more than 180 countries that aims to protect the world's plant resources from the spread and introduction of pests. The Convention's goals are achieved through international phytosanitary certificates, prepared and issued in accordance with guidelines and requirements defined by the International Standards for Phytosanitary Measures (ISPMs). These certificates are issued through government departments or officials empowered by the National Plant Protection Organization (NPPO) for every country that exports or imports plants and plant-based products. The phytosanitary certification process was previously a slow paper-based system that was likely to result in lost or erroneous permits. However, in 2014, the Ninth Session of the Commission on Phytosanitary Measures (FAO, 2014) transitioned to ePhyto. Adoption of this new electronic system has dramatically streamlined procedures, enabling permits to be directly and rapidly transferred between NPPOs of various countries with less fraud and error (FAO, 2021).

Technical justifications for phytosanitary measures are determined in part by the incidence or status of regulated pests within the country of origin (CDFA, 2021). Optimal compliance requires a cohesive approach that incorporates a variety of pre-border pest management strategies to eradicate or suppress excluded weeds, pests, and diseases. In some cases, plants, plant products, and seeds must be verified as treated prior to export. Depending on the type of commodity and importing country's requirements, treatments can include water (i.e., washing), grinding, cold, heat, pressure, irradiation, and vacuum (physical treatments) or fumigation, pesticide drenches, aerosols, and fogging (chemical treatments or exposure to elevated levels of carbon dioxide) (Armstrong et al., 2014; New Zealand Ministry for Primary Industries, 2020b). Current phytosanitary conditions for logs exported from New Zealand require debarking or fumigation with either methyl bromide or hydrogen phosphide (New Zealand Ministry for Primary Industries, 2020a). Pests that infest cereals are typically controlled by traditional disinfestation techniques that include fumigants, irradiation, extreme temperature treatments, and biorational approaches, or may involve newer techniques such as nonthermal (cold) plasma and metabolic stress disinfestation and decontamination (Pai et al., 2018; Paul et al., 2020).

The North America Plant Protection Organization (NAPPO) develops science-based regional standards to promote safe trade and protect agricultural, forest, and other plant resources against regulated pests. In some cases, regulatory restrictions are only applied to certain areas during specific seasons or times of the year. NAPPO Regional Standard for Phytosanitary Measures 33 (NAPPO, 2021) provides risk management guidelines that prevent entry and establishment of the Asian gypsy moth (AGM) in areas free of this pest, such as North America and New Zealand. According to this standard, vessels are regarded as "high risk" for 12 months after traveling to ports where AGM occurs, particularly during times of the year when female moths are flying or AGM larvae are ballooning. All "high risk" carriers must have a Certificate of Freedom issued by a recognized inspection body prior to docking in New Zealand, or the vessel can be detained 4 nautical miles at sea pending completion of a thorough offshore inspection (New Zealand Ministry for Primary Industries, 2021b).

USDA oversees operations to detect, control, eradicate, suppress, prevent, and/or retard the spread of plant pests in the U.S. under the authority of the Plant Protection Act (7 U.S.C. 7701). The Act is enforced by the Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) (APHIS, 2021e) and prohibits importation or exportation of plants without a special permit. In New Zealand, the Biosecurity Act 1993 designated the Ministry for Primary Industries (MPI) as the NPPO responsible for protecting that nation's environment, economy, health, and socio-cultural values from harmful organisms (*Biosecurity Act 1993*, 1993). MPI relies on the ePhyto system (New Zealand Ministry for Primary Industries, 2021c) to manage phytosanitary certificates (New Zealand Ministry for Primary Industries, 2020c) and defines Import Health Standards for all plant-based imports to New Zealand, as required by the Biosecurity Act 1993 (New Zealand Ministry for Primary Industries, 2020e). Import Health Standards are generally equivalent to the Quarantine Pre-Shipment (QPS) protocols followed in the U.S., except the New Zealand standards also apply to some inanimate commodities such as sea containers, machinery, and cars (New Zealand Ministry for Primary Industries, 2020h).

Preparedness Plans

Recognizing the significant and lasting consequences of biosecurity lapses, most governments have enacted legislation specifically directed at prevention and response to foreign species and pests (*Biosecurity Act 1993*, 1993; Genovesi et al., 2014; National Invasive Species Act, 1996). In most cases, documented plans are developed in accordance with set standards and regulations that describe diagnostic testing, disinfection, vaccination, and quarantine requirements needed to verify the integrity and safety of various goods and products prior to entry. These requirements must be incorporated into individual plans that align with each country's specific philosophy and approach for managing concerns associated with the quality and integrity of agricultural goods exchanged in commerce. The plans outline formal actions that must be taken at the point of origin, prior to movement, or during transit to ensure commodities meet the receiving country's minimum entry requirements and can be imported safely.

Preparedness plans often outline surveillance and response efforts aimed at detection and pre-border defense measures to mitigate risks and challenges that are posed by foreign species of plants, animals, and pests. New Zealand's national biosecurity strategy defines the roles and accountabilities of various participants who are responsible for reducing incoming threats (New Zealand Ministry of Agriculture and Forestry, 2003) and includes a science-based strategy for collecting evidence and other technical data needed to support the country's expanding biosecurity goals (New Zealand Ministry of Agriculture and Forestry, 2007). Many New Zealand research efforts to protect the nation's valued terrestrial plants (i.e., agricultural, amenity, native, nonnative, etc.) from biosecurity risks at the border are coordinated by a voluntary alliance of research organizations, universities, government departments, and primary industries called the "Better Border Biosecurity" (B3, Better Border Biosecurity, 2021). New Zealand's national biosecurity strategies were refreshed in a "Biosecurity 2025" direction statement (New Zealand Ministry for Primary Industries, 2020d), and a 2012 revision of the New Zealand Biosecurity Act 1993 provided the foundation for Government Industry Agreements which provide a more comprehensive approach for addressing biosecurity readiness and response (GIA, 2021). Many New Zealand industries have formally signed these agreements, which: 1) identify biosecurity risks of greatest concern to each industry; 2) define actions to minimize the probability of introduction; 3) review preparations for incursions that do occur; and 4) clarify expectations for sharing costs and decision-making authority. Details of how the New Zealand government responds to pest incursions have been defined in a comprehensive policy statement (New Zealand Ministry of Agriculture and Forestry, 2008). This broad set of policies recently served as the basis for another ambitious initiative known as "Predator Free 2050," designed to purge New Zealand of well-established nonnative pests (e.g., rats, stoats, and possums) that are harmful to native fauna (e.g., birds) by 2050 (Owens, 2017; Predator Free NZ, n.d; Russell et al., 2017.).

The U.S. has developed similar plans to deal with emergencies involving foreign pests. The New Pest Response Guidelines (NPRGs) were developed by APHIS PPQ to describe federal response team actions during the first 30 to 60 days after the identification of any new pests capable of causing significant damage and destruction to domestic plant resources. These Guidelines include: 1) information relevant to pest identification in the field; 2) the pest's biology; 3) methods for conducting a delimiting survey; 4) eradication and control options; and 5) a summary of knowledge gaps. The National Plant Disease Recovery System (NPDRS) supports these efforts by developing a series of written preparedness plans under the advice and guidance of an external panel of experts that detail response and recovery efforts for a variety of plant pathogens and diseases affecting major agricultural commodities. The content of the NPRG and NPDRS may appear similar; however, each document has unique scientific objectives intended for a specific group of end users. The NPRG focuses on plans designed to limit the spread and ultimately eradicate new pests, whereas the NPDRS provides timely and accurate information on a variety of plant pests and diseases, emphasizing control actions that growers can implement immediately during an outbreak. The U.S. government has also established the National Plant Health Emergency Management Framework, a comprehensive control strategy intended to preserve the health and safety of U.S. crops through a coordinated system of emergency preparedness, pest exclusion, response, and recovery programs designed to eliminate or mitigate the risk of invasive plant pest introductions (APHIS Plant Health Emergency Management Framework, 2019).

On the veterinary side, the U.S. has established a robust national network of veterinary diagnostic laboratories (National Veterinary Services Laboratories) and focused response plans to address specific foreign animal diseases and pests (e.g., the North American Plan for Animal and Pandemic Influenza, USDA APHIS Foreign Animal Disease Preparedness and Response Plan) (DHHS, 2017). These control efforts are managed through federal, state, and local partnerships that coordinate surveillance resources and efforts during investigation of suspect cases that are identified and reported through a network of private and governmental oversight systems. General mitigation strategies, reflecting the most current threat and post-incident recovery information for a range of frequently encountered incidents, can be used as blueprints to quickly develop highly specific supplemental plans needed to respond to unanticipated disease and pest outbreaks, disasters, and other urgent situations (Harper, 2020).

Pre-Clearance Offshore Plans

Controlling the spread of animal diseases is an economically sound management decision for private producers and those involved in global commerce. Government agencies often design regulatory programs intended to minimize biosecurity risks during the handling, shipment, and quarantine of imported animals using the same management practices adopted by the commercial livestock and poultry industries. Efforts focus primarily on ensuring the effectiveness of sanitation and disinfection protocols and having confidence in the health status of incoming animals and related products (i.e., meat, cheese, animal feeds, germplasm, etc.). The U.S. swine industry serves as a prime example, routinely applying comprehensive biosecurity measures that include: 1) rigorous personal hygiene procedures for visitors and employees at production facilities; 2) sanitation of transport vehicles and equipment; 3) quarantine and testing of all incoming animals; and 4) filtration and treatment of incoming air (Silva et al., 2018). These procedures are well-documented in the literature and have been shown to significantly reduce the risk of airborne viruses, such as porcine reproductive and respiratory syndrome (PRRS) virus, at commercial breeding and gestation facilities (Dee et al., 2010; 2012).

Pre-border biosecurity screening programs require access to current and reliable epidemiological data to accurately assess risks associated with imported animals and their products. This information is gathered through national and regional surveillance programs. Coordination of these efforts between countries can sometimes be difficult, due to variations in the testing capacity and diagnostic accuracy of participating laboratories. Even a minor deviation in test results or data interpretation can severely compromise efforts to protect susceptible animal and plant populations from high-consequence pathogens and may have devastating impacts on food security and the economy (see Table 3). For this reason, many programs incorporate duplicate testing requirements and redundant practices as part of an enhanced quarantine program, which is strategically designed to compensate for potential lapses and minimize the likelihood that vulnerable indigenous populations will be exposed to a foreign pathogen or pest without advance warning (Jamieson et al., 2016; Pharo, 2006).

Table 3. Estimated costs of foreign animal disease outbreaks in the U.S.

Disease	Projected Economic Consequences
FMD	\$12.9-\$14 billion during the first year (Paarlberg et al., 2002)
ASF	\$15 billion over 2-years (Carriquiry et al., 2020)

USDA created the National Clean Plant Network (NCPN) to protect the environment and maintain the global competitiveness of U.S. growers of grapes, citrus, berries, hops, roses, sweet potatoes, fruit trees, and other specialty crops by preventing the spread of economically important plant pests and diseases targeting these commodities. Membership is voluntary and includes a network of scientists, educators, state and federal regulators, large and small nurseries, and specialty crop producers. There are 18 NCPN clean plant centers in the U.S. that serve as sources of healthy plant materials. Each center propagates and distributes foundation planting stock that has been tested, treated, and maintained free of designated plant pathogens for U.S. growers. Advisory committees, made up of representatives from research, commodity groups, and state and federal regulatory agencies, provide direction by determining NCPN priorities and initiatives (APHIS, 2021b). To help prevent the spread of pathogens through seed material, USDA APHIS has authorized the National Seed Health System program to support the issuance of Federal phytosanitary certificates for international movement of seed. New Zealand has maintained a seed health certification program to verify the hygiene, viability, and potential performance of imported commercial seed lots since the 1920s (Hampton, 1994). As an additional measure, the National Plant Pest Accord maintains a database of prohibited plant species and monitors the compliance of commercial outlets to ensure plants identified as pests in New Zealand are not propagated or commercially distributed (New Zealand Ministry for Primary Industries, 2021a).

Pesticide use surveys can be useful indicators of aggregate pest pressure and have been proposed as a mechanism to monitor cumulative plant pest incidence within a region or country. Pesticide applications are probably the most common and extensively used pre-border control system for crops, plants in the field, and horticultural plants (potted or bare root), with approximately 2 million tons of pesticide applied annually around the world for this purpose (Sharma et al., 2019). However, studying pesticide use patterns to identify specific pest outbreaks in real time has limited value due to the amount of time it takes for these reports to be published (Garthwaite, 2015). QPS requirements in the U.S. are determined by the presence or absence of specific pests within the originating country, and some QPS protocols require shipments to be proactively treated with fumigants, such as methyl bromide. Therefore, in cases such as this, an actual pest survey may not even be conducted to verify if goods are infested.

A variety of biological control techniques have also been used to manage pre-border biosecurity threats. One strategy involves deliberate release of microbial pathogens, toxins, parasites, or natural predators that specifically target the pest(s) of interest. These practices can be highly effective. However, a pre-release risk assessment must be completed, with effective safeguards to minimize unintended consequences due to non-specific pathogenicity, parasitism, or predation that could have negative effects on beneficial organisms and/or the environment (Azfar et al., 2015; Barratt et al., 2017).

An example of a successful biological control program is the production and release of irradiated sterile *Cochliomyia hominivorax* (new world screwworm) males to manage infestations in livestock. This practice started in the 1950s and has been used extensively to protect livestock from screwworm predation throughout northern Africa and the western hemisphere (Bushland & Hopkins, 1953). The same technology has been deployed to control the Mediterranean fruit fly (Hendrichs et al., 1995) and other species of fruit flies (Purnell, 2019). Analogous programs that involve planned introductions of natural predators and parasites targeting the pest of interest are also effective control options. Strategic releases of parasitoid wasps, including *Apoanagyrus lopezi* (*Epidinocarsis lopezi*), have been used to control the cassava mealybug since its introduction to Africa and Asia (Herron & Neuenschwander, 1991).

Efforts are in progress to expand these well-established management strategies by incorporating innovative genetic technologies which offer a greater level of precision and efficiency (Alphey, 2016) (refer to the Case Study 1). Options range from the "Trojan female technique" that uses naturally occurring gene mutations to impede male fertility (Wolff et al., 2017) to more advanced genetic manipulations capable of introducing harmful or lethal traits into the species of interest. Gene editing is being used to disrupt or inactivate genes essential for reproduction and survival in mosquito vectors of diseases such as malaria, yellow fever, and Zika (Berube, 2020; Hammond & Galizi, 2017; Li et al., 2020a; Owens, 2017; Williams et al., 2020). Very similar applications show promise for controlling invasive weeds (Barrett et al., 2019), vespulid wasps (Lester et al., 2020), and rodents (Owens, 2017). As the use of these technologies continues to expand, numerous precautions must be put into place to prevent unintended adverse effects in wild populations and the ecosystem (Akbari et al., 2015; Krishnan & Gillum, 2017).

Animal feed as another risk factor came to light in the late 1990s, when various countries attempted to prevent the spread of "mad cow disease" by excluding some mammalian-derived protein sources as ingredients in ruminant feeds (Brown, 2001; Doering, 2008). The concept emerged again in May 2013, following introduction of PEDV into U.S. swine herds (Dee et al., 2014). Feed ingredients and prepared diets were not considered important vehicles for pathogen transport and transmission prior to that time, and no standard biosecurity practices were in place even though many countries imported animal feed components on a routine basis.

Links between diet and disease transmission have since been established and raise concerns that U.S. herds could be infected by consuming contaminated feed, originating from countries where regulated diseases are endemic, and sanitation and quality assurance procedures are lax (see Figures 1, 2, and 3). Experimental data has demonstrated that some feed ingredients, particularly soy-based products, support the viability of at least three significant viral pathogens of swine (i.e., CSF, ASF, and Pseudorabies Virus) (Dee et al., 2018; Niederwerder et al., 2020). ASF has been found to survive in a total of 9 distinct feed ingredients, including three soy-based products, choline chloride, three pet diets, pork sausage casings, and complete feed exported from China to the U.S. (Dee et al., 2018; Stoian et al., 2020). Two key factors that influence a pathogen's persistence in feed include the virus phenotypic properties and the feed matrix produced by specific feed ingredients and additives. Some matrices are more conducive to the survival of viruses and capable of supporting several different viruses at the same time.

The U.S. purchases significant amounts of soy-based products from several ASF-positive countries, including China, and the ability to quantify the amounts and types of imported products as well as

Figure 1. Grain drying practices in areas with lenient biosecurity standards

Figure 2. Grain drying practices in areas with lenient biosecurity standards

Figure 3. Grain drying practices in areas with lenient biosecurity standards

the entry ports where the goods are received can yield useful information in predicting risk. A recent study evaluated these parameters for soy-based ingredients (soybeans, soybean meal, soy oil cake, and soy oil), using data from the International Trade Commission Harmonized Tariff schedule website for calendar years 2018 and 2019. The results showed that the U.S. imported 104,707 metric tons (MT) of soy-based products in 2018, with 52.6% (55,101 MT) of that product originating from China and 42.9% (44,775 MT) from the Ukraine. In 2019, the U.S. imported 73,331 MT, with 54.7% (40,143 MT) coming from the Ukraine and 8.4% (6182 MT) from China. At least 80% of the Chinese products entered the U.S. through San Francisco/Oakland, CA and Seattle, WA, whereas nearly all Ukrainian products entered through New Orleans, LA and Charlotte, NC. This information helps authorities to estimate risks associated with various commercial routes that are likely to serve as channels for foreign diseases to enter the U.S. and strategically allocate resources and mitigation efforts to high priority ports of entry (Patterson et al., 2020).

Growing recognition that contaminated feed and feed ingredients can serve as vehicles for the transmission of viral pathogens highlights the need for improved biosecurity policies and procedures directed at this product sector. New Zealand has implemented rigorous processes to manage these threats (New Zealand Ministry for Primary Industries, 2020g). However, Canada and Australia have emerged as global leaders by implementing comprehensive national programs to address risks associated with the importation of plant-based animal feeds.

The Canadian program was implemented in March 2019 and targets specific "high risk ingredients" that include grains, meals, and oilseeds from more than 44 countries identified on the "ASF watch list" maintained by the Canadian Food Inspection Agency (CFIA). Listed countries must obtain a permit to export certain feed ingredients to Canada, which includes submitting written documentation that identifies the country of origin, manufacturing company, audit history, type(s) of product, source(s), intended use (human or animal food), and any mitigation(s) implemented prior to shipment. If approved, the cargo is received at a specialized holding facility located in a designated "secondary control zone" near a port and held at a constant temperature for a specified interval (i.e., 20°C for 30 days) to enhance viral decay, before being released to commercial milling systems inside the country. The watch list and extended storage criteria are subject to change as new information becomes available.

The official program for importing processed plant-based animal feed into Australia was released by the Department of Agriculture, Water, and the Environment in November 2020, and targets specific pathogens (ASF and FMD). Export companies must apply for a permit, and the manufacturing company must complete a production questionnaire that describes quality control and assurance protocols applied at the plant. These include: 1) proper temperature and pH to neutralize viruses during the manufacturing process; 2) closed tube delivery systems for product movement; 3) single-use totes; and 4) containers that remain sealed throughout the shipment process. Australian officials review each application to determine if additional measures are required. Next steps could include a "desk audit," involving more questionnaires and photographs of the manufacturing facility; additional information on biosecurity protocols and mitigation strategies that lower risk; and the locations of any animal populations near the manufacturing plant. A third-party inspection team may conduct a "site audit" to verify the integrity of the facility, biosecurity protocols, and risk mitigation plans. If a permit is granted, imported goods are visually inspected at the port to determine if any additional treatment is required (i.e., use of feed additives extended storage time, etc.) and if entry is approved or denied.

Reliable testing of bulk commodities and animal feed ingredients for microbial contamination is an inherent challenge, due to the limited availability of validated sampling methods and specific analytical assays capable of detecting viable pathogens. Most conventional methods are time consuming and lack sensitivity (Gopi et al., 2017; Jones et al., 2019b). Relatively small, concentrated "hot spots" of contamination can be easily missed during bulk sampling and may lead to false negative results, allowing excluded pathogens to avoid detection and enter countries free of that agent. This type of error can quickly compromise a country's disease status, with dire and long-lasting consequences on future commerce with global trading partners. For these reasons, imported feed components are not routinely sampled in the U.S. and Australia. However, when there is due cause, the Australian program permits representative samples of certain feed ingredients to be collected and analyzed for potential contamination.

Despite numerous complex challenges, feed surveillance presents unique opportunities for targeted research and innovative strategies that can have profound effects on agricultural biosecurity. The risk of pathogen entry through contaminated feed ingredients is considered relatively low for most agents (Jones et al., 2019a). However, the consequences associated with an unexpected lapse can have lasting and devastating effects on animal agriculture and commerce. The growing complexity of international trade, combined with expanding movement of feed-related products across multiple borders, contributes to unpredictable conditions that are increasingly difficult to manage and track. Fortunately, new technologies are developing at an equally rapid pace and can be leveraged to enhance and refine management of these emerging risk factors at the transboundary and domestic levels (refer to the Case Study 2).

BORDER ACTIVITIES

Border activities include organized surveillance, inspection, disinfection, fumigation, screening, isolation, and other control procedures. These are deliberately performed on incoming goods and commodities at designated ports of entry to specifically prevent invasive species and diseased, infested, or illegally traded agricultural products from entering the destination country. Defense strategies also frequently involve passive surveillance programs to monitor indigenous plants and animals adjacent to the physical boundaries of formal points of entry, for incursions of invasive pests and species from neighboring jurisdictions. Safety zones free of susceptible hosts can be established as protective barriers to minimize the impact of biosecurity lapses and are found around many Canadian and U.S. ports. In some cases, imported animals, plants, and other goods may be moved to a separate "transitional containment facility" located away from the designated port of entry for a specified quarantine period or to undergo additional testing.

Inspections or Audits at Transportation Hubs

Active surveillance programs to monitor incoming agricultural commodities, freight, and international passengers for potentially invasive species and improperly manifested or contaminated products at ports and borders is a critical component of an overall control program. Border officials have dual responsibility for overseeing the legal movement of people and goods through designated borders and ports, while preventing the exchange of prohibited items, illegal contraband, and infested or contaminated products that have not been properly identified. Commonly intercepted goods include exotic meats and animal tissues, game trophy, animal hides, seeds, herbs, plant material including medicinal herbs, live birds and reptiles, and a variety of other banned agricultural products.

In these situations, surveillance refers to the ongoing systematic collection, analysis, and interpretation of outcome-specific data. The resulting information is subsequently used to plan, evaluate, and implement risk-based quality and health standards for all kinds of agricultural products (Thacker, 2000). Monitoring these pathways serves multiple functions, including: 1) improving pest interception; 2) reducing propagule pressure; 3) supporting oversight functions that increase compliance with phytosanitary regulations; 4) decreasing dishonest or fraudulent behaviors that contribute to biosecurity lapses; and 5) accelerating collection of temporal and spatial data tied to regulatory actions to inform ongoing or future biosecurity risk assessments.

Effective surveillance programs require uniform monitoring, accurate data collection, and timely analysis of information, which is critical for identifying biosecurity concerns at multiple critical control points throughout the production and distribution cycle. Continuous information sharing and coordinated efforts within and across borders ensure cohesive and consistent monitoring of the status and movement of agricultural commodities. A reliable system depends on an agile risk assessment process; dedicated financial and technical resources; and well-defined enforcement authority. Every party to the agreement is responsible for developing and implementing discrete plans that fulfill core surveillance obligations. A model has been published (Gottwald et al., 2019) and is available online (Census Travel Interactive Web-App, n.d.) to assist users in predicting entry points for exotic plant, animal, and human pathogens, based on epidemiological characteristics of the agent(s), international travel patterns, and endpoint census data.

Border surveillance programs should be comprehensive and purposely designed to monitor the health status and quality of plants, animals, and products grown domestically, in addition to external goods produced by international trade partners. Successful programs include strategies to overcome logistical challenges encountered by global networks, such as: 1) inadequate human, technical, and financial resources; 2) infrastructure deficiencies; 3) knowledge gaps in scientific methods and operational concepts; and 4) inconsistencies between international policies (Hitchcock et al., 2007).

Surveillance team members should be able to predict when, where, and how a disease is likely to occur, as well as the anticipated impact in terms of virulence (Parnell et al., 2017). The NPPO uses a web-based early-warning system called PestLens to analyze these effects on U.S. agriculture (Meissner et al., 2015). PestLens maintains a web-based platform that is used to collect information on plant health and exotic pest activity for decision-makers and stakeholders and to document mitigation actions taken to protect vulnerable commodities. Implementation of a similar system with international scope, which is equally efficient and cost-effective, would greatly enhance efforts to detect and monitor plant diseases and pests on a global scale (Carvajal-Yepes et al., 2019; Zhang et al., 2019). Other initiatives have included planting sentinel plants in strategic offshore locations to act as early-warning systems for incursions of harmful foreign pests (Mansfield et al., 2019), and establishing public surveillance networks staffed by volunteer citizens who report field sightings of pests and diseases through online platforms such as iNaturalist (Pawson et al., 2020) and iPiPE (NIFA, 2021).

Lapses can have serious consequences. A notable example was the failure to detect introduction of ash dieback in the United Kingdom until after the disease was well-established. Knowing the disease was prevalent throughout Europe should have sparked interest in setting up a proactive biosecurity program in the United Kingdom, which may have prevented the disease's emergence and greatly limited its overall impact (Woodward & Boa, 2013). By contrast, the New Zealand Government maintains formal surveillance programs for a relatively wide range of high-risk pathogens and pests including multiple arboviruses and their vectors; avian influenza and other high consequence animal diseases; several fruit fly species; gypsy moths; and numerous invasive ants. It conducts routine surveillance for these unwanted organisms at air and seaports and other high-risk locations (Acosta & White, 2011; Acosta et al., 2020) and screens animal feed components for potentially dangerous contaminants, including ruminant-based proteins in feeds intended for ruminant consumption (Brown, 2001).

Pressures that contribute to the introduction of foreign pathogens and pests will continue to escalate as the international movement of people and goods expands and trade volume increases. Changes in transportation patterns and systems complicate management of biosecurity risks, and NPPOs must continuously analyze and adapt to shifting global trends by promoting situational awareness and advocating for the development of new and innovative solutions that can address changing biosecurity priorities (Clarke et al., 2018; Delane, 2019). The international cruise ship industry serves as a recent example. Before the Covid-19 pandemic, NPPOs faced a robust leisure travel and tourism sector and had to develop operational procedures to address emergent risks presented by passenger ships (McKirdy et al., 2019). However, cruise ship travel precipitously declined at the onset of the pandemic in 2020, and NPPO's had to quickly refocus their efforts on rising threats caused by significantly increased volumes of international mail and packages due to a rapid upsurge in online purchases and other digital transactions.

Selective introductions of agricultural imports, documented and cleared through a regulatory and policy framework founded on standard biosecurity measures, can slow pathogen and pest transmission. However, in addition to collecting information on the controlled and legal movement of these goods, actions must also be taken to monitor the normal movement of wildlife, birds, and other feral animals that can accidentally contribute to the introduction and spread of restricted pests and pathogens. Even isolated island countries like New Zealand, which is more than 2,000 kilometers from its nearest foreign neighbor (Australia), routinely contends with organisms that have migrated or been naturally dispersed through wind and water (Close et al., 1978; Fox, 1973; Turner & Vijay, 2017). Establishing formal quarantine buffer zones to trap, test, and monitor the health status of wild populations at terrestrial borders and ports can quickly identify invasive pests and diseases commonly transported by these routes. Wireless sensors that collect images of remote insect traps and transmit them to a central monitoring station have greatly improved the accuracy and efficiency of monitoring offshore and pre-border pest populations (Azfar et al., 2015). Both target and non-target populations can be actively or passively surveyed to make sure atypical hosts capable of pathogen transmission are not accidentally overlooked. Migratory birds can be natural reservoirs of Influenza A viruses that cause significant losses in domestic fowl. Understanding their role in the global spread of avian influenza has led to large-scale monitoring of this virus in wild birds, both live and dead, by direct sampling and screening. Surveillance of international migratory routes and bird habitats that extend beyond national boundaries yields useful epidemiological information about the risk of transmission as a result of these dynamic pathways (Lickfett et al., 2018).

In recent years, there has been growing interest in developing a global invasive species monitoring system, equivalent to those used by public health experts to track human diseases that can lead to an epidemic or worldwide pandemic (Carvajal-Yepes et al., 2019; Latombe et al., 2017; Mackenzie et al., 2014; Pagad et al., 2018). These networks could promote agile data exchange between partners that can be used to pre-emptively analyze risk, rather than waiting months or years for individual case reports to be collected, analyzed, and formally published. Their establishment has proven to be an effective and efficient monitoring system that keeps international authorities informed of the spread and severity of various outbreaks (Kelly et al., 2020; Vaz et al., 2018).

Disinfection Protocols and Management of Agricultural Products and Wastes at the Border

Some imported products can be disinfected or sterilized immediately before or upon arriving at the border to ensure significant biological contaminants are cleared or inactivated prior to distribution. More commonly used techniques include microwave irradiation; chemical additives or treatment (i.e., methyl bromide or hydrogen phosphide fumigation of grains and commodities); desiccation; exposure to elevated temperatures or heat; and extended storage or quarantine periods. In general, integrated strategies that merge several independent control methods into a single harmonized treatment protocol tend to yield the best results. Relying exclusively on one method tends to be less prudent and can promote the development of resistance in target pests and pathogens, leading to control failures and unintended biosecurity lapses. Whereas, using a variety of procedures and equipment that have highly specific targets and complementary functions causes less disruption to the environment and helps to minimize risks to workers and live commodities, such as plants and animals.

Some insecticides, herbicides, fungicides, and other chemicals applied for the lethal control of certain pathogens and pests prior to shipping may temporarily suppress the growth of other agents, masking their presence without significantly impacting long-term viability or survival. Sudden oak death, caused by *Phytophthora ramorum*, is an example of an invasive plant pathogen that threatens the coastal forest ecosystem in the U.S. This agent has a wide host range, infecting the foliage and twigs of many popular horticultural species such as rhododendrons, camellias, and viburnum. Pre-emptive treatment of these plants with fungicides prior to transport often conceals the presence of viable *Phytophthora*, allowing infected plants to be shipped and widely dispersed before infection can be confirmed (Tjosvold et al., 2005). Pheromones released by beneficial insects that do not pose biosecurity threats can also mask or conceal chemical markers of agriculturally significant pests, helping them to evade detection during formal screening procedures.

Adding chemicals to products intended for use as animal feed can alter the viability of some viral pathogens. Comparison of 15 different additives (i.e., organic acids, fatty acid blends, formaldehyde-based products, and essential oils) showed pigs consuming feed with an additive had significantly better health and performance following challenges with PEDV, PRRS, and Seneca virus A compared to cohorts fed diets without additives (Dee et al., 2020). Similar results were observed in challenges with ASF (Niederwerder et al., 2020), suggesting the use of a validated additive may be efficacious in lowering risks linked to viral-contaminated ingredients.

Learning more about the half-lives of viruses found in a wide array of biological materials has led to science-based protocols that allow some products to be safely introduced into the U.S. from high-risk countries. This approach, referred to as “Responsible Imports,” relies on a comprehensive risk assessment process that considers: 1) the absolute necessity of importing the material(s); 2) availability of alternative ingredients that can be obtained through other sources (i.e., countries free of regulated diseases); 3) prevalence of specific virus(es) regarded as credible threats; 4) access to reliable half-life data for these agents in various ingredients and substrates; 5) estimated transport times, from supply source to final destination; 6) mitigation methods and strategies to reduce viral load during transit; and 7) storage temperatures and times that will eliminate residual virus from feed ingredients prior to use. The relatively new concept of “feed quarantine” is evolving rapidly, as production companies design storage facilities to safely receive and store incoming products. These practices help to minimize risk and support secure, long-term trade with a wider range of international partners (Patterson et al., 2019).

The same principles can be readily adapted to waste products generated during the transportation process, including contaminated agricultural commodities that are denied entry. All refuse associated with the importation process should be surveyed. This includes shipping materials found in enclosures, containers, and parcels used to transport animals, plants, and related agricultural products, as well as ballast water used to stabilize cargo ships and other maritime vessels during transit. These materials are often regarded as conventional waste or garbage and can be inadvertently overlooked during routine inspections, even though they provide safe refuge to viable microbes, seeds, spores, pollen, eggs, larvae, and some adult forms of invasive species that are often unapparent and can be accidentally released unless properly contained and treated.

Discarded food waste generated on passenger aircraft, ships, and vehicles must also be surveyed. The Codex Alimentarius Commission, jointly administered by the World Health Organization and the FAO, establishes international quality standards for all types of food products (Thomson et al., 2004). However, these standards are focused on a product's safety prior to human consumption and not readily applied to potentially contaminated products at the time of disposal. There is a tendency to view these materials as harmless and inconsequential by-products; however, they can serve as effective conduits for agricultural pests and pathogens without appropriate surveillance and management.

Live insects can also be inadvertently transported in the passenger or cargo space of aircraft. Forced directional air currents at the point of entry can limit their access, and ventilation filtration systems provide an additional layer of protection by trapping airborne pests that successfully evade air curtain barriers. However, in some cases, it may also be necessary to treat the interior surfaces of aircraft with residual insecticide (Pang et al., 2020). Despite these measures, case studies have shown that relatively few hitchhiker pests are successfully found during biosecurity inspections at borders (Turner et al., 2020; Work et al., 2005), and movement of live organisms does take place during air and surface transport of goods and passengers (McNeill et al., 2011; Work et al., 2005).

At minimum, effective controls should include a comprehensive risk assessment process to verify the biosecurity status of incoming materials, proper containment of suspect items, inactivation or treatment protocols for contaminated goods and products, and effective waste disposal procedures. Materials that cannot be safely disinfected should be treated as contaminated and carefully disposed or destroyed, using validated methods or allowed to return to the country of origin where appropriate mitigations can be performed for possible reshipment.

Inspection and Detection Support Systems

Ideally, biosecurity efforts are supported by a cohesive network of inspection and detection systems that rely heavily on an extensive network of international diagnostic and analytical reference laboratories. Resulting data is used to verify the health and quality of plants, animals, and other agricultural commodities that are being transported and exchanged. National authorities can also use this information to monitor disease trends; make decisions relevant to trade; and evaluate the effectiveness of mitigation efforts used to limit the spread of pathogens and pests. The U.S. Foreign Animal Disease Diagnostic Laboratory (FADDL) network is one example of this type of system. FADDL coordinates testing and evaluation of imported animals and their products, including confirmatory testing for exotic agents that could jeopardize the health and welfare of American livestock and poultry (APHIS, 2021c). Similar programs have been developed to support plant health, such as New Zealand's Plant Health and Environment Laboratory (New Zealand Ministry for Primary Industries, 2020i).

Success is largely dependent on the ability of participating laboratories to collect and disseminate accurate information in a clear, unambiguous, and timely manner. Transparent and efficient sharing of epidemiologic and diagnostic test information among the various partners is critical in an integrated global control program. Unfortunately, the rate and accuracy of information exchange continue to be the greatest barriers to progress. Communication lapses and delays have significantly impeded efforts to monitor and respond to the movement of agricultural diseases and pests in areas of the world that are less-developed (Carvajal-Yepes et al., 2019).

Screening

Screening procedures at borders and ports can be improved and refined by applying data collected through a variety of intelligence gathering mechanisms, including domestic and international market surveys; analysis of international commerce trends; contraband interception records; and proactive relationships with industry and liaison groups. Standard compliance monitoring activities at various consumer outlets routinely identify illegally marketed goods sold at distribution centers; flea markets; animal, plant, and insect trade shows; large and small chain stores; roadside vendors; and neighborhood markets. Collected information is analyzed and then used to complete risk-based assessments that help to prioritize resource allocation and inspection priorities (APHIS SITC, 2020).

The inspection process can be further enhanced by using trained detection dogs, pest detection sensors, electronic sniffers, x-ray machines, and other imaging equipment that complements traditional inspection methods (Stephenson et al., 2003). Dogs have an acute sense of smell and can be trained to alert their handlers to the presence of restricted agricultural products in baggage and cargo (Moser et al., 2020; U.S. Customs, 2016; Whyte, 2006). New technologies are being designed to mimic this approach. Artificial intelligent noses (or electronic noses) are being developed to monitor gas emissions for characteristic volatile organic profiles released from diseased or infested plants (Cui et al., 2018). Imaging tools and sensors are also being built to detect thermal gradient variations and unique spectral signatures for agricultural products that are diseased or infested with pests. Fast neutron radiography, alone or in combination with high-energy gamma radiation, enhances detection and discrimination of a wide range of organic materials found in cargo shipments. The range of technological tools is constantly evolving and often builds on military research activities which are focused on mitigation strategies for bioterrorism incidents. Research directed at more efficient surveillance methods, precision diagnostics, improved therapeutic agents, and advanced emergency response efforts continues to expand the range of counter-offensive measures available against accidental or intentional pathogen and pest transmission (refer to Case Study 3).

Diagnostics

Confidence in the inspection procedures; diagnostic and reference laboratory capabilities; and technical accuracy of various trading partners contributes to safe and orderly exchange of agricultural products. Sensitivity and specificity ranges of various analytical tests define whether an assay targets a single specific agent or a broader range of pathogens and pests. Some screening methods may require specimens to be collected and analyzed in a precise way, and this requires careful coordination of resources, sample collection, transport, and testing procedures to yield reliable and consistent results for the agent(s) of interest. Test method selection is often influenced by a specific procedure's efficiency in screening large quantities of plants, animals, and related products. When testing of every product, shipping container, or transport vehicle is not practical, validated sampling strategies must be followed to ensure results that are reliable, accurate, and representative.

Rapid metagenomics diagnostic assays are a rapidly emerging tool in human medicine which show great promise in agriculture. This technology operates on a multiple microarray platform with thousands of probes capable of detecting most known pathogenic viruses and other major disease-causing bacteria, fungi, and parasites. These assays provide accurate and efficient identification for a wide range of primary pathogens in less than 24 hours and can be assembled with additional probes to detect other opportunistic agents of interest that cause co-infections or significantly alter the course and severity of disease (Espindola & Cardwell, 2021). Use of multiple probes across the genome spectrum generates compensatory signals that minimize false negative results caused by faulty probes, significantly improving overall accuracy and efficiency. Successfully adapting this technology to agricultural applications will dramatically expand the scope, scale, and efficiency of diagnostic screening programs for plant and animal commodities.

Post-Entry Quarantine Centers for Incoming Animals/Plants/Products

Quarantine programs help to keep unwanted diseases and pests from entering the receiving country by providing options to physically confine and isolate newly imported plants, animals, germplasm, and products upon arrival. A quarantine program's primary objective is to prevent non-indigenous pathogens and pests from being released into environments favorable for their establishment but may also be warranted if there is a national regulatory effort to suppress, contain, or eradicate an agent's spread within a country's borders. Diseases and pests are considered to have "quarantine significance" if they are not native to the country of concern and known to cause significant economic damage, or when their life cycle suggests they have the capacity to cause extensive damage under the right conditions.

Biosecurity practices followed during quarantine vary in complexity, depending on: 1) the type of agricultural commodity involved; 2) the susceptible host range; 3) the exact pests and pathogens of interest; 4) their geographic distribution; and 5) agent(s) life cycle(s), as influenced by environment, climate, and prevailing agricultural practices. Specific quarantine conditions and procedures are determined by the receiving country's regulatory framework.

In the U.S., imported plant materials and products intended for commercial trade are inspected at the port of entry. This includes live plants, trees, ornamentals, seeds, germplasm, pollen, and plant products intended for human or animal consumption. Goods that show any evidence of infestation or contamination are either treated at the port of entry or moved to a U.S. Customs and Border Protection bonded facility for holding and treatment. The importer must ensure all suspect materials are safely treated within 24 to 48 hours and approved for release, prior to commercial movement. Infested or contaminated goods that cannot be successfully treated may be returned to the country of origin or destroyed and disposed using validated methods.

Animals are often transferred to a quarantine facility at the time of receipt and held for a specified time to ensure they are free of high-impact diseases and pests, prior to release into commerce. Barns, lots, and pastures designated as quarantine facilities must meet strict design and construction specifications to ensure animals and any contaminated waste, including contaminated feed and bedding, will be adequately contained throughout the entire quarantine period. All structures and grounds must be able to withstand intense decontamination and disinfection procedures that are performed before and after every use. Treatments, vaccinations, and disinfection or sterilization procedures may be required to conclusively validate the health and quality of animals and their products prior to release from quarantine. It may also be necessary to repeat diagnostic tests, which were previously conducted in the country of origin, during the quarantine period to allow adequate time to detect latent infections caused by organisms with unusually long incubation periods as well as newly acquired infections or infestations caused by biosecurity lapses during transit.

OPPORTUNITIES

Transboundary risks continue to threaten agricultural biosecurity, despite various control measures that have been implemented at the pre-border and border levels. Foreign pathogens, pests, and species can be introduced during routine commercial activities, through environmental routes, and coincident to illegal shipments. Minimizing their impact depends on early detection and response, placing great value on options that increase the precision, speed, and efficiency of these systems. Success demands innovative and often unique management strategies and technologies. Recent examples have been provided throughout this chapter to illustrate how emerging scientific and technological advances are being adapted to enhance or replace standard control procedures that are currently used in agriculture. Development trends encompass a wide range of disciplines and have led to important procedural and policy shifts in meeting these critical demands.

Genetic engineering provides opportunities to precisely manipulate the genomes of plants and animals in ways that enhance their immunity and resistance to high consequence diseases and pests. These same techniques can also be used to alter insect pests or pathogens in ways that limit or suppress their survival. Developments in synthetic biology are providing an even greater range of options to create novel organisms and biological products that improve the specificity and effectiveness of critical vaccines, molecular diagnostic tools, and cell-based therapeutic interventions.

Key technological advances are also increasing analytical and diagnostic test capacity. The use of nanotechnology has resulted in superior analytical platforms that offer improved detection limits, accuracy, speed, and cost effectiveness. These changes have led to several major advantages, including: 1) enhanced methods for processing aggregate samples and miniaturized assay volumes; 2) higher throughput enabled by automated workflow and systems; and 3) greater portability associated with hand-held, mobile devices that can be easily deployed and operated in a field environment. Sampling procedures are also being expanded using highly selective and non-invasive technologies such as remote spectroscopic, magnetic, and vibration anomaly sensing and imaging techniques. The use of drones and satellites, coupled with these various sensor technologies, facilitates long-range surveillance and detection methods capable of monitoring large fields, standing crops, and bulk commodities.

Mathematical modeling and statistical methods have also helped to refine risk profiling and management strategies. Seamless integration of data from a variety of sources has been made possible by the development of information products that accelerate the categorization and analysis of incoming data; facilitate pattern recognition; and translate the results into practical metrics. A few of these examples include machine-learning algorithms, computational biology applications, and predictive bioinformatics. The net result is an agile decision-making process that complements traditional laboratory methods, offering strategic pathways to validate rigorous sampling methods and support rational development of new diagnostics, vaccines, therapeutics, and other countermeasures.

Tactical solutions are integral to protecting agricultural assets from harmful foreign pathogens and pests. Progress has been made, but continued success depends on timely introduction of cost-effective strategies that are made possible through innovative technologies, products, and systems. Collaborative strategies offering diversity of thought and expertise show the greatest promise in meeting these critical and rapidly changing demands.

CONCLUSION

Border biosecurity has a critical role in reducing risk to domestic agricultural resources, native flora and fauna, human health, and the environment. Previous protections offered by the barriers of time and distance are quickly vanishing as the scope and pace of international commerce and travel continue to expand, raising an appreciation for novel mechanisms of transmission (i.e., feed and feed ingredients) that were not previously understood. The continuum of prevention strategies must include an open-minded thought process, combined with a growing system of complex offshore, pre-border, and border control measures and technologies. Current approaches involve coordination of international efforts and resources; sophisticated disease and pest surveillance; comprehensive inspection and screening programs; and robust communication networks for sharing data and intelligence to deter introduction and spread of nonindigenous species and disease. Ideally, this approach will enhance the accuracy of risk assessments, promote the development of innovative, science-based mitigation techniques, and ultimately bring about a change in philosophy regarding global exchange of agricultural commodities, from one that is primarily driven by price to one that puts equal emphasis on the originating country's pest and pathogen status.

CASE STUDY 1: GENETIC PRE-BORDER CONTROL STRATEGIES FOR NEW WORLD SCREWWORMS

The Challenge

The New World screwworm (NWS), a blow fly found primarily in South America and parts of the Caribbean, is an obligate parasite of warm-blooded animals. Adult female flies deposit hundreds of eggs in the wounds and mucous membranes of livestock that hatch into 2-cm larvae within 8 to 15 hours. The larvae appear like fat, white screws, due to bristly ridges encircling the entire body length, and burrow into the living flesh of animals causing intense pain. The name (*Cochliomyia hominivorax*) roughly translates to "eater of man" in Latin.

This pest has been successfully eradicated in North and Central America through coordinated surveillance and pre-border release of flies rendered sterile through irradiation. The sterile males act as "biological barriers" by mating with wild females which produce eggs that are not fertile. Currently, both males and females are raised and irradiated for field release. Finding technologies that produce only male flies would significantly improve the economics and efficiency of this program (Wyss, 2000).

The Solution

Analysis of the pest's genome has led to the identification of candidate genes that are essential for host-seeking behavior (chemosensory), larval development in living flesh (heat shock protein, immune response), and production of transgenic strains with the potential to markedly enhance genetic control programs (sex determination, germline). Ongoing research efforts have produced the first transgenic lines of NWS that produce only males when raised on special diets. Preliminary fitness and mating assessments of these lines show strong potential for application in the current sterile insect program, providing advanced genetic control options that are more economical and efficient (Concha et al., 2020).

Future research directions include opportunities to: (1) enhance worker safety by developing dominant lethal strains that do not require irradiation to induce sterility and/or (2) lower program costs by introducing additional embryonic lethal strains that lead to selective survival of larvae.

The Outcome

Successful control and eradication of NWS in the U.S., Mexico, and Central America represents one of the greatest international collaborative achievements relevant to animal production and wildlife conservation. For more than 60 years, the program has served as a practical pre-border control program that has effectively suppressed NWS infestations over a wide geographic area without the need for harmful chemicals. Although operating expenses continue to be a significant barrier for program implementation in some parts of the world, evolving genetic technologies show promise as cost effective options for extending eradication and control programs to these areas.

CASE STUDY 2: THE LINK BETWEEN BIOSECURITY AND ANIMAL FEEDS CONTAMINATED WITH PORCINE EPIDEMIC DIARRHEA VIRUS (PEDV)

The Challenge

PEDV, an exotic Coronavirus, was first found in the U.S. in 2013, causing more than 7 million pigs (10% of annual production) to die in the year following its detection. All ages of pigs are susceptible, although suckling pigs less than seven days of age are the most vulnerable. Affected animals develop severe vomiting, diarrhea, and dehydration due to diffuse villous blunting and fusion of enterocytes that often results in high mortality.

The American Association of Swine Veterinarians (AASV), the National Pork Producers Council, and USDA compared more than 100 epidemiological variables between case and control herds during the initial outbreak, and found a significant correlation between PEDV transmission and seven risk factors linked to animal feeds ($p < 0.05$). This data had greater impact during the peak year of the PEDV epidemic because U.S. International Trade Commission Harmonized Tariff Schedule data showed the U.S. had imported 89,469 metric tons of soybeans, 6,633 metric tons of soybean meal, and 21,469 metric tons of lysine hydrochloride from China during that timeframe. Additional evidence confirming the original PEDV strain isolated in the U.S. shared a 99.7 to 99.8% nuclear identity with viruses actively circulating in Chinese herds ultimately led experts to hypothesize PEDV must have been introduced into the U.S. through contaminated feed ingredients imported from this region.

Research Summary

A controlled study published shortly after the start of the epidemic conclusively documented PEDV could be transmitted to naïve pigs through contaminated complete feed and feed ingredients (Dee et al., 2014). Feed samples used in these experiments originated from storage bins supplying commercial farms where index cases of PED had been confirmed. Subsequent studies found the minimum infectious dose was 5.6×10^1 TCID_{50} per gram of feed.

Viability of PEDV in various feed components was also compared and showed virus could be recovered for up to 30 days following inoculation of lysine hydrochloride, choline chloride, Distiller's Dried Grains with Solubles (DDGS), and other porcine by-products and for 180 days from soy bean meal. The study used a Trans-Pacific transportation model to simulate conditions encountered during shipments of grain from China to the U.S. and verified viable PEDV could be successfully transported in five ingredients commonly used in swine diets (i.e., soybeans, soybean meal, lysine hydrochloride, choline chloride and Vitamin E) (Stoian et al., 2020).

The Solution

U.S. swine producers, the AASV, and the scientific community were quick to act on this information. Strategies designed to treat feed and inactivate suspected viral contaminants through chemical mitigation, thermal mitigation, and mechanical reduction (flushing and sequencing) were validated and implemented across American farms. U.S. companies that supply essential feed ingredients originating from China (e.g., B vitamins) also started to scrutinize the manufacturing process, documenting consistent adherence to pH and temperature requirements aimed to eliminate viable ASF virus before shipping to the U.S. Products were stored for specified intervals prior to being transported to feed mills and again at the farm for an extra 30 to 45 days as an additional line of defense.

Despite prompt action by the swine industry, USDA downplayed the critical role of contaminated feed in the PEDV epidemic. The U.S. Government Accountability Office (GAO) concluded USDA did not take sufficient action following the 2013 outbreaks and was not prepared to track and respond to emerging diseases that threaten animal health and food security.

The Outcome

Unfortunately, the risk of introducing foreign animal diseases through contaminated feed ingredients is still largely unresolved and continues to pose a significant threat to U.S. agriculture. Feed risk mitigation research is ongoing, with results freely shared and disseminated between all parties to improve collaboration and communication. The U.S. Government and industry stakeholders have established a national task force to focus on the threat of foreign animal disease introduction through contaminated feeds. There are hopes these efforts will foster consensus between industry stakeholders and governmental agencies, galvanizing the emerging concept of "global feed biosecurity," to mitigate potential pathogen movement via feed.

CASE STUDY 3: SENSING TECHNOLOGY TO DETECT PEANUT SMUT

The Challenge

Peanut smut is a severe fungal disease affecting legume crops (*Arachis spp.*). It is caused by *Thecaphora frezii*, a highly adaptable pathogen that was originally identified in wild peanuts growing in Brazil in

1962 (Carranza & Linquist, 1962) and in commercial peanut fields in Argentina in 1995 (Marinelli et al., 1995).

Peanut smut is spread primarily through wind, contaminated equipment, and movement of products harvested from diseased plants (Rago et al., 2017). Control measures are complicated because *T. frezii* produces spores that remain dormant in the environment for many years. Host plants become infected when floral structures (i.e., pegs) penetrate the soil, releasing enzymes that cause spores to germinate. Infected plants and immature seed pods may not show signs of disease, further delaying detection and allowing infections to become established and spread before control measures can be implemented. Treatment of soil, seeds, and plant foliage with fungicides yields variable results because the effectiveness of different products varies and can be severely compromised by shifts in environmental conditions. Spores often persist longer than chemical residues, with infections emerging up to 40 days after treatment.

The Solution

Several control strategies have been proposed for successful management of this pathogen. Development of smut-resistant germplasm shows promise as a sustainable and durable method, particularly in areas where there is existing disease. Resistance traits have been isolated in wild cultivars and are being selectively transferred to commercial lines through programmed crosses (Bressano et al., 2018).

Surveillance methods to prevent introduction and further spread of the disease are also being improved. Highly accurate polymerase chain reaction (PCR) assays have been developed to detect *T. frezii* and are used to screen harvested products and commodities for contamination (Cazón et al., 2016). However, expanding the use of this technology to the field has been more challenging due to difficulties in collecting representative crop and soil samples over large plots of land that will yield accurate and consistent results.

Remote sensing technology offers many advantages for these types of applications. The methods are precise, efficient, and non-destructive, providing quick and reliable surveys of large fields and crops at the point of production. Applications that are under investigation use a wide range of sensor modalities, including: (1) spectral, multispectral, and hyperspectral imaging to measure reflectance, fluorescence, and emission of radiation as an indicator of plant health; (2) electronic noses to detect characteristic volatile organic emissions from diseased plants and pathogens; (3) nanosensor technology that is capable of rapid, highly sensitive and specific detection of low numbers of pathogens in the field; and (4) light detection and ranging (LiDAR) technology that can analyze standing plant architecture for signs of foliar and soil-borne diseases.

The Outcome

Peanuts are a major food crop with global significance. Sustained production would be severely compromised through the accidental or intentional introduction of smut to other continents. Efforts to treat and eradicate this pathogen have been largely unsuccessful, resulting in a pressing need to validate new field surveillance methods that will accurately identify pathogen distribution in infected crops and commodities before they are transported and potentially contribute to new outbreaks. Advanced sensor technologies, combined with rapid data processing, are quickly emerging as critical management and control tools to prevent further spread of this major peanut pathogen.

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KEY TERMS AND DEFINITIONS

Actionable Pathogens and Pests: A specific disease-causing micro-organism (pathogen) or destructive insect (pest) that initiates a sequence of coordinated interventions and control measures to prevent introduction and dissemination into new territories or vulnerable populations.

Ballooning: (Also called kiting) a process by which some early-stage larvae (i.e., caterpillars), small insects, and other invertebrates are dispersed by releasing gossamer threads that allow them to hang from the forest canopy and become airborne when picked up by wind or electric currents.

Bioexclusion: (Also known as external biosecurity) preventative measures and risk reduction strategies designed to prevent the introduction of new pathogens or pests into a population from an outside source.

Biorational Approaches: An economical and ecologically sound approach to pest control that uses products of natural origin with limited or no adverse effects on the environment or beneficial organisms.

Border: The official boundary of a distinct geographical or political jurisdiction, which serves as a critical control point for regulating or limiting the movement of people, animals, plants, and other goods into and out of a designated area or region.

Established: Refers to (1) a pathogen or pest found regularly in susceptible populations of a country or jurisdiction and regarded as endemic, or (2) invasive species that have been successfully dispersed and are proliferating in a new region or territory.

Exclusion: An organized strategy or formal process for intentionally excluding a specific risk (e.g., pest or pathogen).

Incubation Period: The interval between initial exposure to an infectious organism and the onset of clinical signs.

Metabolic Stress Disinfection: A non-thermal, residue-free decontamination process, involving physical manipulations that generate cycles of expansion and compression forces combined with low vapor concentrations of natural chemicals to simultaneously disinfect and disinfest fresh agricultural products.

Nonthermal Plasma: A disinfection process that relies on energy released from a partially ionized gas maintained at a temperature that is sufficient to inactive biofilms and other microorganisms, but compatible with living tissues, plant material, meat, and other products with fragile surfaces.

Outcome-Specific Data: Specific, measurable data or indicators that track progress toward a targeted outcome or goal.

Pest-Free Status: Refers to an officially identified area or region where a target pest is not found and which is managed and maintained to prevent the target pest's introduction, dispersal, and establishment.

Phytosanitary: Information relevant to the health status and disease control procedures for plants and plant products, especially as they pertain to international trade and commerce of agricultural crops.

Phytosanitary Certificate: An inspection certificate issued by a competent government authority for a shipment of plants or plant materials that confirms the goods have been inspected, treated, and verified free from harmful pests and plant diseases; the certificate must be issued prior to customs clearance for export and import.

Quarantine Period: The amount of time that plants, animals, and/or other goods which have been potentially exposed to an infectious agent must be restricted from movement or kept in isolation to prevent pathogens, insect pests, and other contagious diseases from being dispersed or transmitted into vulnerable populations.

Subclinical Infection: An infection that has no, or minimally recognizable, clinical signs or symptoms.

Virus Phenotype: The observable physical properties and other attributes of a virus that influence its survival, transmission, and virulence.

Zoosanitary: Information relevant to the health status and disease control procedures for animals and animal products, especially as they pertain to international trade and commerce of agricultural crops.

APPENDIX

Acronym List

AGM: Asian Gypsy Moth
APHIS: USDA Animal and Plant Health Inspection Service
ASF: African Swine Fever
CFIA: Canadian Food Inspection Agency
CSF: Classical Swine Fever
DDGS: Distiller's Dried Grains with Solubles
FADDL: Foreign Animal Disease Diagnostic Laboratory
FAO: Food and Agriculture Organization of the United Nations
FMD: Foot and Mouth Disease
IPPC: International Plant Protection Convention
ISPM: International Standards for Phytosanitary Measures
MPI: New Zealand Ministry for Primary Industries
MT: Metric Ton
NAFMDVB: North American FMD Vaccine Bank
NAIT: National Animal Identification and Tracing
NAPPO: North America Plant Protection Organization
NAVVCB: National Animal Vaccine and Veterinary Countermeasures Bank
NCPN: National Clean Plant Network
NPDRS: National Plant Disease Recovery System
NPPO: National Plant Protection Organization
NPRG: New Pest Response Guidelines
OIE: World Organization for Animal Health, Office of International des Épizooties
PCR: Polymerase Chain Reaction
PEDV: Porcine Epidemic Diarrhea Virus
PPQ: Plant Protection and Quarantine
PRRS: Porcine Reproductive and Respiratory Syndrome
QPS: Quarantine Pre-Shipment
TAD: Transboundary Animal Diseases
$\mathbf{TCID}_{50}$: Median Tissue Culture Infectious Dose
USDA: United States Department of Agriculture

Chapter 5

Surveillance for Early Detection of High-Consequence Pests and Pathogens

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ABSTRACT

Surveillance is one of the core activities of national organizations responsible for human, animal, or plant health, with the goal of demonstrating the absence of infection or infestation, determining the presence or distribution of infection or infestation, and/or detecting as early as possible exotic or emerging pests and pathogens that may be harmful to agriculture and the environment. Surveillance is a tool to establish absence of the pest or pathogen, monitor trends, facilitate the mitigation and control of infection or infestation, provide data for use in risk analysis, substantiate the rationale for sanitary measures,

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and provide assurances to trading partners, producers, and the public. The type of surveillance applied depends on the objectives of the surveillance, the available data sources, resources, and the outputs needed to support decision-making.

INTRODUCTION

Introduced pests and pathogens require strategies for detection, identification, and prevention of spread. These strategies can be applied across regions, states, or international borders. Threats include known, high-consequence pests and pathogens, as well as emerging, unknown threats. Ideally, an integrated, strategic approach to intervention and mitigation is in place prior to introduction of the threat, however there are manifold threats and myriad pathways by which foreign or exotic pests and pathogens may enter a new area or region. This may occur through human-assisted transport of animals and plants, products made from animal or plant materials, contaminated food, migratory wild animals or insects, and even through shipping containers and airport luggage. However, natural spread of pests and pathogens also can occur. Given that weather patterns, especially hurricanes and typhoons, can move birds and insects, or dust and debris, across international borders, movement of pests and pathogens become almost inevitable.

With awareness of potential threats and routes of introduction, what strategies and tactical elements can be applied to detect an introduced pathogen or pest early and rapidly contain the invader? Critically, we need sound **surveillance** strategies, including guidelines for when to look, where to look, how to look, and for timely data analysis and sharing. These strategies include interoperable tactical sciences to look for, and identify, something unusual that could be indicative of some new organism that may pose an agricultural or environmental threat. A global surveillance system for crop diseases has been proposed that would allow sharing of information to facilitate detection of new threats to enable countries and regions to quickly respond to emerging disease outbreaks (Carvajal-Yepes et al., 2019). Global systems also exist to facilitate information sharing and threat detection for animal pathogens through a network of national and international organizations. The **Office International des Epizooties** (**World Organization for Animal Health** [OIE], 2021) coordinates efforts among more than 75 national and regional organizations.

The common analogy for the detection of foreign or exotic pathogens and pests in the United States (U.S.) is the 'needle in a haystack' reference. It is much more complex. The premise is that we only 'know what we know,' but we also must acknowledge that what we 'do not know is much more than what we do know.' Essentially, in many instances, the unknown universe of potential pest and pathogens is far greater than the relatively limited knowledge of those we know cause disease and/or economic damage to agricultural crops, livestock, landscape plants, forest and urban trees, and other aspects of the food and environmental landscape in which we live. What we do know, however, are the characteristics of the populations we are trying to protect. We know location, density, prevailing conditions of climate, operational norms, and opportunities for movement in and out of the area. This knowledge helps determine our strategies.

Design and implementation of an effective surveillance system is critical to early detection, identification, and containment. The goal of early detection is to find and identify pathogens and pests before they become established and cause widespread damage and economic harm to the agricultural, landscape, and environmental sectors (Reaser et al., 2020). The U.S. National Invasive Species Council's National Management Plan (2018) champions the concept that early detection, rapid assessment, and rapid response is a critical second line of defense and provides the greatest opportunity for eradication and cost-effective management. An effective early detection and rapid response program increases the likelihood that invasive species will be found, contained, and eradicated before they become widely established.

To do this, a variety of surveillance strategies have been developed and implemented. These can be categorized into two main strategies, **active (specific)** and **passive (general) surveillance**. There are advantages and disadvantages of both types of surveillance and the selection of active and/or passive surveillance approaches is dependent upon the assumed risk. Passive surveillance is effective because it casts a wide net and can be more easily conducted on an on-going basis and is usually less expensive. However, it may result in underreporting and/or incomplete data. If the risk and/or consequences are deemed low, then passive, lower cost, methods may be more appropriate. These might include use of less specifically targeted **surveys**, remote sensing technologies, or indirect methods such as **monitoring** social media to identify unusual events. Active surveillance should be used to investigate diseases and pests with a high risk to agriculture and the environment and to confidently declare absence of pests and pathogens. Data collected through active surveillance generally provides more accurate and complete information than passive surveillance, however, it can be resource intensive. If the entry risk and consequences of a pest or pathogen to agriculture and the environment are high, it follows that resources should be invested into active surveillance strategies. These may include regular sample collections and testing of specific sentinel populations, or targeted interrogation of first detector communities through surveys or reporting requirements. There is no definitive delineation between active and passive surveillance and some strategies use components of both. One strategy leads to the other, and vice versa, and it can be difficult in practice to distinguish a given approach as active or passive (Table 1). The effectiveness and efficiency of any surveillance strategy should be evaluated based on the probability of detection of the agent in a particular scheme, and the accuracy of guaranteeing detection, containment, and ultimate freedom from the disease or pest.

The data gathered by the surveillance system is ultimately subject to analysis to describe the pest or pathogen status, generate hypotheses about the risks, and model potential spatial and temporal spread (Figure 1). This information feeds back to inform decisions about how well the systems are working and how to effectively manage and mitigate the threat. These aspects, including risk assessment, are covered in other Chapters. This Chapter explores both animal and plant surveillance strategies, identifies complementary strategies, and shows where surveillance strategies may be specific to either the animal or plant world.

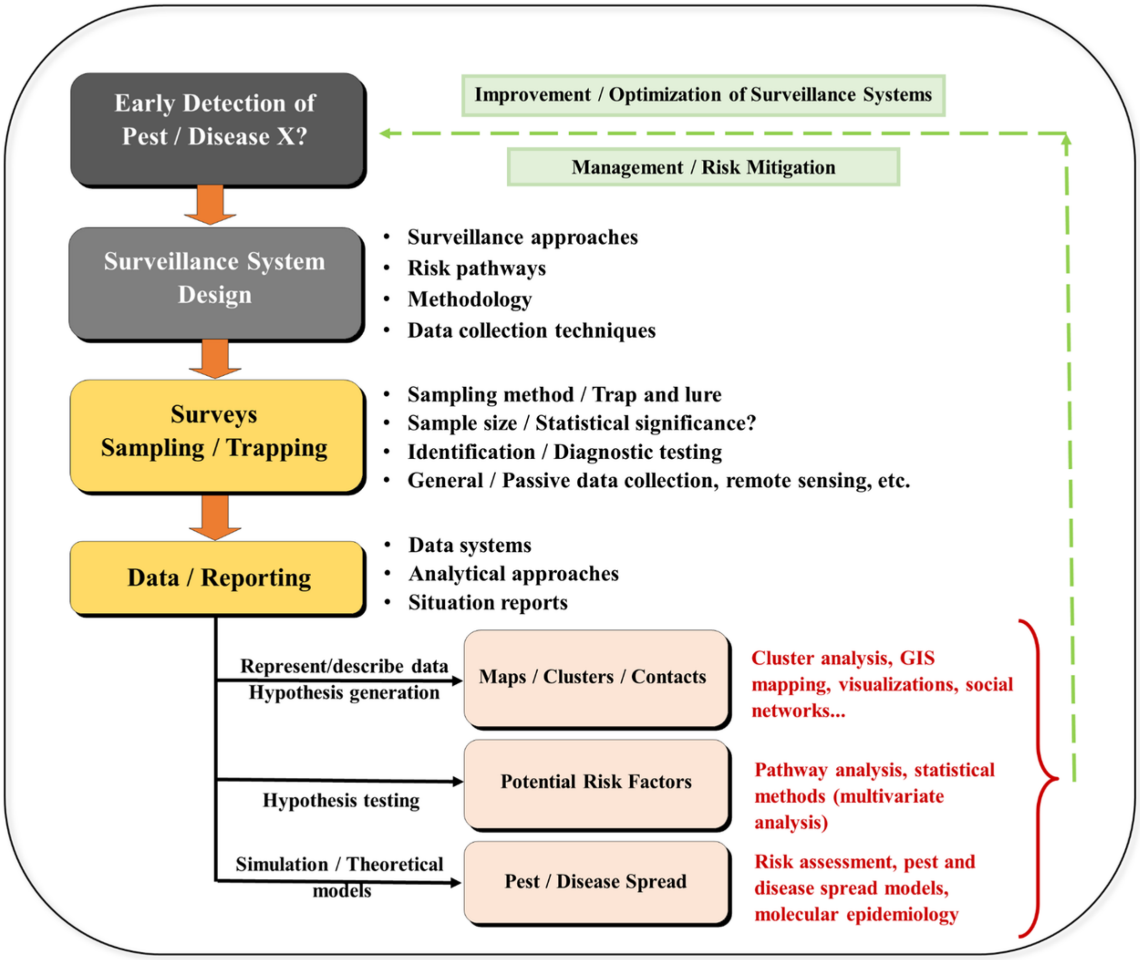
Surveillance results provide data on the:

- Detection of new pests and pathogens in an area,
- Distribution of a pest or pathogen,
- Establishment and maintenance of pest-free areas and pest-free production sites,
- Determination of the status of a pest in an area for reporting to other countries,
- Changes in a pest population,
- Delimitation of a pest population in an area,
- Eradication of a pest in an area, and
- Effectiveness of pest management in an area

Table 1. Various strategies to conduct surveillance

Surveillance Approach	Brief Description and Considerations	Advantages	Disadvantages	Examples Animal / Plant
Structured Interviews	● Use of closed or structured questionnaires and interviews for data collection ● It is essential to know: a. what information is required b. how to capture that information c. how to structure that information so that it can be easily digitalized and analyzed	● Quick contact with large number of people ● Easy to create, code, and interpret ● Easy to standardize	● Fixed format; thus, difficult to examine complex epidemiological scenarios or risk factors	● Reports of NAHLN Laboratories ● State Veterinarians ● Local Veterinarians
				● Nursery Inspectors ● Master Gardeners ● Landscapers ● Reports from NPDN labs
Sentinel Surveillance	● Health status of a population of animals or plants is periodically assessed ● Sentinel animal population, crop field plot, or habitat (reporting unit) selected based on the high probability of detecting the target pathogens and/or pests	● Rapid ● Economic	● Depends on the quality of the sentinels ● May not be effective for rare diseases ● Not population based	● Testing milk produced by a dairy for the presence of a pathogen
				● Defined areas in crop fields that are assessed for the presence of a target pathogen
Syndromic Surveillance	● An investigational approach where health indicators are monitored to detect outbreaks of disease or pests earlier than would otherwise be possible with traditional methods ● Use of clinical, diagnostic, or health-related information that precedes confirmatory diagnosis of specific disease or pest conditions ● Alternative approach focuses on detecting individual atypical cases, where a new disease or pest that shows symptoms or signs the clinician or diagnostician cannot link to a known pathogen or pest	● Easy ● Economic ● Sustainable	● Potential "false alarms" (low specificity) ● Limited or no information on "negatives"	● Respiratory congestion, cough, or nasal discharge across multiple animals on a farm or some other reporting unit
				● Analyses of pathogen trends from results of multiple diagnostic laboratories ● Reports of new symptoms in plants
Proxy or Indicator Surveillance	● Like syndromic surveillance, but uses other, non-health-related information (e.g., animal or crop productivity, human movement, social activity)	● Easy ● Economic ● Sustainable	● Potential "false alarms" (low specificity)	● Reduced weight ● Reduced feed intake ● Immobility
				● Reduced crop yield ● Reduced commodity quality
Participatory Surveillance	● Stakeholders have a greater role in shaping animal and plant health surveillance programs ● Uses the existing knowledge that people have about the animals they keep and plants they cultivate, and about the pests and pathogens that cause disease that impact their health and livelihoods ● Uses semi-structured interviews, focus-group discussions, ranking/scoring, exercises, and diverse visualization techniques	● Low cost ● Communities that are at risk engage with the surveillance process ● Can lead to the development of disease control programs that are both acceptable and effective ● Allows for "discovery" of new information	● Data generated can be difficult to code, interpret, and/or analyze without standardization	● University extension ● Continuing education ● State agencies ● Commodity and grower associations ● Industry groups
Post-harvest surveillance	● Sampling/surveys at the slaughterhouse and packinghouse level (usually for food safety purposes). May be just visual (e.g., clinical signs or fruit symptoms) or through tests or surveys. ● Quality analysis of grains and fresh produce	● Easy ● Relative low cost	● Data access ● Potential difficulty tracing back to the farm of origin ● Depends on inspector experience	● Monitoring for signs of infection or inflammation in lymph nodes, liver, spleen, kidneys
				● Commodity mills and packinghouse inspections
Risk-based surveillance	Sampling of high-risk groups: these risk groups might share risk factors or be geographically defined; for example, along border areas or in animal-dense areas.	● Cost-effectiveness ● Same or increased sensitivity with smaller sample size	● Definition and quantification of risk ● Sometimes complex design	● Transportation of animals between locations, especially when animals from more than one location are mixed
				● Pathways along transportation routes ● Previous detections

Figure 1. Summary of some of the most important considerations when designing a surveillance program for post-border detection of high-consequence plant and animal pests and diseases



National and International Organizations Engaged in Surveillance for Introduced Pathogens and Pests

National Plant Protection Organizations (NPPOs) conducting surveillance to detect high-consequence plant pests and diseases follow international guidance and protocols as described in various **International Standards for Phytosanitary Measures** (Adopted Standards [ISPM], 2021). The **Standards** are developed by the Commission on Phytosanitary Measures (CPM, 2021), the governing body of the **International Plant Protection Convention** (IPPC, 2021), and published by the Food and Agriculture Organization of the United Nations (Food and Agriculture Organization [FAO], 2021). For surveillance of plant pests and pathogens, ISPM 6: Surveillance is the guiding international document (ISPM 6, 2019).

As an example, the United State participates in the International Plant Protection Convention (IPPC, 2021) through the North American Plant Protection Organization (NAPPO), which is the phytosanitary standard-setting organization for North America (North American Plant Protection Organization

[NAPPO], 2021). “NAPPO was created in 1976 as a regional organization in accordance to Article IX of the International Plant Protection Convention (IPPC). NAPPO provides a forum for public and private sectors in Canada, the United States and Mexico to collaborate in the regional protection of plant resources and the environment while facilitating safe trade” (NAPPO, 2021). Official Pest Reports and Emerging Pest Alerts are published via NAPPO’s Phytosanitary Alert System (Phytosanitary Alert System [PAS], 2021). Official Pest Reports are provided by National Plant Protection Organizations within the NAPPO region. Emerging Pest Alerts are news items obtained from public sources and are not official communication from NAPPO. Emerging Pest Alerts are early warnings for emerging plant pests that are not present in the North American region.

As defined in ISPM 5: Glossary of Phytosanitary Terms (ISPM 5, 2019), surveillance is defined as “An official process which collects and records data on pest presence or absence by survey, monitoring or other procedures.” Surveillance activities form the basis for regulatory decisions and the establishment and removal of federal and state quarantines on the intra- and interstate movement of goods, the facilitation of bi-lateral trade talks and subsequent requirements for pathogen and pest information with foreign trading partners, and agricultural/crop/animal and environmental/forest management decisions based on pest or pathogen presence, threshold, or absence. With pests and pathogens come costs in one form or another in terms of management or eradication. Surveillance results can reduce, manage, or eliminate these extra costs.

Similarly, general surveillance of pathogens and pests affecting animals involves the regular collection and reporting of observational and surveillance data and relies on the cooperation of many organizations and individuals, General surveillance often is the result of outreach programs that target veterinarians and others in the livestock industry. Often people or groups are directed to report what they see to state extension or regulatory officials, or report through various mobile applications that collect the information.

As in the practices for plant pests and pathogens, surveillance to detect high-consequence animal pests and diseases also follows international guidance and protocols. The Office International des Epizooties (OIE, 2021) is the intergovernmental organization responsible for improving animal health and welfare worldwide. The OIE, established in 1924, is made up of 182 Member Countries and is recognized as a reference organization by the World Trade Organization (World Trade Organization [WTO], 2021). In addition, the OIE has relationships and agreements with nearly 75 other international and regional organizations, including the Food and Agricultural Organization (FAO, 2021) and World Health Organization (World Health Organization [WHO], 2021) of the United Nations, and has offices on every continent. Working through a network of 246 OIE Collaborating Centers and Reference Laboratories across the world and recognized as the sole international reference organization for animal health, information on animal disease control is made available to improve disease control and eradication. An example of these collaborating centers is the Center for Food Security and Public Health (CFSPH) at Iowa State University (CFSPH, 2021). The OIE standards outlined in the **Terrestrial and Aquatic Animal Health Code** (OIE, 2019) are recognized by the World Trade Organization as reference international sanitary rules.

In direct collaboration with their respective Veterinary Services, the Member Countries of OIE agree to report listed animal diseases detected within their respective territories. There are over 100 OIE-listed terrestrial and aquatic animal diseases that carry a specific mandate for reporting whenever they are detected. This information is disseminated through the Disease Information and the World Animal Health Information System Interface (WAHIS, 2013), so countries can take necessary action. The OIE reporting also includes animal diseases transmissible to humans occurring both naturally through deliberate means.

The OIE has five Regional Commissions to address specific problems facing its Members in the different regions of the world. The United States is a member of the Regional Commission of the Americas. The United States actively participates in developing the OIE international animal health standards. Within the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), the Deputy Administrator of Veterinary Services (VS) (APHIS, 2020a), as the Chief Veterinary Officer (CVO), is charged with managing and coordinating U.S. animal health standard-setting activities related to the OIE.

In the United States, USDA, APHIS, VS is the agency responsible to protect animal health. Preventing, controlling, or eliminating animal diseases requires the ability to detect emerging foreign and domestic diseases, monitor trends, risks, and threats around the world, evaluate disease control and eradication programs, and disseminate animal health information to multiple audiences. The national reference laboratory for the United States is operated by the National Veterinary Services Laboratories (NVSL) (APHIS, 2021b) located at Ames, Iowa and the Foreign Animal Disease Diagnostic Laboratory at Plum Island, New York. Testing for certain types of diseases, such as OIE-listed diseases, must be performed at either the NVSL or other approved facilities such as laboratories in the National Animal Health Laboratory Network (NAHLN) (APHIS, 2020c) distributed across the United States.

General Surveillance

General surveillance involves the regular collection and reporting of surveillance or observational data and may rely on the cooperation of many organizations and individuals. Data and information sources may include federal, state, or local government offices, university research and extension, museums, scientific societies and their publications, trade journals, consultants, producers and their organizations, independent specialists and websites, and the public, among others. General surveillance often is the result of outreach programs that target the public for awareness (Crimmins et al., 2017), or specific groups like crop consultants, Master Gardeners, landscapers, or tree experts. Often people or groups are directed to report what they see to state extension, veterinarians, or regulatory officials, or report through various mobile applications that collect the information.

In addition to official notifications, the OIE conducts active search activity for non-official information and rumors relating to animal health and public health. This information is evaluated in the context of the animal health situation prevailing in the country or region concerned and, where appropriate, verified with the Member Country or Territory for the purposes of official confirmation and potential publication. This general surveillance begins with the search for non-official animal health information and rumors disseminated by the media, networks such as Health Canada's Global Public Health Intelligence Network (GPHIN, 2021) and ProMed (2021), and scientific journals and publications. Reports also are collected from OIE Reference Laboratories, which have a mandate to report any positive finding relating to an OIE-listed disease. The search is intended to identify specific health events, including the suspected disease, clinical signs (e.g., high **mortality**), geographical location, and the animal species affected. Further analysis determines relevance of the information and whether it relates to an exceptional event requiring immediate action, confirmatory investigation, and notifications. If the information relates to an animal disease that is already present or to human cases of a zoonotic disease, it is retained for comparison with future reports. Zoonotic diseases are diseases and infections naturally transmitted between vertebrate animals and humans. The U.S. Centers for Disease Control (CDC) One Health Office is an effort to track and prioritize zoonotic disease surveillance (CDC, 2021). If appropriate, notifications include the designated official of the Member Country or Territory who would officially notify the OIE if that had not already been done.

Another example of this type of effort is housed within the Center for Invasive Species and Ecosystem Health at the University of Georgia (Bugwood, 2021). The Early Detection & Distribution Mapping System (EDDMapS, n.d.) began as an effort to aggregate and normalize invasive species observation data among partners focused on different geographic scales, disciplines, institutions, taxonomic groups, and commodities. By not only aggregating the data but working with partners to ensure that the definitions used to describe different attributes of an observation aligned, it became possible to directly compare data sets without having to first resolve differences in terminology.

Once put online, partners working with EDDMapS wanted to be able to continue adding data by accepting reports from both their teams and the public. This led to development of a platform for citizen science reporting of invasive species and other agronomic pests. Reports received are routed to the local teams working on a given taxa. These teams communicate with the reporter, regulatory authorities, and related invasive species management areas. Once verified, they review the online records and take appropriate measures to protect the confidentiality of the locations and reporters. All partners have the option of signing up for alerts in their given areas of interest to keep them informed of ongoing detections. The data are made available for all partners to develop more effective management plans, conduct research to better predict new invasions and spread of existing species, and continue to give a near-real time update of invasive species distribution.

Success within the invasive species arena has led to further expansion to incorporate agronomic pests. While not all the pests currently are considered invasive, many were at one time newly introduced species, and after many years the goal of eradication or containment became untenable. This combined with efforts of the Integrated Pest Information Platform for Extension and Education (iPiPE, 2021, Isard, 2015) and its predecessor, ipmPIPE (Isard, 2006b), to provide an infrastructure with tools, information products, and expert commentary for the detection and management of pests that threaten U.S. crops.

Rather than attempting to be the one solution for all users, and having to deal with competition between different commercial platforms, EDDMapS forms the basis for a National Pest Observation Repository (NPOR, n.d.) Multiple platforms including FarmDog (2021) and myFields (n.d.), which are specifically aimed at crop consultants and growers, contribute data alongside platforms such as iNaturalist (n.d.). These partners unite with the tools provided by EDDMapS through AgPest Monitor (n.d.) to provide distinctly branded notifications to both invasive species and agriculturally focused audiences. Data products, models, and risk analysis tools are made available to all partners contributing data so they may improve their decision management process.

USDA, APHIS, VS, works with a national network to carry out surveillance activities in the United States, including the NAHLN laboratories, the National Animal Health Monitoring System (NAHMS) (APHIS, 2021a), and the National Animal Health Surveillance System (NAHSS) (APHIS, 2020e). NAHMS conducts national studies on the health and health management of United States domestic livestock populations. These studies are designed to meet the information needs of the industries associated with these commodities, as identified by people within those industries. NAHSS is a system created to detect events and trends related to animal health. Comprehensive and Integrated Surveillance (CIS) within the NAHSS is based on having an integrated and coordinated database for alerts and decision analysis. National plans for CIS for multiple high-impact pathogens of animal species in the United States and supporting resource documents are published by USDA, APHIS, VS (APHIS, 2020a).

In general surveillance of high-consequence animal diseases and pests, veterinary practitioners play a key role. Veterinarians interacting with owners of livestock or pets are often the first line of defense against emerging and exotic animal disease. It is the role of the veterinary practitioner to educate owners, to be aware of unusual clinical signs, to be aware of current disease outbreaks or threats, and to immediately report possible diseases of concern to both Federal and State Animal Health Officials. Veterinarians are required by law to immediately report to the APHIS Veterinarian-in-Charge (APHIS, 2020i) and the State Animal Health Official (USAHA, 2021) all diagnosed or suspected cases of a communicable animal disease for which APHIS has a control or eradication program, and all diagnosed or suspected cases of any animal disease not known to exist in the United States.

Similarly, for plant pests and pathogens, the National Plant Diagnostic Network (NPDN, 2021) is a network of plant health diagnostic laboratories in all 50 states and four territories with the goals to provide high quality diagnostics and to ensure effective and timely communications with regulatory partners, other diagnostic and regulatory labs, and the plant health communities they serve. The NPDN was established in the wake of the terrorist attacks on September 11, 2001, with USDA funding to "protect national plant health by quickly detecting and accurately identifying plant pests and pathogens and effectively communicating these diagnoses to stakeholders and clientele." The NPDN contributes to the safeguarding of U.S. agricultural and natural ecosystems by providing early detection and identification of plant pests and diseases, enhancing diagnostic and detection capabilities, improving communication among federal, state, and local agencies, and delivering educational programs regarding the threats posed by their introductions. As veterinary practitioners play a key role in animal health, plant diagnosticians play that same role for plant health as they are in a prime position to recognize an 'out of the ordinary' pest or pathogen in their State or Region and alert State and federal regulatory agencies to its occurrence. The NPDN labs also partner with state and federal survey efforts by performing validated diagnostic procedures for pests and pathogens of regulatory concern, thus adding capacity for survey efforts and national, state, and local responses to disease and pest outbreaks.

Specific Surveillance

Specific, or active, surveillance is a structured process where specific pests of concern are targeted in surveys over a defined area and period using standard methodology. Pest- or pathogen-specific information is collected to determine the characteristics of a population (size, density, range, etc.) or to determine if a species is present or absent in an area within the timeframe of the surveillance. As the case with both plant and animal pathogens, the disease caused by the pathogen often is the focus of the survey. Here we will refer to pathogen populations with the understanding that they also refer to populations of diseased individuals.

There are three types of specific surveillance activities: **detection**, **delimiting**, or **monitoring surveys** depending on the objectives of the specific surveillance program. These surveys may be developed for pests or pathogens in relation to one or more areas, sites, hosts, pathways, or commodities. This chapter deals specifically with surveillance for early detection when the pest or pathogen is either absent or very rare. A detection survey is conducted in an area over a specified time frame to determine if a pest or pathogen causing disease is present or absent. These surveys may target foreign pests and/or **endemic** pathogens not known to occur in an area, and invasive or established pests or pathogens not known to be present in an area. Often, the purpose of this type of surveillance is to document absence of a pest or disease caused by a pathogen for phytosanitary purposes that will facilitate market access, trade and the movement of crops, forest products, animal products, and other commodities and goods within a country or internationally. The overall goal is to document the status of a pest or pathogen as either present or absent.

An example of a specific surveillance program structured on the early detection strategy is the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Cooperative Agricultural Pest Survey (CAPS) program (Cooperative Agricultural Pest Survey [CAPS], 2021b, Animal and Plant Health Inspection Service [APHIS], 2020g, Kalaris et al., 2014). Similar programs exist in just about every country, and although they may be structurally different, all attempt to adhere to the principles of surveillance in ISPM 6 (2019). The CAPS program is a federal and state partnership that conducts science-based national and state surveys targeted at specific exotic plant pests, diseases, and weeds identified as economic and environmental threats to U.S. agriculture and/or the environment and is an essential element in providing a continuum of checks from offshore preclearance programs, domestic port inspections, and surveys in rural and urban sites across the United States.

The goals of the CAPS program in the United States are to keep agriculture and natural resources safe and to satisfy other countries that the United States' agriculture is safe. To accomplish these goals, the program provides a survey profile of presence/absence data on exotic plant pests in the United States deemed to be of regulatory significance through early detection and surveillance activities. This documented information serves as the basis of APHIS' regulatory efforts and pest management programs that preserve economic opportunities for farmers (i.e., interstate commerce and international trade) and safeguard U.S. agricultural and natural resources. Refer to the CAPS Resource and Collaboration website for more detail on structure, function, and survey guidance (CAPS, 2021b).

The CAPS Program strengthens APHIS' emergency preparedness efforts through the early detection efforts aimed at discovering these pests before they spread and become pest emergencies. These activities are accomplished primarily under USDA funding that is provided through cooperative agreements with state departments of agriculture, universities, and other entities. Resulting survey data support the development and expansion of export markets by identifying pest-free regions that allow the continued export of commodities from different areas of the country.

Similarly, the USDA Forest Service also conducts early detection surveys through their Early Detection and Rapid Response (EDRR) program (Rabaglia, 2019). The goals of the program are to detect, delimit and monitor newly introduced exotic bark and **ambrosia beetles** at selected high-risk forest areas and quickly assess and respond to newly detected infestations. The introduction and establishment of non-native species through raw wood products, solid wood packaging material, or live plants has already had profound ecological effects on forests across the country. The EDRR team, consisting of Forest Service, APHIS, university, and state representatives, has developed a framework for implementing a national, interagency detection, monitoring, and response system for these insects within the forest environment, and complements APHIS and Invasive Species Counsel efforts in the agricultural, landscape, and aquatic environments.

For animals, Comprehensive Integrated Surveillance (CIS) plans (APHIS, 2020a) call for regular testing of specific populations and/or subpopulations of animals in a geographic area determined to be at-risk for a specific pathogen introduction. At-risk areas might be large concentrations of livestock that move in an out of the area or have opportunity for contact with wildlife. An example would be domestic swine operations with proximity to feral swine populations. Animal disease reporting systems can be supplemented with participatory methods, surveys, and specific sampling. Examples include inspection of animals at slaughterhouses/abattoirs; surveillance of sentinel units involving regular testing of one or more animals of known health or immune status in a specified geographical location; clinical observations of animals in the field; systematic analysis of health data, including **morbidity** and mortality rates, production records, and other parameters indicative of changes to the health of an animal population. These data can be supplemented with surveillance of wildlife populations including road-kills, wild animal meat markets, sanitary inspection of hunted animals, morbidity and mortality observations by the general public, wildlife rehabilitation centers, wildlife biologists and wildlife agency field personnel, farmers and other landholders, hunters, and conservationists. Analyses used in surveillance may arise from clinical observations, production records and rapid field and detailed laboratory assays. Various features of these methods are described in other Chapters, and specifics are based on the surveillance objectives, epidemiology, risk of introduction, etc., as listed in the OIE Terrestrial Animal Health Code (OIE, 2019). ISU_FLUture (2021) is an interactive web-based tool developed to provide diagnostic information on Influenza A Virus infection in swine. There is a database of test results, metadata collected from surveys submitted with diagnostic samples, and virus genome sequences collected at the Iowa State University Veterinary Diagnostic Laboratory. The goal is to find trends in the data that will allow for informed decisions regarding influenza and swine health.

Surveillance Strategies and Questions to Answer

A surveillance program, whether for plant or animal pests and pathogens should first consider the objectives of the surveillance, and then develop strategies to achieve those objectives. A system to detect any introduced pathogen or pest should consider all animal or plant species susceptible to the infection or infestation in the region of interest, including wildlife and weeds that may be pest and pathogen reservoirs. In cases where the aim is to detect a known pathogen, the surveillance strategy can be active and highly targeted. Detection of unknown or newly emerging pathogens may be more passive. Regardless of the overall surveillance strategy, multiple assessments and knowledge must be acquired and infrastructure put in place to conduct the surveillance. A multi-pronged approach may include:

- A structured, transparent assessment process to identify threats;
- An assessment of the biology and epidemiology of the pest or pathogen (e.g., pathogenesis, **vectors**, transmission pathways, seasonality, environmental factors);
- An assessment of the risk of introduction and spread;
- Knowledge of husbandry and crop practices, production systems, and disease prevention and control measures;
- Development of cost-effective, scientifically sound survey methods and protocols;
- Development and validation of diagnostic tests and identification methods;
- Providing approved, standardized survey materials (traps, lures, etc.);
- Conducting the actual surveys;
- Decisions on data collection methods;
- Timely reporting and compiling of pest or pathogen survey results through an approved database;
- Ensuring that the data collected are valid and of high quality; and
- Notification of significant pest detections through established protocols.

To carry out its mission and achieve its goals, a surveillance program must answer some fundamental questions with scientific answers that are common to most specific surveys. These are:

- What pests and pathogens to target and how to assess or prioritize their potential risk;
- Where to carry out surveillance activities, and when;
- How to survey and what methodology and tools to use;
- How to collect and record surveillance activities and results; and
- How and where to share the information obtained?

What Pests and Pathogens to Target

The first task to complete when contemplating a surveillance program is to determine which pests and/or pathogens to target. For early detection surveys, where the pest or pathogen is not known to occur domestically, one needs to look beyond one's borders for pests and pathogens that may be reported and/or on the move in different regions of the world. For plant pests and pathogens, the PestLens (PestLens, n.d.) and NAPPO's Phytosanitary Alert System (PAS, 2021) are components of that analysis, along with reports and communications from collaborators in other countries. For animal pests and pathogens, the World Animal Health Information System Interface (WAHIS, 2013) provides summary analysis of disease status in countries around the world. The USDA Animal and Plant Health Inspection Service provides analysis of present, future, and emerging animal health threats, including epidemiological and economic impact modeling.

Likewise, The Program for Monitoring Emerging Diseases (ProMED, 2021), a program of the International Society for Infectious Diseases (ISID, 2018), is an important and publicly available system conducting global reporting of infectious disease outbreaks. The goal of ProMED is to "identify unusual health events related to emerging and re-emerging infectious diseases and toxins affecting humans, animals, and plants." A multidisciplinary global team of subject matter experts produce reports and provide commentary in a variety of fields, which are then emailed to subscribers. Subscribers to these reports are often the first to be aware of disease outbreaks. For example, reports of outbreaks of **Severe Acute Respiratory Syndrome** (SARS), **Ebola**, and the early spread of **Zika** virus were first reported by ProMED.

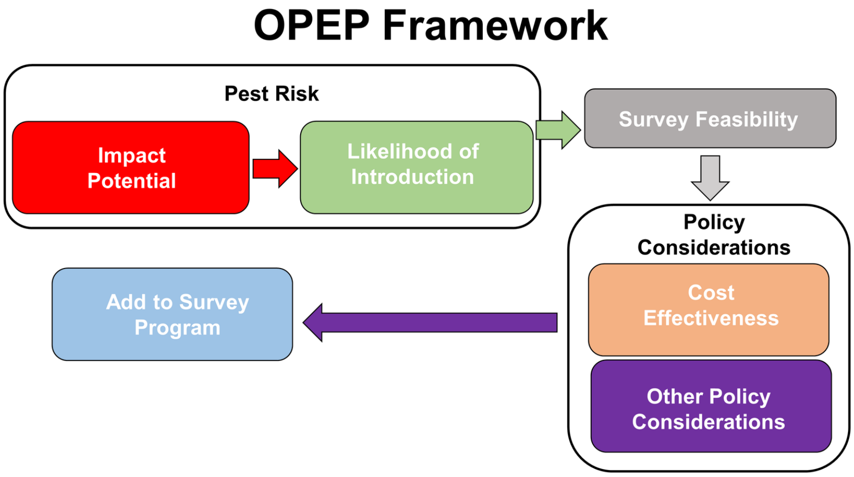
PestLens is an early-warning system that supports the analysis of which pests and/or pathogens to target by collecting and distributing new information on exotic plant pests and providing a web-based platform for documentation and reporting. A team of PestLens analysts with expertise in entomology, plant pathology, weed science, and technical communication systematically collects, evaluates, and summarizes relevant pest information, both from online sources and contributed by system users. When summarizing news items, the PestLens team places them into a plant health context and includes pertinent biological background information. A weekly e-mail notification is sent to PestLens subscribers that includes new distribution records, new host records, new pest descriptions/identifications, significant outbreaks, weed naturalization events, new pathogen/vector relationships, and research of regulatory or phytosanitary interest. Sensitive information is not distributed in the weekly notification. However, it is made available through the PestLens web system to designated USDA representatives via a login. While PestLens was developed for USDA, its audience now extends to a wide range of international plant protection officials.

How to Assess or Prioritize Potential Risk

There are a several internationally agreed-upon frameworks used to assess risk. Two examples of pest and pathogen risk assessment frameworks are outlined in the International Standards for Phytosanitary Measures No. 2, *Framework for pest risk analysis* (ISPM 2, 2019) and in the OIE, *Terrestrial Animal Health Code* (OIE, 2019). Even when potential pests or pathogens of importance have been identified, with limited resources and literally hundreds—if not thousands—of known threats, one of the most fundamental challenges countries face is deciding which of these pests and/or pathogens to target.

While risk analyses are covered in more detail in other Chapters, it is important to understand that different strategies may be employed under the same framework, and to support a strategy, the combination of biological, epidemiological, geographical, statistical, economic, and sometime political and policy sciences work together within a given approach to inform risk. One such example is the **Objective Prioritization of Exotic Pests** (OPEP) model. This model categorizes pest and pathogen threats based on the likelihood of causing serious impacts in the U.S., and the likelihood of being introduced (Figure 2). The process is evidence driven, comparable across different types of organisms, flexible in that it can be used to compare risk by region and host, and defendable in that it uses methods that have been tested and validated statistically.

Figure 2. Objective Prioritization of Exotic Pests (OPEP) Framework



One of the most important changes in how the U.S. prioritizes pests is the intentional focus on ensuring survey resources go towards the pests most likely to cause serious impacts. To this end, two models were developed, one for arthropods and one for plant pathogens, to determine the characteristics most predictive of impact. Each model was developed using the same process the USDA used to develop its Weed Risk Assessment model (APHIS, 2019; Caton et al.; 2018, Koop et al., 2012). A set of yes/no and multiple-choice questions were developed that may be predictive of impact.

Using the arthropod model as an example, the questions that were developed covered:

- Biology and natural history, including natural dispersal, reproduction mode (e.g., sexual, parthenogenic), number of generations, **oviposition**, **fecundity**, **diapause**, use of mating or aggregation **pheromones**, and phenotypic variability,
- Pest damage including whether the pest typically attacks healthy vs. stressed hosts, the ability to vector potential plant pathogens, type of damage (e.g., visible, internal changes, loss of vigor, reduced production, mortality), plant parts damaged, and what other species use the same resources (feeding guild).
- Research and management (e.g., evidence of controls, types of controls, amount of research conducted, magnitude of damage, types of host systems, efforts at eradication).

First, non-native arthropods and pathogens were identified *that were introduced* to the United States. Each pest on the list was then analyzed *as if it were <u>not</u> present* in the United States. In other words, each pest was evaluated using information from outside the United States. Question-specific guidance was developed to minimize variance among the analysts (all questions were approached from a similar standpoint).

A separate team of entomologists and economists classified each pest in terms of its *<u>observed</u>* impacts in the United States. Observed impacts were classified into five (5) groups: very high impacts, high impacts, medium impacts, low impacts, and very low impacts. Because the amount of damage was not available for every pest, especially those that have been present in the United States for a long time, also included were "level of management and control costs" and "amount of research" conducted on the pests as proxies for damage when that information was not available. Thus, the final criteria for observed impact class included:

- Severity of unmitigated damage
- Frequency of severe outbreaks
- Impact on production practices
- Environmental and social impacts
- Level of management and cost control
- Amount of research

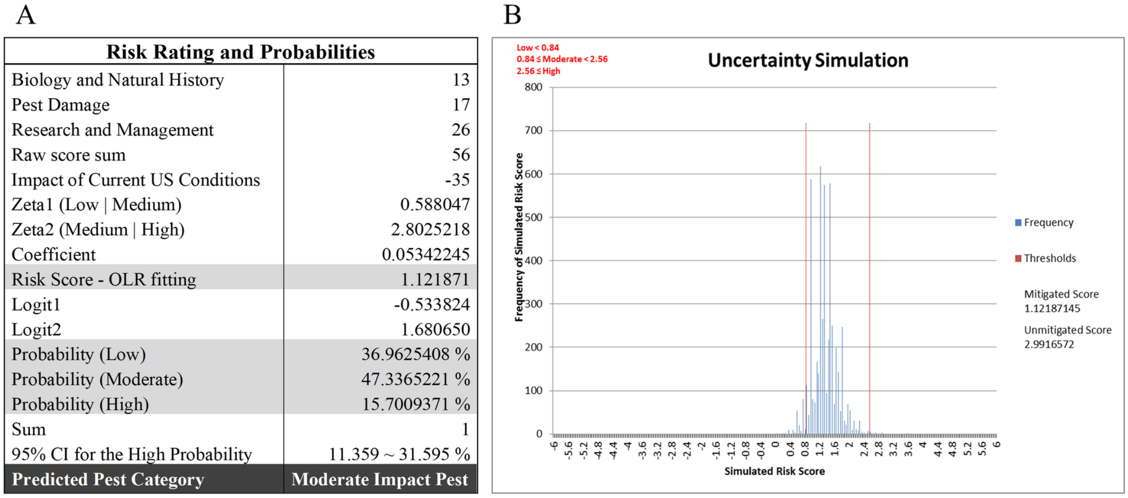
A statistician compared results of the assessments to the actual observed impacts in the United States and tested how well each question predicted observed impact. Non-predictive questions were removed using the following methods:

- Entropy based techniques
- Maximized mutual information scores
- Non-parametric tests including Chi-square with contingency analysis and Kruskal-Wallis tests

The questions left in the model were weighed by their predictive power. Questions that were the most predictive received the largest weight. Additionally, the production practices in the United States, particularly control practices already in place for other pests, will greatly influence the additional impact a pest will have should it become established. Therefore, questions also were added that consider how effective the control methods and practices already in place for other pests would be at controlling the pest being evaluated. The model was *validated by assessing additional pests* established in the United States using the methods described above, and re-testing some questions that were not predictive.

The current model is housed in Microsoft Excel. Ordinal logistic regression was selected based on model usability within the development platform (Excel). Results are provided as probabilities for a given pest being a high, moderate, or low impact pest (Figure 3A). Uncertainty was considered through a Monte Carlo simulation with 5000 iterations where alternate answers were used (Figure 3B).

Figure 3. OPEP model outputs. **A.** *Example output of the regression model in Excel. The risk score is determined based on how analysts answer the questions in the excel template. The percent probability a pest will have a low, moderate, and high impact are the results of the logistic regressions.* **B.** *Example output of a Monte Carlo simulation of uncertainty*



Risk Rating and Probabilities	
Biology and Natural History	13
Pest Damage	17
Research and Management	26
Raw score sum	56
Impact of Current US Conditions	-35
Zeta1 (Low | Medium)	0.588047
Zeta2 (Medium | High)	2.8025218
Coefficient	0.05342245
Risk Score - OLR fitting	1.121871
Logit1	-0.533824
Logit2	1.680650
Probability (Low)	36.9625408 %
Probability (Moderate)	47.3365221 %
Probability (High)	15.7009371 %
Sum	1
95% CI for the High Probability	11.359 ~ 31.595 %
Predicted Pest Category	**Moderate Impact Pest**

The result is a list of pests and pathogens grouped by risk score and uncertainty. Pests that have the greatest likelihood of causing serious impacts and of being introduced are given the highest priority for survey. Three categories with 10 Risk Groups have been developed to prioritize pests and pathogens. Regardless of the model used, arthropod or pathogen, the outcome of the analyses will place all pests into one of these Risk Groups (Table 2). Over time the list of pests and pathogens has the potential to become very large as more species are analyzed. It is up to the organization funding and conducting the surveys to determine the number of pests in the Risk Groups that will be the targets of the surveys. Some situations may call for a more restricted prioritized list based on resources and capacity.

The introduction of a pest or pathogen into a new area can be defined as the entry of that pest or pathogen and results in its establishment (ISPM 5, 2019). However, establishment requires the pest to find 1) a suitable host and climate and 2) a mate or host for reproduction (if necessary). Because of the difficulty of predicting likelihood of establishment, the United States has opted for a qualitative approach to evaluating likelihood of introduction. Only pests and pathogens with predicted high or moderate impacts are considered further because a low impact pest or pathogen that is highly likely to be introduced into

Table 2. OPEP model outputs place organisms into Risk Groups based on Predicted Impacts

Risk Group	Predicted Impacts
A	Predicted to be a high impact pest in the United States (low uncertainty)
B	Predicted to be a high impact pest in the US, but with a higher degree of uncertainty
C	Probability of being a high impact pest vs moderate impact pest is roughly equivalent (w/in 10 percentage points)
D	Predicted to be a moderate impact pest, but probability of being high impact is >20% (cause high impacts when environmental conditions are right)
E	Predicted to be a moderate pest, but with a high degree of uncertainty
F	Predicted to be a moderate pest
G	Probability of being a moderate vs. low impact pest is roughly equivalent (within 10%)
H	Predicted to be a low impact pest, but with high uncertainty
I	Predicted to be a low impact pest
J	Undetermined-- Cannot be evaluated through OPEP; Pest does not cause damage in its native range and has not been introduced outside of native range, or pest has not been introduced outside its native range and closely related species are high impact pests

the U.S. is not relevant for prioritizing survey resources. Pests and pathogens with a higher likelihood of being introduced are elevated in the overall prioritization, while pests and pathogens that are less likely to be introduced are given a lower priority.

A pest's priority is elevated due to likelihood of introduction when one or more of the following situations exist:

- The pest is present in Mexico, Canada, or the Caribbean and capable of natural spread into the United States.
- There is evidence in recent literature that demonstrates that a pest has recently been introduced to new areas and is spreading.
- Introduction and eradication of the pest has previously occurred in the United States.

A pest's priority is reduced based on likelihood of introduction if one or more of the following situations exist:

- Pest spread requires a vector that is not present in the United States
- Pest requires more than one host, and it seems unlikely that all will be present in an area where the pest would enter to allow for establishment.
- Pest establishment has not occurred outside of its native range.

As outlined in the OIE Terrestrial Animal Health Code (OIE, 2019) there are four components to import risk analysis, hazard identification, risk assessment, risk management and risk communication. The steps of risk assessment involve entry or release assessment, describing biological pathways for release of a threat agent and estimation of the probabilities of these under various relevant conditions; similar to plants, what is the likelihood of introduction and/or what is the likelihood of spread? Scenarios might include evaluation of animal species, types of veterinary services, and surveillance and control methods available. Exposure assessment describes the pathways necessary for exposure of humans/animals to the threat agents released and estimation of these probabilities. Factors to consider here include potential vectors of the pathogen, impacts of cultural or geographic characteristics. Consequence assessment is the description of the relationships between exposure to threat agents and consequences of those exposures in both biological and economic terms, such as impact on trade. The overall risk assessment, then, should be based on an iterative process of entry (or release) assessment, exposure assessment, and consequence assessment under current local conditions over time. It is important to note that risk can be estimated in different ways, such as using climate suitability models as outlined below to evaluate climate-based risk, a different but complementary approach.

Where to Carry Out Surveillance Activities

When an exotic species has been predicted to be a high impact pest if it were to become established, the next question to ask is *where* surveys should be conducted. Detection surveys are a key defense against new exotic pests and pathogens but are only applicable when the **prevalence** or abundance of the agent is low (or zero). This means that simple random sampling, in which each individual, trap, or area has an equal probability of being inspected, would have to proceed at exceptionally high levels to either reliably detect the presence or declare the absence of the agent. This issue can be overcome by preferentially sampling hosts known to be at a higher risk of containing the agent. Although the terminology describing such approaches varies both within and between disciplines, they are commonly termed "targeted" or "risk-based" approaches (Hoinville et al., 2013; ISPM 6, 2019). Whilst the central tenet of these surveillance activities is that not all hosts or geographical areas are equal when it comes to surveillance value, there are a wide variety of ways in which this is translated into practice. Sampling sites may be selected based on their spatial location, as has been commonplace for plant pests and pathogens for some time – generally manifesting as increased surveillance close to ports of entry or known infected areas (Bulman, 2008; Carter, 1989; Lance, 2003). In these cases, pathway models of trade movements are commonly used to quantify the incursion risk when planning such surveillance activities (Douma et al., 2016; Kalaris et al., 2014; Magarey et al., 2011), although recent approaches using large datasets of movements also have shown promise (Gottwald et al., 2019). Another approach is to consider selecting individuals based on the attributes of the hosts themselves (Stärk et al., 2006). This is commonly achieved by using statistical models to identify epidemiological "strata" within a population and quantify their relative risk of infection (Doherr & Audigé, 2001; Hadorn & Stärk, 2008; Martin et al., 2007). Approaches based upon methods such as this have been used to plan surveys to declare the absence of pests and pathogens of both animals (Calistri et al., 2018; EFSA, 2012) and plants (Lázaro et al., 2020).

Climatic Approach to Risk

When considering the spatial distribution in surveillance activities, modelling and mapping the potential distribution of threatening pests is used to quantify the risk of establishment in a new area (Lantschner et al., 2019; Srivastava et al., 2019). Identifying which regions are at highest risk of invasion can improve early detection and rapid response measures by providing information on where to concentrate surveillance resources and efforts to detect the pest or disease (Reaser et al., 2020). InterSpread Plus® (ISP) and the North American Animal Disease Spread Model/Animal Disease Spread Model (NAADSM/ADSM) are the most used applications to simulate animal disease spread and control (APHIS, 2020b).

Climate is typically the dominant variable for risk mapping because of its usefulness as a regional-scale indicator of environmental suitability for organisms (Sutherst, 2014). Spatialized temperature and precipitation data are freely available from databases such as WorldClim (Fick & Hijmans, 2017; WorldClim, 2020), CliMond (CliMond, n.d.; Kriticos et al. 2012), and PRISM (Parameter-elevation Relationships on Independent Slopes Model) (Daly et al., 2008; PRISM, 2021). Risk of establishment also may be influenced by anthropogenic factors (e.g., eradication efforts and assisted dispersal) and biotic factors (e.g., host availability, natural enemy limitations, and interspecific interactions (Lantschner et al., 2019). However, incorporating these factors into risk models is complicated by a lack of understanding of the mechanistic underpinnings of their effects, and how they may change in new environments (Lantschner et al., 2019; Srivastava et al., 2019).

Pest risk maps of modelled climate suitability may be generated via correlative or process-based approaches (reviewed in Lantschner et al., 2019; Srivastava et al., 2019). Process-based distribution models include parameters that describe how environmental factors directly influence the development and survival of a species, such as temperature thresholds and stress limits (Evans et al., 2016; Kearney & Porter, 2009). Conversely, correlative models involve statistically linking spatial environmental data to species distribution records, and do not require knowledge of the mechanistic links between climate and the biology of a target organism (Elith & Leathwick, 2009). While correlative models are more widely used than process-based models, they are thought to be less reliable in predicting a species' potential distribution in novel climates (Evans et al., 2016; Kearney & Porter, 2009).

As an example, the CLIMEX software (Hearne Scientific Software, Melbourne, Australia) is a widely used modeling tool for pest risk mapping (Lantschner et al., 2019). The CLIMEX model uses a process-based approach to give an overall measure of the suitability of a location for long-term persistence by a species (Sutherst, 2014; Kriticos et al., 2016). It considers multiple factors that either contribute to or limit growth and survival of a species, including heat and cold stress, wet and dry stress, and the minimum number of heat units (degree-days) that must accumulate to support reproduction (Kriticos et al., 2016). CLIMEX model parameters are fine-tuned, and the model is fitted using observations from the species' known geographical distribution, although laboratory collected data may help with parameterization (Sutherst 2014; Kriticos et al., 2016).

Pest risk maps of modelled climate suitability are typically based on **climate normals** because these data are available at global scales, whereas real-time (current) climate data are limited to certain countries or regions (e.g., PRISM data is generated solely for the continental United States, CONUS). This facilitates developing a model for a species whose current range may extend across several countries, and then predicting the potential distribution in a new region. However, biosecurity practitioners should consider that a model based on averages of historical climate may produce unrealistic predictions of present-day climatic suitability due to rapid climate change in recent years, and that climate averages may essentially erase signals of extreme weather events (e.g., hard freezes, heat waves) that may influence suitability. See for example, changes in distribution of important tick vectors of disease (Marques, et al., 2020; Saleh, et al., 2021).

Considering Epidemiology and Ecology When Planning Surveillance

The entry and spread of pests and pathogens are dynamic processes, and as such the performance of surveillance activities is affected by the epidemiological and ecological factors underpinning these temporal and spatial dynamics. Considering these first in a nonspatial context, one characteristic of early stage spread of new pests and pathogens in areas with large numbers of susceptible hosts is exponential growth. This means that as the levels of the agent increase, the probability of detecting the agent for the first time would be expected to initially increase (as the pest becomes more widespread) and then decrease (as the probability of having failed to detect earlier decreases). This pattern allows one to predict the expected level of an agent at either the time of first detection or in the absence of any detections given knowledge of the initial exponential growth rate of the agent, the test performance (the diagnostic sensitivity and the duration of the lag period before detection is possible), and the intensity of sampling (Alonso Chavez et al., 2016; Bourhis et al., 2019; Mastin et al., 2019; Mastin et al., 2021b; Parnell et al., 2012, 2015). These same ideas also can be applied to heterogeneous systems in which the agent moves between different "strata", such as a vector-borne pathogen moving between a stratum of plant or animal hosts and a stratum of arthropod vectors. In these cases, a key additional requirement is an estimate of the relative prevalence in each stratum during early stage spread. This can be obtained from analysis of a compartmental model of pathogen spread between hosts and vectors – thereby relaxing the requirement for empirical estimates of the prevalence, which may be challenging to obtain for an exotic pets or pathogen (Mastin et al., 2017; Mastin et al., 2021b). These methods allow the estimation of the prevalence of infection in hosts at the time of first detection or in the absence of any detections for a given sampling effort amongst hosts, vectors, and therefore the sampling effort required to detect infection before a given host prevalence is reached. Interestingly, the total required sample size generally is minimized when a single stratum (the one with the highest prevalence of infection during early stage spread) is selected for surveillance (Ferguson et al., 2014; Mastin et al., 2017), and this stratum can be identified analytically from a relatively small number of epidemiological parameters (Mastin et al., 2017; Mastin, et al., 2021b).

As well as increasing in number following initial entry, new pests and pathogens also would be expected to spread spatially. A limitation of using potential establishment to target surveillance is that it does not account for the process of entry and onward spread. That a particular location is suitable for a pest does not mean that the pest has a high likelihood of arriving there. Conversely, that a particular location has a high risk of entry does not mean that establishment and spread will take place. These issues would be expected to be particularly pronounced for plant pathogens, where the distribution of susceptible hosts would be expected to have a considerable impact on pathogen spread, but also for many vector-borne pathogens for which spread patterns may be influenced by environmental heterogeneity. Wind-borne arthropods, mosquitoes, midges, and/or ticks can quickly spread high-threat animal pathogens including protozoal and viral pathogens (Yanase et al., 2020; Folly et al., 2020). Pest entry and spread models can be combined with data on host distribution and climatic suitability to predict where and when disease will occur, as well as provide estimates of how much surveillance is required to achieve early detection.

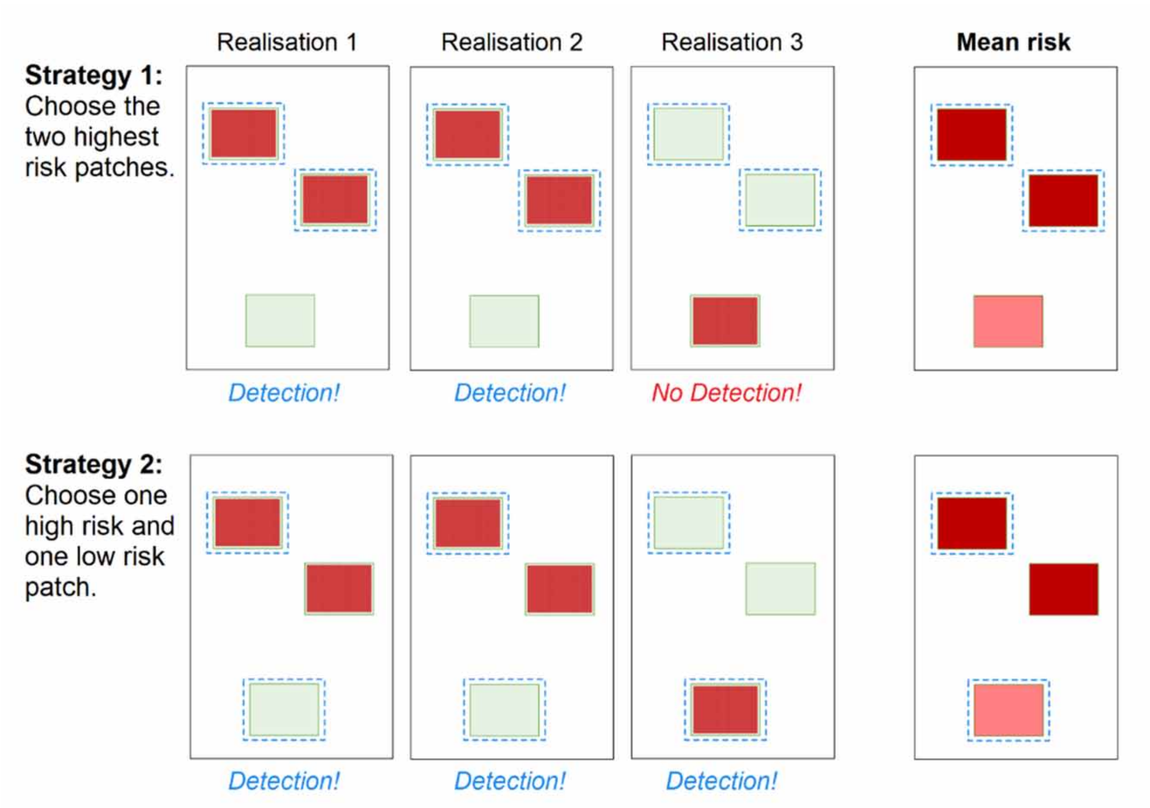
The risk of spatial spread to any given location will be influenced by agent dispersal patterns, the distribution of suitable hosts, and the environmental suitability, as well as the site of entry. These considerations have been captured in a risk-based sampling framework developed to identify where to target surveillance efforts for plant pathogens (Parnell et al., 2014). Under this approach, the region of interest is divided into grid cells, and for each the product of the probability of infection (determined by the dispersal characteristics of the pathogen and the landscape connectivity) and the predicted size of an epidemic if entry did occur (as captured by the basic reproduction number, R_0, which would be expected to be associated with the host density in the cell along with other pathogen-specific factors) is estimated. The use of this method was demonstrated for the economically important plant pathogen, *Candidatus* Liberibacter asiaticus (the cause of the citrus disease huanglongbing; HLB) in Florida (Parnell et al., 2014). Risk-based survey methodologies such as this have been used routinely in Florida since 2006, not only for HLB, but for a range of citrus pests and pathogens (Gottwald et al., 2007; Parnell et al., 2014, 2007). These approaches have been expanded and are currently conducted multiple times per year across the citrus acreage in all citrus producing states (Florida, Louisiana, Texas, Arizona, and California) (McRoberts et al., 2019).

A consideration when planning surveillance is how well a particular surveillance strategy performs in terms of achieving a given surveillance aim. Although methods based on host or site-specific "risk" provide a flexible and transparent methodology for the identification of where best to place surveillance efforts, they do not explicitly quantify how well surveillance performs. Additionally, effectively capturing "risk" can be challenging, since it could relate to a variety of processes, including the potential for establishment (such as climatic suitability or the host density), the probability that an agent actually enters and establishes (which also requires consideration of host connectivity and agent spatial spread

Figure 4. Impact of correlation in site status on detection probability

Only three patches are considered and represent patch status as a dichotomous variable (infected, shown in red (dark), or uninfected, shown in green (light)) rather than considering dynamic trends over time. Over the three simulated outcomes (realizations) considered, the top two patches become infected more frequently than the lower one and would therefore likely both be selected if site selection was based on infection risk (selected sites have a dotted outline). However, if we assume that infected patches will always be detected if visited, sampling these patches will consistently fail to detect infection in the 3rd outcome. Instead, selecting one of the top two patches and the lower patch maximizes the overall detection probability

Source: Mastin et al., 2020



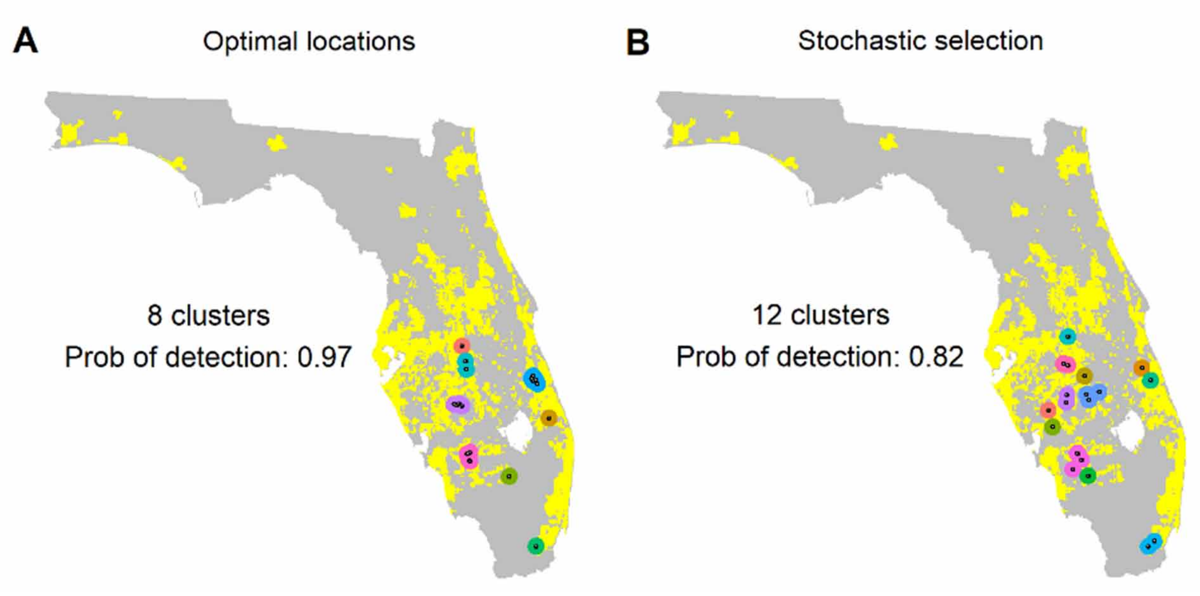
patterns), and the magnitude of the impact if the agent establishes. The question then arises whether an "optimal" arrangement of sites which achieves a given surveillance aim can be identified without quantifying risk. To do this, we need to consider both the performance of the detection method (which will affect the relative value of surveillance in any given site) and the **stochasticity** and variability in the entry and spatial spread of the agent (which will result in the status of certain sites being correlated with each other). As these will impact upon each other, they must be considered simultaneously. This is represented in Figure 4, which shows two different sites selection strategies – one in which sites are selected purely on risk (Strategy 1), and one in which sites are selected from distinct "risk clusters" which capture the correlation in infection status (Strategy 2). While Strategy 1 would be expected to maximize the mean number of detections per outcome, the mean probability of detection per outcome is lower than Strategy 2. While the above is true when the ability to detect infection in a selected site is high, Strategy 1 may give a higher detection probability than Strategy 2 when the ability to detect infection in a selected site is low as infected sites may be missed.

Stochastic spatial simulation models can be used to capture the degree of spatiotemporal correlation in site infection during early stage spread, allowing us to capture the concepts shown in Figure 4. These models can be parameterized using a relatively small number of variables relating to agent entry and spread, either based upon available data or adjusted to reproduce expected spatiotemporal spread dynamics and run up to a predefined maximum prevalence or abundance. The ability to detect infected sites can be estimated given some knowledge of the sampling effort per site and the performance of the detection method used (diagnostic sensitivity and detection lag). From these, the probability of detecting the agent in any given site over the course of early stage spread, and the probability of at least one detection for any given selection of sampling sites (i.e., a particular "survey arrangement") can be estimated. By running multiple simulations, the variability in the spread of the agent through the landscape, and by extension the variability in the detection probability for any given survey arrangement, can be captured. The mean of these outcome-specific detection probabilities provides a single metric – the "mean detection probability" – for each survey arrangement which captures both the expected correlation and variability in site status and the ability to detect a pest or pathogen.

While it is relatively easy to estimate the mean detection probability for any given survey arrangement, the question of what this arrangement should look like is more challenging. Due to the sheer number of potential locations in a typical landscape, the number of possible combinations of selected sites is likely far too high to consider enumerating all possible survey arrangements. Instead, a computational optimization routine to approximate the arrangement of sites which maximizes (or minimizes) a carefully chosen "objective function" can be used (Mastin et al., 2020). In the case of early detection surveillance, the mean detection probability provides a useful objective function, but alternatives could be used, according to the particular surveillance aim in question (Mastin et al., 2021a). As this method considers surveillance in isolation of any control strategies which would be required following detection, economic considerations associated with control are implicitly captured by selecting an economically acceptable maximum level of the pest or pathogen, although some economic considerations such as the estimated costs of moving between sites could be captured by adjusting the objective function used in the optimization step. Alternatively, other methods are available for the investigation (Rimbaud et al., 2019) or optimization (Bussell & Cunniffe, 2020; Epanchin-Niell et al., 2012; Mehta et al., 2007) of combined surveillance and subsequent control activities.

As well as offering valuable theoretical insights into surveillance strategy, such as the impact of the diagnostic test performance on the survey arrangement and the differences between optimal and conventional site selection methods, optimization-based surveillance planning has direct applied value. As the method quantifies spread in real-world landscapes and the performance of the detection method, optimal survey arrangements can be precisely targeted, and the impact of epidemiological uncertainties explored. Although not yet evaluated in the field, when using the example of HLB, the optimization method outperformed all other approaches based on quantification of "risk" within sites, such as probability of pathogen entry, host density, and the product of these, as represented in Figure 5 and was resilient to parameter misspecification. Indeed, many of the suggested surveillance sites correlated with sites found to be infected during the early stages of spread, suggesting that approaches such as this could have considerable value in planning and implementing surveillance strategies.

Figure 5. Performance of optimized sampling (A) in contrast to conventional site selection strategies (B). These plots show the distribution of 20 sites selected using the optimized approach assuming a diagnostic sensitivity of 0.5 (A) and selected with a probability proportional to the mean end prevalence. Clusters are defined as sites within 20 km of each other. Shaded areas represent citrus production.
Source: Mastin et al., 2020



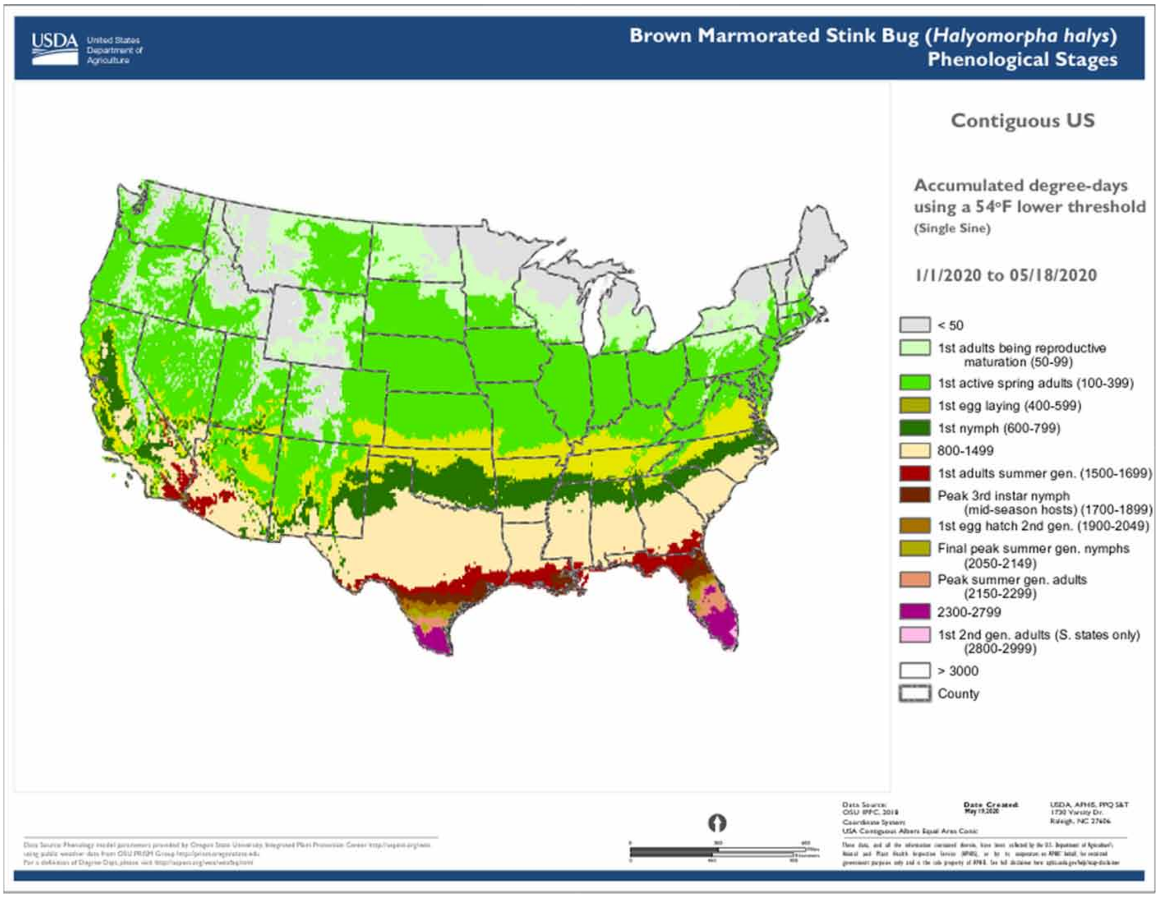
When is the Appropriate Time to Carry Out Surveillance Activities?

Following the question of *where* to conduct surveillance is *when* to conduct surveillance activities. Knowing when to deploy resources can improve the timing of surveillance, e.g., when to deploy traps or collect samples, and thus conduct surveillance activities in a more efficient and economic manner. The case study on **vesicular stomatitis** explores this concept in terms of animal health.

Modeling the **phenology** (seasonal activities) and life-cycle development of the target organism is a standard method used to improve the timing of surveillance. Classically, a two-dimensional (temperature and time) heat unit is the primary driver of phenology models. Most phenology models measure heat units as degree-days, which are calculated using daily minimum and maximum temperatures (T_{min} and T_{max}, respectively) and the lower (and sometimes upper) developmental threshold of a target species (Pruess, 1983; Wilson & Barnett, 1983). Degree-day accumulation is tracked over a daily time step to predict the timing of transitions to life stages and stage-specific events (e.g., first adult flight and first egg laying). The simplicity and ability of degree-day models to accommodate multiple species with varying life-histories have made them popular decision support tools for detecting and managing invasive pests (Coop & Barker, 2020; Crimmins et al., 2020).

A host of phenology modeling desktop software and web platforms have been developed to support decision-making related to preventing the establishment and spread of invasive pest species in the United States (reviewed in Coop & Barker, 2020). These offer users an opportunity to model the phenology of multiple species at single locations (site-based model) or across a certain area (spatialized model). Web-based phenological mapping platforms for pests include Michigan State University's Enviroweather (2021), uspest.org (2021), Oregon State University's **D**egree-**D**ays, **R**isk, and **P**henological Event Map-

Figure 6. Example degree-day lookup table map using the brown marmorated stink bug (Halyomorpha halys) model from the SAFARIS APHIS, PPQ Field Operations Weekly map series, The map uses a degree-day lookup table to associate cumulative degree-days with predicted life-stages present across CONUS on 5/18/2020. Thus, it provides a "snapshot in time" of phenology model predictions for a specific date and pest.



ping (DDRP) platform (Barker et al., 2020; DDRP, 2021), the USA National Phenology Network (Crimmins et al. 2017; USA-NPN, 2021), and the Spatial Analytic Framework for Advanced Risk Information Systems (SAFARIS, 2020), among others.

Phenological maps (i.e., a spatialized model) are typically of greater use to biosecurity practitioners than site-based models because surveillance activities occur across numerous localities. For example, the USDA, APHIS, CAPS program supports and oversees national and state surveys targeted at species of foreign and invasive plant pests. Maps that predict the timing of phenological events such as first spring flight are important for detection and monitoring program because they facilitate timely surveys and trap placement (Barker et al., 2020, Crimmins et al., 2020). These predictions are also relevant to eradication or management of established pests, because optimally timed operations (e.g., pesticide applications, sterile insect releases, and pheromone mating disruption) are more effective and cost-efficient.

Degree-day lookup table maps provide a "snapshot in time" of phenology model predictions for a specific date. When generated over multiple days or weeks, they provide a gradually changing view of the current or near-future status of pest phenology. For example, USA-NPN Pheno Forecast maps (USA-NPN, 2021) have versions that include 7-day National Digital Forecast Database (NDFD, 2021) forecasts to provide a 1-week "look ahead" prediction. Another example is the SAFARIS PestCAST (SAFARIS, 2020) maps that include a 1-month forecast that use the same 7-day NDFD forecast followed by three weeks of recent 20-year average PRISM data. As an example, Figure 6 shows a degree-day lookup table map for the brown marmorated stink bug (*Halyomorpha halys*) produced by SAFARIS for a weekly map series (FO Weekly). Predictions of where first adults are reproductively mature and active on any given date could help ensure that surveillance activities are optimally timed in the spring.

The workflow of generating a degree-day lookup table map involves using gridded T_{min} and T_{max} data from PRISM or other sources to calculate degree-day accumulations between a start date (usually January 1, although some models use other start dates) and a specified end date. Degree-day lookup tables are then used to associate degree-day accumulations with life stages, and output maps depict the results with color tables and legends. The simplicity of this approach and its applicability to multiple organisms has sustained its use for years.

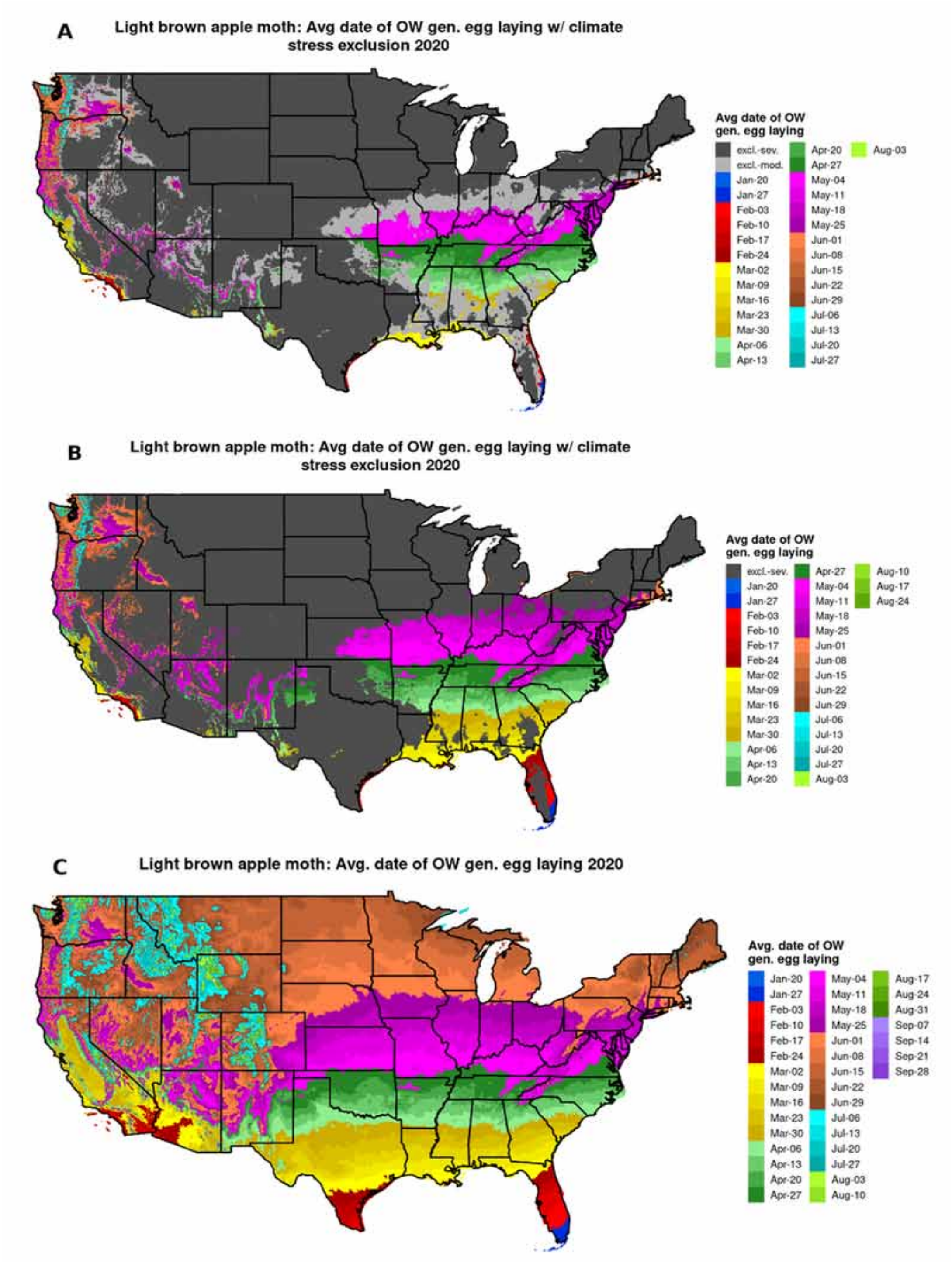
In contrast to a degree-day lookup table map, a phenological event map (or Pest Event Map; hereafter, PEM), depicts the *dates* on which accumulating degree-days reach a value (target degree-day total) that corresponds with a phenological event for a **poikilothermic** organism, an animal whose internal temperature varies considerably (Barker et al., 2020; Grevstad & Coop, 2015). As an example, Figure 7 shows PEMs produced by DDRP which depict the average oviposition date by the overwintering generation of light brown apple moth (*Epiphyas postvittana*) for 2020.

Pest Event Maps could be considered a more operational (tactical) product than degree-day lookup table maps because predictions may be generated weeks or months into the future. For example, a decision-maker may want to start planning their trap-setting operations several weeks before the estimated date of first spring flight. Additionally, dates are straightforward to interpret, which facilitates direct communication of operational support and the comparison of year-to-year variations of events.

Integrating Predictions of Where and When

Although degree-day models have been widely used for many years, they have not integrated information on risk of establishment and spread, which would offer answers to the questions of both where *and* when to survey for one or even multiple pests. Consequently, biosecurity practitioners would need to find or

*Figure 7. Example phenological event maps for light brown apple moth (Epiphyas postvittana) produced by DDRP for 2020. All three maps depict the predicted average oviposition date by the overwintering generation, but only maps (**A**) and (**B**) integrate predictions of climatic suitability. Map (**A**) depicts both moderate and severe climatic stress, whereas map (**B**) depicts severe stress only.*



develop two or more models to address each question separately. This situation, however, is changing. The DDRP platform, developed by researchers at Oregon State University, integrates mapping of phenology and climatic suitability to predict both where and when to expect insect pests (Barker et al., 2020).

DDRP's model outputs include gridded (raster) and graphical (map) predictions of the risk of establishment based on two levels of climate stress, number of generations, PEMs for up to four life stages and generations, and more traditional generation and stage maps (i.e., degree-day lookup table maps). Currently, the platform is being used to produce regularly updated (every three days) model outputs for CAPS Priority Pests, which are available at DDRP's homepage (DDRP, 2021). While DDRP is currently designed to model insects, its open-source code (link available at DDRP, 2021) could be modified to model other types of temperature-dependent pest organisms such as non-insect invertebrates, plants, plant-pathogenic bacteria and fungi, and insect plant and animal virus vectors.

The DDRP platform uses a process-based modeling approach in which degree-days and temperature stress are calculated daily and accumulate over time to model phenology and climatic suitability, respectively. Models are typically run using observed (recent or current) and future (forecast or recent average) temperature data to provide within-season decision support. Oregon State University has been using 4 km spatial resolution T_{min} and T_{max} from the PRISM database for real-time data, and either monthly-updated, daily-downscaled NMME (North American Multi-Model Ensemble) 7-month forecasts (Kirtman et al. 2014), or recent 10-year average PRISM daily data for forecast data.

Pest Event Maps for light brown apple moth (*Epiphyas postvittana*) produced by DDRP for 2020 illustrate the benefit of integrating predictions of phenology and climatic suitability (Figures 7A and 7B). If deployed as an operational product, a PEM that does not include climate stress exclusions (Figure 7C) could wrongly convince surveillance program directors to include areas that are not conducive to survival. For example, CAPS have conducted numerous surveys for light brown apple moth in Great Lakes and New England states in recent years but did not detect the species. This should be the case, as climate suitability models including DDRP have indicated, that establishment, and therefore detection, is highly unlikely because these areas are too cold for establishment. Using an integrated map as a standard product provides managers with knowledge of where to focus surveillance operations in the spring and could allow a significant amount of trapping personnel and resources to be re-directed to other species.

For an invasive species, areas that are not excluded by either moderate or severe temperature stress in DDRP (e.g., Figure 7A) are at highest risk of establishment because of increased population survival. Areas under moderate stress may represent temporary zones of establishment, and areas under severe stress do not allow for even short-term establishment. However, using two levels of stress may also provide a way to represent uncertainty for estimating the potential distribution. To potentially avoid under-predicting the risk of establishment, the potential distribution could be defined as areas not under severe climate stress as opposed to defining it using both stress levels (e.g., Figure 7B).

The focus of DDRP on real-time and forecasted risk of establishment at fine spatial scales (e.g., a single state or region) differs from programs like CLIMEX, which were designed to predict suitability based on coarse-scale (10′ and 30′ resolution) global climate data sets of averages of historical conditions, or on future projections from global circulation models. DDRP generates maps at a user-specified frequency, providing insight into how establishment risk changes over the growing season, and how extreme climate events such as a hard freeze may affect suitability. The climatic suitability map for the last day of the year (day 365) under investigation is usually of most interest because it provides insight into the potential distribution for an entire growing season.

Each model developed for DDRP, like all models, requires verification and testing, often referred to as validation. However, DDRP has primarily been used to model pests that are not (yet) present in CONUS (i.e., many of the CAPS Priority Pests), which hinders validating a model for this region. One exception is light brown apple moth, which has been established in California since at least 2006. Barker et al. (2020) used three population monitoring data sets for California to test the hypothesis that DDRP can correctly predict the timing of first spring egg laying and the generation length of the species. In general, they found support for this hypothesis, although there was variation in prediction error across years and regions. They also found that DDRP correctly predicted climatic suitability at most locality records for California.

DDRP models could be fitted and validated using climate data for the native or long-established range of a pest species. For example, Barker et al. (2020) used occurrence records and daily gridded T_{min} and T_{max} data (2005−2016) for Brazil to help fit a DDRP climatic suitability model for the small tomato borer (*Neoleucinodes elegantalis*) in that country. Similarly, the E-OBS daily climate dataset (Cornes et al., 2018) may be useful for fitting and validating DDRP climatic suitability models for pests in Europe. However, daily temperature data similar to PRISM are not currently known to be freely available for most countries. One solution to this issue is to use the CLIMEX program to help fit a DDRP climatic suitability model. Briefly, this involves running DDRP with historical PRISM T_{min} and T_{max} 30-year average data that matches the time-schedule of CLIMEX's climate data, and then fine-tuning DDRP parameters to maximize similarity between DDRP's and CLIMEX's predictions of temperature stress accumulation and the potential distribution in CONUS (Barker et al. 2020). Recently developed quasi-global daily temperature data sets such as CHIRTS-daily (Verdin et al., 2020) may also offer opportunities for fitting and validating DDRP models for species in any part of the world.

The use of temperature (T_{min} and T_{max}) as the sole environmental input in DDRP has sufficiently predicted the potential distribution for the insect species modeled thus far, as evidenced by validation analyses for two species (Barker et al., 2020). This finding suggests that using a relatively simple approach of temperature stress accumulations achieves a parsimonious balance between model simplicity and complexity. However, incorporating the effects of moisture stress into the climatic suitability model process would allow DDRP to model new categories of invasive pest threats including plant pathogens and weeds, and may increase model accuracy for moisture-sensitive insects.

Going forward, the development of climate data at higher temporal and spatial resolutions, both for the world and for CONUS, and the addition of non-temperature parameters to DDRP, should improve prediction reliability and, therefore, decision support. Some efforts have already been made in adding photoperiod-cued diapause parameters to DDRP, which has improved prediction accuracy for estimates of development and **voltinism** in three species of weed biological control insects (DDRP, 2020; Grevstad et al., in press). Modeling tools such as DDRP will be an important resource for guiding the design and implementation of surveillance operations for introduced pests, helping to achieve the goal of early detection.

Signs and Symptoms

When conducting detection surveillance, field observers must be trained to recognize the signs and symptoms that indicate a potential animal or plant health abnormality. However, the terminology of signs and symptoms differ depending whether one is referencing animal or plant disease.

Animal Disease

- A **sign** is a health issue that can be observed. It is an objective, observable phenomenon or evidence of disease that can be identified by others. A sign is objective. A veterinarian can usually diagnose a medical condition more easily if there are observable signs, e.g., a fever or a blister.
- A **symptom** is a health issue that is apparent only to the patient and cannot be observed by a veterinarian. It is a subjective experience that cannot be identified by anyone else but is a subjective description by the patient of a health issue. With animal disease, patients cannot describe the symptoms they are feeling, therefore diagnoses are made on observable signs.

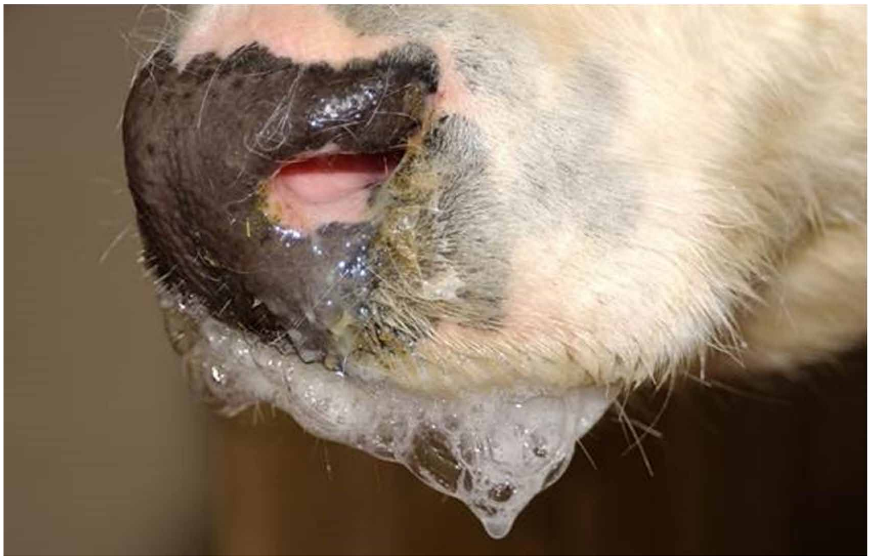
Plant Disease

- A **sign** is the physical evidence of the pathogen or pest. Fungal **fruiting bodies** are a sign of disease. Powdery mildew on a lilac leaf is the sign of the actual parasitic fungal disease organism. Bacterial canker of stone fruits causes gummosis, a bacterial **exudate** emerging from the **cankers**. The thick, liquid exudate is primarily composed of bacteria and is a sign of the disease, although the canker itself is composed of plant tissue and is a symptom. Signs are actual physical evidence of the occurrence of the pathogen in association with the unhealthy plant material.
- A **symptom** of plant disease is a visible effect of disease on the plant. Symptoms may include a detectable change in color, shape, or function of the plant as it responds to the pathogen. Leaf **wilt** and spots, with or without **necrosis** (the death of cells or tissue, usually accompanied by darkening to black or brown color) and **chlorosis** (the failure of chlorophyll development, caused by disease or a nutritional disturbance; fading of green plant color to light green, yellow, or white) are typical symptoms of plant pathogens causing disease. One does not actually see the cause of disease, but rather a result of the presence of the pathogen or pest.

Animal Pathogens

As stated previously, one of the first steps in surveillance of animal pathogens usually involves clinical observation and recognition of disease signs caused by the pathogen, followed by laboratory testing. In some cases, different diseases cannot be distinguished by the signs alone (Figure 8). The clinical signs of vesicular stomatitis infection primarily occur in cattle, horses, and pigs. Signs follow a typical viral incubation period of 3 to 7 days with an initial febrile period followed by **ptyalism** in cattle and horses (Bennett, 1986; Knight & Messer, 1983; Reif, 1994). Lesions of the oral mucosa include raised, blanched, and rarely fluid filled vesicles. The dorsal lingual surface often is affected but the gingival surfaces, palate and mucocutaneous junctions may also exhibit lesions (Reif, 1994). Vesicles are very short-lived and rupture leaving ulcerations and erosions. Lesions often coalesce to form large, denuded areas of oral mucosa with the presence of epithelial tags. Vesicular and/or ulcerative lesions outside of the oral mucosa occur on the snout of pigs, teats of cattle and coronary bands of pigs, cattle, and horses. Teat lesions are not as common as oral lesions but in cattle may be associated with severe mastitis. A similar pattern of clinical signs is seen in vesicular stomatitis, **Foot-and-Mouth Disease**, contagious **ecthyma**, poxvirus and pseudocowpox, and only laboratory testing can definitively discriminate one of these diseases from the other.

Figure 8. A wide range of important animal diseases are the so-called "vesicular" diseases, including vesicular stomatitis and foot-and-mouth disease (FMD). Excessive salivation followed by the appearance of blister-like lesions is seen in vesicular stomatitis, FMD, contagious ecthyma, poxvirus, and pseudocowpox. Only laboratory testing can definitively discriminate one of these diseases from the other.
Source: Photo source: United States Department of Agriculture



It is critically important to test any animal with clinical signs quickly to identify which pathogen is causing illness. Veterinarians and livestock owners who suspect an animal may have vesicular stomatitis or any other vesicular disease should immediately contact State or Federal animal health authorities. Diagnosis of the disease cannot be made based on clinical signs. Testing of samples at a facility approved by the U.S. Department of Agriculture's National Veterinary Services Laboratories (APHIS, 2021b) may be required. In either case, having the right diagnostic assays, be it isolation and culture, biochemical assays, immunoassays, or various molecular assays, is critical to the successful outcome of pathogen surveillance. Diagnostic methodologies and assays are covered in detail in another chapter of this book, so we leave it to the reader to fully invest themselves of this topic because it is so critical to a successful outcome.

Plant Pathogens

Surveillance of plant pathogens mainly involves visual observation and recognition of disease symptoms caused by the pathogen. Symptoms can include **blight**, **blotch,** canker, chlorosis, **defoliation**, **dieback**, **exudate**, **flagging**, **gall**, **leaf spot**, **lesion**, **mosaic**, **necrosis**, **ringspot**, **scorch**, **shot hole**, **stunting**, wilt, and others as well. Often, two or more symptoms may be present on a plant or population of plants. For example, symptoms of the **select agent** *Ralstonia solanacearum* race 3 biovar 2 on geraniums include wilting, chlorosis, and necrosis (Figure 9). Signs of a pathogen also may be visible, and can include **mildew**, **mycelium**, **sclerotia**, **spores**, **pustules**, and other fruiting bodies of the pathogen. Taken together, these can be diagnostic, or at least point the diagnostician in the right direction. However, as with animal disease, diagnostic testing is needed to correctly identify the pathogen causing the disease. For instance,

Figure 9. Advance wilting and abnormal leaf yellowing symptoms (chlorosis) caused by the select agent Ralstonia solanacearum race 3 biovar 2 on zonal geraniums.
Source: The Wisconsin Department of Agriculture, Trade and Consumer Protection

other races of *R. solanacearum* may cause similar symptoms, but only race 3 biovar 2 is categorized as a select agent, so it is important to get the diagnostics right and not rely completely on symptoms.

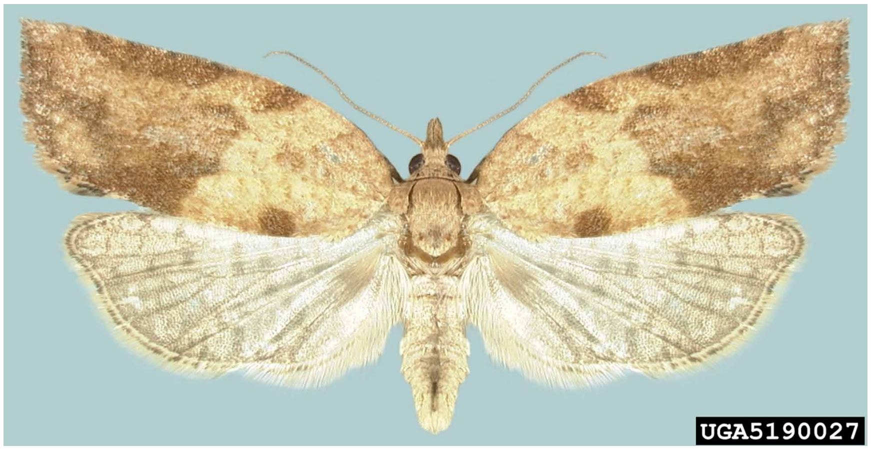
Pathogen Vectors

An important aspect in the surveillance of certain plant and animal pathogens is that some pathogens are vectored primarily by insects and other organisms. The surveillance program needs to be cognizant of this fact when planning for surveillance and may need to adjust resources to focus on the vector (Ferguson et al., 2014; Mastin et al., 2017; Mastin et al., 2021b). Ambrosia beetles vector several major plant pathogenic fungi that cause major diseases in tree species. The classic example in the United States is the spread of Dutch elm disease caused by the fungus *Ophiostoma ulmi* and vectored by three species of elm bark beetles. More recent are the emergence of Laurel wilt on redbay and avocado caused by the fungus *Raffaelea lauricola* and vectored by the invasive redbay ambrosia beetle *Xyleborus glabratus*, thousand cankers disease on walnut caused by the fungus *Geosmithia morbida* and vectored by the walnut twig beetle *Pityophthorus juglandis*, and Japanese oak wilt caused by the fungus *Raffaelea quercivora* and vectored by the oak ambrosia beetle *Platypus quercivora*, among others. A major disease threatening *Citrus* spp. is Citrus greening or Huanglongbing (HLB). This disease is caused by the bacterium *Candidatus* Liberibacter asiaticus and is vectored by the Asian citrus psyllid *Diaphorina citri*. Lethal yellowing of palms is caused by the phytoplasma *Candidatus* Phytoplasma palmae and is vectored by the planthopper *Haplaxius crudus*. Potyvirus plum pox virus is transmitted to stone fruits by about 20 different species of aphids. Many different genera of viruses are transmitted to a wide range of hosts by aphids, leafhoppers, planthoppers, whiteflies, thrips, and mites. Similarly, insect vectors are critical in consideration of animal disease introduction and spread. Blackflies, sand flies, and biting midges are the primary vector species transmitting Vesicular Stomatitis viruses. These relationships complicate the surveillance effort, but they must not be forgotten lest the surveillance results be compromised.

Insect Pests

Surveillance of insect pests and vectors create other unique challenges. Insects can be extremely mobile and fly long distances, e.g., spruce budworm (*Choristoneura fumiferana*) adults can easily disperse 20 km (Greenbank et al., 1980), or they can be more sedentary and move about slowly. Human assisted movement can be a critical factor in the dissemination of insects, e.g., European gypsy moth (*Lymantria dispar dispar*) and spotted lanternfly (*Lycorma delicatula)* or movement can be through fresh produce, soil, nursery stock, or green waste and conveyances, e.g., light brown apple moth (Figure 10). Other than a robust quarantine and inspection program, placing specific traps, with or without chemical attractant lures, is the most common methodology for insect surveillance. This takes advantage of the insect's mobility to go to the trap on its own accord if the right combination of trap, trap color, and chemical attractant is known. The challenge then is to bring together all these variables in a trap design coupled with the chemical attractants to employ an effective and efficient surveillance tool that is not cost prohibitive depending on the scope of the planned surveillance. The development of an effective and efficient trap and lure combination involves the bringing together the sciences of insect ecology, behavior, and chemistry.

Figure 10. Epiphyas postvittana, Light brown apple moth
Source: Photo by Natasha Wright, Braman Termite & Pest Elimination, Bugwood.org https://www.invasive.org/browse/detail.cfm?imgnum=5190026



Providing that adequate resources are available to get started, research to develop an attractant is needed. Collaboration with researchers in locations of the pest's origins now needs to be established and certified and permitted containment facilities need to be established. Once funding, research collaborations, a containment facility, and permits are in place, initial work can begin to collect insect and host material in both the native and local invasive range. Work can begin establishing effective rearing protocols involving host plant materials, artificial diets, and the correct environmental conditions. A healthy population of the target species is critical to the work that is yet to come.

Learning how the target species behaves in response to stimuli gives valuable insights into how it finds a host or a mate in the environment. The collection of volatiles emitted by various host plants, or different parts of plants, is an important first step to study the role of the host plant in the behavior of the target species. One of the most often used behavioral bioassays in chemical ecology research to study how arthropods locate their hosts involve the use of a device called a Y-tube **olfactometer** (Calatayud et al., 2014). This bioassay tests possible sensory stimulation responses with the observation of how the target species responds to the stimulation. It can be used to determine the attractiveness of plant volatiles to the target species, to pheromones from its own species, and/or **kairomone**s from other species. Wind, or flight tunnels also have been used to test the response of the target species to host plants and other stimuli (Cardé & Hagaman, 1979). These bioassays are important to determine specific compounds that the target species responds to for incorporation into a potential lure.

The collected natural volatiles and extracts are made of numerous compounds. To determine the compounds that make up a promising volatile or extract, an analytical method known as **gas chromatography–mass spectrometry (GC-MS)** is employed (Millar & Haynes, 2012). A GC-MS chemical analysis can identify different substances within the active insect and plant compounds. A comparison can be made between the composition of male and female collected volatiles, as well as among those of various hosts. Purification and/or synthesis of compounds of interest can then be conducted using the appropriate chemical methodology.

The antennae on insects are important sensory appendages. On the surface of the antenna, there are morphologically and functionally different types of **sensilla** that are sensitive to various chemical and physical stimuli. For instance, insects oscillate their antennae vigorously when they detect attractive odors such as sex pheromones. Behavioral studies are then conducted to determine what purified compounds cause a reaction of the insect's antennae using observation, electrodes, or other technology (Millar & Haynes, 2012). This research further narrows down the list of potential candidates that may be incorporated into a potential lure.

A short list of antennally-active compounds is further analyzed using GC-MS, **nuclear magnetic resonance** (NMR), and microchemical reactions. Nuclear magnetic resonance is used to study the physical, chemical, and biological properties of matter. For our purpose, it is used in combination with chemical tests to determine molecular identity and structure of the antennally-active materials. This knowledge will point to the chemical pathway for the synthesis of the active material if it is not commercially available. Behavioral studies are once again conducted to determine the efficacy of the synthetic attractants. At this stage, studies on the synergism of several compounds are tested along with various ratios of the attractants.

At the end of this research, one should have a good idea of what compounds and materials, and at what concentrations, will attract the target species and be suitable for lure development and field testing. For example, the lure for summer fruit tortrix moth (*Adoxophyes orana*) contains four **semiochemicals**: (*Z*)-9-Tetradecenyl acetate (Z9 -14: Ac), (*Z*)-11-Tetradecenyl acetate (Z11-14: Ac), (*Z*)-9-Tetradecen-1-ol (Z9-14: OH), and (*Z*)-11-Tetradecen-1-ol (Z11-14: OH) (CAPS, 2021a). Other species may require multiple lures. A semiochemical is a chemical compound that is released by an organism that influences the behavior or physiology of the same or different species. Therefore, the purpose of this line of research is to find a chemical, or set of chemicals, that when placed in a trap will attract the target species to the trap, and thus be caught. The more specifically efficacious the lure, the better the results will be.

The next step in the process is to determine the formulation of the lure. One needs to determine what is to be added to the lure mixture to maximize stability, shelf life, and field life, and under what conditions the lure mixture will remain efficacious (Nielsen et al., 2019). The choice of a release matrix also will need to be considered. This is the physical structure that will hold the lure formulation for storage, shipping, and attachment to a trap. For the *Adoxophyes orana* lure, the dispenser or container is a rubber septum (Figure 11). Other examples of dispensers are termed polysleeves or bubble caps, all with semipermeable membranes that allow the volatiles to escape. There are others as well. While the rubber septum is the most common, the choice of dispenser may be determined by the chemical properties of the lure formulation. Studies will then be conducted to evaluate the rate of release of the volatiles to determine if the concentration of the released volatile and how the concentration of the released volatile degrades over time. This is important information that will determine how long a lure remains viable, and when it needs to be replaced.

Figure 11. Rubber septa used to hold lure formulations
Source: United States Department of Agriculture

Experimental trap design is the next step in the process. Shape, size, and color all are part of the overall effectiveness of the development program. Knowledge about the flight pattern of the target species, what shapes it is attracted to, and the ability of the species to escape the trap are all important considerations in effective trap design. With the advent of 3D printers and the correct software, different aspects of a design can be tested and modified in a relatively short period of time. Perhaps the most important aspect of trap design is the color or color combination. For some species, it makes no difference, but for others it makes a large difference. Behavioral studies can be conducted to determine a response to different colors, or shades of a color. These can be conducted in flight tunnels. **Electroretinogram** studies also can be conducted where electrodes are inserted into the insect's retina, and an electrical response can be measured in response to different color stimuli. For example, with emerald ash borer (EAB, *Agrilus planipennis*), a purple-colored trap is more efficacious when placed on the trunk of an ash tree, whereas a light-green colored trap is better suited when trapping for EAB in the tree canopy (Crook et al., 2009).

Once potential trap and lure formulations have been identified, field testing needs to take place in the target species' native range, to determine if it will attract the target species. The collaborators identified previously will be an enormous help in setting up, monitoring, and collecting results. Analysis of the results may lead to modifications to the trap design and/or reformulation of the lure, and further field tests to address the modifications. This will be an iterative process until an effective trap design and lure formulation are determined for the target species.

The last step before a trap and lure can be deployed in a surveillance program is the procurement of the trap and lure. If a trap is not already commercially available, then a Statement of Work (SOW) will need to be developed with the exact description and dimensions of the trap for a manufacturer to accurately produce the traps in the quantity needed. Whether this needs to be put out for bid, or singularly contracted, will be determined by the organization developing the trap and/or conducting the surveillance. Likewise, for the lure. In many instances in USDA, APHIS, the semiochemicals are purchased separately and formulated inhouse to specifications, and then added to the release matrix or dispenser. In other instances, the semiochemicals may not be readily available from commercial sources. A contract or other form of agreement may need to be developed with a researcher or company to synthesize and deliver the ingredients or the final lure to the desired specifications. The contract will specify whether the complete, packaged lure in the dispenser will be the product delivered, or just the chemical lure to be placed in the dispenser either inhouse or elsewhere.

The Importance of Standard Methodology

The documentation of negative data in a surveillance program is extremely important. While a positive detection often comes from outside an ongoing surveillance program and from various sources using different methodologies, the implementation of standard and scientifically based survey methodology is the cornerstone of valid negative data. Negative, or absence, data from surveillance strengthens and facilitates trade and the safeguarding of agriculture and the environment. Therefore, all surveillance programs should strive to ensure that all negative data is valid and consistent. This is the end result of putting the necessary effort into determining what pests and pathogens to target, determining what pests and pathogens are of primary importance, where and when to conduct surveillance activities for high priority pests and pathogens, and to develop standard surveillance methodology so that surveillance results are directly comparable across state, regions, and countries. It is then vitally important that all these facets of the surveillance program be properly documented so that agricultural producers and trading partners accept the surveillance results.

To ensure consistency and standardization across the country, the CAPS program in the United States developed the Approved Methods for Pest Surveillance (CAPS, 2021a). These methods, including specific trap and lure combinations and diagnostic tests, are the only survey methodology accepted by the CAPS program to report valid negative, or absence, results. As with any surveillance program designed to survey for pest and pathogens not known to be present, the CAPS program strives to ensure that all negative data is valid and scientifically sound (ISPM 6, 2019). Likewise, the NAHLN has established and implemented standards to ensure confidence in diagnostic test results, both domestically and internationally, including quality management systems, standardized testing, training of personnel, assurances of secure communication, and preparedness (APHIS, 2021j).

Where and How to Store and Maintain the Data

The final question to ask when establishing or reviewing a surveillance program is where or how to store the resulting survey results and other information. While the topic is at the end of this chapter and is the final destination or repository for the data, it likely should be one of the first questions asked of the surveillance program, and not be an afterthought. Determining what data and information to collect, what is needed for further analyses, and how to present and share the information are all critically important. Applicable privacy laws that include and restrict sharing of Personal Identifiable Information (PII) and organizational data sharing policies need to be considered as well. The data management initiative should be developed in parallel with the surveillance aspects of the program and should complement and support the surveillance.

As an example, in the United States the CAPS surveillance program was established in the early 1980s. By the mid-1980s, an early version of National Agricultural Pest Information System database (NAPIS, 2021) was developed at Purdue University. The NAPIS database now houses over 5.44 million summarized survey result records for 6,394 insects, pathogens, weeds, mollusks, and biological control organisms. Access to the NAPIS database is controlled and role-based to preserve the integrity of the stored information. Rules and approval processes have been developed to prevent changing the data but allowing corrections to be made to ensure that the information is valid. One of the most important system rules implemented validates negative data entry against the approved surveillance methods for each pest prioritized for survey (CAPS, 2020, 2021a). Negative data not conforming to the approved method is not accepted into the database (confirmed positive data can be entered from any source or methodology). The NAPIS database validates the incoming information in that it not only checks that the required fields contain the correct type of information, but that the required fields contain the correct information. In this manner, the approved surveillance methods are enforced, resulting in validated negative data. The data collection, maintenance, and storage systems developed over time along with the surveillance program to meet the program's needs.

Sharing the Results of Surveillance

Sharing information and data outside of one's organization can be problematic based on local and organizational privacy laws, regulations, and rules, and the data sharing policies of the organization. Memoranda of understanding that spell out data sharing policies may need to be signed between and among cooperators and outside organizations where each side agrees to protect the information. Yet, the sharing of information and data does need to occur to promote cooperation and facilitate trade. Cooperators and trading partners will need to review and evaluate the data. Therefore, it is necessary to have valid data backed by scientifically sound surveillance methodology, and to have it available for review.

Following the example from above, summarized results from the NAPIS database are shared with cooperators, trading partners, and the public through the Pest Tracker (2021) website. The Pest Tracker website contains survey results summarized at the county level for over 140 pests and pathogens. The website contains pest and pathogen information, county-level survey maps for the last 10 years for most pests, state-specific information to include planned surveys for the current year, and contact information, as well as outreach material and videos. The Pest Tracker website is the forward-facing outreach component of the United States surveillance program and NAPIS database. In 2020, there were 7,437 sessions of non-United States users with 11,260 page visits to the Pest Tracker website (personal communication). The Pest Tracker website fulfills the United States' obligation to make survey data available under the IPPC (2021) and ISPM 6 (2019).

The National Plant Diagnostic Network (NPDN) collects all diagnostic data from across the United States and stores it in a National Repository (Stack et al., 2006). Diagnostic laboratories in every state receive sample from client growers and state surveillance authorities. The diagnostic data feed into a centralized data system. The first time a pest or pathogen name comes up in a state, an alert is automatically sent to an APHIS PPQ identifier, adding a layer of passive surveillance to the active surveillance of the CAPS program and the NAPIS database.

On the animal side, the NAHSS system (APHIS, 2020e) gathers data from a veterinary diagnostic laboratory network, including the NAHLN laboratories (APHIS, 2020c), stores and manages the data, and provides analyses to decision makers. NAHSS includes passive and active surveillance for reportable diseases, including those reported to the World Organization for Animal Health (OIE) as well as those diseases covered by federally funded surveillance programs. In addition, the surveillance system includes monitoring for diseases that States are not mandated to report. In all cases, this data gathering involves survey methodology to detect, delimit, and monitor for specific threats. Part of NAHSS is the National Animal Health Reporting System (NAHRS) (APHIS, 2020d) which gathers data from participating State animal health officials on the presence of the National List of Reportable Animal Diseases in the United States (APHIS, 2020f). The United States meets its OIE reporting obligations using a variety of sources including NAHRS reporting, CFSPH reporting, foreign animal disease reports, national program disease surveillance reports, and others. Zoonotic disease affecting humans as well as other animal species are also tracked by the U.S. Centers for Disease Control (CDC, 2021). These reports are available through OIE (OIE, 2021).

CONCLUSION

The focus in an early detection surveillance program is to determine if a pest is present or absent in order to facilitate market access, trade and the movement of crops, forest products, animals, animal products, and other commodities and goods within a country or internationally. This chapter has summarized various components, topics, and issues that are part of an early detection surveillance program and has focused on two broad categories of surveillance: general or passive, and specific or active surveillance. These are broadly defined as gathering information from all sources and surveys targeting specific pests and pathogens, respectively. Within these two approaches to surveillance, there are multiple variations on the themes. Table 1 lists several of these different variations. Developing and deploying any of these approaches to surveillance will depend on the goals, resources, funding, and other variables available to the surveillance program. The authors suggest the reader to investigate these various approaches to understand the breadth of surveillance options that have been developed. The authors then challenge to reader to expand on what was presented and to develop surveillance programs that are more effective and efficient in achieving the overall goal of detecting and identifying foreign or exotic pests and pathogens once they pass your borders.

CASE STUDY: VESICULAR STOMATITIS (VS)

vesicular stomatitis must be considered one of the most enigmatic animal diseases of the western hemisphere and provides a particularly salient example of the influence of the environment on the introduction and subsequent expansion of disease into new areas. vesicular stomatitis viruses (VSV) are members of the family Rhabdoviridae which include viruses that infect vertebrates, invertebrates and many plant species (Wagner & Rose, 1996). Although there are many members of the *Vesiculovirus* genus, two are of particular interest in the United States, vesicular stomatitis virus - New Jersey serotype (VSV-NJ) and vesicular stomatitis virus – Indiana serotype (VSV-IN). Timely detection of disease emergence requires access to target populations, a network of laboratories to diagnose and differentiate disease, and training and awareness programs for individuals involved in handling animals and animal products for identification and reporting. Once there is a report, a timely response and investigation of suspected cases to confirm and to acquire accurate knowledge of the situation for further action is essential.

vesicular stomatitis is a disease of the western hemisphere with areas throughout South America, Central America and Mexico considered endemic. A review of vesicular stomatitis in Mexico showed that cases occurred in every year between 1981 and 1995 with both serotypes identified in most years. This review also indicated that vesicular stomatitis has a national distribution in Mexico although most cases occur in the southern states of Chiapas, Tabasco, and Veracruz. The central area of Mexico also is considered endemic although at a lower level and the northern area of Mexico is more similar to the southwestern United States with sporadic cases occurring. Outbreaks of vesicular stomatitis in the U.S. are sporadic and although once proposed to occur in a 10-year cycle, have occurred in a total of 6 years between 2010 and 2020. Outbreaks in the U.S. can have significant impacts on trade and due to the inability to distinguish vesicular stomatitis from Foot and Mouth Disease in pigs and cattle, requires extensive monitoring and investigation of positive premises by animal health regulatory agencies.

vesicular stomatitis viruses are classified as arboviruses with blackflies (Simuliidae), sand flies (Lutzomyia), and biting midges (Culicoides) as the primary vector species. Flight ranges of these insect vectors vary but none would be adequate to explain the often-large distances observed between either individual or clusters of infected premises. Backward trajectories of winds were examined for vesicular stomatitis outbreaks in 1982 and 1985 (Sellers & Maarouf, 1990). Findings from the trajectory analysis suggest the feasibility of infected insects being transported for long distances on wind currents and subsequently landing on non-infected premises many miles from the infected premises or cluster of premises. vesicular stomatitis virus is just one example, numerous pathogens exhibit lifestyles involving multiple animal hosts and insect or arthropod vectors adding significant complexity to surveillance.

With the primary mechanism of transmission of VSV by insects and arthropods, the influence of the environment could be expected. Observational, landscape scale studies conducted during outbreaks or retrospectively have identified environmental factors associated with livestock premises housing infected animals including distance of animals to various water body types, access to pasture, precipitation, and others (Hurd et al., 1999; McCluskey et al., 2003). For example, Elias et al. (2018) investigated the contributions of hydrological factors on the emergence of VSV in the western United States. Their results indicated that vesicular stomatitis positive premises were detected near stream networks. Overall, 72% of positive premises were located within 1 km of rapidly moving freshwater habitats and all index cases occurred after peak annual streamflow, with 89% occurring after streams returned to baseflow. This finding supports developing early warning surveillance for vesicular stomatitis through stream and streamflow monitoring in locally relevant geographic areas. Knowledge of the timing of streams return to baseline flows can be used to increase communications to livestock owners about insect control on individual animals and in the livestock housing environment.

Peters et al. (2020), using spatial distribution models based on human-guided machine learning, and constructed using geo-referenced harmonized maps, investigated environmental and epidemiological variables that described the relationship between vesicular stomatitis occurrence and the environment. They examined two events that were temporally and phylogenetically distinct, the first a disease incursion year (2004) and the second a disease expansion year (2005). They then tested the identified relationships identified in additional incursion and expansion years, 2014 and 2015 respectively, at a local and landscape/regional scale. Emblematic disease ecology drivers were identified (climate, drought, hydrology, land surface properties, pedology, topography) and within each driver, variables expected to influence the disease events were selected. The results presented in this study were extensive and according to the authors "yield new insight into factors governing the spatial patterns in vesicular stomatitis occurrence." Greater than 470 variables within the identified drivers were investigated with hydrology, vegetation, and climate important drivers in both 2004 and 2005, elevation important in 2004, and soil properties important in 2005. Veterinarians and regulatory officials can use these results to develop early warning strategies and enhanced communications with livestock owners about biosecurity measures and mitigations.

CASE STUDY: SOYBEAN RUST (SBR)

Detection

Soybean rust (SBR) is caused by the fungus *Phakopsora pachyrhizi* Sydow. The disease is spread by **urediniospores** transported long distances by winds (Miles et al., 2003). If they are protected from ultraviolet radiation by cloud cover, the spores may remain viable in the air for many days (Isard et al., 2006a). Soybean rust causes moderate to severe soybean (*Glycine max* Merrill) yield loss wherever established (Sikora et al., 2014). The fungus produces foliar lesions on soybean, kudzu, and other legume hosts, reducing photosynthetic area and potentially causing premature defoliation. Severe yield losses can result if defoliation occurs during the mid-reproductive growth stages of soybean (Bromfield, 1984).

Soybean rust was first reported in Japan in 1902 and was confirmed throughout much of southeast Asia by 1934 (Bromfield & Hartwig, 1980). In the early 1990s, it was found in Africa (Pretorius et al., 2001). The disease was detected in Paraguay in 2001 (Morel et al., 2004) and spread throughout South America over the next 3 years. During this period, the USDA began preparing for a U.S. incursion of SBR by supporting education, training, and surveillance programs, enhancing diagnostic facilities, backing offshore fungicide evaluation trials, funding risk assessments, and searching breeding materials for novel sources of host plant resistance (Livingston et al., 2004). The sense of urgency stemmed not only because *P. pachyrhizi* had demonstrated aerial spread among other major soybean growing areas, but also because soybean is produced on about 30 million hectares annually in the U.S., with a value between $18 and 32 billion (NASS, n.d.).

In 2003, research groups began developing models to assess the risk and pathways of *P. pachyrhizi* incursions into the United States. An aerobiology process model (Integrated Aerobiology Modeling System (IAMS)) was specified for the SBR system to simulate spore production, escape of spores from the crop canopy, turbulent transport and dilution in the atmosphere, survival of spores while airborne, deposition of spores into a soybean crop, and colonization of a soybean crop (Isard et al., 2005). Field research was conducted during January and February 2004 in Paraguay to parameterize spore production, escape, and survival stages (Isard et al., 2006a). Historical meteorological data (1999-2004) were then used to simulate daily spore movement from the infected soybean production region in southern Brazil and Paraguay. The IAMS model output revealed that aerial movement and deposition of viable spores in the U.S. was not likely to occur from this region. However, simulations indicated that the pathogen was likely to spread into areas north of the equator in South America. Model runs were also conducted for days between July and September in the historical dataset for soybean production areas in Venezuela, Roraima State in Brazil, Guyana, and Suriname assuming that soybean rust infestations were present in these regions. The output from these simulations revealed that deposition of viable spores would occur primarily in Central America and that the deposition zone shifted further north with the **Intertropical Convergence Zone** as summer progressed, extending as far north as southern and eastern Mexico. There were two tropical weather systems in the five-year record that resulted in direct transport to and deposition of viable spores in the southeastern United States.

The IAMS model was then configured to run on real-time and forecast meteorological data for days during the 2004 tropical cyclone season in the Caribbean basin. In July 2004, an advanced SBR infestation was found in central Columbia (Isard et al., 2005). The IAMS simulations indicated that transport and deposition of viable SBR spores from northern South America to the U.S. could only have occurred in association with one tropical cyclone that season, Hurricane Ivan. A second aerobiological transport model using short-term climate model output also forecast the possible incursion of SBR spores into North America from the Columbia source region in association with Hurricane Ivan (Stokstad 2004). Output from the IAMS model became the basis for the USDA Economic Research Service assessment of the risk of a SBR incursion and its potential impact on U.S. agriculture (Livingston et al., 2004).

In autumn 2004, SBR was found infesting soybean fields in Louisiana (Schneider et al., 2005). The IAMS simulations conducted the evening of the discovery showed that airflows converging into Hurricane Ivan as it made landfall along the Gulf Coast had likely transported viable rust spores to the southeast United States from an infected source area in Columbia. The model output indicated that spores from the Rio Cauca source area released on September 7-9 remained viable during transport and were deposited in rain between September 15-18 in the southeastern United States. A model generated map delineating regions of spore deposition associated with the hurricane was provided to the USDA APHIS Soybean Rust Rapid Response Team and was used successfully to scout for the pathogen. *Phakopsora pachyrhizi* was confirmed by polymerase chain reaction (PCR) methodology (Frederick et al., 2002) at numerous locations in nine states in the Mississippi River valley and southeast United States within three weeks of the initial discovery and was identified but not confirmed at many other locations in the same region. Most of the positive observations corresponded to the area of spore deposition predicted by IAMS output (see Figure 5 in Isard et al., 2005). Subsequent scouting along the Gulf of Mexico revealed that soybean rust was overwintering on kudzu.

Monitoring to Determine if Establishment Occurred

During the 2004/2005 winter, the USDA implemented a coordinated framework for providing soybean growers with decision support for managing SBR including surveillance, reporting, prediction, management, and outreach components (Isard et al., 2006b). The USDA APHIS and Risk Management Agency (RMA) funded SBR monitoring allowing real-time reporting and mapping of the disease, and the development of predictive models of disease spread. APHIS and the Cooperative State Research, Education, and Extension Service (CSREES, now the National Institute of Food and Agriculture (NIFA)) provided funds to the IAMS team for the construction and operation of an Information Technology platform (Soybean Rust Information System; later named the Integrated Pest Management Pest Information Platform for Extension and Education (ipmPIPE)) to integrate soybean rust monitoring, database construction, aerobiological modeling and communications to stakeholders (Isard *et al.*, 2006b). The framework received unprecedented support from growers, national and state soybean commodity associations, agricultural businesses, and USDA agencies.

In spring 2005, soybean "sentinel" plots were established in North America to monitor for the disease (Giesler & Hershman, 2007). **Sentinel plots** were planted earlier than local commercial soybeans to provide early warning of disease and utilized a variety of soybean maturity groups to prolong monitoring throughout the season. Observations from sentinel plots were supplemented by "mobile scouting" in commercial soybean fields and neighboring kudzu sites. Mobile scouting became the predominant surveillance choice at northern latitudes once it was determined that SBR rarely occurred in the continental interior of North America.

Disease observation data were collected with a standard protocol and uploaded into the ipmPIPE database. High fungicide efficacy requires applications before the SBR reaches 5% **incidence** in the lower-canopy foliage (Dorrance et al., 2008), and thus timely communication is paramount for successful disease control. The primary use of the observation data was to populate a map on the ipmPIPE public website indicating presence of SBR in counties linked to state-specific commentary on risk and disease management. A secondary use of the data was to parameterize daily predictive models for the aerial transport and deposition of SBR which aided mobile scouting and recommendations of fungicide applications for soybean farmers.

SBR was monitored on a weekly basis during the growing season throughout the soybean growing region in the decade following its incursion into North America. Thirty-five states and five Canadian provinces established soybean sentinel plots in 2005. The number of sentinel plots in North America peaked at 984 in 2008 and expanded to include plots in soybean production areas in Mexico. By 2012, the number of plots had declined to 285 (Sikora et al., 2014), and continued to decline thereafter. Although monitoring for soybean rust continues in a few southern states today, the nationwide network and information system is no longer functional. This decline primarily occurred because SBR did not become a significant problem in the continental interior of North America. Ten years after its incursion, soybean rust had been reported in 20 states and Ontario, Canada (Sikora et al., 2014).

Due in large part to the sentinel plot system, management of SBR in North America has been highly effective. Observations of SBR in sentinel plots in southern states during the early growing season trigger timely monitoring and targeted fungicide applications in local commercial fields controlling the disease until pod set in late summer. These applications keep inoculum levels low in the southern United States. Thus, throughout most of the growing season, even when weather is conducive to northward transport of spores, the risk of soybean yield loss in the large continental interior production region of the United States and Canada is minor (Isard et al., 2011). Over time it was determined that in the south, fungicide applications were no longer necessary to protect yield once soybeans reach the late bean fill stage. However, because the pathogen often thrives and spreads aggressively in commercial fields in the late fill stages, soybean rust has been observed in the northern growing region late in the growing season (Isard et al., 2011). By late August and September when this movement occurs, most soybean plants within the continental interior are in their late stages of development and yields are no longer reduced by the disease. In short, the soybean rust sentinel plot network coupled to the ipmPIPE information delivery system enabled control of soybean rust in North America. Timely control practices in southern soybean fields suppress buildup of inoculum and delays subsequent spore movement northward, protecting the substantial acreage of soybean produced in the North American continental interior.

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KEY TERMS AND DEFINITIONS

Ambrosia Beetles: Beetles of the weevil subfamilies Scolytinae and Platypodinae (Coleoptera, Curculionidae), which live in nutritional symbiosis with fungi. The beetles excavate tunnels in dead or stressed trees in which they cultivate fungal gardens, their sole source of nutrition.

Blight: A sudden, severe, and extensive spotting, discoloration, wilting, or destruction of leaves, flowers, stems, or entire plants.

Blotch: Dead areas of tissue on the foliage, irregular in shape, and larger than leaf spots.

Canker: A plant disease characterized (in woody plants) by the death of cambium tissue and loss and/or malformation of bark, or (in non-woody plants) by the formation of sharply delineated, dry, necrotic, localized lesions on the stem.

Chlorosis: The yellowing or whitening of green plant parts as a result of chlorophyll breakdown or production failure.

Climate Normals: A 30-year average of a weather variable for a given time of year.

Defoliation: The loss of leaves from a plant, whether normal or premature.

Delimiting Survey: Surveillance conducted to determine the boundaries over an area where a pest has been detected or considered to be established or invasive.

Detection Survey: Surveillance conducted in an area over a specified period of time to determine if a pest is present or absent.

Diapause: A physiological condition or state of restrained development and reduced metabolic activity which cannot be directly attributed to unfavorable environmental conditions. Visual consequences of diapause in postembryonic stages includes lowed or suspended growth, differentiation, metamorphosis, or reproduction.

Dieback: The progressive death of shoots, leaves, or roots, beginning at the tips.

Ebola: Ebola virus disease (EVD), formerly known as Ebola haemorrhagic fever, is a rare but severe, often fatal illness in humans. The virus is transmitted to people from wild animals and spreads in the human population through human-to-human transmission.

Ecthyma: A skin infection characterized by crusted sores beneath which ulcers form.

Electroretinogram: A diagnostic test that measures the electrical activity of the retina in response to a light stimulus; used to measure photoreceptor responses in insect retinas to different wavelengths of visual stimuli so they can identify the most attractive colors, patterns, and intensities.

Endemic: Referring to a disease or condition regularly found among a particular people or in a certain area.

Epizootics: Widespread, rapid occurrence of a disease affecting many individuals or a large proportion of an animal population at same time.

Exudate: A liquid excreted or discharged from diseased tissues.

Fecundity: The ability to produce an abundance of offspring or new growth.

Flagging: An isolated, wilted, or necrotic branch with dead leaves attached.

Foot-and-Mouth Disease (FMD) or Hoof-and-Mouth Disease (HMD): An infectious, and sometimes fatal, viral disease that affects cloven-hoofed animals, including domestic and wild cattle, buffalo, sheep, goats, and swine. FMD has severe implications for agricultural trade and farming and ranching, since it is highly infectious and easily spread through contact with contaminated equipment, vehicles, clothing, and feed, and by domestic and wild predators. Its containment requires considerable efforts in vaccination, strict monitoring, trade restrictions, quarantines, and the culling of both infected and healthy (uninfected) animals.

Fruiting Body: Various shaped structures that contain the spores of a fungus.

Gall: An abnormal swelling or localized outgrowth produced by a plant as a result of attack by a fungus, bacterium, nematode, insect, or other organism.

Gas Chromatography-Mass Spectrometry (GC-MS): An analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample; separates chemical mixtures (the GC component) and identifies the components at a molecular level (the MS component).

Hypha (pl. hyphae): A single, tubular filament of a fungal thallus or mycelium; the basic structural unit of a fungus.

Incidence: The frequency at which individuals within a specific population develop a given symptom or quality.

International Plant Protection Convention (IPPC): An intergovernmental treaty signed by over 180 countries, aiming to protecting the world's plant resources from the spread and introduction of pests, and promoting safe trade. The Convention introduced International Standards for Phytosanitary Measures (ISPMs) as its main tool to achieve its goals, making it the sole global standard setting organization for plant health. The IPPC is one of the "Three Sisters" recognized by the World Trade Organization's (WTO) Sanitary and Phytosanitary Measures (SPS) Agreement, along with the Codex Alimentarius Commission for food safety standards and the World Organization for Animal Health (OIE) for animal health standards. https://www.ippc.int/en/.

International Standards for Phytosanitary Measures (ISPMs): Standards are adopted by the Commission on Phytosanitary Measures (CPM), which is the governing body of the International Plant Protection Convention (IPPC). Standards provide guidance to contracting parties in meeting the aims and obligations of the Convention. The intention of ISPMs is to harmonize phytosanitary measures for the purpose of facilitating international trade. ISPMs can cover a wide range of issues, including surveillance, pest risk analysis, establishment of pest free areas, export certification, phytosanitary certificates, and pest reporting. The IPPC encourages adoption of these standards, but they only come into force once contracting (members) and non-contracting parties to establish requirements in national legislative instruments. Compares with the Animal Health Codes on the OIE website. https://www.ippc.int/en/core-activities/standards-setting/ispms/.

Intertropical Convergence Zone: A narrow zone near the equator where northern and southern air masses converge, typically producing low atmospheric pressure.

Kairomone: A chemical substance emitted by an organism and detected by another of a different species which gains advantage from this, e.g., a pest seeking a host.

Leaf Spot: A plant disease lesion typically restricted in development in the leaf after reaching a characteristic size; an obvious, defined lesion or area of diseased tissue, on a leaf.

Lesion: A localized diseased area or wound.

Mildew: A thin coating of mycelial growth and spores on the surfaces of infected plant parts.

Monitoring: An ongoing process to verify an event; an official ongoing process to verify phytosanitary situations, e.g., monitoring survey.

Monitoring Survey: Surveillance designed to verify the status and various characteristics of an existing pest population within a defined area; Ongoing survey to verify the characteristics of a pest population (ISPM 5).

Morbidity: The state of being symptomatic or unhealthy for a disease or condition. It is usually represented or estimated using prevalence or incidence (Hernandez & Kim, 2020).

Mortality: Mortality is related to the number of deaths caused by the health event under investigation. It can be communicated as a rate or as an absolute number (Hernandez & Kim, 2020).

Mosaic: A disease symptom characterized by nonuniform coloration, with intermingled normal, light green and yellowish patches, usually caused by a virus.

Mycelium: A mass of hyphae constituting the body (thallus) of a fungus.

Necrosis: The death of cells or tissue, usually accompanied by darkening to black or brown color.

Nuclear Magnetic Resonance (NMR): A spectroscopic technique to observe local magnetic fields around atomic nuclei; an analytical chemistry technique used in quality control and research for determining the content and purity of a sample as well as its molecular structure; used to identify monomolecular organic compounds.

Objective Prioritization of Exotic Pests (OPEP): Developed by USDA, APHIS to prioritize exotic pests according to the impacts they are likely to have if introduced into the United States. Separate models were developed and validated for arthropods and plant pathogens (including nematodes). Risk criteria consist of questions focused on biology and natural history, pest damage, research, and management elsewhere in the world. Control measures and production practices in place in the United States are also considered when predicting the potential impacts. Questions require objective, documented evidence from primary scientific literature and are statistically weighted based on their ability to predict impact. Each model predicts the likelihood each organism will cause high, moderate, or low impact (as defined by APHIS) in the United States. The results of each assessment are used to develop a prioritized list to help focus resources on those pests that are most likely to cause significant impacts.

Office International des Epizooties (OIE): See World Organization for Animal Health.

Olfactometer: Devices used to present odor stimuli in a standardized laboratory setting; a device used to study insect behavior in presence of an olfactory stimulus.

Oviposition: The deposit or laying of eggs.

Phenology: The study of the timing of recurring biological events and the causes of their timing with regard to weather and climate.

Pheromone: A chemical substance produced and released into the environment by an animal, especially a mammal or an insect, affecting the behavior or physiology of others of its species; a secreted or excreted chemical factor that triggers a social response in members of the same species.

Poikilothermic: An organism with variable body temperature that fluctuates with and is similar to the temperature of its environment: a cold-blooded organism.

Prevalence: The proportion of the population with a given symptom or quality.

Ptyalism: A condition that causes the overproduction of saliva.

Pustule: A small, blister-like elevation of epidermis formed as spores emerge.

Ringspot: A disease symptom characterized by yellowish or necrotic rings enclosing green tissue.

SARS: See Severe Acute Respiratory Syndrome.

Sclerotium (pl. Sclerotia): A vegetative resting body of a fungus, composed of a compact mass of hyphae with or without host tissue, usually with a darkened rind.

Scorch: Any symptom that resembles the result of flame or fire on the affected part, often seen at the margins of leaves.

Select Agent: Biological agents and toxins that have been determined to have the potential to pose a severe threat to public health and safety, to animal and plant health, or to animal or plant products; regulated through the Federal Select Agents Program.

Semiochemical: A chemical substance or mixture released by an organism that affects the behaviors of other individuals; a pheromone or other chemical that conveys a signal from one organism to another to modify the behavior of the recipient organism.

Sensillum (pl. sensilla): An arthropod sensory organ protruding from the cuticle of exoskeleton, or sometimes lying within or beneath it.

Sentinel Plot/Population: A survey methodology that consists of plots of land with a specific crop or plant species or defined populations of animals that are routinely or consistently monitored for the presence of a pest or pathogen.

Severe Acute Respiratory Syndrome (SARS): A viral respiratory disease caused by a SARS-associated coronavirus. It was first identified at the end of February 2003 during an outbreak that emerged in China and spread to 4 other countries.

Shot Hole: Small fragments of leaves falling off and leaving small holes in the leaf tissue.

Sign, Animal: A health issue that is an objective, observable phenomenon or evidence of disease that can be identified by others.

Sign, Plant: An indication of disease from direct observation of a pathogen or its parts (contrasts with symptom).

Spore: A reproductive structure of fungi and some other organisms, containing one or more cells; a bacterial cell modified to survive an adverse environment.

Standard: Document established by consensus and approved by a recognized body that provides for common and repeated use, rules, guidelines, or characteristics for activities or their results, aimed at the achievement of the optimum degree of order in a given context.

Stochasticity: By random chance or probability.

Stunting: The reduction in height of a vertical axis resulting from a progressive reduction in the length of successive internodes or a decrease in their number; abnormally small plant growth.

Surveillance: An official process which collects and records data on pest presence or absence by survey, monitoring, or other methods.

Surveillance, Active: Proactive targeting of specific plant or animal populations over a period of time to collect detailed information on the health status of the population; An active search for cases or occurrences of the pest or disease.

Surveillance, Passive: There is no active search for cases. It involves passive notification by surveillance sites and reports are generated and sent by local staff or the public; Information and data is gathered from all sources.

Surveillance, General: A process whereby information on pests of concern in an area is gathered from various sources. Sources may include national or local government bodies, research institutions, universities, museums, scientific societies (including those of independent specialists), producers, consultants, the general public, scientific and trade journals, unpublished data, and the websites of other National Plant Protection organizations (NPPOs) or international organizations (ISPM 6, https://www.ippc.int/en/publications/615/).

Surveillance, Specific: A process whereby information on pests of concern in an area is obtained over a defined period. Organizations actively gather specific pest-related data. Specific surveillance includes surveys that are conducted to determine the characteristics of a pest population or to determine which species are present or absent in an area (ISPM 6, https://www.ippc.int/en/publications/615/).

Survey: An official procedure conducted over a defined period to determine the presence or absence of pests, or the boundaries or characteristics of a pest population, in an area, place of production, or production site (ISPM 5, https://www.ippc.int/en/publications/glossary-phytosanitary-terms/).

Symptom, Animal: A health issue that is apparent only to the patient and cannot be identified by anyone else; a subjective description by the patient of a health issue.

Symptom, Plant: An indication of disease by reaction of the host, e.g., canker, leaf spot, wilt (contrasts with sign).

Terrestrial and Aquatic Animal Health Code: Provides standards for the improvement of animal health and welfare and veterinary public health worldwide, including through standards for safe international trade in terrestrial animals (mammals, reptiles, birds, and bees) and their products. The health measures in the *Terrestrial Code* should be used by the Veterinary Authorities of importing and exporting countries to provide for early detection, reporting, and control agents that are pathogenic to animals or humans, and to prevent their transfer via international trade in animals and animal products, while avoiding unjustified sanitary barriers to trade. Compare to the International Standards for Phytosanitary Measures on the IPPC website. https://www.oie.int/en/what-we-do/standards/codes-and-manuals/

Urediniospore: The asexual, dikaryotic, often rust-colored spore of a rust fungus, produced in a structure called a uredinium; the “repeating stage” of a heteroecious rust fungus, i.e., capable of infecting the host species on which it is produced.

Uredinium (pl. uredinia): The fruiting body (sorus) of a rust fungus that produces urediniospores.

Vector: An organism or object that transports or transmits a pest, parasite, or pathogen from one area or host to another place or host.

Vesicular: Pertaining to or consisting of vesicles or small sacs or bladders.

Vesicular Stomatitis: A virulent disease of livestock in New World caused by any of several arboviruses assigned to Rhabdoviridae. Transmission to humans by phlebotomine sand flies (*Lutzomyia* spp.) implicated during epizootics.

Voltinism: Pertaining to organisms with many generations in a year or season. Term often applied to Lepidoptera, Diptera and other insects of economic importance.

Wilt: The drooping of leaves and stems from lack of water (i.e., inadequate water supply or excessive transpiration); a vascular disease that interrupts normal water uptake.

World Organization for Animal Health (OIE): The need to fight animal diseases at global level led to the creation of the Office International des Epizooties through the international Agreement signed on January 25, 1924. In May 2003, the Office became the World Organization for Animal Health but kept its historical acronym OIE. The OIE is the intergovernmental organization responsible for improving animal health worldwide. It is recognized as a reference organization by the World Trade Organization (WTO), and in 2018 has a total of 182 Member Countries. The OIE maintains permanent relations with nearly 75 international and regional organizations and has Regional and sub-regional Offices on every continent. The organization is placed under the authority and control of a World Assembly of Delegates consisting of Delegates designated by the Governments of all Member Countries. https://www.oie.int/about-us/

Zika: Zika virus is a mosquito-borne flavivirus that was first identified in Uganda in 1947 in monkeys. It was later identified in humans in 1952 in Uganda and the United Republic of Tanzania.

Zoonoses: Diseases and infections naturally transmitted between vertebrate animals and humans.

APPENDIX

Acronyms

ADSM: Animal Disease Spread Model
AHP: Analytical Hierarchy Process
APHIS: Animal and Plant Health Inspection Service
CAPS: Cooperative Agricultural Pest Survey
CDC: Centers for Disease Control and Prevention
CIS : Comprehensive and Integrated Surveillance
CONUS: Continental United States
DDRP: Degree-Days, Risk, and Phenological Event Mapping platform
NPN: National Phenology Network
EDDMapS: The Early Detection & Distribution Mapping System
EDRR: Early Detection & Rapid Response
FAO: the Food and Agriculture Organization of the United Nations
GPHIN: Global Public Health Intelligence Network, Health Canada
iPiPE: Integrated Pest Information Platform for Extension and Education
IPPC: International Plant Protection Convention
ISID: International Society for Infectious Diseases
ISPM: International Standards for Phytosanitary Measures
NAADSM: North American Animal Disease Spread Model
NAHLN: National Animal Health Laboratory Network
NAHRS: National Animal Health Reporting System
NAHSS: National Animal Health Surveillance System
NAPPO: North American Plant Protection Organization
NDFD: National Digital Forecast Database
NPDN: National Plant Diagnostic Network
NVSL: National Veterinary Services Laboratories
OIE: Office International des Epizooties (World Organization for Animal Health)
OPEP: Objective Prioritization of Exotic Pests
PAS: Phytosanitary Alert System
PRISM: Parameter-elevation Relationships on Independent Slopes Model
SAFARIS: Spatial Analytic Framework for Advanced Risk Information Systems
USDA: United States Department of Agriculture
WAHIS: World Animal Health Information System Interface
WHO: World Health Organization of the United Nations
WTO: World Trade Organization

Chapter 6

Surveillance Design After Initial Detection

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ABSTRACT

Surveillance is the collection, analysis, and dissemination of information to support prevention and mitigation of pest and pathogen impacts across natural and managed health systems. Surveillance provides an informational foundation for the risks posed by the organism, current status of the outbreak, directing limited resources, and effectiveness of management actions within the context of a response. Each response may have a series of management goals to accomplish over time and the information needs to support each goal will vary. Surveillance must be appropriately designed to align with the response goal and be well supported by risk assessment information on the biology of the invasive pest/pathogen, biology of the host or host system, pathways of introduction and spread, types and magnitude of impact, etc. This chapter proposes a generalized framework as a starting place for designing surveillance schemes using core design factors and how to effectively narrow parameterization of these factors within the context of a response goal.

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INTRODUCTION

Surveillance may be defined as an organized system of sample design data collection and reporting intended to gather information about a hazard within a certain likelihood in a timely manner. Plans describing complete surveillance systems are often developed to address multiple objectives for a single hazard and include hazard background, budget details, and communication plans in addition to one or more surveillance schemes. Here we focus on designing surveillance schemes for a single objective. In the previous chapter, we were introduced to the concept of surveillance for the early detection of plant and animal pests and diseases, which we collectively define in this chapter as "hazards". As the previous chapter states, the objective of early detection is to find the hazard before it becomes established, resulting in ecologic and economic harm to the agricultural or ecological system. However, this chapter focuses on surveillance *after* the initial detection and the application of biological knowledge to guide that surveillance (i.e., risk-based surveillance). Unlike early detection, there may be multiple post-detection response goals and the surveillance designed to support those response activities will need tailoring for each goal. Also, there may be multiple surveillance activities that occur simultaneously. It is not possible to adequately cover all aspects of surveillance design in a single chapter, nor is it realistic to propose a prescriptive guide that fits all situations. Thus, the intent of this chapter is to illustrate the critical thinking skills of the design process, define a set of core factors to consider in each scheme, and describe how to rapidly adapt a scheme to any post-detection hazard response situation for plant, animal, or ecological health systems. While this chapter provides a common set of core factors and discusses how each varies according to objective, the overarching tenet is the same: the purpose of surveillance design is to support the post-detection goal(s) and narrow the focus such that collection of information is optimized for the response goals for the hazard.

The chapter begins with the context of how surveillance occurs within a set of response activities (Figure 1). Once a hazard is detected, the responding agency, department, or program will have a high-level mission to accomplish regarding the hazard (e.g., pest or pathogen eradication). The response implemented depends on a number of variables, including previous experience with a similar type of hazard, expected consequences of the hazard, point of entry, first-detection numbers, susceptible hosts, at-risk livelihoods, ecosystems, and businesses, knowledge of environmental conditions for survival and spread, and resources available. Because of the large number of variables, no response will be the same for different hazards or even for different incursions of the same hazard. Each response will have multiple distinct goals with activities tailored to achieve each goal.

Surveillance does not directly achieve a response goal. Surveillance has its own objectives aligned to the response goal (Table 1) and provides supporting information to the overall response such as guiding the response activities or estimating success of the response goal. The design of a surveillance scheme will depend on the upstream choices (Figure 1) and existing information. It is the complexity of response goals and uniqueness of activities that imply a need for both agility and flexibility when designing surveillance. Response scenarios are primarily used for fast spreading, high-consequence hazards to illustrate how different response goals drive different surveillance objectives. Not all these response goals and the associated surveillance objectives are necessary with every new introduction. There are also response goals and surveillance objectives not listed in Table 1 here that may be appropriate for certain hazards.

There must be basic information readily available on the first detection before designing surveillance of any kind, including the identification of a hazard and its host system. A hazard is a biological agent with the potential to cause an adverse effect in natural or agricultural ecosystems. A hazard may be a plant

Figure 1. Conceptual diagram of the structure of a response following first detection and the relationship of surveillance within the context of a response. Surveillance is one of many simultaneous activities, and it usually follows cyclical development and iteration of the design as new information is made available. Surveillance designs (or schemes) and outcomes will vary based on the response goal.

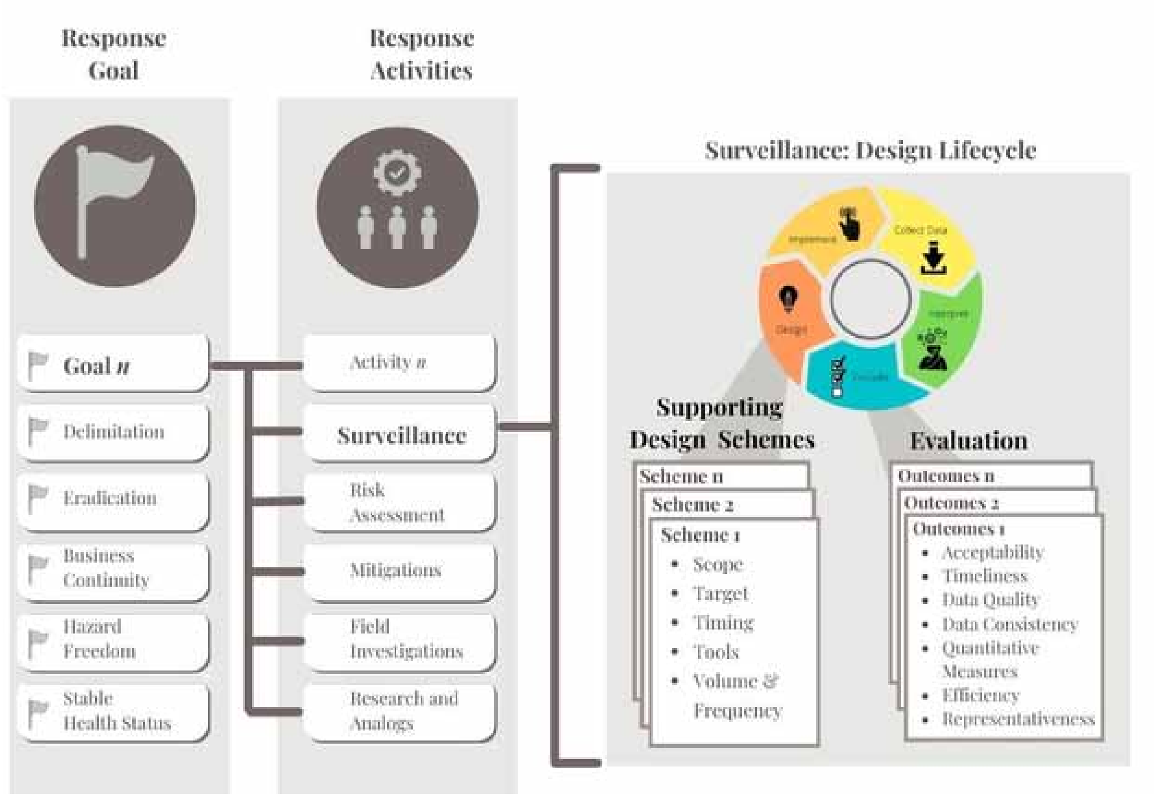


Table 1. Common response goals and associated surveillance objectives for a fast spreading, high-consequence hazard

Response Goal	Description	Surveillance Objective
Delimitation or bounding incursion	Place boundaries on the extent of infection or infestation. Often accompanied with quarantining and other movement restrictions.	Determine which farms, premises, or habitats are within the exposed or infected area and which are outside of the boundary.
Business continuity during an outbreak	Provide a structure for moving product, materials, and equipment without spreading the hazard. Activities often include strict biosecurity measures, treatment, cleaning and disinfecting, holding times and other specific mitigation actions.	Provides evidence that product, material, or equipment has a reduced probability of spreading the hazard (due to biosecurity measures and/or other mitigations) while allowing continued business operations.
Eradication	Reduce the occurrence of and eventually remove the hazard. Activities include quarantining, treatment, depopulation, cleaning and disinfecting, and other mitigations.	Detect all remaining cases to direct where additional control measures are needed.
Hazard freedom	Open trade by demonstrating eradication has been achieved or no hazard existed.	Demonstrates the effectiveness of eradication measures by providing evidence that a hazard does not exist, often for trade purposes.
Stable health status management	Manage an endemic hazard at a steady or stable state of control.	Detects changes that might warrant a response to maintain a stable state.

pest (including weeds, bacterial, fungi, nematodes, insects, mites, and viruses), animal disease-causing agents (bacteria, viruses, protozoa, prions, parasites, and other organisms), or ecologically damaging pests or agents that include all of the above plus invasive plants or animals. None of these organisms are hazards by themselves; they must be placed in a context or setting. This may include a specific host plant, animal species or population, or specific habitat being impacted by the hazard. For the purposes of designing surveillance, we must identify the primary host system for the hazard response. What is considered the "primary" host system may depend upon the focus of the agency driving the initial detection and subsequent surveillance. For example, with highly pathogenic avian influenza (HPAI), animal health officials would be concerned with domestic poultry as a primary host whereas wildlife officials would be concerned with wild bird populations as hosts. An intermediary host would be an alternate host system which is not the primary focus of the surveillance but plays a role in the introduction, maintenance, or spread of a hazard. For hazards with intermediary host systems, it is common (and often desirable) for different agencies to coordinate surveillance efforts to contain a biosecurity threat, based on their combined expertise and jurisdiction over different host systems.

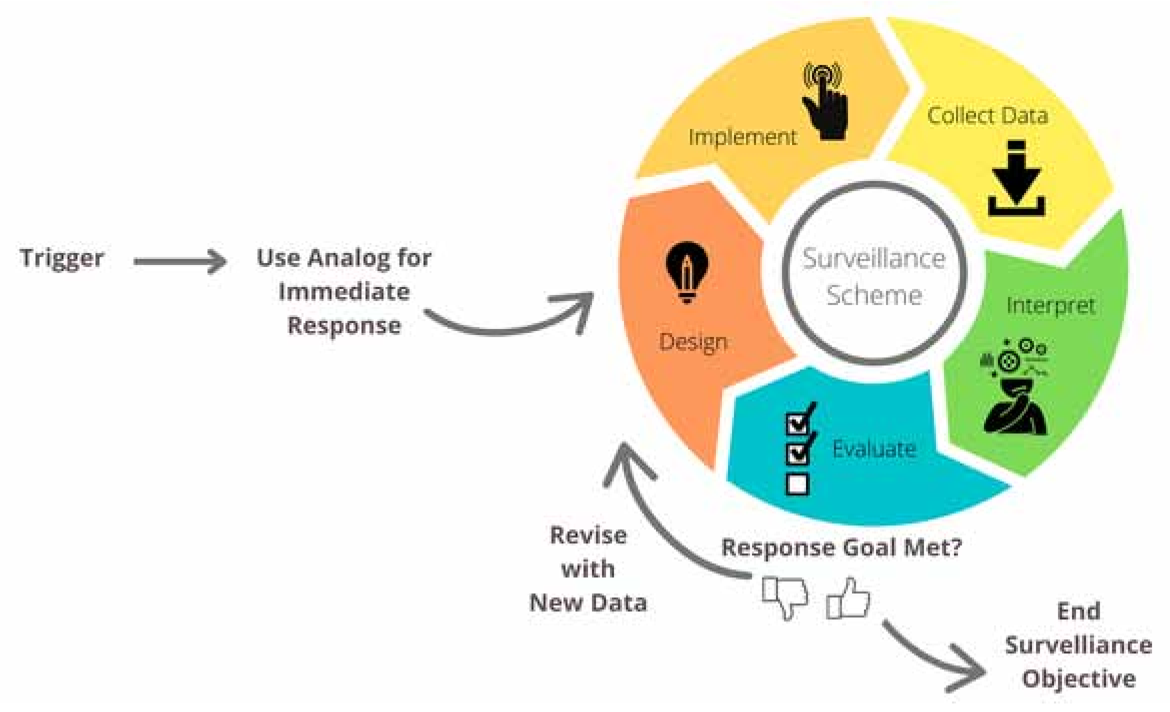
A surveillance scheme defines the parameters and scope for conducting surveillance. Information gathered from surveillance itself, as well as from risk assessments and other investigative work inform the design factors which in turn define the scheme. This information forms the basis for risk-based surveillance (Stärk et al., 2006), similarly called "target analysis" by the U.S. National Invasive Species Council (Morisette, Reaser, Cook, Irvine, & Roy, 2020). For a rapidly spreading high-consequence hazard, timeliness of surveillance design is paramount due to the fast-moving nature of an emergency response. In these situations, an initial surveillance scheme may be drawn from prior knowledge of earlier or similar outbreaks (analogs), so surveillance may be rapidly implemented. As new information becomes available from the surveillance or other response activities such as risk assessments or investigative work, the design of the surveillance scheme may be adjusted "on the fly" to better target the emerging hazard and the surveillance objective. For other types of hazards (e.g., slower spreading, lower consequence) there may be time to gather the necessary background information to develop a more robust surveillance scheme from the start. *The purpose of this chapter is to provide the framework for designing an appropriate surveillance scheme for different response goals and surveillance objectives using a common set of design factors.*

The effectiveness of a surveillance activity will depend on how well it aligns with the objective(s). For example, surveillance that targets host with obvious observable signs for the hazard cannot be used to estimate the prevalence or extent of spread of the hazard without additional information on the occurrence of those observable signs in both the general population and the population affected by the hazard. Surveillance depending on a combination of lack of reporting from observational surveillance with periodic active sampling to detect disease in highly likely subpopulations would not be an effective way to support eradication efforts. Choice of measurement tool may also impact the effectiveness of the surveillance. Measurement tools are very specific to the hazard-host system but can also be specific for the objective. An animal diagnostic test that detects presence of antibodies which occur because of an animal's immune response to the infection would not be an appropriate tool for an objective focused on detecting early infections such as delimitation. A diagnostic test for the virus itself rather than the immune response to the virus would be more appropriate. It is evident that the biology or epidemiology of the hazard is key to designing surveillance. It is impossible to detail all the different factors, variations, and uncertainties in designing surveillance for every potential hazard. Therefore, this chapter provides a generalizable framework for designing a surveillance scheme for the essential core of surveillance design, leaving room to iterate and adjust with specifics that will vary by application.

ITERATIVE SURVEILLANCE DESIGN PROCESS

The introduction section briefly mentioned how surveillance design is by necessity an iterative process (Figure 2). Once a hazard has been detected, surveillance often needs to be put quickly into place to support the response actions needed to delimit, contain, and mitigate the hazard. There often isn't time to do thorough research, deep analyses, and a full risk assessment. Therefore, an analog may be used to substitute biological information for the hazard of interest. Once more information becomes available, the surveillance scheme may be adjusted to more appropriately target, for example, the risk associated with entry pathways or the biology of the hazard (Cheng et al., 2020). This information is updated in the surveillance scheme, which is then implemented in the field. The cycle of updating the surveillance plan with new risk information, an updated surveillance scheme, and subsequent implementation may continue. Some portions of the cycle in Figure 2 will be addressed in more detail in subsequent sections of this chapter.

Figure 2. Iterative process of designing and updating a surveillance scheme based on available information and how lessons learned from implementation may feed back into cycles of reassessment and adjustment of the surveillance scheme



Surveillance Design Factors

During iterations of surveillance design, considerations such as information regarding the host, environment, biology, ecology, epidemiology, etc., must be incorporated to appropriately target surveillance for the objective. This can be an extremely complex balance among the host-hazard system, the mathematical optimization of the surveillance, and practical considerations. Using the initial detection(s) and any available risk assessment information, there are five design factors that, when combined, guide the design of a complete surveillance scheme: 1) defining the scope of the surveillance by location or population of interest, 2) targeting surveillance to a sublocation or subpopulation for efficiency, 3) selecting the best timing of surveillance relative to the life phases of the hazard, life phases of the primary host system, seasonality, and/or weather, 4) using the correct or best tool for collecting samples or making measurements, 5) determining the volume or how much surveillance to conduct and the frequency or how often to conduct the surveillance to best achieve the surveillance objectives given the other factors. All these design factors are present in every surveillance scheme and factors may be defined as a direct result of the surveillance objective or given a practical limitation rather than as a choice by the planner. This set of design factors are not exhaustive of the additional and nuanced factors which may warrant consideration when designing surveillance. These five factors can be considered the minimal set.

Factor 1: Define the Scope of a Surveillance Design by Location and Population of Interest

When a hazard enters a primary host system of interest, the first step is to define the scope of the surveillance to a manageable set of locations or populations that could potentially harbor the hazard. Imagine this as the total extent that could contain the hazard or all the populations which could be impacted before further narrowing the focus of the design. Location may be defined in terms of a geographical or political area unit (such as a county or state boundary) due to ease of defining and implementing regulatory actions or location may be defined to specific sites such as farms, business operations, or some buffered area around a site of invasion. The scope can also be narrowed to what population (or species) is impacted by the hazard within that area and has relevance to the agency as a primary host for surveillance. However, the scope of the area should consider any vectors by which the hazard may spread (if applicable). Practically, this also sets the reference frame from which logistical resources need to be leveraged for conducting the surveillance and what levels of government resources are involved (local, state, multi-state/federal) to ensure success of a surveillance objective.

For example, during an outbreak of highly pathogenic avian influenza (HPAI) in birds, animal health officials might focus on populations of susceptible animal species of primary interest to their mission: chickens, turkeys, and duck. Let's say that a single detection on a farm in the eastern United States occurred. An immediate quarantine and survey area may be established around the farm (Brown et al., 2018) delineating what is known as the infected zone (USDA, 2017). An additional buffer around the infected zone completes the survey area known as the control zone (USDA, 2017). A monitoring zone may be established on a county or more regional level due to the number of susceptible farms and fresh waterbodies that attract migratory birds.

Factor 2: Targeting Surveillance to a Sublocation or Subpopulation for Efficiency

Within a defined area or population for surveillance, there may be too many potential locations or subjects to sample effectively. Depending upon the objective of the surveillance, systematic or random sampling within the entire extent or across all the population may not detect the hazard because there are differences in how the hazard is expressed across sublocations or subpopulations (i.e., the hazard does not occur uniformly). In these cases, efficiency is gained by focusing on a sublocation or subpopulation defined by differential risk from the rest of the population of interest (Stärk et al., 2006). Differential risk might be based on susceptibility, likelihood of entry, pathways of spread, where hazard thrives (highest prevalence), likelihood of establishment, hazard survival, consequence, observable impact, or other distinctions that increase chances of hazard detection or increase the value of information gained through surveillance. In other words, surveillance is targeted to where and how the hazard is most likely to occur to increase the likelihood of detecting it or we target surveillance based on consequences or other factors to increase likelihood of capturing the information needed to support the response goal.

Factor 3: Selecting the Best Timing for Surveillance

The most efficient surveillance plans collect samples and take measurements when the hazard (or information about the hazard) is easiest to measure in the host system or at the highest risk of spread, such as during host movement. The best time may be a function of the life phases of the hazard, the life phases of the primary host system, season, current weather conditions, or a combination of these. The response goal driving the surveillance objective may also dictate timing.

For example, many agricultural pests are poikilotherms meaning that their growth and development are dependent upon temperature. Temperature-based models, known as degree-day models, predict the timing of different insect life stages based on temperature over time using studies researching base temperatures and developmental thresholds (Baskerville & Emin, 1969; L. Wilson & Barnett, 1983; Zalom, Goodell, Wilson, Barnett, & Bentley, 1983). Degree day models may be used to predict the timing for mitigations or surveys based on when the life stage is expected to occur: examples include larval stage for area-wide chemical treatment or adult stage for detection and ongoing monitoring of population levels.

Factor 4: Using the Correct or Best Tool for Collecting Samples or Making Measurements

Often, there are multiple tools available with different characteristics from which to select. Not only does the surveillance planner need to select the best tool for the objectives, but the characteristics of the selected tool is important in determining how much and how frequent to sample or take measurements. The sensitivity and specificity of a diagnostic test, population size, and hazard prevalence is important in determining the volume of sampling required (Cannon, 2001). For trap designs in plant pest or ecological applications, the ability of the trap to capture the hazard depends on the effective distance of the lure (Byers, Anderbrant, & Löqvist, 1989) and density of traps (Turchin & Odendaal, 1996). Unlike diagnostic tests, traps do not have a measure of sensitivity or specificity. However, they may have measures of efficiency such as the proportion of population captured given a trap density (Adams et al., 2017; Turchin & Odendaal, 1996).

For example, emerald ash borer is an invasive wood boring insect that attacks ash trees. It is a cryptic, hard to detect pest because the larvae burrow under tree bark. Lure traps for emerald ash borer vary in effectiveness from 25-100% (EFSA et al., 2020) depending the trap type (funnel, double-decker, prism) and lure type (manuka oil, (3Z)-hexanol, (3Z)-lactone, or a combination). These traps are designed to detect adults once they have emerged from the tree, but, by then, the damage is already done, and the beetles are spreading to attack new trees. Detection to delimit and contain spread would ideally focus on trees actively being attacked by the larvae. New emerging technologies such as acoustic sampling of the vibrational frequencies of larval feeding (Sutin et al., 2018; Sutin et al., 2019) or e-nose volatile organic chemical detection methods have emerged as non-invasive and relatively sensitive methods for detection at this stage of invasion (A. D. Wilson, Forse, Babst, & Bataineh, 2019).

In animal health surveillance, diagnostic tests are specific to not only the hazard and the host, but also to the specimen type (e.g., blood or tissue sample) and the timing of sampling relative to initial exposure (e.g., during active infection or post-recovery). Some diagnostic tests can only be performed once an animal is dead, while some can be performed on live animals but may involve invasive procedures. Often, when the hazard is unknown, a variety of tests are used to narrow down the cause until a determination is made. In some cases, tests may not yet be available for a particular specimen type or even for a specific hazard. The choice of diagnostic test for a given surveillance depends on availability, biology, practicality, and sometimes on the surveillance objective.

Factor 5: Volume and Frequency of Samples

The question of how much to sample (i.e., volume of sampling) is related to the true value of the measure (the parameter) of interest and how precisely estimates from the measurements need to be (Lohr, 2019). Sampling frequency affects the likelihood of detection because of timing, but also the usefulness of the information captured from surveillance as time passes. The more quickly the situation changes, the more quickly the usefulness of previously captured information fades (Schwermer, Reding, & Hadorn, 2009). Sampling volume and frequency are affected by choice of the subpopulation, timing, and tools selected and these two factors impact each other. When sampling occurs where and when the hazard is easiest to find, the sampling volume can be reduced when the objective is detection at the population level (Williams, Ebel, & Wells, 2009). For some objectives, such as finding the last cases at the end of an eradication response, sampling volume needs to be increased where and when the hazard is most likely to be found. Using the most accurate measurement tools also reduces sampling volume. If the hazard-host system is slow changing, sampling effort can be spread through time to reduce the required resources at any one point in time. On the other hand, practicality may dictate the need to sample over an extended period of time to satisfy the surveillance objectives (Hadorn, Rüfenacht, Hauser, & Stärk, 2002). While low volume sampling events repeated in time can result in the same overall volume of sampling as a high-volume sampling conducted at one time, the number of samples required for a single sampling event and the need to repeat a sampling event are not interchangeable.

As an example, consider a viral infection in animals living in groups (i.e., herds, flocks, pens, houses) on farms. After an initial detection, surveillance to support delimitation requires at least one round of surveillance on farms with species susceptible to the virus. The volume of sampling is typically defined by a prevalence threshold. Prevalence is the percentage of the population with the hazard. Surveillance might be designed to detect a 2% or 5% prevalence, for example, and the volume of sampling for a 2% prevalence threshold is much greater than for a 5% threshold. The exact number depends on the surveillance objectives, the sensitivity and specificity of the test, how false positive and false negative results are handled (Cannon, 2001). During the outbreak, as control and eradication measures are carried out, repeated rounds of surveillance must be conducted on farms with susceptible species to support the delimitation goal because, as long as there is active spread, the information gained from a sampling event in the past loses value. Contrast this with a business continuity surveillance scheme that might require a set volume of testing each of two days in a row to demonstrate negligible risk of viral spread through animal or product movements. In this case, the repeat testing does not serve the purpose of maintaining information through time, but rather to achieve detection of a very low prevalence threshold (Cameron & Baldock, 1998).

DEFINING FACTORS BY SURVEILLANCE OBJECTIVE

Different surveillance objectives require choosing design factors that are appropriately focused to accomplish the objective. This is perhaps the subtlest and "tricky" aspect of surveillance design: matching selection of design factors to the objective in a way that supports the surveillance objective for a specific hazard-host system. In Table 2, we provide a high-level summary of the design factors by objective for comparison and easy reference.

Subsequent sections of this chapter provide illustrative examples using this table to enhance understanding of the linkages between design factors and objectives. Plant and animal examples will illustrate how surveillance can be designed to support specific response goals using the design factors. The examples are hazard/species-specific and are written to help distinguish between the use of the factors for different objectives; therefore, application of design factors described here may not reflect what may have been implemented in recorded outbreaks.

Delimitation Example: Bacterial Leaf Streak Disease

For the delimitation response goal, the objective of the supporting surveillance is to determine which farms, premises, or ecosystems are within the area being affected by a pest or pathogen and which are outside of the affected area. In 2016, a bacterial leaf streak (BLS) disease was discovered affecting sweet corn on farms across 51 counties of the United States (U.S.) (Broders, 2017). The causative bacterium was *Xanthomonas vasicola* pv *vasculorum* (Xvv). It was formerly known to occur only in its endemic region in South Africa, where the pathogen primarily affects sugarcane. Using this historical example, the following approach could be used to determine which corn farms are affected by the Xvv pathogen. Using climate data on temperature and precipitation, a climate suitability analysis could provide a risk map showing which areas of the U.S. would most likely support disease persistence based on preferred growing conditions. This analysis would return a very large region covering the midwestern United States stretching down into Texas but could be further refined by known corn production areas (scope). However, this is far too large a region to reasonably sample within a growing season. Research into the epidemiology of this disease shows that the bacterium thrives in semi-arid areas with monsoonal rain events that spread the spores to new locations (An et al., 2019; Ortiz-Castro et al., 2020). Armed with this information, surveillance could be further targeted to corn production areas that utilize center-pivot irrigation (Lichtenberg, 1989; Turkington et al., 2004) and corn monocultures (T. Hartman, Harbour, Tharnish, Van Meter, & Jackson-Ziems, 2020) because bacterial spores would have ready access to

Table 2. Summary of targeting surveillance design factors by survey objective

Objective	Design Factor	Targeting the Design Factor
Delimitation		
	Scope	Host(s) or population(s) affected by the hazard and a spatial extent (or multiple extents) which encompasses the known affected area(s). Each surveillance area should be a homogeneous unit or the design should account for heterogeneity between areas.
	Target	Sublocations that increase detection likelihood such as along the pathways of spread and where the hazard tends to be most prevalent. Subpopulations that are differentially affected by the hazard, such as a life stage or gender. Targeting can reduce volume of sampling needed to achieve detection at fixed prevalence threshold.
	Timing	Stage in the hazard or host lifecycle when it is most easily detected (i.e. highest prevalence). For delimitation timing may be driven by the urgency of the response (i.e. conduct surveillance immediately!).
	Tool	Highly sensitive, appropriate tool or combination of tools.
	Numbers or Volume & Frequency	High to moderate volume to statistically support survey with low to moderate prevalence threshold and frequency determined by host/hazard timing of emergence (such as population doubling time, incubation period, etc.) and by the consequences of false negative results tempered with knowledge that there are control measures in place to reduce spread and that surveillance will be ongoing until eradication is achieved.
Business Continuity		
	Scope	Single site with a spatially buffered area of hazard freedom with focus on the host, commodity, or material that needs to be moved and could potentially spread the hazard through normal business activities.
	Target	Targeted to subpopulations or locations with highest risk of hazard occurrence, such as potential biosecurity weaknesses, high human traffic, movements in/out, etc.
	Timing	Immediately prior to movement or periodically to maintain hazard free status.
	Tool	Very high tool sensitivity
	Numbers or Volume/Frequency	Volume is driven by acceptable level of risk given the consequences of spread but is typically a very high volume (very low prevalence threshold). Frequency for periodic testing (see *Timing*) will depend on the probability of introduction given biosecurity and other mitigations.
Eradication		
	Scope	Cast a wide spatial net to look for remaining few cases, but narrow scope to locations with highest likelihood of occurrence based on what is known from prior infestation.
	Target	Narrow the scope to sublocations like areas previously impacted by the hazard (where the hazard was prevalent). Target subpopulations most likely to display clinical signs/symptoms. Better to sample rare populations/locations than revisit others.
	Timing	When the hazard is most prevalent according to life stage, seasonal weather, host availability, etc.
	Tool	Highly sensitive tool to increase likelihood of detection
	Numbers or Volume/Frequency	Enough samples to detect the hazard at half (or lower if possible) the expected prevalence level of what it would be if the hazard was an endemic population (generally resulting in large volumes). Frequency should optimize probability of detection for hidden or rare cases.
Hazard Freedom		
	Scope	The broader population and/or geographies, ensuring representative sampling of all risk subgroups such as previously affected areas/populations, along pathways in case new introductions are occurring, different life or growth stages, and hazard-free areas/less susceptible hosts.
	Target	Same as *Delimitation* and *Business Continuity* if there is equal exposure between target groups and the rest of the population. When target groups and other subpopulations different in exposure, hazard freedom surveillance must include surveillance in populations that may not have a high risk of hazard occurrence to provide statements about the entire population.
	Timing	More likely to be systematic with a variety of tools to ensure that lack of detection is not a function of poor timing. Post-outbreak, surveillance for Hazard Freedom must occur only after a sufficient amount of time has passed to allow a detectable reoccurrence
	Tool	Highly specific, so that false positives do not risk trade implications.
	Number or Volume/Frequency	Same as Delimitation. Lower sample sizes or less frequent sampling occurring over a longer duration; frequency justified by control and eradication efforts, ongoing delimitation surveillance during the outbreak, and a lack of previous findings. Longer duration ensures no subclinical/cryptic hazards going undetected as well as a large volume because of the longer duration.
Stable Health Status		
	Scope	Same as *Hazard Freedom*
	Target	When prevalence changes are the only focus, target like *Delimitation* and *Business Continuity.* For estimation of characteristics, sampling must be representative not risk-based to ensure all subpopulations are represented
	Timing	Systematically sample over time to record changes in characteristic being tracked
	Tool	Appropriate ecological sampling tool, trap, or harvesting approach to that increase chances of capturing the necessary information. Hazard detection may not be the only measurement of interest and different tools may be required to capture other data. Measures of health status such a population size are often more useful here than hazard detection. Balance of measurement sensitivity and specificity.
	Numbers or Volume/Frequency	Volume necessary to support precision level for the estimate being inferred from the survey. Higher precision requires higher volume of samples. If the estimate needs to be tracked over time, then increased frequency of sampling will also increase the volume of samples collected. The frequency will most likely be determined by biological characteristics that are expected to change with time and how precisely it needs to be measured per unit time.

moisture and continuous host availability (target). Within these high-risk fields, samples could be collected from corn plants displaying visible leaf streak symptoms of long streaks of brown or orange lesions between leaf veins (target). Leaf samples would most likely be collected and appropriately stored (tool) once from each candidate farm during the peak growing season for corn during the V7 growth phase (timing) (Terra Hartman & Jackson-Ziems, 2017).

Using molecular diagnostic methods (tool) (Mahuku, 2004) the samples would be tested to identify the pathovar or specific genomic strain (Lang et al., 2017; Stulberg et al., 2020). Southern-most farms would be sampled first and progress northward, following the progression of the growing season so that all candidate locations may be sampled before harvest (timing). As irrigation would be expected to spread the disease throughout a field, there would be less concern about detection at very low prevalence in each field. Therefore, given the sensitivity and specificity of the diagnostic method, enough samples would be collected from each field for an assumed moderate level of prevalence (such as 10%) because of the targeted approach. For initial delimitation, one round of testing (frequency) across the corn producing areas in the U.S. would provide the desired information about the extent of infestation for that year. If treatments are available for BLS, subsequent surveillance schemes may be needed to support eradication activities. If not, then growers may be limited to implementing alternate management practices (tillage, irrigation method, disease resistant varieties, rotating crops, etc.) and monitoring the health status of corn and disease incidence until new technology and management tools become available (Ortiz-Castro et al., 2020).

Business Continuity Example: Avian Influenza

For the business continuity response goal, the objective of the supporting surveillance is to ensure that a given "business" can continue despite the presence of the hazard. Here, "business" is not strictly engaging in commerce but the more general regular operations. In 2015, highly pathogenic avian influenza (HPAI) was detected in commercial poultry flocks across multiple states in the U.S. A summary of response activities can be found in the USDA-APHIS HPAI Response (USDA, 2016) and one response activity is to support business continuity and issuing movement permits. While specific business continuity guidelines were available for some types of commercial premises during the 2014-2015 HPAI Outbreak (Hennessey et al., 2010; MN, 2021), the description below illustrated how the core factors apply and is not intended to represent actual business continuity guidance provided for commercial poultry operations during this historic outbreak or for future outbreaks.

Surveillance is only one part of supporting business continuity. Biosecurity practices, such as having areas where employees can only enter after showering, truck and equipment cleaning procedures, and viral reduction treatments for products and waste are also important activities that play a role in business continuity. Surveillance is used to determine if infection levels are sufficiently low to present negligible risk of spread and would only apply to those premises seeking movement permits (scope). As with delimitation, dead or unhealthy birds and bird in cages living near the sick or dead birds should be sampled because of the need to detect at a very low prevalence as early as possible in an outbreak (target). Targeting of a high prevalence subpopulation reduces sampling while still allowing inference back to the entire population because the dead, sick, and healthy birds are housed together. Surveillance should occur just prior to movement (timing). A test detecting avian influenza virus using a diagnostic test (tool) such as the real time RT-PCR (Spackman et al., 2002) would be best because active (not past) infection is of concern for business continuity and because the real-time RT-PCR is a highly sensitive test (Spackman et al., 2002). Detections by real time RT-PCR should be followed with a serological subtyping of the virus to determine pathogenicity because not all avian influenza strains are highly pathogenic (W. O. f. A. H. OIE, 2021) (tool).

Surveillance for business continuity usually requires detection at a much lower prevalence threshold than delimitation or hazard freedom. This is because there's only one shot at surveillance before things move and the consequence of moving infected animals or materials without sufficient mitigations is to spread the virus to other premises. Exactly how low is necessary depends on what is being moved and what mitigations are available. The risk of spread by moving eggs from an infected farm is different from the risk of spread by moving live animals from an infected farm. For example, it may be sufficient to demonstrate a prevalence threshold of 5% or less (volume of testing approximate 60 to 65 birds without considering targeting) combined with cleaning of the product for movement of eggs, while movement of live birds directly to slaughter might require demonstrating that infection is in less than 2% of the group of birds to be moved (volume of testing approximately 150 to 180 birds without considering targeting).

Another approach to designing surveillance that requires a low prevalence threshold is to test and delay movement for one or two days, retesting after the delay (using a combination of volume and frequency) allowing time for the disease to spread and be detected on the second round of testing given all negative results on the first round. This works well for animal products such as eggs as well as movement of waste and equipment which may be easier to hold in storage compared to live animals.

Targeting dead and sick birds can substantially reduce the volume of sampling required because 2% prevalence among all birds in a house might result in 20% prevalence or higher among the dead and sick birds in that house. Depending on the size of the poultry flock, it may be possible to sample all dead and sick birds providing a very high probability of detecting one infected bird within the subpopulation of sick and dead using a relatively small sample size.

Eradication Example: Pink Bollworm

Pink bollworm (*Pectinophora gossypiella*) is a major pest of cotton. The larvae of this pest feed upon seeds and destroy cotton fibers within the developing boll, reducing crop yield and quality. Pink bollworm was first discovered in Texas in 1917, spreading across several cotton-producing states of the United States. A domestic federal quarantine was implemented in 1955 for Arizona, Arkansas, California, Louisiana, New Mexico, Oklahoma, and Texas to prevent further spread. Due to extensive eradication and control efforts involving transgenic Bt cotton (which produces insecticidal proteins from the bacterium *Bacillus thuringiensis* or Bt), mating disruption using the gossyplure pheromone rope dispensers (Lykouressis, Perdikis, Michalis, & Fantinou, 2004), adaptive management practices, and through sterile insect release (Tabashnik et al., 2021), the federal quarantine was lifted for all but Arizona, California, New Mexico and Texas by 2003 (USDA, 2018a). Ten years later, surveys returned zero occurrences of pink bollworm, indicating successful eradication. Four additional years of confirmatory surveys were required (USDA, 2009) before pest freedom could be declared in 2018 by U.S. Secretary of Agriculture Sonny Perdue (Perdue, 2018). Using this historical example of a successful pest eradication, we analyze the literature and reframe the available information into surveillance scheme factors to support the goal of eradication.

Surveillance for eradication means finding all remaining cases or occurrences of the pest on a national level, not a single field or geographic area. This requires casting a wide net, thorough sampling of affected areas, and verifying ongoing pest-free status of unaffected fields or areas in addition to the case finding. This can be very costly, so the key to efficient eradication surveillance seeks to reduce sampling for verification of free areas (free zones) and increase sampling to detect remaining cases requiring mitigation. Pink bollworm prefers cotton for growth and lifecycle development but may also invade 46 other plant species (especially okra) in North America (Shiller, Noble, & Fife, 1962); therefore, the geographic scope of surveillance extends across host areas with particular focus on cotton. Pink bollworm is a poikilotherm, meaning that its growth and development is also affected by suitable climate conditions. Using phenology models (Abou Hadid, 2011), the geographic scope may be further restricted to frost-free, cotton-producing areas (Fife, 2014; Gutierrez, D'Oultremont, Ellis, & Ponti, 2006) where overwintering larvae are more likely to survive (scope). Through prior delimiting surveys and extensive mapping, surveillance would be targeted to known infected areas as well as those with historical or sporadic outbreaks or higher potential for introduction/spread. Since pink bollworm can migrate long distances, cotton fields are in constant risk of infestation/reinfestation. Ideally, surveillance to find remaining cases would focus on larval stages of pink bollworm, prior to adult emergence by randomly cutting bolls from flowering cotton plants in natural cotton fields and inspecting them for the presence of larvae (target, timing, and tool). IPPC recommends targeting 4 cotton fields per 4800-6000 ha and sampling cotton bolls weekly until field harvest (International Plant Protection Convention, 2016) (volume and frequency). If larvae are present, then control measures such as mating disruption and/or sterile insect release can be potentially mobilized before adult emergence.

In addition to surveillance focused on detecting remaining cases, detection surveys may also occur in previously eradicated or unaffected fields to verify pest-free status to ensure that the pest hasn't escaped eradication efforts. In addition to visual surveys, pheromone traps using gossyplure pheromone lures (Hummel et al., 1973) are used to detect adult life stages of pink bollworm to monitor effectiveness of eradication measures and insect population levels (targeting, timing, and tool). The density of pheromone traps (tool) are determined by the effective area radius of the pheromone lure, complexity of topography, and flight characteristics of the insect (Byers & Naranjo, 2014). Trap density is generally 1 trap/30-40 acres of Bt cotton, but higher densities of 1 trap/10 acres (volume) were used for natural cotton (USDA, 2009). Pheromone traps were typically deployed at the "pin square" stage of cotton development or the first flower development and monitored weekly until harvest (timing and frequency).

Hazard Freedom Example: Infectious Salmon Anemia

Infectious salmon anemia (ISA) is a disease that primarily affects Atlantic salmon but has also been found in other related species such as brown trout. The hazard is a pathogenic virus (ISAV) from the family *Orthomyxoviridae* and is a World Organization of Animal Health (OIE) pathogen of international concern (OIE, 2021b). In October 2011 researchers detected and reported ISAV genetic sequences in a sockeye salmon caught off the coast of British Columbia, although the reports were not confirmed by the Canadian Food Inspection Agency. However, the reports lead to a multiyear surveillance effort to demonstrate hazard freedom in the Pacific Northwest region of the U.S. (Gustafson et al., 2018). Populations of interest included free-ranging Pacific salmon and trout and all active Atlantic salmon farms exposed to marine environments in Alaska and Washington (scope). Sampling was focused on collecting fish showing physical characteristics of disease or recently dead fish and special attention was given to steelhead trout because this species has demonstrated a particular susceptibility to the ISAV. Salmonids were also sampled during spawning when they are likely to be more susceptible to infection (targeting). Surveillance was conducted over 3.5 years including sampling in multiple seasons (timing). Tissues (e.g., heart, gill, and kidney) were collected from fish and tested for the presence of ISAV ribonucleic acids (RNA) using a validated set of standardized operating protocols and testing was conducted at more than one laboratory (tool). Because of previous reported detections it was important to sample tissues with the greatest likelihood of containing viral RNA and a testing protocol with high sensitivity and high specificity to demonstrate that detection or failure to detect was not the result of an imperfect test. International guidelines recommend a prevalence threshold for detection of 2 percent or lower (OIE, 2021a) and to this surveillance was designed to detect with a greater than 0.95 probability at a 1 percent prevalence threshold in any of the regional populations (volume) on an annual basis (frequency). This means that the sample size required to meet these thresholds was collected for each population each year (or to the extent that was possible) in each region. The broad coverage of species, geography, and time, the focus on susceptible species, physical characteristics, and life-stage, along with sampling at a volume to detect a low prevalence threshold for each population annually for multiple years to provided support for the absence of ISAV despite some laboratory reports of ISAV in the region.

Stable Health Status Example: North American Gypsy Moth

North American gypsy moth (*Lymantria dispar dispar*) is a forest pest that can feed on more than 300 tree species, causing extensive ecological and economic impacts through foliar defoliation in the larval stage of development. North American gypsy moth (NAGM) was imported to the U.S. from Eurasia in 1869 to attempt breeding a hardy silkworm species. However, NAGM escaped captive breeding into the surrounding forest habitat where it established and rapidly began to spread. There are three means or pathways of spread: larval instars float on silken threads (“ballooning”) to find host trees, male moths fly in search of non-flying females for mating, and the translocation of egg masses long distances by hitchhiking on campers, boats, vehicles, lawn furniture, and other outdoor objects that can move with a household. With these various means of spread, unchecked NAGM populations can spread between 13-20 km/year. Initial control efforts were insufficient to eradicate this pest. More than 100 years later, NAGM is now established in 20 northeastern states as well as neighboring Canadian provinces. Seventy-five million acres of hardwood forests have been defoliated since 1970 but 75% of the U.S. remaining forests are still at stake (STS, 2021).

In 2000, the U.S. Congress authorized funding for a new federal program called “Slow the Spread”, implemented by the U.S. Forest Service in 2005. The Slow the Spread, or STS, program combines intensive surveillance with control operations to reduce the spread rate and maximize the longevity of forest health and productivity. The STS program states that their premise is to identify and eradicate newly introduced populations along the spread front (which resulted in lowering spread rate by more than 50%). We present available information on this pest and surveillance methods published by STS regarding NAGM so that it may be reframed within the proposed framework. The STS program includes surveillance that fits into what we’ve called stable health status surveillance. The surveillance objective is to detects changes in the hazard/host system that requires a response to maintain a stable state, for example, in the case of the NAGM example, spread beyond the current infestation front.

Although an extensive area is infested by gypsy moth and it has potential for long-range movement, surveillance for stable health through the STS program is limited to a 100-km wide, 1200-mile long swath along the active spread front (scope). The boundary of the spread front is defined as a moth catch density of 10+ moths per trap. This 100-km wide monitoring area is known as the “transition zone” between the infested and uninfested area and STS employs surveillance and treatment to prevent new colonies of NGAM from establishing within the transition zone. More than 300 species of trees and shrubs are affected by NAGM, which means that targeting susceptible host species isn't very helpful. In cases where there are specific susceptible species for a pest or where there is observable damage by the pest, sampling could focus on these species or areas of unhealthy trees that could be due to the pest (target).

The STS program uses pheromone traps (typically what are known as "milk carton" traps) baited with 500 µg of synthetic sex pheromone called (+)-disparlure (tool). Traps are hung from tree canopies 1.5 meters above ground (cite PPQ ops manual). Delta may be used for lower moth densities but lose efficacy after approximately 12 moths (USDA, 2007). The base detection grid is 1 trap/km^2 (volume). If moths are detected in the same location for at least two years, then a higher density delimiting grid is established (16 traps/km2 or 250m spacing) to delimit "potential problem areas" and next year eradication activities (USDA, 2007). This monitoring of population levels allows the STS program to quickly respond with targeted eradication activities and heightened surveillance along the spread front.

The timing of trap deployment is important; so that traps are in the field just before and just after active flight season. As insect growth and development is temperature dependent, phenology models are used to predict the adult life stage (i.e., moth flight) which varies geographically (Régnière & Nealis, 2002). Five days are added to the date bounds to account for model prediction uncertainty. Traps may be checked weekly and replaced if the trap is full or the trap is missing/damaged. Traps will be removed, and moths sent for identification at diagnostic labs. Each year, the STS program deploys over 130,000 pheromone traps (volume and frequency) between its detection and delimiting grids. This intensive surveillance program has been very effective in monitoring NAGM population levels along the spread front and reducing spread rate into new, uninfested areas.

An interesting feature of pheromone trapping is that these can serve a dual purpose. Prior research has shown that effective area radius (Byers et al., 1989) of a pheromone trap and trap spacing less than 250 meters affects the ability of a moth to follow the pheromone trail back to a trap or a mate (Elkinton & Cardé, 1988; Sharov, Thorpe, & Tcheslavskaia, 2002), resulting in lower trap catches (or mating success). High density trapping can be used to disrupt mating success although other methods are typically used to disrupt mating (Tcheslavskaia et al., 2005) but would bias results if the intended purpose was for surveillance to estimate population size. Thus, it is important to keep in mind whether the purpose is to use traps as a control activity or gather information as part of a set of surveillance objectives and determine if a multi-purpose use for the traps is appropriate or if they can only serve one purpose.

DATA COLLECTION AND MANAGEMENT

Data collection and management systems refers to the information technology (IT) infrastructure that supports the acquisition of surveyed observations, their transfer to a central processing location, their analyses, interpretation, and presentation, and their distribution to appropriate audiences (Demchenko, Zhao, Grosso, Wibisono, & Laat, 2012; Fukaya, Kusumoto, Shiono, Fujinuma, & Kubota, 2020; Salvador-Meneses, Ruiz, & Rodríguez, 2017 Wallace, et al., 2020). Such an infrastructure evolves over time as new devices, tools, methods, and knowledge become a reality.

Data collection begins with the type and method of collecting observations. Observations can be made by human eye, in situ measurement, onsite filming, capture and counting, and remotely sensed imaging. They can include not only pests and diseases of interest, their hosts and evidence of their direct and indirect damage to the environment, but also demographics that place the disease and host observations in context of time and place. Observations are ubiquitous to any type of surveillance system design. In the not-too-distant past, observations were written down on paper and manually archived in cabinets. Today, nearly all observations are recorded electronically using mobile devices, such as a laptop or cell phone. Application (app) software on these devices provide custom formats for data entry and file transfer protocols to transmit observations via the Internet from the field to a central processing location.

Central processing locations can be local, regional, or national. In recent years, entities responsible for data collection and management have moved from local servers to cloud computing, which are hosted by regional and national centers. These centers have communication trunks with redundant cabling that support multi-tiered Internet networks, arrays of computers for data storage and processing, physical and software security, management software for data reception, processing, and transmission, and file transfer protocols for the exchange of data among users having authorized access (Jadeja and Modi, 2012). Cloud computing allows users to purchase computer resources on a demand basis without a long-term investment in local servers. With the appropriate mobile device, users can access and manipulate field-collected observations from any place that has Internet access. Furthermore, cloud computing allows users to remotely analyze, interpret, and present observations in custom formats, which can be distributed to target audiences in real-time or by scheduled release.

Data management, or more specifically, the management of surveillance observations including diagnostic results, requires highly trained individuals and custom software support. Even the simplest of presentations, such as displaying observations as data pins on a landscape map, require “back end” and “front end” development (Miller and Sytsma, 2014 or Strode 2012). Back end development refers to highly trained individuals who can program in languages such as PHP, Java, .Net, and Python for server-side or database processing. Front end development refers to similarly trained individuals who can program in languages such as HTML, CSS, jQuery, and JavaScript for graphical use interfaces and website development.

Back end development is concerned with the ingestion of observations transmitted from the field and their storage on cloud computers. The ingestion includes not only their recorded values but also their global positioning system (GPS) coordinates, when available, and any images. After arranging copies of the observations into custom file formats, back end development can further process observations as input into models, machine-learning algorithms, and other programs that create derivative data for decision making. Back end development can combine the original observations and/or their derivative data with other compatible data sets, such as georeferenced, remotely sensed images, climate data, or data from another hazard/host surveillance.

There are two major concerns with ingested observations from the field: specifically, data quality and validation. Inevitably, there are going to be errors in observations, starting at the point of collection and continuing through any phase of transmission to final storage. For example, a biological sample could be taken of a hazard in the field and then sent by mail to a lab for identification. After being analyzed in a lab, the sample results are recorded on a local computer and then transmitted electronically to a central processing center. Once at a center, the results are combined with other data about the hazard of interest. All along this stream of data movement, be it collection, analysis, or storage, there are different handlers, computers, protocols, and labelling practices. Each step invites error, which, in the end will affect the quality of final products received by a user.

The second concern of data validation is subtler. There is always the question whether an observation is truly representative of a hazard, be it its identification or behavior. In other words, is the data being collected addressing the data needs of a surveillance plan? Validation of data requires strict standards for their collection along with frequent checks for integrity as an observation moves from the field to storage. Furthermore, other evidence supporting the final results could be used in validation before data are passed to front end development.

Front end development is focused on the generation of products that are accessible through a website interface or transferred to a user via an application programming interface (API). Products can be in the form text, graphs, tables, landscape maps, images, videos, or audiocasts. They can be formatted for a number of devices that are popular with users. Products can be scheduled or automatically released according to a user-specified criterion. For example, a text message can be automatically sent to users' cell phones alerting them that a hazard was detected during a surveillance operation. The message can include details of the detection, such as the name of the observer, his/her surveillance role, the recorded species, the affected host, form of capture, number of individuals, their location, their time of discovery, and any confirmation diagnostics. In another example, a product could be a landscape map depicting the seasonal, regional tracking of a known disease.

Front end development in some ways can be more challenging than the back end because the types of products and their form of delivery must be tailored for the organism of interest and the targeted audience. Furthermore, front end products must be sent in a timely manner, be easy to understand, and be supportive of pre- and post-detection surveillance strategies. Successful data management systems will integrate different sources of data (utilizing both back and front-end development) to provide holistic analyses and data visualizations. These integrative systems bring added value for enhanced decision making beyond single stream data systems, in addition to more opportunity for cross-cutting standardization and quality control thus increasing the confidence of the data.

It is important to note that the IT infrastructure that constitutes data collection and management systems is always evolving. Computers, which are at the heart of the infrastructure, are increasing in storage and processing speeds, and are becoming cheaper without expanding their footprint. Back end and front-end development will not only see new languages, such as Julia (Bezanson, Karpinski, Shah, & Edelman, 2012), but existing languages will become more feature rich and offer more libraries. Furthermore, the operating systems and application software on mobile devices will become more sophisticated in the number and kinds of tools offered users for collecting observations. With each passing year, surveillance strategies will benefit from ever-improving data collection and management systems.

DATA INTERPRETATION

Interpretation of invasive pest or disease observations and their derivative data is an important component in any surveillance program. Like other aspects of surveillance, interpretation has guidelines based on past successful approaches for eradicating or containing regulated or non-regulated hazards. These guidelines are jointly published by the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and the World Organization for Animal Health (OIE). They include the correct language for the regulatory reporting of the presence or absence of a hazard, especially for their economic impact on international trade.

In this section, the focus of interpretation will be the tools for making sense of data generated in a surveillance program. All these tools have the one common goal of providing meaning to data so that an economically important hazard can be controlled after initially detected in the field. While text, tables, graphs, and maps in hardcopy form have been a mainstay for interpreting the results of a surveillance program, in recent decades, there has been a shift to online tools. Online tools, such as business intelligence dashboards, have electronic versions of traditional text, tables, graphs, and maps, but also many audio (e.g., podcast) and video (e.g., YouTube) forms to communicate surveillance results. These online tools typically feature dynamic graphs or other visualizations which update with user interaction. This dynamic component of online tools encourages higher digestibility of complex data through interactivity, allowing the user to explore and investigate patterns in data with button clicks and drop-down selection boxes. While there is an ever-expanding number of creative ways to provide online interpreted results, only the most popular ones are covered in this section.

It can be argued that a cartographic map is one of the oldest and singularly most important tools for interpreting surveillance data. A cartographic map gives an instant picture of the geographic location of a hazard within the context of its surroundings (Kraak & Ormeling, 2020). Besides location, a map can include other features relevant to a hazard, such as an at-risk host or likely avenues of movement. Cartographic maps have similarly migrated from hard copy to online mapping, providing the user tools to zoom into or out of a region, add/remove layers of interest, and calculate distances.

Business intelligence dashboards are becoming increasingly popular for managing field-collected observations and coupling them with decision-support systems. Dashboards are computer-generated displays of data that are accessible over the Internet either through a website or an application (app) and present metrics and visualizations so that data can be easily interpreted to support a course of action in response to a hazard (Ioannou et al., 2019; Yousefinaghani, Dara, Poljak, & Sharif, 2020). Dashboards allow individuals from different government and industrial sectors to coordinate their surveillance and mitigation efforts while providing users an integrated picture of hazard activity and measures for judging the effectiveness of resources allocated to combat a particular hazard. For example, a web-based dashboard developed in China (Yao, Zhu, Yun, Peng, & Li, 2017) allows field scouts to share observations and create a real-time surveillance picture of locust movement and behavior.

Interactive decision models are part of a decision-support system accessible through business intelligence dashboards (Franklin et al., 2017; Hedgebeth, 2007). Decision models allow online users to explore “what if” scenarios of hazard spread. By employing knowledge of how similar invasive species behaved in the past and applying population-dynamics algorithms, modeled predictions can be made to predict behavior and mechanisms of spread. These predictions are complete with uncertainty analysis and decision thresholds for implementing further surveillance measures and mitigation strategies.

Like data, interpretation can occur at multiple scales depending on the hazard, its points of entry, behavior, and economic importance (Leibold et al., 2004). As such, surveillance decisions must account for legal and social constraints at regional, state, and local levels and not just the economic impact on business communities. Lastly, interpretation of surveillance results must always be mindful of the resources available to control a particular pest and that there is a limited time window for an effective response.

SURVEILLANCE EVALUATION

Surveillance design, implementation, and evaluation are all critical parts of surveillance cycle. Earlier in the chapter, a framework for designing specific surveillance schemes to support specific response goals was described; however, the most carefully planned surveillance scheme may need adjustment (or even fail) when implemented due to unforeseen factors. A common example of using analogs for a new or unknown hazard so that surveillance can be quickly implemented was also detailed. Therefore, it is necessary for a surveillance schemes to be continuously reexamined after implementation to evaluate its ability to reach the predetermined surveillance objectives and adjust the system as needed (Figure 2).

There are many excellent publications devoted entirely to surveillance evaluation (Drewe et al., 2015; Peyre et al., 2019) which list more than 20 aspects to consider during an evaluation. These publications address evaluations of complex surveillance systems. Recall that a surveillance system often addresses multiple surveillance objectives for a single hazard and includes hazard background information, budget details, and communication plans in addition to describing all the surveillance schemes required to address the identified objectives. To adequately evaluate a surveillance system, six factors were identified as universally vital for successful surveillance scheme operations. These factors are:

Acceptability - Participation in the surveillance system among stakeholders.

Timeliness - Duration of time between steps in a surveillance system.

Data quality and consistency - Accurate data collected from all populations with consistent data standards so that data has meaning.

Quantitative measures of detection capabilities - Calculations performed based on the sample size, population size, prevalence, and other factors to provide analytical insight into each surveillance scheme's ability to meet objectives.

Efficiency - Value gained compared to the amount of time, money, and human resources dedicated to surveillance in each scheme.

Representativeness - The degree to which the characteristics of the target population is reflected in those included in sample collection for each scheme.

Acceptability

Acceptability, the participation of stakeholders (Klaucke et al., 1988; Peyre et al., 2019), is vital to the success of any surveillance system and can affect many of the other attributes listed above. Without a high degree of participation, there may be insufficient data to draw generalizable conclusions and data quality, representativeness, and efficiency will suffer. The conclusions may be narrow in inference or limited in application.

Acceptability can often be difficult to evaluate and must be done through both a quantitative and qualitative approach (Klaucke et al., 1988). While some surveillance system plans define the stakeholders and, therefore, participation can be directly measured (e.g., 95% of known producers have submitted specimens for testing), others target broad populations complicating any calculated acceptability. Often, acceptability can only be measured through reporting incidence, improvements when compared to previous activities, or simply identifying the number communications and extent of outreach. Measured rates of acceptability typically carry more weight, but the qualitative evaluation of acceptability can provide great insight. For example, if the implementation of an emerging disease surveillance system has sparked concern and conversation among producers, acceptability may be measured by the increased awareness and participation in passive surveillance.

This vital trait is often overlooked when designing surveillance; however, without participation there will be no data and therefore, no conclusions can be made. Compromises must be made to assure stakeholder participation and data quality. One method to maintain stakeholder participation is to provide timely feedback (Halliday et al., 2012; Smolinski, Crawley, Olsen, Jayaraman, & Libel, 2017). Monetary or diagnostic incentives are routinely used as a method to stimulate initial participation, but maintaining participation can be achieved through information sharing and increased cooperation (Smith et al., 2010).

Timeliness

Timeliness should be measured in days or hours and can impact acceptability and the usefulness of each surveillance scheme and the surveillance system as a whole (Klaucke et al., 1988) . For any surveillance objective, timely reporting of data to the stakeholders or the competent reporting authority is essential. There are multiple important measures of time for surveillance schemes and more complex surveillance system involving more than one scheme. In a scheme that relies on laboratory testing of field collected samples, there are 4 primary measurements that need to be considered: 1) time from sample collection to laboratory submission 2) time from laboratory submission to testing 3) time from testing to reporting to responsible party, and 4) time from reporting to public release. These measurable time periods, of course, may vary between surveillance schemes, but the core idea remains: in order for the data produced from a surveillance scheme to be useful, it must be delivered in a timely manner to those making the administrative decisions necessary to meet the objectives. If a hazard detection is reported too late, it can completely derail an eradication effort. For a surveillance system comprised of multiple schemes, timeliness can be a factor depending on the extent to which each scheme depends on or informs other schemes. As with any complex system, if parts of the system fail because necessary pieces fail to be in place in time for subsequent actions, the system will fail.

Data Quality/Consistency

Accurate and complete data is necessary to make valid claims from any surveillance scheme and depending on what is being sampled, can be assessed through qualitative or quantitative measurements. Many elements can affect data quality and consistency. For example, are the individuals collecting or testing samples properly trained? Are the tests validated with the specific sample type? Is the mechanism for reporting designed to minimize human error? Are samples collected sporadically or year-round? All these questions and more can impact the data quality and consistency and can therefore impact the effectiveness of each surveillance scheme. Conclusions made based on inaccurate data can lead to harmful or inefficient responses and loss of trust in the system. Another issue that may be unique to surveillance is the separation between data collected in the field and data coming from further analysis of samples in a laboratory. Ensuring that field data and laboratory data on the same unit are connected correctly in the databased is not trivial and a common source of erroneous data. Unfortunately, there is no single solution to poor data quality as issues can stem from surveillance design, sample collection, sample handling, data input, test quality, or limitations of the data integration and storage system. These issues can be difficult to identify and should be intensely analyzed while designing the surveillance scheme and data management systems prior to implementation to avoid the need for backtracking to identify the source of the issues.

Quantitative Measures of Detection Capabilities or Measures of Precision

This factor encompasses many different potential measurements including detection capabilities, positive predictive value, negative predictive value, estimate precisions, or specificity and sensitivity of the scheme (Klaucke et al., 1988; Peyre et al., 2019). While all these calculated values are important and could be included in any evaluation, it is not necessary to independently evaluate each measure separately. Different objectives might drive the need for a different quantitative measure. A scheme designed to detect a hazard may rely on a detection capability (prevalence that could be detected if present), while a scheme designed for hazard freedom might benefit from assessing the positive and negative predictive value. Surveillance for stable health status may depend on estimating prevalence or population sizes and measuring the precision of the estimate would be most important. Sometimes data availability may limit the ability to use these measures in a generalizable way and results may simply be descriptive statistics rather than inferential. The quantitative assessment can provide concrete values that determine the probability of hazard detection or the number of hosts required to be sampled before a hazard will be identified or estimated with a desired level of precision.

This type of analysis can be very powerful and convincing for outside authorities as long as the calculations are performed properly. Often these calculations are viewed as the primary evidence for a successful surveillance scheme; however, measures of detection capabilities cannot be interpreted in a vacuum. Despite the results of these calculations, if the data quality, acceptability, or representativeness is poor, then these calculated values do not stand alone to indicate surveillance scheme value. It is always important to evaluate all attributes of the surveillance system or a surveillance scheme together for a comprehensive understanding of the quality of the conclusions reached.

Efficiency

Efficiency can be defined as the extent to which surveillance objectives can be achieved without wasting resources. The efficiency of a surveillance scheme may be measured through quantitative or qualitative methods. When using quantitative methods of evaluation, economic analyses are a beneficial method for determining the value generated by the scheme compared to the amount of money spent. Evaluating efficiency answers the question: is the surveillance information gained worth the cost of implementing the surveillance scheme?

Economic analyses are powerful tools to determine efficiency; however, monetary value is not always the driver of surveillance. Systems designed for the public good often place less weight on the cost of a surveillance system and focus on the benefits gained compared to the time and resources dedicated to the surveillance system. Limited resources must be distributed where they are most needed or where they will have the most significant impact. This may include devoting more resources to one surveillance scheme within a system compared to another scheme. It also drives the need to examine whether separate surveillance schemes can be combined to address more than one objective. In this chapter, we've separated schemes for different objectives to reduce complexity and to ensure mindful design. Once schemes for different objectives are designed, combining schemes where possible is recommended. The designer should always keep track of what features of the design support each objective so that future adjustments don't result in collecting data that no longer supports one of the original objectives. With all of this in mind, qualitative assessments of efficiency are equally important for those systems where an economic analysis is simply not feasible. While the money can still play a role in this analysis, the time and effort dedicated to the system are the primary considerations. Regardless of the method of evaluation, the core question remains: are the findings valuable enough to justify the resources required?

Representativeness

For a surveillance scheme to successfully address the objectives, the results must be applied to all members of the population defined in the scope of the surveillance. For this to be possible, the hosts sampled must provide an accurate depiction of that population. For example, if the population is 20% male and 80% female, those animals sampled should display similar demographics. While this seems simple at the surface, representativeness may be necessary across a wide range of demographics including age, geographic, temporal, breed/species, gender, production type, sample size, and much more. And because targeting is a core design element to improve efficiency, ensuring representation while targeting can be confusing. When using a targeted subpopulation, it is important that inference can be made back to the full population defined in the scope through the concept of equal or uniform opportunity for exposure. Suppose that females are more likely to present clinical signs of the infection and therefore are valued as a target group. Although prevalence or disease presentation is lower in the males, there must be an equal or uniform opportunity for exposure in the males allowing us to make inference that no disease in the females directly implies no disease in the males. If males and females live mostly separately and are only exposed to each other for a very short time during mating season which coincides with little or no opportunity for spread of the infection, targeting females may be insufficient to make claims about the male population without also collecting samples from the males. Knowledge of the hazard, the host system and the factors used for targeting is essential for making appropriate statements about representativeness in these complex surveillance schemes.

As you can imagine, perfect representativeness is incredibly difficult to achieve, but it is important to evaluate as poor representativeness can lead to results that are non-generalizable. Non-generalizable results cannot be applied to the desired population described in the scope, only to a subpopulation, sometimes so narrow in scope that result is not useful. Using the example above, if exposure was not uniform between males and females and our surveillance resulted negative tests for 90 females and 10 males, we may be able to claim no detection in the females, but would have insufficient evidence to make such a claim in the males.

Evaluating the attributes given in this section provides a full picture of the overall value of a surveillance scheme. These attributes are not intended to provide a comprehensive evaluation of a surveillance system. They focus primarily on the surveillance schemes within the system and represent a condensed list of the most vital factors that should be considered. There are many other parts of a surveillance system, such as a communication plan, and other evaluation attributes for consideration along with numerous resources available providing detailed guidance on the application of each attribute. Some such resources include RISKSUR Evaluation Tool (Peyre et al., 2019), SERVAL (Drewe et al., 2015), and CDC (CDC, 2021).

FUTURE OPPORTUNITIES

Scientific improvements in detection tools, computing, and analytics are driving advancements in surveillance and other fields of study. However, we caution the reader that any discussion of future technologies or opportunities are assumed to occur within the context of a complete, robust design that addresses all the basic elements that we have summarized in previous sections of this chapter. Surveillance is like a web or a network of information and processes that is only as robust as the weakest link. Therefore, the implementation of a new and highly sensitive detection tool is only effective if the data generated from that tool is appropriately collected, managed, analyzed, and reported in a timely fashion to aid decision making and response actions. Otherwise, the investment in the new technology has very little gain in improving surveillance. A thoughtful approach to identifying opportunities to improve surveillance for any hazard would be to exercise the surveillance design in mock trials or exercises. Mock exercises simulate the emergence of a hazard and walk participants through the process of surveillance, mitigations, reporting, and all the inter-related activities of a response. Such exercises help probe the design for weaknesses and identify gaps for improvement before a hazard occurs. These gaps should help prioritize next steps and opportunities for improving surveillance. With that context in mind, we summarize the different emerging technologies and critical information needs that could drastically impact the future of surveillance.

Basic research to address knowledge gaps

With any emerging hazard, the most immediate need is relevant information on the biology, ecology, epidemiology, host range, reproductivity/transmissibility, etc. As noted in the chapter, surveillance plans are often formulated using analog species in risk assessments which then inform the parametrization of the surveillance scheme. The probability of a surveillance scheme achieving its objective will rely heavily on how well-informed it is with the relevant biology and interactions in its host system. In plant health systems, the integrated pest management (IPM) strategy relies upon such knowledge to optimize crop production and reduce insecticide or herbicide treatments and a similar approach to understanding host/ hazard interactions can inform surveillance. We have given numerous examples of leveraging crop and insect phenology for timing surveys. In animal health systems, information on transmission and spread is crucial for parameterizing models to predict emergence of clinical signs, disease incidence, and temporal windows for mitigations. Directing resources toward basic research for emerging diseases is always a priority, as this informs basic parameterization across multiple factors of a surveillance scheme.

Improved Detection Tools

In surveillance, the detection tool needs to be appropriate to the hazard and the surveillance objective. It is often the case that tools are implemented because they are broadly accessible, inexpensive, and easy to deploy or maintain. However, there may be situations in which the deployment of a tool for detection does not match the biology or environmental constraints, resulting in a lowered probability of detection. For example, wood boring pests are often monitored using pheromone traps and pheromone traps attract the adult life stage of the insect. However, most of the lifespan of wood boring insects is spent beneath bark in larval life stages, when the pest is actively doing damage to its host. In these stages, the insect is cryptic (difficult to detect) so surveys may focus upon the adult life stage for convenience. Delimitation surveys have the objective of determining which locations are within an affected area and which locations are outside the affected area (Table 2). Surveys that use pheromone traps in a delimitation survey miss the crucial stage of infestation, and instead sample when adults are actively searching for new hosts. Detector dogs have been shown to be effective detecting pests actively attacking trees by picking up on the volatile organic compounds released by the trees when under stress. Similarly, detector dogs have demonstrated high sensitivity to detecting disease in people and animals, increasing early detection and limiting spread. However, detector dogs are expensive to train and have limited duration in the field. New emerging technology called electronic noses ("e-noses" or "e-sniffers") are sensors that detect VOCs and compare to known profiles for detecting pest or pathogen presence. These types of sensors could rapidly narrow the targeting of surveillance to stressed plants or animals for further diagnostic screening and have the added benefits of lower cost of deployment and longer duration. Other examples of technology for improving the timing of detection for the surveillance objective include diagnostic tests for specific animal tissues that show presence of infection before more typical blood or tissue samples.

Internet of Things

Earlier, the importance of integrating all the pipelines and information processes that link to surveillance was discussed. The concept of Internet of Things (IoT) describes physical objects embedded with sensors, processing ability, software, and other capabilities such that information is exchanged seamlessly over the internet or other communications network (Tzounis, Katsoulas, Bartzanas, & Kittas, 2017). As private citizens increase adoption of IoT technologies such as integrated voice commands, WiFi appliances, cleaning robots, security systems, remote monitoring, etc, in their homes, there is the potential for agricultural systems to adopt a similar approach where farmers can monitor and control aspects of the monitoring system to maximize the health and productivity of their crop or herd. In precision agriculture applications, robots are already being used to scan fields for pests and pathogens, increasing ease and rapidity of detection (Cubero, Marco-Noales, Aleixos, Barbé, & Blasco, 2020; Rey, Aleixos, Cubero, & Blasco, 2019). The next step toward IoT is the integration of pest management with detection sensors (Esposito, Crimaldi, Cirillo, Sarghini, & Maggio, 2021). In addition to monitoring specifically hazards, watching for small but wide-spread changes in feed intake and production can provide early warning for and indirect measures of hazard incursion.

There remain many challenges with adoption of IoT in agriculture: accessibility and affordability of sensors like radio frequency identifications (RFIDs), the networking and intercommunications of the sensors, and the applications which can handle the diversity of sensors and communications protocols (Tzounis et al., 2017). For example, RFIDs are an older technology that is useful for traceability of animals or plant products, but widespread adoption is limited because of the cost and accessibility of the scanners. Decreasing the cost of RFID scanners would vastly improve the adoption rate of this technology and allow increased traceability of pests and diseases. As with all technology, new sensors and tools will have more meaningful impact on biosecurity if the technology is broadly accessible, available, and affordable.

The IoT concept has potential for increasing timeliness and standardization (i.e. quality) of data flows because most of the processes are intentionally integrated and are usually designed to be modular (and thus customizable to the customer). While the upfront cost of such systems may be expensive, the implementation of integrative surveillance technologies can return greater efficiency in the long term through reduction of time and manual labor.

Collaboration and Acceptability

Emergency response often involves multiple governmental agencies as well as industry. Therefore, safeguarding agriculture is a responsibility than intersects both the public and private sectors. However, data collection is not readily shared across sectors because the information may be considered proprietary or confidential. There are opportunities to increase data transparency in plant and animal health surveillance through bilateral data sharing agreements, anonymization of data, or transformation of data that shows deviations from a baseline. Transparency does not necessitate sharing of raw data. If there are agreements that can be drawn as to the necessary information and some innovative thinking about how to communicate it, there is more potential for greater coordination and fewer points of vulnerability in an emergency response.

Point of care (POC) systems that focus on tests that can be performed in the field by the producers themselves are an example of new technology that could improve early detection but that are limited by lack of development in the area of trust. Use of these new technologies are limited by the need for regulatory authorities to maintain control of information. False positives can have a severe detrimental effect on regional and national economies and the chain of custody of sensitive information within a regulatory entity is well defined to prevent such economic impacts. Well-defined collaborative agreements need to be developed and accepted before POC system would be a viable option for early detection.

CASE STUDY: ASIAN GYPSY MOTH

Background

Gypsy moth is a serious forest pest, affecting hundreds of species of trees present in North America. The European gypsy moth (*Lymantria dispar dispar*) was introduced to the U.S. in 1869 and has since established in the northeastern United States. This pest feeds on over 300 species of deciduous species, but prefers oak, alder, and poplar (Montgomery & Wallner, 1988). Large infestations of gypsy moth in larval stages can completely defoliate a tree, leaving it susceptible to disease or attack by other pests. Repeated defoliation over two or three years can lead to death of large sections of a forest (Savotikov, Smetnik, & Orlinski, 1995). USDA spends approximately US$10-13 million each year to slow the spread of the European gypsy moth (Tobin, 2008) into new areas. Gypsy moth defoliates an estimated 700,000 acres of forest each year (USDA, 2016a). These defoliation events cause hundreds of millions of dollars in economic losses to homeowners, recreation, and the timber industry (Gottschalk, 1990; Payne, White, McCay, & McNichols, 1973). While European gypsy moth is a continuing threat to North American forests, the Asian gypsy moth is a more serious threat.

Asian gypsy moth is a pest complex of defoliator moths (*Lymantria dispar asiatica*, *Lymantria dispar japonica*, *Lymantria albescens*, *Lymantria umbrosa*, and *Lymantria postalba*) with native geographic distributions in Russian, China, and Japan. The Asian gypsy moth (AGM) is a generalist, affecting up to 600 host tree species (double that of EGM), including both deciduous and coniferous species. The main mechanism of spread is larval ballooning, where the first larval instars release a silken thread and float through the air to nearby host trees to feed. Unlike the related European gypsy moth subspecies, African gypsy moth females can also fly (up to 25 miles), increasing its invasive spread potential.

Asian gypsy moth (AGM) does not occur in the U.S., but several incursions have occurred through the transport of egg masses on cargo ships. AGM moths are attracted to light sources, so the abundant lighting at seaports draw female moths to gather and lay eggs on ships docked at the port. Females can lay up to 1500 eggs per egg mass, attaching the egg mass to any surface on a cargo vessel, including walls, machinery, rigging, and tarps. As this is a known pathway, cargo ships originating from AGM-affected countries must have certification that they are free of AGM before arriving in U.S. ports (USDA, 2016b). Even with international cooperation to ensure AGM-free shipments, egg masses are incredibly difficult to detect so the potential for introductions remain. The earliest known incursion to the United States occurred in the port of Vancouver, British Columbia in 1991, with subsequent detections in Washington and Oregon (Savotikov et al., 1995). The largest AGM introduction occurred in 2015, such that several detections of Asian gypsy moth occurred across Oregon and Washington. In this case study, we review the response to the 2015 AGM incursion in the Pacific Northwest.

Phase 1: Risk Assessment

After detections were reported in various locations in Oregon and Washington from annual detection surveys, an expert panel of scientists formed a technical working group to review AGM pest biology and known information on the detections for the purpose of making recommendations on the survey design and treatment of the AGM incursion. Fortunately, the well-studied European gypsy moth (EGM) subspecies (*Lymantria dispar dispar*) is an excellent analog for the AGM species. However, important distinctions remain: the wider host range and the ability of females to fly. At the time, it was assumed that AGM phenology was similar to EGM but subsequent studies have demonstrated some differences (Gray & Keena, 2019; Limbu et al., 2017; Trotter, Limbu, Hoover, Nadel, & Keena, 2020).

The technical working group (TWG) gathered information regarding lab diagnostics on the captured moths. Genetic sequencing suggested different populations sources from East Asia, the Asian mainland, and Japan, from which the TWG inferred that the detections were the result of multiple introductions. Additionally, the detections were scattered across the two states across multiple geographic locations (Tacoma, Kent, Nisqually, Puget Sound, and Portland/Vancouver) therefore the moths did not appear to have naturally spread from a single source population or release point. In some of the reported locations, multiple moths were detected. Given the trap density in these locations and low detection rate of the traps (~2-12% moth capture rate depending on the trap density), it is likely that there were reproducing populations in some of the areas. Given that AGM females were likely in these areas, the uncertainty about source populations, and the agency's eradication policy, the TWG recommended an aggressive delimiting and treatment program (USDA, 2015).

Phase 2: Delimitation and Eradication

The USDA-APHIS-PPQ Asian Gypsy Moth Survey (AGM) and Response Guidelines (USDA, 2014) is the response manual that provides the technical and general guidelines for detection, delimitation, and eradication of Asian gypsy moth. As Asian gypsy moth is a univoltine pest (one generation per year) and there is a short season in which to mitigate, the surveillance plan simultaneously delimited the population while monitoring efficacy of eradication. Insecticide treatments occurred in the spring during the caterpillar stage while subsequent surveillance occurred in the fall, targeting adult moths. The surveillance objective was to detect any Asian gypsy moth that may have escaped the treatment area so that next season treatments could be adjusted accordingly.

The response manual states that the treatment area should encompass a minimum of half a mile in diameter from the detection (total area of 1 square mile). Some detection sites were in sufficient proximity to each other (Table 3) that the TWG recommended a combined treatment area with a minimum bounding area of a ½ buffer around the combined detection area (Port of Tacoma and cross-border locations between Vancouver, WA and Portland, OR).

Due to the generalist nature of the pest, targeting surveillance to particular tree hosts was not feasible. Therefore, a systematic detection grid of pheromone traps was used in the surveillance. Surveillance to support delimitation and eradication occurred within the same year of treatment (to detect and delimit any adult moths that escaped spring treatments) and surveillance continued for two years to monitor population levels and ensure eradication success. The AGM response manual recommends 49 traps per square mile in the core surveillance area around the area of treatment (USDA, 2014). However, given the large combined treatment areas, the TWG increased the range of the core surveillance area from 2

Table 3. Areas of treatment and proposed level of surveillance for each location with Asian gypsy moth detections

Location	# AGM detected	Area of Btk Treatment	Trap Arrangement		
			Trap Group	*Miles from Detection*	*Trap Density (sq/mi)*
Kent, WA	2	640 acres	Core Extended	0-3 3-6	36 25
Tacoma Port, WA	4	7000 acres	Core Extended	0-3 3-6	36 25
Gig Harbor, WA	1	600 acres	Core Extended	0-3 3-6	36 25
Nisqually, WA	1	640 acres	Core Extended	0-3 3-6	36 25
Lacey, WA	1	640 acres	Core Extended	0-3 3-6	36 25
Vancouver, WA	1	800 acres	Core Extended	0-3 3-6	36 25
Portland, OR	2	8000 acres	Core Extended	0-3 3-6	36 25

to 3 miles but reduced trap density from 49 to 36 traps per square mile (USDA, 2015), so that detection efficiency remained sufficiently high while reducing trapping costs over the wider geographic area.

The timing of deployment for the traps was based on European gypsy moth (EGM) phenology (Régnière & Nealis, 2002), specifically when adult moths emerge and male moths fly in search of females for reproduction. Moth development is based on the accumulation of heat units, so warmer days lead to faster insect development to adult life stages. EGM is a very well-studied species in North America, which serves as an analog for understanding AGM development and timing of adult flight. Gypsy moth produces a single generation per year, making it easier to target the timing for treatment of larval stages or trapping of adult stages. However, AGM is a pest complex that is native to a different geography and climate conditions which may result in adaptations and biological differences from EGM (i.e. uncertainty in predictions using EGM models). The TWG recommended adding a one-month buffer before and after the phenology model predictions for adult flight to account for uncertainty in model predictions and weather variability (USDA, 2015). Areas of treatment and surveillance were reduced for waterways and in some cases, access to tribal lands. State departments of agriculture had the option to increase surveillance effort above the recommendations, such as increasing density of traps.

The preferred tool for AGM surveillance is a pheromone trap, attracting males over several miles in search of the female emitting the sex pheromone. Gypsy moth pheromone lures contain 0.5 milligrams of disparlure in either a PVC-coated string or laminated plastic strip. These dispensers provide slow release of the attractant into the air over a period of several months. The lures are used in delta or milk-carton traps. Delta traps are used outside of areas that are generally infested with gypsy moth, where catch is expected to be less than 10 moths per trap (USDA, 2014). Due to the longevity of the lure, a single trap can last an entire trapping season. Traps are hung in trees 1.5 meters from the ground from host trees. Traps are checked biweekly and only replaced if the trap is full or damaged (USDA, 2014). A trap is considered negative if no Asian gypsy moth has been captured over the course of the flight season. A summary of the surveillance scheme for the eradication of Asian gypsy moth is shown in **Table 4**.

Table 4. Surveillance design for the delimitation and eradication of Asian gypsy moth

Factor	Description
Scope	Target treatment and surveillance within 6-mile merged buffers around detections (core plus extended surveillance areas).
Target	Hang trap within any tree canopy, 1.5 meters from ground.
Timing	Based upon adult life stage (flight) of European gypsy moth phenology, with 4-week buffer around estimated flight time to account for uncertainty
Tool	Disparlure-baited milk carton or delta traps
Volume	Trap density 36 traps/mi in core area (0-3 miles) and 25 traps/mi in extended trap area (3-6 miles)
Frequency	Continuous trapping through flight season, with follow-up trapping in subsequent two years. Trap checks minimum every 2 weeks for potential trap replacement if full.

Several treatments are approved for controlling gypsy moth populations, but *Bacillus thuringiensis* var. *kurstaki* (Btk) is a preferred treatment. Btk is a targeted bacterial insecticide that is lethal to larval instar stages I and II. It is applied every 4-10 days aerially but may have effects on nontarget caterpillars. Btk has a short half-life in the field and is approved for use in organic agriculture and in organic gardening (USDA, 2014).

Washington State Department of Agriculture applied Btk three times to seven sites totaling almost 10,500 acres in April and May of 2016 when gypsy moth was estimated to be in its caterpillar stage (WSDA, 2016). Oregon similarly applied Btk to the Portland side of a cross-border treatment area three times in the spring of 2016 (ODA, 2018). Neither Washington or Oregon caught Asian gypsy moth in 2016 or 2017 traps, indicating that the aerial insecticide treatments were effective and that no new introductions occurred. The eradication of the 2015 AGM invasion was considered successful. USDA continues annual detection surveys for gypsy moth nationally as there is always the potential for new introductions.

CASE STUDY: RABBIT HEMORRHAGIC DISEASE

Background

Disease and pest outbreaks can vary in size, scope and complexity, ranging from a detection and successful eradication, to a widespread infestation that takes root and becomes endemic. Rabbit hemorrhagic disease virus (RHDV) is an example of a hazard that was detected on US soil and rapidly became endemic. RHDV is an acute, highly contagious viral disease of rabbits with mortality rate of up to 90% among naïve populations. There are two known antigenically distinct serotypes of RHDV: RHDVa and RHDV2 (OIE, 2018). RHDVa has a short incubation period of approximately 1-3 days and a mortality rate of 80-90%, however, the host range is limited to European rabbits. RHDV2 has a slightly longer incubation period (3-5 days) and a mortality rate of 5-70% (OIE, 2018). Infection primarily occurs through the fecal-oral route; however, insects are suspected to be an important route of mechanical transmission (Asgari, Hardy, Sinclair, & Cooke, 1998; McColl et al., 2002). This case study will focus on an outbreak of RHDV2 which has a wider host range and is known to cause disease in both domestic and wild North American rabbits.

There are two primary tests used for detection of RHDV2 in the U.S.: antigen enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) (USDA, 2018b). Liver tissue is required for the antigenic tests and therefore all antigenic diagnostics are performed post- mortem (USDA, 2020). Due to the short incubation period, high mortality, and possibility of cross reactions with native caliciviruses, antemortem testing is not considered to be valuable in epidemiologic or disease control efforts(OIE, 2018). Prior to March 2020, there had been a few incursions of RHDV2 into the U.S.; however, much like the Asian Gypsy Moth, these events were contained and the disease was eradicated (USDA, 2020). The most recent, and ongoing, RHDV2 outbreak was first detected on March 24, 2020 although it is suspected to have begun before that time based on information collected in the disease investigation.

The events described in this case study do not precisely represent the events that occurred during this outbreak. The description of some events was added or altered for educational purposes.

Phase 1: Detection and Delimitation (March-July 2020)

In March 2020, a local New Mexico pet rabbit owner experienced a mortality event leading to the third detection of RHDV2 in the U.S. since 2018 and the first in the southwestern U.S. (USDA, 2020). Prior to this detection among domestic rabbits in the southwestern U.S., there had been reports of regional wild rabbit die-offs; however, the cause was unknown. Wild rabbit death events are not uncommon and the usual culprits, including plague (*Y. pestis*) and tularemia, were suspected. As with any initial detection, the first goal is answering the question "where is the disease and where is it not?" As such, the delimitation process began, and expanded testing was initiated in the region among both domestic and wild rabbits. Approximately 10 days later, the first positive detection in wild rabbits occurred. By the end of April, RHDV2 had been detected in domestic and wild rabbits across 5 states. This event rapidly expanded from a single detection to a multi-state, multi-species outbreak, complicating plans for containment and eradication. Although the hope of eradication faded quickly, containment efforts were implemented concurrently with ongoing delimitation efforts. Unfortunately, the rapid influx of information experienced in this outbreak is relatively common. Rarely is an outbreak as simple as a single detection and eradication. What sets this outbreak apart, however, is the prolonged period of delimitation due to the rapid spread and continued detections of RHDV2 in new regions.

The delimitation phase of this outbreak followed the outline described above. Factors such as scope, target, timing, tools, volume, and frequency were evaluated, and an action plan was generated. Additionally, the virulent nature of RHDV2 was accounted for in the design by targeting animals with clinical signs. RHDV2 infected rabbits show clinical signs (death) within days, reducing the risk of spreading disease through subclinical infections. For this reason, surveillance was limited to those rabbits exhibiting clinical signs. Additionally, due to the limited target subpopulation (rabbits with compatible clinical signs), the scope was expanded to nation-wide.

As RHDV2 had already been detected in two distinct populations (pet rabbits and wild rabbits), two separate surveillance plans were needed to capture the variations in population dynamics. The mechanism for disease introduction into the region was unknown contributing to the challenge of understanding disease spread. Complicating things further, tracing animal movements was not a useful tool for supporting delimitation of the disease. To effectively apply core factors for surveillance design, we must understand key characteristics of both the populations at risk and the disease.

Pet Rabbits

Pet rabbits are typically kept in more controlled environments with limited exposure to other lagomorphs and increased exposure to humans. Some pets are kept indoors, while others are kept outdoors within enclosures. Regardless of the animal's housing or exposures, the increased human interaction increases the likelihood of detection. After all, it is much easier to detect a mortality event when the rabbits are kept within a limited enclosure. Finally, gaining access to pet rabbits, for sample collection and testing, is simple and can be performed by a private veterinarian.

Wild Rabbits

Wild rabbits have a much wider range of exposures than pet rabbits. They are not contained within controlled environments and are exposed to other wild lagomorphs and wildlife. Some wild lagomorphs live in social groups and have regular interaction with each other. Most importantly, identification of infected wild rabbits and access for sample collection and testing are very difficult. Unlike pet rabbits, wild rabbits do not have veterinarians and are only identified as ill when someone (public or government) finds dead rabbits. As is the case with most wildlife populations, this could result in a combination of organized trapping, sampling, and testing campaign as well as opportunistic sampling of sick and dead rabbits reported by vigilant residents. In the case of RHDV2, only follow-up on reported deaths were useful. This limitation typically meant there was a limited convenience sample available of the population we were trying to sample.

Table 5. Rabbit hemorrhagic disease virus (RHDV2) Surveillance Design for Delimitation.

Factor	Domestic Rabbit	Wild Rabbit
Scope	Nation-wide prioritizing counties/states in which disease was previously undetected OR epidemiologic links.	Nation-wide prioritizing neighboring counties/states in which disease was previously undetected. Scope narrowed with information about host range of susceptible wild rabbits.
Target	Rabbits exhibiting clinical signs (death)	Rabbits exhibiting clinical signs (rabbits found dead)
Timing	Immediate	Immediate
Tool	Visual observation of case compatible clinical signs including sudden death followed by testing liver samples with PCR or ELISA	Visual observation of case compatible clinical signs including found dead followed by testing liver samples with PCR or ELISA
Volume	All case compatible samples outside of known infected zone	All case compatible samples outside of known infected zone
Frequency	All cases with compatible clinical signs included sudden death	All cases with compatible clinical signs (if observed) AND rabbits found dead

Table 5 describes the surveillance design for the delimitation phase of the RHDV2 outbreak by factor. You will notice that no specific timing nor frequency is provided during the delimitation phase. This is due to the rapid death of infected rabbits and the fact that the goal of delimitation is to detect a single case within a designated area. As such, resources are focused on those animals that have the highest risk of testing positive: those with clinical signs. For pathogens with an extended incubation period, multiple

testing rounds (typically separated by the duration of the incubation period) would be recommended to increase the probability of detection as animals may not test positive until an extended time post infection. The tools applied to delimitation for RHDV2 incursion include two diagnostic tests. Those used should be very sensitive as false negatives may result in inaccurate delimitation.

This outbreak presents unique challenges because of the variability in housing for the at-risk populations. Pet rabbits are not typically housed in large facilities with a lot of other rabbits. With other populations, you may run the risk of missing the disease if you don't test a certain number of animals (i.e., testing a specified sample size within a flock of 10,000 chickens). In this scenario, however, if a home has three rabbits, and one of them is showing clinical signs, it is much easier to test all rabbits within the epidemiologic unit. For this reason, the volume of testing did not include a number or confidence level. Rather, we aimed to test all case compatible samples outside of the known infected zone. For larger operations with numerous dead rabbits, the recommendation to submit samples of all case compatible animals was adjusted to limited submissions to nor more than five.

In wild rabbit populations, there may be many dead rabbits available for testing although suitable samples may be limited because of scavenging and decay. For a disease with mild clinical signs, trapping, sampling, and testing a specified number of rabbits would be necessary to determine if a particular population of wild rabbits was infected. In the case of RHDV2, death in the wild populations is common and thus a strategy of sampling all suitable dead rabbits found was employed. One limitation on this strategy was resources were prioritized to those regions where RHDV2 had not already been detected.

By the end of June, the delimitation phase had detected RHDV2 in 9 states, totaling more than 175 infected domestic premises in 8 states and wildlife detections in 7 states (USDA, 2021). Hazard responses may occur on a national level; however, sometimes a state level-response is more appropriate. In this case, rabbits are not expressly regulated federally, therefore, they fall under state jurisdiction. Within each state, jurisdiction varies based on local government organization and structure. Some states classify rabbits as livestock, while others have no regulatory authority or regulations related to domestic rabbits. Wild rabbits and hares, however, are regulated by the state's natural resource agency. As a result, each state has the opportunity to design their own surveillance and response plan to the RHDV2 outbreak, fragmenting a coordinated national response. To establish a comprehensive national response, working groups were created with state wildlife agencies, state wildlife veterinarians, state veterinarians, USDA, and the U.S. Department of the Interior. No states were obligated to participate, however, all states with confirmed cases did. These working groups established testing protocols that prioritized testing suspect cases in new counties or states and implemented control measures to reduce spread of disease. The control measures included cancelling all pet rabbit shows within 250 miles of an RHDV2 detection and limiting animal movement (ARBA, 2020).

Delimitation is still ongoing today, and suspect cases continue to occur in other states.

Phase 2: Identifying Health Changes (July-present)

The rapid spread of RHDV2 between March and July, and its persistence in both domestic and wild species despite control measures, severely hindered any hopes of eradication. Once the extent of the outbreak was determined many states began vaccinating pet rabbits to control spread. International declarations of disease freedom require strict adherence to OIE guidelines. A declaration of freedom from RHDV2 must be followed by evidence that the virus has not been detected for 12 months, and that there has been no vaccination campaign during that time. Unfortunately, eradication and freedom of disease were out of reach. As such, RHDV2 was declared stable in affected states and a surveillance system designed to identify health changes was implemented. While affected states have moved into a new phase of the outbreak, delimitation continues nationwide in states in which no cases have been detected.

Table 6 describes the strategy employed to track RHDV2 status in affected states. The events this system aims to capture detections in domestic rabbit, detections in wild lagomorphs, detections in endangered or threatened species, and detections in new species, while monitoring for large spikes in detections in endemic areas.

Table 6. Rabbit hemorrhagic disease virus (RHDV2) surveillance design for identifying health changes.

Factor	Domestic Rabbit	Wild Rabbit
Scope	Within states with confirmed cases	Within States with confirmed positive case AND bordering states
Target	Rabbits experiencing clinical signs OR rabbits found dead	Rabbits experiencing clinical signs OR rabbits found dead
Timing	Immediately when clinical signs are observed OR prior to animal movement or participation in shows	Immediately when clinical signs or dead rabbits are observed
Tool	Examination by qualified personal to determine case compatible clinical signs followed by testing liver samples with PCR, ELISA, or histologic examination	Testing liver samples with PCR, ELISA, or histologic examination
Volume	All cases are tested. Sample size was not determined by a detection threshold.	All observed cases are tested Sample size is not determined by a prespecified detection threshold. Sample size is limited in some states by costs.
Frequency	Only one round of tests required for each premises	Only one round of tests required for each population

Declaring the outbreak stable allowed for a few valuable changes to be made to the response (delimitation to stable health status). First, if a disease is considered endemic, it no longer must be treated with the rigid diagnostic procedures that accompany a foreign animal disease. This allows for expanded laboratory capacity and no longer requires that samples be tested at the Foreign Animal Disease Diagnostic Laboratory (FADDL), reducing the burden on national reference laboratories. Additionally, the location in which this surveillance plan operates (scope) is restricted to states with previous detections. While delimitation continues in non-endemic areas, the surveillance strategy to identify health changes aims to obtain data to recognize high level trends among at risk populations in endemic regions. For example, the detection of a large increase or decrease in case numbers, or seasonal patterns, is valuable data that can be used to prevent future spread to new naïve areas. Future surveillance for the purpose of stable health status will include monitoring wild susceptible rabbit population sizes rather than focusing on hazard detection.

Declaring an animal disease endemic in domestic populations can often have severe international trade impacts. For example, when highly pathogenic avian influenza is detected in a commercial poultry flock there are often significant restrictions on what poultry products trade partners will accept from the U.S. The U.S. rabbit industry has very little international exports (USITC, 2021), suggesting minimal trade consequences for declaring RHDV2 endemic. RHDV2 is continuing to spread to new populations in new states despite movement restrictions and careful disease detection protocols. New detections continue to occur, and it is unknown when a homeostasis will be met.

Business Continuity

While devastating to lagomorph populations, RHDV2 has little to no impact on the current U.S. rabbit industry and international trade; therefore, the nationwide RHDV2 outbreak has not required a business continuity phase. However, for the purposes of this chapter, one hypothetical scenario in which business continuity may be needed is described.

In this hypothetical scenario, suppose a large-scale domestic rabbit producer with 50,000 rabbits located within an endemic state would like to continue exporting an untreated meat product internationally. The international trade partner is skeptical of products coming from a geographic region where RHDV2 has been detected; therefore, a surveillance plan must be designed and implemented to provide confidence that the products being exported are RHDV2 clean. The goal of this system would be to provide evidence that this production facility is free from RHDV2 and accordingly, the scope of the surveillance would be within the facility in question. Dead rabbits and some rabbits in cages near dead rabbits would be targeted and timing would be a function of shipping date. The diagnostic tests would not change from previously discussed surveillance, but volume of testing would be designed to a specific prevalence threshold and probability of detection perhaps according to the trade partner specifications. This surveillance might be conducted each time a shipment was due. Alternatively, a facility may be able to establish freedom according to the next hypothetical scheme and reduce the need for frequent repeated testing.

Establishing Freedom

There is not one correct method to establishing freedom within a geographic area or individual facility. There are multiple options that meet the necessary statistical requirements with varying degrees of practicality that must be considered. One option at the individual facility level is outlined below again as a hypothetical surveillance scheme that is not currently being used.

To declare freedom from disease within an endemic region, the facility must first establish a quarantine to assure the risk of introduction is reduced. Next, there must be active surveillance performed on apparently healthy rabbits as well as those with a higher risk of disease. Without testing all rabbits within the production facility, it is statistically impossible to be 100% confident there is no disease and without an antemortem test available, testing all rabbits is simply not an acceptable option for the producer.

An acceptable disease prevalence in which to detect disease must be determined. RHDV2 spreads rapidly through rabbit populations, especially those in close contact. Consequently, it can be assumed that if disease was present, it would not fester at a low prevalence. Rather, it would be expected to observe a sudden high rate of mortality among rabbits in the facility. This, in theory, would allow for the selection of a higher disease prevalence for sample size calculations. Unfortunately, there are no known cases of an RHDV2 outbreak in a large-scale production facility and it is unclear how the virus will behave. The international trade partner has made it clear that they will only accept minimal risk based on available literature. As such, we must test 313 of the 50,000 rabbits to be 95% confident that there is no disease at a 1% prevalence.

The producer has identified a group of 350 rabbits that are ready for slaughter or will be ready soon. Every rabbit sent to slaughter will be tested until 313 rabbits have been submitted for testing. Provided all 313 rabbits are sent to slaughter within a few days of each other, this degree of testing should be acceptable. For this testing strategy to provide sufficient evidence that the herd is free from disease at the time of slaughter, one must assume that all rabbits in the facility share the same exposures. Keep in mind, if there were an antemortem test available, there would be no need to wait for rabbits to reach slaughter. Instead, they could be tested immediately and be returned to the production unit (**Table 7**).

Maintaining Freedom

Table 7. RHDV2 surveillance design for business continuity.

Factor	Domestic Rabbit
Scope	Strictly contained within the business requiring a freedom status
Target	All rabbits on farm
Timing	Dependent on timing of animal movements
Tool	Strict quarantine and testing liver samples with PCR or ELISA
Volume	A representative sample sufficient to claim freedom with a 1% design prevalence (313) followed by all on farm mortalities
Frequency	Comprehensive initial testing with weekly follow-up tests (maintain freedom)

The expansive testing strategy above provides a snapshot in time of the disease status at the time of sample collection; however, the outbreak has continued. To assure recognition of continued freedom, testing must continue as well. All animal production facilities, unfortunately, have some degree of mortality and RHDV2 is likely to cause a high degree of mortality. Therefore, it can be assumed dead rabbits are more likely to be infected with RHDV2 and are more valuable samples. To maintain a freedom status, the facility must continue to perform diagnostic tests on rabbits found dead in the facility. In effort to reduce the impact of testing on the farmer, we may suggest testing of five rabbits found dead each week. The continued testing over time will add significant value to maintaining a claim of freedom.

Although technically not a part of the surveillance plan, a robust biosecurity system must be implemented to reduce the risk of introduction. This allows for the test results to maintain value for an extended period. In other words, if the daily risk of introduction is very high, diagnostic test results will only be valuable for 1 day, because after 24 hours, the risk of a new disease introduction is high. If the risk of introduction is very low due to enhanced biosecurity, the value attributed to the test results will be maintained longer. Biosecurity is vital for international recognition of freedom status, especially when located in a region in which disease has been detected.

This case study is in direct contrast to the Gypsy Moth case study above. Some hazard events can be contained and eradicated, while other times detection occurs too late, and the hazard has become too widespread to be contained. It is important to understand that this is not a failure of the responders nor the state or federal governments. A combination of factors including rapid spread and infection of a pervasive and social wildlife species made this outbreak impossible to contain. Such is the challenge of dealing with a contiguous and ubiquitous wildlife population. Ultimately, the goal of eradication had to be discarded for a more realistic and useful goal of detecting health changes.

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KEY TERMS AND DEFINITIONS

Business Continuity: Ensuring that an organization’s critical functions are not interrupted or that the business-critical functions will be quickly restored to operational status.

Case: A detection that is suspect, presumptive positive, or confirmed positive for the hazard itself or for host exposure to the hazard. Some forms of detection (such as environmental samples) may indicate potential of the hazard but are not indicative of active spread.

Case Finding (Also Delimitation): Detection of the occurrence of a hazard within an ecological system with the intent to detect geographic boundaries.

Confidence Level: Probability that the true parameter value falls within the estimated interval.

Consequences: Social, economic, and biological impacts incurred from a hazard.

Control Zone: A buffered around and inclusive of the infested/infected area within which surveillance and control operations occur. The control zone ensures that the hazard is contained if it spreads beyond the infected/infested zone.

Delimitation (Also Case Finding): Detection of the occurrence of a hazard within an ecological system with the intent to detect geographic boundaries.

Eradication: Elimination of a hazard to a known detectable level such that mitigations are no longer required.

Exposure: Contact of a host or host system with a hazard.

FAO: Food and Agriculture Organization of the United Nations.

Free Zone: Area outside of the control zone which is considered hazard-free and not actively (or perhaps intensively) surveyed.

Hazard: A biological agent with the potential to cause an adverse effect in natural or agricultural ecosystems.

Hazard Freedom: Achievement and maintenance of eradication of a hazard.

Host-Hazard System: The biology and interactions between two distinct organisms, a pest or pathogen (hazard) and the host (plant or animal) upon which the hazard sustains itself, and the impact of those interactions within an ecological context.

Infected or Infested Zone: A buffered area around presumptive or confirmed positive detections of a hazard. Infected may reference pathogen-based hazards whereas infested may reference hazards that are insect pests or weeds.

IPPC: International Plant Protection Convention.

Likelihood: The chances of a hazard or some other event occurring.

Monitoring: The collection of information for the purpose of assessment of the progress and success of a land use (or fishery management) plan. Monitoring is used for the purpose of enforcement and of revising the original plan, or to gather information for future planning.

OIE: World Organisation for Animal Health (formerly the Office International des Epizooties).

Outbreak: The occurrence of a case or cases in excess of what would normally be expected in a defined community.

Population of Interest: The population upon which a study is based, is actively being managed, or from which inference is being drawn.

Prevalence: Proportion of hosts affected by a hazard.

Production facility: A licensed premise, business, or foreign manufacturing site where any step in preparing agricultural products occur.

Risk: A combination of the likelihood of occurrence and the consequences of the occurrence.

Risk Assessment: Evaluation of the risk associated with a hazard.

Risk-Based Surveillance: The application of risk analysis to inform which health hazards and outcomes matter most, the result of which (risk) is used to prioritize and resource surveillance activities to efficiently achieve the surveillance goal.

Scheme: Collection of factors, risk information, and other parameterizations that make up a surveillance design for a particular objective.

Scope: The geographic extent and populations potentially at risk from a hazard.

Sensitivity: True positive rate, the probability of detecting a hazard when it is present.

Specificity: True negative rate, the probability of not detecting a hazard when it is absent.

Stable Health Status: Health metrics of interest for a population (disease prevalence, population size, birth rate, growth rate, milk production, etc.) maintain a steady rate with a normal level of random fluctuation over a sustained period of time.

Subpopulation: A fraction of a population which has common characteristics of interest in the study.

Surveillance: An organized system of sample design and collection, testing, and reporting intended to collect information about a hazard with a certain likelihood or level of precision in a defined period of time.

Susceptible: A (host) population or subpopulation at risk of becoming affected by a hazard.

Target: The subpopulation or sublocation potentially affected differentially by the hazard, and from which information needs collection.

Target Analysis: An approach that provides the structure of first making the strategic decisions related to identifying the target (who or what) and goal (desired outcome) and then making sound operational decisions that guide surveillance to the right place (where), right time (when) and right tools (how).

Chapter 7

Training, Tests, and Tech:

Deployment of Diagnostic Tools for Biosecurity

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ABSTRACT

Diagnosis of disease is a process of hypothesis, investigation, and synthesis. Regardless of whether a human, animal, or plant is afflicted, the process of diagnosis is strikingly similar. Positioned on the biosecurity continuum between surveillance and response, early and accurate diagnosis is critical to effective mitigation and management of disease. Infectious diseases have the potential to spread among animal or plant populations, jump species barriers, and result in epidemics and global pandemics. Additionally, zoonotic infectious agents can also significantly impact human health on a mass scale. It is critical that infectious diseases be identified and detected in a timely fashion to prevent spread. This chapter will delve into the resources and supporting activities for that process, demonstrated via case studies from animal and plant systems, illuminating similarities and differences in the diagnostic process tools that can be mobilized and enhanced for biosecurity.

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INTRODUCTION

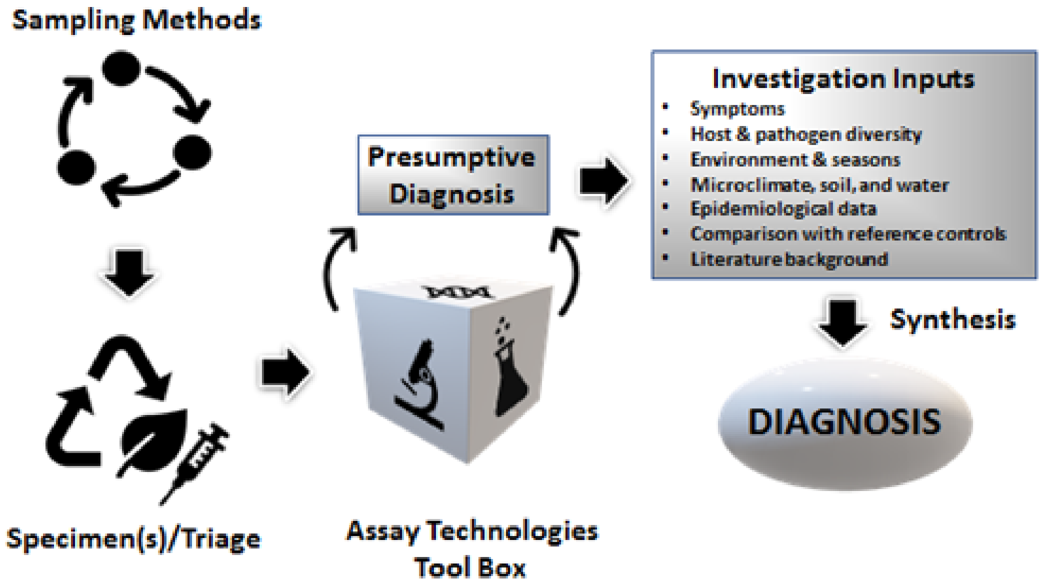
At its most basic level, diagnostics is the process of figuring out what is causing a problem so that a solution can be prescribed. In humans, this might entail visiting a family physician who listens to a patient's description of the problem and symptoms, observes the signs, orders a test swab of throat and nasal passages, and then concludes that the patient has a bacterial sinus infection. The patient then receives a prescription for rest, to support the body's immune system response, and antibiotics, to kill the bacteria causing the current infection. The process deployed by the physician included collection of information from the patient and synthesis of those observations with data from tests as well as education in human medicine and knowledge of current diseases spreading in their community. Animal and plant practitioners use the same process, and even many of the same tools, for the benefit of farmers, ranchers, gardeners, and pet owners, all with the end goal of healthy plants and animals.

Diagnosis of disease is a process of hypothesis, investigation, and synthesis that encompasses hard science skills of investigation and testing hypotheses, as well as soft skills of conversation and imagination. Regardless of whether a human, animal, or plant is afflicted, the process of diagnosis is strikingly similar, even if the occasional term is different. For example, in plant diagnostics, symptoms are the observable plant response to disease, while signs are the evidence of the causal agents, but in human diagnostics, symptoms are what the patient reports, while signs are anything the clinician observes. Since these terms are often used interchangeably in practice, the authors will hew towards the animal and human practitioner for this chapter. Positioned on the biosecurity continuum between surveillance and response, early and accurate diagnosis is critical to effective mitigation and management of disease. The authors will focus on the diagnosis of infectious diseases in plants and animals. Infectious diseases have the potential to spread among animal or plant populations, jump species barriers and result in epidemics and global pandemics (Dhama, 2020; Lau, 2015; Lau, 2017; Mahy, 2000; Marano, 2007; Riedel, 2006; Wong, 2007). These can potentially cause severe economic losses to associated industries at a local or global scale. Additionally, zoonotic infectious agents can also significantly impact human health on a mass scale (Marano, 2007). It is critical that infectious diseases be identified and detected in a timely fashion to prevent spread. Diagnostic methods may be as simple as observing unique clinical signs or as complicated as employing advanced and cutting-edge technologies such as whole genome sequencing, metagenome-based diagnostics etc. This chapter will delve into the resources and supporting activities for that process, demonstrated via case studies from animal and plant systems. The authors will focus on the agricultural systems served by diagnosticians, illuminating similarities and differences in the diagnostic process tools that can be mobilized and enhanced for biosecurity.

BACKGROUND

Diagnosis is distinct from detection and identification. In the previous chapter, detection was defined and described. In this chapter, the authors will focus on the next step: diagnosis of the causal agent once an outbreak has been detected. Identification of an organism is a step in the diagnostic process; several identification tools may be deployed during the process and then synthesize the results to arrive at a final diagnosis (Figure 1). The technology deployed by diagnosticians changes over time, reflecting developments in related sciences and the growing body of knowledge about biological and physical systems. Visual observation of host and pathogen morphology was the first tool, and is still critical today. Many diagnostic services rely solely on microscopy, especially in resource-poor situations, and it is adequate for the majority of routine diagnoses at the local level, especially when coupled with experiential knowledge of local hosts and common diseases. Diagnostic techniques have expanded from observation with our own eyes to many types of microscopy and scanning technologies. The ability to “see” an organism is still critical to the identification of the organism, and microscopy remains the gold standard for many diagnosticians. However, morphology is not adequate for the differentiation of many organisms, especially for regulatory or biosecurity issues. In those cases, one might identify suspect tissues to sample via visual observation, but then deploy additional serological (Enzyme-linked Immunosorbent Assay, ELISA) or molecular tools (Polymerase Chain Reaction, PCR). Many diagnostic techniques and technologies are used in common between animal and plant diagnostic laboratories. From gross examination of specimens to microscopic examination of whole or thin sections looking at cellular structure and changes, from culture of viable organisms (bacteria, virus, fungi) to more advanced molecular nucleic acid (DNA or RNA) techniques such as polymerase chain reaction and, most recently, genetic sequencing. While the targets may differ, the equipment and processes are recognizably the same. There are of course some significant differences as well. Detection of antibodies or other biomarkers produced in response to exposure to a pathogen is a mainstay in animal diagnostics, with no equivalent in the plant diagnostic laboratory. The definition of gold standard for the tool depends on the organism and the specificity to which it must be identified. The deployment of such tools depends on the situation that calls for the diagnosis.

Figure 1. A flow chart of diagnostics from sampling methods in the field or collecting specimens during clinical triage, through testing using predetermined target assay technologies, or a sequence of assay methods if of unknown cause to a presumptive diagnosis, synthesis, and final diagnosis



Sampling

Sampling is a critical step in the diagnostic process. It is essential that appropriate specimens in adequate amount, numbers and frequency be collected in order to obtain an accurate and reliable diagnosis. Diagnostics start with the sample, and sampling varies as broadly as the hosts and the pathogens that infect them and signs that may be produced. Diagnosis of a sample is limited by the sample itself. Sampling is usually performed by someone at the source - on the farm, at the international or state border, in the feedlot, etc. While many diseases have similar signs, the organism's location within tissues is not consistent or necessarily similar between diseases. For this reason, sampling should be guided by the diagnostician or other experts in the field so they may recommend best sampling practices to increase the odds that the sample will contain the necessary tissue for diagnosis. For instance, a leaf spot disease obviously requires fresh leaves with the spot symptom, but a wilting plant may require roots, crown, and/or stem tissue to be examined. Often, an initial consultation with a trained diagnostician including images of the affected individual will help guide collection of an optimal sample, resulting in faster and more accurate diagnoses. Sampling for phytosanitary or surveillance programs may require a certain number of samples, depending on the population; this is discussed in more detail in the detection chapter. Pathogens are also found infecting a variety of host organs in which they accumulate, as well as the environment. In any case, it is preferred to follow sampling method procedures that are statistically validated. In the absence of such procedures, the collector should make an effort to sample a number of specimens representative of the disease or phenomenon. The collection method of the specimens and subsequent manipulation is also important to the integrity of the specimen components, such as nucleic acids or proteins, since these are required for subsequent specimen preparation and analyses. For this reason, sampling procedures must be developed with the possible pathogen and its diagnostics in mind.

The statistics that guide surveying large populations to detect rare biosecurity targets are very different from the process that guides diagnostic sampling for common diseases in a given location. In this chapter, the authors will concentrate on samples resulting from either presentation of disease or those derived from surveillance. The choice of diagnostic method may not change depending on the number of samples; it may be more important whether the disease is routine and can be diagnosed by the practitioner with traditional methods, or whether the target is regulated and the diagnostic process is dictated by standardized and regulated protocols.

Regardless of whether the target is a regulated organism, the entire process of diagnosis is dependent upon the sample - the specimen type (tissue, exudates, etc.), the quality of the sample, the progression of signs, and the stability of the specimen and pathogen in the time between sampling and testing. The pathogen, disease, and test to be conducted determine the sampling location in the affected individual. These factors may not always be obvious. It would seem reasonable to sample from symptomatic areas - wilted leaves, for instance - but the causal agent of the wilt may be distal from the symptom, often in the stem or roots for a wilt disease. Additionally, sample collection directly from affected systems can be destructive and it may be necessary to seek alternative sites that are easily accessible. Lung tissue may be best for confirming a respiratory virus, but nasal or throat swabs are obtainable by the practitioner without the need for surgery. In some cases, direct sampling is not necessary, as tests have been developed to detect products of the pathogen or disease, such as antibodies produced in response to infection. For all of these reasons, sampling should be guided by knowledge of the pathogen and the test(s) to be conducted. While the symptom may guide a practitioner, a practitioner knowledgeable of the disease and test should guide the sampling to ensure that the appropriate type, amount, and quality of tissue is collected. Timing of sampling also should be considered. If the sample is degraded by disease or environmental issues, or if the sample is too small or not representative of the pathogen, diagnostic testing may fail or test results may be invalid. The practitioner must take all of this into account to ensure sampling is precise and adequate. Any time sampling must be repeated, time is lost, and the disease likely continues to progress without treatment.

Forensic Diagnostics

Diagnostics are critical in forensic analyses, which blend epidemiology, pathology, analytical, and criminal sciences to support investigations regarding diseases and their causative pathogens. Diagnostics within forensics provide unbiased, rigorous, and traceable scientific methodology and evidence to determine the pathogen origin, biosecurity pathway(s) of movement, as well as the possible role of human intent, which may lead to criminal attribution. Diagnostics play a relevant role when applied to support trace-back strategies for agricultural biosecurity, which includes science-based policies, measures, and regulatory frameworks for reacting to and managing risks associated with food, agriculture, forestry, and environmental protection (UN, 2002).

Diagnostic Networks

Local knowledge of hosts and their common ailments is useful for diagnosing endemic diseases, those normally found in a geographic area. But pathogens and pests don’t respect political boundaries, so diagnosticians need to be able to identify something new or unusual. Working knowledge of both common and new diseases is developed through experience in the field or lab as well as knowledge transfer between diagnosticians and subject-matter experts during professional meetings and relevant publications. Networks of laboratories and diagnosticians exist globally to foster communication of findings as well as support for diagnosis (Miller et al., 2009). A particular strength of diagnostic networks is the ability to maximize expertise such that a single diagnostician or lab does not have to be an expert in everything, they can call on a known expert for assistance. The availability of resources is what often defines the breadth of services an individual lab can offer, though the scope of the lab is defined by the local need and expertise of the diagnostician. Networks such as the National Plant Diagnostic Network (NPDN) and National Animal Health Laboratory Network (NAHLN) in the US, the Caribbean Plant Diagnostic Network (CPDN) and the Caribbean animal health network (CaribVET) in the Caribbean, the Canadian Animal Health Surveillance Network (CAHSN) and the extensive network of plant clinics supported by the Centre for Agriculture and Bioscience International (CABI) all leverage expertise across states and countries in support of diagnostics. Many of these networks are deployed by government agencies, and make funding available to support both infrastructure and diagnosis at the local level. The NPDN, for example, is a cooperative agreement between the USDA National Institute for Food and Agriculture (NIFA) and individual land-grant university diagnostic programs organized into five regions (Figure 2). NIFA also partners with the Animal and Plant Health Inspection Services (USDA-APHIS) to support the NAHLN laboratories across the US (Figure 3). These programs are often funded by multiple means, leveraging federal and state funds with sample fees and grant projects. As globalization of trade impacts the movement of pathogens and pests, global efforts must be made to prepare for and manage diagnosis of new pest incursions. These networks are actively working to share detection protocols and diagnostic information to protect agricultural biosecurity worldwide. The current efforts of the Global Surveillance System (Carvajal-Yepes et al., 2019) aim to tie these many networks together in the hopes of even better communication.

The development of networks of diagnostic laboratories has facilitated the standardization of diagnostic methods and interpretations. These networks establish standard operating procedures (SOPs) which prescribe the reagents, equipment and protocols used for diagnosis of a disease. For example, each of the forty-seven NAHLN laboratories approved to test for Foot and Mouth Disease uses the same SOP developed by the National Veterinary Services Laboratories (NVSL). More than a dozen NPDN labs are APHIS-approved to use the same SOP for detection of *Phytophthora ramorum*, the causal agent of Sudden Oak Death. A benefit of networks is the ability to deal with rare or routine disease detection and diagnosis, as well as readiness to process high numbers of samples from routine surveillance or biosecurity emergencies. Internationally, the World Organization for Animal Health (OIE) coordinates a network of animal diagnostic reference laboratories as well as establishing international standards for animal disease testing protocols (Anon., OIE, 2018). On the plant side, the World Trade Organization's (WTO) International Plant Protection Convention (IPPC) is a plant health treaty signed by over 184 countries to protect plant health and promote safe trade by setting standards

Figure 2. The National Plant Diagnostic Network (NPDN) regions
Source: (WWW.NPDN.ORG)

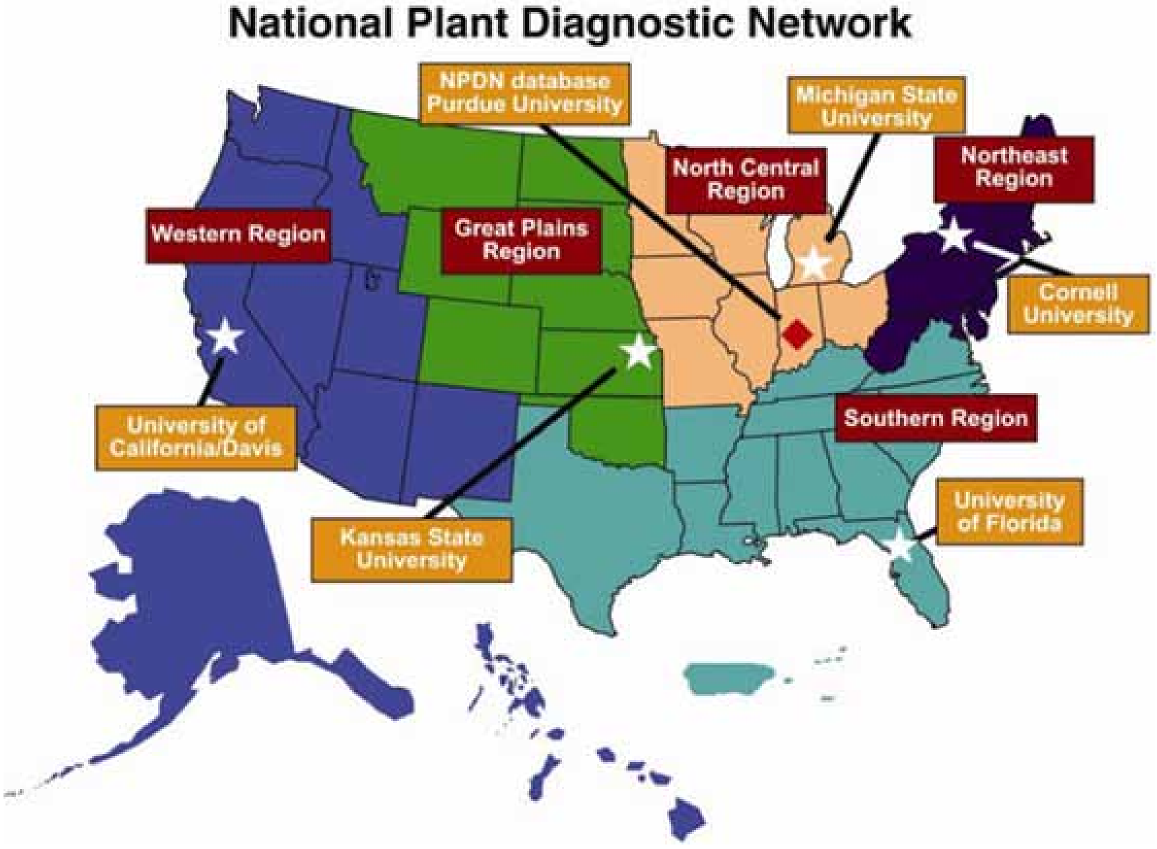
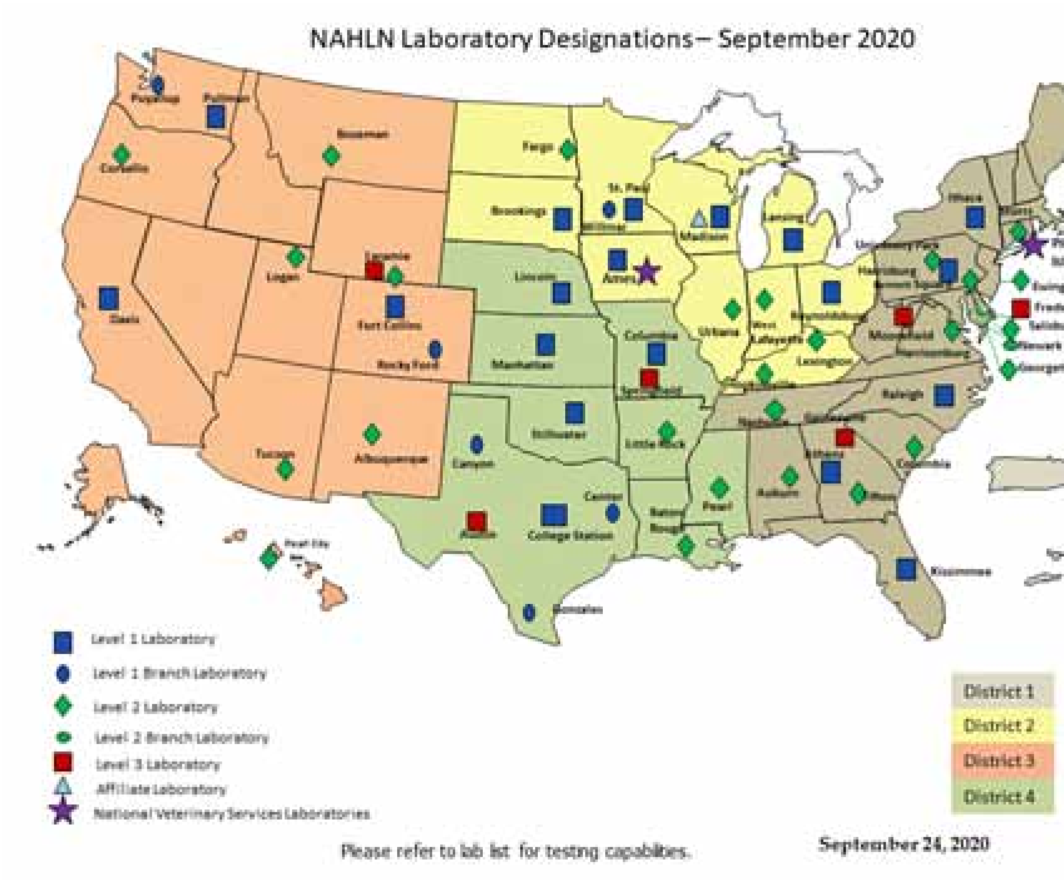


Figure 3. The National Animal Health Laboratory Network (NAHLN)
Source: (https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/lab-info-services/nahln/CT_National_Animal_Health_Laboratory_Network)



and encouraging capacity building to support diagnostics within those standards. The Convention introduced International Standards for Phytosanitary Measures (ISPMs) as its main tool to achieve its goals, making it the sole global standard setting organization for plant health, but the IPPC does not itself support or network the laboratories themselves. The IPPC supports efforts to find donors and governmental aid to loosely network governments with subject-matter experts and groups such as the Consultative Group on International Agricultural Research, the NPDN, and CABI's network of plant clinics. In an effort to concentrate on diagnostic capacity in developing countries, the International Plant Diagnostic Network (IPDN) was a US Agency for International Development, Integrated Pest Management Collaborative Research Support Program (IPM CRSP) funded program operating in 13 countries in Africa, Central America and South Asia (Miller et al., 2010). Based on the NPDN concept in the US and drawing on its expertise, the IPDN focused on the human resources aspect of diagnostics, training dozens of plant health professionals and providing a mechanism to encourage communication between diagnosticians. However, once the grant ended, the program was not sustainable through years of governmental shifts and personnel turnover in the participating countries. As of the writing of this handbook, there is a chance of a global plant network through the Global Surveillance

System proposal to establish and sustain a coordinated strategy of communication and harmonized diagnostics (Carvajal-Yepes et al., 2019). This program is focusing on sustainability and leveraging of regulatory, industry, academic, and NGO entities to ensure participation, regulation, and data sharing are supported by each participating country. The success of the US networks (NPDN and NAHLN), operating continually since 2002, may lie in the leveraging of the partnership and infrastructure of the land-grant institution in every state, sustained through the federal funding structure of each network program. For the NPDN, USDA enacts a cooperative agreement with each of the five regional centers, who in turn sign cooperative agreements with each of their states/territories. The cooperative agreement requires participating institutions to demonstrate support, whether financial or in-kind, encouraging sustainability of the program locally and nationally. For NAHLN, USDA supports agreements with individual laboratories to identify expectations and fund infrastructure needs.

Laboratory Infrastructure

The diagnostic laboratory in animal systems is an analytical laboratory, serving the needs of the licensed clinical practitioner or regulatory diagnostician, usually a veterinarian in a public or private practice who sends samples or specimens to the analytical lab and then interprets the results of the tests and prescribes therapeutic interventions or a regulatory response. This clinical practitioner is also the starting point of communication about new or high-impact detections. In plant diagnostics, specimens or samples are sent to the diagnostic lab by the grower or person in charge of plant care. The clinical practitioner in this case is a diagnostic professional in the laboratory, who talks to the submitter, triages the sample, tests hypotheses, and performs the interpretation and prescription of therapy. Because of the differences in how the laboratory and practitioner interact, the NAHLN and NPDN labs are distributed differently.

At least one NPDN lab exists in every state, nearly always located at the main land-grant university, which is the institution in the state responsible for agricultural education and extension service to its citizens. The NPDN currently has more than 70 active laboratories trained to detect and diagnose routine and high-impact diseases and pests (see map at NPDN.org). The USDA-APHIS Plant Pathogen Confirmatory Diagnostic Laboratory (PPCDL) in Laurel, Maryland, is responsible for regulatory confirmation of new or high consequence diseases detected by any one of the NPDN or state department of agriculture labs.

The 60 NAHLN laboratories comprise entities across the US affiliated with either or both universities and state departments of agriculture. In addition, the network includes Federal laboratories associated with agencies outside of APHIS. The NAHLN is served by NVSL for official confirmation of regulatory testing, especially for high consequence foreign animal diseases.

All of these labs require physical infrastructure, in addition to the skilled personnel who staff them. A basic plant diagnostic laboratory can diagnose most routine samples with a relatively short list of equipment and a modest laboratory space. A fully functional analytical lab for identification of not only common but new or emerging pathogens requires a more extensive dedicated infrastructure. These expert-level labs should have a plan for replacement and adoption of specialized equipment to stay current with technological advances that enable the speedy and specific identification required of new or high-risk organisms. Laboratories at this advanced level must also be involved in development and validation of new diagnostic methods and equipment. Finally, biosafety must be considered to protect those who work in diagnostic labs as well as the environments around the labs. Basic precautions against accidental exposure to infectious agents should be documented and all staff trained. Given the wide range of immunocompromising issues such as diabetes, pregnancy, autoimmune disorders, etc., all cultures and specimens should be handled as though they may be infectious to humans. The number of cases documenting seemingly innocuous plant pathogens as human pathogens is growing, and includes Pythium, a common plant pathogen that can cause pythiosis in humans and animals, and several common bacteria that can infect through the eye (Presser & Goss, 2015; Szczotka-Flynn et al., 2010). Gloves, face shields, and containment of specimens are simple but effective safety precautions. The WHO defines biosafety levels and a short training on all four levels is available on the website of the US Centers for Disease Control (CDC). The CDC also publishes the *Biosafety in Microbiological and Biomedical Laboratories* handbook, considered a gold standard reference for good biosafety practices in laboratories (DHHS, 2020). Labs that will receive samples limited to their state and without zoonotic potential may be able to function with Level I biosafety, though care should be taken to avoid accidental exposures of all pathogens as a basic protection. Level II is appropriate and adequate for most endemic and non-regulated pathogens, and many plant and animal diagnostic labs operate predominantly at this level. However, for those labs that need to handle potentially exotic, zoonotic, or otherwise high-risk pathogens in their samples, Biosafety Level III is recommended. The critical aspects of the permits and regulations that underpin these levels are containment of potential airborne or water-borne pathogens, protection of the diagnostic staff, and the ability to render the pathogen nonviable to protect against any escape.

Laboratory Funding

Funding for labs is nearly as varied as the diseases they test for. Depending on the clientele they serve and the institution in which they are housed, the labs are funded through a combination of testing fees, institutional support, state budget dollars, industry contributions, and federal or state grants. Dependability of funding is important in ensuring a lab or a network can detect new diseases or adopt new methods. The diagnostic personnel must be able to avail themselves of up-to-date and relevant training, replace aging or obsolete equipment, and adapt to new protocols needed by their clientele for biosecurity. However, adoption of new methods may require purchase of equipment or supplies that don't fit the annual budget. In these cases, partnering with research or training projects may allow the lab to acquire the training and equipment necessary for diagnostics, while sustaining equipment maintenance and consumables with fees from the service side of the lab. In those labs that charge for their services, fees make up a substantial portion of the annual budget, often more than half for animal diagnostic labs. However, fees alone cannot sustain a diagnostic program that must be responsive to public health issues and horizon-scanning for new and emerging diseases.

The NPDN and NAHLN programs fund a small portion of diagnostic needs based on annual Congressional appropriation levels. This funding typically is used to support personnel, equipment and supplies, but it is not enough to completely fund any single diagnostic program. This relatively small amount is meant to encourage the cooperative nature of the participation agreement with the institution and state that houses the lab. The institution has to demonstrate real support for their diagnostic lab and personnel prior to the signing of the original network agreement, and every new agreement since then. This allows the lab and the institution or state to seek ways to leverage the federal grant dollars with other revenue streams to allow the lab to serve the changing needs of its clientele and fully participate in the NPDN or NAHLN.

Diagnostic Workflow

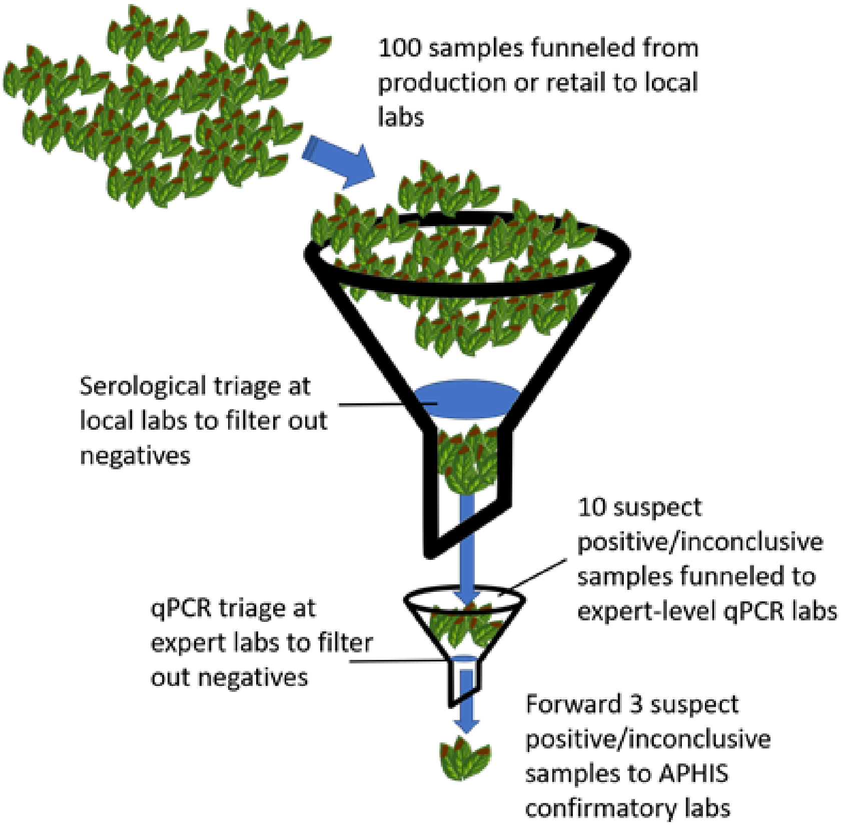
The typical workflow for sample diagnostics begins with the clinical practitioner's observation of signs. This may occur in the field, in the veterinary office, or the plant diagnostic lab. These signs, timing of signs onset and history of any related issues are combined with a practitioner's experience and training to form testable hypotheses. Whether the tests happen in the practitioner's hands, or the practitioner interprets tests run by a lab doesn't matter as much as their training, experience, and access to resources and references. Either way, it is important that the practitioner have a working knowledge of common local diseases, and some training in biosecurity to detect something new. The first level of tests may include visual observation at a macro and microscopic level. Subsamples of tissues or other targets are then selected and collected for more definitive testing such as serology or microscopy. Neither serology nor microscopy are likely to be confirmatory for the specific identity of a new or regulated organism but are often enough to diagnose common local diseases and prescribe management. If serological or morphological tests cannot discriminate the causal agent, further methods such as pathogen culture or those using nucleic acid detection will be employed. Considerations for the choice of method include fitness for purpose, which may change depending on the economic or epidemiological impact of the diagnosis and its management. It is critical that both the diagnostician and the clinical practitioner understand the test's limitations so they can make appropriate decisions. Routine diagnostic testing may lead to confirmatory testing if a regulatory pathogen is suspected at the triage level. In regulatory diagnostic situations, results of tests conducted at the triage lab, even PCR, may need to be confirmed by additional testing at the USDA-APHIS confirmatory lab, either the PPCDL for plant pathogens or the NVSL for animal pathogens, even if the initial triage laboratory is a state department of agriculture regulatory laboratory. A specific and standardized chain of communication and sample testing is followed to ensure confidential, secure, and timely notifications and accurate diagnoses. Confirmatory testing must be done by the official regulatory lab responsible for such testing because regulatory response may be initiated based on the test results.

High-throughput Capacity

Disease outbreak events may result in the collection of thousands of samples over a short period of time. In the best-case scenario, these samples are collected according to a standardized protocol designed specifically for the organism of interest. Additionally, the availability of testing capacity should be determined prior to the collection of samples so that no single lab is overwhelmed with samples. When sample number surpasses testing capacity, samples may degrade before they can be tested, resulting in potential misdiagnoses and/or lack of test-based information. Since such biosecurity events often require rapid response to delimit and mitigate, sample turnaround and result communication must be efficient. Diagnostic networks can be valuable in disseminating standard protocols, controls, and communications, while sharing the testing load. However, there are some procedures and organisms where only a very small number of laboratories have the equipment, permissions, or expertise to handle sample diagnosis. In these cases, testing capacity should be designed into the sampling and testing system at the outset. The USDA's NAHLN and NPDN laboratories have responsibilities for specific diseases, and are supported, in part, through federal funds to maintain that capacity.

The USDA's NPDN assesses network capacity for overall routine samples, plus specific capacity for biosecurity organisms on an annual basis. USDA-APHIS-Plant Protection and Quarantine (PPQ) National Plant Protection Laboratory Accreditation Program (NPPLAP) has three programs that support standardized diagnostic testing for plum pox virus, *Phytophthora ramorum*, and the citrus greening pathogens. On the animal side, USDA-APHIS includes the National Veterinary Services Laboratories (NVSL), providing national reference laboratory testing for diseases_in swine, poultry, cattle, horses, and other animal systems. These national systems ensure that state-level diagnosticians have access to standardized and validated protocols, appropriate vetted controls, and a documented and accessible system of triage and expert laboratories. This funnel-and-filter system (Figure 4) spreads out screening or triage-level testing among many local labs. These labs can determine negatives quickly, which allows the local regulatory officials to release holds on material for sale, minimizing damage to the industry. Suspect positive samples are then funneled to USDA-approved labs for confirmatory testing. The distribution of these labs across the US means that samples can be processed quickly and no single lab is overwhelmed with samples. These labs filter out all negatives and communicate screening results with the state officials. A small fraction of samples suspected to be positive are then forwarded to the regulatory expert-level labs for confirmatory testing. Any positive tests at this level initiate regulatory response such as destruction

Figure 4. "Funnel and filter" method for high-throughput sample screening and confirmatory diagnosis on a network-wide level
Source: (Adapted from Smart et al. 2020)



and tracing efforts. In this system, the local labs filter out negative samples to serve industry and local clientele and the regulatory labs confirm positives and initiate response, ensuring appropriate activities based on legal roles and responsibilities.

Diagnosis of New or Emerging Diseases

Clinical signs can be the primary basis of diagnosis during disease outbreaks or in the case of diseases endemic to a region. Such diagnoses may be less accurate but necessary due to limitations of test resources, economic reasons or timeliness. Also, downstream management and treatment practices of other diseases on the differential diagnosis list may not be significantly different, making a specific diagnosis less valuable. For example, in many developing countries parvovirus and canine distemper infections in dogs are endemic and commonly diagnosed based on typical clinical signs such as diarrhea and neurological signs. Viral diseases in plants are often diagnosed by signs alone, especially when field management is the goal. Many fungal plant diseases are likewise diagnosed by characteristic plant symptoms and a simple microscopic identification of expected spores or other fungal signs. Even though advanced diagnostics are available, access and affordability of such technology might be challenging in resource limited laboratories. No single laboratory can test for all possible diseases or pathogens. Diagnostic laboratories primarily employ tests designed and validated to detect and identify the disease pathogens that are expected to occur in the animal or plant host species and geographic area that the laboratory serves. The goal in diagnostics has been to develop tests that are both highly sensitive and highly specific. Highly specific tests though are more likely to miss a new or emerging disease pathogen, even one that is closely related, though not identical, to the pathogens that the laboratory currently has validated tests for. For many years, non-specific tests such as bacterial cultures or viral cell cultures were the mainstay for detecting novel pathogens. However, not all pathogens can or will grow in the artificial growth media or cell lines routinely used in the laboratory. Even those that will, still have to be identified by further characterization work, which can be arduous, time consuming and not always successful.

The newer molecular testing technologies (e.g. gene sequencing, metagenomics) avoid the need to have a viable pathogen that can be grown, instead directly detecting the genetic material of the organism, even if it is non-viable. These techniques are also usually pathogen agnostic with unlimited multiplexing capability. A single procedure is used for generating data that is analyzed for detecting any and all organisms within a sample. With this technology, detection and identification are largely merged into a single process. As an added bonus, reading the genetic material of the pathogen can also provide other important functional information such as antibiotic resistance or toxin production capabilities. While imperfect, these techniques are currently favored by laboratories when the need arises to further explore a disease problem that “walks like a duck and quacks like a duck” but isn’t a duck, or at least not a duck seen before. As these technologies become more understood and mainstreamed, the cost per sample, turnaround time, throughput capacity and challenges of test result data analysis will cease to relegate their use to only a small subset of cases where the usual test methods have failed to identify the cause of a disease. This technology is evolving very rapidly and will almost certainly become routinely used in the diagnostic toolkit of the near future.

Quality Assurance in the Diagnostic Laboratory

Multiple factors may influence the choice of a diagnostic laboratory for testing including cost, turnaround time, convenience or ease of use, but the most important of all, is accuracy or reliability of the testing results. The reliability of laboratory testing rests on the foundation of:

- Use of validated test methods
- Adequate and current training of staff
- Periodic proficiency testing to ensure ongoing competency
- A formal documentation process, the Quality Management System (QMS)
- Auditing and accreditation of the laboratory by national or international independent organizations

Figure 5. Factors influencing the reliability of a laboratory

Use of Validated Testing Methods

It is critical that all test assays used in a laboratory have clear, complete documentation of the evaluation of the performance and reliability of the assay. The type and extent of the evaluation of the assay's performance will vary depending on whether it is an assay developed *de novo* in the laboratory (validation) or is merely the implementation of a commercially available kit or previously validated, published assay or modification of such (verification).

Validation of a *de novo* assay typically occurs in stages. The first stage, **analytical validation**, is usually part of the development process and involves determining the performance of the assay when used on artificially contrived samples (e.g., adding a controlled amount of the assay target to the typical sample matrix) made in the development laboratory, often referred to as a "spiked sample". The second stage, **diagnostic validation**, consists of running the assay on samples naturally derived from actual field conditions or experimentally infected animals or plants. To evaluate the performance of a new assay on field samples, the results of the newly developed test are compared to the results of the same samples tested for the same target pathogen using another fully validated test method, often referred to as a “gold standard test” for that target pathogen. This second stage of validation is critical due to the much higher variability in the makeup and condition of field samples compared to laboratory created spiked samples. The validation process includes various statistical measures of performance. The most common are Sensitivity (the ability to detect the target if it is truly present) and Specificity (the ability to not falsely detect the target if it is truly absent). For more in-depth detail on proper test validation, see *Chapter 1.1.6, Principles and Methods of Validation of Diagnostic Assays for Infectious Diseases* in the *OIE Terrestrial Manual, 2018* (Anon OIE, 2018; Cardwell et al., 2018).

Verification is a simpler, less comprehensive process of determining if the test assay performs the same in your laboratory as the manufacturer of the kit or the published validation literature indicates. This also applies to re-assessing the performance of an assay that has been modified, up to a point. This verification usually involves running the test on a smaller number of already well-characterized field samples than required for full validation.

During the initial development and validation of an assay, it is also important to develop a set of **controls**, both positive and negative, that can be run alongside the assay routinely. These controls provide both intra-assay and inter-assay, ongoing indication of the performance of the assay. Depending on the assay, these controls may be run once for every sample, once for each batch of samples run at the same time or at least periodically, to ensure that the performance of the assay has not changed beyond acceptable limits. For some assays, the results for the controls are actually used as the benchmark to interpret the results for the sample run.

Going hand in hand with validation or verification of an assay is the formulation of the **standard operating protocol (SOP)** that will be the ongoing standard for the routine execution of the assay. The SOP specifies all steps and critical influences on the performance of the assay, reagents, equipment calibration and use, environmental factors and more, including interpretation of assay results.

Staff Training

The human component of the laboratory is still the most critical in ensuring the quality and reliability of the testing conducted. It is not sufficient to train staff initially, they must be kept current in their area of testing responsibilities through continuing education and their performance routinely evaluated through blinded proficiency testing and other means. Documentation of staff training is essential.

Proficiency Testing

Even with day-to-day quality control efforts, it is important that periodic evaluations are made to independently determine the proficiency of the staff and the accuracy of the testing conducted. Proficiency testing, using well characterized, blinded samples provided usually by an independent, certified or accredited source, is an accepted way to demonstrate this. While it is ideal to demonstrate proficiency for each individual staff member that conducts testing, in some situations it may only be feasible or necessary to evaluate proficiency at the laboratory level. Proficiency testing can also be used to rapidly increase the number of staff certified to perform a critical test method needed during a disease outbreak response.

Documentation

A general principle of quality assurance is that if it is not written down, it does not exist. Comprehensive, logical documentation of all the processes involved in testing operations, from SOPs for individual tests, to quality control records, staff training records, equipment maintenance logs, and etc. are the corpus of the quality management system (QMS) and the tangible "proof" of quality. Whether the documentation exists in hard copy or electronically, it is the touchstone for all testing processes and must therefore be kept up to date and strictly adhered to. Such documentation is also useful for resolving disputes of or questions about a test result and serves as an historical record.

Accreditation and Certification Standards

There are formal, independent, nationally- and internationally-recognized quality management systems (QMS) which define the minimum policies, practices and procedures considered adequate to prove the quality and reliability of laboratory testing results. Users of diagnostic laboratories should always inquire as to the QMS or standards the laboratory operates under. Laboratory accreditations or certifications should be prominently displayed on the laboratory website or otherwise publicly available.

The most widely recognized standards setting body is the International Standards Organization (ISO, www.ISO.org). The ISO creates unbiased standards for a variety of testing and business activities. The relevant ISO standard for animal and plant diagnostic laboratory accreditation is **ISO/IEC-17025**.

There are two types of ISO standards, those used for accreditation and those for certification. These two terms are often used interchangeably and can be confusing. Under ISO, **accreditation** is for individual activities (e.g., an individual test or test method), whereas **certification** applies to the activities of an entire organization (e.g., the laboratory). The distinction is important when assessing what a non-ISO QMS really means, as use of the accreditation and certification terminology is inconsistent. Is it focused on assuring the quality of a specific test' performance and results (accreditation) or does the QMS provide more general assurance of the operational protocols and processes of the organization (certification)? Some QMS are hybrids, containing standards for both an organization's operations and individual activities. Each country may have one or more ISO-accredited auditing organizations capable of providing the external evaluation of a laboratory's successful compliance with the ISO/IEC 17025 standard and awarding accreditation.

In addition to the ISO international standard, there are national level accreditation or certification programs, often for regulatory testing and administered by regulatory agencies or national professional organizations. In the US, the USDA specifies and enforces quality standards for regulatory testing in both the NAHLN and the NPDN testing networks as well as other regulatory testing. The NAHLN and NPDN quality programs are both based on the ISO/IEC-17025 standard. National professional organizations such as the American Association of Veterinary Laboratory Diagnosticians (AAVLD.org) and the National Plant Diagnostic Network (NPDN.org) encourage professional development and certification. The NPDN has a high-level laboratory certification program called the System for Timely, Accurate & Reliable Diagnostics (STAR-D), and a base-level called CORE. The CORE standard aims to institute best practices in all NPDN laboratories. Neither of the NPDN certification levels are strictly for regulatory purposes, but instead cover all testing in a lab. USDA-APHIS administers the National Plant Protection Laboratory Accreditation Program (NPPLAP), which certifies diagnosticians to run molecular tests for three regulated organisms: Plum pox virus, *Phytophthora ramorum*, and *Ca. Liberibacter asiaticus* and *americanus*. All NAHLN laboratories are required to have their QMS audited by a third-party auditing body to document that they meet requirements based on the ISO/IEC-17025 standard. In all cases, the concept is to standardize testing and increase confidence in test results at the lab level and beyond.

Test Performance Parameters

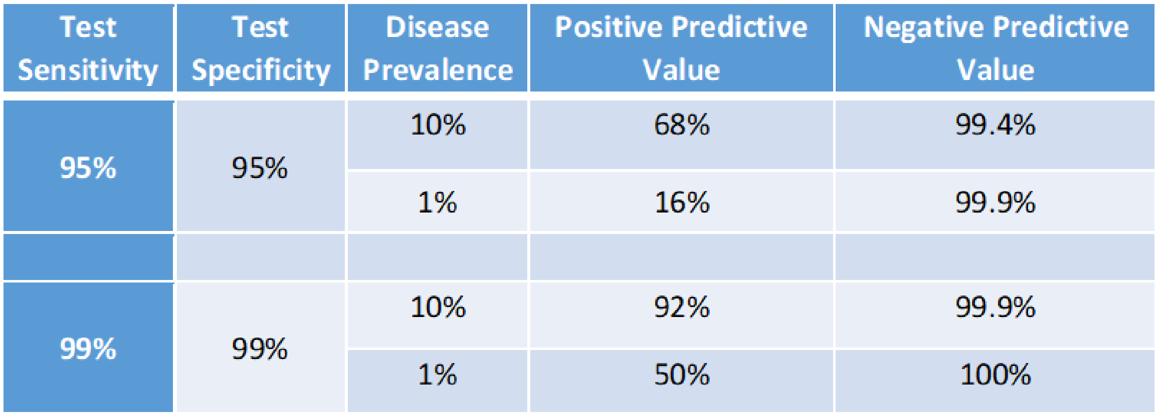
Limit of Detection is the smallest amount of the target that can be consistently detected by the assay. This is important when the test target is unequally distributed in host tissues, when only a small amount of the pathogen can cause disease, or when there is zero tolerance for the presence of the pathogen due to regulatory requirements.

Sensitivity is the ability of the assay to detect the target if it is truly present and is usually expressed as a percentage. An assay that is 99% sensitive would therefore only miss the target pathogen (false negative) in 1/100 times it is run. It is important to keep in mind that as sensitivity increases, specificity of the test may decrease.

Specificity is the ability of the assay to not falsely detect the target if it is truly absent, i.e., how well the assay can differentiate between closely related organisms or targets, such as species, subspecies, or strain of an organism. Differentiation between species or subspecies is required for quarantine and biosecurity determination. Specificity is also expressed as a percentage and an assay that has a 95% specificity would be expected to misidentify (false positive) the target of the assay 5/100 times it is run. It is important to keep in mind that as specificity of a test increases, sensitivity may decrease.

Predictive Value of an assay is a statistical measure of how reliable a positive or negative result is. The predictive value is greatly affected by the prevalence of the disease in the population being tested. Even a highly sensitive test can have a low positive predictive value if the prevalence of the disease is near zero in the population being tested. Likewise, negative predictive values are unreliable when the prevalence of the disease approaches 100% in the population (Figure 6).

Figure 6. Effect of disease prevalence on positive and negative predictive values



Test Sensitivity	Test Specificity	Disease Prevalence	Positive Predictive Value	Negative Predictive Value
95%	95%	10%	68%	99.4%
		1%	16%	99.9%
99%	99%	10%	92%	99.9%
		1%	50%	100%

Fitness for Purpose

A key element in the development, validation, and application of diagnostic testing methods is deciding what the purpose(s) of utilizing a given test in a given situation may be (Anon., OIE, 2018). There are multiple purposes that testing may be employed for including:

Detection of pathogen presence – consideration must be given to what detection level of pathogen presence (prevalence) is necessary. This can vary considerably depending on whether the purpose is merely control of a disease, which may allow for some ongoing level of disease, or actual eradication of a disease, which cannot tolerate any level of disease occurrence. If control is the objective, a test with a lower sensitivity of detection may still be a useful tool as long as that sensitivity level matches up with the lowest tolerable level of disease detection in the program. If, on the other hand, eradication is the goal, then tests with 100% sensitivity must be the mainstay of the diagnostic program.

Certification of pathogen absence – since the purpose in this case is to provide certainty that the pathogen is not in the animal or plant, then the diagnostic workflow must include testing with both 100% sensitivity and 100% specificity. Ideally this would be accomplished with a single test method that has these performance characteristics. Often though, this may require a combination of tests run in serial fashion which, when combined, achieve this level of both sensitivity and specificity. Such test combinations are often referred to as Screening Tests and Confirmatory Tests (see below).

Pathogen detection in an individual – accurately characterizing the infection status of an individual plant or animal generally requires the highest level of performance in the tests used. An alternative, when there is no test or tests that can reach the needed levels of performance, can be to test multiple samples from the same individual, using samples taken all at the same point in time or samples from successive time points.

Pathogen detection in a population – generally multiple units (animals, plants) of a population are tested for this purpose, either at a point in time or across a span of time. Depending on the detection goal (presence vs. absence) and the acceptable level of uncertainty (disease control vs. eradication), a test with less-than-ideal performance characteristics may well be acceptable.

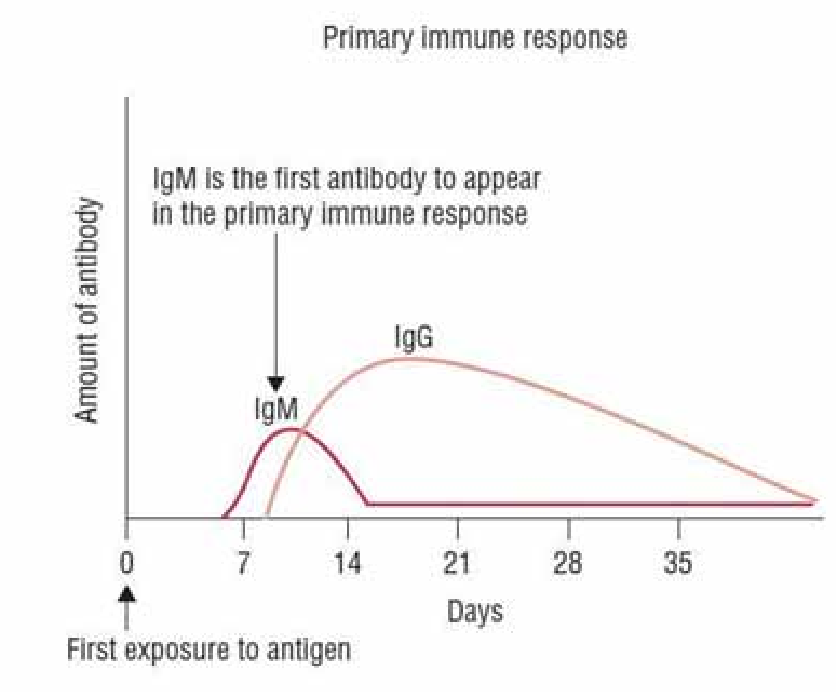
Detection when pathogen is at low prevalence – The known or expected prevalence of a disease in a population or population unit has a significant statistical impact on the acceptable performance characteristics for a test. The predictive value or reliability of a positive test result is especially affected by low prevalence of a disease. Under these conditions, even a highly performing test has a significant probability of producing false positive results (Figure 6). This emphasizes the importance of test results to always be interpreted in the context of all other known factors about the population or individual being tested.

Detection when disease is at high prevalence – When a disease is present at a very high prevalence, the statistical significance of a negative test result becomes less reliable and again, must be interpreted alongside of what is known or suspected concerning the prevalence level (Figure 6).

Direct pathogen detection - most diagnostic tests are formulated to directly detect a pathogen or pathogens. Many of these tests not only detect but are targeted to a very specific pathogen or group of pathogens, providing identification as well. In some cases (e.g., cultures) detection and identification require more than one step or test to accomplish. Fitness to directly detect a pathogen is critically affected by the pathophysiology of the pathogen which may determine appropriate sample types, locations or timing of the sampling. For example, urine or blood in animals or leaves in plants may be easy samples to collect, but the pathogen of interest may not appear at all, only for a very limited time or at very low levels in that sample type.

Indirect pathogen detection – in some cases it may be easier or more appropriate to look for indicators of a response to a pathogen, rather than the pathogen itself. This may be because the pathogen is difficult to detect directly due to limited spatial or temporal distribution within the host, or at least within a readily accessible sample of the infected host. Such indirect biomarkers may also be used as an indicator of either the degree or duration of response of the host to the pathogen, which may be useful in the interpretation of the timing of the infection or the host's ability to fight off the pathogen. The most common biomarker in animals are antibodies produced by the host' immune system, which can be highly specific to the pathogen and thus provide both detection and identification. There are several classes of antibodies (e.g. IgM, IgG) and they are produced at different times post-infection and last for different durations of time once produced (Figure 7). These characteristics can be used to determine, in a relative way, the probable time of onset of infection and whether, at the time of sampling, the infection is in an acute or chronic phase.

Figure 7. Typical antibody response time curves for IgM and IgG after infection
(Source: https://microbenotes.com/differences-between-primary-and-secondary-immune-response/)



Other biomarkers, such as the acute phase proteins (e.g., C-reactive protein, Serum amyloid A, haptoglobin), while non-specific as to the identity of the pathogen involved, serve as indicators of both the strength of the host response to the infection as well as the stage of the infection, acute vs. chronic (Eckersall and Bell 2010).

Detection of unknown diseases – perhaps the most challenging purpose for testing is the detection of a new or emerging disease or pathogen. Such diseases arise when either a previously unknown disease is recognized for the first time, a previously known disease is introduced to a new geographic location or a previously known disease mutates into a new form, one that may not be detected by the assays designed to detect the “old” variant of that disease. An understanding of the specificity of the tests being used is crucial in this case. Highly specific tests may completely miss a pathogen that is closely related, but not identical to the original target organism for that test. A test with less specificity may cross react to a limited degree with a different, related pathogen, giving some indication that further exploration is needed. Historically, tests with broad specificity (the ability to detect a wide range of pathogens, e.g., bacterial culture, viral cell culture) have been relied upon for first detection of an unanticipated new pathogen. These have limitations though as not all pathogens are detected equally or grow equally well, or at all, in the culture media or cell lines that might routinely be used in the diagnostic laboratory. The nucleic acid and gene-based tests (e.g., polymerase chain reaction (PCR), next generation sequencing (NGS), whole genome sequencing (WGS), meta-genomics) can potentially overcome the drawbacks of culture methods as they rely on direct detection and identification of pathogen genetic material without requiring that the organism even be alive, much less capable of replication to increase the target numbers. Detection of a new pathogen is often only the first step with further work required to fully identify or characterize the novel organism.

Screening test – a test that is intended for rapid, cheap screening of large numbers of samples often will sacrifice some degree of specificity in order to achieve the highest degree of sensitivity possible. Since this choice will tend to increase the rate of false positive results, such screening tests are almost always part of a test series so that any sample positive on a screening test is then retested with a highly specific, but often less sensitive, second test. The sample is only considered a true positive if it is positive on both tests. Screening tests are often optimized to support high throughput of testing so that large quantities of samples can be tested easily and quickly.

Confirmatory Test – a confirmatory test is a secondary test used on a sample that has previously tested positive, usually with a screening test as described above. The confirmatory test can be just a repeat of the original or screening test, but it is often a completely different test with a different test method, technology or performance characteristics. While screening tests are often optimized for high sensitivity, confirmatory tests are usually optimized for high specificity. The results of the confirmatory test are usually the final determinant of the test result.

Further information about and applied examples of fitness for purpose can be found in the OIE *Terrestrial Manual, 2018, Chapter 1.1.6, Principles and methods of validation of diagnostic assays for infectious diseases* (Anon., OIE, 2018).

Other Critical Testing Parameters

Cost - Cost of testing, both to the laboratory and to the sample submitter, can often be a controlling factor in whether a test is available from the laboratory and whether it is agreed to by a submitter. For the laboratory, this cost includes not only the expense of actually running a test on a sample, but also the cost of maintaining that capability (equipment, reagents, staff, building overhead), particularly if the test is not in high demand. For the submitter, there must be sufficient value in the results of diagnostic testing, economic or otherwise, to incentivize the submission of samples. Often this is because the diagnostic testing is to protect the health of a large number and therefore value of plants or animals. In some cases, however, an individual animal or plant may have such high genetic value that even a relatively expensive diagnostic assay is worth running for the protection of that individual.

Throughput - The testing throughput of a laboratory is the number of tests that can be completed in a given unit of time (hour, day, week). Turnaround time may be measured as either the time it takes to complete the test method on a sample or, more realistically, as the time elapsed between when a sample arrives at the laboratory and the final results of the test are sent to the submitter. Both of these measures of timeliness are influenced by a variety of factors including the type of sample and its condition, sample processing required before testing, the testing method or protocol, reliance on biologic processes (e.g., cell growth in culture), and the suitability of applying automation to the testing process. Even the use of standardized sample containers can have a significant impact (e.g., a specific size test tube instead of whatever vial or jar the submitter has laying around), especially for automated testing workflows.

Scalability - The ease of expanding the capacity of testing protocols (scalability) is an important consideration prior to deployment of an assay. In addition to the issues associated with throughput on an individual lab level, scalability introduces additional considerations such as availability of reagents and consumables, location of vetted equipment and trained personnel, and accessibility of controls and standards. Finally, distribution of samples during a surge should be planned to ensure no single lab is overwhelmed with samples and all samples can be processed in a timely manner. Discussion of potential maximum sample number and timing is crucial to preparedness for scaling up a test.

Reporting - Perhaps the most under-appreciated part of the diagnostic testing workflow is the results reporting process. The focus tends to be primarily on the content of the report. Laboratory accreditation standards such as those of the International Standards Organization (ISO-IEC 17025) specify minimum requirements for the content in reporting, from unique identification of samples and testing methods to specification of the testing laboratory and laboratory staff responsible for the testing. Outside of those minimum requirements for reporting, the level of detail contained in a report may be tailored to the level of technical sophistication of the target audience or recipient. A critical oversight however has been a lack of specification, standards or guidance on formatting and layout of results in reports to ensure clear, unambiguous communication and avoid misinterpretation of the results. Diagnostic reports may contain only the raw, primary results of the testing conducted or may include some limited additional interpretive comments by diagnosticians. A missed opportunity is to also include additional result data aggregation or statistical analyses that would add value to the results and provide significant additional information for improved decision making. For animal testing, clinical diagnosis and determination of treatment recommendations is left to the licensed clinical veterinary professional associated with the submission. In the plant world, laboratory professionals often fulfill both the role of diagnostician and of clinician, interpreting test results and making treatment recommendations for the client.

Diagnostic Toolbox

Many different types of diagnostic tests are employed currently to identify the underlying cause of an overt disease problem or to determine if a potential disease-causing agent is present in a sample (Bolboaca, 2019, Boonham, 2013, Buchan, 2014, Busin, 2016, Cunha, 2015). Table 1 lists some of the more commonly used methods along with a brief description of the operational principles of the test.

Figure 8 differentiates some of the common testing methods based on their performance characteristics. Breadth refers to the range of different target pathogens or their biomarkers the method can detect. All of these factors, plus fitness for purpose, play a crucial role in the decision of which method is best applied in any diagnostic situation.

Table 1. Commonly used methods and their operational principles

Type of Diagnostic Test		**Principles**
Immunoassays		
Agglutination (slide agglutination, bacterial agglutination, particle agglutination, hemagglutination, latex agglutination)	Antigen-antibody reactions result in the formation of visible clumps (agglutination)	**Plants** - D.G.A. Walkey, N.F. Lyons, J.D. Taylor. An evaluation of a virobacterial agglutination test for the detection of plant viruses. Pl. Pathol., 41 (1992), pp. 462-471 **Animals** - Castillo Y, Tachibana M, Kimura Y, Kim S, Ichikawa Y, Endo Y, Watanabe K, Shimizu T, Watarai M. Microplate Agglutination Test for Canine Brucellosis Using Recombinant Antigen-Coated Beads. Int Sch Res Notices. 2014 Oct 28;2014:348529. doi: 10.1155/2014/348529. PMID: 27355048; PMCID: PMC4897435.
Complement Fixation (**CF**)	Antigen-antibody reactions will prevent destruction of red blood cells	**Animals** - Olafson PU, Thomas DB, May MA, Buckmeier BG, Duhaime RA. Tick vector and disease pathogen surveillance of nilgai antelope (*Boselaphus tragocamelus*) in southeastern Texas, USA. J Wildl Dis. 2018 Oct;54(4):734-744. doi: 10.7589/2017-09-239. Epub 2018 Jun 4. PMID: 29863973.
Enzyme-linked immunosorbent assay (**ELISA**)	Uses either stationary antibody to detect antigen or vice versa, with an enzyme to amplify the detection signal	**Plants** - Loconsole G, Potere O, Boscia D, Altamura G, Djelouah K, Elbeaino T, et al. Detection of Xylella fastidiosa in olive trees by molecular and serological methods. Journal of Plant Pathology. 2014; 96(1):7– 14. **Animals** - Olafson PU, Thomas DB, May MA, Buckmeier BG, Duhaime RA. Tick vector and disease pathogen surveillance of nilgai antelope (*Boselaphus tragocamelus*) in southeastern Texas, USA. J Wildl Dis. 2018 Oct;54(4):734-744. doi: 10.7589/2017-09-239. Epub 2018 Jun 4. PMID: 29863973.
Fluorescent antibody (**FA**)	Labeled antibody binds to target antigen and emits detection signal under fluorescent light	**Plants** - Awaludin N, Abdullah J, Salam F, Ramachandran K, Yusof NA, Wasoh H. Fluorescence-based immunoassay for the detection of Xanthomonas oryzae pv. oryzae in rice leaf. Anal Biochem. 2020 Dec 1;610:113876. doi: 10.1016/j.ab.2020.113876. Epub 2020 Aug 1. PMID: 32750357. **Animals** - Muraro LS, Souza AO, Leite TNS, Cândido SL, Melo ALT, Toma HS, Carvalho MB, Dutra V, Nakazato L, Cabezas-Cruz A, Aguiar DM. First Evidence of *Ehrlichia minasensis* Infection in Horses from Brazil. Pathogens. 2021 Feb 25;10(3):265. doi: 10.3390/pathogens10030265. PMID: 33669023.
Immunohistochemistry (**IHC**)	Primary antibody binds to target antigen and this complex is visible using a secondary antibody to the primary antibody that produces a detection signal when attached.	**Plants** – Saggaf MH, Ndunguru J, Tairo F, Sseruwagi P, Ascencio-Ibáñez JT, Kilalo D, Miano DW. Immunohistochemical localization of *Cassava brown streak virus* and its morphological effect on cassava leaves. Physiol Mol Plant Pathol. 2019 Jan;105:67-76. doi: 10.1016/j.pmpp.2018.06.001. PMID: 31007375; PMCID: PMC6472608. **Animals** - Gonzales Viera OA, Crossley B, Carvallo-Chaigneau F, Blair E, Rejmanek D, Erdoğan-Bamac Ő, Sverlow K, Figueroa A, Gallardo RA, Mete A. Infectious Bronchitis Virus Prevalence, Characterization and Strain Identification in California Backyard Chickens. Avian Dis. 2021 Jan 5. doi: 10.1637/aviandiseases-D-20-00113. Epub ahead of print. PMID: 33400768.
Lateral flow assay	A variation of the ELISA technique using capillary flow of the liquid sample across the fixed antibody	**Plants** - Ahmed FA, Larrea-Sarmiento A, Alvarez AM, Arif M. Genome-informed diagnostics for specific and rapid detection of Pectobacterium species using recombinase polymerase amplification coupled with a lateral flow device. Sci Rep. 2018 Oct 29;8(1):15972. doi: 10.1038/s41598-018-34275-0. PMID: 30374117; PMCID: PMC6206099. **Animals** - Zhuang L, Gong J, Ji Y, Tian P, Kong F, Bai H, Gu N, Zhang Y . Lateral flow fluorescent immunoassay based on isothermal amplification for rapid quantitative detection of Salmonella spp. Analyst. 2020 Mar 21;145(6):2367-2377. doi: 10.1039/c9an02011j. Epub 2020 Feb 7. PMID: 32031182.
Radioimmunoassay (**RIA**)	Detection of antigen-antibody complexes utilizing radioisotopes as detection labels	**Plants** - P.W.G. Chu, P.M. Waterhouse, R.R. Martin & W.L. Gerlach (1989) New Approaches to the Detection of Microbial Plant Pathogens, Biotechnology and Genetic Engineering Reviews, 7:1, 45-112, DOI: 10.1080/02648725.1989.10647856 **Plants** - Weeks I. Sturgess M, Brown RC. Woodhead JS. (1986). Immunoassays using acridinium esters. Methods in Enzymology 133,366-387 **Animals** - Christensen CM, Zarlenga DS, Gasbarre LC. Ostertagia, Haemonchus, Cooperia, and Oesophagostomum: construction and characterization of genus-specific DNA probes to differentiate important parasites of cattle. Exp Parasitol. 1994 Feb;78(1):93-100. doi: 10.1006/expr.1994.1009. PMID: 8299764.
Serum/virus neutralization (**SN/VN**)	Detection of antibody that successfully prevents viral infection of cells	**Animals** - Lee H, Kim EJ, Cho IS, Song JY, Choi JS, Lee JY, Shin YK. A serological study of severe fever with thrombocytopenia syndrome using a virus neutralization test and competitive enzyme-linked immunosorbent assay. J Vet Sci. 2017 Mar 30;18(1):33-38. doi: 10.4142/jvs.2017.18.1.33. PMID: 27297411; PMCID: PMC5366300. **Animals** - Gauger PC, Vincent AL. Serum Virus Neutralization Assay for Detection and Quantitation of Serum Neutralizing Antibodies to Influenza A Virus in Swine. Methods Mol Biol. 2020;2123:321-333. doi: 10.1007/978-1-0716-0346-8_23. PMID: 32170698.
Agar gel immunodiffusion (**AGID**)	Detection of antigen-antibody complexes using diffusion through agar gel	**Plants** - Rivera C, Pereira R. Identificación del virus del mosaico del maíz, un rhabdovirus, en Costa Rica [Identification of the corn mosaic virus, a rhabdovirus, in Costa Rica]. Rev Biol Trop. 1994 Aug;42 Suppl 2:105-9. Spanish. PMID: 7701083. **Animals** - Iwasaki R, Nakagiri Y, Yaguchi Y, Oguma K, Ono M, Horikita T, Sentsui H. Survey of bovine foamy virus infection among cattle in Japan and comparison with bovine leukemia virus infection. J Vet Med Sci. 2020 May 20;82(5):615-618. doi: 10.1292/jvms.19-0592. Epub 2020 Mar 20. PMID: 32201403; PMCID: PMC7273609.
Mass Spectrometry Methods		
Matrix-assisted laser desorption ionization-time of flight (**MALDI-TOF**)	Detection and identification of biomolecules (e.g. DNA, proteins, peptides) following laser vaporization, particle dispersion and generation of a mass spectrum to compare to a reference library database	**Plants** - Sindt NM, Robison F, Brick MA, Schwartz HF, Heuberger AL, Prenni JE. MALDI-TOF-MS with PLS Modeling Enables Strain Typing of the Bacterial Plant Pathogen Xanthomonas axonopodis. J Am Soc Mass Spectrom. 2018 Feb;29(2):413-421. doi: 10.1007/s13361-017-1839-0. Epub 2017 Nov 27. PMID: 29181812. **Animals** - Dieckmann, R., Malorny, B.: Rapid screening of epidemiologically important *Salmonella enterica* subsp. enterica serovars using whole-cell matrix-assisted laser desorption ionization-time of flight mass spectrometry. Appl. Environ. Microbiol. 77(12), 4136–4146 (2011) **Humans** - Sharma P, Diene SM, Thibeaut S, Bittar F, Roux V, Gomez C, Reynaud-Gaubert M, Rolain JM. Phenotypic and genotypic properties of Microbacterium yannicii, a recently described multidrug resistant bacterium isolated from a lung transplanted patient with cystic fibrosis in France. BMC Microbiol. 2013 May 3;13:97. doi: 10.1186/1471-2180-13-97. PMID: 23642186; PMCID: PMC3655929.

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Table 1. Continued

Type of Diagnostic Test		Principles
Microscopy		
Electron microscopy (**EM**)	Utilizing a beam of accelerated electrons to generate high-resolution images of cellular and pathogen ultrastructure	**Plants** - Zechmann B, Graggaber G, Zellnig G. Microwave assisted rapid diagnosis of plant virus diseases by transmission electron microscopy. J Vis Exp. 2011 Oct 14;(56):e2950. doi: 10.3791/2950. PMID: 22025001; PMCID: PMC3227200. **Animals** - Lakshman M. Application of conventional electron microscopy in aquatic animal disease diagnosis: A review. J Entomol Zool Stud 2019;7:470–5.
Light microscopy	Utilizes regular light and magnifying objectives to visualize small objects	**Plants** - Cleary, M.R., Daniel, G. and Stenlid, J. (2013), Light and scanning electron microscopy studies of the early infection stages of *Hymenoscyphus pseudoalbidus* on *Fraxinus excelsior*. Plant Pathol, 62: 1294-1301. https://doi.org/10.1111/ppa.12048 **Animals -** Daaboul GG, Freedman DS, Scherr SM, Carter E, Rosca A, Bernstein D, et al. (2017) Enhanced light microscopy visualization of virus particles from Zika virus to filamentous ebolaviruses. PLoS ONE 12(6): e0179728. https://doi.org/10.1371/journal.pone.0179728
Phase microscopy	Utilizes differences in refractive index to facilitate the visualization of transparent structures such as unstained cells	**Plants** - Thomas P, Swarna GK, Patil P, Rawal RD. Ubiquitous presence of normally non-cultivable endophytic bacteria in field shoot-tips of banana and their gradual activation to quiescent cultivable form in tissue cultures. *Plant Cell, Tissue and Organ Culture.* 2008a;93:39–54. **Animals** - Torres-Anjel MJ, Guevara A, Sandino R. The use of phase contrast microscopy in the diagnoses of dermatomycoses. Indian J Dermatol. 1973 Jan;18(2):31 passim. PMID: 4575802.
Molecular Assays (Nucleic Acid Based)		
Clustered regularly interspaced short palindrome repeats (**CRISPR**)	Utilizing CRISPR enzymes to selectively cleave segments of DNA for detection	**Plants** – Kang H, Peng Y, Hua K, Deng Y, Bellizzi M, Gupta DR, Mahmud NU, Urashima AS, Paul SK, Peterson G, Zhou Y, Zhou X, Islam MT, Wang GL. Rapid Detection of Wheat Blast Pathogen Magnaporthe Oryzae Triticum Pathotype using Genome-Specific Primers and Cas12a-mediated Technology, Engineering, https://doi.org/10.1016/j.eng.2020.07.016 **Animals** - Ren M, Mei H, Zhou J, Zhou M, Han H, Zhao L. Early diagnosis of rabies virus infection by RPA-CRISPR techniques in a rat model. Arch Virol. 2021 Apr;166(4):1083-1092. doi: 10.1007/s00705-021-04970-x. Epub 2021 Feb 5. PMID: 33544254; PMCID: PMC7862975.
In situ hybridization (**ISH**)	Detection and localization of specific segments of DNA in a histologic tissue section	**Plants** - Ellison MA, McMahon MB, Bonde MR, Palmer CL, Luster DG. In situ hybridization for the detection of rust fungi in paraffin embedded plant tissue sections. Plant Methods. 2016 Jul 27;12:37. doi: 10.1186/s13007-016-0137-3. PMID: 27471544; PMCID: PMC4964054. **Animals** - Liu W, Zhang Y, Ma J, Jiang N, Fan Y, Zhou Y, Cain K, Yi M, Jia K, Wen H, Liu W, Guan W, Zeng L. Determination of a novel parvovirus pathogen associated with massive mortality in adult tilapia. PLoS Pathog. 2020 Sep 24;16(9):e1008765. doi: 10.1371/journal.ppat.1008765. PMID: 32970777; PMCID: PMC7588064.
Loop-mediated isothermal amplification (**LAMP**)	Amplification and detection of target DNA at a constant temperature	**Plants** - Stehlíková D, Beran P, Cohen SP, Čurn V. Development of Real-Time and Colorimetric Loop Mediated Isothermal Amplification Assay for Detection of *Xanthomonas gardneri*. *Microorganisms*. 2020; 8(9):1301. https://doi.org/10.3390/microorganisms8091301 **Animals** - Upadhyay L, Chaturvedi VK, Gupta PK, Sunita SC, Sumithra TG, Prusty BR, Yadav AK. Development of a visible loop mediated isothermal amplification assay for rapid detection of Bacillus anthracis. Biologicals. 2021 Jan;69:59-65. doi: 10.1016/j.biologicals.2020.11.004. Epub 2020 Dec 10. PMID: 33309531.
Polymerase chain reaction (**PCR**)	Amplification of target nucleic acid (usually DNA) utilizing a series of heating (denaturation) and cooling (annealing) cycles. Can be quantitative (**qPCR**), or, with an extra step, amplify RNA (**RT-PCR**)	**Plants** - Bilodeau GJ, Martin FN, Coffey MD, Blomquist CL. Development of a multiplex assay for genus- and species-specific detection of Phytophthora based on differences in mitochondrial gene order. Phytopathology. 2014 Jul;104(7):733-48. doi: 10.1094/PHYTO-09-13-0263-R. PMID: 24915428. **Animals** - Clouthier SC, Schroeder T, Bueren EK, Anderson ED, Emmenegger E. Analytical validation of two RT-qPCR tests and detection of spring viremia of carp virus (SVCV) in persistently infected koi Cyprinus carpio. Dis Aquat Organ. 2021 Feb 25;143:169-188. doi: 10.3354/dao03564. PMID: 33629660.
Recombinase polymerase amplification (**RPA**)	Amplification and detection of target DNA and RNA at a constant temperature	**Plants** - Zhang S, Ravelonandro M, Russell P, McOwen N, Briard P, Bohannon S, Vrient A. Rapid diagnostic detection of plum pox virus in Prunus plants by isothermal AmplifyRP® using reverse transcription-recombinase polymerase amplification. Journal of Virological Methods.Volume 207, 2014, Pages 114-120 https://dx.doi.org/10.1016/j.jviromet.2014.06.026 **Animals** - El-Tholoth M, Branavan M, Naveenathayalan A, Balachandran W. Recombinase polymerase amplification-nucleic acid lateral flow immunoassays for Newcastle disease virus and infectious bronchitis virus detection. Mol Biol Rep. 2019 Dec;46(6):6391-6397. doi: 10.1007/s11033-019-05085-y. Epub 2019 Sep 23. PMID: 31549369; PMCID: PMC7089497.
Western blot analysis	Detection and characterization of specific proteins separated according to their molecular weight	**Plants** - Wieczorek P, Budziszewska M, Frąckowiak P, Obrępalska-Stęplowska A. Development of a New Tomato Torrado Virus-Based Vector Tagged with GFP for Monitoring Virus Movement in Plants. Viruses. 2020 Oct 20;12(10):1195. doi: 10.3390/v12101195. PMID: 33092281; PMCID: PMC7588970. **Animals** - Desoubeaux G, Pantin A, Peschke R, Joachim A, Cray C. Application of Western blot analysis for the diagnosis of Encephalitozoon cuniculi infection in rabbits: example of a quantitative approach. Parasitol Res. 2017 Feb;116(2):743-750. doi: 10.1007/s00436-016-5343-4. Epub 2016 Dec 13. PMID: 27966020.
Whole genome sequencing (**WGS**)	Determining the complete DNA sequence of the entire genome of an organism	**Plants** - Raths R, Peta V, Bücking H. *Duganella callida* sp. nov., a novel addition to the *Duganella* genus, isolated from the soil of a cultivated maize field. Int J Syst Evol Microbiol. 2021 Jan;71(1). doi: 10.1099/ijsem.0.004599. Epub 2020 Dec 3. PMID: 33269999. **Animals** - Kagambèga A, Hiott LM, Boyle DS, McMillan EA, Sharma P, Gupta SK, Ramadan H, Cho S, Humayoun SB, Woodley TA, Barro N, Jackson CR, Frye JG. Serotyping of sub-Saharan Africa Salmonella strains isolated from poultry feces using multiplex PCR and whole genome sequencing. BMC Microbiol. 2021 Jan 19;21(1):29. doi: 10.1186/s12866-021-02085-6. PMID: 33468047; PMCID: PMC7814607.

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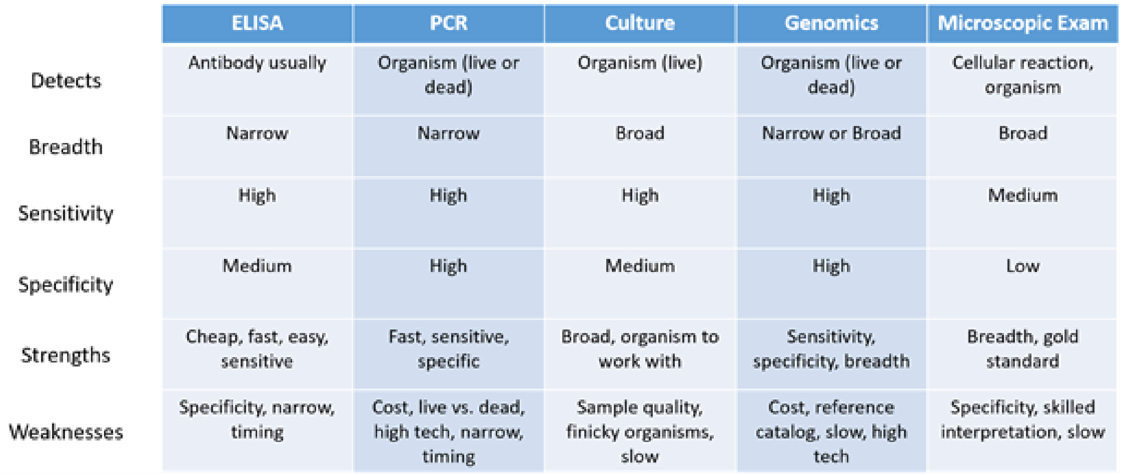
Table 1. Continued

Type of Diagnostic Test		Principles
Next generation sequencing (**NGS**)	Determining the DNA sequence of a subset or section of the genome of an organism	**Plants** - Pieck ML, Ruck A, Farman ML, Peterson GL, Stack JP, Valent B, Pedley KF. Genomics-Based Marker Discovery and Diagnostic Assay Development for Wheat Blast. Plant Disease. 2017 Jan;101(1):103-109. https://doi/10.1094/PDIS-04-16-0500-RE **Animals** - Dimitrov KM, Sharma P, Volkening JD, Goraichuk IV, Wajid A, Rehmani SF, Basharat A, Shittu I, Joannis TM, Miller PJ, Afonso CL. A robust and cost-effective approach to sequence and analyze complete genomes of small RNA viruses. Virol J. 2017 Apr 7;14(1):72. doi: 10.1186/s12985-017-0741-5. PMID: 28388925; PMCID: PMC5384157.
Culture		
Bacterial or fungal culture	Growing bacteria or fungi on/in artificial media, followed by one or more morphological, biochemical or other techniques (e.g., MALDI-TOF) to identify the organisms.	**Plants** – Zhang J, Liu YX, Guo X, Qin Y, Garrido-Oter R, Schulze-Lefert P, Bai Y. High-throughput cultivation and identification of bacteria from the plant root microbiota. Nat Protoc. 2021 Feb;16(2):988-1012. doi: 10.1038/s41596-020-00444-7. Epub 2021 Jan 13. PMID: 33442053. **Humans** - Sharma P, Gupta SK, Diene SM, Rolain JM. Whole-genome sequence of Chryseobacterium oranimense, a colistin-resistant bacterium isolated from a cystic fibrosis patient in France. Antimicrob Agents Chemother. 2015 Mar;59(3):1696-706. doi: 10.1128/AAC.02417-14. Epub 2015 Jan 12. PMID: 25583710; PMCID: PMC4325762.
Viral culture	Growing viruses in artificial preparations of live cells, followed by one or more techniques (e.g., EM, FA) to identify the viruses.	**Plants** - Andika, I.B.; Wei, S.; Cao, C.; Salaipeth, L.; Kondo, H.; Sun, L. Phytopathogenic fungus hosts a plant virus: A naturally occurring cross-kingdom viral infection. Proc. Natl. Acad. Sci. USA 2017, 114, 12267–12272 **Animals**- Helenius, A., Kartenbeck, J., Simons, K. & Fries, E. (1980). On the entry of Semliki Forest virus into BHK-21 cells. Journal of Cell Biology 84, 404-420.

Figure 8. Examples of differing performance characteristics of test methods that should be considered in the appropriate application of a test



	ELISA	PCR	Culture	Genomics	Microscopic Exam
Detects	Antibody usually	Organism (live or dead)	Organism (live)	Organism (live or dead)	Cellular reaction, organism
Breadth	Narrow	Narrow	Broad	Narrow or Broad	Broad
Sensitivity	High	High	High	High	Medium
Specificity	Medium	High	Medium	High	Low
Strengths	Cheap, fast, easy, sensitive	Fast, sensitive, specific	Broad, organism to work with	Sensitivity, specificity, breadth	Breadth, gold standard
Weaknesses	Specificity, narrow, timing	Cost, live vs. dead, high tech, narrow, timing	Sample quality, finicky organisms, slow	Cost, reference catalog, slow, high tech	Specificity, skilled interpretation, slow

Interpretation of Diagnostic Results

The following general principles for interpretation of diagnostic test results can be used for many types of testing. However, if there is any doubt or confusion over the interpretation of the results from a specific test or tests, the best course of action is always to contact the laboratory that performed the tests for assistance to be sure results are not misinterpreted.

Presence – in many cases, any detection of a known disease-causing agent or substance is enough to be interpreted as a significant finding. Examples include the rabies virus, *Brucella abortus* or *Ralstonia solanacearum* bacteria, all of which are subject to a mandatory regulatory reporting policy.

Amount – Some disease-causing agents, substances, or markers may be detected in and considered a normal finding for clinically healthy animals and plants. In these cases, the amount of the agent, substance or marker detected is critical to know to determine whether it is a likely cause of the clinical illness observed. This requires that a test provide not just an identification of the possible agent, but also a quantitative measure of the amount detected. The amount reported can be categorical (small, large) or an absolute numerical measure. For these situations where not just the presence but the amount of an agent matters to the interpretation, the laboratory will often provide a reference range to compare the test result to. This reference range represents the level(s) of the agent considered to be within the normal variation of findings for clinically normal animals or plants.

Timing – A single sample and test of that sample typically represents the disease status of an animal or plant at the discrete point in time that the sample was collected. If the sample was collected very shortly after the disease agent was introduced, the level of the agent can be too low to detect, either directly or through the response to the agent (e.g. antibody levels as a biomarker). Similarly, if the sample is collected very late in the clinical course of the disease, the amount of agent or response biomarker may have fallen off to the point where it is no longer detectable, even though that agent was the cause of the clinical disease. Additionally, secondary or opportunistic infections may mask or outcompete the primary causal agent over time.

It is often useful to have samples tested from at least two-time points, several days to a few weeks apart. With two points of measurement, whether of the amount of agent present or the level of response biomarker, a much clearer picture can emerge of where in the course of the disease the animal may be. For example, a second sample that has higher levels of agent or biomarker usually indicates a disease trajectory that is still rising from a relatively recent introduction, whereas a second measurement that is lower indicates the disease is waning and was likely not recently introduced. Testing of two samples from the same subject is often referred to as “paired sampling” or “acute and convalescent sampling” and is a powerful, but frequently underutilized technique in individuals. In crops, multiple samples over time are common to track disease progression.

Data Management

Documenting, managing, and communicating sample and test result information is important for routine diagnoses and critical during biosecurity events. The information associated with a sample includes data such as sample submitter, collection site, collection date, species sampled, sample type, tests and results, diagnosticians involved, and other communications. Laboratory Information Management Systems (LIMS) organize sample metadata and processes. The most basic LIMS simply track samples with unique identifiers and organize the tests and sample submission information within the lab performing the tests. Advanced LIMS can organize protocols, test data, sample information, handle fee billing and suggest or initiate communications and actions within a network of diagnosticians or laboratories. Some systems also assist in notifications and communications with clientele, diagnosticians, other laboratories, subject-matter consultants, and regulatory authorities. It is also important to note what a LIMS is not designed to do. A LIMS is not, and should not be expected to be, a data mining or data analysis tool. Those functions require the export of LIMS data to other appropriate tools.

For legal purposes, the LIMS is usually the data source of record and is expected to be secured to provide confidentiality of client information, except as provided by law. Commonly, exceptions are granted for the laboratory to share information with appropriate regulatory authorities. For sample submissions that are, or become, subject to legal proceedings, the LIMS may also serve as the chain-of-custody record, providing proof in a court of law of the movement of samples into and through a laboratory, who may have had access to the samples, how the samples were handled, what tests were performed, and the ultimate disposition of the samples.

It is increasingly important for the LIMS to include the capability to transfer information to other institutions and applications. Precise, unambiguous communication is critical to diagnostics. Whether it is describing the technology, methods, and protocols used to make a diagnosis, or the findings of the diagnostic investigation, using an agreed upon standard terminology ensures all parties involved in the communication have a clear understanding of what was done, how it was done, and what the results were. Great efforts have been made to construct internationally recognized standards for diagnostic terms and methods. An example of the first is the Systematized Nomenclature of Medicine (SNOMED, https://www.snomed.org/), containing terms that cover anatomy, diseases, findings, procedures, microorganisms and more. A standard for describing testing methods used is the Logical Observation Identifiers, Names and Codes standard (LOINC, https://loinc.org/). A further useful standard is the Health Level 7 messaging protocol (HL7, http://www.hl7.org/) which establishes the framework for communicating between software applications, essentially prescribing which pieces of information, in what format, are necessary to ensure precise communication. Likewise, the European Plant Protection Organization (EPPO) has standards for terminology, reporting, and database interoperability via a set of codes, which are also used by the North American Plant Protection Organization (NAPPO). In the US a domestic database, the National Agricultural Pest Information System (NAPIS), contains and reports data from the Cooperative Agricultural Pest Surveys, and its codes are used as the basis for the NPDN's National Data Repository (NDR). All NPDN laboratories use a prescribed data standard to collect a subset of information from every sample and upload it to the NDR. Similarly, the NAHLN laboratories electronically message test results to a central database housed within USDA-APHIS. Each laboratory utilizes LOINC and SNOMED codes within the HL7 message framework to quickly provide testing data to regulatory officials. This data is primarily used for surveillance program management and is essential during an outbreak situation to inform decision-makers with almost real-time information used to direct regulatory action. The NPDN is currently building a library of diagnostic protocols and a standard for validation and publication of plant diagnostic protocols is nearing completion, with protocols to be published in the journals Plant Health Progress and the open access PhytoFrontiers.

Communications

Notification of results of routine detections may be simple, just an email from the diagnostician to the sample submitter. Communication of a potential regulatory or biosecurity detection however, requires adherence to a communication chain designed to ensure only and all those who need to know the information are included in the communication. The NPDN Chain of Communication protocol, for example, describes the steps using generic titles (e.g. diagnostician, laboratory director, department chair, State Plant Health Director or APHIS Program Manager). Tabletop exercise activities encourage those in the chain to designate the individual associated with that title in official laboratory documentation, including phone numbers, email addresses, and other pertinent information useful during a detection emergency (Stack, 2006). Figure 9 outlines one such communications chain.

Figure 9. The National Plant Diagnostic Network exercise program to assist each state in developing preparedness plans. Each exercise is based on a scenario to ensure the timely and secure communication of information and samples that will result in a rapid and accurate diagnosis and a rapid and appropriate response. (APHIS = Animal and Plant Health Inspection Service; LGU = Land Grant University)
(Source: Stack et al. 2006)

OPPORTUNITIES AND FUTURE DIRECTIONS

Succession-planning

Diagnosticians are practitioners of plant, animal, or human medicine; they interact with the patient, observe symptoms and signs, interpret test results, synthesize information, and prescribe disease management. A good diagnosis is dependent on the work of a good diagnostician; therefore, the authors stress the need for training programs and professional development to ensure a steady pipeline of trained professionals. Rather than reinventing the wheel though, the authors recommend supporting existing programs and growing new ones to fill gaps in the pipeline.

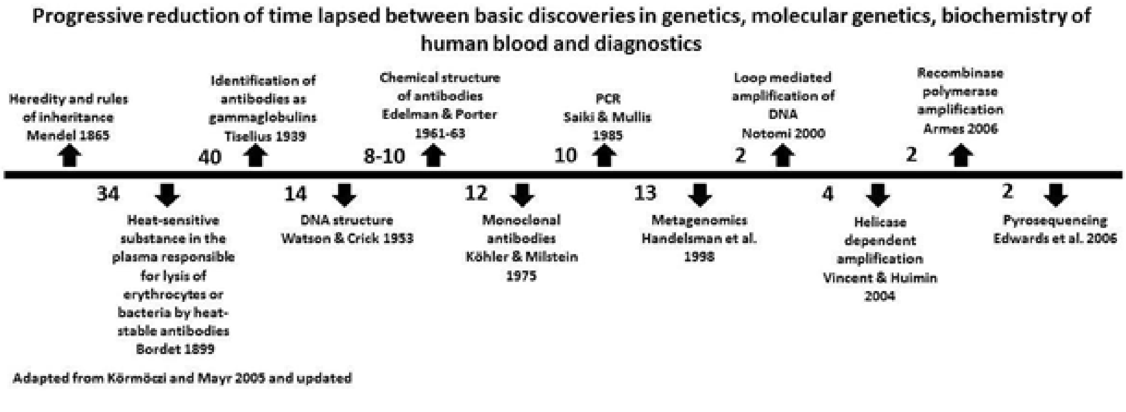
The pipeline starts in K-12 by promoting sciences that support diagnostics in areas such as agriculture, pathology, biology, chemistry, mathematics, and also communication and ethics. Development of pathology- or diagnostic-specific case studies and activities for K-12 classrooms can encourage young scientists. In the US, 4-H (https://4-h.org) involves elementary and secondary schoolchildren in hands-on sciences, including plant and animal care, but also including community involvement. The American Phytopathological Society has developed an Education Center (https://www.apsnetorg/edcenter) with peer-reviewed laboratory lessons focused on plant health for K-12 and beyond. The UK has provided science-related resources for K-12, including many with a focus on plant and animal sciences (https://www.stem.org.uk/resources/collection/137043/working-animals). Experiential learning for secondary school students includes innovative programs to attract students to STEM fields through summer research. Experiential learning for grad students includes rotations in related fields and fellowship programs to support scientific investigation. The USDA National Institute of Food and Agriculture supports this effort through the National Needs Graduate and Postgraduate Fellowship Grants Program Funding Opportunity. Training in the critical thinking process of diagnosis, though, requires experience in a clinical setting. Internships in diagnostic laboratories or other clinical operations should be encouraged. Courses in diagnostics should include symptom recognition and disease progression, pathogen biology, biochemistry and biology that underpins the diagnostic tests, interpretation of test results, and implications for disease management. Since the value of a diagnostician is in recognition and interpretation, studies in diagnostics and medicine should emphasize discussion and problem-solving activities over rote memorization.

The practice of diagnostics in any clinical setting is one of continual engagement and challenge. Although the need is recognized for continual training in new techniques and treatments, dissemination of that training is often inconvenient and even overwhelming for diagnosticians. Attendance at continuing education opportunities and meetings is required to obtain the credits needed to maintain licenses and improve one's knowledge and skills, but these are on top of daily work hours or in place of them, without a good way to rebalance the workload. As diagnosticians leave the field due to retirement, the workload for remaining diagnosticians increases. Demand for diagnostic services is on the increase, so caseloads for individual practices are creating the perfect storm of critical need against diagnostician burnout. Networking laboratories together can help to rebalance diagnostic load and allow breathing room for training and personal care. This can keep diagnosticians in the game longer and encourage them to train their eventual replacements. Without this "future-proofing", each generation of diagnosticians has a steep learning curve and a significant portion of trained professionals who burn out before they can reach their potential. Succession planning should be part of the structure of a practice or laboratory, but it will take a concentrated effort from the government down to the individual laboratories to formalize and sustain on a national level.

New Technology

Scientists develop new methods to manage complex diagnostics of new organisms in new environments and hosts, alternatives for mass processing of samples or data, multiplexing targets, and need for simplicity, test sensitivity, specificity, flexibility, portability, simplicity of use, and affordability. At present, point

Figure 10. Time between major discoveries in sciences related to diagnostics
(Source: Adapted from Körmöczi & Mayr 2005 and Ochoa-Corona 2011)



of care assays are rapid, portable, and easy to perform, but limited in the number of assays available. However, the time between big advancements in detection technologies is shrinking, bringing new tech to the lab in record time. A brief examination of the time elapsed between basic discoveries in genetics, molecular genetics, and biochemistry of human blood shows a progressive reduction of the time between cornerstone discoveries and inventions to approximately 1/3 after comparing dates registered from 1865 to the last 60 years (Fig. 10) (Körmöczi & Mayr, 2005). For example, 15 years after the invention of the polymerase chain reaction (PCR) in 1985, a breakthrough of isothermal DNA amplification techniques occurred between years 2000 and 2006. Loop mediated amplification of DNA (LAMP) was reported in 2000 (Notomi et al., 2000), followed by Helicase Dependent Amplification (HDA) in 2004 (Vincent & Huimin, 2004) and recombinase polymerase amplification (RPA) in 2006 (Piepenburg et al., 2006)

The assessment of the problem and its context may indicate methods or existing technologies may be adapted, improved, or combined calling for innovation rather than invention (Iyigun, 2006; Ballor & Claar, 2019; Kerr, 2010; Saaty & Rokou, 2017; Bonetto, 2020). In general, improvements in technological productivity often come after the initial invention as the product goes through innumerable minor modifications and alterations. For example, ELISA was invented by Engvall and Perlmann in 1971 and subsequently experienced multiple innovations and adaptations which made available to scientists a large number of ELISA variants accompanied by the generation of novel and specific antibodies. The application of ELISA to plant pathogens occurred by the mid 70's (Voller et al., 1976; Clark & Adams, 1977). Similarly, the polymerase chain reaction (PCR) experienced further innovation and adaptation to satisfy multiple applications after being invented (Saiki et al., 1985, 1988).

Another opportunity for diagnostics is the application of the rapidly developing tools of data science and analytics to the vast store of data generated by the diagnostic laboratories. There is a tremendous amount of value to be added and insights to be gained from the systematic aggregation and analysis of such data. Going beyond just the generation of individual case findings to provide evidence-based support for informed decision-making regarding disease occurrence, spread, control and the directions needed for further disease and diagnostic research and development efforts will be invaluable. An example is the Electronic-probe Diagnostic Nucleic-acid Analysis (EDNA, Stobbe et al., 2013), which provides the framework for a new sequence-based detection system that cancels the need for assembly of High Throughput Sequencing (HTS) data and eliminates 'big-data' bioinformatics challenges. HTS suffers from a large amount of computational time and power needed to identify a pathogen sequence from the obtained HTS dataset. EDNA allows rapid identification and simultaneous characterization of multiple specific pathogens and changes the role of HTS data from a query to the queried database relative to other tools. EDNA uses pathogen-specific sequences, known as electronic probes (e-probes), to detect specific viruses or organisms in metagenomic data. E-probes have been validated and generated using either complete or partial pathogen genomes (Bocsanczy et al., 2019). Although developed with plant pathogens (RNA virus, a DNA virus, bacteria, fungi, and an oomycete), the technology would be also useful in metagenomic sequences from vertebrates (Stobbe et al., 2013; Espindola et al., 2015; Espindola et al., 2018). Recently, to make EDNA easier to operate by non-skilled bioinformatic operators, an online graphical user interface named MIFI© (Microbe Finder) was created.

Existing tools such as syndromic surveillance and geospatial/temporal analyses have so far only been applied to diagnostic laboratory data in limited cases, but should become a standard, widespread practice. These data are also the critical fodder for Machine Learning and Artificial Intelligence algorithms that may reveal patterns and insights that can alert us to disease trends quicker, allowing interventions to also start sooner. Achieving such capabilities will require sustained investment in data management and analytics expertise and infrastructure. Building such an infrastructure should be done in a thoughtful, nationally, and internationally coordinated manner to ensure the greatest benefit from that investment.

Novel Diagnostic Methodologies

There are additional opportunities for the application of existing or new technologies from other scientific disciplines such as physics, chemistry, materials science, and engineering to the advancement of diagnostic capabilities. The potential for the application of nanotechnology, microfluidics, spectroscopy, machine learning and artificial intelligence is just starting to be realized in human diagnostics and will no doubt benefit animal and plant diagnostics similarly in the next decade.

Diagnostics based on spectral analyses show promise for detection of pathogens directly, or by detection of infected individuals. These new methods include Raman spectroscopy, CARS spectroscopy (Stryker, 2019), and variations on nucleic acid and protein detection (Buchan, 2014). Point of care (POC) diagnostic platforms based on isothermal nucleic acid amplification protocols (eg: Loop-mediated isothermal amplification or LAMP), lateral flow assays, and CRISPR (clustered regularly interspaced short palindromic repeats) based diagnostics are gaining prominence. On-site screening for pathogens directly at the source/point of entry/exposure will not only speed up detection but also help in reducing risks associated with shipping potentially contaminated materials to remote laboratory testing locations. A main advantage of these technologies is that they can simplify the diagnostic process by circumventing the need for expensive instruments such as thermocyclers and fluorescent plate readers.

Hyperspectral sensors and Raman spectroscopy are new technologies able to detect with high sensitivity chemical changes in the plant host, including the early release of volatiles, and may detect the presence of pathogens or microscopic arthropods before the damage they inflict is visible. A hyperspectral sensor can identify unique and specific spectral signatures associated with plant diseases (Mahlein et al., 2016). Raman spectroscopy provides information about molecular vibrations, chemical structure of the plant tissue, and can also detect plant pathogens (Farber & Kurouski, 2018; Egging et al., 2018). Multispectral crop imaging can detect environmental stress of plants, which enhances disease scouting accuracy (Grieve et al., 2015). Matrix-assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry is another impactful technology that has been widely adopted in recent years for bacterial identification based on protein profile signatures. Another significant development in the recent decades has been the advances in novel technologies for nucleic acid sequencing. The cost of sequencing genetic material continues to decrease and the sequencing cost of a human genome has fallen from $100M in 2001 to around $1K presently. The introduction of portable sequencing platforms such as the Minion (Oxford Nanopore) platform has been a disruptive innovation that can hasten the adoption of nucleic acid sequencing into routine diagnostics even in resource limited settings.

There are however, several significant hurdles to overcome between the initial experimental, proof-of-concept research environment and implementation of a new technology or assay in the routine environment of the diagnostic laboratory. Some of these hurdles are discussed earlier in this chapter under test development and validation, e.g., quality assurance, cost per sample, turnaround time and throughput. Many promising new diagnostic assays have never been implemented in the routine workflow of a frontline diagnostic laboratory due to failure to clear one or more of these critical, practical hurdles. Regardless, close relationships and collaborations between researchers and diagnosticians should be encouraged and researchers educated on the parameters that need to be included in development efforts.

CASE STUDY: PLUM POX VIRUS

Introduction

The relevance of diagnostics science from initial detection to response and eradication declaration is particularly showcased by the interesting case study of Plum pox virus (PPV) disease in the United States. Plum pox is the most devastating viral disease of stone fruit trees from the genus *Prunus*, which includes peaches, apricots, plums, nectarine, almonds, and sweet and tart cherries. Wild and ornamental species of *Prunus* may also become infected by some variants or strains of PPV, and their identification, location, and testing is also important because of their epidemiological significance. (Levy et al., 2000). Early detection of PPV is important because there is no cure once it gets established in an orchard. Once infected, trees with the virus and those in a 50-meter radius need to be removed and destroyed because PPV does not kill trees. If the trees are left to stand, the tree will remain as a reservoir for the virus.

Plum pox disease is caused by the virus species ***Plum pox virus*** (PPV), a linear single stranded RNA virus in the order *Patatavirales*, family *Potyviridae*, genus *Potyvirus*. The virus is transmitted by aphids and during vegetative propagation. PPV can be transported long distances in infected plant material to new locations, and can also be transmitted from infected trees by grafting or budding. Although PPV does not kill trees and poses no danger to human or animal health, it reduces the yield and marketability of stone fruits by causing acidity, deformities, and undesirable cosmetic damage as seen in Figure 11 (Atanassov, 1932; Levy et al., 2000).

Figure 11. Plum pox virus (PPV) infected fruit
(Source: John Hammond, USDA Agricultural Research Service, Bugwood.org)

How it is Diagnosed

Plum pox virus is routinely diagnosed at the screening level via ELISA, with confirmation done by reverse-transcriptase PCR. The USDA-APHIS NPPLAP administers the certification required to clear or confirm the pathogen in budwood, imports, and orchards (Wetzel et al., 1991; Cambra et al., 1994; Candresse et al., 1998; Levy et al., 2000).

How it is Spread

Plum pox virus is spread by the transplant of infected plant propagules and nursery stock. Insufficient monitoring of stone fruit before transporting the fruit from one location to another is also a cause for spread. Asymptomatic fruit and plant material may accidentally pass inspection. Plum pox virus spreads over a short distance by aphid vectors (Gottwald et al., 1995). The disease symptoms were first reported in Bulgaria in 1916-1917 (Atanassoff, 1932). Since then, the disease has spread in several European countries, and it was detected for the first time in North America in 1999, in a Pennsylvania orchard. PPV-D, the only strain of PPV detected in the United States, was originally described from Europe, is not the most virulent of plum pox strains, and does not appear to infect cherry species (Levy et al., 2000).

Cost to Industry

The economic impact of Plum pox virus on the global plum, peach, and apricot industry is estimated at over 12 million USD/year (Cambra, 2011). A study from 2006 estimates the economic loss in previous years to the peach industry from the PPV-D strain in the United States and Canada to be about 4.8 million euros (about $6.1 million USD) from the removal of 264,000 trees in Canada and 190,000 trees in Pennsylvania (Cambra et al., 2006). Widespread outbreaks of the virus could lead to increased cost for consumers due to a decline in production and exportation.

How it was Managed

The only way to manage the disease is to destroy all infected trees, which brings with it significant economic losses. To prevent the potential destruction of the $6.3 billion stone fruit industry in the United States from plum pox disease, the USDA quickly issued an emergency declaration and provided funding and support for the eradication of PPV across the country. Methods and techniques implemented to control and prevent plum pox spreading included field surveys, enforcement of domestic and international quarantine regulations, removal and destruction of infected trees, use of pox-resistant varieties (if available), maintenance of healthy nursery stock, increasing insecticide sprays for the aphid vector, and oversight of aphid travel patterns and wild and ornamental species of *Prunus* (Mumford 2006).

Conclusion

Thanks to the emergency declaration and the collaborative efforts among USDA ARS, APHIS, and farmers, efforts aimed at eradication of PPV have ended after nearly 20-years (Halbrendt, 2020). The successful eradication of PPV in the U.S. could not have been achieved without the cooperation of fruit growers, state and federal regulators and researchers, and homeowners. However, PPV could make a comeback. To address this continuing threat, USDA-APHIS is currently taking steps to ensure PPV does not reappear in the United States. This includes examining budwood for clean propagative material and monitoring stone-fruit-growing states, such as Pennsylvania and New York, for potential outbreaks of PPV. This successful PPV monitoring program includes the use of trained canines to detect PPV in orchards and in budwood used for plant propagation with more than 99 percent accuracy, with no need for destructive sample collection and subsequent laboratory processing unless necessary. The PPV monitoring program also closely inspects imported plants for signs of the disease (Jiang, 2019).

CASE STUDY: PORCINE EPIDEMIC DIARRHEA

Introduction

One of the major challenges in the swine industry is maintaining a healthy herd of animals. Disease outbreaks will not only impact local and regional business profitability but can also lead to loss of valuable animal stock, result in national and global sanctions and embargoes on animal products and in rare instances affect human health. Trade restrictions can significantly impact the whole industry and even the economy of nations.

Routine methods alone may not be ideal to diagnose uncommon, emerging, re-emerging or novel diseases. Testing methodologies based on advanced technologies such as nucleic acid sequencing can yield valuable information in such situations. An example is the 2013 outbreak of Porcine epidemic diarrhea virus (PEDV) infection of swine herds in the US (Stevenson, 2013; Goede 2016). While PEDV infection had been reported in Europe and Asia, it had not been reported in the US prior to 2013. PEDV is a single stranded, positive sense RNA virus belonging to the Alphacoronavirus genus (Family Coronaviridae). PEDV is very contagious and mainly transmitted by the fecal-oral route. The virus preferentially infects intestinal epithelial cells and causes their destruction once entry is gained. This ultimately leads to life threatening diarrhea (Jung, 2015).

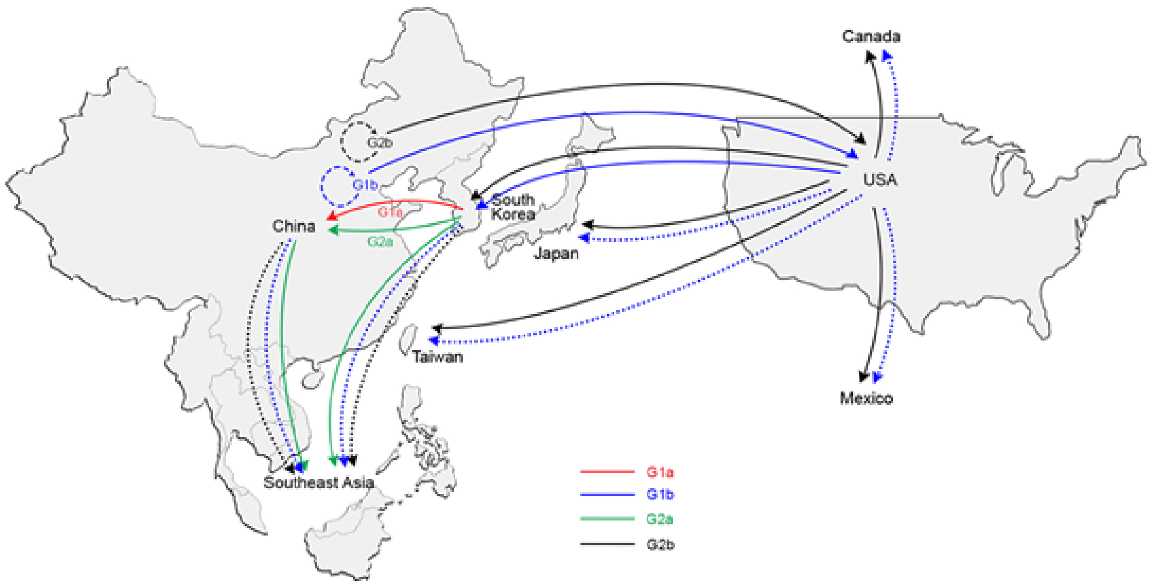
How it is Diagnosed

Based on clinical signs and the nature of the outbreak, several NAHLN laboratories received samples and initially considered Transmissible Gastroenteritis Virus (TGEV) as the likely cause. However, pathogen-specific PCR testing for TGEV turned out to be negative. Further evaluation at the NAHLN laboratories using electron microscopy showed the presence of coronavirus like particles in fecal samples. Based on this finding, a generic Coronavirus PCR was performed, and fecal samples from all farms tested positive. Nucleic acid sequencing of the PCR amplicon showed 98 to 99% identity to PEDV strains from China. Based on next generation sequencing techniques, whole genomes of PEDV strains from the individual farms were found to have 99.9% similarity with each other. The diagnosis of PEDV was confirmed officially at the NVSL. The advanced sequencing and diagnostic technologies used not only helped in identifying the etiological agent, but also provided valuable information regarding provenance and source of the original infection.

How it is spread

In the early half of 2013, severe outbreaks of diarrhea and vomiting were reported in pigs of multiple swine farms in Iowa, USA (Stevenson, 2013) (Figure 12). PEDV is believed to have originated in the UK and Belgium in the 1970s. Later the virus was identified in Asia. The US strains showed highest genetic similarity to strains reported from China in 2012.

Figure 12. Potential global routes of transmission of different PEDV subgroups
(Source: Lee. Virology Journal, 2015, 12:93)



Cost to Industry

The US global pork export market was valued at $7 billion in the year 2019 (Source: U.S. Census Bureau Trade Data - BICO HS-10, U. S. Agricultural Export Yearbook, 2019). Significant losses were reported with animals of all ages being infected and resulting in more than 95% mortality in suckling piglets. PEDV has not yet been eradicated from the US and has already resulted in the deaths of millions of piglets and an ongoing cost of millions of dollars.

How it was Managed

A multi-pronged approach has been adopted for ongoing control of this disease including, induction of herd immunity in gilts with subsequent transfer to piglets through colostrum, restriction of animal movement from infected premises, thorough disinfection of premises and personnel and strict biosecurity on farms.

CONCLUSION

Although the industry was not prepared for an outbreak of the scale that was witnessed, technological advances in NAHLN laboratories helped quickly and accurately identify the etiologic agent. Global and local emergence of highly contagious diseases in animal populations should be closely monitored and evaluated. Appropriate risk assessments should be performed and necessary biosecurity measures implemented in a timely fashion.

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KEY TERMS AND DEFINITIONS

Antibody: A molecule produced by an organism in response to the presence of a foreign substance, organism (bacteria, virus, fungus) or antigen as a defensive mechanism.

Antigen: A foreign substance or organism, in whole or in part, capable of eliciting an immune response such as antibody production.

Biomarker: A measurable substance in an organism whose presence is indicative of response to some phenomenon such as disease, infection, or environmental exposure.

ELISA: Acronym for enzyme-linked immunosorbent assay, a test method generally used to detect antibodies in serum or other fluid samples, but may also be built to detect antigens.

Forensic(s): The science of investigation and diagnosis of the cause(s) of death or injury in criminal or legal cases.

Gene Sequencing: A method of determining the order and number of amino acids that make up the chain of a genetic molecule or gene, which provides a unique identification (DNA or RNA).

Laboratory Information Management System (LIMS or LIS): Software used to capture, maintain, and transmit information pertaining to the specimens submitted to the laboratory, testing performed and the results of those tests and to disseminate a report of those results. A LIMS may also include financial functionality to support calculating fees for testing, sending invoices to clients and tracking accounts receivable.

Metagenomics: The study of a collection of genetic material (genomes) from a mixed community of organisms.

Molecular: Testing method based on detection of nucleic acids (DNA or RNA).

Morphology: The gross physical appearance of an organism.

Multiplexing: An assay design that allows for the detection of multiple targets, usually representing multiple pathogens or biomarkers, at the same time.

Pathogen: An organism capable of causing clinical disease in a host.

Pathophysiology: The effect(s) of a pathogen on the normal functioning (physiology) of the host plant or animal.

Phytosanitary/Sanitary Rules: Rules and regulations governing the health status of plants (phytosanitary) or animals (sanitary) in commerce.

Polymerase Chain Reaction (PCR): A molecular test method utilizing an enzyme to rapidly make copies of DNA or RNA, usually coupled with a mechanism to specifically target and identify the nucleic acid sequence of a known pathogen.

Proficiency Testing: Determining the competence of an individual or laboratory to correctly perform a test, usually done by having the individual or laboratory run the test on a carefully constructed set of samples that they do not know the true status of (blinded) and comparing their answers with the known true status of the samples as determined by a reference laboratory.

Serological: Testing method utilizing serum or occasionally plasma, usually to detect antibodies or other biomarkers, occasionally to detect antigen.

STEM: Acronym for science, technology, engineering, and mathematics areas of knowledge.

Triage: Performing an initial assessment of a situation or clinical submission and deciding what further course of action is warranted to best serve the diagnostic needs of the case.

Whole Genome Sequencing (WGS): Determining the sequence of amino acids for the entirety of the genetic material of an organism.

Zoonotic: A disease capable of being transmitted between animals and humans, in either direction.

Chapter 8

Initial Emergency Response:
Organizational Structure and Coordination

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ABSTRACT

This chapter focuses on emergency response following an initial detection of an invasive plant pest or foreign animal disease (FAD) as well as the regulatory authority utilized to initiate a response. Many emergency responses will be associated with crops and livestock on farms and ranches while others may involve nurseries, forests, wildlife, and exotic animals in various urban and rural locations. The incident command system framework is typically utilized to organize response efforts. Standard response preparedness and mobilization will be discussed with a consideration of the multitude of internal and external influences that can impact the strategy and tactics used during an outbreak. Sources of emergency funding and the critical need to manage public perception and information are also explained.

INTRODUCTION

Emergency response to animal and plant disease outbreaks encompasses much more than eliminating an infectious agent or pest. Responses are rapidly changing and complex, often involving multiple

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organizations. Decisive actions must be taken to contain, control, mitigate, or eradicate invasive pests and infectious agents. Emergency preparedness well in advance of a disease incursion supports rapid detection and diagnosis, mobilization of emergency personnel, treatments and recovery. Responses must be conducted in an environmentally sensitive manner with the cooperation of impacted industry and community stakeholders.

Emergency responses are carried out in a variety of rural and urban locations. Depending on the agent, control efforts may focus on a single type of operation such as a farm or ranch while other responses concentrate on a wide variety of locations ranging from nurseries to urban forests, warehouses and even suburban backyards. Millions of taxpayer dollars may be spent to protect livestock, crops, poultry, trees, wildland plants, grains or spices. In addition, the protection of human health cannot be overlooked. According to the Centers for Disease Control and Prevention, scientists estimate that more than six out of every ten known infectious diseases in humans can be spread from animals, and three out of every four new or emerging infectious diseases in humans come from animals (United States Centers for Disease Control, 2017).

Each response is unique and can be quite different even for the same disease or pest dependent on timing, location and other factors. In most cases, responding regulatory agencies, industries, and other groups organize utilizing the Incident Command System (ICS). The ICS structure creates a highly flexible yet systematic framework from the initiation of a response through to its conclusion. During a complex response, Incident Management Teams plan and guide activities within each ICS functional discipline which allows responders to focus on assigned tasks. There are a multitude of internal and external influences that determine the effectiveness of a response and how leadership approaches decision making. To succeed, emergency responses need to be accepted by the communities they occur in or impact. Communication and understanding public perception can be among the most challenging aspects of a response.

In this chapter, we describe emergency response processes for animal and plant disease outbreaks used in the United States (U.S.). However, similar response protocols are used globally, especially in areas with relatively advanced biosecurity systems including Australia, New Zealand, Canada, the United Kingdom, and France. However, outside of these nations, most countries do not have the resources and infrastructure to train and use ICS. Many international non-governmental organizations like the Food and Agriculture Organization (FAO), Red Cross and Red Crescent use the ICS and foster its basic concepts and structures in countries where they are active and respond or provide aid. The FAO has produced a series of locally relevant response guidance documents, termed Good Emergency Management Practices (GEMP) for use by developing country governments responding to animal disease outbreaks. The principles and practices inherent in the ICS are even more important when the responding entity are resource restricted.

AUTHORITIES FOR RESPONSE

The authority to initiate and conduct emergency disease response activities can be complicated as federal, state, tribal and local jurisdictions may overlap. All levels and types of government may influence response decisions because these entities often have regulatory and legal responsibilities requiring them to be involved in the decision to initiate a response and to support and manage tactical response actions. In the U.S., the Code of Laws of the United States of America (USC) and the Code of Federal Regulations (CFR) are codified authorities representing different stages of the legislative process. The USC provides the general and permanent statutes of the United States, which are passed by Congress and signed by the President. Executive branch agencies then interpret the USC, developing detailed regulations in the CFR. The CFR is developed through a public rulemaking process (United States Department of Agriculture, Animal and Plant Health Inspection Service, 2016b).

For plant disease incidents, the Plant Protection Act (PPA) (Title IV., Pub. L. 106-224, 114 stat. 438.7 USC 7701-7772) provides the Secretary of Agriculture the authority to initiate an emergency response. The PPA and emergency declarations, codified in Federal regulations, serve as the foundation for flexible programs for protecting the United States against exotic plant pests. The United States Department of Agriculture (USDA) has broad authority to take actions against threatening pests and to promulgate or modify existing regulations when necessary. The PPA provides the authority to regulate the movement of plant pests and their carriers, into or through the United States and to take emergency measures pending promulgation of quarantines and regulation (USDA Animal and Plant Health Inspection Service, 2010).

In a foreign animal disease (FAD) incident response the USC and CFR provide policy, via statutes and regulations, for the USDA. Interim regulations can be implemented—in the event of an outbreak—to prevent the spread of disease (United States Department of Agriculture, Animal and Plant Health Inspection Service, 2016c). The Animal and Plant Health Inspection Service (APHIS) receives its permanent and general regulatory authority for a FAD response from the Animal Health Protection Act (AHPA) 7 USC 8301 et seq. The AHPA enables the U.S. Secretary of Agriculture to prevent, detect, control, and eradicate diseases and pests of animals, including foreign animal and emerging diseases, in order to protect animal health, the health and welfare of people, economic interests of animal agriculture and related industries, the environment, and interstate and foreign commerce in animals and other articles. The term “animal” means any member of the animal kingdom except a human (7 USC 8301-8302). The Secretary is specifically authorized to carry out operations and measures to detect, control, or eradicate any pest or disease of livestock, which includes poultry (7 USC 8308) and to promulgate regulations and issue orders to carry out the AHPA (7 USC 8315). The Secretary may also prohibit or restrict the importation, entry, or interstate movement of any animal, article, or means of conveyance to prevent the introduction into or dissemination within the United States of any pest or disease of livestock (7 USC 8303-8305). Title 9 of the CFR provides detailed USDA APHIS administrative regulations for the control and eradication of animal diseases, including FADs and emerging animal diseases (United States Department of Agriculture, Animal and Plant Health Inspection Service, 2016c). In the event a FAD involves wildlife, USDA APHIS works closely with federal, state, tribal and local wildlife agencies.

State authority is derived in a similar manner as federal authority through statute from a state legislative body and regulations from a state’s executive branch. The authority over plant disease-related incidents is handled by APHIS State Plant Health Director (SPHD) in cooperation with the State Plant Regulatory Officer (SPRO) based with the state or territory department of agriculture. In some states, such as California, local agricultural officials at the county level are involved in the response. State authority in a FAD is typically held by the State Animal Health Official (SAHO). The SAHO in the majority of states is the State Veterinarian, and most quarantines utilized in an outbreak are state quarantines specific to the state where the infection occurs. Depending on the location and scope, tribal as well as local laws and regulations may also apply.

RESPONSE INITIATION

Before a plant or animal emergency disease response is initiated, there must first be surveillance and identification of the disease agent or the general nature of the disease if the cause remains unknown. General surveillance for plant and animal diseases is conducted in a variety of ways and may be classified as active or passive. For plants, caretakers such as farmers, horticulturalists, arborists or foresters, serve as a first line of evaluation. Detection becomes challenging when a plant disease impacts areas with little to no active management, such as wildlands.

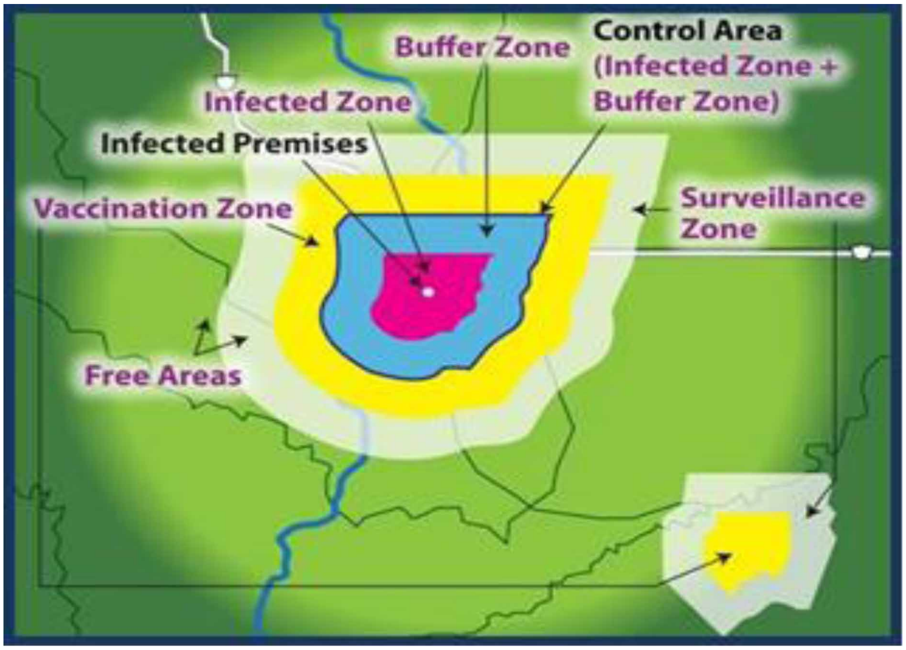
In the animal realm, animal owners along with their veterinarians are on the front lines for initial detection of a FAD in domestic animals. Veterinarians or animal owners, suspecting a FAD in animals, report suspicious cases to the SAHO or USDA in their state. Following a report, a trained Foreign Animal Disease Diagnostician conducts an investigation of the affected animals and collects additional samples for testing. Wildlife presents a unique set of obstacles depending on the management of the area. If a disease or pest presents similar clinical signs or behaves similarly to a common endemic disease, accurate identification of the agent may take as long as several years.

In the United States, plant diseases are typically first identified at state departments of agriculture or extension/university laboratories. All samples of new pathogens are then confirmed by the USDA APHIS Plant Protection and Quarantine – Science & Technology Beltsville Laboratory. State diagnostic labs may then be trained and certified to confirm the new pathogen by the APHIS National Plant Protection Laboratory Accreditation Program (NPPLAP) laboratory. For animal diseases and parasites, confirmations are made at either the USDA APHIS Foreign Animal Disease Diagnostic Laboratory (FADDL) or National Veterinary Services Laboratory (NVSL). FADDL and NVSL are the national reference laboratories for USDA program diseases and FADs. These laboratories serve as the only sites in the United States that can determine the FAD status of the country and provide official results for notification to our trading partners and other international entities.

Following identification and confirmation, international, state, tribal, and domestic industry stakeholders are notified by USDA officials. For animal diseases, Office International des Epizooties [Office International of Epizootics, World Organization for Animal Health (OIE)] notification occurs immediately. This notification most often results in international export restrictions of animals and animal products by trading partners. Domestically, interstate movement restrictions are typically placed by those states without the disease. Depending on the disease, a nationwide stop movement order may be placed for susceptible species. For example, for a Foot and Mouth Disease detection a 72-hour stop movement order would be placed nationwide (USDA APHIS, 2014b). In the case of plant diseases, an Emergency Action Notice is issued by USDA at the infested site, stakeholders are notified and quarantines, handling and movement restrictions are put in place.

When notification occurs for an animal disease, State Departments of Agriculture often enter into a Unified Command under the Incident Command System with their federal counterparts in the affected state. Depending on the size and circumstances of the disease incident, in some cases, states may choose to take the lead on response efforts. Emergency disease response is initiated and coordinated with affected premises owners, industry partners, and other stakeholders. Initially the index premise (IP) is quarantined while decisions are made on the strategy and tactical actions to effectively eliminate the threat. Quarantines and movement controls are an important regulatory intervention to control and contain a FAD. In an incident, State and/or Federal quarantines may be implemented. Federal quarantines and movement restrictions may be instituted to prevent disease transmission and to control interstate and international movement of infected animals and contaminated animal products. States implement quarantines to prevent disease transmission, and may restrict the intrastate movement of animals, animal products, equipment, and other items. Typically, a state will quarantine an IP, and the state and/or Unified Incident Command will establish a Control Area—where movement controls are implemented—around that IP. If a Federal quarantine is implemented, the quarantine area is commonly the Control Area (Figure 1).

Figure 1. A depiction of all zone and area designations surrounding an Infected Premises. Infected Zone – Pink. Buffer Zone – Blue. The Infected Zone and the Buffer Zone together comprise the Control Area. Vaccination Zone – Yellow – which can be located inside or outside of a Control Area, as seen on this map. A Vaccination Zone may be either a Protection Vaccination Zone or a Containment Vaccination Zone. Surveillance Zone – White. Free Areas – are all areas not included in any Control Area.
Source: USDA; illustration by Dani Ausen, Iowa State University



For plants, the incident response is similar, with the ‘vaccination zone’ becoming a potential ‘treatment zone’. USDA APHIS issues Emergency Action Notices, prohibiting or restricting movement of the host materials while corrective action is taken at the site of infestation. Infected and adjacent plants, and associated materials are likely to be destroyed, some of which may have been shipped to another location, as in nursery stock. Due to the elusive and cryptic nature of many plant pathogens and the wide range of environs in which an outbreak can occur, the response must be tailored to each specific event. The response in a forest is vastly different than one in a grower’s field or an establishment. Identification of the full host range of the pathogen can be challenging if the pathogen is new to science. Aside from live plants and the soil in which it is planted, plant products (e.g., wreaths, compost, and firewood) must be evaluated for potential to transmit the pathogen, and those materials must be regulated. Collaboration between federal, state, and tribal governments, as well as cooperation from the affected industry is necessary.

Defining the extent of the disease is a critical initial action taken during an emergency disease response. Based on the epidemiology of the disease, surveillance is conducted in a prescribed control zone or area around the index premise. For most plant diseases, visual surveys and sampling are implemented around the infested site, the zone area determined by the nature of the pathogen. Wind- or waterborne pathogens will have a greater area designated for survey than a soilborne pathogen. Soilborne pathogens present a challenge as they may be moved on soil adhering to vehicles, tools, shoes, nursery stock and other items. Each pathway of movement must be investigated and managed. Trace-forward and trace-backward investigations of potentially infected products are conducted. For example, pathogens found on nursery stock are tracked back to the origin of the material, where inspection and sampling will occur. At that premises, all related species and known pathogen hosts which had been sold and shipped within the previous six months will be tracked, inspected and sampled. This can become difficult for plants sold from retail outlets, where traceability is rarely possible. Adding to the challenge, some plant diseases do not exhibit obvious symptoms, such as Plum Pox Virus in some stone fruit varieties. In the case of insect-vectored diseases, such as Huanglongbing (HLB, citrus greening, caused by *Candidatus* Liberibacter spp.) carried by Asian citrus psyllid (*Diaphorina citri*), surveillance of the vector is essential and often completed through the use of traps baited with pheromone attractant.

For most animal diseases, evaluation of animal and human movements from the index premises along with area surveillance is conducted to evaluate the extent of disease spread as well as to define the point of disease introduction. Animal movements are evaluated as trace-ins or trace-outs. Animal movements into the population, such as new purchases or introductions, are considered trace-in type movements. Animals leaving the premises, such as for shows or sales, would be considered trace-outs. A typical surveillance control area is a 5 km or 10 km zone surrounding the index premises. Surveillance may be conducted in a variety of ways ranging from surveys of homeowners and testing of susceptible species to collection of samples from public areas. For animal diseases, alerts are sent to local USDA-accredited veterinarians in areas outside established control zones. These alerts heighten awareness amongst the veterinary community, as well as affected animal owners.

COMMUNICATION

Communication between numerous authorities is critical to successful emergency response as federal, state, tribal, and local jurisdictions may have varied priorities and interests. Communication to the public and industry stakeholders is also particularly important at the initiation of an emergency disease outbreak.

Developing an effective public message and delivering it through multiple channels can establish early cooperation from impacted stakeholders. In the case of Unified Command, a Joint Information Center (JIC) would be established to coordinate information management and help ensure the public hears “one voice” with clear and correct information from the responding agencies. Inclusion of the general public in surveillance activities, such as a citizen scientist program, may increase the surveillance area and reduce disease spread through human movement. Effective public messaging can also influence lawmakers and others involved in the decision-making process for funding an emergency disease outbreak.

At the initiation of an emergency disease outbreak, officials from state agencies along with their federal colleagues in the state will often manage response activities with a Unified Command structure with assistance from industry stakeholders. However, if the response expands geographically or continues for an extended period of time, authorities may request an organized State or USDA Incident Management

Team (IMT) to manage the response activities. In some disease responses, such as the 2014-15 Highly Pathogenic Avian Influenza (HPAI) outbreak that occurred in fifteen US states and Canada, the complex response activities were managed by multiple IMTs (United States Department of Agriculture, Animal and Plant Health Inspection Service, 2016a).

Governmental agencies on the national, regional, state and local level may be required to respond due to laws, regulations, and programs that protect national, state, and local economies. Public and societal health and food security are also of utmost importance. Governmental management of plant and animal disease outbreaks are also often tied to requirements of international trade agreements and membership in organizations such as OIE.

ICS BASICS

Within regulatory responses to serious plant and animal diseases there are different levels and types of governmental response. Policy leaders manage high-level strategy and their respective interstate and international portions of the response. Federal, tribal, state, and local agencies with responsibilities to implement program disease management are typically involved in the initial tactical response to these disease incursions. Initial response activities, which may or may not be led by state authorities, typically focus on mitigation of disease spread, limiting economic damage, and maintaining adequate animal welfare. Effective responses rely on the implementation of well thought out plans by subject matter experts using proven organizational schemes. In the United States, emergency responses to animal and plant pathogens use the Incident Command System (ICS) to coordinate and manage the response.

As a result of the September 2001 terrorist attacks on the World Trade Center and the Pentagon, Homeland Security Presidential Directive #5 was issued in February 2003 with direction to establish a single, comprehensive national incident management system. In March 2004, the United States Department of Homeland Security issued the National Incident Management System (NIMS) which integrates the Incident Command System (ICS). With this issuance, it was mandated that NIMS (and thus ICS) must be utilized to manage emergencies in order to receive federal funding. ICS is the standardized incident management system for all federal, state, and local agencies.

As defined by the National Wildfire Coordinating Group, the Incident Command System (ICS) is a standardized on-scene emergency management concept specifically designed to allow its user(s) to adopt an integrated organizational structure equal to the complexity and demands of single or multiple incidents, without being hindered by jurisdictional boundaries. ICS was developed in the early 1970s by the Firefighting Resources of California Organized for Potential Emergencies (FIRESCOPE) and since that time has been used for all manners of all-hazards incidents and planned events.

ICS is a modular system that easily expands and contracts with the scope and complexity of the incident or event. ICS is applicable for incidents involving single jurisdiction/single agency, single jurisdiction/multi-agency or multijurisdictional/multi-agency incidents. It is based on the following components that allow for effective incident management:

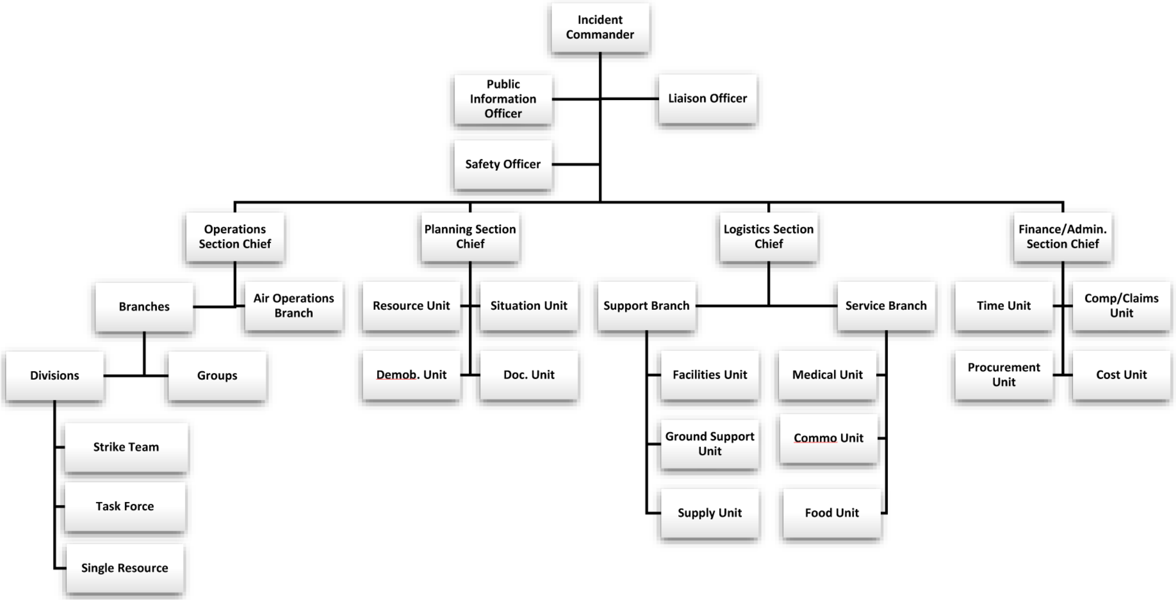
- Common Terminology
- Modular Organization
- Integrated Communications
- Unified Command Structure

- Incident Action Plans
- Manageable Span-of-Control
- Pre-designated Incident Facilities
- Comprehensive Resource Management
- Measurable Objectives
- Establishment and Transfer of Command
- Chain of Command/Unity of Command

ICS utilizes five main functional areas for the management of an incident or planned event. Commonly known as Command and General Staff, the five functional areas are: Command Staff, Operations Section, Logistics Section, Planning Section, and Finance/Administration Section. The Command Staff is comprised by the Incident Commander, Public Information Officer, Liaison Officer, and Safety Officer. A Section Chief is responsible for the oversight of each section within the General Staff. Each section is then further subdivided with standardized subordinate roles. Sample ICS Organizational Charts are in Figures 2 and 3.

Figure 2. A simple ICS structure for use to respond to a small, uncomplicated incident

Figure 3. A complex ICS structure for use in a large emergency response



Simple ICS Organization

Each functional area has specific duties related to incident management. A short description of duties for the Command and General Staff roles is as follows:

- Incident Commander – The Incident Commander (IC) is responsible for the overall management of the incident. The IC establishes incident objectives, orders necessary resources, and makes assignments. The Incident Commander must have training and experience commensurate with the assigned incident's complexity. In the Incident Command System, the IC is the one position that is always filled. In the event that Command and General Staff positions are not filled, the IC is responsible for those functions until a qualified individual is assigned to the incident.

NOTE: While all incident resources are subordinate to the Incident Commander, the Incident Commander is subordinate to the Agency Administrator from the Agency with jurisdictional authority where the incident is occurring. In many cases the IC receives a Delegation of Authority from the Agency Administrator which allows the IC to operate in an environment which may be outside of their normal scope-of-employment.

- Liaison Officer – The Liaison Officer is the Incident Management Team's point-of-contact for representatives from cooperating agencies or incident stakeholders.
- Public Information Officer – The Public Information Officer, under the direction of the Incident Commander, develops accurate incident information for release to the general public. The PIO also serves as the point-of-contact for information requests from the media, agencies, cooperators, and stakeholders.
- Safety Officer – The Safety Officer is responsible for assessing and implementing mitigations for the hazardous and unsafe situations that jeopardize the safety of incident personnel.
- Operations Section Chief – The Operations Section Chief is responsible for the implementation and oversight of the incident's tactical ground and aviation operations. The Operations Section Chief is also directly responsible for the development of the Incident Action Plan.
- Logistics Section Chief – The Logistics Section Chief is responsible for providing all incident ground resources support needs including ordering personnel and equipment, establishing incident facilities, transportation, supplies, incident medical services, communications, and feeding.
- Planning Section Chief – The Planning Section Chief manages incident data regarding assigned personnel, equipment, and the situation status. This position is also responsible for producing the Incident Action Plan, assembling incident documentation and other related information along with resource demobilization. The Planning Section Chief determines and facilitates the Incident Management Team meeting schedule and the incident planning process.
- Finance/Administration Section Chief – The Finance Section Chief is responsible for all financial aspects of the incident. This includes tracking personnel and equipment time, procurement of supplies and necessary incident support, Compensation/Claims, and producing incident cost summaries.

On small or emerging incidents, one individual may fulfill all of the Command and General Staff roles. However, as an incident expands it becomes necessary to staff each function, unit leaders, and other ICS positions with qualified individuals to effectively manage the incident as it increases in complexity.

The NIMS Guideline for the National Qualification System defines Incident Complexity as the incident criteria determined by the level of difficulty, severity, or overall resistance faced by incident management or support personnel while trying to manage or support an incident to a successful conclusion or to manage one type of incident or event compared to another type. Complexity is a major consideration in determining the size and makeup of the organization necessary to successfully manage an incident.

Incident complexity is rated from Type 5 (least complex) to Type 1 (most complex). Complexity Type is based on a pre-determined set of incident characteristics and conditions which are evaluated as needed from the initial response through the eventual close of the incident response. Examples of characteristics or conditions to determine incident complexity may include:

- Impacts to life, property, and the economy
- Responder and public safety
- Potential incident duration
- Political sensitivity
- Incident extent and/or jurisdictions involved
- Availability of resources

Some commonality of incident characteristics and conditions for determining complexity exists across the broad spectrum of disciplines which use ICS for incidents and events. For example, Type 3 incident characteristics common to all disciplines would be an incident duration lasting multiple operational periods with several types and kinds of resources committed. However, each response discipline will have unique evaluation criteria specifically identified to assist in determining the necessary level of response. For a FAD response the number of resources needed to implement a “Stop Movement” order may be one characteristic to identify complexity.

The ICS should be established at the time of the initial response to an incident. Early implementation of ICS has positive implications on incident management for the duration of the event. Early implementation also allows for a seamless transfer of command if necessary.

When a response occurs, the Incident Commander should initiate the size-up process to determine incident objectives, strategy and tactics, resource needs, and incident potential. Items to consider in sizing up the incident include, but are not limited to:

- Location
- Incident extent
- Potential extent and duration
- Values-at-risk
- On-scene resources
- Additional resource requirements
- Safety hazards
- Socio-political implications
- Current and expected weather conditions
- Current tactics or control actions.

Each size-up factor should be documented on an ICS-201 Incident Briefing form or other similar format. This information can serve as a basic Incident Action Plan for briefing incoming resources and serve as final documentation should the incident be mitigated by the initial response. Another important step in the size-up process is to map the incident. The map should include important incident features such as the area involved, transportation routes, values-at-risk, premise locations, or closure areas. The incident should also be subdivided on the map into logical geographic Divisions to aid in resource deployment.

Size-up also provides the basic inputs necessary to conduct a Complexity Analysis. The Complexity Analysis may reveal that a higher level of incident management is warranted due to factors such as significant incident growth potential or duration. If transfer of command or if incident management organizational growth will occur there are measures that can be taken to ensure a smooth transition from initial response to extended response:

- Establish an Incident Command Post (ICP) – The ICP will serve as a focal operating point for the incident management team.
- Establish Check-In Locations and Staging Areas – This will allow an opportunity to provide incoming resources a briefing on incident details and their assignment duties.
- Determine Incident Objectives, Strategy, and Tactics – Objectives for the incident is a description of the desired outcome using specific, measurable, achievable, realistic and time-limited results. Strategy is the general plan to accomplish incident objectives. Tactics are the ‘nuts and bolts’ of how the chosen strategy is implemented.
- Organize the incident into Divisions and/or Groups and assign supervision - This is a primary consideration for organizing the incident and helping mitigate span-of-control issues. Divisions are geographic in nature where Groups are functional. A Division is a portion of the incident area with clearly delineated borders. A Group performs a specific incident function, such as Disease Surveillance, anywhere within or near the incident boundaries.
- Develop a communications plan – Establish how incident communications will take place. If by two-way radio, assign frequencies for each Division/Group. Develop a contact list of assigned personnel if communications between resources and incident management occurs by cell phone. In many cases both two-way radios and cell phones will be used for communications purposes.
- Order necessary resources to begin or extend operational capability – Provide the date and time needed, check-in location, and any special instructions related to the assignment. Consider ordering an Incident Management Team if the incident complexity warrants. Expect that the following operational period may be the earliest that resources may arrive on-scene due to notification procedures, travel time, availability, etc.
- Prepare to brief incoming resources on the current and expected incident situation, incident objectives, strategy, tactics, safety hazards, assignment, operational period, etc.
- Track resources for accountability – Record where resources are deployed, the resource information, when they were assigned, and any other pertinent resource data. Data concerning the currently committed resources is extremely valuable to an incoming Incident Management Team.

As incident objectives are met, the incident management organization is scaled accordingly to address any remaining tasks or to close out the incident. Demobilization should be planned and organized to minimize logistical issues for incident resources returning to their home unit.

Incident Command Teams are extremely valuable in providing the organizational structure necessary to manage all manners of emergency incidents and planned events. In some cases, an Incident Management Team may be assigned to an incident which falls outside of their training and experience. In these instances, the IMT should enlist assistance and guidance from discipline-specific Subject Matter Experts (SME) in order to develop the strategy and tactics for mitigating the incident. For example, an Incident Commander and Operations Section Chief may be highly trained and qualified for a foreign animal disease incident, but would need plant pathology SMEs to properly manage the response to an outbreak of Sudden Oak Death (*Phytophthora ramorum*) across a large geographic area.

In-depth ICS training is available on-line through the Federal Emergency Management Agency. Go to https://training.fema.gov/ for a list of available courses.

FUNDING

Despite the fact that emergency response is not a planned event, the United States has established mechanisms to provide emergency funds to cover response costs. The cost of implementing an emergency response varies depending on the commodity, disease, extent of the outbreak, and other factors. The authority to transfer federal funds for plant and animal disease emergency response is contained in both annual Congressional appropriations and in the Code of Laws of the United States of America or U.S. Code. The Plant Protection Act (7 USC 7701-7772 and 7781-7786) and the Animal Health Protection Act (7 USC 8301-8317) provide the authorities for APHIS to conduct animal and plant health monitoring and surveillance, and to regulate and enforce such programs, including pest and disease management and eradication (USDA APHIS, 2020c).

Federal emergency response funding may be available from contingency funds administered by the USDA APHIS. However, if the funding amount needed is beyond what is available through contingency funds, a Declaration of Emergency may be issued by the U.S. Secretary of Agriculture to request a transfer of Commodity Credit Corporation (CCC) funds or other U.S. Department of Agriculture funds to APHIS (USDA APHIS, 2017a).

The CCC is a wholly-owned government corporation with the authority to incur up to US$30 billion in outstanding debt to the U.S. Treasury. CCC funds are also used to respond to natural disasters and finance farm commodity, trade and conservation programs. The CCC repays the funds it borrows from the Treasury through periodic congressional appropriations so that its US$30 billion debt limit is not depleted (Stubbs, 2019).

Along with the emergency declaration, federal regulations may be issued (e.g., an interim rule to contain a pest). An emergency declaration may also be issued for a state by a governor, state department of agriculture head, or other appropriate governmental official to authorize funds for activities to arrest, control, eradicate, or prevent spread of a disease, and related expenses including indemnity for producers (USDA APHIS, 2017a). In the U.S., significant financial support for emergency response is provided to APHIS via Plant Protection Act Section 7721. APHIS in turn provides funding to external cooperators – mostly State departments of agriculture, but also academic institutions and other Federal agencies – as partners in response.

OUTSIDE INFLUENCES ON EMERGENCY RESPONSE AND PUBLIC PERCEPTION

Emergency response to animal or plant diseases requires extensive cooperation and coordination between regulators and many segments of society. However, the nature of emergency disease response— the need for quick, often destructive action that may bypass public involvement and the declaration of an emergency — may increase public suspicion and erode public cooperation.

To support emergency response, trust — a belief in the reliability and ability of the responders and the benefit of the response — is needed among regulators, affected owners and producers, industry leaders, and the public. Since trust takes time to develop, interactions to develop partnerships are essential in advance of new incursions. Emergency response relies on publicly funded preparedness support such as surveillance, laboratories, and trained response personnel. Through these activities relationships are developed. Relationship building should be an on-going effort well before there is a need to respond.

While based on science, emergency actions for plant and animal diseases are often accompanied by political actions such as required approvals from elected or appointed officials (e.g., state governors, U.S. Secretary of Agriculture). Funding allocations for larger programs may be set out in State and Federal legislation because emergency response can cost millions or reach into the billions of taxpayer dollars (USDA APHIS, 2020c).

Building a Coalition to Support Emergency Response

To initiate an emergency response to a pest or disease, State and Federal regulators, each with overlapping authorities, must agree to a set of rules and actions for prevention, containment, control or eradication. On the local level, county, city and extension personal are often called upon to maintain public safety and infrastructure, and facilitate access for surveys and other response actions. Alignment among federal, state and local entities in an efficient manner can hasten the response and enhance public trust in the regulatory response. The more that agencies, organizations, educators, and researchers unite behind the emergency response the higher the likelihood of public acceptance and support (Burgiel, 2020). For emergency response to succeed many levels of government and various jurisdictions need to buy-in, and understand their roles and responsibilities (National Association of State Foresters, 2004). The implementation of Unified Command, as described in the ICS, is a key element of achieving success in a complex animal or plant disease response.

While emergency response actions may occur in nurseries or feedlots, the interventions reverberate across society into affected communities, to consumers of the commodity, animal rights advocates, and environmentalists. The response actions taken often involve preemptive destruction of host animals or plants through means which may trigger intense emotional reactions from some segments of society.

Developing stakeholder support can be challenging because the various parties involved in or concerned about emergencies may have different values, communication patterns, and disparate or conflicting interests. Politicians in the areas subject to quarantine or eradication may be hesitant to support emergency actions because it may disrupt trade and be expensive to implement (European and Mediterranean Plant Protection Organization, 2019). Owners of animal or plant hosts subject to new regulation may not agree to the destruction of their property for the greater good of industry (Gottwald, 2007) or others in distant areas. Compound those concerns with confusion generated by the need to constantly reissue protocols as the relevant science evolves (i.e., development of new diagnostic methods or identification of additional hosts) – and the difficulties of sustaining support can overwhelm even the most proficient and dedicated responders.

PUBLIC PERCEPTION

While all emergency response actions rely on public cooperation, responses to diseases where the hosts occur in yards and lands of multiple ownerships serve as dramatic examples of the importance of public perception to support successful emergency response. These cases from Florida and California highlight some of the complexities that may arise.

Asian citrus canker eradication, 1995 to 2006 in Florida. Asian citrus canker (ACC) caused by bacteria, *Xanthomonas axonopodis* pv. *citri* (*Xac*), had been declared eradicated in Florida in 1992, but reappeared in 1995 in Dade County on residential citrus south of the Miami International Airport (Gottwald et al., 2002). An eradication campaign was launched by USDA, APHIS and the Florida Department of Agriculture and Consumer Services with the formation of the joint State/Federal Citrus Canker Eradication Program (CCEP). The eradication protocols were based on an epidemiological study of ACC spread that determined the need to remove all presumably exposed trees within a 579 m radius of a known infected tree (Gottwald et al., 2001).

The program became mired in legal battles between the CCEP and residential homeowners who felt that the regulatory actions protected the citrus industry at the expense of residential citrus tree owners. Numerous injunctions and appeals led to eradication delays. As the legalities of eradication were debated, Hurricane Wilma struck in 2005 and significantly spread the disease. After 10 years and expenditure of US$1 billion the regulatory agencies, citrus industry representatives, and research scientists concluded that the pathogen had become widespread in Florida and terminated the eradication program. In 2006, USDA declared that citrus canker eradication was no longer feasible, which was followed by the Florida House of Representatives issuing a decision to halt the program (Gottwald, 2007).

Virulent Newcastle Disease eradication, 2018-2020 in California. The eradication of Virulent Newcastle Disease (vND) in Southern California first detected in 2018 and declared eliminated in June 2020, involved 476 positive premises, including four commercial poultry facilities, in six California counties (USDA APHIS, 2020d). Eradication required the depopulation of over 1 million domestic poultry and other birds (Hagerman and Marshall, 2020). The virus spread among backyard flocks, some of which comingled at live bird markets and exhibitions. Mandatory euthanasia of infected and exposed poultry and other birds, including pet birds, was required. (California Department of Food and Agriculture, 2020).

The trade consequences of the infestation were limited due to the disease's prevalence in non-commercial, small-holder populations, primarily backyard poultry flocks. But the backyard infestations complicated response and increased the types of problems the public and regulators had to face. Successful eradication relied on community cooperation to help stop the spread, including self-reporting of sick birds and no transport of birds beyond the quarantine area.

New infestations were caused by residents moving infected birds from within the vND quarantine area to another county and inadvertent distribution of infected birds at a retail feed and pet store. In response, feed and pet stores in parts of Southern California were prohibited from selling chicks for Easter. Multiple disasters had to be balanced, the California State Veterinarian provided guidance for flock evacuations during the Saddleback Ridge Fire in October 2019 stating that under mandatory fire evacuation orders, the vND quarantine requirements prohibiting bird movement were temporarily lifted and bird movement should be done in a manner that minimizes the disease threat. Despite these complications, vND was declared eradicated in June 2020 (California Department of Food and Agriculture, 2020) at a cost of over US$28 million in federal funds (USDA APHIS, 2020c).

Communications to Support Emergency Response

Hagerman and Marshall (2020) in a review of animal disease preparedness and response in the western United States concluded that public perception of disease occurrence needs to be factored into response strategy selection, particularly in the rapidly changing digital world. The authors point out that preparedness involves more than just readying responders for interventions at production facilities. Preparedness also involves preparing to respond when the public demands answers and reassurances about the safety of U.S. food supplies and details of humane animal handling – all in a world where information from both official and unofficial sources is just a tap away on a mobile device.

For emergency response, rules or requirements governing the agency with jurisdiction may dictate what and how information is shared with various audiences, but in many cases, there are few set rules or requirements for what information is shared with a particular audience. Typically, USDA APHIS first shares information internally and then with state and federal partners, then with industry and the public. Information may be shared via in-person meetings, conference calls, issuance of field talking points, press releases, social media and more formal routes such as State Plant Representative (SPRO) policy decision letters, North American Plant Protection Organization (NAPPO) alerts, and World Trade Organization notices (U.S. Department of Agriculture, Animal Plant Health Inspection Service, 2017a). Also notified are the US Animal Health Association, National Association of State Departments of Agriculture, World Organization for Animal Health (OIE) and other organizations in animal disease outbreaks.

As a matter of practice, when designing a response, it is beneficial to consider how diverse populations value and interact with the regulated plant or animal hosts because their practices and beliefs may drive the need for different actions (Estévez et al., 2015).

Notification and negotiations are required for actions that may affect officially recognized tribes in the US. These requirements are described in several Executive Orders (12898 - 1994; 13175 - 2000; 13647 - 2013). USDA APHIS is required to provide opportunities for tribes to participate in policy development including animal and plant disease quarantines to the greatest extent practicable and permitted by law.

The *Phytophthora ramorum* (sudden oak death) quarantine is a good example of the need for emergency response to reflect the values and traditions of diverse peoples, such as American Indians. Tanoak (*Notholithocarpus densiflorus*), a primary host for *P. ramorum,* is prized by American Indians for its acorns while many commercial foresters consider it a weed that competes with more economically valuable conifer species (Bowcutt, 2015). Interventions that remove tanoak are viewed differently by each group (Alexander et al., 2017). Additionally, many *P. ramorum* host plant species are gathered by American Indians for use in cultural activities or household items, presenting a risk of pathogen spread (California Oak Mortality Task Force, 2006).

Building Public Support and Involvement

Numerous communication and consultation actions are needed to support emergency response, especially if a significant public attitudinal and behavioral change is needed. In 2019, the European and Mediterranean Plant Protection Organization (EPPO) adopted standard PM 3/86 (1) “Raising public awareness of Quarantine and Emerging Pests" to enable quick response to plant pest emergencies. The standard provides a comprehensive description of communication needs and methods, as well as risks, such as raising expectations, inappropriate or inaccurate media reports, impacts on local and international trade, and others (European and Mediterranean Plant Protection Organization, 2019).

The following may improve public perception and facilitate public participation in disease and pest prevention.

- Scenario based table-top exercises and explanations of roles and responsibilities for hypothetical outbreaks can help partners become acquainted and prepare to rapidly respond.
- Creation of one science-based website for information explaining pest impacts, treatments, biology, monitoring, and diagnostic techniques. Include photographs and information targeted to various user-groups, professions and educational levels.
- Citizen and professional advisory panels may support ongoing interaction between regulators, scientists, and the public.
- Citizen-science monitoring or treatment programs can directly involve the public.
- Regulatory staff training in effective approaches to public interaction including the importance of trust and relationship development. Topics may include effective listening, unconscious bias, and the importance of prompt replies to public inquiries, consistent messaging, and developing community relationships.
- Outreach across disciplines, sectors, knowledge systems, and people with a diversity of lived experiences.
- Strengthen links between emergency response program leaders and social scientists to evaluate strategies to improve public cooperation.

Public support of emergency response is essential to the effectiveness of regulatory policies and programs. Ongoing, concerted public engagement, education, and outreach is needed to bolster plant and animal health and invasive species prevention and provide a foundation for emergency response (Klapwijk et al., 2016). Maintenance of broad support requires sensitivity to the social constructs that underlie people's expectations and fears. Consideration of the cultural, sociological and emotional factors that influence the way regulatory actions and information are interpreted is needed to garner support from an increasingly fragmented and distracted citizenry.

FUTURE OPPORTUNITIES

In our globalized world, plant and animal diseases and pests can spread rapidly across borders. Dependable and robust diagnostics, timely sharing of scientific information, development and implementation of biosecurity measures and notification, and the development of response capabilities are needed. The implementation of these measures is imperative to prevent transboundary spread and minimize impact of invasive species on agricultural production, local and regional economies, ecosystem services, natural resources, and to agrarian communities.

Identification and implementation of best practices are essential. Specific best practices identified in this chapter include:

- Rigorous implementation of appropriate biosecurity measures to exclude pathogens and pest introductions at points of entry into production areas and systems, and along all points of the supply chain.
- Familiarity and adoption of the Incident Command System to prepare for and manage incident disease/pest incursion responses
- Research on rapid detection and improved biosecurity measures for all points of the supply chain
- Development of resistant plant and animal varieties
- Development of big data programs to detect and predict outbreaks and emergence of significant plant and animal pathogens and pests. One example of this is the University of California, Davis PREDICT project developed as part of USAID's Emerging Pandemic Threats (EPT) program.

Also needed, as noted in OIE & FAO's Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs, 2004), are:

- Commitment to implementing existing international standards,
- Increasing access to adequate and sustainable human, financial or technical resources,
- For FADs, development of vaccines and other specific biologics for emerging diseases.

CASE STUDY: SUDDEN OAK DEATH - RESPONSE TO AN EMERGING PLANT PATHOGEN, *PHYTOPHTHORA RAMORUM*

The Challenge - Background

Sudden oak death was first recognized in the greater San Francisco Bay Area in the mid-1990s by arborists, appearing as bleeding cankers on oaks (*Querus* spp.) and tanoaks (*Notholithocarpus densiflorus)* which died suddenly. In 2000, the cause of the tree mortality was determined to be *Phytophthora ramorum* Werres, De Cock & Man in't Veld, thought to be new to science when first described (Rizzo et al., 2002). The pathogen was then isolated from rhododendron container plants in a Santa Cruz County nursery. Subsequently, researchers recognized that the pathogen had been found previously, in 1993, in Europe on *Rhododendron* and *Viburnum* associated with nursery production areas (Werres et al., 2001). Eventually over 100 host species would be identified including ferns, herbaceous plants, shrubs and trees (U.S. Department of Agriculture, Animal Plant Health Inspection Service, 2020a).

As noted by a Government Accountability Office review (2006) several years had elapsed between *P. ramorum*'s arrival to the U.S. and its discovery, thereby giving the pathogen time to become established in the environment before control programs began. By 2001, the disease was recognized as killing trees in eight Northern California coastal counties (California Oak Mortality Task Force [COMTF], 2020) and on 16 ha in Curry County, Oregon (Goheen et al., 2002). From 2000 - 2002, international and local quarantine regulations were issued to prevent pathogen spread (COMTF, 2020; Frankel, 2008). Despite the quarantines, in 2004, more than two million host nursery plants were inadvertently shipped from a few Southern California and Oregon nurseries that contained *P. ramorum* infected plants. Potentially infected plants were shipped to over 5,000 unique establishments nationwide (Jones, 2006) and infected plants were detected in 176 locations in 21 states (Kliejunas, 2010; USDA APHIS, 2014a). By 2009, more than 68 countries had implemented quarantines. (Sansford et al., 2009) (Figure 4).

Figure 4. Tanoak mortality caused by sudden oak death, Phytophthora ramorum, along the northern California coast
(Photo source: US Forest Service, Pacific Southwest Region, Forest Health Protection)

Challenges to Response

Completely new to the scientific community, all aspects of the *P. ramorum* program had to be developed, including emergency response approaches, diagnostics, surveys, and prevention and management strategies. The lack of scientific information also made risk assessment and disease forecasting difficult.

P. ramorum was shown to be both airborne and soilborne (Davidson, 2005). The importance of aboveground spread via windblown rain or wafted infected leaves, as well as potential spread via movement of infested forest soils and potting media, demonstrated a complex disease etiology that made control difficult.

Many products could potentially carry the pathogen and were subject to quarantine regulation, so disparate industries and interest groups were forced to work together to prevent spread: forestry and fire protection agencies, nurseries, utilities, compost manufacturers, Christmas tree growers, spice producers, floral producers, municipal waste haulers, tribes and arborists.

The California outbreak occurred in the densely populated San Francisco Bay Area with abundant parks, open space areas and forested communities, many of which were not actively managed. By the time the causal organism was identified, the disease was widespread and impacted lands under many different ownerships.

Solutions

To design and implement a coordinated response and provide one source of science based information, the California Oak Mortality Task Force (COMTF) was formed under the joint leadership from the of US Forest Service, Pacific Southwest Region and California Department of Forestry and Fire Protection to bring together all concerned parties, including federal, state and local government, tribes, industries, and researchers.

The COMTF website (www.suddenoakdeath.org) collated science-based information: postings included an online, GIS map of wildland tree infection; disease chronology; symptoms gallery; and forest and nursery best management practices tailored for specific user groups.

Over time, improvements in molecular diagnostics, use of standardized protocols, quality control for state and federal laboratories, and the creation, in 2002, of the National Plant Diagnostic Network increased the speed and accuracy of pathogen testing. The ability to conduct genetic fingerprinting elucidated the pathways for long-distance pathogen spread on nursery stock. Four lineages have been identified with several introductions in Oregon and California wildlands (Grunwald et al., 2012 & 2019).

The Outcome

Since the disease was first recognized in California forests in the mid-1990s, the sudden oak death pathogen has killed an estimated 50 million trees in California and Oregon (Cobb et al. 2020). In the U.S., the pathogen has been detected in waterways in ten states, often associated with run-off from infested nurseries (Chastagner et al., 2010; COMTF, 2019a, 2019b) and in a botanic garden in Washington state (Strenge et al., 2017). However, outside of California and Oregon there have been no detections on oaks or other tree species in wildlands in the U.S. With continuing nursery detections, *P. ramorum* has severely impacted the nursery industry; some nurseries have stopped producing host plants or been forced out of business, and production costs have increased (Grunwald et al., 2019; Suslow, 2004) (Figure 5).

Figure 5. Camellia nursery stock bagged for destruction by deep burial due to P. ramorum infection. Under the U.S. quarantine, diseased and adjacent plants are destroyed
(Photo source: Nawal Sharma, California Department of Food and Agriculture)

CONCLUSION

The emergency response to *P. ramorum* demonstrates the difficulty of having to design a quarantine and emergency response for an emerging pathogen, when the full distribution and host range for the pathogen are unknown. Despite quarantine establishment in 2002, interstate shipments of potentially infected nursery stock in 2004 triggered a near complete shutdown of all horticultural plant commerce along the West Coast and the regulation of all nurseries with host plants in California, Oregon and Washington (Jones, 2006).

The sudden oak death outbreak increased awareness of the need for improved nursery sanitation and provided the impetus for the Systems Approach to Nursery Certification (SANC) program (https://sanc.nationalplantboard.org/) and the Canadian Nursery and Landscape Association's Clean Plant program (http://www.cleanplants.org/).

Experience gained from response to *P. ramorum* has improved response to subsequent invasive species detections since plant pathogen diagnostic laboratory standards and quality control procedures were strengthened.

The use of the incident command system proved helpful for initial response but scientists and others unaccustomed to rigid management systems found it hard to accept their position in the hierarchy.

The emergency response for *P. ramorum* highlights the need for cross disciplinary, interagency communications. Organizations could be better prepared for future emergency responses by understanding their roles and responsibilities and what assistance they can expect from agencies with overlapping jurisdictions (National Association of State Foresters, 2004).

CASE STUDY: 2016-2017 NEW WORLD SCREWWORM OUTBREAK IN FLORIDA

The Challenge - Background

New World screwworm (NWS), a reportable transboundary (foreign) animal disease in the U.S., is caused by larvae of the fly *Cochliomyia hominivorax* that infest and feed on the living tissue of warm-blooded mammals including humans and on rare occasion birds. Most maggots feed on decaying tissue, but NWS female flies lay their eggs in small wounds such as tick bites, injection sites, and newborns' navels. NWS flies can also deposit eggs on mucous membranes such as nasal passages and external genitalia. Once hatched, the larvae burrow into the animal's live tissue to feed. The resulting condition in the host is called myiasis. As the larvae grow, they deepen and damage the wound to the extent that severe infestations, if left untreated, are almost always fatal to the host.

NWS appeared in the southwestern U.S. in the mid-1800s and became a significant problem in the southeast in the 1930s, costing livestock producers millions annually. Infestations continued into the 1950s, until the sterile insect technique (SIT) was developed by USDA's Edward Knipling and Raymond Bushland. This technique created sexual sterility in male NWS flies through gamma ray exposure to the pupae leading to sperm chromosomal damage. As NWS only mate once, when irradiated sperm fertilized a wild female's egg, the resulting embryo died (Dyck et al., 2006).

In July 2016, severe skin and tissue wounds caused by fly larvae infestation (myiasis) were observed in a road-killed Key deer (*Odocoileus virginianus clavium*), an endangered sub-species of white-tailed deer. As the summer progressed, several more Key deer were euthanized after being found with myiasis. A handful of domestic animals in the Florida Keys had also been seen with myiasis from July to September 2016. On September 29, 2016, the National Key Deer Refuge in Big Pine Key, Florida, contacted the Florida Department of Agriculture and Consumer Services (FDACS) regarding these cases of myiasis. Florida Department of Agriculture and Consumer Services (FDACS) immediately initiated a Foreign Animal Disease Investigation and submitted larvae from an infested deer to the National Veterinary Services Laboratory (NVSL) in Ames, IA for identification. NWS was confirmed by NVSL on September 30, 2016.

The number of known Key deer mortalities associated with NWS rose from 30 in September to nearly 100 in October, peaking around October 9, with 30 mortalities that week. There were confirmed animal infestations on 6 different Florida Keys and NWS flies were detected on additional Keys. As the situation in the Florida Keys appeared to be stabilizing, on January 6, 2017, NVSL confirmed an infestation of a stray domestic dog found on the mainland, in Homestead, Florida. This dog was the only confirmed NWS case outside the Florida Keys. In total, 145 cases were reported: 128 presumptive cases and 17 confirmed cases of NWS in the Florida Keys, with 135 of those positive cases observed in Key deer. No production livestock were ever affected. (USDA APHIS Veterinary Services Response, 2017b) (Figure 6).

Figure 6. Tusklike mandibles protruding from the screwworm larva's mouth rasp the flesh of living warm-blooded animals. A wound may contain hundreds of such larvae
(Photo source: USDA photo by John Kucharski)

Challenges to Response

As a Foreign Animal Disease in the U.S., NWS must be reported to state and federal animal health officials. The delay in NWS identification from July to the end of September 2016 complicated and expanded the response efforts. In August 2016, U.S. Fish and Wildlife Service (USFWS) staff at the National Key Deer Refuge saw an unusual increase in buck mortality, approximately twice the normal rate in late summer, typically caused by road strikes or natural causes. Interviews with local veterinarians revealed that highly unusual myiasis had also been observed in a few pets in the Florida Keys in July and August 2016, near the National Key Deer Refuge. None of the domestic animal cases had been reported to state animal health officials by examining local veterinarians.

Following the confirmation of NWS, high mortality rates of Key deer continued in October, rising to nearly 100 in that month alone. NVSL continued to confirm cases of NWS submitted by Unified Incident Command (IC) personnel; because of the high number of Key deer infested, on-site NWS subject matter experts also made the determination of presumptive positive cases in some situations without further confirmation from NVSL. From November 20 on, all presumptive cases were submitted to NVSL for confirmation and cases continued to be detected until January 10, 2017.

The failure to recognize and report animals with severe myiasis in species including Key deer, dogs and domestic swine by wildlife officials and local veterinarians delayed response activities by three months, increased the risk of NWS being spread to the continental U.S. and resulted in the death of numerous endangered Key deer (Skoda et al., 2018).

Solutions

The complex multi-jurisdictional and multi-agency eradication of NWS from the Florida Keys in 2017 is an example of a "One Health" approach to a serious economic and zoonotic threat. This approach couples the skills and science from human and veterinary medicine and with emergency management to respond to and manage the increasingly complex issues we face in a globalized world.

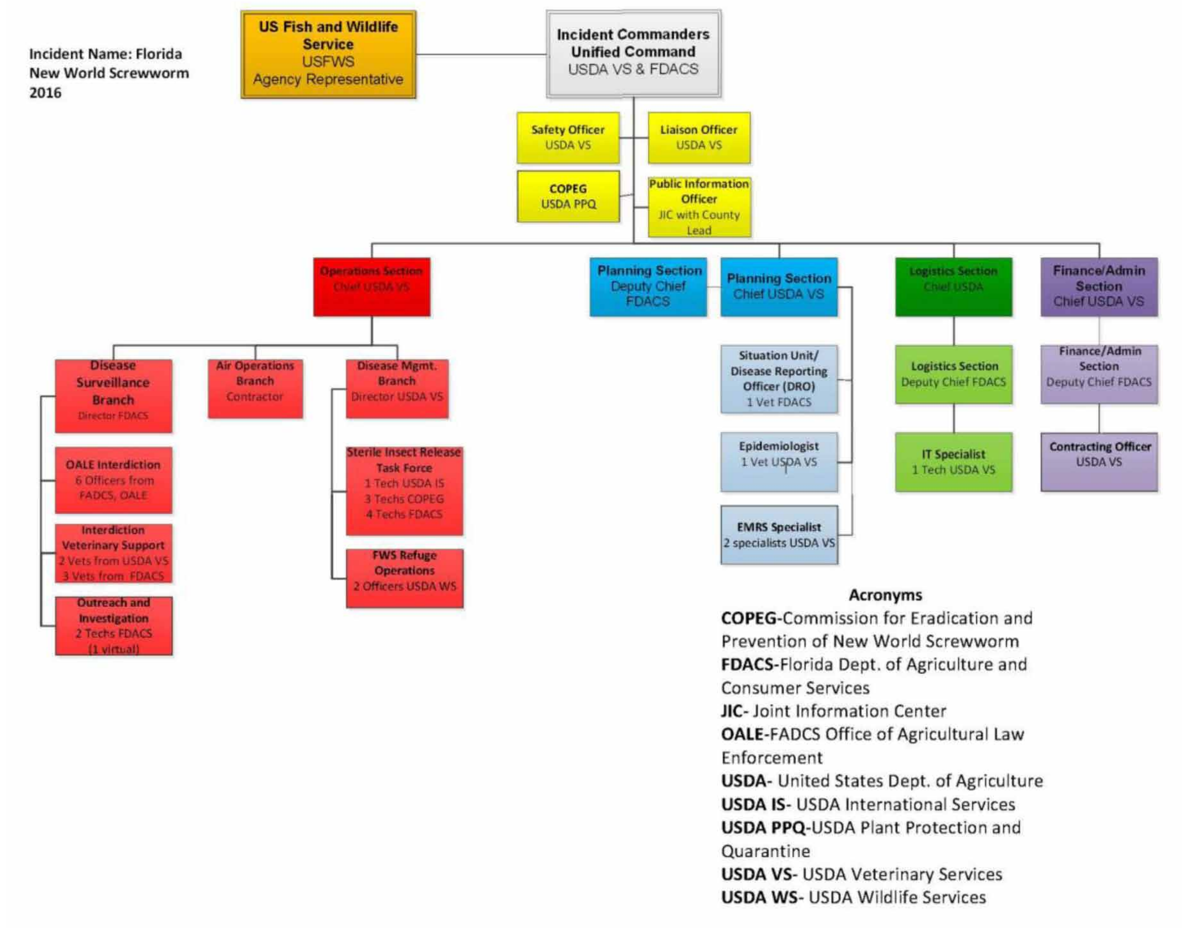
The use of the ICS and the immediate formation of a Unified Command fostered efficient and effective collaboration among responders from many agencies and jurisdictions. This response also combined personnel serving multiple rotations on site in the Florida Keys with some working virtually.

Communication with the multicultural community of the Keys to provide critical public health and disease information required a culturally sensitive and multi-lingual approach. Community involvement, and concern for NWS on Key Deer and support of the response was critical to eradication success.

The Outcome

This incident response lasted from October 1, 2016 until May 1, 2017; NWS was declared eradicated by March 31, 2017. The ICS was used to organize the response with a Unified Incident Command and ICP in Marathon, Florida run by the Florida Department of Agriculture and Consumer Services (FDACS), Monroe County, and USDA APHIS. Overall, there were 539 deployments by 350 personnel; 95 percent of these deployments were on-site in Florida (the remainder were virtual). A unified JIC was virtually established to distribute public information. FDACS personnel led this unified JIC with PIOs from FDACS, USDA Legislative and Public Affairs (LPA), Monroe County, and USFWS. An APHIS National Incident Coordination Group (ICG) and a Multiagency Coordination (MAC) Group provided further support for resource allocation and policy guidance and was staffed by up to 35 personnel. Commodity Credit Corporation money was not requested for the NWS response. APHIS spending on this incident was approximately US$3.2 million (USDA APHIS, 2017) (Figure 7).

Figure 7. Florida New World Screwworm 2016 ICS organizational chart example
Source: USDA APHIS Veterinary Services



CONCLUSION

Following detection of the Florida Keys NWS incident, the rapid initiation of an effective Unified Incident Command between the Florida Department of Agriculture and Consumer Services (FDACS), Monroe County, FL, and USDA APHIS limited both the disease area and its impact on animal health. Only six Florida Keys had confirmed or presumptive cases of NWS in wild or domestic animals, along with the one case found on the Florida mainland, south of Miami. During the outbreak, no humans or production livestock became infected. After diligent and expansive use of the SIT along with intensive surveillance, including in remote locations, APHIS declared NWS eradicated from the U.S. on March 23, 2017.

The success of this response is due in large part to effective collaboration of USDA APHIS Veterinary Services, USDA APHIS International Services, USDA APHIS Wildlife Services, USDA APHIS Plant Protection and Quarantine, USDA Legislative and Public Affairs, multiple other Federal partners, in addition to state and local personnel facilitated by a highly effective Unified Incident Command as well as excellent subject matter expertise in NWS biology and application of SIT (USDA APHIS, 2017b).

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KEY TERMS AND DEFINITIONS

Accredited Veterinarian: A veterinarian licensed in the state of origin and approved by the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), to perform certain functions of federal and cooperative state-federal programs in accordance with the provision of Title 9 Code of Federal Regulations (CFR) §160 through §162.

Agency Administrator: The official responsible for the management of a geographic unit or functional area. The managing officer of an agency, division thereof, or jurisdiction having statutory responsibility for incident mitigation and management (Source: NWCG Glossary of Wildland Fire, PMS 205).

Animal: Any member of the animal kingdom except a human.

Citizen Science: Scientific research conducted, in whole or in part, by nonprofessional scientists.

Commodity Credit Corporation: The Commodity Credit Corporation (CCC) finances invasive species and natural disaster emergency response, as well as farm commodity, trade and conservation programs. The CCC is a wholly-owned government corporation with the authority to incur up to US$30 billion in outstanding debt to the U.S. Treasury.

Complexity Analysis: An analysis of current and expected incident conditions performed at the onset and periodically during an emergency in order to identify the appropriate incident management organization necessary to meet incident objectives.

Control Area: For animal diseases, the area where movement controls are implemented around the index premise. The control area may include an infected zone and a surrounding buffer zone.

Cooperator: A federal, tribal, state, or local agency that participates with another agency(s) in planning and conducting fire or emergency management projects and activities.

Demobilization: Release of resources from an incident in strict accordance with a detailed plan approved by the incident commander.

Division: The ICS organization level between the Branch and the Task Force/Strike Team. Divisions are used to divide an incident into geographical areas of operation. Divisions are established when the number of resources exceed the span-of-control of the Operations Section Chief.

Emerging Animal Disease: Any terrestrial animal, aquatic animal, or zoonotic disease not yet known or characterized, or any known or characterized terrestrial animal, aquatic animal disease in the United States or its territories that changes or mutates in pathogenicity, communicability, or zoonotic potential to become a threat to terrestrial animals, aquatic animals, or humans.

Endemic Disease: A disease considered to be constantly present and/or the usual prevalence of a disease or infectious agent in a population within a geographic area.

Foreign Animal Disease (FAD): A terrestrial or aquatic animal disease or pest, not known to exist in the United States or its territories. (A more complete definition is available in APHIS FAD Framework: Response Strategies [FAD PReP Manual 2-0] https://www.aphis.usda.gov/animal_health/emergency_management/downloads/documents_manuals/fadprep_manual_2.pdf)

Incident Command System (ICS): A standardized on-scene emergency management concept specifically designed to allow its user(s) to adopt an integrated organizational structure equal to the complexity and demands of single or multiple incidents, without being hindered by jurisdictional boundaries.

Incident Coordination Group (ICG): In USDA APHIS, an ICG is responsible for supporting an Incident Command and Area Command in acquiring resources, formulating policy options, and assisting in development and implementation of response and recovery strategies for a FAD outbreak.

Incident Management Team: The Incident Commander and appropriate Command and General Staff personnel assigned to an incident (Source: NWCG Glossary of Wildland Fire, PMS 205).

Index Premise: Site of the first confirmed positive case for an animal disease.

Joint Information Center (JIC): A facility established as the central point of contact for news media and interested parties to coordinate emergency incident communications activities at the scene of an incident. Public information officials from all participating federal, state, tribal and local agencies co-locate and coordinate communications at a joint information center.

Multiagency Coordination (MAC): The APHIS MAC Group offers guidance on the most efficient way to allocate resources during an animal health event. Specific responsibilities vary from disease to disease, but the general functions of the APHIS MAC Group include incident prioritization, resource allocation and acquisition, and identification and resolution of issues common to all parties.

National Incident Management System (NIMS): The Federal Emergency Management Agency (FEMA) oversees the National Incident Management System (NIMS) which defines operational systems that guide how personnel work together during incidents for all levels of government, nongovernmental organizations, and the private sector.

One Health: The One Health Initiative (https://onehealthinitiative.com/) is a movement to forge co-equal, all-inclusive collaborations between physicians, osteopathic physicians, veterinarians, dentists, nurses and other scientific-health and environmentally related disciplines, including the American Medical Association, American Veterinary Medical Association, American Academy of Pediatrics, American Nurses Association, American Association of Public Health Physicians, the American Society of Tropical Medicine and Hygiene, the Centers for Disease Control and Prevention (CDC), the United States Department of Agriculture (USDA), and the U.S. National Environmental Health Association (NEHA).

Operational Period: The period of time scheduled for execution of a given set of tactical actions as specified in the Incident Action Plan. Operational periods can be of various lengths, although not usually over 24-hours.

Span-of-Control: The supervisory ratio of from three-to-seven individuals, with five-to-one being established as optimum.

Staging Area: Locations set up at an incident where resources can be placed while awaiting a tactical assignment on a three (3) minute available basis.

Stakeholder: An individual, group, or organization affected by an emergency or other situation with a vested interest in the incident outcome but not directly involved in the incident response.

Trace-Backs: Backwards tracing means tracing back to the source of the product, to find the origin of infection or contamination. Trace-backs are required in many federal or state quarantines (regulations). May also be used synonymously with trace-ins.

Trace-Forwards: Trace-forwards investigate forward to identify everywhere that infection or contaminated plants or animals may have been shipped. Trace-forwards are required in many federal or state quarantines (regulations). May also be used synonymously with trace-outs.

Unified Incident Command: A unified incident command (UIC) is an emergency response structure that brings together the incident commanders of the major organizations involved in the incident in order to coordinate an effective response, while at the same time allowing each to carry out their own jurisdictional, legal, and functional responsibilities.

Vaccination Zone: Areas where vaccines are administered as part of an emergency response to an animal disease. The vaccination zone can be set up for containment (Containment Vaccination Zone, CVZ) or protection (Protection Vaccination Zone, PVZ).

Values-at-Risk: The elements of a community or natural area considered valuable by an individual or community that could be negatively impacted by an emergency situation or the mitigating operations. These values can vary by community and can include diverse characteristics such as homes, specific structures, water supply, power grids, natural and cultural resources, community infrastructure, and other economic, environmental, and social values.

Zoonotic Diseases (Also Known as Zoonoses): Diseases caused by germs that spread between animals and people. Zoonotic diseases may be caused by viruses, bacterial, parasites and fungi.

APPENDIX

Abbreviations

APHIS: Animal Plant Health Inspection Service
FADD: Foreign Animal Disease Diagnostician
USDA: United States Department of Agriculture
OIE: World Organization for Animal Health

Chapter 9

Response and Recovery Tactics

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ABSTRACT

The policy and resource infrastructure required to manage agricultural and environmental pest and pathogen incursions evolve and strengthen over time. Animal and plant health responses involve the highly coordinated efforts of various entities. Governments partner with state and territory officials, subject matter experts, and representatives of the commodity(ies) that are/may be impacted by the invasion. Short-, intermediate-, and long-term animal and plant health incident management tactics may change over time depending upon multiple conditions and externalities that will be described in this chapter. Results to response may range from fully successful eradication to learning to live with the pest and deregulation. Although the scope, timeframe, and consequences of events can vary, actions taken in response to the identification of an exotic plant or animal pest, disease, or condition are designed to minimize economic and environmental impacts, ensure trade and food security, assure business continuity, and avoid social unrest.

INTRODUCTION

The United States (U.S.) faces, on an ongoing basis, a diverse set of threats and hazards that adversely impact row crops, specialty crops, forests, public gardens and park lands, individual animals and herds,

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or large integrated commercial networks of animal producers. The opportunity for disease spread within a commercial network is different than for a single producer since producers in large commercial networks often share trailers, employees, or utilize uniform contracts from outside vendors. For these entities, disease spread often occurs across large geographic boundaries and are not localized to one city, county, parish, or state. The livelihood of those engaged in commercial agriculture, forestry, or horticulture is jeopardized by an infectious or communicable animal or plant disease occurrence. A widespread animal disease could be disruptive to the U.S. economy and impact national food security and supply. Plant diseases and pests can economically damage a wide range of plant systems and natural resources or alter landscapes. For example, chestnut blight (*Cryphonectria parasitica*) eliminated a major component of eastern U.S. forest ecosystems and Dutch elm disease (*Ophiostoma ulmi* and *O. novo-ulmi*) has killed elm trees altering the landscape of streets, urban forests, parks and the natural environment in more than 25 states since the 1930s (D'Arcy, 2000) (Figure 1).

Figure 1. Elm trees once lined city streets across the U.S., but they were all killed by an introduced fungus (Image from: Bailey-Howe Library, University of Vermont).

Although the scope and consequences of events can vary, actions taken in response to the identification of an exotic plant or animal pest, disease, or condition are designed to minimize economic and environmental impacts, ensure trade and food security, assure continuity of operations, and avoid social unrest. This chapter will describe the complex ecosystem and decision processes of response to novel, potentially high consequence incursions.

Response Authorities, Partners, and Players

Within the global biosecurity system, the U.S. is a member country of the World Organization for Animal Health (OIE) whose main objective is to control epizootic diseases and prevent their spread. The OIE is recognized as a reference organization by the World Trade Organization (WTO) and promotes transparency by informing governments on the occurrence and distribution of specific animal diseases and zoonoses. The U.S. is also a member of several Phytosanitary organizations, including the North American Plant Protection Organization (NAPPO), the International Plant Protection Convention (IPPC), and the United Nations Food and Agriculture Organization (FAO).

Within the country, response to pest and disease outbreaks requires the coordinated efforts and assistance of various public and private organizations (Annelli, 2006; FAO, 2011; Gilpen, et al., 2009). Every day, local, state, tribal and federal governmental agencies work together with private industry to coordinate subject matter experts (SMEs), share resources, integrate tactics, and act collaboratively to implement control tactics.

The availability of experienced and trained responders is essential to achieving a favorable outcome to any animal or plant health event. By their very nature, animal and plant health emergency incidents are unplanned and occur sporadically, it is incumbent upon animal and plant health agencies to provide opportunities for personnel to interact with and assist producers with mitigation and remediation of adverse health events, both routine and those which are required by law. These opportunities provide benefits to the agricultural community and to responders alike. Experience gained while conducting programmatic activities provides a broad-based set of transferrable skills and a foundation for responders to draw upon during an emergency deployment.

At the national level, there are legal underpinnings which grant the U.S. authorities responsibility to respond to incursions of high consequence animal and plant pests and diseases (DHS, 2002; DHS, 2003; DHS, 2004; DHS, 2011; DHS, 2018). Acts of the U.S. Congress established Animal Health Protection and Plant Protection authorities to respond to incursions of foreign animal diseases (FADs) and exotic plant pests and pathogens in the U.S. (Congress, 2002). These Acts authorize the United States Department of Agriculture (USDA) to restrict the importation, entry, or further movement in the U.S., or order the destruction or removal of animals (including livestock), plants and related conveyances, and facilities for reasons of pest or disease control. The Acts also authorize related activities pertaining to exportation, interstate movement, cooperative agreements, enforcement and penalties, seizure, quarantine and disease, and pest eradication.

Accordingly, in the past 10 years, the USDA Animal and Plant Health Inspection Service (APHIS) has deployed response teams with the expressed purpose of eradicating or controlling multiple exotic diseases and infestations. Although far from exhaustive, Table 1 provides a short list of examples of recent animal and plant health responses.

In the U.S., national preparedness includes the National Incident Management System (NIMS) which outlines a comprehensive approach to incident management and policy and procedures for protection, prevention, mitigation, response, and recovery. The NIMS Incident Command Structure (ICS) is a standardized all hazards - all risk approach to managing crisis response operations as well as non-crisis events with principles that can be applied to all types of incidents. The Department of Homeland Security (DHS) requires all federal departments, including the USDA to adopt and fully implement the NIMS ICS (Annelli, 2006; DHS, 2019; Gilpen, et al., 2009; USCG, 2014).

The National Plant Disease Recovery System (NPDRS) was developed by the USDA to provide an additional framework for significant plant diseases of high-impact commodities (DHS, 2004; USDA APHIS PPQ, 2020). There are currently more than 30 disease specific NPDRS recovery plans that provide details about the pathogen, management methods, and areas where research and additional information may be needed to manage and recover from an infestation (USDA ARS, 2021).

Table 1. Examples of responses to incursion of exotic plant and animal pests and diseases into the United States

Disease or Insect pest	Agent	Family	Response method	Affected species	Reference
Highly Pathogenic Avian Influenza	Virus	Influenza A (Orthomyxoviridae)	Depopulate	Poultry	Dargatz, et al., 2016; Wells, et al, 2017
New World Screwworm	Insect	*Cochliomyia hominivorax*	Sterile fly biological	Multiple species	Hennessey, et al., 2017
Vesicular Stomatitis	Virus	Rhabdoviridae	Test and quarantine	Equine/Bovine	APHIS VS, 2021
Contagious Equine Metritis	Bacterium	*Taylorella equigenitalis*	Test and treat	Equine	Erdmann, et al, 2011
Brown rot of potato	Bacterium	*Ralstonia solanacearum* (race 3 biovar 2)	Test and eradicate	Solanaceae /multiple	Goldner, 2020
Asian Long-horned beetle	Insect	*Anoplophora glabripennis*	Monitor, contain, & eradicate	Multiple hardwood tree species	Meng, et al., 2015
Plum Pox Virus	Virus	Potyviridae	Monitor & eradicate	Stone fruits	APHIS PPQ, 2021
Mediterranean fruit fly	Insect	*Ceratatis capitata*	Monitor, quarantine, sterile fly	Most fruits	USDA APHIS, 2003
European Cherry fruit fly	Insect	*Rhagoletis cerasi*	Monitor, quarantine	Cherry	Carroll & Herrmann, 2017
Citrus greening disease	Bacterium	*Candidatus Liberibacter asiaticus*	Monitor, vector control	Citrus	Citrus Research Board, 2010

At the state and territorial levels, the infrastructure and authorities for pest or disease response resides with the state Departments of Agriculture, which house State and Territory Plant Health Regulatory Officers (National Plant Board, 2014); USDA APHIS counterparts, the State Plant Health Directors (APHIS, 2021c) and the State Animal Health Directors (APHIS, 2021d). These officials oversee state-level pest and disease detection and regulatory activities, and coordinate survey activities between government agencies, public and private sector organizations. States, tribes, and territories in the U.S. vary with respect to relevance of commodities and resources that can be allocated to low-priority commodities. As a NIMS response is being stood for a new pest or disease detection, state and territorial officials are part of response teams to the extent that they have resources available.

Every plant and/or animal foreign disease or pest-related emergency directly impacts growers and producers, managers of nurseries, public gardens, and small- and large-scale industrial enterprises. The U.S. is comprised of over one billion acres of urban, suburban, and rural lands under production, ranging from household level to integrated industrial systems. When an outbreak is occurring, those with most at stake must be involved in discussions and provide input into the decision-making process.

Additionally, when preparing for a response, many different types of personnel will be involved.

Case/Site Managers are assigned to serve as a single point of contact for producers/owners of an infected or infested premise. These individuals are a source of information and coordinate the depopulation, disposal, pathogen elimination and environmental sampling on the premises. The site manager maintains the line of separation that distinguishes the "clean" side from the "dirty" side on an infested location. Biosecurity efforts are controlled by the site manager who monitors the inventory of contractual workers and equipment that is used on an infested premise. The site manager controls movements of vehicular traffic, materials, and personnel on and off the premises to ensure adherence to biosecurity standards and procedures. A site manager is generally assigned to a single infested facility during an event. A plant health safeguarding specialist can fill this role during plant health emergencies.

Epidemiologist or Science Officers provide epidemiologic assessments on movement and potential spread of the pest or pathogen to assist in developing mitigation strategies to control disease spread. The epidemiologist performs as a multidisciplinary asset with a focus on data collection, data integrity, and data analysis. Team members who serve in this capacity have additional education requirements and specific experience and may be recruited from academia or other program areas.

Composting/Disposal Specialists are required to have extensive hands-on training under the supervision of a composting specialist or university faculty member whose area of expertise is compost management. They serve as a subject matter expert during an event and may be an internal employee of USDA or an independent contractor. A plant health safeguarding specialist fills a similar role during plant health emergencies. Risk or financial management specialists assist producers with the indemnity and reimbursement or insurance claims processes, assisting with documentation and submissions.

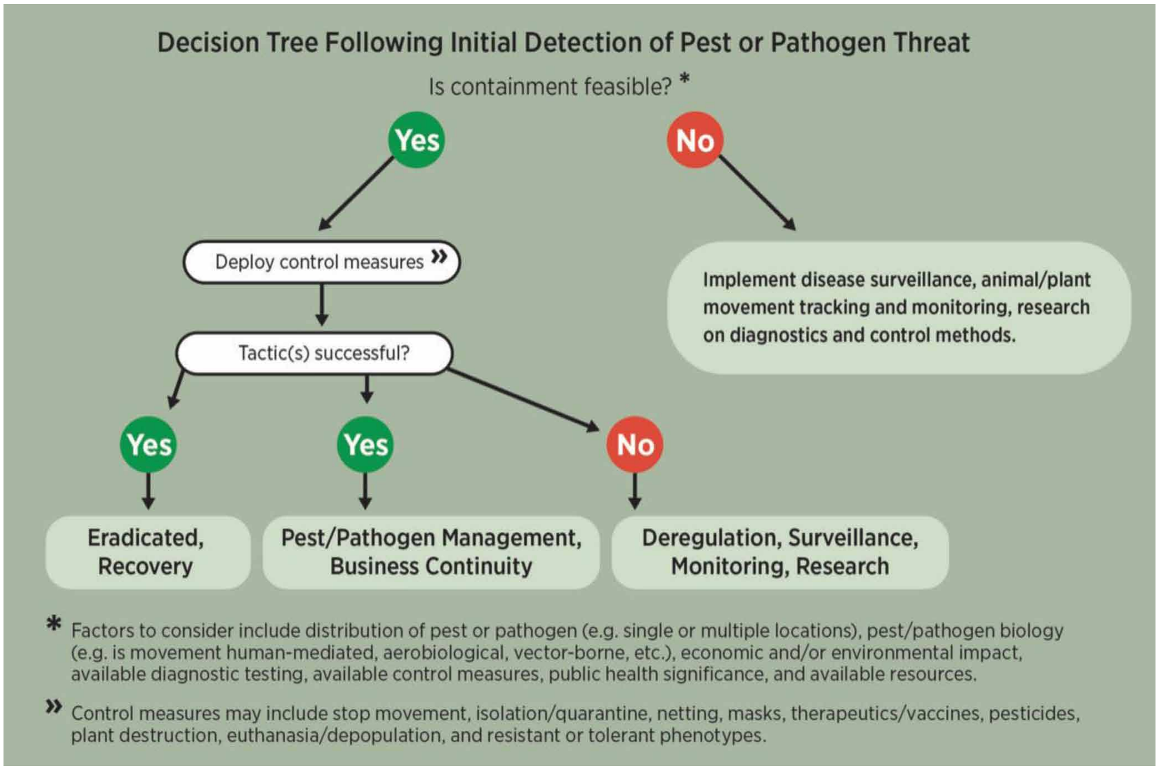
Response Decision Parameters

Many factors must be taken into consideration during a plant or animal health incident response. APHIS, state authorities, tribal officials, and invited subject matter experts weigh factors such as the biology, distribution, economic and/or environmental impact, available diagnostic testing, and public health significance of the pest or pathogen when determining whether to move forward and allocate funding for its control. The animal and plant pathogen may be new to the U.S. or previously unidentified. Decisions may be made that there should be no Federally coordinated response. It may be determined that the pest or pathogen does not pose a significant threat to plant or animal health or is not a significant trade issue (i.e. trading partners do not consider the pest important enough to threaten trade of a commodity). Or, in some cases, the problem is too widespread to be effectively managed, in which case the decision may be to maintain surveillance and monitoring. For example, West Nile virus (WNV) was initially identified in the U.S. in 1999 and caused fatal encephalitis in animals, birds, and humans (Roehrig, 2013). The initial U.S. cases occurred in New York City, but quickly spread due to mosquito transmission of this virus. Of the bird species affected, the American crow (*Corvus brachyrhynchos*) was particularly susceptible to fatal illness. The occurrence of WNV in mosquitoes and wild bird populations precluded the implementation of immediate control measures. Moreover, public health capabilities were limited due to eroded funding and the lack of available diagnostic tests. In the face of these significant barriers to controlling the virus, the strategy employed was disease surveillance and monitoring in parallel with research aimed at developing accurate diagnostic tests and treatment options. By 2002, cases of WNV had been confirmed in more than 40 states in the U.S. and involved regions of Canada. The westward spread the virus to encounter a new, more efficient mosquito species which facilitated the expanded geographic establishment of the virus.

The decision about how to respond to an animal or plant pest or pathogen is important and usually has extensive input from the affected industry. When the consequences to the industry are perceived as severe, it favors a decision to respond and attempt to eradicate. In some cases, the most appropriate response may be for the affected industry to directly manage the issue, with minimal regulatory intervention from federal, state, or local authorities. For example, porcine epidemic diarrhea virus (PEDV) was initially detected in the U.S. in 2013 and rapidly spread throughout commercial swine herds, resulting in high mortality of infected piglets (Jung et al, 2020). In response to the virus threat, the USDA issued a Federal Order requiring disease reporting, surveillance, herd monitoring, movement tracking, and support for epidemiological investigations, diagnostic test development, and field virus analysis (USDA, 2014). However, disease was largely managed by commercial swine companies and involved enhanced biosecurity practices and decontamination of transport trailers and feed trucks. Herd immunity was achieved through controlled exposure of sows to field virus since no PEDV vaccines were available at the time (Jung et al., 2020).

Decisions about pest and or disease incursion into wild populations, wild animals, important tree species, national forests and parklands, etc. are not directly related to trade, nor to a specific industry. The protection of these high-value assets generally falls on governmental agencies. However, the vast amount of land and environmental resources under governmental authority are difficult to closely monitor, increasing the likelihood that a pest or pathogen may have become widespread at the time of detection. In such cases, an asset-based approach may be employed to manage invasive species and rely on the cost-benefit ratio for controlling the threat. Several factors must be considered to determine the feasibility of containment and eradication, including the biology of the organism, epidemiology of spread, and how widespread the outbreak is at the time of discovery (Figure 2).

Figure 2. A simplified overview of factors that drive decisions about what kind of response may occur



* Factors to consider include distribution of pest or pathogen (e.g. single or multiple locations), pest/pathogen biology (e.g. is movement human-mediated, aerobiological, vector-borne, etc.), economic and/or environmental impact, available diagnostic testing, available control measures, public health significance, and available resources.

» Control measures may include stop movement, isolation/quarantine, netting, masks, therapeutics/vaccines, pesticides, plant destruction, euthanasia/depopulation, and resistant or tolerant phenotypes.

Biology of the Invasive Organism – Is Containment Feasible?

The decision about what tactics to use in response to an invasive organism initially considers the biology and lifecycle of the organism and epidemiological questions concerning survival, effective reproductive rate, and mechanism of dissemination. Highly effective reproduction rates lead to more rapid spread, adding urgency to the response decision-making. Mechanism of dissemination or spread is one of the most pivotal pieces of information. Understanding how the organism moves, or is moved, is key to effective response determination (Table 2, Figure 2).

Table 2. Control options relative to organism dispersion mechanism

Mechanism of spread	Movement	Infectious particle/carrier	Control options
Aerobiology – long distance spread	Flying, wind carried, air currents, dust	Air-borne spores, Aerosolized virus, Flying insects/vectors, birds	Containment difficult to impossible Barriers – nets, mesh
Human mediated	On germplasm or produce	Seed, semen, live plants & animals, feed, produce, etc.	Quarantine, Stop movements, Eradication
Human mediated	On transport	Containers, packing materials, baggage, on boats, in airplanes, on equipment	Fumigation, Decontamination, Containment, Eradication
Contagion – short distance spread	From host to host	Rain splash, body fluids, sneeze/cough, air currents	Isolation, Quarantine, Depopulation

Distribution and Scale of Outbreak when First Detected

Scale and location of an outbreak can have an impact on the decision about what tactics will be deployed in response. Delays in detection and response can lead to an expansion of the outbreak to neighboring fields or grower operations, additional states, or territories, and from one country to another (Annelli, 2006). If the outbreak is confined to a single area the chance for containment and eradication improves. If it has spread to multiple locations /producer operations, the response requires greater coordination. Therefore, once an exotic plant pathogen or foreign animal disease has been detected and diagnosed, an ICS will be set up initially under emergency provisions to organize personnel, assess extent of the outbreak and develop a comprehensive and appropriate response plans.

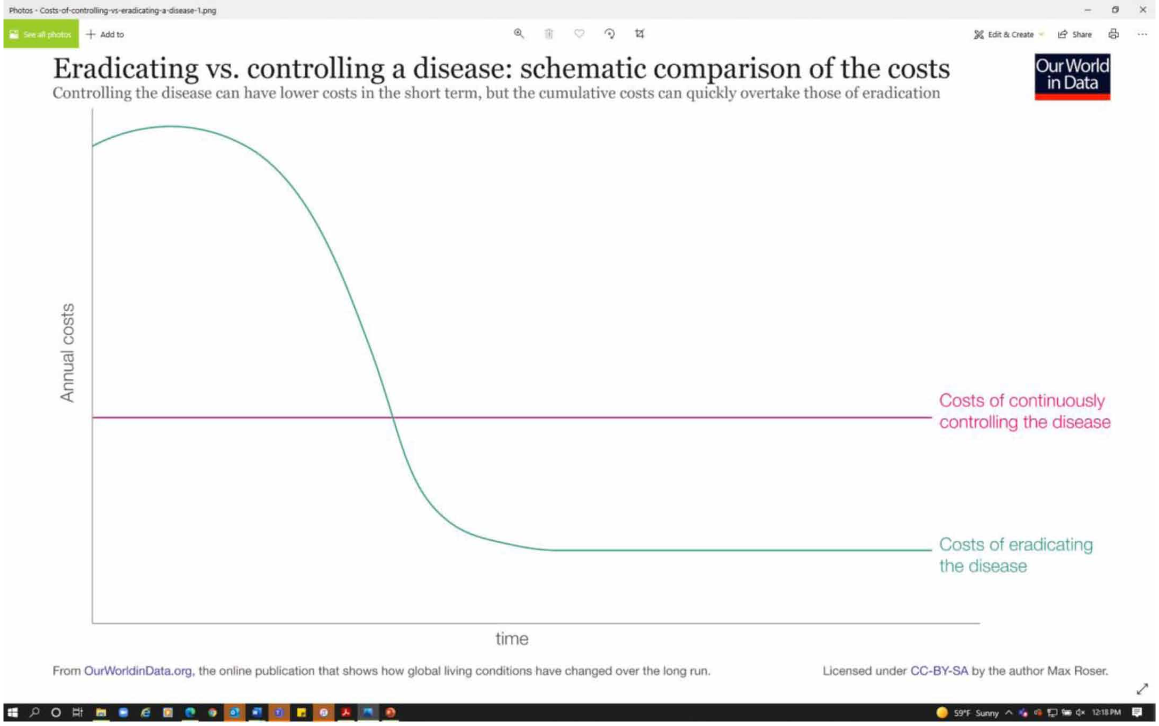
Timeframes of Response and Recovery

Response plans can occur over different timeframes. Tactics such as **eradication**, **isolation/containment**, **stop movements** at the state level, and **quarantine** to stop spread from infected to non-infected areas are informed by early actions during emergency response, delimiting surveys, and economic considerations.

The short-term response tactics deployed early in an outbreak are designed to attempt to eliminate the problem altogether or limit spread to contain it in place (Annelli, 2006; FAO, 2011; Hand, 1982). Plant and Animal health incidence response emergencies attempt to be completed in less than a year (see Case Study 1 *Ralstonia solanacearum* Race 3 pathovar 2).

However, the timeframe of a response is often driven by cost (Figure 3). The initial cost of any effective control and mitigation, may be less than attempting to eradicate, but in the end the cumulative cost will be higher than if a successful eradication had been waged. If/when eradication is successful, the cost reverts to prevention to precluding reintroduction.

Figure 3. Comparison of costs over time for eradicating or continuously controlling an invasive species



Eradicating vs. controlling a disease: schematic comparison of the costs

Controlling the disease can have lower costs in the short term, but the cumulative costs can quickly overtake those of eradication

From OurWorldinData.org, the online publication that shows how global living conditions have changed over the long run.

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The types of operational activities conducted in response to an animal or plant health emergency are comparable in many ways. Regardless, the technical expertise required, the necessity to use standardized policies, the need for operational strategies and disease specific risk mitigations are similar and independent of the operational window. Animal and plant disease emergencies often require an immediate or near-immediate response, with state or national oversight, to minimize serious and immediate socio-economic impacts and public health consequences. Delays in response could lead to an expansion of the outbreak to additional states or territories (FAO, 2011). Large scale animal health eradication campaigns, emergency responses are often conducted in a compressed time frame – months to 1-2 years instead of decades. For example, viscerotropic velogenic Newcastle disease (VVND), contagious equine metritis (CEM), and highly pathogenic avian influenza (HPAI) were eradicated within one to two years as was *Ralstonia solanacearum* Race 3 from greenhouses in the U.S. on numerous occasions.

Nevertheless, responses can last for much longer time spans – often decades – depending on the biology of the pest or pathogen. In the U.S., the bovine tuberculosis eradication campaign began in 1917 and remains in effect. This program has been heralded as a success by reducing human illnesses and deaths (Olmstead et al, 2004). Similarly, the bovine brucellosis disease eradication campaign began in

1956 and has reduced disease-related losses in U.S. cattle from an estimated US$400 million in 1952 to less than US$1 million today (USDA, APHIS, VS. 2020). Legacy plant pest and disease eradication programs include witchweed, boll weevil, and Golden Nematode. These USDA APHIS Plant Protection and Quarantine (PPQ) programs have been occurring for more than 40 years with exceptional compliance based on statutory quarantines (USDA, APHIS, 2020; National Cotton Council, 2021; USDA APHIS PPQ, 2012).

Regardless of the operational window, tactics selected, or technical expertise required, the necessity to use standardized policies, the need for operational strategies, and disease-specific mitigations are similar requirements whether plant or animal.

Operational Activities Conducted in Preparation for Animal and Plant Health Responses

The response to the animal or plant health event requires conducting many different types of activities at the field level. A summary of mission-critical priorities is listed below and represents the most common activities conducted by APHIS animal or plant health officials with their State and local counterparts in response to an animal or plant health emergency, regardless of the species involved or specific disease condition being addressed.

Initial Activity 1: Biosecurity Assessment. As a first order of business, biosecurity requirements must be established by experts. In this case, biosecurity refers to efforts to keep diseases and the pathogens that carry them – viruses, bacteria, fungi, parasites, and other microorganisms – contained within the infested premises while protecting the responders on the ground.

- **Structural biosecurity:** measures used in the unique physical construction and maintenance of animal confinement or plant growth facilities: coops, pens, poultry houses, barns, family farms, commercial farms, nurseries, fields, groves, orchards, ensuring that there are avenues for ingress/egress to premises, vehicles, and other types of facilities, or equipment that require appropriate sanitation measures. These could be disinfestation solutions that must be driven or walked through to enter the facility.
- **Operational biosecurity:** establishment of management practices, procedures, and policies that are appropriate to prevent or limit pest or pathogen introduction and spread and ensuring these procedures are consistently followed and enforced. This also includes biosafety measures to assure that the responders have appropriate personal protective equipment (PPE) and operating procedures to protect themselves and to avoid becoming fomites themselves.

Good biosecurity practices prevent the incursion of animal or plant diseases onto or out of a facility and can reduce the impacts to agriculture or the environment. Biosecurity is everyone's responsibility but during an emergency response the job of overseeing the implementation of biosecurity measures is delegated to the Site Manager. Specific guidance and recommendations for management of biosecurity risks for an animal health and plant health events can be found at USDA APHIS 2020a and USDA APHIS 2020b.

Initial Activity 2: Assessing the Scope of the Situation

Delimiting surveillance has been discussed in a previous chapter describing how samples are collected and tested to confirm location of the causal agent. This information is need while planning and carrying out epidemiological investigations and tracing at-risk animals or susceptible plants populations. If the pest or pathogen is in a single location versus multiple locations, it can impact which response tactics could be most useful (Figure 2). Epidemiological assessment of the mode of dissemination and how quickly the invader will spread is also information needed when making implementation decisions (Figure 2, Table 2).

Conducting quarantine and movement control depends on local conditions and cooperation among multiple partners. To support continuity of business clear delimitation of area/enterprises that are ‘free from’ infection or infestation must be differentiated from those that have infections or infestations. This involves safeguarding animal welfare and uninfected/uninfested plant material during response operations. Containment and movement control tactics may require permitting and will require continued monitoring.

Initial Activity 3. Communications

Managing information from the infested field or facility up through appropriate channels to the national level. For USDA, this is normally handled through the legislative and public affairs group, who coordinate and communicate with state, local, and industry stakeholders. Additionally, in the short-term, early in an outbreak, managing public perceptions is critical. Communications plans will include the 3 C’s: confidence, containment, and compensation for affected growers and/or animal husbandry operations

OPERATIONAL ACTIVITIES CONDUCTED DURING PLANT AND ANIMAL HEALTH RESPONSE

No Regulatory Response

In some cases, the decision is made to not have a formal or government-led response. For animal diseases that are foreign to the U.S., response actions are based on the need to maintain or regain disease freedom. For those diseases that are new or emerging, the decision to respond or intervene is often based on the anticipated economic repercussions to international trade and whether the industry being impacted supports the response activity. But, in some cases, the affected industry wants to manage the situation. For example, the APHIS response to the occurrence of porcine epidemic diarrhea virus (PEDV) and swine enteric coronavirus diseases (SECD) was limited in scope due to the industry’s desire to pursue a non-regulatory approach to disease control (USDA APHIS, 2014 and 2016).

Decisions to conduct a formal regulatory response or not, and how to respond are based on many factors including the lifecycle of the organism, alternate or reservoir hosts prevalence, whether the industry can implement control strategies. It simply may not be possible to contain or eradicate some pests or pathogens, and therefore they are quickly deregulated. Sometimes, there is no robust and easily deployable detection method making detection of an incipient infestation unlikely. An example of this includes PEDV coronavirus carried in feed ingredients (Dee, et al., 2014; Pascik, et al., 2014). In other cases, the pest or pathogen spreads too rapidly to be able to contain. Examples include Asian Soybean

Rust, the brown marmorated stinkbug; West Nile virus spreading in mosquitos and birds (Kistner, 2018; Roehrig, 2013; Schnepf, 2005). In these cases, decisions were made to not regulate or treat the pest or pathogen at a federal level.

Data Management and Permitted Movements

Responding to animal and plant health incidents requires a data system that is both widely available to state and industry cooperators and which supports various methods of collection from numerous data sources. Currently, USDA-APHIS -Veterinary Services utilizes the Emergency Management Response System 2.0 (EMRS2) to support data collection, management, and analysis of routine investigations of foreign animal diseases (FADs), surveillance and control programs, state specific disease outbreaks, and national animal health emergency responses (all-hazards) (USDA APHIS VS, 2021). The PPQ also has many data management systems that are being developed and pulled into the Integrated Plant Health Information System or IPHIS to work closely with the NPDN to ensure that all plant samples have the appropriate chain of custody and that notifications of diagnostic results occur promptly and accurately.

The use of movement controls is a critical component to disease management as it prevents spread of disease to other susceptible populations. For animals, the permitting process is conducted mainly by state and industry representatives and utilizes the EMRS2 digital portal to facilitate requests and approvals. In general, industry representatives can request a plant or animal permit be issued for a regulated article such as an animal, group of animals or an animal- or plant-derived product (eggs, meat, milk, fruit, vegetables, firewood, wood chips) or any potentially infected/infested plant material, to allow that item to move interstate from within a surveillance zone, quarantine boundary or control zone to another state (See Citrus Canker case study 2). There is also the ePermit and eFile systems which allow users to get electronic phytosanitary permits. These are mainly used at this point for international commodity movement.

Premises that request a permit and receive approval for the movement will have satisfied a set of biosecurity criteria and completed laboratory testing to ensure that the plants, animals, or plant- or animal-associated products do not represent a risk for pest or pathogen spread. The permit is time dependent and based on recent survey or inspection results. The digital portal allows for ease of access and an opportunity for the receiving state to review and approve the movement. APHIS is an intermediary in this transaction, helping to establish movement criteria and providing for a transparent process for permit application and approval, used mainly for phytosanitary permits (ePermit and eFile) (USDA Pers Comm).

Quarantine, Containment and Movement Control at the Farm Level

The use of field-level quarantines has been an important and effective method to reduce the movement of numerous plant pests, especially those that are exclusively soil-borne such as golden nematode (*Globodera rostochiensis*), pale cyst nematode (*Globodera pallida*), and the exotic witchweed (*Striga asiatica*). Once detected in a field, any equipment (from a farm implement to a van that is checking a cellular communications tower) that moves out of a field must be pressure washed and steam sanitized prior to leaving a field (USDA APHIS PPQ, 2012). Similarly, one of the initial responses following a high-consequence pathogen of food-producing animals (e.g., poultry, swine, beef cattle, and dairy cattle) and equine species is to restrict their movement. In the U.S., this is achieved by federal, State, and/or Tribal authorities issuing a Stop Movement Order as a measure to control the spread of pathogens into non-infected livestock and poultry. This action requires the coordination of these agencies, along with all public and private stakeholders, as discussed in the previous chapter. Recent examples of the use of Stop Movement Orders for the containment of animal pathogens include PEDV, VVND, and HPAI.

In some cases, a campaign to stop movement depends on effective communication with the public, and awareness and compliance by individuals. The don't move firewood campaign that the USDA funds through The Nature Conservancy has been an important mechanism to reduce the movement of firewood from areas where potential pests occur (Figure 4).

Figure 4. Example of literature used in the Don't Move Firewood campaign. Photo by dontmovefirewood.org

An invasive and tree-killing insect, the emerald ash borer (EAB), is active in Boulder County. The emerald ash borer kills all ash trees and can easily be spread by moving firewood.

BUY IT WHERE YOU BURN IT.

HOW YOU CAN HELP:

- Leave firewood at home! Don't bring it with you for camping or traveling.
- Buy firewood near your destination, buy only what you need, and burn it completely before leaving.
- If you have already brought wood with you, burn it all before leaving. Do not take it home, and do not leave it for the next person.

Visit BoulderCountyEAB.org for more information.

DONTMOVEFIREWOOD.org

This campaign uses outreach to encourage the use of firewood from the areas where people camp rather than bringing the firewood from their homes. Plant pests that can be moved on firewood include *Lymantria dispar dispar*, Asian Long-horned beetle, spotted lantern fly, and emerald ash borer to name a few. By encouraging campers and other outdoor enthusiasts to only use firewood from local sources, the potential for plant pest movement is reduced.

Eradication with Compensation for Animal and Plant Losses

The Animal Health Protection Act and the Plant Protection Act grants the Secretary of Agriculture the authority to carry out operations and measures to detect, control, or eradicate any exotic plant or animal pest or disease. These measures may include, but are not limited to, the removal or destruction of infected or potentially infected and exposed animals. While APHIS has the authority to order destruction of livestock, it rarely exercises this option, preferring instead to utilize State or Tribal authority to quarantine or restrict livestock movements.

When eradication is attempted, responders and extension personnel provide resources and guidance for mass euthanasia (depopulation) or plant destruction and include individuals with subject matter expertise in the best methods for carcass or plant disposal. If eradication is relatively quick and successful, experts and agencies provide guidance, options, and contracted support for cleaning and disinfection (pathogen elimination).

The methods utilized to achieve depopulation are diverse. For animals, the procedure selected for euthanasia depends on the species, the size and configuration of the operation, and cost. Euthanasia is performed in a way that eliminates or minimizes pain and distress. Several resources outline the practical considerations and approaches for the depopulation and disposal of poultry (Poultry Industry Council, 2016), cattle (Shearer, 2018), and swine (American Association of Swine Veterinarians, 2020).

Tree removal is often conducted in the attempt to eradicate pests and pathogens of trees. In many cases, this requires entry onto private property. When tree disease eradication is underway, APHIS works closely with State, Tribal and local authorities and communications with the public are a priority to assure that people:

1. Allow program officials access to their property to inspect trees and remove any infested trees that are found,
2. Hire companies that have compliance agreements with the eradication program for working on host trees, and
3. Never move wood out of quarantined areas because it can spread the pest and other tree pests and diseases. State and federal officials monitor the movement of wood within and around regulated areas to enforce the quarantine. They may issue fines to individuals and businesses that do not comply with the regulations.

Trees that are felled are often chipped or mulched to avoid the temptation of moving wood for home or campsite use. For crop diseases, the farm operators are advised by extension personnel about which pesticide or fumigant will be most effective. In some cases, a defoliant herbicide might be used to attempt to stop further inoculum development of the plant pathogen to reduce spread.

In the U.S., the Federal government compensates individuals for a variety of property losses. In some cases, this compensation extends to the removal of healthy animals to mitigate the spread of infectious or communicable diseases. The USDA has used indemnity payment to incentivize herd owners to depopulate animals and thereby contain the spread of infectious or communicable diseases. During an emergency event, quickly reducing the susceptible population is sometimes necessary to remove healthy animals that would otherwise contribute to the spread of disease. Conceptually, indemnity payments will influence both public and private behavior. Within the ICS structure, the position of Field Reimbursement Specialist (FRS) is restricted to animal agriculture and the activities they are responsible for can become a choke point for operational activities down steam if an agreement for reimbursement cannot be made in a timely manner (Costa & Akdeniz, 2019; Kuchler & Hamm, 2000; USDA APHIS VS, 1984).

Indemnity for plant health events occurs when the Secretary of Agriculture declares an "Extraordinary Emergency" (USDA, 2000) and Commodity Corporation funds are made available. The Commodity Credit Corporation (CCC) is a U.S. government-owned and operated entity that was created to stabilize, support, and protect farm income and prices (Farm Services Agency, 2012). It provides funding for a wide variety of activities in support of U.S. agriculture, including providing funding related to livestock and plant pest programs and disaster relief. Additionally, crop insurance is available for commodities supported by the USDA Risk Management Agency (RMA). The RMA serves America's agricultural producers through risk management tools to strengthen the economic stability of agricultural producers and rural communities. The RMA is committed to increasing the availability of federal crop insurance as a risk management tool and manages the Federal Crop Insurance Corporation (FCIC) to provide crop insurance products to America's farmers and ranchers. Approved Insurance Providers (AIP) sell and service Federal crop insurance policies in every state and in Puerto Rico through a public-private partnership with RMA (RMA, 2021).

Although such crop insurance protection is not common, it was offered prior to soybean rust (*Phakopsora pachyrhizi*) detections in 2004. Therefore, if growers bought the insurance and had losses associated with soybean rust, (if the growers could document appropriate prevention measure) they could be compensated for any losses caused by soybean rust.

When determining the amount of compensation paid to the owner, the government does not need to consider how the property was intended to be used, only the Fair Market Value (FMV) or price that the property would sell for on an open market (Kuchler & Hamm, 2000). The USDA does not provide compensation to owners based on a replacement cost, the actual cost to replace an animal at its pre-loss condition. Rather, replacement cost equates to the price of a new animal similar in type and production as the one removed. Replacement costs do not reflect or consider whether the animal was resident in an infected herd or group or whether the animal is subject to a decrease in value due to disease or lost opportunity. Guidance for determining the current range in indemnity values are calculated by economists and shared with the IMT. During an outbreak, the task of determining indemnity values on any given premises is delegated to the on-site FRS.

Management, Therapeutics and Vaccines

In many scenarios, the decision is to manage the pest or pathogen, rather than try to eradicate it. Also, when eradication attempts have been unsuccessful, and the animal or plant pest or pathogen persists, tactics will have to shift to longer-term management or recovery. How is the decision made to switch from eradication to management? The methods and reasons vary, but they are science-based, and consider how well the pest or pathogen can be detected in incipient populations, how widespread the pest or pathogen has become, and how well the potential mitigation methods in use are working (including how expensive the methods might be). The citrus canker case study below is an example of an eradication program that changed to a management program.

Therapeutics and vaccines may be available and integrated into the response plan. In the U.S., the USDA maintains the National Veterinary Stockpile (NVS) to provide animal vaccines, antiviral therapeutics, containment supplies and equipment, and response support to assist States, Tribes and Territories during a foreign animal disease treat (USDA APHIS, 2021). The NVS was established in 2004 to protect the U.S. food supply by strategically placing stocks of these countermeasures in various regions of the U.S., with the expressed goal of deploying these critical veterinary resources within 24 hours of a foreign animal disease incursion. Examples of the types of materials that can be deployed include bulk PPE, sample packaging and shipping supplies, disinfection and decontamination supplies, animal handling equipment, and euthanasia/depopulation equipment and supplies. Response support services include utilizing private-sector heavy equipment through USDA contracts. Additionally, the NVS provides foot and mouth disease vaccine assistance to Canada and Mexico through the North American Foot and Mouth Disease Vaccine Bank. In plant health, there may be resistant varieties, pesticides, and/or biological control options. The availability and logistics of deploying control options is taken into consideration as response is planned and coordinated.

Another example of where a federal management program works in collaboration with states, industry, and other federal agencies is the *Lymantria dispar dispar* moth "Slow the Spread" program. The tactics of the APHIS *L. dispar dispar* program are to define the extent of the *L. dispar dispar* infestation, to eradicate with insecticides isolated *L. dispar dispar* populations outside the quarantine area, and to contain on-going artificial *L. dispar dispar* spread of the moth beyond the infested area through effective quarantines. Spread occurs because of both natural flight of the moth and the attachment and transport of egg masses on vehicles. APHIS *L. dispar dispar* quarantine and containment efforts regulate known host material movement from infested areas to other parts of the U.S. (USDA APHIS PPQ, 2021b).

Biological control, or biocontrol, has long been part of the management of plant and animal pests. Classical biocontrol involves the use of parasitoids, or other pathogens of the pest or pathogen of concern, to greatly reduce the pest or pathogen populations. Biological control cannot be used as an eradication method because the biocontrol methods require the living pest. All biocontrol treatments that are used by the Federal government must go through extensive non-host testing and environmental assessment prior to any release into the environment. This assessment must be reviewed by the U.S. Fish and Wildlife services and other Federal agencies (as well as Tribal and state regulatory and environmental officials) to ensure that no threatened or endangered species may be affected by the release of the specific biocontrol organisms. There is also a required public comment period when a notice is published in the Federal Register and all comments responded to by the regulatory agency in advance of planning release of parasitoids or other biological control agents. Biocontrol typically involves the mass-rearing of the approved parasitoid and inundative releases in the areas where the pest or pathogen occur.

De-escalation and Recovery after Successful Response Actions

The declaration of "eradicated" for an animal or plant pest or disease means that no pest or disease can be detected in the animal or plant population that was once present and the reason for the initial emergency. Post-eradication, additional monitoring is typically conducted, dependent on the pest or disease. Surveillance activities are maintained in the affected area until the pest or pathogen can no longer be found. Then the target area can be declared 'Free From' disease. All surveillance is based on the best available science for the pest or disease. If a pest was eradicated, monitoring is conducted normally for 3 potential generations based on the life cycle of the pest. Why 3 life cycles? At low population densities, the pest may be present, but very difficult to detect. However, after three generations, almost every pest surveillance system is sensitive enough to detect the pest or pathogen infestation more than 95% of the time (Novy, 1991; USDA APHIS, 2003; USDA APHIS, 2017). If an animal or plant disease eradication has occurred, an appropriate number of samples that can detect the pathogen must be taken again for several potential pathogen lifecycles (sample size is determined by modeling, what level of infestation/infection is attempting to be detected, what confidence l level is needed, the pathogen or pest biology, and sensitivity and robustness of the sampling/survey methodology). Once the eradication period has elapsed (which is different for every plant and animal pest or pathogen) when no new detections of the pest or pathogen, the eradication is officially declared completed. This is normally announced through the Federal Register and directly to the trading partners. At this point trade from the area can be normalized again.

Deregulation

There may be times when regulatory quarantines or other regulatory program activities are deemed no longer effective to prevent the spread of a particular animal, plant pathogen, or pest. Communication is a very important aspect of deregulation. Normally when deregulation is being considered, a notice of an intent to deregulate the pest or pathogen program is published in the Federal Register with a specific time frame (typically between 30 and 90 days) where comments regarding the deregulation are accepted. USDA must then respond to every comment received during the comment period in writing in the Federal Register. Often, if the program is controversial or has received much attention, there can be thousands of comments that must be responded to. Once the comments are responded to, a final rule is put out addressing all comments and, if deregulation has been warranted, stating when that deregulation will occur (again, normally 30-90 days after the rule is published). Communication of this and other parts of the deregulation process are important, however, USDA personnel must be careful about what is communicated with stakeholders during periods of time when deliberations of the regulatory actions are occurring. Discussions directly related to regulatory activities and what will or will not be done (i.e., continued regulation or deregulation of a pest) is considered *ex parte*. *Ex parte* communications are illegal when regulatory considerations are being promulgated.

CONCLUSION

Biology is not black and white. Rather, responses to animal and plant health emergencies are 256 shades of grey. Situational flexibility is required, and control or eradication campaigns must be constantly monitored to see if success metrics are being met. Activities may shift and new plans devised based on the cost and efficacy of on-going campaigns. Prevention is a better bet in biosecurity. Once a biosecurity breach has occurred, the outcome can be very costly. Response and eradication programs occur once everything else has failed. Once an invasive species has gained a foot-hold, it is difficult to get the genie back into the bottle.

What can go wrong with response tactics? Communications failures are one of the most common problems. When communications are off, the entire incident response can be derailed. There is a myriad of examples that would require a full chapter to elucidate the deleterious effects of poor communication with the public and other stakeholders. Inability or unwillingness to comply by the public, coordination failures, logistics failures, and the inability to change programmatic direction when new scientific information becomes available are all things that can go wrong during a response. Often, plant and animal incidents that involve exotic pathogens or pests may be acting differently than they do in their natural environment. As such, the information available about the pathogen or pest in our environment may be limited. When control tactics fail, scientists must evaluate why in order to advise policy makers to develop a new strategy.

Emergency response is like a fire department. While it is much more cost-effective to prevent fires (say with working smoke detectors in a building), and everyone should be prepared for household fires when the fire is out, the fire department is the best way to contain the blaze. The response to animal and plant health incidents are analogous, with response officials in federal and state agencies filling the fire department role. The tactics discussed and outlined in this chapter exemplify these response mechanisms. The following case studies provide further examples of the same.

CASE STUDY 1: *RALSTONIA SOLANAREARUM*

Ralstonia solanarearum is a bacterium that causes vascular wilts on many different species of plants across a wide range of plant families. *Ralstonia* bacteria are motile in water and can move into water systems to invade plants. Several different races of the species are known (a total of 5) and most races attack plants that grow in the tropics and subtropics. One race of *R. solanacearum* (Race 3) is known to occur in temperate regions and is also known to cause a vascular wilt on tomatoes and potatoes. This race also is endemic in many tropical regions and is known to cause a vascular wilt on geranium (*Pelargonium* spp). Since most of the US geranium production system is offshore in many of these same tropical areas where Race 3 is endemic, the introduction of this pathogen into the geranium production system is a constant threat. In 2004, a significant introduction of *Ralstonia* on geranium cuttings that came to the US resulted in a new offshore Pelargonium production certification program that worked closely with producers to reduce the potential of *R. solanacearum* Race 3 on geranium cuttings (USDA APHIS PPQ, 2021). This program has effectively reduced the annual influx on *R. solanacearum* race 3 biovar 2, but in April, 2020, the pathogen was detected in wilting geranium cuttings in Michigan. The geranium cuttings were Fantasia 'Pink Flare' and Fantasia 'Salmon' cuttings from a large production facility in Guatemala. The cuttings were eventually traced to 650 greenhouse locations in 44 states. A total of more than 621,000 infected and exposed plants were destroyed. This destruction stopped the infestation, and the nurseries were disinfested to ensure that the pathogen was no longer present.

From the first detection in April to declaration of eradication in June 2020, during the height of the COVID-19 pandemic, this was an incredibly well coordinated event. This would not have been possible without the tracing efforts that were established in 2004 after a previous *R. solanacearum* outbreak on geranium cuttings. As a result of that outbreaks, the offshore geranium production certification program was started, meaning that all geranium cuttings produced offshore can be traced back to the greenhouse and even the bench they were produced on (USDA APHIS PPQ, 2021a).

CASE STUDY 2: CITRUS CANKER

Citrus canker (CC) is caused by *Xanthomonas axonopodis* pv. *citri*. The bacteria are not harmful to humans but are spread by wind-driven rain and cause raised lesions on leaves of many citrus varieties. While the disease is mainly a leaf spotting and fruit blemishing disease, when conditions are suitable, infections can also include severe defoliation, shoot dieback and significant fruit drop. Citrus canker was originally introduced into the State of Florida in 1912 on infected trifoliate orange (*Poncirus trifoliata*) seedlings from Japan and consequently spread to Alabama, Georgia, Louisiana, South Carolina, and Texas (Gottwald, et al, 2002; Whiteside, 1988). It was eradicated in Florida by 1933, and from the rest of the U.S. by 1947. Citrus canker reappeared in Florida in 1986, was eradicated again, then reappeared in Miami-Dade County near the airport in 1995 (Gochez, et Al., 2020; Gottwald et al., 2001; Gottwald, et al., 2002a; Gottwald, et al., 2002b; Schubert, et al., 2001; USDA APHIS PPQ, 2002; Vogel, 2001). The costs of eradication, quarantine, tree replacement and treatment have reached close to $1 Billion in FL alone (Gottwald, 2000).

For the 1995 CC outbreak, emergency response was conducted by a cooperative federal/state citrus canker eradication program that included USDA APHIS and the Florida Department of Agriculture and Consumer Services (FDOACS) (Gottwald, et al., 2001; Gottwald, et al., 2002a; Gottwald, et al., 2002b; Schubert et al., 2001). This outbreak followed a similar ICS structure, with unified commanders and multi-area command centers where strike teams were sent out to survey the mainly residential area for citrus canker. Research by the USDA Agricultural Research Service had determined that 95% of all citrus canker bacteria spread during rain events moved less than 579 meters (1900 ft) (Gottwald, et al, 2001). Part of the 1995 CC eradication program—one of the most impactful parts—became known as "the 1900-foot rule", where all trees within 1900 ft of a citrus tree that has citrus canker were considered exposed to citrus canker (Gottwald, et al, 2001). Previous CC eradication programs conducted by the state and APHIS required all citrus trees within 150 feet of a known positive tree be removed. At the height of the eradication program, deployed strike teams would fell and chip an average of 5000 citrus trees per day (Vogel, 2001).

The initial infestation of citrus trees near the Miami Dade Airport likely occurred in 1992 or 1993. By 1995 when the infestation was detected, citrus canker had spread to 14 square miles in the first two years of the epidemic centered in a Miami-Dade residential area near the Miami International Airport (Gottwald, et al, 2001). This spread was due mainly to conducive environmental conditions that included hurricanes and other tropical systems and storms with high winds and wind-driven rain on a frequent basis, but also due to eradication work stoppage through injunctions imposed due to litigation (Gottwald, et al., 2002). Even though the lawsuits eventually failed, the three years of injunctions combined with significant storm events through south Florida resulted in exponential spread of citrus canker through the region. Between 2000 and 2004 at least 12 tropical events hit south Florida, including 4 hurricanes in 2004 (Charley, Frances, Ivan, and Jeanne) (NOAA, 2021). By 2002, the quarantine area around the original Miami-Dade infestation covered more than 1500 square miles Gottwald, et al, 2002b). Once citrus canker became so widespread, eradication was determined to be no longer possible. Destruction of exposed trees ceased; the USDA and FDOACS personnel conducting the eradication program were demobilized (USDA APHIS, 2018). Currently, the entire state of Florida and parts of Texas and Louisiana are regulated for citrus canker. Regulation involves the use of permits to move citrus fruit and nursery stock from the quarantine areas.

Citrus canker has had devastating effect to the $9 billion citrus industry in Florida, more than $400 million having been spent on attempted eradication alone (Gochez, et Al., 2020; Gottwald et al., 2001; Gottwald, et al., 2002a; Gottwald, et al., 2002b; Schubert, et al., 2001; USDA APHIS PPQ, 2002; Vogel, 2001).

The current Citrus Canker regulations (7 CFR 301.75) were originally promulgated December 13, 1985 and have been amended dozens of times to update the regulated area, regulated articles, and many other provisions (eCFR, 2021). Once the decision was made to move from an eradication program in Florida to a management program, APHIS worked closely with the FDOACS personnel to implement the change.-

CASE STUDY 3: MEDITERRANEAN FRUIT FLY (MEDFLY)

Every year, fruit flies are found in the US. The Mexican Fruit Fly (*Anastrepha ludens*) often is found in the Lower Rio Grande Valley in citrus crops. The Oriental Fruit Fly (*Bactrocera dorsalis*) is sometimes found in California. No fruit fly, however, causes more concern than the Mediterranean Fruit fly or Medfly. Medfly can infest more than 300 hundred varieties of fruits, vegetables, and nuts, including almond, apple, apricot, avocado, bell pepper, cherry, coffee, eggplant, fig, grape, grapefruit, kiwi, lemon, lime, mango, nectarine, orange, olive, papaya, peach, pear, persimmon, plum, pomegranate, tangerine, tomato, and walnuts (Szyniszewska & Tatem, 2014).

The first recognized U.S. Medfly outbreak was detected in Hawaii in 1910, and it later became established there (NAPIS, 2019; Szyniszewska & Tatem, 2014). Although Medfly has been periodically introduced to the U.S. mainland, beginning with Florida, since 1929, successful eradication programs have prevented it from becoming an established pest in the Continental United States, mainly through preventative release programs (PRP) and active response to Medfly introduction through close and consistent monitoring programs (Enkerlin, et. Al., 2017; USDA APHIS, 2014). These PRPs use eclosion facilities in California and Florida that rear pupae that are produced in the Guatemala MOSCAMED facility (Enkerlin, et al., 2017). These two eclosion facilities are used to dispense approximately 650 million adult irradiated Medflies (sterile) into more than 2300 square miles of high-risk Medfly outbreak areas in California and Florida (USDA APHIS, 2014) .

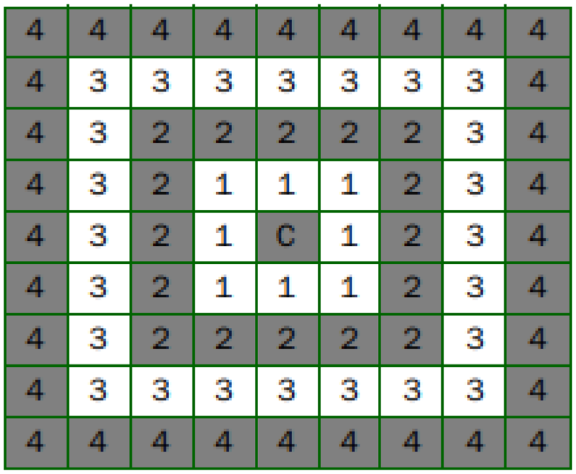
The MOSCAMED program began in 1975 with larvae imported from the International Atomic Energy Agency to a vacant aircraft hangar in Guatemala City. The program is an international cooperative agreement between The United States, Guatemala, and Mexico (Enkerlin, et al., 2017). The program has advanced to produce a temperature sensitive line of only male flies to be sterilized. When the sterilized males mate with wild females, the resultant eggs are non-viable (Enkerlin, et al., 2017). In 1980, during Medfly outbreaks, the Los Alamitos, California Facility received 1.73 billion sterile flies and the Florida Facility received 3.1 billion sterile flies from the MOSCAMED facility in Chiapas, Mexico. Subsequent to these eradication programs, the eclosion facilities have taken a preventative release program strategy, by releasing a relatively low number of sterile flies in high-risk areas, 35 million sterile flies per week from Los Alamitos and 102 million per week from Sarasota (Enkerlin, et al., 2017).

Sterile insect technique was first developed to control the New World Screwworm *Cochliomyia hominivorax* (Coquerel). The New World screwworm is the only known Diptera that parasitizes the living flesh of warm-blooded animals (including humans). In the 1930s, ARS scientist Edward Knipling and Raymond C. Bushland began looking for alternative methods to control this screwworm. After World War II they began looking at the potential for radiation to be used to control Screwworm. After six months of work, they showed that 2500-5000 roentgens of x-rays would effectively sterilize screwworm pupae but not adversely affect adult behavior (FAO, 1989; Novy, 1991; Smith, 2009). The researchers used a 36 km^2 island in Florida with a screwworm infestation as an experimental site. Releasing 38 sterile male flies per sq.km per week for several months as much as 80% of eggs were sterile (FAO, 1989; Novy, 1991; Smith, 2009). However, migration of mated females from the mainland made proving the theory elusive. Eradication was shown on the island of Curaçao (in the Caribbean), in 1954 after just 6 months of releasing 300 sterile flies per sq. km (FAO, 1989; Novy, 1991; Smith 2009; USDA APHIS, 2017). Once proven as an effective eradication technique, the sterile insect technology was developed for numerous other plant and animal pests, including fruit flies. Additional information on the New World screwworm is presented in detail in Chapter 8.

All fruit fly outbreaks have emergency response triggers and guidelines that include the thresholds for delimitation, the duration of the delimitation (typically based on a number of generations of the species, 21-30 days in tropical weather conditions), the number of flies required and the eradication area radius, and the number of flies and radius required for quarantine (USDA APHIS, 2020c). For Medfly, a single fly that is captured in a trap triggers a delimitation, which greatly increases the number of traps deployed in the area (USDA APHIS, 2003, 2020c).

The initial trap density in a high-risk area is 5 Jackson traps per square mile. When an infestation is detected, the core 1 square mile area increases from 5 to 100 Jackson traps per square mile within 24 hours of the initial identification of Medfly (Figure 5) (USDA APHIS, 2003). The first ring around the infested area includes 50 baited Jackson traps per square mile in the next delimitation mile outside of the core (**Figure 5**), 25 baited Jackson traps in the #2 ring, 20 baited Jackson traps in the #3 ring and 10 baited Jackson traps in the# 4 ring. In total, instead of 405 Jackson Traps in the 81 square miles where a single adult Medfly is detected, 1700 traps are deployed for delimitation of the infestation. During delimitation, traps are serviced daily in the core. If an additional Medfly is detected, traps are continued to be serviced every day in the Core area and every two days in the #1 ring for the first week (USDA APHIS 2003).

Figure 5. This configuration illustrates the appropriate array pattern for placing Jackson traps in the field, where C = the core area; 1 = the 8 square miles around the core area; 2 = the next 16 square miles outward; 3 = the next 24 square miles; and 4 = the outermost 32 square miles
Source: USDA APHIS 2003



4	4	4	4	4	4	4	4	4
4	3	3	3	3	3	3	3	4
4	3	2	2	2	2	2	3	4
4	3	2	1	1	1	2	3	4
4	3	2	1	C	1	2	3	4
4	3	2	1	1	1	2	3	4
4	3	2	2	2	2	2	3	4
4	3	3	3	3	3	3	3	4
4	4	4	4	4	4	4	4	4

Often, fruit is inspected on the property or near to where Medfly is detected, to ensure it has not become infested (USDA APHIS, 2003). Infested fruit often have small circular oviposition scars but may not have any external signs. In an infested area, up to 100 fruits of preferred hosts may be sampled by inspectors (USDA APHIS, 2003). As part of the eradication program, the core areas where the Medflies were detected receive treatment of a mixture of insecticide and a protein/sugar bait to eliminate the adult stage. Once the bait/treatment schedule is completed, sterile medflies are released in large numbers to disrupt the breeding cycle (USDA APHIS, 2003). If no other adults are captured in traps after 3 generations (typically 90 days, depending on the temperature) the area is declared to be free from the pest.

Both the initial infestation and the declaration of eradication are announced in the Federal register. Also, the quarantine states what commodities cannot be moved outside of the quarantine area without treatment, inspection, or certification.

CASE STUDY 4: WEST NILE VIRUS IN U.S. HORSES

In late August 1999, an equine practitioner on Long Island, New York, U.S.A, reported to the New York State Veterinarian the detection of an 18-case cluster of neurological illness among horses residing on 13 premises (Trock, et al., 2002). The presenting signs varied in severity for the affected animals, but they consistently had acute onsets of rear limb ataxia. Only one animal had an elevated temperature (102.8°F) and most continued to eat. Five animals tested positive for Equine Protozoal Myelitis (EPM) using a Western Blot technique; four from cerebrospinal fluid (CSF) samples and one from a serum sample. Two animals had elevated equine herpesvirus-1 (EHV-1) serum neutralization (SN) titers; one with a declining titer on a sample taken 25 days after the test. Treatment included antibiotics, analgesics, steroids, and thiamine; some animals were also administered pyrithiamine/sulfadiazine (Daraprim) and Baycox (toltrazuril) (USDA APHIS VS, 1999).

Before and concurrent with this outbreak, an outbreak of arboviral encephalitis among humans was recognized in New York City and neighboring counties in New York State. Additionally, local health officials observed increased fatalities among New York City birds, especially crows, and were alerted by officials of the Bronx Zoo of the deaths of a cormorant, two captive-bred Chilean flamingoes, and an Asian pheasant. The human cases were initially attributed to St. Louis encephalitis (SLE) virus based on positive serologic findings in CSF and serum samples using a virus-specific IgM-capture enzyme-linked immunosorbent assay (ELISA) (CDC, 1999). Necropsies performed on the birds at the zoo revealed varying degrees of meningoencephalitis and severe myocarditis. Tissue specimens from these birds and a crow with pathologic evidence of encephalitis from New York state were sent to the U.S. Department of Agriculture APHIS National Veterinary Services Laboratories (NVSL) in Ames, Iowa for identification and characterization (CDC, 1999).

The New York State Veterinarian was aware of ongoing public health investigations being conducted closer to New York City and the similarities in clinical presentations between the human and equine cases. He therefore requested the assistance of the USDA-APHIS with an investigation into the neurologic cases occurring among horses on the northern end of Long Island, NY. Under the direction of the USDA APHIS Veterinary Service (VS) Eastern Regional Director, a team of state and federal Foreign Animal Disease Diagnosticians (FADDs), including an epidemiologist with extensive experience with equine infectious diseases and a pathologist from NVSL were deployed to investigate the cluster of equine cases. The decision to send a five-member strike team in lieu of a larger complement of responders was based on a need to deploy quickly with a cadre of veterinarians with specialized experience and a desire to maintain a low public profile to avoid the introduction of bias into survey data collection and to maintain the privacy of owners with affected horses.

Once on-site, each of the 13 affected premises and one other were visited by members of the strike team and either the owner or manager was interviewed to obtain demographic information, clinical signs, onset, and duration of illness of the affected horses. A survey instrument was used to identify risk factors for equine neurologic disease and included questions related to management practices and exposure to insect and wildlife vectors. Active case finding was implemented that included phone contacts with veterinary practitioners in the area to determine whether other unreported cases of equine neurologic illness had been identified. Convalescing animals and images of animals showing clinical signs were examined. Blood samples were obtained from ill horses as well as from a subset of other animals, including cows, swine, sheep, goats, chickens, and ducks raised at the Cornell University Research Laboratory to determine possible exposure risk to other species as well as to supplement general surveillance efforts (USDA APHIS VS, 1999).

An effort was made to collect samples from all horses which were either commingled or on a premise co-owned or managed alongside case animals. Thirteen samples, ten serums and three CSF, collected by the local practitioner who reported the cluster were included in specimens sent to both NVSL and the Center for Disease Control and Prevention (CDC), Vector-borne Laboratory in Fort Collins, CO for processing. For all horses residing on affected premises, serologic testing for EHV-1, Eastern/Western Equine Encephalitis (EEE/WEE), and EPM was requested to rule out other causes of neurologic illness (Trock, et al., 2001).

A case was defined as a horse with onset of illness since August 1, 1999 who resided on a premise on Long Island, New York accompanied by any one of the following symptoms or signs: fever (>102.0°F), lethargy, anorexia, ataxia, staggering, weakness to paralysis of the hind legs, dysphagia, depression, flaccid paralysis of the lower lip, impaired vision, head pressing or acute death (USDA APHIS VS, 1999).

Of the 18 case animals, eight (44%) died or were euthanized while the remaining 10 animals improved with supportive care. Vaccination practices for most owners of affected horses follow a spring/fall schedule of flu, EEE/WEE, tetanus, and EHV-1 and, while most horses on affected premise were not up to date on immunizations for EEE/WEE, 9 (50%) case animals had received EEE/WEE within the previous 6 months; two within 75 days of disease onset. In 1999, resources for laboratory diagnosis of flavivirus infections were limited but subsequent to the investigation, the cause of the outbreak was initially based on ruling out other neurologic illness but was subsequently confirmed as a West Nile-like virus based on the identification of virus in human, avian, mosquito and equine samples (CDC, 1999, USDA APHIS VS, 1999).

West Nile Virus (WNV) is one of the best examples of a prototypical emerging pathogen with its introduction into New York state in 1999 and subsequent spread to all 48 contiguous United States over a period of a few years. To date, WNV is considered endemic throughout the country and is the leading cause of arboviral disease in humans and equids in the United States (Wimberly et al., 2014).

The USDA-APHIS response to this incursion and the support provided to the Office of the New York State Veterinarian demonstrates some basic tenets of the ICS system. The event was scalable to the extent that only a few responders were deployed in support of the outbreak, yet they were able to meet the needs of the requesting agency. It incorporated personnel from different agencies within a common management structure which provided logistical, laboratory and administrative support for the response. While the long-term financial burden of WNV to equine owners in the US is undetermined, a survey conducted by the USDA, Veterinary Service, Centers for Epidemiology and Animal Health in the wake of viral spread across the US in 2002 estimated the cost of treatment, loss of use, and prevention for 843 equine owners in Colorado and Nebraska at US$3.9 million. For the 1,478 cases of equine WNV, the estimated cost of treating mild, moderate, and severe cases totaled US$490,844 or approximately US$332 dollars per case horse (USDA APHIS VS, 2002). Since 1999, more than 25,000 cases of WNV encephalitis have been reported in the U.S. horse population (AAEP, 2021) suggesting that treatment costs alone would have approached US$8.3 million for these cumulative cases.

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KEY TERMS AND DEFINITIONS

APHIS: Animal and Plant Health Inspection Service. The agency within the United States Department of Agriculture responsibility for regulatory actions and responding to plant and animal health emergencies.

Authority: The entity or person designated to deal with matters associated with regulations and rules set forth in Code (FAO, 2007).

Biocontrol: The intentional introduction and establishment of an organism to control a pest or disease (FAO, 2007).

Biosecurity: Measures aimed at preventing the introduction and/or spread of harmful organisms to animals and plants to minimize the risk of transmission of infectious disease.

Bioterrorism: The intentional release or dissemination of an agent such as viruses, bacteria, fungi or toxins by a person, or group, to cause a disease or pest infestation for the purpose of causing economic damage, loss of the commodity and to induce panic into markets and the public to produce terror or as a means of biological warfare.

Chain of Custody: Method of ensuring that samples can be traced from collection, through shipping, to diagnostics and results. This ensures that the samples have legally been in possession or transit at all times.

Commodity Credit Corporation: A government-owned and operated organization created by Congress in 1933 to provide rapid access to funding for response to animal and plant health emergencies.

Depopulation: To remove all organisms in a given area, usually through euthanasia or destruction of the organisms to reduce the likelihood of pest or pathogen spread.

DHS: Department of Homeland Security.

Eradication: A treatment to completely eliminate a pest from a given area (FAO, 2007).

Euthanasia: From Greek for “Good Death,” to humanely end the life of an organism.

***Ex parte*:** A Latin legal term that means to communicate with one group while deliberations are still occurring.

Exposed: In this chapter, exposed means that an organism has likely come in contact with a pathogen or pest and may be infected or infested, but at too low a level to be detected by the currently available detection methods.

Homeland Security Presidential Directive (HSPD): A specific type of Presidential Executive Order that directs federal agencies in the executive branch to perform specific tasks related to the security of the US.

Incident: An occurrence of an exotic plant or animal health emergency to which USDA APHIS must respond.

Incident Management Team: Trained teams of personnel who quickly respond to animal, plant health or other types of emergencies.

Indemnity: Payment of money for a financial loss.

Infected: When a living organism has a pathogen that is causing disease.

Infested: When air, water or premises or other non-living materials contain a pathogen or pest that can cause disease to specific organisms.

National Incident Management System: Part of the National Response Framework developed by DHS and FEMA to provide guidance to first responders to emergencies, hazards, threats, or disasters.

National Plant Board: A non-profit organization made up of all State Plant Regulatory officials in the United States (including Guam and the Commonwealth of Puerto Rico).

National Response Framework (NRF): Framework to help the US and other entities respond to all types of emergencies and disasters and includes the Emergency Support Functions.

Phytosanitary: Relating to the health of plants, measures for the control of plant diseases.

Premises: Location where animals or plants are kept prior to dispersal.

Quarantine: Official containment of regulated articles for treatment, inspection, or other regulatory activities.

Regulated Articles: Any animal, plant, animal product, plant product, storage place, packaging, conveyance, container, soil and any other organism, object or material that could spread or contain a regulated pest or pathogen.

Sanitary and Phytosanitary Standards: Documents approved by a recognized body, that provides, for common and repeated use, rules, guidelines or characteristics for activities or their results related to plant and animal freedom from pests or pathogens (FAO, 2007).

State Plant Regulatory Officials: A state employee responsible for overseeing, enforcing, and maintaining plant regulatory activities within a state.

Sterile Insect Technique: An integrated pest management technique that uses low dose radiation to sterilize insect pupae such that the insects produce non-viable eggs.

Chapter 10

Tactical Applications of Microbial Forensics in Agricultural and Environmental Biosecurity

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ABSTRACT

The secure and continuous production of agricultural commodities and food security are key to U.S. national security. The introduction of foreign-origin or emerging animal, plant, and human diseases by intentional acts of espionage, terrorism, biological warfare, or criminal activity can lead to severe consequences for domestic and international agricultural markets, the economic security of the agricultural community, food safety and food security, and the credibility of responsible state and federal agencies. Early public, animal, plant health, law enforcement, and intelligence assessments and investigations of suspected or confirmed intentional threats are critical additions to existing interagency prevention, response, and management protocols. Forensic microbiology, a multidisciplinary science, is essential to the nation's readiness for responding to a potentially criminal, intentional, or otherwise nefarious incident in the agricultural sector (plant or animal), and of eventual supporting attribution and the prosecution of the perpetrators.

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1. INTRODUCTION

The secure and continuous production of agricultural commodities and the assurance of access to food products are key components of U.S. national security. The introduction of foreign-origin or emerging animal, plant, and human diseases can occur by natural incursion, accidental introduction, or intentional acts of terrorism, biological warfare, or criminal activity. These threat agents, which are mostly biological, can lead to severe consequences for domestic and international agricultural markets, the economic security of the agricultural community, food safety and food security, and the credibility of responsible state and federal agencies (FBI Weapons of Mass Destruction [WMD] Directorate, 2017; FEMA, 2019b).

Biological disease agents (exotic or non-endemic pathogens, pests, or toxins) affecting livestock, wildlife, crops, forests, and rangelands can impact human health in direct and indirect ways (Destoumieux-Garzon et al., 2018; Fletcher et al., 2006; Fletcher et al., 2020). Plant diseases can impact food security significantly for both humans and livestock and highly transmissible **zoonotic diseases** could have severe public health consequences. For example, commercial swine are commonly infected with swine influenza viruses that are usually different from seasonal human influenza viruses. While rare, influenza viruses can spread from pigs to people and from people to pigs. When an influenza virus that normally circulates in animals (but not people) is detected in a person, it is designated a “variant influenza virus.” As of September 2021, ten human infections with a novel influenza A virus, which has been prevalent in swine, have been reported in the United States. Only one of those infected had no known connection to swine (Centers for Disease Control and Prevention [CDC], 2021; Program for Monitoring Emerging Diseases [ProMED], 2021).

Highly transmissible biological disease agents of either plants or animals can spread exponentially beyond the initial point-source of introduction. Characteristics to be considered when attempting to forecast rate of spread include natural or genetically manipulated pathogenicity, transmissibility or disease contagion, environmental impact on the pathogen life-cycle and efficiency of its dissemination, and agricultural production/marketing activities. (Federal Emergency Management Agency [FEMA], 2019b). These features and characteristics must be factored into threat assessments and mitigation plans.

Agricultural biosecurity is an issue of concern and attention for nations around the world, and efforts to mitigate threats to agricultural production are, in many respects, international in scope. Although this chapter focuses largely on systems, networks and approaches used in the United States, these elements of biosecurity should be considered within the larger context of the global agricultural infrastructure.

a. Terrorism, Criminal, and Other Intentional Actions Against Agriculture

Due to current world threats, U.S. agriculture, law enforcement, and intelligence agencies must rule out intentional introductions when assessing and initiating investigations for unusual, atypical, suspicious, or otherwise unexplained disease incidents (FBI & CDC, 2018; FBI WMD Directorate, 2017; FEMA, 2019b).

The use of chemical, biological, radiological or nuclear (CBRN) agents and hazardous materials provides a spectrum of potential tools and methods available to state or non-state adversaries. These considerations are critical for disease threat modeling, introduction event risk assessments, and **attribution** of suspicious activities and disease incidents. State and federal agricultural agencies regularly develop risk assessments for the more predictable and likely means of introduction. Predictive and planning tools are often based on recent experiences or historical disease incidents. Agencies prepare, train, and perform interagency response exercises based on knowledge derived from prior cases. Unfortunately, many agencies plan and train only for the severity of incidents for which they have the existing capability. It is rare to exercise complex, severe situations that could exceed agency resources to the point of failure or prepare for criminal or terrorist acts (FEMA, 2019b).

The expected disruptive effects of a high consequence disease would be amplified beyond those of a natural incursion or accidental introduction if the source or means of introduction is determined to result from an intentional action by an individual or group. Unanticipated methods of dissemination and introduction, multiple initial outbreaks in widely separated geographic locations, the unexpected, simultaneous use of multiple pathogens, and the detection of disease agents with altered transmissibility or pathogenicity could overwhelm existing response capabilities of responsible agencies. The effective use of well-designed disinformation operations through social media and other mass information techniques could have profound negative effects on response operations and government agency credibility [see Case Study 1]. Unlike the planned response to commonly recognized disease agents, the additional threat scenarios, unanticipated security requirements, and the requirement for simultaneous disease response, law enforcement, and intelligence operations by state and federal agencies could strain existing response capabilities (FEMA, 2019b).

A biological incident due to a high consequence pathogen, regardless of origin, can provide an opportunity for exploitation by domestic extremists, embedded elements of foreign terrorist groups, intelligence agencies or individuals. Opportunistic acquisition of otherwise unavailable biological materials can lead to their dissemination into secondary populations, species, and geographic areas beyond the initial point of introduction, confounding epidemiological analyses.

Suspected or confirmed intentional food and agriculture incidents could trigger exaggerated fear, anxiety, and psychological responses in individuals and the public (FEMA, 2019b). Uncertainty and distrust in the availability of safe food supplies could have pervasive sociological effects and challenge modeled and planned-for response and support capabilities of the interagency Incident Command System (ICS) response protocols (Brown et al., 2005, DHS, 2006; FEMA, 2011).

b. Threat Actors and Motivations

Historically, the primary threats to livestock, crops, and natural ecosystems have been accidental or natural introductions of foreign animal diseases or exotic plant pathogens and pests. Preparation for these threats has resulted in the development of response protocols, training scenarios, and exercises that focus on these recognized risk factors but often overlook responses to possible intentional incidents. When intentional incidents are included in risk modeling exercises, they often are presented as acts of "**agroterrorism**" by politically or philosophically motivated extremist groups or individuals. However, additional threat actors who would benefit from the disruption of the agriculture sector must also be considered. What are their motivations and goals? What do they have to gain against an adversary, and what methods are available to them?

The assessment of suspicious incidents should include the consideration of 'bad actors,' both domestic and foreign. These may include state-sponsored espionage, sabotage, and biological warfare by adversary governments and foreign intelligence services. Others with motive could be state-supported terrorists or "proxy" foreign fighters organized, trained, and funded by adversary governments. Non-state sponsored incidents may be perpetrated by foreign terrorist organizations, domestic terrorists and extremists. Economic sabotage might motivate corporate, criminal, or foreign economic competitors.

Other non-state sponsored motivations could include "insider threats" from disgruntled or recruited employees, domestic activists wishing to draw attention to a cause, or even hostility among neighbors. (FBI WMD Directorate, 2017). Additionally, the rapid development of advanced genetic and computing techniques, artificial intelligence, and critical agricultural biotechnology has increased efforts to steal **trade secrets** and **intellectual property** by competitors or adversary governments for both economic and geopolitical advantage. These threats emphasize the importance of incorporating law enforcement and intelligence communities early during the assessment and investigation of agricultural incidents and the need for attribution capabilities (FEMA, 2019a).

c. The Need for Forensic Sciences

An early foundation for **microbial forensics** began in 1996 with the creation of the Hazardous Materials Response Unit (HMRU) of the Federal Bureau of Investigation (FBI), which responded to hundreds of cases (mostly hoaxes, such as "white powder" cases). Several Department of Energy National Laboratories as well as the U.S. Army and Navy medical laboratories began to support HMRU. The first concept publications on microbial forensics, which appeared in the early 2000s (Budowle, 2003; Budowle et al., 2003; Murch, 2001;), allowed for quicker and more robust improvements in science and resources following the anthrax attacks of 2001.

Box 1. The Amerithrax Example

Beginning in 2001, an extensive investigation involving the FBI, CDC, and multiple state, local, federal law enforcement, intelligence, public health, and other emergency response agencies determined that letters containing highly refined anthrax spores had been intentionally manipulated, processed, and mailed to targeted individuals. There were severe public and psychological health effects, extensive contamination of buildings and infrastructure, and the exposure, infection, and deaths of non-targeted individuals. The novel situation of a severe public health threat possibly implemented by a criminal or terrorist actor required rapid and simultaneous disease control responses and epidemiological investigations by the CDC and local and state public health assets.

Simultaneously, the FBI, U.S. intelligence agencies, local, state, and federal law enforcement, and hazardous materials and first responder teams had to investigate, disrupt on-going threats, and perform attribution of the incidents. These efforts required extensive counterterrorism, counterintelligence, and criminal investigations, multiple hazardous evidence crime scene investigations, and the development of new techniques to determine the sources and the means of manipulation and dissemination of the anthrax materials. Continuous input and information sharing between the joint public health and law enforcement teams was critical to determining the source, motivations, and technical capability of the perpetrator(s).

In response to the assessments and lessons learned from the joint CDC and FBI led Amerithrax investigation, a series of joint investigative concepts were established by the FBI, CDC, and later USDA through a series of memoranda of understanding and the development of FBI-USDA sponsored "Joint Criminal-Epidemiological Investigations" courses.

Applications of microbial forensics in the investigation of animal and plant diseases soon followed. The United States government was moving to enhance and strengthen our capabilities to protect, respond to, mitigate and recover from potential criminal actions against elements of the nation's critical infrastructures, including our agricultural food production and distribution systems (Fletcher et al., 2006). Until the early 2000s it was unusual for most in the plant health community to consider the possibility that a plant disease might be the result of an intentional introduction or other criminal action.

A team of plant pathologists having expertise in disciplines essential to microbial **forensic science** (including diagnostics, molecular biology and genetics, biochemistry, pathogen biology and ecology, evolution, and epidemiology), with guidance from forensic specialists, worked to identify existing plant pathology capabilities, developed for normal diagnostic and mitigation efforts but important also to forensics. In doing so, they identified gaps in awareness, scientific knowledge, and technical rigor in the application of forensic science to plant disease investigation and made recommendations for the development of a robust capability in forensic plant pathology (Fletcher et al., 2006). Around the same time, there was recognition of the need for building forensic capacity for investigation and reporting related to animal diseases that might have resulted from intentional pathogen introduction (McEwen et al., 2006). Among veterinary applications was the use of molecular epidemiology and bacterial genotyping to answer questions about the origin of a strain of *Mycobacterium bovis* causing bovine tuberculosis in the British Isles (Smith, 2011).

The aims of this chapter are to review current literature on applications of microbial forensic sciences in the agricultural sector, to detail specific requirements and standard procedures for forensic investigations in crop and livestock settings, and to provide a series of case studies illustrating microbial forensic science in practice. The chapter is designed for use in upper-level coursework for those having some background in agricultural sciences. It also will be useful for professionals in law enforcement, security positions, forensic sciences, agriculture and others whose responsibilities may include preventing, detecting, responding to and investigating potentially criminal occurrences related to the agricultural enterprise.

2. TACTICAL SCIENCES FOR FORENSIC INVESTIGATION OF DISEASE OUTBREAKS IN AGRICULTURAL SETTINGS

In what sense is microbial forensics a 'tactical science'? Tactical sciences for agriculture are inter-related scientific efforts that, independently and/or in coordination, support and protect a nation's food production and distribution functions from threatening pests and pathogens, as well as chemical contaminants and other disasters (USDA NIFA, 2017). Microbial forensics is an essential component of readiness for response to a potentially criminal, intentional or otherwise nefarious incident in the agricultural and environmental sectors (plants and/or animals), and for critical **evidence** collection for eventual attribution to, and **prosecution** of, the perpetrators.

a. Comparing Major Objectives of Agriculturalists and Law Enforcement Personnel

State and Federal law enforcement, intelligence, and animal-plant health agencies share many common goals (FBI WMD Directorate, 2016; FEMA, 2019b):

- Identify existing and emerging threats to the public and to agricultural/environmental resources
- Evaluate vulnerabilities and the security of potential targets
- Prevent deliberate attacks against the agriculture sector
- Develop joint tools and techniques to recognize and report the **trigger**s or indicators of suspicious or possible intentional activities against agricultural/environmental infrastructure targets
- Perform attribution of suspicious, atypical, unexplained threats or disease incidents to determine the origin, the means of introduction, and whether they are naturally occurring, accidental, or intentional introductions

At the same time, each of these sectors is guided by unique legislative mandates, missions and goals, stakeholder concerns, and other drivers. Individual interests and concerns associated with the agricultural sector and the law enforcement sector are summarized in Table 1 and further described in the following sections.

Table 1. Primary objectives and procedures in a plant disease outbreak response, for agricultural specialists and law enforcement officers or forensic investigators

Agricultural specialists	Law enforcement/forensic investigators
Design and implement sampling and diagnostic protocols for **delimiting** and on-going surveillance	Assure security of crime scene and evidence
Delineate extent of outbreak- local, state, regional, and whether it has spread or is spreading	Assure security of evidence collection, packaging and transport to establish **chain of custody**
Identify diagnostic labs having appropriate personnel, materials and instrumentation, and accreditation to process samples in possible surge scenarios	Thoroughly investigate and fully document the crime scene
Implement secure sample collection, transport and chain of custody transfer procedures	Implement secure sample collection, transport and chain of custody transfer procedures
Implement proper and thorough biological sample laboratory analysis, interpretation of results, and timely and effective reporting	Implement proper and thorough forensic sample (crime scene) laboratory analysis, interpretation of results, and timely and effective reporting
Implement **trace forward/trace back** protocols per USDA APHIS Guidelines	Complete a robust investigation to support perpetrator identification and to understand motives, methods and means
Identify and implement **containment** or control measures	**Exonerate** individuals not responsible
Assess current and future economic damage	Communicate and coordinate with pertinent agencies during field and forensic investigations
Identify potential human health risk, risk to animal population and species, impact on interstate and international trade; economic and environmental impact of disease response efforts, food safety	Provide support to prosecutors and other authorized personnel

b. Objectives and Procedures of Agricultural Specialists in Plant Disease Outbreak Response

To illustrate the elements of the agricultural outbreak response we use the example of response to a plant disease. Outbreaks or infestations of plant diseases may occur in such diverse settings as crop production fields, orchards, nurseries, greenhouses, storage facilities, and shipping containers in ports of entry. Barring announcement of a criminal act, county, state, and United States Department of Agriculture (USDA) agricultural specialists would be the first to be summoned to the site of a suspected outbreak. Protocols in place for highly regulated plant pathogens such as those listed by the USDA Animal and Plant Health Inspection Service (APHIS) as Select Agent Plant Pathogens (CDC & USDA, 2020) would dictate immediate notification and consultation with federal law enforcement agencies. While the objectives of agricultural specialists and law enforcement are both focused upon identifying the source of the introduction of a pathogen, protocols applied by the two groups early in an outbreak may diverge in purpose and scope. Agricultural specialists would be focusing on identifying the disease and pinpointing the foci of infection across a production field, nursery etc., while law enforcement officials would focus on securing the location(s) and evidence collection before sample materials can be contaminated. These efforts may not be complimentary and must be coordinated from the outset to ensure that the objectives of both groups are met.

Identify, design and implement sampling and diagnostic protocols for delimiting survey. Early efforts in a plant disease investigation focus upon development of a sampling plan and identification of relevant existing protocols for diagnostic laboratories. These steps may proceed rapidly if the pathogen is quickly identified or may be delayed while identification is confirmed by USDA. Once the causal agent has been identified definitively, the sampling plan is developed using experts in the biology and epidemiology of the pathogen to model the rate of spread from individual infection foci, and thus set the sampling distances from these focal points. In some cases, detailed sampling, chain of custody and diagnostic protocols have been developed and enhanced using knowledge gained from past outbreaks, *e.g. Ralstonia solanacearum* Race 2 biovar 3 and *Phytophthora ramorum* (USDA APHIS, 2008, USDA APHIS 2020).

Delineate extent of outbreak- local, state, regional - and whether spread or movement is occurring. Delimiting surveys, designed to provide the data required to outline the extent of an outbreak, may take days to weeks to complete. In many cases **traceback** protocols (see below) may be in place early in an outbreak to identify whether there may be additional foci to investigate. The situations encountered with field, nursery or port introductions require very different methods and the analysis and interpretation of very different datasets in order to determine whether and how disease is spreading, or pathogen movement is occurring.

Identify diagnostic labs having appropriate personnel, materials and instrumentation to process samples in possible surge scenarios. Once the projected extent of the affected area has been determined and the intensity of sampling from the outbreak have been planned, state and regional laboratories within the National Plant Diagnostic Network (NPDN) or National Animal Health Laboratory Network (NAHLN) are contacted to evaluate staffing, diagnostic infrastructure capacity and capability. Diagnosticians and technicians in other accredited laboratories with documented proficiency in specific pathogen diagnostic protocols are identified for potential surge sample receipt. Resources are directed from state and federal agencies to provide equipment, diagnostics, and reagents and to augment labor costs to ramp up sample diagnostic, analysis and interpretation capacity. Ideally, the process of planning the sampling design and numbers of samples to be collected is done in communication with the laboratory network to assure that the flow-through of samples can be timely and sustained. A sudden surge of samples can quickly overwhelm laboratory processing capacity.

Implement secure sample collection, transport and chain of custody transfer procedures. Once sampling has been initiated, standard operating procedures are implemented to ensure that individual samples are clearly labeled, securely packaged using Department of Transportation guidelines, stored at proper temperature, tracked and transported for analysis.

Implement trace forward/trace back protocols per USDA APHIS Guidelines. The goal of trace forward/trace back investigations is to determine the extent to which the pathogen has moved from the site of introduction and/or to trace pathways and modes of movement of the pathogen back to the origin source. Detailed protocols, drawn from sales and transportation records, have been developed for high consequence pathogens of ornamental plants originating from offshore (*Ralstonia solanacearum* Race 2 biovar 3) and domestic production (*Phytophthora ramorum*) facilities. Ornamental plants may move to local or national retailers, often including large garden centers in big box stores, and usually with traceable sales records. Field production trace studies present greater challenges, as grain or seed may move into local, state and regional storage facilities before moving to mills or ports, while fruit and vegetable crops may move to multiple packing houses and then on to grocery chains.

Identify and implement containment or control measures. Once the extent of the outbreak has been determined, state and federal regulatory agencies may impose stop movement orders or shipping bans and implement state, regional, or national **quarantines.** Public outreach measures, often initiated to keep the public informed and cooperative, facilitate the success of quarantine measures. Within the quarantine zone, in some cases, destruction of crops, residues and weedy reservoirs may be needed to ensure containment of the pathogen. These may be accomplished by mechanical plowing, **rogueing** (removal) of infested trees and/or the application of chemical defoliants. Animal disease outbreaks may call for depopulation campaigns at the local, regional or national level, depending upon the anticipated risk and consequences, and the scope of the initial detection and delimiting surveys.

Assess current and future economic damage. Inspectors and investigators from county, state and USDA agencies are tasked with identifying the direct crop value losses from an outbreak and determining whether reimbursement is needed for producers and downstream production facilities. In addition, economic losses due to impacts on export markets and downstream value-added agricultural products and by-products, (*e.g.* animal feed, processed food, food and feed additives) are identified and quantitated.

c. Objectives of Law Enforcement Personnel

Prevention of criminal and terrorist threats and incidents. A critical responsibility of law enforcement is to prevent and disrupt intentional targeting and attacks against the production of animal and plant commodities and natural ecosystems. Interagency efforts to enhance biosecurity and threat awareness, and to recognize and respond to possible deliberate incidents, are essential. The development of outreach, training, and exercise programs are defined in several agreements and directives between law enforcement, public health, agriculture and natural resource agencies, academia, and the private sector (FBI WMD Directorate, 2016; FEMA, 2011; FEMA, 2019b).

Security of crime scene and evidence. Once a crime scene is suspected or identified, securing the scene and potential evidence within the confines of that location are tantamount to a successful investigation and assignment of culpability for a crime. If the primary or associated scenes have not been secured, then evidence associated with that scene could be considered contaminated or suspect by prosecutors, defense attorneys and jurists, leading to the exclusion of some or all of the evidence from the investigation or trial (see also sections 11. Crime Scene Recognition; 12. Forensic Evidence; 13. Crime Scene and Evidence Preservation).

Security and integrity of evidence collection, packaging and transport. It is crucial that forensic evidence and biological samples collected at crime scenes be processed by trained, qualified professional crime scene investigators who prioritize the security and integrity of the "**chain of custody**". This term refers to secure and fully documented evidence collection, packaging and transport, from the crime scene location(s) to the sites of evidence analysis (most often one or more laboratories). Ensuring the security and integrity of the chain of custody continues within the laboratory as well as at any other site having access to the evidence (see also section 3.13. Chain of Custody).

Thorough crime scene investigation, including full documentation. All potentially relevant (**probative**) evidence should be properly collected and deposited in the appropriate, pristine container. Each piece of evidence observed and collected must be fully documented with a description of the item, and where and how it was collected. The presence of hazardous materials or evidence may necessitate the use of personnel protective equipment, biosafety procedures, and certified personnel. The physical evidence itself, as well as the information and documentation, are considered evidence. Evidence collection must be unbiased, with no assumptions about who might be culpable in any manner. It should be collected to best represent the event, supporting efforts to answer the key questions: "What, When, Why, How, Where and Who?" Forensic field investigation can help the "rule in, rule out" process as law enforcement personnel seek to identify those responsible and narrow possible sources of forensic evidence. The burdens of proof of "reasonable suspicion" and "probable cause" can be eased significantly by rigorous crime scene investigation. It is important to understand that evidence will be used by investigators and legal counsel, as well as in courts of law, if introduced and thoroughly examined as part of the trial process. Failure to follow prescribed methods and requirements could result in the exclusion of evidence before or at trial.

Proper and thorough laboratory analysis, interpretation of results and timely and effective reporting. Laboratory analysis must be conducted thoroughly and properly by qualified experts in concert with the requests of investigators and legal counsel. The goal is to fully "exploit" the evidence by using appropriate and fully validated methodologies that will assist the "customers" in answering the key investigative questions noted above. Safety of personnel and security of the evidence, documents, instrumentation and facilities are paramount. The documented results are interpreted within the limits of what the science can provide. The analyses are to be unbiased and rigorous, and their interpretations completely and clearly communicated. All steps must be conducted as defensibly as possible; if any laboratory contributions are probative for a case or trial, they will be challenged and possibly excluded. Faulty evidence analysis, interpretation or reporting could negatively impact the investigation or trial. It is not the responsibility of forensic personnel to "solve the case" but rather contribute as much as possible to solving the case no matter the outcome.

Robust and thorough investigation to identify perpetrator(s), motives, methods and means. The many components and sources of information in a properly conducted investigation are woven together dynamically through the entire process. Ultimately, the investigators seek to provide their "customer" with the maximum amount and best types of evidence to meet the trial standard of a conviction that is "beyond a reasonable doubt." Ideally, evidentiary analyses support attribution decisions. That is, they lead, with a preferably high degree of scientific certainty, to the conclusion that the evidence originated from one source. With cases involving hazardous microbial evidence, it may not be only that evidence that contributes to "the forensic story". Forensic evidence is often only part of a total battery of evidence that is crafted into the proponent's case for presentation and argument at trial. For any evidence important to an attorney's case, those testifying (including both field and forensic investigators and analysts) should expect rigorous and deep examination of all steps, methods, requirements, results, interpretation and reporting.

Exoneration of individuals not responsible. The goal of law enforcement investigators and forensic examiners is to utilize forensic evidence and information collected at a crime scene or through other investigative methods to identify the perpetrators, techniques, chemical, biological, radiological/nuclear or explosive materials, and dissemination devices and link those evidentiary findings to the actions of a perpetrator(s). Of equal importance in the investigation is to determine the motivation, purpose, methods, and desired effects. The use of approved investigative techniques and forensic procedures serves to progressively confirm the identity of or eliminate possible suspects as well as identify the methods and materials used (FBI WMD Directorate, 2017).

As more evidence is found and examined and investigative information from suspects, witnesses, and electronic techniques is collected and analyzed, individuals or groups that are not linked to the incident can be removed from consideration.

Communication and coordination with pertinent agencies during field and forensic investigations. When suspected major crimes or terrorism events are identified, the information must be shared with other pertinent agencies (law enforcement at the local, state, and Federal levels) for best overall collaboration to bring the perpetrators to justice and protect the public. For local, state and Federal law enforcement agencies dealing with serious crime, these procedures have been in place for many decades. Over the past 15 years in several countries, such cooperation has been extended substantially to a host of agencies that either are stakeholders or can provide value to the investigation. In the United States, for example, partnerships between the Federal Bureau of Investigation (FBI), U.S. Army and Navy Medical and Medical Service Corps and the Centers for Disease Control and Prevention (CDC) have been expanded to include agencies that need or can develop knowledge further through specialized laboratories and companies contracted for such purposes. Networks of coordination and protected communications among these parties support investigations and operations to aid the decision-making process, from tactical to strategic. Since "attribution" is a major goal of the U.S. biosecurity policy, appropriate and timely coordination and information sharing is expected. See also 7. Joint Investigations.

Provision of assistance and support to prosecutors and other authorized personnel. Often, forensic personnel assist attorneys' pretrial preparation by explaining how evidence was collected, analyzed and interpreted. They also explain the limits of what the science is capable of determining. Within a network of cooperating agencies or entities, the communication and explanation of these aspects may expand considerably, resulting in improvements in the science or its applications, as well as increasing value to investigators and decision-makers at all jurisdictional levels. The **Daubert ruling**, adopted to address the reliability of expert witness testimony, emphasizes reliability of information as established by peer reviewed publications, acceptance by the professional scientific community, validation of the technique, science and limitations, and the presence of an established error rate (Daubert, 1993; Kiely, 2005).

CASE STUDY 1 – QUICK DECLINE SYNDROME AFFECTING ANCIENT OLIVE TREES IN APULIIA, ITALY

Demonstrates the importance of careful investigatory methods and the power of microbial strain discrimination via validated laboratory assays for supporting forensic investigation of a devastating plant disease.

Introduction: The Pest, Source of Origin, Life Cycle, Level of Damage it Causes

Xylella fastidiosa is a gram-negative bacterium, distinct strains of which cause severe diseases of orange, grape, almond and shade trees. The bacterium colonizes the **xylem**, inhibiting water conduction in the infected host, leading to "scorched" appearance of mature leaves. It is commonly transmitted by insects such as sharpshooters and spittlebugs and thrives in warm, dry Mediterranean climates.

In 2013, an outbreak of unknown origin and cause produced severe desiccation and wilt of mature olive trees (olive quick decline syndrome or OQDS) in the olive production areas of the Apulia Region of the Salerno peninsula in Italy. This outbreak was traced, by plant pathologists at the Institute for Sustainable Plant Production in Italy, to *X. fastidiosa*, a pathogen that had not been identified previously in the EU. Subsequent genetic characterization of the strains recovered from olive trees identified the pathogen as *X. fastidiosa* subsp. *pauca (Xfp)* (Giampetruzzi *et al.,* 2019), which was then confirmed by **Koch's postulates** to be the causal agent (Saponari *et al.,* 2019).

Following European Plant Protection Organization (EPPO) guidelines, the Regional Council of Apulia, the Italian National Plant Health Service, the European Food Safety Authority and the European Commission prescribed a quarantine zone and recommended containment and removal of infected trees, along with measures to control the spittlebug vector to attempt containment and disease eradication.

Soon, however, environmental groups and local olive producers became resistant to the implementation of these procedures. The popular media were also critical of the measures, quoting activists and growers claiming government and scientific incompetence in the identification of the causal agent and development of quarantine procedures. The media published reports of a scientific plant pathology workshop held in 2010 to train researchers on *Xylella* detection in which certain *X. fastidiosa* strains had been brought for use by workshop participants (and destroyed after the workshop was completed per EPPO regulations) (Guglielmi, 2017). This was followed by accusations that the scientists responsible for the discovery and identification of the causal agent of OQDS had purposefully released the *X. fastidiosa* causal agent, linking them to a plot to replace the region's olive trees with genetically modified (GMO) trees (Guglielmi, 2017). Italian prosecutors brought criminal charges against the scientists, interrogating them and confiscating laboratory materials, computers and notebooks.

Forensic investigation vs. global pathway analysis

Because the origin of, and responsibility for, OQDS in Italy were the subjects of a criminal case, forensic investigation would be an appropriate next step. Critical evidence would include whether the bacteria isolated from Italian olive trees were similar or identical to other characterized strains of *Xfp.* Such a determination would best be made by molecular comparison of the genomic DNA of strains from Italy with that from strains isolated in other parts of the world. However, publicly available information does not reveal whether such tests were conducted at the direction of court officials in this case, or used in the criminal investigation, because the charges against the scientists were later dropped amid allegations of prosecutorial incompetence and lack of evidence.

Despite the pre-trial dismissal of the criminal case, however, the origin of the Italian *Xfp* strains remained a critical question for plant health officials and scientists who needed this information to understand the disease epidemiology. Specifically, this included the pathway(s) by which the bacteria were moving, to avoid repeat introductions. **Multi-locus sequence typing** (Elbeaino *et al.,* 2014) indicated a very close relationship between the Italian olive *Xyella* with a Costa Rican isolate of *Xfp* (Nunney *et al.,* 2014). Previous reports from Central and South America had identified a strain of *Xfp* infecting olive in that region. It was subsequently hypothesized that the pathogen was introduced inadvertently into Italy in olive **budwood** from Costa Rica. Had they been used in court proceedings these data would have been instrumental in exonerating the scientists accused of introducing the pathogen during the 2010 Xylella Workshop.

Conclusions

Although strict containment measures were finally initiated in 2015, the result of OQDS in southern Italy has been a devastating impact on the centuries-old €3.2B olive industry. The disease spread across the region for several years while the courts were investigating the case and is now present in southern EU olive production areas in Spain and France. A recent Master's thesis focusing on the media's role in the disaster (Guglielmi, 2017) concluded that the blame for the inability of EU and Italian regulatory authorities to implement measures to contain the disease can be placed on the media, environmental anti GMO zealots, local law enforcement, and a scientifically uninformed and skeptical public.

3. INVESTIGATIVE COLLABORATION

In most nations of the world, responsibilities for protecting and responding to threats against critical infrastructures are divided among several agencies, each having designated, distinct but complementary roles. In the U.S., for example, Lead Federal Agencies (LFA) have the primary responsibility to protect and respond to threats against critical infrastructure functions. The United States National Response Framework and the National Infrastructure Protection Plan assigns them legal authorities for implementation, regulation, and enforcement (Brown et al., 2005; DHS, 2006; FEMA, 2011). Additional federal agencies are tasked to support the LFAs with specific, unique resources and emergency response capabilities under interagency Emergency Support Functions (FEMA, 2016; FEMA, 2019a; U.S. Department of Health and Human Services, 2019). Similar collaborations and interactions, necessary for effective biosecurity, are in place in other nations as well. Interagency partners in all of these countries have developed official directives, policies, guidelines, sector specific plans, memoranda of understanding, and other tools for the prevention, joint investigation, and response to potential public health and agricultural threats (Table 2). These are implemented by the development of joint threat recognition, information sharing, investigation, and response training and exercise programs by governments along with private sector and academic partners (FBI & CDC, 2018; FBI WMD Directorate, 2017).

Table 2. Joint Investigation Concepts

Concept	Component(s)
Establish interagency relationships between law enforcement, intelligence, and agricultural agencies	• Communication plans • **Call trees**
Enhance prevention techniques	• Evaluate agriculture sector biosecurity, physical security, and employee screening measures
Improve threat awareness	• Interagency agricultural threat working groups • Outreach programs with interagency partners
Develop suspicious incident reporting programs	• Triggers and **tripwire**s
Enhance surveillance and detection	• Unusual disease incidents • Suspicious or criminal activity
Develop joint response protocols	• Tripwire alert notification • **Threat credibility evaluation** (TCE) (threat assessment) • Joint information sharing • Joint investigations - interviews

Depending on the nature of the incident and the degree of threat to investigators, the public, or plants and animals, investigative partners may include law enforcement and defense officials, public, animal and plant health specialists, diagnostic labs, research entities, etc., within the U.S. For example, the FBI Laboratory Division in Quantico, VA, performs forensic examination and analysis only on evidence that is not contaminated with CBRN materials. For potentially contaminated evidence, the FBI established formal relationships with multiple laboratories in the Laboratory Response Network (LRN) system for forensic analyses (CDC, 2019). LRN laboratories have the expertise to perform analyses with approved equipment, qualified personnel, validated assays, and accepted practices.

For testing evidence and materials suspected of being contaminated with biothreat materials including Select Agents, biological toxins, and emerging pathogens, the FBI has formal agreements with the CDC, the Association of Public Health Laboratories (APHL), the USDA, and state and local public health laboratories. Evidence and samples are field screened for chemical and radiological/nuclear contamination before submission to LRN public health laboratories.

The FBI can perform forensic analysis on unknown biological materials at the National Bioforensic and Analysis Center, Fort Detrick, Maryland. The FBI also maintains Hazardous Evidence Analysis Team (HEAT) facilities at partner National Laboratories, where trained FBI analysts can perform traditional forensic techniques on evidence contaminated with chemical, radiological, or nuclear materials in a facility that protects the examiners from exposure.

For suspected or known chemical materials and contaminated evidence, samples are submitted to the HEAT facility at the U.S. Army Edgewood Chemical and Biological Center, Aberdeen Proving Ground, Maryland. Evidence and samples collected at a suspected radiological or nuclear crime scene are submitted to the HEAT facility at the Savannah River National Laboratory, Aiken, SC. For evidence contaminated with unknown, emerging, or suspected Foreign Animal Diseases (FAD), the FBI has HEAT capability at the Plum Island Animal Disease Center (PIADC), Foreign Animal Disease Diagnostic Laboratory (FADDL), Greenport, NY. Forensic analysis of explosive materials is performed at the FBI Terrorist Explosive Device Analytical Center (TEDAC), Redstone Arsenal, Huntsville, Alabama. Evidence of a high consequence plant pathogen must be evaluated by expert identifiers at the USDA Laboratory Science and Technology National Center for Applied Plant Protection in Beltsville, MD.

4. TRIGGERS: INDICATORS OF INTENTIONAL ACTS

Law enforcement and first responders use the term "**trigger**" to describe the first signs or indicators of unusual behavior, activities, threats, or incidents that are recognized as atypical or suspicious. Primary categories of triggers can be classified into two types. "Overt triggers" are non-specific threats, public declarations of a crime or intent to commit a crime, manifestos, social media releases, increased activity of protests against specific targets, or claims of responsibility for visible acts. "Covert triggers" are actions that may be recognized only by threat reporting from intelligence sources, recognition of operational planning and development activities, or evidence of an attack through the detection of high-consequence plant and animal pests or diseases. Additional key indicators are unusual or suspicious activities or behavior that indicate planning, preparation, and dissemination of threat agents, acts of espionage, or sabotage, detection of changes in expected or baseline disease patterns which can be identified by effective biosurveillance in livestock, crops, wildlife or human populations, or law enforcement and intelligence reports of the evidence of espionage, criminal, or terrorist activity) (FBI WMD Directorate, 2016; FEMA, 2019b).

Agriculture sector agencies must regularly perform systematic evaluations of atypical or suspicious disease incidents and differentiate between (1) expected background levels or within normal variations of endemic pathogens, (2) natural introductions along environmental and aerobiological pathways, animal migrations, or spillover from wildlife or wild plant species, (3) accidental or inadvertent introduction through contaminated materials or human activities, and (4) intentional introductions resulting from criminal enterprise, terrorism, or sabotage (FBI & CDC, 2018; FBI WMD Directorate, 2017, FEMA, 2019b).

Although accidental or natural disease introductions are commonly suspected, epidemiologists are challenged to equally evaluate suspicious triggers for all possible means of introduction and rule them in or out. In these situations, the inclusion of law enforcement and intelligence assets is critical to evaluate the possibility of intentional acts. These relationships and protocols should be in place before such situations arise. Early recognition and timely communication concerning threats and possible intentional incidents are key elements of Joint Investigations to facilitate a rapid assessment and response.

The development of "**tripwire** initiatives" that encourage reporting of suspicious triggers are tools to prompt law enforcement and agriculture sector partners to initiate information sharing protocols as soon as they are recognized. During the course of daily operations, agriculture agencies perform epidemiological investigations of suspected or confirmed disease incidents while law enforcement and intelligence agencies investigate and analyze reports of suspicious or criminal activities. As a result, both professions may recognize different unusual incidents that could be the initial indicators or triggers of a developing threat or attack (FEMA, 2019b). Specific triggers can be associated with the animal-plant health sector or to the law enforcement and intelligence sectors. (FBI & CDC, 2018; FBI WMD Directorate, 2017) (see also Section 11. Crime Scene Recognition (Table 4).

Animal-plant health triggers include (FEMA, 2019b)**:**

- Presumptive or confirmed laboratory diagnosis of high-consequence foreign animal diseases (FAD), emerging animal or zoonotic diseases, and exotic plant pathogens, pests, invasive species and toxins.
- Initial investigations of syndromic signs or symptoms of highly suspicious animal and plant diseases.
- Simultaneous or progressive outbreaks of an emerging and foreign animal or exotic plant diseases or pests in multiple locations with no apparent epidemiological link.
- Unusual increase in the number of sick or dying animals or plants above baseline expected normal levels.
- Large numbers of animals with similar, unexplained clinical signs or disease or mass morbidity or mortality incidents.
- Multiple unusual or unexplained diseases in the same animal, herd or crop.
- Disease with an unusual geographic or seasonal distribution or atypical signs and clinical presentation; altered response to pesticides, vaccines, antibiotics or other therapeutics.
- Similar genotypic lineage among pathogens from geographically distinct sources.
- Identification of an unusual, atypical, genetically engineered, or antiquated strain of a biological agent.
- Unexplained increase in incidence of endemic diseases or atypical clinical signs.
- Simultaneous suspected or confirmed outbreaks of the same disease in animals and humans

Law enforcement and intelligence triggers include (FEMA, 2019b)**:**

- A public declaration of a threat, crime, or the intention to commit a crime against U.S. agriculture.
- Increased overt activity or protests against a specific agricultural target or targets.
- Suspects apprehended with agriculturally significant biological agents, dissemination devices, bio-processing equipment, or equipment for preserving and transporting biological agents.
- Notifications of unusual activity at agricultural production or research sites.

- Intelligence or indications that an individual, group or organization is unlawfully in possession of any animal or plant disease agent.
- Identification or seizure of literature pertaining to the production or dissemination of animal or plant disease agent.
- Intelligence or field assessments that indicate a credible threat against agriculture is being planned, initiated or ongoing.
- Law enforcement or CBRN-E investigations and crime scene operations that discover the presence of animal or plant biological or chemical agents (e.g., clandestine laboratory detections).

5. NOTIFICATION AND INFORMATION SHARING

Rapid recognition and reporting of suspicious triggers allow the timely evaluation of potential threats and need for joint responses. Effective notification and information sharing tools include efforts to establish initial incident reporting protocols and standard operating procedures (SOPs). Also important are the designation of interagency points of contact or liaison officers for 24/7 communication, development of on-call duty rosters and regular verification of phone numbers and email addresses, and development of interagency agreements and memoranda of understanding. It is critical to build interagency threat working groups, such as FDA Rapid Response Teams or agriculture sector working groups. Such groups should have representation from FBI, local, state, and federal law enforcement, emergency management, public health, USDA, FDA, State Departments of Agriculture (SDA), the private sector and academia. They also should implement regular situation reports (SITREP) for Incident Command staff (e.g., weekly phone or web-based updates) and develop threat reporting checklists for law enforcement, public health, and agriculture agency on-call staff to collect the most critical, pertinent information from first responders, the public, and interagency partners (FBI WMD Directorate, 2016; FEMA, 2019b).

With effective notification and information sharing, law enforcement and intelligence agencies can compile and correlate existing intelligence from different partners. Agriculture agency emergency management and law enforcement personnel can coordinate to identify and preserve crime scenes and collect forensic evidence, and to prevent or disrupt on-going operations. Similarly, animal and plant health agencies and the agriculture sector will be able to strengthen biosecurity, physical security, and cybersecurity of agriculture facilities, evaluate and enhance biosurveillance and response capabilities, and notify staff and field personnel of possible safety and security threats (FEMA, 2019b).

Although initial reports and information may be incomplete, interagency partners should not hesitate to report the initial incident or intelligence triggers before definitive explanations or diagnoses are available, preferably within hours. Early notifications allow intelligence and subject matter experts to begin threat assessments and query sources for linkages to similar reports or threats actors, and possibly identify previously unrecognized incidents before severe effects can develop.

After the initial notifications, sustained information sharing may uncover previously unreported information, identify new contacts and sources, and additional resource and asset requirements needed for joint operations.

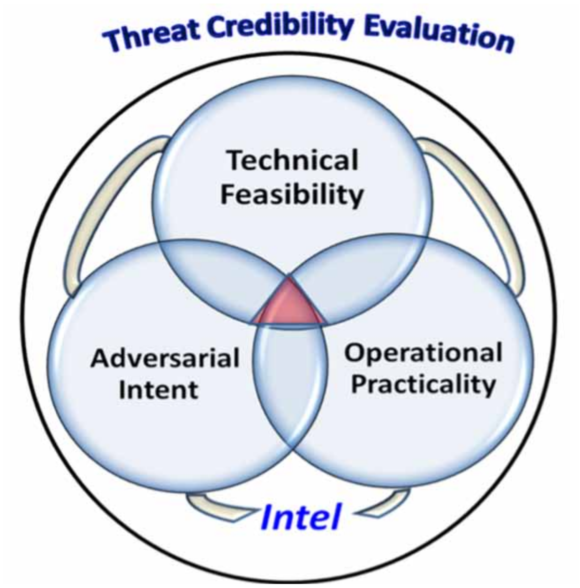
6. THREAT CREDIBILITY EVALUATIONS (TCE)

FBI WMD Coordinators are the key contacts for the development of interagency working groups and joint investigations. Coordinators are required to initiate an FBI-managed "**threat credibility evaluation**" (TCE) conference calls as a quick and efficient tool to assess and determine if a suspicious biological threat or incident is credible and what further law enforcement and intelligence actions are required. TCEs provide critical information necessary for immediate and further joint operations, including a summary of the reported incident and introduction of key participants. They also include an initial SITREP in which on-scene responders and headquarters personnel report the status of disease investigation and response operations, laboratory results and law enforcement investigations, and what has been done so far (Figure 1).

TCEs also begin attribution for suspected sources and methods of introduction (accidental, naturally occurring, or intentional), potential threat actors, motivations, goals, and desired effects. They share unclassified intelligence assessments, identify and request additional information and support assets, develop a joint investigation plan and goals, assign tasks and set up a schedule for follow-on calls and updates.

The information provided by the on-scene responders and the subject matter experts from the responsible public health and agriculture agencies is a critical component of the TCE process. Per protocol, three critical considerations are evaluated in light of available intelligence information to build a valid risk assessment (Figure 1): (1) **technical feasibility** – does a threat actor have or can acquire the expertise, technical knowledge, and access to WMD materials necessary to plan and implement some type of intentional operation; (2) **operational practicality** – if the technical feasibility is credible, is the plan operationally sound and can it actually be successful; and (3) **behavioral resolve or adversarial intent** – does the threat actor have the commitment and willingness to follow through with threats or planned actions (FBI & CDC, 2018; FBI WMD Directorate, 2016; FBI WMD Directorate, 2017; FEMA, 2019b).

Figure 1. FBI WMD Threat Credibility Evaluation Process



7. JOINT INVESTIGATIONS

If the threat assessment process indicates a suspected or likely intentional incident has occurred, joint investigations between law enforcement and animal-plant-public health agencies can be initiated. These processes are more effective if teams have prior training and a common understanding of the differences in authorities, policies and protocols (FBI & CDC, 2018; FBI WMD Directorate, 2016;-FBI WMD Directorate, 2017; FEMA, 2019b):

Criminal and epidemiological investigation protocols. Law enforcement personnel follow strict crime scene investigation, evidence handling-collection, and criminal investigation procedures; and agriculture or public health investigators follow official disease investigation protocols to collect clinical or environmental samples and diagnose diseases or chemical intoxications (FBI & CDC, 2018; FBI WMD Directorate, 2016; FBI WMD Directorate, 2017; FEMA, 2019b).

Clinical or forensic laboratory support. Animal, plant, or public health diagnostic laboratories perform microbiological, toxicological and genetic analyses, **necropsy**, clinical and gross pathology examinations, and other clinical diagnostic tests to identify disease pathogens and clinical disease syndromes. The FBI Laboratory Division and partner state and federal forensic laboratories perform traditional and genomic forensic analysis of evidence collected at crime scenes or other locations for linkages to possible criminal acts or terrorism. Test results and documentation of suspected WMD threats agents from a public health or animal-plant disease diagnostic laboratory may confirm that a crime has been committed and will be considered evidence in court cases (FBI & CDC, 2018; FEMA, 2019b).

Joint interviews of witnesses, victims, employees. USDA, CDC, SDAs and public health epidemiologists perform interviews to gather information needed to diagnose disease outbreaks and determine the origin and means of introduction. FBI and law enforcement officers perform interviews of the same individuals interviewed during the disease investigations to gather evidence on motivation, or intelligence of threats or suspected criminal or terrorist acts. Interviews of an individual may be conducted jointly with health and law enforcement officers present to gather the respective information (FBI & CDC, 2018).

Disease control and crime scene operations. SDAs, USDA, and/or in some cases public health agencies will establish quarantines, movement restrictions, and **control zones** (**CZ**) to prevent the further spread of diseases and allow effective disease control operations. The FBI and law enforcement will establish official crime scenes to limit access and protect critical evidence from damage or contamination by unauthorized personnel, vehicles, animals, perpetrators, and environmental threats. Crime scenes and any evidence within the restricted zone may also be contaminated with CBRN-E materials and pose a threat to investigators, witnesses, victims, and forensic laboratory personnel. Limiting access and environmental controls can reduce the risk of exposure and spread of contamination outside the crime scene and, in the case of animal or plant disease incident investigations, a potential crime scene may be located within a quarantine or biosecurity control zone. Criminal investigators, equipment, and materials will be required to follow USDA or SDA disease control and decontamination requirements for movements into and outside the biosecurity CZ (FBI & CDC, 2018; FEMA, 2019b)).

8. FBI AND LAW ENFORCEMENT CRIMINAL INVESTIGATIVE PROCEDURES

The FBI has multiple roles and responsibilities and is required to operate within the laws that govern investigations and further prosecution. The FBI follows specific investigatory guidelines and protocols for criminal, counterterrorism, and counterintelligence investigations and hazardous evidence (CBRN-E) crime scene investigations. Currently, they do not have the authority or responsibility for animal-plant disease epidemiological investigations and control operations; routine site security, traffic, and movement control operations; or remediation of contaminated crime scenes or premises. Operating in these conditions requires early and sustained coordination with all the local, state, and other federal agencies involved.

FBI and law enforcement investigations specifically require teams to perform intelligence risk and threat assessments, and to interview leads, witnesses, and victims. They also must differentiate credible from non-credible threats (hoaxes), recognize overt from covert threats and perform risk assessments at crime scenes to identify and mitigate hazards and threats to the safety of investigators. Later, these responders collect and process forensic evidence, provide testimony in court and assist criminal investigations of law enforcement partners.

Initial information may be insufficient to determine if an incident is a criminal, sabotage, or terrorism incident so FBI, USDA, and SDA investigators will be required to perform risk assessments and joint investigations based on three risk categories:

- **No Risk**

Assessment: Initial epidemiological investigations identify credible explanations for suspicious disease outbreaks. **Investigation:** APHIS and state agricultural investigators continue epidemiological investigations and manage disease control operations. Law enforcement investigations are not necessary unless further information suggests intentional acts.

- **Possible Risk**

Assessment: Information suggests the incident may be intentional. No credible explanation for the occurrence of the disease has been identified. **Investigation:** FBI intelligence analysts search databases and sources for relevant intelligence reporting. USDA APHIS continues epidemiological investigations to determine the source and means of introduction. FBI, APHIS and the Office of the Inspector General (OIG) coordinate joint information sharing and threat modeling.

- **Likely Risk**

Assessment: Reasonable belief the disease incident was intentional. Presumptive or confirmed laboratory diagnosis of high consequence animal and plant diseases. No credible explanation for the occurrence of the diseases has been identified. FBI intelligence suggests a nexus to a threat actor (terrorism, criminal, adversary country). **Investigation:** FBI and USDA Office of the Inspector General initiate a joint criminal, counterterrorism, counterintelligence investigation. An FBI Joint Operations Center is established as needed. Ongoing intelligence collection and assessment operations continue. FBI and USDA establish Unified Command operations as needed (FBI & CDC, 2018; FEMA, 2019b).

9. CRIME SCENE INVESTIGATIONS

To assure that investigators follow uniform procedures to ensure that any discoverable information and evidence that is collected, processed, and analyzed is suitable for submission for prosecution in a **court of law**, FBI crime scene investigations follow official operational guidelines and the following protocol:

FBI Twelve Step Crime Scene Investigation Process

- Preparation
- Approach scene
- Secure and protect scene
- Initiate preliminary survey
- Evaluate physical evidence possibilities
- Prepare narrative description
- Depict scene photographically
- Prepare diagram/sketch scene
- Conduct detailed search and collect physical evidence
- Record evidence
- Conduct final survey
- Release scene and transport evidence to a designated laboratory

The potential use of WMD agents to attack agricultural targets increases the risk that crime scene(s) are contaminated with hazardous materials and are a risk to the health and safety of investigators as well as forensic examiners. The FBI performs risk-based assessments using current intelligence and preliminary site surveys to screen for CBRN-E and plan appropriate investigation protocols (Table 3).

Table 3. Types of Crime Scene Investigation Protocols

Type of crime scene	Type of threat	Goal
Standard	No CBRN	*Collect and protect forensic evidence from inadvertent contamination/compromise
CBRN-E or hazardous materials	CBRN-E or hazardous materials	*Utilize FBI 'hazardous evidence' procedures to prevent exposure and protect investigators *Crime scene sites and forensic evidence may be contaminated with dangerous CBRN-E materials *Suspicion or evidence of explosives or precursors requires clearance by Bomb Tech personnel
Agricultural	Biological CBRN threat	*Prevent the spread of disease agents outside of the crime scene and protect investigators from biological threats and zoonotic diseases

10. AGRICULTURAL CRIME SCENE INVESTIGATIONS

Regardless of the target, threat actor, or motivations; the intentional use of WMD agents, espionage, terrorist, or criminal operations against agricultural targets will likely create recognizable crime scenes.

Furthermore, the technical and operational requirements for development, planning, preparation and implementation of such actions will likely generate triggers or indicators of suspicious activities and valuable forensic evidence. **Operational requirements** for intentional actions include (1) the **perpetrator** (having sufficient expertise, capability and motivation), (2) **access and/or ability** to acquire CBRN-E threat agents, (3) **agricultural target** (livestock, crops, public lands, biotechnology assets, laboratories, markets, processing, transportation, state-federal personnel and facilities), (4) **operations** (planning, development, buildings and facilities, logistics, personnel), (5) **access to the site**, and (6) **means of dissemination.**

Due to the harsh environment, on-going farming operations, crime scenes and forensic evidence maybe minimal and fragile, and may go unnoticed or be found commonly on farming operations. Crime scene sites may be small areas within a larger restricted biosecurity or quarantine zones managed by the industry, USDA or state departments of agriculture. Investigators may need to decontaminate equipment and personnel to prevent animal or plant pathogen transmission outside the control zone. Sites located in remote areas with rough terrain and poor roads will have limited access, while continuity of operations for livestock or crop production can interfere with the ability to protect the crime scene and forensic evidence from damage or contamination. Quickly isolating and restricting access to the possible crime scene area will require coordination and support from the facility staff and workers. Agricultural facilities are often hazardous work sites due to dangerous equipment, livestock, poor maintenance protocols, exposure to biosafety risks and environmental contamination from manure storage facilities, farm chemicals and electrical hazards, etc. Crime scene investigators are encouraged to follow guidance from facility personnel and state or federal agriculture specialists to ensure safe operations.

The likely use of biological agents against agricultural targets has been used for the development of operational response planning guidelines and exercise planning (e.g.: the FEMA Food and Agriculture Incident Annex [FEMA, 2019b] and Foreign Animal Disease response planning tools and exercises [USDA, 2021]).

11. CRIME SCENE RECOGNITION

The recognition of a crime scene depends on situational awareness of personnel arriving first, who may identify some type of irregularity or unusual activity, damage, or physical materials resulting from nefarious actions. In an agricultural environment, employees are often the individuals most likely to differentiate normal from abnormal. The veterinarian or plant disease specialist responding to a report of an unusual animal or plant disease incident may be the first to recognize the possibility that an intentional threat or incident has occurred.

Factors that affect the location and appearance of a crime scene depend on actions taken at the scene, the type of facility and physical layout, the type of target (e.g., livestock, crops, or employees), or the type of CBRN-E materials used. Awareness of threats or suspicious activity reports, intelligence of threat group or individual activities, and types of targets will help investigators know where to look for a possible crime scene. For example, CBRN-E materials could be disseminated or spilled in animal concentration or holding areas, into seed and water supplies, or in agricultural fields or other crop growing areas. Other indications include signs of human or vehicle activity such as tire tracks, evidence of break-ins, broken locks, cut wire, theft or vandalism, and suspicious vehicle activity.

Sources of threat agents and locations where CBRN-E materials can be purchased, stolen, stored, tested, or manipulated could show signs of criminal activity and provide a source of forensic evidence. Examples include clandestine laboratories, storage facilities, home and farm supply stores, research and diagnostic laboratories, as well as commercial or restricted sources of chemical and biological materials. Valuable evidence could be found at locations where materials or equipment have been discarded or left behind by perpetrators during or after their deliberate operations (see also Section 4. Triggers: Indicators of Intentional Acts.)

There has been at least one effort to create a tool by which early responders (farmers, Cooperative Extension personnel, local law enforcement personnel, or others) who might be unfamiliar with critical elements of a potential crime in an agricultural setting, could judge whether a disease outbreak was naturally or intentionally incited, and whether forensic investigation is needed (Rogers, 2011; Rogers et al., 2009). Recognition of anomalies - unusual or unexpected features - of an outbreak or incident that cause the observer to consider whether it might have been intentionally caused, may be the triggers that lead to a divergence in the nature, sequence or timing of normal response steps. The earlier that notification of official responders occurs, the more likely it is that the incident can be successfully attributed. To assist early-responding investigators in assessment of whether a crop disease outbreak might have been caused by an intentional pathogen introduction, justifying further investigation, a decision tool, the Crop Bioagent Introduction Intent Assessment Tool (CBIIAT) was developed and experimentally validated in the field (Rogers, 2011; Rogers et al., 2009). Major trigger criteria (Table 4) related to disease symptoms, timing, possible vector involvement, weather, development and previous history of the area, as well as social and political factors and traditional forensic evidence collection, were assigned

Table 4. Major Anomalies/Trigger Factors in the Crop Bioagent Introduction Intent Assessment Tool

Evidence type	Description
Geographical distribution	Has this disease been seen (commonly or occasionally) in this area before?
Pathogen	Does this pathogen normally occur in this area? Is it presenting in the seed lot from which this crop was planted?
Vector (if relevant)	Does the vector insect occur naturally in this area?
Spatial distribution	Is the infection pattern in the field or orchard typical of this disease? Does the pattern suggest epidemiological evidence related to the pathogen source or in-field dissemination?
Weather	Were weather conditions in the area favorable for pathogen survival (and vector survival, if vector-transmitted) at the time of infection?
Temporal	Did this disease outbreak occur at a time of year that is typical for this disease? Is the symptom severity typical for this time of year?
Field history	Has this disease been found in this field before? Was the field tilled before planting?
Surrounding areas	Is the disease (or other anomalous symptoms) present in nearby fields, in **volunteer plants**, or in alternate host plant species?
Crop history	What crops were planted in this field in past years? If different from this year's crop, were the previous crop species susceptible to this disease?
Human activity	Can all evidence of human activity in the field or on the farm be accounted for by the farm staff?
Physical evidence	Are there unexplained footprints, vehicle tracks, discarded equipment (sprayers, gloves, masks, containers, etc) or trash in the affected area?
Motive	Do interviews reveal any possible motive for an intentional pathogen introduction (economic competition, revenge, making a political statement, etc)?

weighting values based on the degree to which that factor would influence the decision. The weighted factors were incorporated into a step-wise decision-support worksheet into which the investigator would enter an assessment value for each, multiply by the appropriate weighting factor, and then calculate a final score that could be compared to score groupings indicating probable intent (highly likely, likely, doubtful, or unlikely).

CASE STUDY 2 – SALMON BLOTCH OF ONION IN ISRAEL

Illustrates the use of the Crop Bioagent Introduction Intent Assessment Tool (CBIIAT), which has been useful in numerous agricultural biosecurity training exercises for agricultural and law enforcement personnel.

The Challenge

An unexpected outbreak of salmon blotch of onion, caused by the fungus *Fusarium proliferatum (Fp),* occurred in southern Israel in the mid-2000s. Although there was no indication that the occurrence of the disease was not natural, the incident served as an opportunity for researchers who had developed and laboratory-tested a variety of microbial forensic tools, to assess and validate the use of these tools in a real-world plant disease setting (Fletcher et al. 2017; Moncrief et al., 2013; Moncrief et al., 2014; Moncrief et al., 2016).

Tactical Elements

Introduction: The Pest, Source of Origin, Life Cycle, Level of Damage Caused

The *Fp* - onion **pathosystem** offers an excellent case-study of an epidemic chain involving plant, animal and human health (Fletcher et al. 2017). The fungus, an opportunistic pathogen of humans that produces an array of mycotoxins (e.g., fumonisin, fusaproliferin and moniliformin) harmful to humans and animals, is a food and feed safety concern. *Fp* is also a plant pathogen and **endophyte** with a very wide host range across many plant families. Distributed globally, *Fp* was first reported in southern Israel in 2005-6 in infected onion bulbs, where its salmon-pink colored spore masses on the onion surface gave rise to the disease name (Figure 2). The pathogen has since been isolated from corn, garlic, cucumber, tomato, watermelon, and pumpkin throughout Israel. *Fp* invades both **sets** (young bulbs, harvested while very small to be planted later in other locations for the production of mature onions) and inflorescences. It may be disseminated in onion seeds at rates of 0.1-10%. The pathogen has also been isolated from many volunteer plants in and adjacent to cultivated fields. Effective management of *Fp* requires an integrated approach, including the production of pathogen-free seeds and sets, supplemental treatments during crop production, and a validated system of traceback.

Figure 2. A. Salmon colored blotches (spores) on cultivated white onion in southern Israel. B. Vegetative growth of F. proliferatum on potato dextrose agar

Forensic Approaches and Technologies Used

Goals of the work were to determine the source of the *Fp* isolate(s) causing salmon blotch of onion in southern Israel, and whether previously developed forensic tools could help to judge whether the outbreak could reasonably have been due to an intentional, nefarious introduction of the pathogen. The investigation incorporated a variety of forensic approaches, and the results were assessed using a version of the CBIIAT (Rogers, 2011; Rogers et al., 2009) in which the criteria and circumstances had been adapted to this scenario.

- **Hypothesis.** A true forensic investigation would not normally include the formulation of a hypothesis. However, since this was a scientific exercise we hypothesized that the *Fp* strains causing salmon blotch in southern Israel originated from the **onion set** farms in northern Israel from which the sets were obtained for commercial planting.
- **Farmer interviews: Field and crop history, sociological and political factors.** Farmers in both northern and southern Israel were interviewed to determine cropping history including crop rotations, onion cultivars grown, agronomic practices used (soil amendments, solarization, irrigation, planting dates, weather data, etc.). Farmers also provided information on farm biosecurity practices and barriers, and personnel (permanent and temporary employees) and neighbors or competitors. There was no evidence of unusual activity, conflicts or disputes, political tensions in the region, or other possible motives for intentional pathogen introduction.
- **Evidence collection.** Investigators followed stringent protocols in the field, during transit, in storage and in the laboratory, to prevent evidence contamination (gloves, sterile tools, boot-cleaning, etc), preserve the chain of custody (record-keeping on sample collection, storage locations, handlers, etc), and to maintain the integrity and usefulness of the samples (storage conditions).
- **Physical evidence.** Onion fields and surrounding areas (an adjacent date palm plantation, a nearby roadside, irrigation sources, weedy field borders, and farm offices, barns and storage areas were examined for evidence of unauthorized entry, unexpected equipment or supplies (spraying apparatus, discarded gloves or masks, etc), or other unusual features.

- **Epidemiology.** Disease delimiting surveys were performed in northern Israel, where onion sets are grown, as well as in southern Israel where the majority of commercial onion production takes place. Onion seeds imported for planting to produce sets, onion sets purchased by southern Israeli onion farmers, and a variety of potential alternate *Fp* hosts (weeds, native plants, etc) and environmental niches in and around the onion fields were also tested.
- **Pathogen genetics and population biology.** Due to *Fp*'s significant diversity, effective strain discrimination required the development of a typing tool based on strain-specific genetic elements that could reveal pathogen relationships, evolutionary history and geographical distributions. *Fp* isolates from multiple countries were characterized using **simple sequence repeat (SSR)** primers (Moncrief et al., 2013; Moncrief et al., 2014; Moncrief et al., 2016). Significant isolate variability within a single country and even a single field allowed the placement of fungal isolates into clear groupings, clarifying the spatial and temporal distribution of *Fp* in Israel.

Forensic Assessment - Results

The SSR profiles of all *Fp* isolates from commercial production farms in southern Israel were highly similar to one another but significantly different from those of isolates from the set production farms in the north, suggesting that the northern-grown onion sets were not the source of the salmon blotch *Fp* found in southern production fields (Moncrief et al., 2013; Moncrief et al., 2014). Close similarity between SSR profiles from mature onion bulbs and those isolated from soils in the surrounding area, together with the knowledge that *Fp* survives in soil for several years, were consistent with a hypothesis that field soil was the likely source of the outbreak fungus. Use of the CBIIAT decision tool, which evaluated historical, epidemiologic, sociological and physical evidence as well as the genetic data, indicated that the likelihood that the outbreak was the result of an intentional introduction was very low. Although the decision tool assessment suggested that this disease outbreak was natural, the tool should be tested also on other onion fields that are naturally infected with *Fp* (Moncrief et al., 2013; Moncrief 2014) as well as on an onion field that was intentionally inoculated with the fungus for comparison.

Conclusion

The use of the CBIIAT decision tool to evaluate the case of salmon blotch of onion in Israel proved useful and could be adapted to other plant-pathogen models. Use of the tool could be further validated by having other subject matter experts, Extension personnel, local growers and law enforcement agents use the tool during training exercises, as was done in a field study reported by Rogers (2011).

12. FORENSIC EVIDENCE

The definition of forensic evidence is *"any physical thing that can be used in a court of law to convict a person of a crime"*. The concept for the use of evidence in criminal investigations is based on **Locard's Exchange Principle** – "*When a subject of a crime comes into contact with the scene, they will bring something into the scene and leave with something from the scene; every contact will leave a trace"* (Mistek et al., 2018).

The criteria for basic evidence handling protocols are to identify, collect, preserve, document, and submit physical evidence for forensic examination (Table 5), including documents, photographs, witness statements, and laboratory test results from epidemiology investigations by animal-plant health agencies. These materials are admissible in court and require approved Chain of Custody procedures. Of the different types of evidence used during criminal investigations, **direct** and **trace** evidence are the categories most critical to confirming the presence of CBRN-E agents and identify the links between the perpetrator, WMD materials, and the tactics, techniques, and procedures used.

Table 5. Types of Evidence

Evidence type	Description
Direct	Documents, records, physical evidence, notes, computer data, videos, other case-related information
Circumstantial	Facts, if proven, allow the investigator to draw conclusions; often has the same value as direct evidence
Trace	Microscopic particles detected chemically or physically, fingerprints, DNA, hair, fibers, tool/dye marks, bio-agent residue
Hearsay	Statements from unavailable source or third party
Eyewitness testimony	Observations by on-scene persons

In joint criminal and epidemiological investigations, law enforcement and animal, plant, and public health agencies are tasked to explain suspicious disease incidents and determine the origin, method of introduction, and whether or not there is a nexus to criminal, terrorist, or espionage operations. Law enforcement evidence links the criminal or terrorist act to a perpetrator and helps determine their motivations, goals, and how to disrupt their operational plans. Epidemiological teams collect diagnostic samples from affected animals, plants, or the environment to identify a disease, its characteristics, means of introduction and transmission, and develop response operations.

Law enforcement and epidemiological investigators use agency specific evidence or diagnostic sample collection protocols (Table 6). Understanding what is evidentiary for both teams can help identify and protect evidence early in an investigation and prevent inadvertent damage or loss of materials.

Table 6. What is Evidence in Law Enforcement Investigations?

Forensic Evidence	Diagnostic Evidence
• Dissemination equipment • Tools, weapons, vehicles, • Discarded PPE, microbiological production material and equipment • Maps, documents, intel reports • Personal effects of perpetrator • Foot/vehicle tracks • Latent prints, DNA, hairs/fibers, tool & die marks, handwriting analysis, cyber/digital/electronic media • Epidemiological investigation reports and laboratory findings • Microbial/genomic forensics	• Clinical history • Farmer interview and notes • Epidemiological investigation findings and laboratory submission forms • Diagnostic samples (lesions-necropsy samples, blood, serum, feces, other tissues and animal/plant materials) • Environmental samples (soil, water, feed, bedding, etc) • Official laboratory results and clinical diagnosis • Microbial/genomic forensics

Regardless of what type of material or information is collected, all categories of evidence require strict adherence to chain of custody protocols to prevent loss, physical damage, contamination, loss of identity, and to establish credibility for use in disease and criminal investigations.

13. CRIME SCENE AND EVIDENCE PRESERVATION

In an agricultural crime scene location, routine operations and environmental conditions can quickly destroy critical evidence. Reports of threats, suspicious activities, break-ins, thefts, vandalism, and suspicious human activities from the initial interviews by animal-plant agency investigators may be first indication that a crime scene exists and that evidence may be collected. The key is to recognize normal from abnormal and report suspicious incidents as quickly as possible.

Guidelines to protect crime scenes and evidence. A consistent rule of thumb is that evidence should be left in place and handled as little as possible; improper contact and handling can destroy trace evidence:

- To avoid damage, move about carefully and don't step on items; when possible, it is preferable to avoid entering a crime scene.
- If evidence or suspicious items are at risk of being destroyed; move or protect the items from further damage.
- Document photographs and descriptions of any suspected evidence.
- Prevent intentional destruction or pilferage of a potential crime scene or suspected evidence.
- Gain control of the suspected crime scene as soon as possible by limiting access by people, vehicles, farm equipment, or livestock and wild animals.
- Use rope, portable fencing, parked vehicles, crime scene tape, or post signage to delimit the crime scene.
- Inform employees and staff to prevent unauthorized access.
- Maintain awareness of and track movement into and out of a crime scene location.
- If evidence must be handled or moved, wear latex or nitrile exam gloves to prevent cross-contamination and touch the items in areas that will lessen the chance of damaging latent fingerprints, DNA, or other trace evidence.

CASE STUDY 3 – WHO KILLED THE COWS?

Shows the importance of crime scene protection and management.

Background

Early on a Sunday morning in the spring of 2008 a regulatory veterinarian (RV) received a call from the State Animal Health Official (SAHO), who reported about 35 dead or dying cows in a rural county in their District, and said that law enforcement was already on the scene. He added that an **agrocrime** or agroterrorism couldn't be ruled out. Twelve hours had elapsed from the time cows began dying until an animal health official had been notified. The producer did not have a regular veterinarian, so the local County Extension Agent had requested one to check the cows, several of which had very recently calved (with others due to calve in the coming weeks). Early that morning, one of the deputies had ordered the producer to separate the newborn calves from their mothers. When the RV arrived at the farm, sheriffs and deputies from surrounding counties were already on the scene (Figure 3). Several had relayed the story of the dead cows to friends and neighbors, and a number of onlookers had arrived, parking at the side of the road and attracting more onlookers. Several people, including the producer and his family, had entered the cow pasture, but no one was wearing personal protective equipment (PPE). Two of the family dogs were roaming freely in and out of the pasture. The local veterinarian was already performing a necropsy on one of the cows. The thoracic and abdominal cavities were opened as tissue samples were being collected and bagged by an assistant. Both were wearing gloves, but no other PPE. No one person seemed to be in control of the situation.

History

There had been thundershowers in the area the night the cows began dying. The producer and his spouse had gone out of town, but their adult son reported that he had arrived at dusk to feed the cows. The son took a bag of cattle feed from his vehicle, emptied it into the trough and counted the cows. All were accounted for, and he noted that many were about to calve. After the cows had eaten the initial feed, he fed them from another bag that he believed to be feed. Within 30 minutes, they began to bellow loudly, as if in pain. Many laid down and began to have violent seizures, and many died.

Figure 3. Dead cows near a feed trough in the pasture. Note roadside cars, a truck and several people, not wearing personal protective equipment, near the cows

Response

The RV asked a deputy sheriff stationed at the driveway entrance to tell all non-essential personnel to leave, but very few left. A cleaning and disinfection line was set up by the RV (Figure 4) and a regulatory animal health technician who had arrived. Regulatory personnel sprayed down tires and undercarriages of trucks that left the pasture. Gloves and boot covers were provided to those who remained. The owner was concerned about the calves penned in a separate pasture, most of which were not yet grazing and had not eaten since the night before. Several cows were still alive but were inaccessible to the calves.

Figure 4. "Clean-dirty" station where visitors can decontaminate themselves and their vehicles with provided sanitizing solutions, brushes and towels before leaving the pasture

Investigation

The RV interviewed the producer and his family members, learning that all of the cattle were purebred Angus. There had been no recent herd additions other than the newborn calves. All the cattle had previously undergone deworming and vaccinations per industry and veterinary recommendations. There had been no previous history of unexplained bovine deaths or illnesses. The cows typically grazed Bermudagrass pastures supplemented with feed purchased locally. The new calves were all still nursing. Water was obtained from the producer's deep well and a creek running through the property. No noxious weeds were found in the pasture or along the fence lines. The owner reported that none of the cows had been febrile, dyspneic (difficulty breathing), hemorrhaging, off feed or had diarrhea. None had any signs of hemorrhage from mucus membranes, which can be a symptom of anthrax. Feces in the pasture appeared normal, except near the dead cows where there was evidence of recent diarrhea. All of the cows were still near the feed trough when they went down. Some were having seizures and the son felt it was unsafe to approach them; however, he had immediately contacted his parents, a neighbor, and the local County Extension Agent. The parents returned late in the evening, but it was not until the next morning when the County Agent called a local veterinarian and the SAHO.

Tainted feed was considered a possible cause of death, but there was confusion between the veterinarian and law enforcement about the chain of custody over the bags from which the son had fed the cows and how they should be handled in case they became evidence in a crime investigation. Local law enforcement ultimately left it up to the Department of Agriculture, and the State Veterinarian directed the State Inspector deliver the feed sack to the State Seed Lab to test for contaminants.

Diagnostics and Results

The RV noted that many of the dead cows were bloated (post-mortem bloating is expected) and had diarrhea, as well as foam coming from their nostrils and mouths. Since the potential causes could have been deadly to the RV and other humans and animals, a whole carcass was transported to the local Veterinary Diagnostic Lab. The pathologist immediately aspirated ocular fluid, which can be tested for a number of toxins. The necropsy was performed, with pathologists wearing appropriate PPE. The cause of death was declared to be acute carbamate toxicity, likely from contaminated feed. Carbamates are cholinesterase inhibitors, neurotoxins that cause the accumulation of acetylcholine in neuron synapses leading to nausea, vomiting, diarrhea, sweating, dizziness, ataxia, respiratory failure, seizures and death, if left untreated. Aldicarb (Temik®), a nematicide used on crops such as cotton, peanuts and potatoes, is also toxic to mammals and fish. It is easily absorbed through the skin, gastrointestinal tract, and any other route of exposure, and ingestion of a small amount can be fatal.

Law Enforcement Investigation

The RV was interviewed 48 hours later by the State Bureau of Investigation Agent. USDA APHIS and SAHO were kept in the loop. Because the State Veterinarian suspected this could have been an agrocrime or terror event, the State Department of Agriculture consulted with the State Bureau of Investigation. It was determined that the farmer's son had mistakenly fed Temik® to the cows during the second feeding. Neither the cattle owners nor their son recalled having ever kept Temik® on their farm, or how the pesticide could have ended up in the feed bag. The State Bureau of Investigation also interviewed the owner and personnel of the feed mill where the owner purchased the feed but found no evidence of wrongdoing and no charges were filed.

Lessons Learned

Neither the RV nor local and state law enforcement had had any previous experience working together in a possible crime scene. During this incident, neither instituted the Incident Command System (ICS) to lead the incident. Instead, each led his own field of technical expertise but did not coordinate the response. Neither conducted a threat assessment. The veterinarian and animal health technician did establish basic biosecurity for those in and leaving the pasture, but the vet did not seek contact information for those who may have been at risk of exposure of a toxin or pathogen, nor was there a communication plan with law enforcement. After this event, and after having participated in the FBI APHIS Criminal-Epidemiological Investigations course, the state and federal veterinarians now understand that each state has a FBI WMD Coordinator who could assist in such investigations, the importance of having contacts or relationships with law enforcement before an event occurs, and that joint interviews and chain of custody management should be coordinated at the onset of an investigation.

14. CHAIN OF CUSTODY

Chain of custody is the *documentation of collection, transfer, receipt, custody, and final disposition of evidence*; simply put, investigators are required to maintain an unbroken written record that tracks the status and control of any evidentiary materials collected from a crime scene or during an investigation. Proper chain of custody procedure requires establishing and maintaining an **evidence logbook** and a **secure storage plan.** An **evidence custodian** must be assigned to account for the whereabouts and security of materials at all times until handing over custody to next recipient. Chain of custody documents are discoverable in court and any break in the continuous verification of the location and status of evidence can invalidate the evidence for prosecution purposes (FBI & CDC, 2018). For FBI investigations, the official chain of custody begins at the time of collection or upon receipt from another individual or other agency and until it is officially transferred to an approved forensic laboratory, evidence storage facility, or partner organization. During court proceedings, the FBI cannot vouch for the procedures used by another agency prior to the transfer of chain of custody to the FBI.

Animal, plant, and public health disease investigations are required to follow agency approved guidelines for diagnostic sample collection and chain of custody management from the point of collection, through the transportation to and receipt by an approved diagnostic laboratory. If the FBI or another law enforcement agency were to request diagnostic or laboratory samples as evidence in a criminal investigation, the approved chain of custody procedures normally used during official disease investigations would be acceptable. Chain of custody for biological samples requires prior communication between the shipper and the receiver. The sample must be bio-secured, a container within another container, for shipping. A federally recognized carrier must deliver the sample into the hands of the laboratory official, who will open the package in a biosafety cabinet.

Figure 5. Example of evidence collection packaging

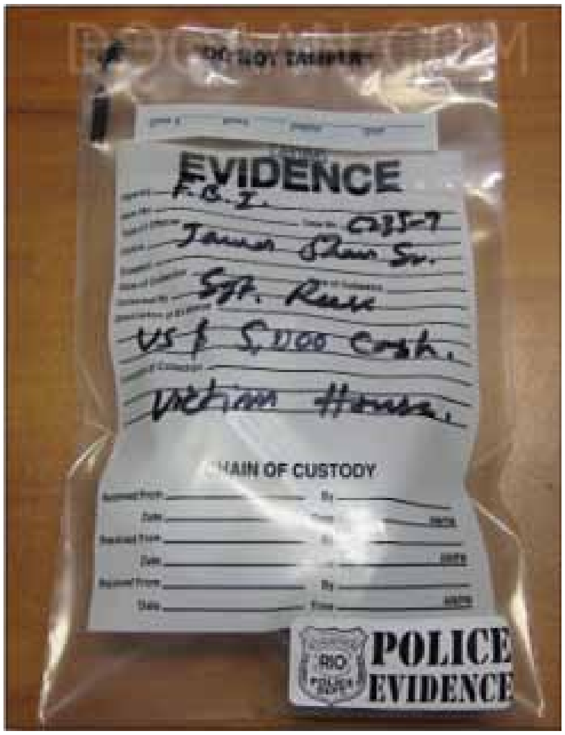
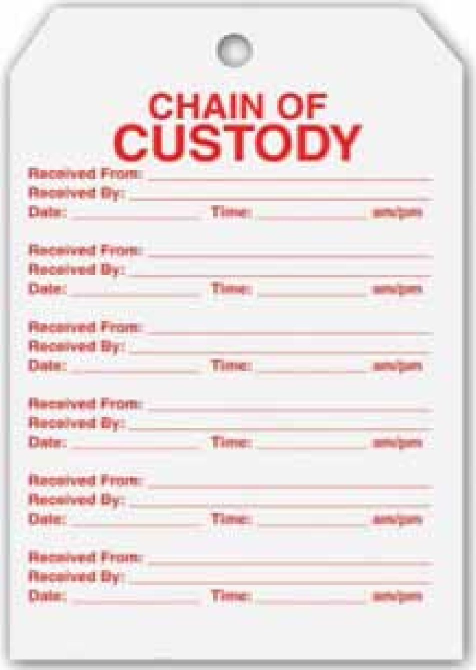


Figure 6. Example of sample chain of custody form



CHAIN OF
CUSTODY

Received From: ____________________
Received By: ____________________
Date: ____________ Time: ____________ am/pm

Received From: ____________________
Received By: ____________________
Date: ____________ Time: ____________ am/pm

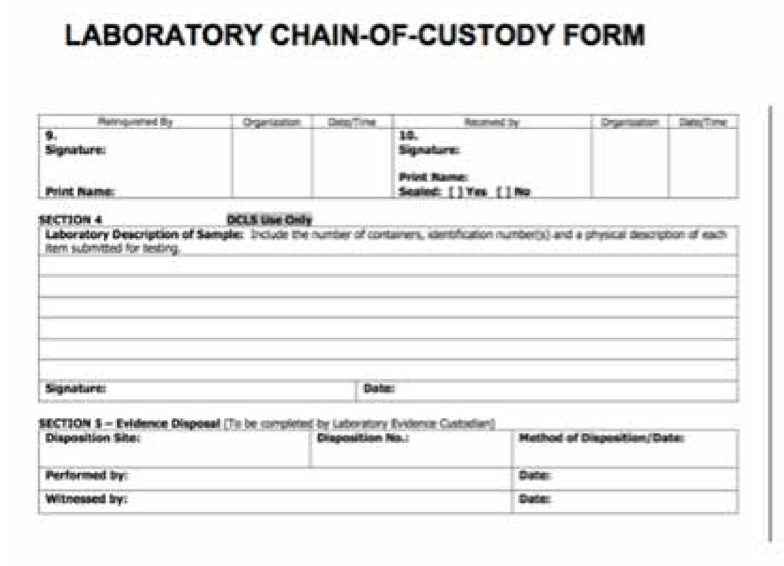
Received From: ____________________
Received By: ____________________
Date: ____________ Time: ____________ am/pm

Received From: ____________________
Received By: ____________________
Date: ____________ Time: ____________ am/pm

Received From: ____________________
Received By: ____________________
Date: ____________ Time: ____________ am/pm

Received From: ____________________
Received By: ____________________
Date: ____________ Time: ____________ am/pm

Figure 7. Example of sample chain of custody form



LABORATORY CHAIN-OF-CUSTODY FORM

Relinquished By	Organization	Date/Time	Received by	Organization	Date/Time
9. **Signature:** **Print Name:**			**10.** **Signature:** **Print Name:** **Sealed:** [ ] Yes [ ] No		

SECTION 4 **DCLS Use Only**

Laboratory Description of Sample: Include the number of containers, identification number(s) and a physical description of each item submitted for testing.

Signature: **Date:**

SECTION 5 – Evidence Disposal (To be completed by Laboratory Evidence Custodian)

Disposition Site:	**Disposition No.:**	**Method of Disposition/Date:**
Performed by:		**Date:**
Witnessed by:		**Date:**

Investigators should consistently use standardized sample collection methods (Figure 5) and chain of custody forms (Figures 6, 7); on the spot substitutions or variations in the forms could invalidate future evidence. Variations or discrepancies with the techniques used for sample collection, processing, and analysis from approved SOPs can prevent the use of the materials as evidence. Diagnostic and forensic laboratories are required to maintain internal chain of custody for materials received from field investigations, through the stages of the forensic or diagnostic analysis, and to final disposition.

15. CONCLUSION, OPPORTUNITIES AND FUTURE DIRECTIONS

Over the past two decades the field of microbial **forensic science** has emerged as a distinct subset of the forensic sciences, its evolution shaped by need. Actual or threatened criminal events can be investigated with rapidly changing tools and scientific capabilities in the detection and characterization of bacteria, viruses, fungi, and other microbes, as well as for the toxins and other harmful metabolic products that they produce. The 2001 FBI-led ‘Amerithrax’ investigations of anthrax spores sent through the U.S. Postal system were a strong driver for the development of new science and technology supporting microbial forensics. Applications of this field quickly expanded beyond cases involving harm to humans. Applications of microbial forensics to crimes in the agricultural sector – on farms and ranches, indoor production units, processing plants, import-export systems, and other components of our food production and distribution systems – continue to increase and to contribute to effective responses to criminal events affecting crops and livestock.

In this chapter, we have discussed the fundamental aspects of the need for, and applications of, microbial forensic sciences in the agricultural sector. We have described specific requirements and standard procedures for forensic investigations in crop and livestock settings, and used a series of agricultural case studies to illuminate the practice of microbial forensic science in these settings.

There is every reason to expect that the field of microbial forensics will continue to evolve in response to new demands, technology and opportunity. Some areas of effort pertaining to technology, tools, and education/training that would benefit this science in the future are noted below.

a. **Technology**
   - Incorporation of emerging technologies for fine-level discrimination among closely related microbes
   - Applications of microbial population biology assessment technologies to understanding population diversity and ecology, as relevant to forensic investigation
   - Greater standardization (and comparability) among laboratories in the selection of technological assays used for microbial identification and comparisons
   - Greater standardization in the levels of precision, repeatability and accuracy that are deemed acceptable for forensic purposes and admissible in a court of law
   - Enhanced methods for determining exposure of humans to zoonotic diseases through improved epidemiological interviews

b. **Tools**
   - More effective tools to evaluate whether a disease outbreak was likely to have been naturally incited, due to accidental causes, or due to an intentional introduction
   - Increased encouragement and incentives for farmers and ranchers to prepare, maintain, and enforce strict biosecurity practices within their production units
   - Enhanced methods for determining exposure of humans to zoonotic diseases through improved epidemiological interviews

c. **Education, Training and Exercising**
   - More opportunities for training and exercising in both field responses and laboratory investigation
   - Targeted education, training and exercising of law enforcement personnel in issues relevant to the agricultural enterprise, from farm to fork
   - Improved awareness of reportable symptoms of emerging or foreign animal diseases, reporting symptoms to regulatory veterinary authorities, mitigation and containment of disease outbreaks (veterinarians, veterinary technicians, livestock and poultry producers, Cooperative Extension agents, agricultural and veterinary schools, and animal production industry should all be made aware of the potential symptoms, and to whom they should be reported)
   - Improved awareness and reporting, among public health officials, of zoonotic and emerging animal diseases
   - Expanded awareness, on the part of crop and livestock producers and early responders, of triggers and tripwires for contacting law enforcement

Forensic science is not a stand-alone discipline but rather is the result of targeted blending of many other sciences: classical microbiology, microbial genomics, population biology, phylogenetics, epidemiology, microbial ecology, bioinformatics, human forensics, veterinary science, plant pathology, entomology, food microbiology, microbial detection, diagnostics and many more. Further strengthening of microbial forensics will depend upon the continued interactions and cooperation among scientists and practitioners in all these disciplines.

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KEY TERMS AND DEFINITIONS

Agrocrime: Any offense impacting animals and crops, the inputs used to raise them, or their products that is classified as a crime as per a country's civil and penal codes.

Agroterrorism: The intentional release of biological agents, toxins, hazardous materials for the purpose of harming or killing animals or plants and the disruption of the agriculture infrastructure with the intent to intimidate or coerce a government or civilian population to further political or social objectives.

Attribution: Determination, to a scientifically sound and statistically defensible degree, of who is responsible and culpable for a crime through the applications of elements of science, law, policy, law enforcement, public and agricultural health, and the media.

Budwood: Bud-bearing branch segments used for grafting onto another plant rootstock.

Call Tree: A layered, hierarchical communications chain involving specific individuals, allowing for expeditious dissemination of information and coordination of next steps.

Chain of Custody: An unbroken, clearly documented, chronological record of the custody, transfer, storage, and disposition of physical or electronic evidence.

Containment: Steps taken after the initial introduction of a pathogen or pest to limit the movement of the agent and the spread of disease.

Control Zone: A potential crime zone or other area of quarantine or biosecurity focus in which investigators and operators must follow USDA or SDA disease control and decontamination requirements for entry and exit.

Court of Law: A hearing or trial presided over by a judge or magistrate in civil or criminal cases.

Crime: An action or failure to act that is prosecutable by the state and punishable by law.

Daubert Criteria: In the United States, the rule of evidence with respect to the admissibility of court testimony by an expert witness.

Delimiting Survey: A survey designed to identify the extent and range to which a pathogen or disease has spread, for purposes of disease management and containment.

Endophyte: An organism, often a fungus or a bacterium, that resides within a plant for at least a portion of its life without causing apparent disease.

Evidence: A body of facts, information, or material supporting a determination of whether a belief or proposition is true or valid.

Exonerate: Free from a legal accusation or charge and from attendant suspicion of blame or guilt.

Forensic Science: The use of scientific means to analyze physical crime evidence for assessment of the innocence or guilt of a specific suspect.

Forensic Microbiology: A scientific discipline in which evidence from a terrorist action or biocrime involving a microorganism or biological toxin is analyzed for use in criminal attribution.

Intellectual Property: An invention or idea resulting from creativity to which one has legal rights and which may qualify for a patent, copyright, trademark or other protection.

Koch's Postulates: Specific criteria that, when met, demonstrate that a microbe is the causal agent of a disease.

Multi-Locus Sequence Typing: Characterization of microbial species through the DNA sequences of internal fragments of several different housekeeping genes.

Necropsy: Postmortem dissection and examination of the body of an animal to determine cause of death.

Pathosystem: A subset of an ecosystem in which the components include a host organism and an associated pathogen or parasite.

Perpetrator: An individual who carries out a crime or otherwise harmful or immoral action.

Probative: Adjective describing something that demonstrates or provides proof.

Prosecution: The process of legal court action against a person charged with a crime.

Quarantine: The temporary isolation of a person, animal or plant that may be harmful to others due to exotic origin or exposure to a disease agent or pest.

Rogueing: The removal of infected or infested plants from an agricultural setting for purposes of disease containment.

Set (Onion Set): A small, young onion bulb planted for the production of mature bulbs.

Simple Sequence Repeat (SSR): Segments of DNA in which short motifs are repeated several to many times in tandem; SSR patterns can be used to characterize and discriminate among different populations of a microbial species.

Threat Credibility Evaluation (TCE): Assessment, by the FBI and/or other law enforcement personnel, of available intelligence and/or case information to determine the credibility of a threat; to include technical feasibility, operational practicality, and adversarial intent.

Trace Forward: Determination of the path on which a pathogen or pest will move in the future.

Traceback: Determination of the source of a pathogen or pest.

Trade Secret: A type of intellectual property consisting of information having actual or potential independent economic value, which may be protected to maintain its secrecy.

Trigger (With Respect to Reporting a Suspicious Incident): The first signs or indicators of unusual behavior, activities, threats, or incidents that are recognized as abnormal or suspicious.

Tripwire: An observation, occurrence, or other factor that leads to a decision on the part of an observer to notify authorities.

Volunteer Plant: A plant that germinates and grows without having been planted by humans, often from seed or vegetative plant parts remaining at the end of a season.

Xylem: Water-conducting tissues of a plant.

APPENDIX

List of Acronyms

APHIS: Animal and Plant Health Inspection Service (USDA)
CBRN: Chemical, Biological, Radiological, Nuclear
CBRN-E: Chemical, Biological, Radiological, Nuclear and Explosive
CDC: Centers for Disease Control and Prevention
CBIIAT: Crop Bioagent Introduction Intent Assessment Tool
CZ: Control zone
DHS: Department of Homeland Security
EPPO: European Plant Protection Organization
FBI: Federal Bureau of Investigation
FDA: Food and Drug Administration
FEMA: Federal Emergency Management Agency
GMO: Genetically modified organism
HMRU: Hazardous Materials Response Unit (FBI)
NIFA: National Institutes for Food and Agriculture (USDA)
OIG: Office of the Inspector General
OQDS: Olive quick decline syndrome
PPE: Personal protective equipment
RV: Regulatory veterinarian
SAHO: State Animal Health Official
SDA: State Department of Agriculture
SITREP: Situation report
SOP: Standard operating procedure
TCE: Threat credibility evaluation
USDA: United States Department of Agriculture
WMD: Weapons of Mass Destruction Directorate (FBI)

Chapter 11

Looking to the Future:
Advancing Tactical Sciences for the Biosecurity Toolbox

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ABSTRACT

Advances in technologies, increased globalization, impacts of changes in climate and land use on food production practices, and the expanding world population will continue to exert significant pressures on global biosecurity systems. The world must be prepared to face novel biosecurity threats, whether a consequence of natural pest and pathogen emergence or an intentional or unintentional release into a community. It is imperative that public and private sectors develop comprehensive and innovative strategies to mitigate these ever-evolving threats rapidly and effectively. This chapter reviews several opportunities that currently exist in global biosecurity of animal and plant systems with the hope that it will provide researchers, health experts, educators, and first responders with the awareness and impetus to adopt biosecurity tactics that enhance preparedness, reduce risk, and prevent catastrophic outcomes.

INTRODUCTION

Advances in technologies, increased globalization, impacts of changes in climate and land use on food production practices, and the expanding world population will continue to exert significant pressures on global biosecurity systems. The world must be prepared to face novel biosecurity threats, whether a consequence of natural pest and pathogen emergence or an intentional or unintentional release into an environment, and whether agricultural or natural, or rural or urban. It is imperative that public and private sectors develop comprehensive and innovative strategies to mitigate these ever-evolving threats

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rapidly and effectively. As our scientific understanding of existing pests and pathogens as well as novel biology of new and emerging pests and pathogens expands, biosecurity practices of today may prove to be inadequate to manage the vulnerabilities of tomorrow.

This book has examined the continuum of biosecurity tactics in animal and plant systems, with the goal of driving informed decisions by policy makers, health experts, first responders, researchers, and educators to enhance preparedness, reduce risk, and prevent catastrophic outcomes. The intent of focusing on the tactical sciences was to highlight that animal and plant system biosecurity scientific principles are broadly overlapping. Economic considerations, processes that promote genetic variations, risk analyses, prevention tactics, diagnostics, surveillance, response, and microbial forensics run parallel in plant and animal biosecurity systems but are essentially interoperable.

The first chapter of this book was dedicated to the economic considerations of an effective biosecurity system. Globally, an estimated 95 million people entered extreme poverty in 2020 because of the COVID-19 pandemic, and an additional 80 million people became more undernourished relative to the pre-pandemic levels (Jackson et al., 2021). The global economic cost of the COVID-19 pandemic may surpass US$20 trillion dollars; this highlights the tremendous financial burden of a reactionary response to a global disease threat, estimated at US$3-4 billion/year (Pimentel, et al., 2020). Moving forward, the investment of a small fraction of this cost may be sufficient to adequately plan, prepare and promptly mitigate the next pandemic threat. However, while learning from the COVID-19 pandemic, threats to the agricultural enterprises and natural ecosystems by invasive species have not stopped.

Several opportunities to enhance global biosecurity have been highlighted in the previous chapters of this book and fall into the following 4 categories:

1. Technology
2. Networking/Cooperation/Engagement
3. Standardization among global partners
4. Education and capacity development

Technology

Emerging scientific and technological advances have the potential to enhance or even replace standard pest and pathogen detection, diagnosis, and control procedures. When intellectual silos are broken down and biologists, chemists, physicists, engineers, and social scientists interact, new, often transformational, technologies emerge. While this chapter attempts to review the technological landscape, it is safe to say that completely different and unforeseen opportunities will arise in the coming decade.

Data Science, Modelling, and Risk Analysis

Tools of data science and analytics currently exist that have the potential to mine large data sets. Artificial intelligence and machine learning have been optimized to such an extent that millions and millions of data points can be simultaneously interrogated to instantaneously detect patterns and utilize mathematical modeling to successfully predict the likelihood of future events. Comprehensive data on human, animal and plant movement, particularly origin-destination data, can be utilized to predict the spread of pests and pathogens. Sophisticated data systems can utilize syndromic surveillance and geospatial analyses for early detection and tracking of disease threats. For example, a novel predictive modelling framework was developed for the spread of COVID-19 and accounts for human interactions, physical distancing, age, infection severity, and infection duration (Ganesan et al., 2021).

Along the same lines, modelling for plant and animal disease and pest analysis are advancing as climatic and transport systems data are enriched, as highlighted in Chapters 3 and 5 in this book (Baker, et al., 2020; Mastin, et al., 2020; Lantschner, et al., 2019; McCluskey, et al., 2003). Modelling of pest or pathogen movement is intrinsically tied to measured environmental parameters. Climate-sensitive variants of pests and pathogens emerge around the world. As was shown in Chapter 2, evolutionary responses to environmental change are unpredictable, but certain to occur (Moran & Alexander, 2014). Improved granularity in awareness of strains and variants would be a product of better collaboration and data sharing around the world.

Establishment and spread of pests and pathogens are closely linked to climate (weather of a region averaged over a long time-period) and weather (short term atmospheric conditions). One of the greatest sources of uncertainty in this epoch is climate change. Climate change and extreme weather events create challenges to knowing how pests and pathogens will move or where they would flourish. Models to aid in planning and decision-making use data that is collected in a scale ranging from crude (large) to fine (granular).

Resources have been dedicated to better capture of climatic data for use in modelling biological activity in the context of climate (Kriticos, et al. 2012, 2016; Fick & Hijmans, 2017). At the largest scale, the Intergovernmental Panel on Climate Change (Masson-Delmotte, et al., 2021) provides data analysis to forecast broad global trends in the long range (a 5-year cycle) to support proactive planning. In the U.S. and most other countries, government agencies at national and regional scales, and universities at still smaller scales, support station networks that collect weather observations in real-time (National Weather Service, 2021, Mesonet, 2018). Weather data observed at stations on a sub-hourly, hourly, and daily basis are used to identify weather systems, recognize extreme events, and construct climate records. The same data when interpolated into grid values are used as input into numerical forecast models (National Weather Service, 2021).

Point and grid weather observations and the forecasts generated from them are interpreted by agricultural models to provide predictions of pest and pathogen occurrence and movement (Olatinwo and Hoogenboom, 2013). Other weather-driven, agricultural models support grower decision making, such as for pest control and irrigation scheduling (Donatelli, 2017; Linker and Sylaios, 2016). Today, the highest grid resolution for weather data is on the order of 1 kilometer. As remotely sensed weather data from satellites, airplanes, and drones becomes routinely integrated with grid ground observations, model results at field and sub-field resolutions (i.e., 100 to 10 meters) will be a reality (Dado, et al., 2020).

Agricultural models driven by this future field-scale weather data will make precision applications possible and support other fine-scale management actions. Future agricultural models, when combined with biosensors and technologies such as the Internet of things (IoT), robots and unmanned aerial vehicles, can bring on a new era of information and machine-driven farming (Itzhaky, 2021). In terms of biosecurity, the future integration of models and technologies can facilitate the safe and economical reporting for inaccessible areas or potentially infected premises while preserving biocontainment (Priye et al., 2016).

Surveillance, Detection and Diagnostics

At points of entry into an area, ports, airports, and borders, advances in biosensor technology and the expanded use of biosensors during domestic or international travel have the potential to allow for the real-time detection of pests and pathogens prior to arrival at a port or border.

At ports and borders, where large quantities of commodities are entering daily, technology that would allow simultaneous testing for multiple kinds of pests or pathogens of quarantine concern in the same sample would significantly improve testing throughput. As discussed in Chapter 5, time is a critical constraint to screening in-coming materials, and current technologies are too slow to be practicable. With Next Generation Sequencing (NGS), it is theoretically possible to analyze a sample for every known pathogen or pest of concern within an hour of receiving the sample. The development of accessible and economical point-of-care tests has potential to minimize the bottleneck associated with widespread diagnostic testing and the subsequent spread of infectious agents that may occur while waiting for test results. The development of high-throughput nucleic acid sequencing and bioinformatics tools for data mining is on the verge of adoption for rapid and economical testing for pathogens of humans, animals, and plants (Espindola & Cardwell, 2021; Ochoa-Corona, et al., 2019).

Clustered regularly interspaced short palindromic repeats (CRISPR)-based diagnostics are being explored for nucleic acid query for molecular signatures of pathogens in tissue (Goottenberg, et al., 2017). Nanotechnology holds promise for expanding our analytical platforms as well as use in diagnostic assays that facilitate the rapid detection of pests and pathogens (Laroui, et al., 2013). Expansion of social media platforms for information sharing has enhanced the potential for early detection and monitoring of pest and disease outbreak by volunteers and community scientists. Such advances will revolutionize the speed, accuracy, and wealth of information collected during disease outbreaks (Carvajal, et al., 2019).

Pest and Disease Management

The COVID-19 pandemic has demonstrated that advances in synthetic biologics can lead to better vaccines, cell-based therapeutics, and diagnostic assays (Anand et al., 2021; Nakagami, 2021). Transitioning from conventional vaccines to synthetic biologics enables the rapid development of vaccines and biologic therapeutics to combat emerging diseases. Until COVID-19, it previously took several years to develop and deploy a vaccine for a novel, unexpected pathogen. With mRNA technology, this process took less than 2 years. Ever increasing understanding at the molecular level opens new opportunities for vaccines, and other biological based controls.

The average time of development and deployment of new therapeutic drugs or treatments is 10-12 years in the U.S. (Dabrowska & Thaul, 2018). Prior to clinical trials with actual patients, biopharmaceutical companies spend 3-4 years conducting laboratory and animal studies to demonstrate the biological activity of the drug against the target disease and to prove that it is reasonably safe. Three-dimensional tissue printing is opening opportunities for testing safety and efficacy of therapeutics and vaccines (Murphy & Atala, 2014). Not only does it provide a life-like matrix for testing, but it reduces dependence on animal models for testing. This technology has potential to shave years and expense off the therapeutics development pipeline.

Biological control of pathogens and pests has gained a foothold among control options for plant and animal diseases. Microbial biological control agents interact with the host, the targeted pathogen, and the resident microflora. Throughout this book there have been examples of how biological control has been deployed. Mechanisms of biocontrol include hyperparasitism, niche displacement, and/or introduction of a plasmid or genetic code for production of antibiotics or antifungal metabolites. This will evolve as we move forward with the arrival of NGS of metagenomics and metatranscriptomics. This technology will help scientists drill further into processes and interactions in complex communities of microbes, with the aim to find novel organisms or communities that suppress disease (Kohl et al., 2019). There is the growing realization that we can't fully understand how biological control works without considering the microbial community and interactions in the host matrix. Molecular research into microbiomes and their interactions with each other and their host will greatly enhance the ability to move from a reactive to a proactive approach, for example in designing agroecosystems that are more resilient to biological invasions.

The newest advent in biological control technology is called 'gene drive'. It is a natural process that drives a particular suite of genes through a population (Callaway, 2017). The technique can employ adding, deleting, disrupting, or modifying genes and then increasing the probability that the specific allele will be transmitted to progeny. Proposed applications include exterminating pathogen transmitting insects like mosquitos that transmit malaria, dengue, and zika or for controlling invasive species, or eliminating pesticide resistance (Benedict, et al, 2008; Redford, et al. 2019). Trials are occurring in Brazil to control the Fall Army Worm, *Spodoptera frugiperda*, with gene drive biological control (Oxitec, 2021). The Fall Army Worm has caused significant devastation and impact on food security after it jumped from the Americas into Africa (Van den Berg, et al., 2021). Although a new technology is possible, that does not necessarily mean that it should be deployed. There are significant bioethical and safety concerns about using gene drives, but the technology is certainly something that could be deployed to exterminate invasive species. If the drive could be time or location limited, and controllable, there might be less consternation. Nevertheless, to eliminate disease carrying mosquitos and/or the Fall Army Worm, that we have been powerless to stop, are objectives that would provide incredible benefits to humanity and alleviate overwhelming suffering and disease and the associated costs.

Traditional plant and animal breeding processes can take up to 10 years to develop and deploy a new disease-resistant variety or genetically superior herd. Genetic engineering can be quickly leveraged to enhance animal immunity and plant resistance to pests and pathogens, thereby altering the survival and successful transmission of pests and pathogens (Dong et al., 2019). Nevertheless, there is decided public uncertainty about genetic engineering of food plants and animals in the laboratory, but new tools allow rapid selection of desirable traits from within the target plant or animal host. Genetic marker-assisted plant breeding is a technology that has gained traction as more genotypic information and fully assembled and annotated genomes have become available. This technology significantly speeds up the development of tolerant/resistant cultivars by decreasing the number of cycles of crossing and selection needed (Das et al, 2017).

Ultimately, technological advances may assist agricultural producers and country regulatory authorities transition from a reactive approach to a proactive stance. Nevertheless, no matter how much effort goes into prevention, early detection, and rapid response, there will always be unexpected invasive pests to which we must react.

Networking/Cooperation/Engagement

Timely sharing of scientific information is key to virtually every biosecurity operation around the world. Regardless of the scale of an outbreak, effective and coordinated communications are needed. International, regional, and national networks have been developed for human, animal and plant outbreak communication, with the aim of providing global surveillance systems for endemic and exotic pests and pathogens (Table 1) (from IPPC Pest Outbreak and Reporting working group).

Table 1. Examples of systems for the coordination and communication of disease outbreak data

Information system	Scope	Type of information	Access
Global Animal Health	Global	Animal disease outbreak	https://empres-i.apps.fao.org/
World Animal Health	Global	Animal disease outbreak	https://wahis.oie.int/#/home
Epidemic Intel Open source	Global	Horizon scanning – human health	https://www.who.int/initiatives/eios
EFSA	Europe	Horizon scanning – all health	https://medisys.newsbrief.eu
Eu Plant Protection Org	Europe	RPPO quarantine	https://www.eppo.int
Epidem Surveillance Vegetale (One Health France)	Europe	Epidemiologic surveillance	https://plateforme-esv.fr
Crop Watch Africa	Africa	Surveillance app: plant health	https://www.cropwatch.africa
Plantwise - CABI	Africa	Crowd-source data modelling	https://www.plantwise.org
Data Reporting Tool (DaRT)	Africa	Info coordination for biodiversity	https://dart.informea.org
Pacific Plant Protection System	Islands	KoBo Toolbox for coordination	https://www.spc.int/pld
Austral. Emergency plant pest incursion response and alert	Australia	NPPO	https://www.outbreak.gov.au
North American Phytosanitary alert	U.S., Canada, Mexico	Regional pest reporting system	https://www.pestalerts.org
PestLens	U.S.	Horizon scanning - global	https://pestlens.info
SINAVIMO	Argentina	Crowd-source data capture	https://www.sinavimo.gob.ar

There are certainly more information systems than those presented in Table 1. However, these are presented to make the point that while such systems are proliferating, they tend to be disconnected. There are two global efforts at collecting surveillance data for animal and zoonotic disease, but there is no corollary for plant systems. A Global Surveillance System for crop diseases has been proposed and discussed (Carvajal, et al., 2019), but resources have not been identified to put it in place. Under the International Plant Protection Convention, up to 183 member nations are obligated to report when they have an outbreak of a high consequence pest or pathogen. However, compliance is spotty and focuses on only organisms of quarantine significance. **All pests and pathogens are local somewhere, but they are exotic somewhere else.** If only pests of quarantine significance are reported, then all the rest of the pests and pathogens in the world are in a black box. The absence of real-time data sharing on a global level means that pests or pathogens emerging in one part of the world can threaten another. It is easier to prepare for what is known so that the response can be proactive rather than reactive.

Coordination of diagnostic assay development and validation is another area that needs development on a global scale. European laboratories, the European and Mediterranean Plant Protection Organization, and diagnostic companies have joined forces in a project funded by the European Union called

Valitest (Trontin, et al., 2021). The objectives of this project were to improve validation procedures and strengthen interactions with the different stakeholders in plant health to improve diagnostic capabilities.

However, in the United States, there is a lack of coordination and strategy for comprehensive plant and animal diagnostic assay development and validation. Diagnostic laboratories and assay developers have no formal system to communicate with each other. There are numerous discrete, disconnected, yet critical, reference collections that are not widely known or easily accessible. Thousands of nonregulated pathogens are routinely diagnosed with assays that were not fully or consistently validated, often offered by for-profit commercial entities that also lack reporting structures through regulatory channels. Increased standardization and coordination of diagnostic assay validation will increase confidence in assay results, leading to better decision-making that supports disease management as well as safeguards the movement of plants and plant products. Strengthening protocols and a framework for validation in all laboratories will strengthen the accuracy of diagnostic methods.

Standardization

Global standardization represents one of the greatest opportunities to protect animal and plant systems from pests and pathogens. Standardization of methodologies for assessing, validating, and reporting various parameters is needed. The World Trade Organization Sanitary and Phytosanitary measures Agreement names the IPPC as the international body for standard setting in plant health, as it does the OIE for animal health (WTO, 2021). Nevertheless, there are many tactical areas that are not necessarily tied to trade for which no standards exist. For example, there are currently there are no shared standards on how to assess the economic impacts of biosecurity breaches. Similarly, there are no standards for reporting disease losses, therefore scientific studies do not report pest or pathogen damage in a consistent manner, making it difficult to analyze and compare data sources. Animal and plant diagnostic laboratories utilize a variety of commercial and home-grown software applications to record, report, and store data. Except for select high-consequence pathogens, veterinary diagnostic laboratories in the U.S. generally have limited capabilities for the real-time sharing of the detection of important endemic pathogens and diseases. Laboratory information management systems (LIMS) now have the capabilities for rapid aggregation and analysis of diagnostic trends and geospatial data, as well as the ability to network with other software applications to safely transfer data within these networks and thereby facilitate the generation of knowledge (McCluskey, 2021). However, the lack of standardization of test and data nomenclature remains a problem in veterinary diagnostic laboratories. The implementation and utilization of universal names and codes can unlock the potential for data mining at large scale (Martin, 2021).

Education and Capacity Development – the Human Resource Pipeline

A successful biosecurity system hinges on data collection, education, training, strict compliance, and periodic reviews with necessary modifications to the system. Education entails a broad swath of individuals, including private and public entities as well as policy makers. Unfortunately, the diversity and competing interests of stakeholders can hinder prevention and biocontainment efforts. Additionally, misinformation and conflicting opinions may preclude policy makers from arriving at the appropriate risk management decisions. The differing views and opinions highlight the critical need for evidence-based management approaches.

An understanding of the principles of biosecurity should start with childhood education and continue into adulthood. A recent review of biosecurity education at universities in the U.S., Europe, and Japan revealed limited opportunities and recommended the following measures to fill this gap: develop new biosecurity courses, add biosecurity modules to existing biology courses, incorporate biosecurity topics into existing bioethics courses, and develop online courses (Land, 2018). Despite the recognition for biosecurity and biosafety education by global leaders, resources committed to ensure adequate education and training are often lacking. The establishment of formal biosecurity-specific courses and majors in colleges will provide a pipeline for future generations of agricultural and environmental scientists, animal and plant diagnosticians, clinical investigators, emergency responders, thought leaders, and policy makers. Additionally, training and professional development courses are needed that include regional, national, and international expertise; these activities align with practices recommended by the World Health Organization (World Health Organization Guidance Document, 2010).

However, capacity development is broader than addressing individual skills. It also has to do with how the different parts of the biosecurity "system" work together to produce system-level outcomes. So, capacity includes organizations and systems as well as that of individuals. The IPPC capacity development strategy says, "National Phytosanitary Capacity is defined as: the ability of individuals, organizations and systems of a country to perform functions effectively and sustainably in order to protect plants and plant products from pests and to facilitate trade, in accordance with the IPPC." This was based on a United Nations Development Program general definition of capacity (UNDP, 2015).

Education and capacity development needs include continual capacity assessments, scientific educational curricula, training curricula, simulations, internships, operations, protocols, and guidelines manuals. These educational and capacity development components are needed specific to each aspect of the biosecurity continuum. From PhD to field practitioner, the biosecurity pipeline needs prepared subject matter experts and practitioners for horizon scanning, risk assessment, pathway analysis and epidemiology, sampling and surveillance, bioinformatics and advanced data analytics, diagnostics, emergency responses, data management and communications systems. All of these must be coordinated and linked together to achieve biosecurity. The needs are real and ever-present, but at the writing of this book, largely left to chance.

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KEY TERMS AND DEFINITIONS

CRISPR: A technology that can be used to edit genes. It is a way of finding a specific bit of DNA inside a cell and then editing to alter the DNA.

Hyperparasitism: The parasitic habit of one species preying upon another parasitic species.

APPENDIX

Acronyms

FAO: Food and Agriculture Organization of the United Nations
OIE: Office International des Epizooties (1924-2003); World Organization for Animal Health (2003-present).
EFSA: European Food Safety Authority
CABI: Centre for Agriculture and Bioscience International
SINAVIMO: Sistema Nacional de Vigilancia y Monitoreo of Argentina

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About the Contributors

Kitty F. Cardwell 127 NRC, Oklahoma State University Stillwater, OK 74074 Appointments Dr. Cardwell is the as Director of the Institute of Biosecurity and Microbial at Oklahoma State University. She is a Professor of Microbiology as applied to Biosecurity and Microbial Forensic Sciences and a research scientist at the frontier of disease diagnostic technologies. She moved to Oklahoma from Washington DC where in 2012-2013 she did a professional detail as a Senior Advisor to the World Bank, working at the interface of child health and agriculture in Africa. From 2001-2016, Dr. Cardwell was a National Program Leader with specialization in Biosecurity at the National Institute of Food and Agriculture in Washington, DC. Before going to Washington, Kitty was: a research scientist at the International Institute of Tropical Agriculture (IITA), in Ibadan, Nigeria; in a private agricultural Business, (Rice Farming) in Villavicencio, Colombia; and a Peace Corps Volunteer in Nicaragua and Colombia. Professional Preparation University of Texas, Austin, TX Botany and Microbiology BA (Honors), 1976 Texas A&M University, College Station, TX Plant Pathology & Microbiology Ph.D. 1989 American University, Washington, DC Executive Leadership Program, 2013 -2014.

Keith L. Bailey currently serves as a Senior Pathologist at Charles River Laboratories. He is a diplomate of the American College of Veterinary Pathologist and has 17 years' experience as a comparative and diagnostic veterinary pathologist at the University of Illinois and Oklahoma State University, including 5 years as Director of the Oklahoma Animal Disease Diagnostic Laboratory. He is a past-President (2018-2019) of the American Association of Veterinary Laboratory Diagnosticians (AAVLD) and has served on numerous AAVLD committees. Additionally, Dr. Bailey has served as a toxicologic pathologist in the biopharmaceutical industry for 8 years.

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Bruce Akey is a nationally recognized leader in veterinary diagnostics. He is a former veterinary diagnostic laboratory Director for the Virginia Department of Agriculture and Consumer Services, the New York State Veterinary Diagnostic Laboratory/Animal Health Diagnostic Center at Cornell University, and the Texas A&M Veterinary Medical Diagnostic Laboratory. His responsibilities encompassed the complete range of laboratory operations, quality assurance, and research and development efforts. A graduate of the College of William and Mary (BS, Biology), he also attended the University of Florida (MS, Parasitology) and University of Minnesota (Doctor of Veterinary Medicine).

Charles (Chuck) Bargeron has been with the University of Georgia for 22 years where his work focuses on invasive species and information technology. He has a B.S. and M.S in Computer Science. Websites that he designed have been featured twice in Science Magazine and have received over 1.7 billion hits since 2002. Chuck developed the infrastructure behind Bugwood Images which runs the ForestryImages.org and Invasive.org websites. Recently, Chuck has focused on mapping invasive species and tools for Early Detection and Rapid Response using EDDMapS and smartphone applications. He has led the development of 73 smartphone applications including the first apps for the U.S. Forest Service and National Park Service. He was appointed to the National Invasive Species Advisory Council in 2013 and elected as Chair in 2017. Chuck has been an invited speaker at over 300 regional and national conferences and co-authored over 62 journal articles and outreach publications. Chuck is the current president of the North American Invasive Species Management Association.

Brittany Barker, Ph.D., currently is a Research Associate with the Oregon Integrated Pest Management (IPM) Center at Oregon State University. Dr. Barker earned her Ph.D. in Biology at the University of New Mexico, completed a postdoctoral fellowship at the University of Arizona, and worked as an Ecologist with the U.S. Geological Survey. Her research focuses on how past and current environmental perturbations such as climate change, fires, and the introduction of non-native species have influenced animal and plant population dynamics. Dr. Barker is developing science-based, ecologically-informed site and spatialized models that can help agricultural decision makers with managing and monitoring pests, their crop hosts, and their natural enemies. Some of her other research efforts have focused on using genomic data to trace the origins of pest invasions, identify signatures of adaptation in biocontrol insects, and explore the impacts of past climate change on the evolution of island species. Dr. Barker's skills include expertise in the R programming language, remote sensing data analysis, modeling, population genetics, and species distribution modeling.

Rosslyn Biggs was raised in southwestern Oklahoma. She attended Oklahoma State University where she completed a bachelors of science degree in Agricultural Economics in 2001. Dr. Biggs graduated from Oklahoma State University College of Veterinary Medicine in 2004. Upon graduation she spent three years as a mixed animal practitioner in Chickasha, Oklahoma. She joined USDA APHIS Veterinary Services in 2007 as a Field Veterinary Medical Officer. In 2015, Dr. Biggs joined the APHIS VS Veterinary Export Trade Services as Assistant Veterinarian In Charge managing international exports of live animals and animal products in Texas, Oklahoma, Louisiana, Mississippi, Arkansas and Missouri. She is a Plum Island trained Foreign Animal Disease Diagnostician and previously served on an APHIS VS incident management team. She has served as a responder to multiple incidents ranging from Oklahoma tornado response to disease eradication taskforces including bovine tuberculosis, virulent Newcastle disease and highly pathogenic avian influenza. Dr. Biggs made the move to Oklahoma State University as an assistant clinical professor in summer of 2019. She currently serves as Director of Continuing Education for the College of Veterinary Medicine and Beef Cattle Specialist for Oklahoma Cooperative Extension. She and her husband have two girls and maintain farming and ranching operations in Stillwater and Chickasha, Oklahoma.

John Bowers, Ph.D., currently serves as the USDA, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, National Policy Manager for Domestic Data. Dr. Bowers previously served for 13 years as the National Policy Manager for Pest Detection with primary responsibility for national management and coordination of the Cooperative Agricultural Pest Survey (CAPS) Program. The CAPS program conducts science-based national and state surveys in all 50 states and three territories that targeted specific exotic plant pests, diseases, and weeds identified as threats to U.S. agriculture and/or the environment. Dr. Bowers received a B.S. in Botany from the University of Maryland, a M.S. in Plant Pathology from Rutgers University, and a Ph.D. in Plant Pathology from the University of Florida. He held postdoctoral research appointments in the Departments of Plant Pathology at the University of Wisconsin, Ohio State University, and the University of Minnesota, as well as with USDA-ARS' Floral and Nursery Plants Research Unit in Beltsville, MD. He then held a position with USDA-ARS' Alternate Crops and System Laboratory in Beltsville. His research focus concentrated on the interaction of biological and environmental factors and variables on the microbial ecology, epidemiology, etiology, and biological control of soil-borne plant pathogens and associated microflora in a variety of cropping systems. He is the author or co-author on 31 peer-reviewed scientific publications. Dr. Bowers then served with the Maryland Department of Agriculture (MDA), where he was responsible for the supervision, operation, and management of the state's Plant Pathology Laboratory before transitioning to APHIS.

Corrie Brown, DVM, PhD, DACVP, is a Josiah Meigs Distinguished Professor and also a University Distinguished Professor at the University of Georgia, where she teaches in the College of Veterinary Medicine. She holds a DVM from the University of Guelph and a PhD in comparative pathology from the University of California at Davis. Brown has authored four books on transboundary animal diseases, field diagnostics, and biosecurity in resource-poor settings. She has served on many national and international expert panels about animal health and has received numerous awards for her efforts, including the AVMA International Award, the Student AVMA National Teaching Award (twice), a Fulbright Award, and the AAVMC Service Award.

Russ Bulluck earned his Ph.D. in Plant pathology from North Carolina State University in 1999. He specialized in the areas of plant pathology, mycology, soil ecology and nematology. After a post-doctoral fellowship at the University of California, Russ was hired as a Risk Analyst at NCSU, in December 2002, working with USDA APHIS PPQ. Russ began his federal career in the Science and Technology group before taking a position in Policy, then in Field Operations. Russ has worked on more than 40 different plant health emergencies and has evaluated "select agents" for potential anthropogenic bioterrorism introduction.

Jeff Chang received his BS in Genetics and Cell Biology from the University of Minnesota and his PhD in Genetics from the University of California, Davis. After completing a postdoctoral position at the University of North Carolina, Chapel Hill, he joined the faculty at Oregon State University. His research program investigates the mechanistic, evolutionary, and ecological bases of plant-bacterial interactions.

Janie Chermak is a Professor of Economics at the University of New Mexico, where she's been a faculty member since 1995. She's an applied microeconomist, specializing in natural resources and dynamic optimization. Her multidisciplinary research focuses on production, consumption, and conservation of resources and includes energy, water, wildfires, and invasive species. Her research has been published in the Journal of Environmental and Economic Management, Ecological Economics, Economic Inquiry, Journal of Environmental Management, and Applied Environmental Letters. Her current research is funded by the National Science Foundation, and New Mexico's Environmental Department.

She has advised over 40 graduate students with placements in academia, governmental agencies, national laboratories, and industry. She is the department's chair and former graduate director.

Leslie Cole currently serves USDA APHIS VS as a Veterinary Medical Officer, Foreign Animal Disease Diagnostician (FADD) and is the Emergency Coordinator for Arkansas and Oklahoma. In that position, she works with stakeholders from other government agencies, private practitioners, industry and non-governmental organizations to improve our readiness to respond to incidents and disasters that impact animals and our food supply. Leslie was chosen as an instructor for a USDA International Services/US Department of State capacity building training on animal disease management and investigation for the Malaysian Department of Veterinary Services in Kuala Lumpur, Malaysia in 2016. Leslie received her Doctor of Veterinary Medicine from Oklahoma State University in 1986. Dr. Cole is Board Certified in the American College of Veterinary Preventative Medicine (ACVPM). Leslie serves as Planning Section Chief on one of Veterinary Services National Incident Management Teams and is a certified ICS instructor. Dr. Cole's practice experience includes state and Federal regulatory medicine and mixed and small animal private practice. She is a member of the Oklahoma Veterinary Medical Association, the American Veterinary Medical Association and US Animal Health Association (USAHA). Leslie serves as volunteer unit co-lead for the Oklahoma Medical Reserve Corp's State Animal Response Team. As Planning Section Chief (PSC) for one of USDA Veterinary Services 5 national Incident Management Teams, Leslie has served as PSC in several national Foreign Animal Disease Outbreak responses. These deployments include over 100 days deployment to the 2015 Highly Pathogenic Avian Influenza outbreak response, the 2016 Missouri Avian Influenza outbreak response, 2 deployments to the 2017 Texas Fever Tick outbreak response on the Texas/Mexico border. In December 2018 she was deployed for 3 weeks and serve as Branch Planning for the Butte County CA, Camp Fire Animal Sheltering Branch on a FEMA mission assignment. In April 2019 she was deployed for 3 weeks to the CA virulent Newcastles Disease emergency response as Commercial Depopulation Branch Chief.

Gericke Cook (she/her) is an interdisciplinary scientist (ecology, mathematics, GIS) at the Centre for Epidemiology and Animal Health in USDA Veterinary Services. She earned a double-major B.S. in biology and mathematics from Western Kentucky University and pursued a PhD in ecology at Colorado State University without the final award. She has worked for USDA for 15 years across both plant health and animal health programs, first supporting operational survey work GIS mapping and developing data collection applications. She transferred to plant health scientific support in 2012 developing predictive models of invasive pest introduction and establishment using climate models, habitat niche models, and human movement networks. In 2017, she joined Veterinary Services as a mathematical statistician supporting risk analysis and developing analytical tools to support entry models and to inform animal health surveillance.

Leonard Coop, Ph.D., is an Associate Professor (Practice) in the Horticulture Department and Associate Director of the Oregon IPM Center at Oregon State University. Dr. Coop is an Entomologist working in the areas of pest and disease phenology and risk modeling, spatial modeling, and decision support systems to support IPM and invasive species management. Dr. Coop also supervises the USPEST.ORG website (https://uspest.org/) which hosts over 160 models that support agriculture and pest management decision making nation-wide.

Allard Cossé, Ph.D., is a Supervisory Agriculturist at the USDA, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, Science & Technology Forest Pest Methods Laboratory. Dr. Cossé, has 40 years of research experience with expertise in chemical ecology, analytical chemistry, organic chemistry, neurophysiology, electrophysiology, and entomology. He has over 70 scientific publications over a broad range of insect taxa. Dr. Cossé has a MSc in Biochemistry, a MSc in Biology, and Doctorate in Biology from the Univ. of Amsterdam, The Netherlands. He received a Ph.D. in organic chemistry/biology from the Technion – Israel Institute of Technology, Haifa, Israel.

Joanna Davis, after graduating from the University of Georgia's College of Veterinary Medicine, worked as a mixed animal practitioner in South Georgia for 11 years, owning a practice for six years. In 2001, she was a veterinary responder for the UK Foot and Mouth Disease outbreak. From 2007-2015, she served as a field Veterinary Medical Officer with USDA APHIS Veterinary Services (VS) in South Georgia. Dr. Davis received a graduate certificate in Veterinary Homeland Security from Purdue University in 2012. She has also had the opportunity to travel to the Navajo Reservation in Arizona and Salem, India to participate in veterinary medical missions. She served as the USDA APHIS VS Emergency Coordinator in Georgia and Florida for 4 years, and is now the Training Coordinator and State Federal Liaison for the National Veterinary Stockpile. She has served as Deputy Operations Chief during the Highly Pathogenic Avian Influenza outbreak, and as the Liaison Officer for the 2016-2017 New World Screwworm Outbreak in the Florida Keys. For 9 years, she coordinated and taught the emergency preparedness course to second year veterinary students at UGA's College of Veterinary Medicine. In September 2018, she traveled as a guest lecturer and facilitator for the APHIS FBI Criminology-epidemiology course delivered in Warsaw, Poland. Dr. Davis is currently based out of the USDA APHIS office in Fort Collins, Colorado.

Scott Dee received his DVM, MS, and PhD from the University of Minnesota College of Veterinary Medicine. He is board certified in Veterinary Microbiology. Dr. Dee joined Pipestone Veterinary Services in Pipestone, Minnesota in July of 2011, after 12 years of swine practice in Morris, Minnesota and then 12 years as a professor at the University of Minnesota where he conducted research in the areas of PRRSV transmission and biosecurity. He currently serves as Director of Applied Research for Pipestone Veterinary Service, a business unit that engages in collaborative research with production companies across North America, Latin America, and Asia. His areas of research focus on the transmission and biosecurity of viral diseases of swine.

Sarah Derda is currently enrolled at CSU College of Veterinary Medicine and Biomedical Sciences to obtain a DVM. She earned her MPH in 2021 through the Colorado School of Public Health and plans to pursue a career in the regulatory veterinary medicine field.

Jacqueline Fletcher's (Regents Professor, Emerita, Oklahoma State University) education included a B.S. (Biology, Emory University), M.S. (Botany, University of Montana), and Ph.D. (Plant Pathology, Texas A&M). She is internationally recognized for work on the virulence and insect transmission of phytopathogenic bacteria, agricultural biosecurity and microbial forensics. She established and led the National Institute for Microbial Forensics & Food and Agricultural Biosecurity at OSU, addressing high priority issues in plant pathogen forensics, crop biosecurity and food safety. Dr. Fletcher served as President of the American Phytopathological Society (APS), and following 9-11, she led APS responses and input to national biosecurity initiatives. She was a member of the NASEM Forum on Microbial Threats, and is active on several federal biosecurity advisory panels including the National Science Advisory Board on Biosecurity. Dr. Fletcher is a Fellow of APS and a Fellow of the American Association for the Advancement of Science. Retiring from OSU in 2015, she now serves as a consultant and advocate for agricultural biosecurity and international scientific diplomacy.

Susan Frankel is a plant pathologist and heads Sudden Oak Death Research, at the USDA-Forest Service, Pacific Southwest Research Station (PSW) in Albany, CA. Much of her current work focuses on Phytophthora problems in restoration areas and rare habitats. She is an associate editor for the Canadian Journal of Forest Research. Susan serves as co-chair of the International Union of Forest Research Organizations (IUFRO) Phytophthoras in Forest and Natural Ecosystems Work Party, the Phytophthoras in Native Habitats Work Group, as well as the founding chairperson of the California Oak Mortality Task Force (www.suddenoakdeath.org). Before joining PSW Research, Frankel worked for 15 years as an extension forest pathologist for the USDA Forest Service, Pacific Southwest Region, State and Private Forestry, in Vallejo, California. More information may be found at https://www.fs.fed.us/psw/programs/efh/staff/sfrankel/.

Mark Goeller is Oklahoma's State Forester/Director of Forestry, working for the Oklahoma Dept. of Agriculture, Food and Forestry - Forestry Services Division for thirty-six years. He graduated from Oklahoma State University in 1981 with a Bachelor of Science in Agriculture – Forest Management. Goeller began fighting wildland fire in 1980. He has experience in wildland fire and all-hazards incidents in twenty-one states. He is currently qualified as a Type 1 Incident Commander and Type 1 Operations Section Chief. Since 1998 Mark has served a primary role on multiple Incident Management Teams. Goeller's other incident management-related activities include: current Chairperson of the National Wildfire Coordinating Group Risk Management Committee; National Association of State Foresters (NASF) Representative on the S-520 Advanced Incident Management Steering Committee; past National Governor's Association Representative to the Wildland Fire Leadership Council; and NASF Wildland Fire Committee. Mark is also the past Chairperson of the Southern Group of State Foresters Fire Management Chiefs; former Chairperson of Oklahoma's All-Hazards Standards, Qualifications and Training Committee for Incident Management Teams; and current member of the Oklahoma Incident Management Team Advisory Committee.

Stephen W. Goldsmith, DVM, is a Management Program Analyst in FBI Headquarters, WMD Directorate (WMDD), Chemical-Biological Countermeasures Unit, Washington, DC as an SME for veterinary, animal, plant, and public health biological threat programs. Dr. Goldsmith has been assigned to the FBI Hazardous Materials Science Response Unit (HMSRU), Laboratory Division, Quantico and the WMDD. He is a graduate of the University of Georgia with a Bachelor of Science in Animal Science (1973) and Doctor of Veterinary Medicine (1977) degree. His veterinary career includes private veterinary practice in Georgia and Florida, the Florida Department of Agriculture, State Veterinarian's Office, USDA APHIS Veterinary Services in southwest Georgia, North Carolina, and Bolivia, and served 30 years in the US Army Veterinary Corps in Veterinary, Infantry, and Special Forces Units in Afghanistan, the southern Philippines, and Central and South America.

Niklaus J. Grünwald is a Research Plant Pathologist with the Horticultural Crops Research Laboratory, USDA Agricultural Research Service, in Corvallis, Oregon. He is a courtesy Professor in the Department of Botany and Plant Pathology and the Center for Genome Biology and Biocomputing at Oregon State University. He received his Ph.D. in plant pathology from the University of California at Davis and conducted postdoctoral research at Cornell University. His principal research interests include the ecology, genetics and management of emerging and re-emerging Phytophthora diseases affecting ornamental and nursery crops with a special emphasis on the Sudden Oak Death pathogen Phytophthora ramorum and the Irish famine pathogen P. infestans. More recently, he has started working on projects involving oomycete biodiversity, whole genome sequencing of the genus Phytophthora, and development of computational and bioinformatics tools for comparative genomics, genotyping-by-sequencing, population genomics and metabarcoding. He is currently very interested in developing cyberinfrastructure for characterizing emerging plant pathogens using high throughput sequencing in real-time. Grünwald has served as associate editor, senior editor and editor-in-chief for Phytopathology, editor for Plant Pathology, and currently serves as founding editor-in chief for CABI Agriculture and Bioscience and PhytoFrontiers. He has held numerous leadership positions including chair of the APS Publications Board overseeing all APS journals that launched the new Phytobiomes open access journal. He is a recipient of the 2006 USDA ARS Early Career Scientist of the Year award, the 2007 APS Syngenta award, the 2015 APS Ruth Allen Award recognizing outstanding, innovative research contribution that have changed the direction of research in any field of plant pathology, and became APS fellow in 2016 and AAAS fellow in 2019.

Carrie Harmon has twenty years of clinical plant pathology experience. She earned her B.S. in plant and soil sciences from the University of Massachusetts, her M.S. in plant pathology from Purdue University, and her PhD in plant pathology from University of Florida. Dr. Harmon leads an international plant diagnostic program at the University of Florida that encompasses clinical pathology, diagnostic research, and education, and serves as the regional center of the Southern Plant Diagnostic Network. Dr. Harmon is the current Executive Director of the National Plant Diagnostic Network, which supports quality diagnostics, laboratory accreditation, and assay development and validation.

Susan Harper currently serves as a special scientific advisor at the United States Department of Agriculture (USDA) Beltsville Agricultural Research Center in Beltsville, MD. She earned her B.S. in agriculture at West Virginia University and a D.V.M. at Louisiana State University, and started her professional career in food animal medicine. She practiced for 8 years before enrolling in a post-doctoral residency and masters degree program at the Penn State University College of Medicine, where she continued to serve as an assistant professor for 2 years following graduation. She left academia to pursue a career with the federal government and has served in a variety of research, clinical, policy, and regulatory roles at NIH, FDA, U.S. Department of Veterans Affairs, and USDA. Dr. Harper has achieved board certification in the American College of Laboratory Animal Medicine and the American College of Veterinary Preventative Medicine, and certification as a registered biosafety professional through the American Biological Safety Association. She also serves on AAALAC Council, the National Research Council Standing Committee for the Care and Use of Animals in Research, and is an active member of numerous scientific and professional organizations associated with veterinary medicine, laboratory animal welfare, research safety, and biosecurity.

Hon S. Ip, B.S. (University of Toronto, Microbiology and Parasitology), M.S. (University of Toronto, Zoology), Ph.D. (Rockefeller University, Molecular Biology), is a research scientist and directs the Diagnostic Virology Laboratory at the U.S. Geological Survey – National Wildlife Health Center. Dr. Ip's laboratory works on emerging viral diseases of wildlife including rhabdoviruses in amphibians, reovirus in bats, poxvirus in penguins, herpesvirus in whopping cranes, paramyxovirus in eiders as well as avian influenza and Newcastle Disease viruses. Dr. Ip help create the largest ever surveillance program on wild birds for the Department of the Interior for the detection of avian influenza viruses. It was his laboratory that detected the introduction of highly pathogenic avian influenza into the US. His laboratory has recently focused on coronaviruses of wildlife including experimental and surveillance programs for SARS-CoV-2. His laboratory has identified a diverse array of other coronaviruses in mink farms in the US that might pose future zoonotic threats. His laboratory has been designated by USDA for the diagnosis of Rabbit Hemorrhagic Disease Virus 2 (RHDV2) in wild lagomorphs. In addition to on-going collaborations with the CDC and USDA on One Health projects, a major focus of the laboratory is to seek a better understanding of the relationships between viral genetics and their spatiotemporal distribution in wildlife. He and his colleagues has published over 80 papers on wildlife viral diseases.

Scott A. Isard, Ph.D., is an Emeritus Professor of Aerobiology in the Department of Plant Pathology and Environmental Microbiology and the Department of Meteorology and Atmospheric Sciences at the Pennsylvania State University. Dr. Isard received his Ph.D. in Geography from Indiana University. He is the lead author of the book "Flow of Life in the Atmosphere: An Airscape Approach to Understanding Invasive Organisms". Research interests include Biological and meteorological factors that govern the aerial movement of biota; Aerobiology, integrated pest management, food safety, and biosecurity; and Developing and deploying generic models for agricultural pests.

Kathleen Kosta, with a Bachelor of Science from California Polytechnic State University, San Luis Obispo, has worked as a plant pathologist for 38 years. From 1981 through 1988 she was a lab technician in the California Department of Food and Agriculture(CDFA), Plant Pathology Laboratory which included providing technical help to the California Department of Forestry. From 1989 through 1995, she served as the Nevada State Plant Pathologist for the Nevada Department of Agriculture, working statewide with farmers, foresters, Cooperative Extension and landscape professionals. As State Pathologist, Kathy completed all field and lab work required for plant disease diagnosis, helped develop policy and programs, presented informational seminars and training sessions, as well as represented the state in regulatory issues. From 1996 to 2006, she returned to CDFA as Detection Field Plant Pathologist for CDFA, working closely with California County Agriculture Commissioners, inspecting postentry quarantined plant materials, nursery stock, field inspections and provided training on all aspects of plant diseases. Forestry disease issues were also part of her responsibility. In 2006, Kathy accepted the position of Primary State Plant Pathologist for CDFA serving as advisor and representative of the Department on issues concerning all plant diseases, but continued to work in the field when necessary, particularly on Sudden Oak Death, caused by the pathogen Phytophthora ramorum. In early 2012 she changed positions to work exclusively on that disease, and other newly emerging pathogens. She started the statewide Best Management Program for Ornamental and Native Plant Nurseries and also served as the CDFA/USDA liaison to the National Ornamental Research Site at Dominican University of California in San Rafael, CA. Recently retired, she plans to continue working with people on plant problems and best management practices for landscape industry related businesses.

Joseph LaForest is Associate Director for the University of Georgia's Center for Invasive Species and Ecosystem Health (Bugwood) and leads the Integrated Pest Management (IPM) and Forest Health programs. He joined University of Georgia in 2006 and has focused on developing resources for educating all audiences on IPM and plant biosecurity as well as using technology to enable more effective use and distribute information. He leads system development for various databases and interfaces used by the center for organization and delivery of images, video, presentations, occurrence data, maps, and other user created content. He also serves as Co-Director for the Southern IPM Center and coordinates the Facilitation of Innovation Through Technology (FITT) initiative to encourage communication between systems focused on IPM and Plant Biosecurity while also leveraging the capabilities of each system to maximize the benefit to all stakeholders. In concert with the extension and leveraging of digital resources, he has begun work to improved impact evaluation of digital resources as well as structural analysis of content delivery and exchange networks.

Douglas Luster completed a B.S. Marine Biology/B.A. Chemistry at the University of North Carolina-Wilmington and a Ph.D. in Botany from North Carolina State University. He was a postdoctoral Research Associate at George Washington University and at the former ARS Photobiology Laboratory in Beltsville, MD. Dr. Luster is a Research Plant Physiologist at the ARS Foreign Disease-Weed Science Research Unit (FDWSRU) at Ft. Detrick, MD, and served as Research Leader of that unit (1997-2015) and of the ARS Beneficial Insect Introduction Research Unit, Newark DE (2009-2016). He has participated in numerous activities and committees addressing biosecurity at USDA BSL-3 laboratories, agricultural biosecurity, anti-crop bioterrorism, and plant pathogen threat rating. He initiated the plant pathogen Select Agent biosafety and biosecurity program at FDWSRU while serving as Responsible Official, and currently serves as Quarantine Officer and Alternate Responsible Official at the lab. His research at Ft. Detrick centers on proteomics/protein expression in host-pathogen systems and development of detection immunoassays for emerging plant pathogens. He recently licensed diagnostic reagents for the emerging plant pathogen Phakopsora pachyrhizi, causal agent of Asian soybean rust, to a commercial diagnostics company.

Jerry R. Malayer, Ph.D., is Professor and McCasland Chair in the Department of Physiological Sciences in the College of Veterinary Medicine and Adjunct Professor in the Department of Biochemistry and Molecular Biology at Oklahoma State University. He received BS and MS degrees in Animal Science from Purdue University, the PhD in Animal Science from the University of Florida, and received a National Research Service Award from NIH while a postdoc in the Biochemistry Department at the University of Wisconsin. Malayer is currently the Senior Associate Dean for Research in the College of Veterinary Medicine at Oklahoma State University. He serves on several state-wide councils and committees, including the Oklahoma Governor's Science and Innovation Council and the Health Research Committee of the Oklahoma Center for the Advancement of Science and Technology.

Beatriz Martínez-López, DVM, MPVM, PhD, is professor of infectious disease epidemiology at the Department of Medicine & Epidemiology, Veterinary School, UC Davis and Director of the Center for Animal Disease Modeling and Surveillance (CADMS) since January 2014, a recognized FAO Reference Center for Veterinary Epidemiology. She has more than 100 publications related with the development and implementation of quantitative methods such as epidemiological modeling, risk assessment, geostatistical methods or network analysis to unravel complex epidemiological problems at the wild-domestic-human interface. Currently, she is leading the development, implementation and validation of novel Big Data analytical and visualization tools and their integration into operational, web-based, user-friendly platforms such as the Disease BioPortal (https://bioportal.ucdavis.edu/) to more timely support animal health decisions in livestock and aquaculture industries.

Alexander Mastin, Ph.D., is currently a Research Fellow in Ecological/Epidemiological Simulation Modelling in the School of Science, Engineering and Environment at the University of Salford, Manchester, UK. Dr. Mastin received his Ph.D. in parasite ecology at the University of Salford after studying veterinary science at the University of Liverpool and a master's degree course in veterinary epidemiology at the Royal Veterinary College and the London School of Hygiene and Tropical Medicine. Dr. Mastin since has transitioned into infectious disease epidemiology with a focus on the mathematical and statistical modelling of pathogens and parasites of humans, animals, and plants. His current research uses these models to examine and evaluate how best to conduct surveillance for these organisms: whether to declare absence, detect new incursions early, learn more about an emerging epidemic, or monitor the performance of control efforts. He is also interested in how plant health, animal health, and human health interact, and how epidemiological research within these disciplines can be unified.

Brian McCluskey, Ph.D., is currently Director of Business Development for Trace First Inc. He served in the USDA's Veterinary Services for 29 years before retiring in 2018. He received a Doctorate in Veterinary Medicine from Washington State University in 1987, a Masters in infectious diseases from the University of Florida in 1994 and a PhD in Epidemiology from Colorado State University in 2003. He joined the USDA in 1990 after 3 years in large animal practice and held numerous positions in Veterinary Services including Area Epidemiology Officer, Dairy Commodity specialist, Director of the National Surveillance Unit, Western Region Director, Chief Epidemiologist and Associate Deputy Administrator. He is a Diplomate in the American College of Veterinary Preventive Medicine and holds an affiliate faculty position at Colorado State University. He has published frequently on infectious disease epidemiology and animal health surveillance.

Barry Meade is a native of eastern Kentucky and a 1986 graduate of the Louisiana State University School of Veterinary Medicine. He began his professional career in a mixed animal practice in central Kentucky before joining USDA in 1989. Since then, Dr. Meade has served as a Veterinary Medical Officer (VMO) in Arkansas, and as an Epidemiologist in Florida and Kentucky. In 1992, Dr. Meade was a participant in the Centers for Disease Control and Prevention (CDC) Epidemic Intelligence Service (EIS) program and, more recently, he completed a PhD Veterinary Sciences with an emphasis in epidemiology through the Maxwell H. Gluck Research Center at the University of Kentucky. He has worked internationally in Mexico, Namibia, Canada, the United Kingdom (UK), Grenada and, most recently, Brazil. He served as a technical expert for bovine tuberculosis, avian influenza, and as the USDA lead for assistance with the eradication of Classical Swine Fever (CSF) in the UK. He has conducted in-country audits to evaluate fitness for trade and to assist VS International Trade and Capacity Building (ITRCB) staff within APHIS International Services with the identification of avenues to improve animal health in the Caribbean. Currently, Dr. Meade is in Raleigh, North Carolina and serves as the Assistant District Director for North Carolina and South Carolina.

Heike Meissner is the Associate Executive Director of the Science and Technology (S&T) arm of the United States Department of Agriculture's Plant Protection and Quarantine. Prior to her current position, she served for 3 years as the Director of Plant Pest Risk Analysis in PPQ S&T. She has over 20 years of professional experience in applied phytosanitary and pathway risk analysis, as well as several years of experience in invasive species research. Dr. Meissner's projects, which have earned her recognition within the U.S. federal government and internationally, have taken her on visits of ports of entry, production areas, treatment facilities, and packing houses in 15 countries. Dr. Meissner earned her PhD in Entomology from North Carolina State University and her MS degree in Biology from the University of Würzburg in Germany.

Jeffrey Morisette works at the Rocky Mountain Research Station in Fort Collins as the manager of the Human Dimensions program; which integrates social, economic, and ecological dimensions of resource management. He has over 20 years experience working in the Federal government spanning a wide portfolio, including geospatial analysis, invasion biology, climate change, and collaboration on policy and planning activities. Jeff started his career with 10 years at NASA Goddard Space Flight Center. In 2008, he moved to the U.S. Geological Survey to lead the Invasive Species branch at the USGS Fort Collins Science Center. From 2012 to 2017 he was the director of the Department of the Interior North Central Climate Science Center, which focused on combining climate science, ecology, and human dimensions to inform climate adaptation and mitigation strategies on federally managed lands. Most recently he has served as the Chief Scientist to the National Invasive Species Council. There he worked collaboratively with federal and university research communities to have their efforts inform invasive species policy, planning, and actions. Jeff received a Bachelor of Art from Siena Heights University, a Master of Science in Applied Statistics from Oakland University, and a PhD in Forestry from North Carolina State University.

Randall Murch, PhD, recently retired from Virginia Tech where he spent 16 years and held three different appointments. In January 2021, he was asked to return to the university to participate in a new center in the College of Agriculture and Life Sciences. Prior to his time at Virginia Tech, he was a Research Staff Member, Institute for Defense Analyses, a leading Federally Funded Research and Development Center, located in Alexandria, Virginia. From January 1980 – November 2002, he was employed as a Special Agent and Senior Executive, Federal Bureau of Investigation, where he served in both field and Headquarters assignments. A major component of his FBI career was 10 years in the FBI Laboratory as a forensic practitioner, researcher, department head and deputy director. During his service in leadership in the FBI Laboratory, he created the national program in Weapons of Mass Destruction forensic program which subsequently established the U.S. capability in microbial, chemical, radiological and nuclear forensics. These capabilities, which are extensive in the U.S. and a number of other countries, support investigations and attribution decisions for criminal, national security and policy decision-making. He was one of the leaders who established the new discipline of microbial forensics, for which he has an extensive publication and presentation record as well as other topics. Dr. Randall Murch has a B.S. in Biology (University of Puget Sound), M.S. in Botanical Sciences (University of Hawai'i at Manoa) and PhD in Plant Pathology (University of Illinois at Urbana-Champaign).

Alison Neeley has been an assistant director with the US Department of Agriculture's Plant Pest Risk Analysis unit since 2016. She oversees a diverse portfolio of pathway, spread, and economic analyses dealing with the risks associated with exotic plant pests. Alison holds graduate degrees in entomology from the University of Florida and economics from North Carolina State University. She began her career with USDA in 2002, and in 2004 she joined the Plant Pest Risk Analysis staff where she prepared qualitative and quantitative pest risk assessments and worked on the team that developed new guidelines for conducting commodity import risk assessments. During her tenure she also developed a new method for prioritizing which plant pests should be targeted for early detection surveys, and routinely developed and delivered training materials and presentations on risk analysis for domestic and international audiences.

Francisco Ochoa Corona received his Ph.D. from the University of Florida and is a forensic plant pathologist, specializing in developing and delivering reference diagnostics for exotic, naturalized, and indigenous plant viruses and other phytopathogens of relevance to agricultural biosecurity and microbial forensics. He then served as Principal Advisor, Virology, of the Investigation and Diagnostic Centre (IDC) at Biosecurity New Zealand (BNZ), Ministry of Agriculture and Forestry (MAF), in Auckland, NZ. His work is applicable to plant pathogens that can be intercepted at borders or detected by general surveillance of field settings or within transitional facilities. His current research includes the adaptation and development of novel tools for sampling, pathogen detection, discrimination, and diagnostics. Of particular interest are plant viruses and phytopathogens used as surrogates in biomedicine, prediction of biosecurity threats, monitoring of the disease dynamics of relevant plant pathogens in agriculture, tracking of their global dispersal routes, and delimiting their bio-geographic distribution.

Stephen Parnell, Ph.D., is a plant disease epidemiologist and modeller at the University of Salford in Manchester UK. Dr. Parnell holds a B.Sc. (Hons) in Ecological Science from the University of Edinburgh and a Ph.D. in Plant Disease Epidemiology from the University of Cambridge. Dr. Parnell conducted Postdoctoral work at the US Department of Agriculture, Agricultural Research Service (USDA-ARS) in Florida where he modelled the invasion and spread of exotic pests and diseases of citrus, working closely with the USDA, Animal and Plant Health Inspection Service (APHIS) on surveillance strategies for early detection of invading epidemics. He then spent seven years as a Research Scientist and epidemic modeller at the agricultural research institute Rothamsted Research in the UK before moving to Salford where he is now Reader in Spatial Epidemiology. Dr. Parnell is a member of the European Food Safety Authority (EFSA) Plant Health Panel and a member of the EFSA Working Group on Plant Health Surveillance. He is a current Board member of the British Society for Plant Pathology (BSPP) and former Senior Editor of Phytopathology.

Craig Phillips is a senior scientist in the Weeds, Pests & Biosecurity Team at AgResearch where he has worked for more than 30 years. Much of his research contributes to a New Zealand national research collaboration called Better Border Biosecurity (www.b3nz.org.nz) which aims to reduce the rate that nonnative pests of terrestrial plants are becoming established in New Zealand.

Katie Portacci is a veterinary epidemiologist with over 15 years of risk analysis experience. Her focus is the evaluation of mitigation options for chronic diseases such as tuberculosis and brucellosis in cattle. She has a passion for teaching and continues to teach risk analysis courses for the World Organisation for Animal Health (OIE). Dr. Portacci also collaborates on the development of national scale cattle movement and disease simulation models to assist with epidemiologic investigations, emergency response planning, and optimizing surveillance for both chronic and highly contagious diseases.

Akhilesh Ramachandran, BVSc & AH, PhD, DACVM, earned his BVSc & AH from Kerala Agricultural University in India and his PhD from Oklahoma State University. Dr. Ramachandran's research focuses on diagnostic microbiology and he serves as the section head for microbiology and molecular diagnostics at the Oklahoma Animal Disease Diagnostic Lab. He is studying the antibiotic resistance pattern of common veterinary and zoonotic bacterial pathogens and evaluating the genomic profile of common pathogens for source attribution studies.

Marta Remmenga has a Ph.D. in Statistics from Kansas State University. She taught statistics at both the graduate and undergraduate level at New Mexico State University and served in the University Statistics Center providing statistical consulting services to students and faculty university-wide for 15 years before taking her current job. Marta now works in the area of surveillance to support animal health with USDA-APHIS Veterinary Services.

Joe Russo is a trained agricultural meteorologist who specializes in modeling the effects of weather on agricultural systems. He spent most of his career running a business which provided weather-based simulations in support of university, government, and industry projects and services. Dr. Russo has extensive experience in predicting the movement and development of invasive species at multiple spatial and temporal scales.

David Schimmelpfennig is the Director of the Phytosanitary Advanced Analytics Team at the Animal and Plant Health Inspection Service's (APHIS), Plant Protection and Quarantine division, in the U.S. Dept. of Agriculture (USDA). Formerly, he was the information economics program leader at USDA's Economic Research Service and a senior Fulbright Scholar at the University of Pretoria, South Africa. His research interests are in crop pest early-warning systems, precision agriculture, and private agricultural research. He has been a principal investigator on Rockefeller Foundation projects on pest resistant crops in South Africa. His work has been featured in National Geographic, Smithsonian's National Museum of American History, and Science Magazine. David has a Ph.D. in Economics from Michigan State University.

Poonam Sharma is a Research Assistant Professor and Validation Science Coordinator at the Institute of Biosecurity and Microbial Forensics at Oklahoma State University, USA. She earned her B.S. in Biochemistry, Microbiology, and Chemistry from Rani Durgavati Vishwavidyalaya (Jabalpur, India), her M.S. in Biochemistry from University of Pune (Pune, India), and PhD in Infectious diseases from Aix-Marseille University (Marseille, France). Dr. Sharma has experience working on recombinant protein expression and purification, lung and gut microbiota of cystic fibrosis patients with emphasis on multi-drug resistant bacteria, development of NGS-based diagnostic techniques to study poultry-related viruses, unique characteristics and trends on antibiotic resistance and mechanisms (genes and mobile genetic elements), comparative genomics, functional characterization, and phylogenetic studies of foodborne pathogens. Dr. Sharma is interested in genome sequence analysis to decipher genetic determinants associated with specific phenotypes and to study the antibiotic resistance and virulence mechanisms to understand bacterial/viral pathogenesis and evolution.

Kevin Spiegel graduated from the University of Georgia with a DVM and MPH in 2019.He has worked with the USDA since 2020 helping to protect US agriculture.

Tim Widmer is the USDA/ARS National Program Leader for Plant Health. He is the point of contact for all USDA/ARS projects related to plant health and issues related to crop agbiosecurity. He has been with USDA/ARS since 2000, working previously as a research plant pathologist in Montpelier, France and Ft. Detrick, Maryland. Dr. Widmer received his PhD in Plant Pathology at the University of Florida, Gainesville, Florida.

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