

Almonds

BOTANY, PRODUCTION AND USES

Edited by

Rafel Socias i Company and Thomas M. Gradziel



EBSCO Publishing : eBook Collection (EBSCOhost)
printed on 2/13/2023 12:47 PM via
AN: 2416078 ; Rafel Socias i Company, Thomas M
Gradziel.; Almonds : Botany, Production and Uses
Account: ns335141

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and

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British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library.

Library of Congress Cataloging-in-Publication Data

Names: Socias i Company, R. (Rafel), editor. | Gradziel, T. (Thomas), editor.

Title: Almonds : botany, production and uses / edited by Rafel Socias i Company and Thomas M. Gradziel.

Description: Boston, MA : CABI, [2017]

Identifiers: LCCN 2017002056 (print) | LCCN 2017008640 (ebook) | ISBN 9781780643540 (hardcover : alk. paper) | ISBN 9781780643557 (epdf) | ISBN 9781786392633 (epub)

Subjects: LCSH: Almond.

Classification: LCC SB401.A65 A45 2017 (print) | LCC SB401.A65 (ebook) | DDC 634/.55--dc23

LC record available at <https://lccn.loc.gov/2017002056>

ISBN: 978 1 78064 354 0 (hardback)
978 1 78064 355 7 (e-book)
978 1 78639 263 3 (e-pub)

Commissioning editor: Rachael Russell

Associate editor: Alexandra Lainsbury

Production editors: Lauren Povey/Alan Worth

Typeset by SPi, Pondicherry, India.

Printed and bound in the UK by CPI Group (UK) Ltd, Croydon, CR0 4YY.

Contents

About the Editors	vii
Contributors	ix
Preface	xii
Dedications	xiv
1 Taxonomy, Botany and Physiology	1
<i>Rafel Socías i Company, José M. Ansón and María T. Espiau</i>	
2 History of Cultivation	43
<i>Thomas M. Gradziel</i>	
3 Production and Growing Regions	70
<i>Thomas M. Gradziel, Robert Curtis and Rafel Socías i Company</i>	
4 Almond in the Southern Hemisphere	87
<i>Michelle Wirthensohn and Luis Iannamico</i>	
5 Classical Genetics and Breeding	111
<i>Ignasi Batlle, Federico Dicenta, Rafel Socías i Company, Thomas M. Gradziel, Michelle Wirthensohn, Henri Duval and Francisco J. Vargas</i>	
6 Molecular Breeding and Genomics	149
<i>Carolina Font i Forcada, Raquel Sánchez-Pérez, Iban Eduardo, Shu-Biao Wu and Ángel Fernández i Martí</i>	
7 Late-blooming Cultivar Development	168
<i>Federico Dicenta, Raquel Sánchez-Pérez, Ignasi Batlle and Pedro Martínez-Gómez</i>	
8 Pollen–Style (In)compatibility: Development of Autogamous Cultivars	188
<i>Rafel Socías i Company</i>	
9 Rootstock Development	209
<i>María J. Rubio-Cabetas, Antonio J. Felipe and Gregory L. Reighard</i>	

10	Propagation Techniques	228
	<i>María J. Rubio-Cabetas, Antonio J. Felipe and Mireia Bordas</i>	
11	Orchard Management	240
	<i>Octavio Arquero and Katherine Jarvis-Shean</i>	
12	Environmental Requirements	254
	<i>José M. Alonso</i>	
13	Almond Water Requirements	279
	<i>David Doll</i>	
14	Almond Tree Nutrition	291
	<i>Saiful Muhammad, Sebastian Saa, Sat Darshan S. Khalsa, Steve Weinbaum and Patrick Brown</i>	
15	Almond Diseases	321
	<i>Ana Palacio-Bielsa, Mariano Cambra, Carmen Martínez, Antonio Olmos, Vicente Pallás, María M. López, James E. Adaskaveg, Helga Förster, Miguel A. Cambra, Henri Duval and Daniel Esmenjaud</i>	
16	Almond Pests	375
	<i>Frank G. Zalom, Eva Núñez and Roger A. Baldwin</i>	
17	Almond Harvesting	406
	<i>José L. Espada Carbó and Joseph H. Connell</i>	
18	Chemical Composition of Almond Nuts	428
	<i>Ossama Kodad</i>	
19	World Almond Market	449
	<i>Ned T. Ryan</i>	
20	Processing and Industrialization	460
	<i>Alexis Verdú, Santiago Izquierdo and Rafel Socias i Company</i>	
	Index	483

About the Editors



Rafel Socías i Company is Ingeniero Agrónomo (ETSIA Valencia, 1970), Diplomé en Horticulture (IAM Zaragoza, 1972), Master of Science (University of California Davis, 1974) and Dr Ingeniero Agrónomo (ETSIA Madrid, 1977).

His research has been focused mainly on almond pollination and breeding with the aim of obtaining self-compatible almond cultivars, with late-blooming time and kernels of high quality. He also has been involved in almond rootstock breeding, in developing and maintaining fruit germplasm, and fruit bibliography.

After collaborating with Dr Antonio J. Felipe in the release of 'Guara', his main releases have been 'Belona' and 'Soleta' in 2005, 'Mardía' in 2007 and most recently 'Vialfas'; he has also collaborated in the release of the rootstocks 'Felinem', 'Garnem' and 'Monegro'.

He has been a researcher at the Fruit Growing Department of the present day CITA of Aragón, serving as its head between 1997 and 2013, and is now a Doctor ad honorem of that organization.



Thomas M. Gradziel received a Master of Science degree in 1980 and a PhD degree in 1987, both in plant breeding, from Cornell University. He is a professor and geneticist in the Department of Plant Sciences, University of California at Davis, where his responsibilities include the genetic improvement of almond and processing peach. This breeding programme is among the oldest and largest in the world for these crops and has released numerous commercial varieties and rootstocks which are widely planted in California and internationally. Almond releases include the varieties 'Winters', 'Sweetheart' and 'Kester' as well as the rootstock 'Nickels'. He currently holds the L.D. Davis Professorship, which was established to promote the application of the appropriate biotechnologies to tree crop improvement. Breeding methods employed in his programme include wild germplasm collection, characterization and introgression, epigenetic selection, intra- and inter-specific hybridization, and marker-assisted selection and breeding.

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Preface

From ancient to modern times, almond has been proved to be adaptable and versatile. Almond is an ancient crop, referenced in Greek mythology and the Old Testament. An adaptation to harsh climates combined with an ability to develop a deep and extensive root system has allowed almond to exploit a wide range of ecological niches from dryland, seedling orchards in the Zagros Mountains of Iran where it originated, to the irrigated, highly productive Central Valley clonal orchards of California. Almond is well suited to the mild winter and dry, hot summer conditions typical of Mediterranean climates due to its low chilling requirement, rapid early shoot growth and high tolerance to summer heat and drought. It is the earliest temperate tree crop to flower, when its spectacular bloom after an often dreary winter has made it a symbol of hopefulness worldwide.

The tree can be highly productive and yields a flavourful, highly nutritious and versatile nut, whose easy transportability and long storability facilitate its global dissemination today as in prehistoric times. Its consumption has been extensive in all growing regions, but less well known in non-producing areas, with the exception of northern Europe, where it has been long appreciated in the cuisines of the Germanic and Scandinavian countries. The recent expansion of global trade has opened new markets to almond, and world demand has increased steadily in the past decade; this, in turn, has fostered new plantings and increased production. Despite decreased production in most traditional growing regions, global production has more than doubled in the past 20 years. Most of the increase in production has occurred in California, where a doubling in crop area combined with a tripling of per hectare productivity has occurred.

Demand has been further promoted by ongoing studies demonstrating health benefits from regular almond consumption. Almond has become not just a tasty snack and a delicious ingredient of many confectioneries, but is also a source of compounds important for maintaining healthy metabolic function, as detailed in Chapter 18, because of its high levels of antioxidants, fibre and minerals. There is also a high proportion of oleic acid in the lipid fraction of the almond kernels. Consequently, almond has evolved from a convenient snack food to an important member of an emerging food group increasingly recognized as essential for good health. Almond kernels have historically been recognized as a source of high nutritional and medicinal value, with references in the old medical treatises of Greece, Persia, China and India. Recent studies have documented a wide range of health benefits including possible protection from cancer, obesity, diabetes and heart diseases.

The high nutritive value of almond kernels arises partly from their high lipid content. This constitutes an important source of calories, but does not contribute to cholesterol formation in humans due to a high level of unsaturated fatty acids, mainly monounsaturated fatty acids. While high in calories, consumption is moderated by a high satiety value. In addition, the high protein fraction of almond kernels is of very good quality and readily assimilated.

The popularity of almond and the associated increased demand has required new plantings. Ongoing changes in cultural and climate conditions continue to test almond's versatility as farming systems adapt to the realities of the 21st century. Available resources include a diverse germplasm in regionally cultivated almond and wild species, a well-documented history of cultural innovation in an array of diverse environments, a genome amenable to genetic improvement and a global network of disciplinary expertise pushing the boundaries of what is possible. Many of these new plantings include new cultivars released by modern breeding programmes targeting emerging problems of almond production as well as increased kernel quality. The breeding of self-compatible cultivars has transformed this previously self-incompatible crop, which hitherto required multi-cultivar orchards and supplementary pollinators. Both classical and modern breeding programmes are now being combined to further improve productivity, particularly in the fields of pest and disease resistances. New cultural methods, such as high density plantings, will also be required to realize the full promise of these new production systems. Consequently, new cultivars as well as new rootstocks will need to be developed.

At present, almond is at an exciting turning point, where the production of almond kernels of very high organoleptic and nutritional quality, and produced under sustainable methods, will be required to satisfy the increasing global demand. This volume explores and advances these opportunities.



Dedications

Antonio J. Felipe



Antonio José Felipe Mansergas served as Research Scientist in the present-day CITTA, Aragón, from 1965 until his retirement in 1998. There, he initiated the first fruit breeding programme in Spain in 1974, which became a very successful almond breeding programme. He also initiated a successful breeding programme for stone fruit rootstocks. Both programmes have had significant impacts in the Spanish almond industry as well as in other sectors of the Spanish horticultural sector, such as the peach industry. His cultivars and rootstocks have reached other countries, particularly Argentina and northern Africa. He also served as Head of the Fruit Department at the CRIDA-03 of

INIA, now CITA (1977–1980), and as Coordinator of the National Fruit Research Programme of INIA (1979–1982).

Antonio was born in Zaragoza in 1933 and earned his university degree at the Agricultural School of the University of Madrid. After a few years of work for the Agriculture Ministry in Teruel, he followed the postgraduate horticultural course of CIDADE (Centro de Investigación y Desarrollo Agrario del Ebro) and trained abroad at the Istituto di Coltivazione Arborea of Florence, Italy, and then at the École Nationale d'Horticulture, Versailles, France.

His research work has focused on almond and stone fruit rootstocks, including germplasm collection, cultivar research, plant propagation, graft compatibility, plant breeding and clonal selection. During this time he has been the recipient of many Spanish and European research grants. His almond research has been pioneering as it was the first long-term dedicated breeding effort in Spain. His earlier research examined the low productivity of the Spanish almond orchards of the time, which, he concluded, was mainly due to the occurrence of frosts and deficient pollination. Consequently, his research focused on the search for self-compatible and late-blooming cultivars, as well as for vigorous and resistant rootstocks for better adaptation to different soil conditions.

Antonio established the first almond collection in Spain, representing worldwide almond variability. This was the origin of the present-day almond germplasm repository, and was the model for the Spanish and European Cultivar Register. Because of its extensive variability, this collection has inspired a large number of subsequent studies. Opportunities for self-compatible genotypes and crop improvement in general were studied, with the first crosses of the breeding programme being made in 1974. The first releases from this programme were made in 1987 and included 'Guara', a virus-infected clonal selection of unknown origin which, once cleaned, showed an exceptional agronomic performance. Cultivation of 'Guara' has increased, and it now represents more than 50% of new almond acreage, providing an effective solution for many problems affecting the early industry. 'Guara' has probably been the most successful plant cultivar in Spanish agriculture and established Antonio's expertise in this area. After I joined the almond breeding programme, other cultivars with varying degrees of success were released.

Antonio's research into stone fruit rootstocks initially focused on pollizo plums (*Prunus insititia* L.) with their low suckering, adaptation to heavy soils and graft compatibility with almond. Later it progressed to the selection of peach × almond hybrids, such as the G×N series, most notably 'Garnem', which demonstrated easy propagation, vigour, adaptation to calcareous soils, resistance to root-knot nematodes and red leaves for easier nursery management. His interest in rootstocks culminated in the book *Patrones para Frutales de Pepita y Hueso* (Rootstocks for pome and stone fruits) (Ediciones Técnicas Europeas SA, Barcelona, Spain), recognized as the best Spanish agricultural book of 1989.

Antonio has been involved in many other activities related to his work, including teaching the postgraduate courses of the IAMZ (Instituto Agronômico Mediterrâneo de Zaragoza), organizing many scientific meetings including the 5th National Congress of the Spanish Society for Horticultural Science, cofounding the Groupe de Recherche et d'Études Méditerranéen pour l'Amandier (GREMPA) and providing leadership to many scientific congresses. He – and the two other almond researchers to whom this book is dedicated – clearly showed the three distinctive features of a true agricultural researcher: humility, being conscious of how much he did not know; openness, in sharing his knowledge with everybody; and adaptability, being equally capable of giving a lecture at an international congress or a talk at a growers' meeting. I have always considered my opportunity of knowing and working with all three of these researchers as a great privilege. After his retirement he wrote a reference book for almond, *Almendo. I. El material vegetal* (Almond: I. The Plant Material) (Integrum, Lérida, Spain, 2000), frequently cited in the pages of this volume. Finally, he remains very busy in a family activity: the manufacture of miniature tools to display in doll's houses.

As a young researcher starting my professional career, I valued his thoughtful teaching, not only on almond, but on fruit growing in general. He has been and continues to be a great friend, and the finest example of what our work should be: dedicated service to the growers. Consequently, I fondly dedicate this book to him.

Rafel Socias i Company

Charles Grasselly



Charles Grasselly has had a long and distinguished career not just in his native France, but worldwide. He was first recruited to INRA Bordeaux in 1951 where he worked as assistant of René Bernhard. His early responsibilities included overseeing the peach rootstock breeding programme. Here, he developed methods for the successful vegetative propagation of the enormously successful peach $\times$ almond rootstock GF677, which was released as a peach rootstock in 1960. At the request of Jacques Souty, who was director of INRA (Institut National de la Recherche Agronomique) Bordeaux at that time, Dr Grasselly initiated the INRA almond breeding programme in 1955. Almond and almond improvement remained his primary focus for the rest of his life. It was also a career that was eminently successful. From his first crosses he obtained the three cultivars 'Ferragnès', 'Ferraduel' and 'Ferrastar', which have remained important French and Mediterranean almond cultivars ever since. Dr Grasselly also enjoyed great success in the collection and characterization of new germplasm for almond improvement. This effort involved collections from throughout almond native regions, while at the same time establishing a broad international network of colleagues and collaborators. As the collection became more extensive, it was transferred – along with the hybrid experimentation programme – to the experimental INRA farm in Manduel near Nîmes in 1964. This south-east region of France was the most important area for almond production. In 1971, working with Gilles Olivier, he discovered the self-compatibility of some of the Apulia cultivars, including 'Filippo Ceo', 'Tuono' and 'Genco'. This discovery greatly accelerated the selection of self-compatible almond types in the European almond breeding programmes. Dr Grasselly released 'Lauranne®' in 1989, which became the second most important almond cultivar in France after 'Ferragnès'.

Charles Grasselly was also a great advocate of collaborative research on almond. He participated in the first GREMPA meetings in 1972 and 1974 and subsequently became an active participant and leader of this group. In 1984 he moved his research to Avignon for the creation, with P. Crossa-Raynaud, of the new INRA unit Station de Recherches Fruitières Méditerranéennes. He subsequently organized the very successful 8th GREMPA meeting in Nîmes in 1990. Internationally, Charles Grasselly has enjoyed good and productive collaborations with Dale Kester from UC Davis and Antonio Felipe from CITA Zaragoza. His PhD thesis, published in 1972, represented the first comprehensive study of the heritability of important almond traits; and two technical books in the French language, *L'Amandier*, with P. Crossa-Raynaud (G.P. Maisonneuve & Larose, 1980) and another, *L'Amandier*, published by CTIFL (1997) remain important references to this day.

Along with Antonio Felipe and Dale Kester, Charles Grasselly has come to represent the best in ideals and practice in the genetic improvement of almond and associated rootstocks. He has become a valued colleague and teacher to many in the almond genetics community who strive to follow in his footsteps. This volume is dedicated to him in appreciation for his many contributions.

Henri Duval

Dale E. Kester



Dale Kester joined the Department of Pomology, University of California at Davis, in 1951, where his first responsibility was to take charge of the long-term almond breeding project. An early goal was to complete the evaluation of advanced almond selections as well as of the several thousand almond seedlings from previous controlled crosses. 'Davey' was jointly released from this project in 1953. A second programme to develop a smaller-sized version of the major almond cultivar 'Nonpareil' led to the release of 'Kapereil' (1963) and 'Milow' (1975). While all three cultivars met the requirements for which they were selected, none became widely planted. This early experience convinced Dr Kester that successful cultivar breeding depended upon a comprehensive understanding of the entire range of biological, cultural and marketing characteristics of almond. His subsequent efforts to achieve this breadth of understanding earned him international recognition as a world authority on almond genetics, culture and improvement. These efforts can be divided into three principal areas: the genetics and breeding of almond cultivars and rootstocks, the identification and characterization of clone degeneration with vegetative propagation, and teaching.

Dr Kester's rootstock evaluation and selection programmes included studies on almond/Marianna plum graft incompatibility and the development of peach × almond hybrid rootstocks. Three hybrid rootstocks, 'Hansen 2168', 'Hansen 536' and 'Nickels', have been released, with the last two attaining important commercial use. In collaboration with state-wide county extension personnel, a comprehensive system of cultivar evaluations in Regional Variety Trial plots was established throughout California. These have facilitated the more recent testing and introduction of new almond cultivars including 'Solano', 'Sonora', 'Padre' and 'Winters'.

A major effort, begun in 1954 and continuing throughout the rest of Professor Kester's career, was a series of genetic and physiological studies of noninfectious bud failure. Studies of variability within individual clones resulted in his strategies for clonal source selection for low bud-failure potential. Promising clonal sources (i.e. foundation sources for subsequent nursery vegetative propagation) were identified by a combination of individual tree source selection and subsequent vegetative progeny testing. The following years were dominated by genetic, physiological and epidemiological research into the problem of noninfectious bud failure and possible solutions. By the time of retirement in 1991, new low bud-failure potential clone sources of essentially all almond cultivars except for 'Carmel' had been identified and made available to growers. In 1989, a series of research initiatives in collaboration with department and extension colleagues both detailed a comprehensive model for the development of noninfectious bud failure in almonds, and also defined measures for its control. A key finding was the demonstration of predictable distribution patterns for this epigenetic factor following sexual as well as asexual reproduction. Nursery management practices introduced to control the resulting noninfectious bud-failure disorder included the identification and evaluation of elite source clone selections, and cultural management procedures for stabilizing source clones in subsequent propagations.

As a teacher, Dale Kester served as major professor to 16 MSc and PhD students and is remembered as a patient mentor whose keen insights and sangunity were always available. He published over 111 papers in refereed journals and conference proceedings. Dr Kester's first teaching assignment in the Department of Pomology was a course on plant propagation which led to a long and productive collaboration with Dr Hudson Hartmann, who had also been teaching plant propagation. In 1959, this collaboration resulted in the preparation of a textbook, *Plant Propagation, Principles and Practices* (Prentice-Hall, NJ, USA). This publication has now gone through eight editions, becoming the world standard text and reference in this field, with translations into Spanish, Russian and Italian. In addition, Dr Kester helped to found the Western Region of the International Plant Propagators' Society and served that organization in many capacities including Western Region President in 1997. He also served as the first Chair of the Propagation Working Group of the American Society of Horticultural Sciences. He was a participant in many international symposia on almond, tissue culture and breeding, and was recognized throughout the world for his expertise in these areas. In 1980 he received the Stark Award for pomological research and in 1997 Dr Kester became a Fellow of the American Society for Horticultural Sciences. In recognition of his lasting contributions to pomological research and the education of horticulturalists throughout the world, he was honoured as the Spencer Ambrose Beach Lecturer at Iowa State University in 1998. Dale Kester was truly a scholar and a gentleman who devoted his life to science and who, until his death in 2003 at the age of 81, was a revered teacher in California and the world. He was my mentor and remains my model as both scientist and teacher, so it is with great pleasure that I dedicate this volume to him.

Thomas M. Gradziel

1 Taxonomy, Botany and Physiology

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1.1 Almond Taxonomy

Almond belongs to the genus *Prunus*, which includes all stone fruits, and belongs to the Rosaceae family. Almond is a diploid species with $n = 8$ (Darlington, 1930), the basic ploidy number of *Prunus*. In contrast to the other species of this genus, whose commercial interest lies in their juicy flesh or mesocarp, almond is the only *Prunus* species grown for its seeds, whereas the corky mesocarp is only utilized in animal feeding (Aguilar *et al.*, 1984) or as a manure (Alonso *et al.*, 2012). Thus, almond is often classified more as a nut than as a stone fruit, despite its very close genetic similarities with the other stone fruits, and mainly with peach.

The botanical name of almond has been a subject of diverse controversies and has been changing continuously since Linnaeus began botanical systematics. Although taxonomy aims at unambiguous identification of species, different subjective criteria have led to different classifications, despite the application of objective parameters for species description. Consequently, frequent cases of synonyms for almond and other related species have been – and continue to be – reported. Linnaeus considered *Amygdalus* as an independent genus, classifying the stone fruits in different genera, corresponding to the main crops:

almond (*Amygdalus*), peach (*Persica*), cherry (*Cerasus*), apricot (*Armeniaca*) and plum (*Prunus sensu stricto*). This division seems excessive, particularly if the possibilities of hybridization among different stone fruit species are considered. At present, most of these groups are considered subgenera of the genus *Prunus*, a single genus including all the stone fruit tree species, as the genus *Prunus sensu lato*.

According to Linnaeus' criteria, under which the taxa well known to him were excessively subdivided, almond was designated as *Amygdalus communis* L. (1753, Sp. Pl. 1: 473). This same denomination was also adopted by A.A. von Bunge as *A. communis* Bunge (1833, Enum. Pl. Chin. Bor. 21), although this name is invalid because it is recurrent. The first change was made by P. Miller, who adopted the denomination *A. dulcis* Mill. (1768, Gard. Dict., ed. 8. n. 2), without taking into account the presence of bitter kernels in almond and of sweet kernels in other *Prunus* species, thus making the *dulcis* designation equivocal.

The first inclusion of almond in the genus *Prunus* was made by A.J.G.K. Batsch with the denomination *P. amygdalus* Batsch (1801, Beytr. Entw. Gewächreich 1: 30). This name was widely accepted for a long time and was also adopted by J.S. Stokes as *P. amygdalus* Stokes

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(1812, Bot. Mat. Med. 3: 101), although this is also an invalid name because of recurrence. H.G.L. Reichenbach recovered the wrong denomination of *dulcis*, but in the genus *Prunus* under the name *P. dulcis* (Mill.) Rchb. (1832, Fl. Germ. Excurs. 644), a denomination reactivated more than a century later by D.A. Webb as *P. dulcis* (Mill.) D.A. Webb (1967, Feddes Repert. 74: 24). Finally, another illegal denomination was proposed by G. Arcangeli as *P. communis* (L.) Arcang. (1882, Comp. Fl. Ital. 209).

The same diversity of names, mainly due to consideration of whether or not *Amygdalus* is a genus or a subgenus, is found for the wild species related to almond (Grasselly, 1976; Browicz and Zohary, 1996). The most rational classification is that of Rehder (1940), which considered *Amygdalus* (L.) Focke as a subgenus under the genus *Prunus* L., although this classification was not fully adopted. Browicz and Zohary (1996) proposed a classification of the species related to almond considering *Amygdalus* as a genus. Subsequently, Socias i Company (1998) proposed a classification of all almond species under the subgenus *Amygdalus* (Box 1.1). Unity of the genus *Prunus* has been confirmed at the molecular level by the Genome Database for Rosaceae (2016) and the synteny of traits among all species (Arús *et al.*, 2006).

The most recent phylogenetic studies consider that all almond and peach species must be considered as forming the subgenus *Amygdalus* of the genus *Prunus* (Yazbek and Oh, 2013), despite the preference of some botanists to maintain *Amygdalus* as a genus, mostly based on morphological traits and the inconsistency of considering mesocarp dehiscence as a differential trait (Zohary, 1998).

1.2 Wild Almond Species

The interest in wild almond species emerged in the 19th century and was pursued mainly by botanists (Grasselly, 1976). However, observation of the diversity of fruit species in central Asia in the early 20th century, where not only wild almond species but also wild almond trees could be found (Popov *et al.*, 1929), led to a new interest in these species (see Chapter 2). The origin of the cultivated almond was attributed to

hybridizations involving several of these wild species due to the ease of crossing (Kovalyov and Kostina, 1935), following the theory of species evolution put forward by Vavilov (1930). Hybridizations are frequent among all species of this subgenus, giving rise to hybrid forms receiving a botanical name and creating some confusion about their real classification (Rehder, 1940; Browicz and Zohary, 1996). Among these species, *P. fenzliana* Fritsch, *P. bucharica* (Korsh.) Fedtsch, *P. kuramica* (Korsh.) Kitam. and *P. triloba* Lindl. may have been involved in natural hybridizations, giving rise to the current cultivated almond (Grasselly and Crossa-Raynaud, 1980; Kester *et al.*, 1990). Recent research with molecular markers has reinforced this previous hypothesis (Zeinalabedini *et al.*, 2010).

Wild almond species are mostly distributed in western and central Asia and eastern Europe (Fig. 1.1), although some isolated populations are found in other locations such as southern Mongolia and central China (Denisov, 1988) and central Spain (Felipe and Socias i Company, 1977). In these peripheral regions new hybridizations may have occurred, as could be the case of the wild Mediterranean species *P. webbii* (Spach) Vierh. (Godini, 1979; Socias i Company, 2004) when almond cultivation spread into the Mediterranean region, resulting in unique self-compatible populations found along the northern shore of the Mediterranean Sea from Greece and the Balkans to Spain and Portugal.

Interest in the wild almond species in scion and rootstock breeding emerged later when they were considered as possible sources for some interesting traits, such as self-compatibility, early ripening, vigour control, drought resistance, disease resistance and kernel composition (Kostina and Ryabov, 1959; Grasselly, 1977; Vlastic, 1977; Socias i Company, 1978; Denisov, 1988; Gradziel *et al.*, 2001; Gradziel, 2003). As a consequence, interest developed in maintaining some representatives of these species in research centres, not only as botanical samples, but also as a source of genetic variability and as possible donors of some traits (Grasselly, 1976). Seeds of some populations of these wild species had been introduced into the arboretum in Davis, California (Kester *et al.*, 1990) and in the collections of INRA, France (Grasselly, 1975);

Box 1.1. Classification of almond species (Socias i Company, 1998)

Genus: *Prunus* L. (1735)

Subgenus: *Amygdalus* (L.) Focke

Series: *Icosandrae* Spach (1843)

(Syn.: Subgen. *Amygdalus* Browicz et Zohary)

(i) Section: *Euamygdalus* Spach (1843)

(Syn.: Sect. *Amygdalus* Browicz et Zohary)

Group *Amygdalus*

1. *P. amygdalus* Batsch (1801)

(Syn.: *A. communis* L., *A. dulcis* Mill., *P. communis* (L.) Arcang., *P. dulcis* (Mill.) D.A. Webb). It includes *P. korshinskiyi* Hand.-Mazz.)

2. *P. trichamygdalus* Hand.-Mazz. (1913)

(Syn.: *A. trichamygdalus* (Hand.-Mazz.) Woronow)

3. *P. fenzliana* Fritsch (1892)

(Syn.: *A. fenzliana* (Fritsch) Lipsky)

4. *P. webbii* (Spach) Vierh. (1915)

(Syn.: *A. webbii* Spach, *A. salicifolia* Boiss. et Bal.)

5. *P. haussknechtii* C. Schneider (1905)

(Syn.: *A. haussknechtii* (C. Schneider) Bornm.)

6. *P. zabulica* (Seraf.) SIC, comb. nov. (1998)

(Syn.: *A. zabulica* Seraf.). It includes *A. browiczii* Freitag)

7. *P. kuramica* (Korsh.) Kitam. (1960)

(Syn.: *A. kuramica* Korsh.)

8. *P. bucharica* (Korsh.) Hand.-Mazz. (1913)

(Syn.: *A. bucharica* Korsh.)

9. *P. tangutica* (Batal.) Koehne (1912)

(Syn.: *A. tangutica* (Batal.) Korsh.)

Group *Orientalis*

10. *P. argentea* (Lam.) Rehd. (1922)

(Syn.: *A. orientalis* Duhamel, *A. argentea* Lam.)

11. *P. discolor* (Spach) C. Schneider (1905)

(Syn.: *A. graeca* Lindl., *A. discolor* (Spach) Roemer)

12. *P. elaeagnifolia* (Spach) E. Murray (1969)

(Syn.: *A. elaeagnifolia* Spach, *A. kermanensis* Bornm.)

13. *P. kotschyi* (Boiss. et Hohen.) Náb. (1923)

(Syn.: *A. kotschyi* Boiss. et Hohen.)

14. *P. carduchorum* (Bornm.) Meikle (1965)

(Syn.: *A. carduchorum* Bornm.)

15. *P. mongolica* Maxim. (1879)

(Syn.: *A. mongolica* (Maxim.) Ricker)

(ii) Section: *Chamaeamygdalus* Spach (1843)

16. *P. nana* (L.) Stokes (1812)

(Syn.: *A. nana* L., *P. tenella* Batsch). It includes *P. georgica* (Desf.) and *P. ledebouriana* (Schlecht.)

17. *P. petunnikovii* (Litv.) Rehd. (1926)

(Syn.: *A. petunnikovii* Litv.)

(iii) Section: *Spartioides* Spach (1843)

18. *P. arabica* (Olivier) Meikle (1967)

(Syn.: *A. arabica* Olivier, *A. spartioides* Spach, *P. spartioides* (Spach) C. Schneider)

Continued

Box 1.1. Continued.

19. *P. scoparia* (Spach) C. Schneider (1905)
(Syn.: *A. scoparia* Spach)

Series: *Dodecandra* Spach (1843)
(Syn.: *Lycioides* Spach)

20. *P. lycioides* (Spach) C. Schneider (1906)
(Syn.: *A. lycioides* Spach)
21. *P. spinosissima* (Bge.) Franch. (1883)
(Syn.: *A. spinosissima* Bge.)
22. *P. eburnea* (Spach) Aitch. et Hemsley (1886)
(Syn.: *A. eburnea* Spach, *A. spathulata* Boiss.)
23. *P. brahuica* (Boiss.) Aitch. et Hemsley (1886)
(Syn.: *A. brahuica* Boiss.)
24. *P. erioclada* (Born.) SIC, comb. nov. (1998)
(Syn.: *A. erioclada* Born.)

The following species are considered peach species but also belong to the section *Euamygdalus*:

- P. persica* (L.) Batsch (1801)
(Syn.: *A. persica* L., *Persica vulgaris* Mill.). It includes *P. compressa* (Loud.) Bean (Syn.: *Persica platycarpa* Dcne., *P. vulgaris compressa* Loud, *P. persica platycarpa* Bailey)
P. davidiana (Carr.) Franch. (1872)
(Syn.: *Persica davidiana* Carr.)
P. mira Koehne (1910)
(Syn.: *A. mira* (Koehne) Rickter)
P. fasciculata (Torr.) Gray (1874)
(Syn.: *Emplectocladus fasciculata* Torr., *A. fasciculata* Greene)
P. kansuensis Rehd. (1922)
(Syn.: *A. kansuensis* Skeele)
P. pedunculata (Pall.) Maxim. (1883)
(Syn.: *A. pedunculata* Pall., *A. boissieri* Carr.)
P. triloba Lindl. (1857)
(Syn.: *P. ulmifolia* Franch., *A. lindleyi* Carr., *Amygdalus triloba* Rickter)

but interest in collecting a larger number of examples fostered the Grasselly–Felipe expedition to Afghanistan in 1974 and the establishment of an arboretum in Saragossa, Spain (Felipe, 1984). Even with the reduced number of samples of each species in the different arboreta, it is possible to ascertain the large variability found in these species (Figs 1.2, 1.3 and 1.4), and even within each species (Fig. 1.5).

The scattered references to wild almond species were comprehensively summarized by Browicz and Zohary (1996), where all the species are described and documented. However, these authors considered *Amygdalus* as a genus and not as a subgenus. Despite belonging to different groups and sections, the wild almond species are quite close genetically and remain apart from

the ensemble of almond cultivars (Fernández i Martí *et al.*, 2015). Of all wild species (Box 1.1), only those involved in the origin of almond or utilized in crosses with almond will be mentioned. Thus, from the group *Amygdalus*, only *P. fenzliana*, *P. bucharica*, *P. kuramica* and *P. webbii* will be considered; from the group *Orientalis*, *P. argentea* and *P. kotschyi*; from section *Spartioides*, *P. arabica* and *P. scoparia*; and from the group of peach species, *P. triloba*.

P. fenzliana Fritsch (Fig. 1.6) is a subspinescent shrub 2–3 m tall with an open growth habit. Fruits are rather large (2.5 × 1.5 cm) and tomentose. Nuts are thin and brown, slightly grooved. Kernels are bitter. It grows from north-east Turkey and Armenia to north-west Iran, Azerbaijan and Uzbekistan, growing mainly on rocky slopes

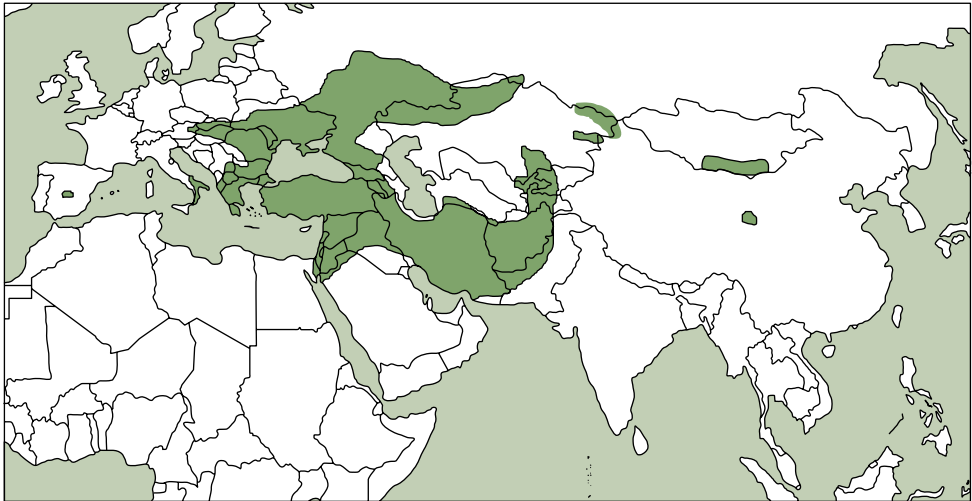


Fig. 1.1. Distribution area of wild almond species in the subgenus *Euamygdalus* Schneid. (Modified from Browicz and Zohary, 1996.)

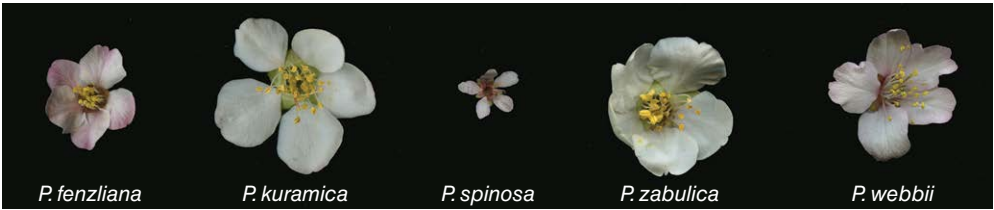


Fig. 1.2. Flower variability among different wild almond species. *P.*, *Prunus*.

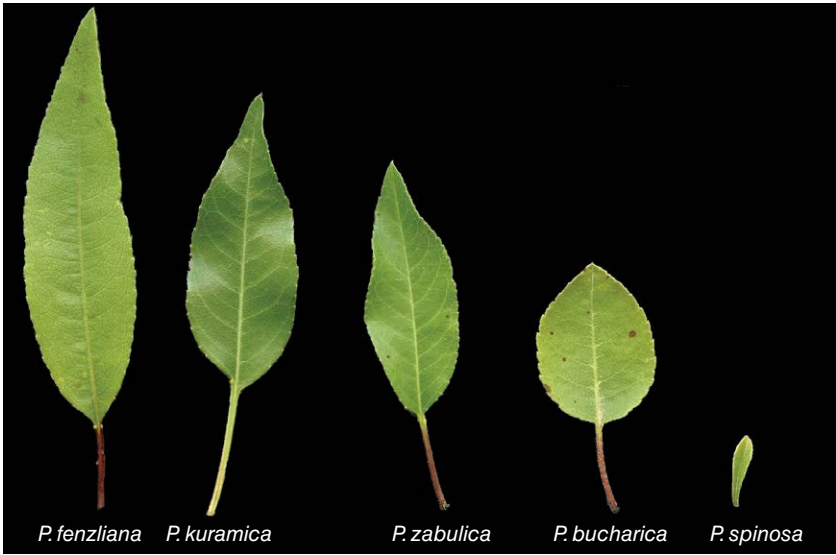


Fig. 1.3. Leaf variability among different wild almond species. *P.*, *Prunus*.



Fig. 1.4. Nut variability among different wild almond species. (From Felipe, 2000.)

between 700 m and 1800 m altitude. It crosses easily with almond and has thus been considered, with other species, to be involved in the origin of almond (Grasselly and Crossa-Raynaud, 1980), although Ladizinsky (1999) considered only *P. fenzliana* as the single most likely ancestor

of almond. This attribution was mostly based on the presence of real wild thickets of *P. fenzliana*, the morphological traits of the species, mainly of the nut and its area of distribution. It has been utilized in almond breeding for late blooming (Grasselly, 1976).



Fig. 1.5. Flower variability among different *Prunus bucharica* accessions.

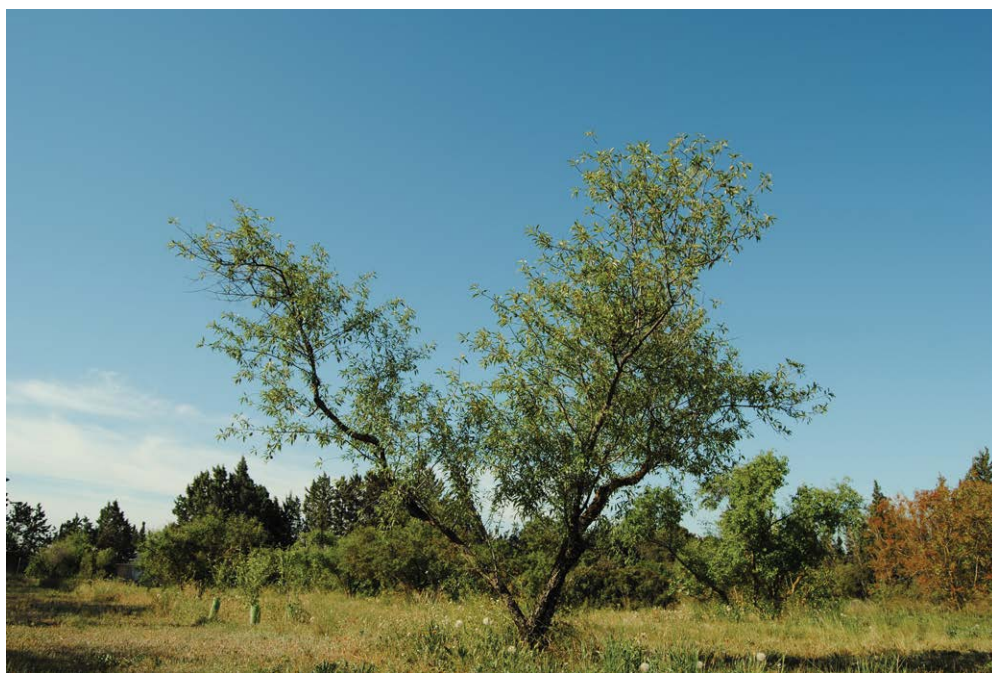


Fig. 1.6. A sample of *Prunus fenzliana*.

P. bucharica (Korsh.) Hand.-Mazz. (Fig. 1.7) is an erect shrub (1.5–4 m tall) or small tree (6–7 m tall) resembling almond, but with tomentose twigs and leaf undersides. Hairiness varies depending on the region, being less hairy in the northern part of its range. Leaves are light green and large. Fruits are large (4×2.5 cm), hairy, slightly asymmetric and pointed, and usually red at maturity, which is early. Thus, it has been utilized in almond breeding for early ripening and late blooming (Grasselly, 1976). Nut is pointed, with smooth surface. Shell is thin, but hard. Kernel is mostly bitter, but some sweet forms have been identified. It is

distributed over the mountain parts of Tajikistan and Uzbekistan, and also extends to south Afghanistan. It grows scattered on stony, sandy or loessy slopes, commonly in the 1000–1800 m altitude range, but fewer are found at lower and higher altitudes. It has been considered, with other species, to be involved in the origin of almond (Grasselly and Crossa-Raynaud, 1980).

P. kuramica (Korsh.) Kitam. (Fig. 1.8) is a shrub or small tree (4–5 m tall) very similar to almond in growth habit, in the morphological traits of twigs, leaves and flowers, and in ripening time. The fruit is small (up to 2 cm long),



Fig. 1.7. A sample of *Prunus bucharica*.

glabrous and dehiscent, showing a peculiar nut, quite flat and sulcate with deep furrows. Kernels are bitter. It crosses easily with almond and has thus been considered, with other species, to be involved in the origin of almond (Grasselly and Crossa-Raynaud, 1980). It is restricted to eastern Afghanistan and northern Pakistan, growing in river valleys and on rocky or gravelly slopes at 1000–1600 m altitude.

P. webbii (Spach) Vierh. (Fig. 1.9) is a shrub or small tree (2–3 m tall), thorny and much branched. Leaves and fruits are relatively small. It is a Balkano-Anatolian species extending to southern Italy and the island of Sicily, and also to central Spain. It grows in open sunny places in limestone rocks or gravelly slopes at altitudes of up to 900 m, rarely higher. It crosses freely with almond (Socias i Company, 2004) and may have been involved in the origin of the Puglia cultivars (Godini, 1979). Ladizinsky (1999) suggested that self-compatible almonds were derived from *P. fenziiana* rather than from *P. webbii*, erroneously concluding that self-compatibility was not naturally found in *P. webbii*, but Godini (2000) clearly demonstrated that *P. webbii* from

the Italian region of Puglia was self-compatible and probably contributed self-compatibility to the Puglia pool of almond cultivars. It has also been utilized as an almond rootstock or in developing almond rootstocks (Vlasic, 1977).

P. argentea (Lam.) Rehd. is a subspinescent shrub (1–3 m tall) characterized by white tomentose shoots, leaves and fruits, which provide the reason for its botanical name. Its flowers show very high frost resistance (Grasselly, 1976). It is a typical near-east species, being the most common wild almond species in north-east Iraq and south and central Anatolia. It grows in open, sunny niches such as sandy hills, loose gravel and rocky slopes at altitudes between 600 and 1200 m.

P. kotschyi (Boiss. et Hohen.) Náb. is also a subspinescent shrub, but smaller than *P. argentea* (30–45 cm tall), with a very dense yellowish-green pubescence covering shoots, leaves and fruits. It is restricted to a small area in northern Iraq, eastern Iran and south-east Anatolia, at altitudes between 1800 and 2500 m, on limestone rocks, boulders and screes. This species, as well as *P. argentea*, belongs to the *Spartioides*



Fig. 1.8. A sample of *Prunus kuramica*.



Fig. 1.9. A sample of *Prunus webbii*.

section and are not so close to almond as the species of the group *Amygdalus*.

P. arabica (Olivier) Meikle is an erect, broom-like shrub, 1.5–2.5 m tall, mainly characterized by stiff, long, permanently green, glabrous, angled branches. It loses the leaves at the beginning of the dry season as a reaction to drought, a trait common with almond. It is a desert species growing on dry bare limestone rocks and sandstone cliffs, at altitudes of 150–1200 m. Its main distribution area covers south-eastern Anatolia, Iraq and Syria, but with single stands also in western Iran, Israel, Jordan and north-west Arabia.

P. scoparia (Spach) C. Schneider is an upright broom-like shrub 3–4 m tall similar to *P. arabica*, but with non-angled branches. It extends in similar ecologies to *P. arabica*, but mostly in Iran and at altitudes over 1200 m. It grows in dry and hot areas, on loose conglomerates and limestone cliffs, loose volcanic rocks, crevices in rock slopes and in clay and sandy soils. *P. arabica*, and *P. scoparia* are early ripening and are considered as possible donors of resistance to drought due to their ecological adaptation and the avoidance of transpiration by leaf fall.

P. triloba Lindl. is a wild peach rather than a wild almond, although it is sometimes called 'flowering almond' and is considered a possible ancestor of almond (Grasselly and Crossa-Raynaud, 1980). It is a shrub or small tree with pubescent young shoots. Fruit is red and hairy, 1–1.5 cm large, with a very hard shell. It originates from China.

1.3 Almond Morphology

Almond is a very heterogeneous species, not only in its morphological and physiological traits (Kester *et al.*, 1990; Socias i Company and Felipe, 1992b), but also in the genetic structure of the different cultivars (Arulsekhar *et al.*, 1986; Arús *et al.*, 2009; Fernández i Martí *et al.*, 2009). This high genetic variability is transcribed into a high diversity of forms and performances, probably linked to the high heterosis of the species, mainly due to the requirement for cross-pollination in most cultivars (Socias i Company, 1990). Additionally, characteristic ecotypes have evolved in different growing regions, very often having been isolated from each

other for centuries (Grasselly and Crossa-Raynaud, 1980). One of the most distinctive ecotypes is found in Apulia, southern Italy, and is mostly defined by late-blooming cultivars with the presence of flower buds mainly on spurs.

Consequently, almond trees may show a high variability in size, shape, vigour, branching habit, growth and fruiting type (Kester *et al.*, 1990). All these traits must be taken into account when describing a cultivar and are classified according to already defined descriptors (Gülcan, 1985), although not all traits have the same importance, mainly depending on the orchard management under which the cultivars are grown. For cultivar registration a reduced number of traits are considered (UPOV, 2016), as well as for germplasm management (ECP/GR, 2016). Almond morphology has already been considered and described in detail (Grasselly and Crossa-Raynaud, 1980; Felipe, 2000).

1.3.1 Root

The root will depend on the rootstock. Therefore, there is not a single morphology for the almond tree root (Fig. 1.10). Additionally, the type of rootstock propagation (seedling, by cuttings or by *in vitro* propagation) and the method of transplanting will affect root development and, consequently, its morphology. The general framework of an almond root is of a reduced number of main roots extending both laterally and vertically giving rise to a large number of small roots terminating with the tips of recently formed fine roots. The root pattern is a generally symmetrical image of the canopy.

Traditional propagation was by seed, and this still continues in some growing regions (Mahhou and Dennis, 1992). In this way a very strong taproot is produced, often reaching very deep levels in the soil. Roots of the almond species are of a yellow-grey colour when young, later becoming brown, but maintaining a rigid structure which renders transplanting problematic. This rigid structure is due to the strong lignification of a relatively small amount of cortical tissues. Almond roots are little fasciculated and very sensitive to asphyxia. This type of root was obtained when rootstocks were

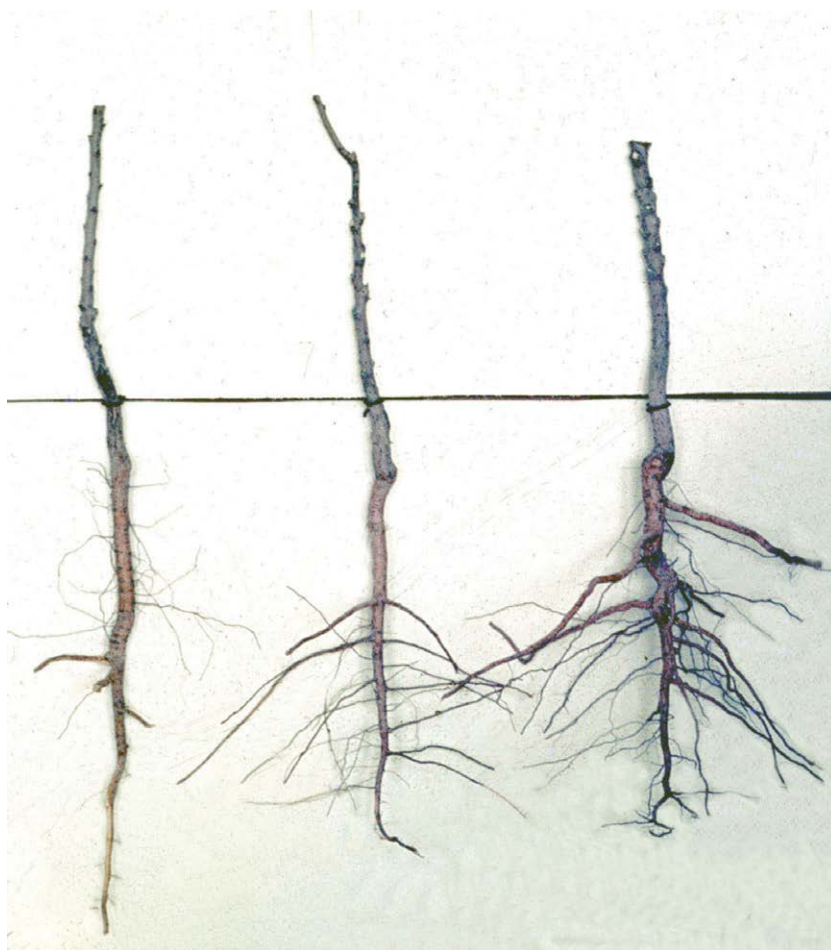


Fig. 1.10. Different morphologies of almond roots. (From Felipe, 2000.)

produced by direct seeding of almond nuts, often bitter, in the orchard, being later grafted *in situ*.

When plants are produced in a nursery on almond seedling rootstocks, the pivoting taproot is cut when transplanting into the orchard and it never recovers, producing only reduced secondary branchings. In all cases the root system mostly spreads in the most superficial levels of the soil, to best profit from rainwater and soil aeration.

At this stage the main requirement for the almond root is to explore an adequate soil volume according to the growing system (non-irrigated or other types of irrigation systems). It must also provide a good anchorage to the tree, mainly to withstand mechanical harvesting and winds in

windy regions. Other requirements are related to each rootstock type and to efficiency of nutrient absorption (Felipe *et al.*, 1998; Chapter 9).

1.3.2 Canopy

The canopy morphology is determined by the cultivar, the vigour induced by the rootstock, the grafting height and the type of pruning applied. In many traditional growing regions the grafting point was very high, with a trunk of approximately 1.5 m where branching started, allowing sheep to graze under the trees. However, the

longer the trunk the smaller the canopy, thus reducing the production capability of the tree because the production level is often related to the volume of the aerial part. On the other hand, a minimum trunk height is required for mechanical harvesting with shakers, since the pincers must have enough space to grip the trunk. Additionally, when an inverted umbrella is utilized for collecting the nuts, it may be difficult to spread the umbrella if the branches are too low. Once the requirements of orchard management have been considered, the trunk length must be as short as possible to reduce the productive effort of the tree.

The different almond cultivars show different growing habits (Fig. 1.11), from drooping as in 'Desmayo Langueta' and other cultivars with similar names (referring to the willow-type branching), to very upright as in 'Fournat de Brézénau'. When the habit is very erect the tree may have a canopy with insufficient spread. This makes orchard management difficult but may be useful in new developments of high density plantings. Generally an upright to spreading habit is preferable, as it facilitates formation of the tree and mechanization of the different operations.

The branching habit is also similarly variable, from cultivars with long shoots to others with spurs. The branching habit is closely related to the fructification type, as flower buds may mostly be found in brindles such as in 'Nonpareil', or on spurs such as in most cultivars from Apulia. From the productive point of view a compensated branching is preferable (Socias i Company *et al.*, 1998), meaning enough annual growth to allow a continuous renewal of the canopy, but with annual growth that is not excessive and so requiring much pruning.

Both the almond trunk and the branches are smooth during the first years. One-year shoots are from light green to reddish brown, depending on the cultivar (Fig. 1.12). With aging, the bark breaks and becomes rough. Both vegetative and flower buds are found on the brindles and the spurs (Fig. 1.13); their appearance and density differ depending on the cultivar (Socias i Company, 1988a; Kodad and Socias i Company, 2008).

Almond leaves are generally smaller, narrower and longer than those of peach, as well as being darker and flatter, although there are

differences among cultivars (Fig. 1.14). 'Desmayo Langueta' leaves are a light green colour as opposed to those of 'Texas', which are dark green. The closely related genotypes show similar morphological traits, including those of leaves (Bernad and Socias i Company, 1998).

1.3.3 Bud and flower

Flower buds are variable in size, shape and colour depending on the cultivar (Fig. 1.15). The number of flowers in a single bud is also a cultivar trait (Fig. 1.16). Cultivars such as 'Desmayo Langueta', 'Nonpareil' and 'Ferragnès' normally have a single flower in each bud, but many Italian cultivars from Apulia, such as 'Tuono', have many buds with twin flowers (Socias i Company, 1983).

Almond flowers are generally perfect and pentamerous, with five sepals, five petals, a variable number of stamens and a single pistil. Deviations from this flower type are sometimes found, and in some cultivars the presence of female sterility with pistil abortion or underdevelopment are important (Socias i Company *et al.*, 1976; Socias i Company, 1983; Socias i Company and Felipe, 1992a; Ben Njima and Socias i Company, 1995). The number of stamens oscillates between 20 and 30, but may reach 40. Frequently several pistils may be found in the same flower receptacle (Fig. 1.17), exceptionally giving rise to the formation of twin nuts: two nuts sealed at their base and with a single peduncle (Fig. 1.18). The style is of variable length (Vasilakakis and Porlingis, 1984; Socias i Company, 1988b) (Fig. 1.19) and may present a bending at the stigma end (Fig. 1.20), with an extreme example in the Majorcan cultivar 'Menut' (Socias i Company and Rallo i García, 1996). This bending is very important as it may allow autogamy in these flowers (Weinbaum, 1985; Socias i Company, 1996).

Flower size is another cultivar trait showing high variability (Fig. 1.21). It has been related to nut size. Petal size and colour are also cultivar traits (Fig. 1.22). Although white is the predominant colour in almond flowers, 'Marcona' is easily distinguished by its relatively small pink petals (Fig. 1.23).

The amount of flowers is a cultivar trait (Socias i Company, 1988a) and may be affected



Fig. 1.11. Different growing habits of almond cultivars. (From Felipe, 2000.)

by alternance in susceptible cultivars. An appropriate flower density is desired to ensure a commercial crop (Kodad and Socías i Company, 2008) and, like the growth habit, flower density is similar in related cultivars (Sarvisé and Socías i Company, 2005).

1.3.4 Fruit

Botanically the almond fruit is a drupe characterized by an outer fibrous layer or hull equivalent to the flesh of the stone fruits. The hull is

made of the pericarp and the mesocarp, and splits at maturity to show the endocarp or shell. The shell contains the seed or kernel, which is the commercial part of the fruit. Nut size is very variable depending on the cultivar and the species (Socías i Company and Felipe, 1992b). Most wild species are characterized by very small nuts (Grasselly, 1976), but some cultivars may also produce fruits of only 2–3 g (Fig. 1.24). The usual fruit weight in almond cultivars ranges from 8 g to 20 g, although some cultivars such as ‘Bartre’ may produce fruits weighing up to 40 g.

The pericarp is pubescent, although some exceptional glabrous forms have been found (Socías i Company, 1993). These forms are of the ‘nectarine’ type and seem to be due to a recessive mutation found in some seedling orchards obtained with seeds produced by pollination of related parents, thus allowing the appearance of the recessive allele in homozygosity. The pericarp is green, although at maturity reddish fruits are found in some cultivars. The mesocarp is generally green-white, sometimes yellowish, and sometimes showing at maturity a reddish inner layer when the mesocarp splits longitudinally at the suture line. Harvesting is usually done at full mesocarp dehiscence, when the shell is fully shown. Mesocarp dehiscence is affected by ambient humidity and the physiological stage of the tree. In cases of extreme drought this dehiscence does not take place and the mesocarp remains completely stuck to the shell. Mesocarp thickness is very variable, from 0.5 cm to 1.5 cm, representing from 38% to 85% of the total fruit dry weight (Godini, 1984a; Alonso *et al.*, 2012), since soft-shell cultivars have thicker hulls (Godini, 1984b). Utilization of the mesocarp as an animal food requires its mixing with nutrient additives (Aguilar *et al.*, 1984; Schirra, 1997).



‘Desmayo Langueta’ ‘Texas’ ‘Bartre’

Fig. 1.12. Different colours of 1-year shoots.



Fig. 1.13. Distribution of flower buds on spurs, on brindles and on mixed shoots in almond.



Fig. 1.14. Different sizes and shapes of almond leaves.



Fig. 1.15. Different shapes and colours of almond flower buds.

The endocarp or shell shows a very large variability among cultivars, from very soft paper shells to very hard stony shells (Fig. 1.25). Their morphology is also very variable, with a large diversity of different modifications such as wrinkles and pores (Fig. 1.26), a more or less pronounced keel at the ventral suture (Fig. 1.27) or a sting at the apex (Fig. 1.28). Shell hardness is related to the shelling percentage, from less than 20% in stony

shells to more than 60% in paper shells, although not to the kernel percentage of the total amount of the fruit, including the mesocarp (Alonso *et al.*, 2012). The preference for each shell type depends on the growing conditions and the prevalent industry in the region. In the Mediterranean region hard shells are preferred since these cultivars seem more adapted to non-irrigated conditions and more resistant to depredation by birds and penetration by insect

larvae damaging the kernel. Furthermore, the nuts can be stored for a long time with reduced problems of becoming rancid or excessively dry, thus allowing their sale throughout the year.



Fig. 1.16. Twin flowers in almond.

The Mediterranean shelling plants are consequently adapted to hard shells and are completely different from shelling plants for soft shells, as found in California and other countries growing similar cultivars, including most growing regions of the southern hemisphere.

The endocarp may be used as firewood because of its very high energetic power; it is sometimes used in the same processing plants, to heat water for blanching kernels. It is also utilized in chipboard manufacture, as active carbon and, reduced to powder, to polish some metals. New research aims to increase its possible use (Socias i Company *et al.*, 2013).

1.3.5 Kernel

The kernel is the commercial part of the almond crop and its size and shape are also cultivar traits (Fig. 1.29). The outer part is the tegument or seed coat, and is very variable in colour and thickness. Its colour ranges from yellow to brown, and it becomes darker with time. The surface may be smooth or wrinkled, and this is also related to its thickness. Seed coat thickness has an industrial importance because elimination of



Fig. 1.17. Different numbers of pistils in a single flower.

the seed coat represents a weight loss of up to 10% at blanching. However, for some uses, a thicker tegument is preferred since this does not adhere too tightly to the kernel, an advantage in roasted almonds whose seed coat is easily removed (as in 'Desmayo Largueta'), and in preparation of different types of flavouring for special appetizers.

The same variability observed in nut weight is reflected in kernel weight, with a range of values from 0.5 g to 1.5 g. For most uses large sizes, generally higher than 1.2 g, are preferred. However, for some special confectioneries very large or very small sizes are chosen, as well as a definite shape. For sugared almonds (peladillas or dragées) and for chocolate almonds large kernels are selected, preferably round to reduce the layer of sugar or chocolate covering the kernel. For chocolate bars, small – and, particularly, flat – kernels are preferred to ensure they are covered by the chocolate and the thickness of the bar is maintained. These cases are, however, very specific and the general trend in the industry is the preference for large kernels to facilitate and cheapen the processes of cracking and blanching.

The presence of double kernels is a cultivar trait (Fig. 1.30) whose expression may vary

depending on the year (Dicenta *et al.*, 1993b) and the region. Consequently, many factors have been suggested to affect the presence of double kernels, whose cause has not been clearly elucidated (Egea and Burgos, 1994; Socias i Company and Felipe, 1994; Asensio and Socias i Company, 1996). Their presence is due to the development and fertilization of two ovules in the ovary (Gradziel and Martínez-Gómez, 2002) when the secondary ovule does not degenerate (Pimienta and Polito, 1982). It is considered a negative trait because the presence of two kernels in a single shell deforms both kernels and the processes of cracking and blanching are rendered more difficult, although the organoleptic quality of the kernels is not affected. Consequently, cultivars producing only single kernels are preferred, although photoelectric cells have recently been designed to separate the double kernels of a sample automatically and quickly. Therefore, cultivars with double kernels but with other interesting characteristics may be successfully grown and marketed.

Some cultivars may produce nuts without kernels (void shells with only the seed coat) or with deformed kernels, as sometimes happens with two seeds inside a single seed coat (Gülcan, 1975).



Fig. 1.18. Twin almond nuts. (Photo by J.A. Fras.)

1.4 Reproductive Physiology

Since the commercial part of the almond crop is a seed, fertilization of the ovule is an unavoidable requirement to obtain a crop. Not only must a large number of flowers be produced, but they must also be efficiently pollinated, since the small size of the almond kernels demands a high number of nuts for a commercial yield (Godini, 2002). The production of a large number of flowers and effective pollination



Fig. 1.19. Different stigma–anther positions in almond.



Fig. 1.20. Pistil bending in almond.

must be maintained throughout the life of the orchard, avoiding any alternance in yield and looking for an adequate equilibrium between production and vegetative growth. These facts stress the importance of the reproductive cycle of almond, initiated by flower bud initiation and ending more than 1 year later with harvest (Fig. 1.31).

1.4.1 Flower initiation

Flower initiation is the process by which vegetative buds become flower buds (Polito *et al.*, 1996). This process takes place during the summer previous to bloom, close to mid-August

depending on the cultivar, and has two main requirements: an adequate developmental stage of the tree and an equilibrated nutrient status. Young trees do not produce flowers, or in a very limited quantity, since they have not yet reached the stage of maturity at which flower initiation may take place. The period during which flowers are not initiated occurs due to two different reasons depending on the type of tree: juvenility in seedlings and unproductive period in grafted plants.

Juvenility is a general phenomenon in fruit trees. It is not interesting from the point of view of production, as normally orchards are made with grafted plants, not with seedlings. It is very important in breeding programmes, where the reduction in generation time is crucial. The juvenile period spans from seed germination until appearance of the first flowers. During this period the aspect of the plants may be quite different from that of the adult trees, as the growth habit is somehow shrubby, with thin, fragile and frequently thorny branches and smaller leaves. Almond is a fruit tree with a very short juvenile period, as some flowers may already appear in the second year after seeding, obtaining normally a general bloom in the fourth year. The length of the almond juvenile period is similar to that of peach and much shorter than that of the pome fruits.

The juvenile traits are more defined when observations are made closer to the tree crown. Consequently, even in adult trees, sprouts from the crown region may show juvenile traits. A reduction in the juvenile period may be looked for in a breeding programme to shorten the generation time and to observe the flowers and fruits quickly. One way of shortening this period is by grafting on adult trees, but taking care to select grafting buds from the outermost part of the seedling tree.

The *unproductive period* runs from grafting to the beginning of flower production in the commercial orchard, and thus effects the possibilities of obtaining a crop. The length of this period is of great economic importance to growers and depends on the cultivar, rootstock, grafting point, planting density and nutrient condition of the tree. Although this period is generally short in almond, it must be taken into consideration in the evaluation of a cultivar. Some varieties, such as 'Marcona', 'Guara', 'Ferragnès', 'Belona' and 'Soleta', are characterized by a very short unproductive period, and they may even show some



Fig. 1.21. Different sizes of almond flowers.

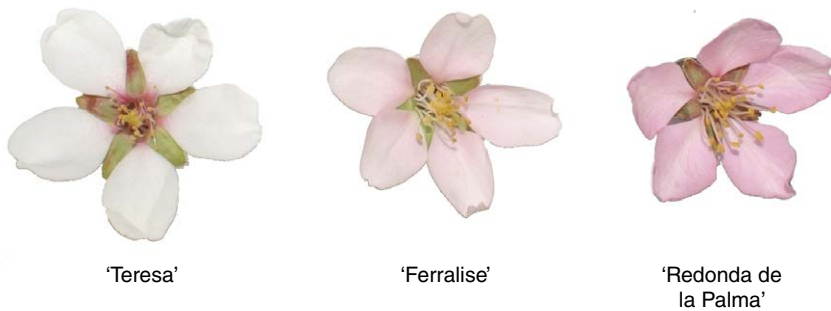


Fig. 1.22. Different colours of almond flowers.



Fig. 1.23. Pink 'Marcona' flowers.

flower buds at the end of their first vegetative year in the orchard. Other cultivars, however, have longer unproductive periods. If the grafting buds are taken from shoots with juvenile traits, such

as watersprouts, the unproductive period may be extended.

The pruning system also affects the length of the unproductive period. Long pruning,

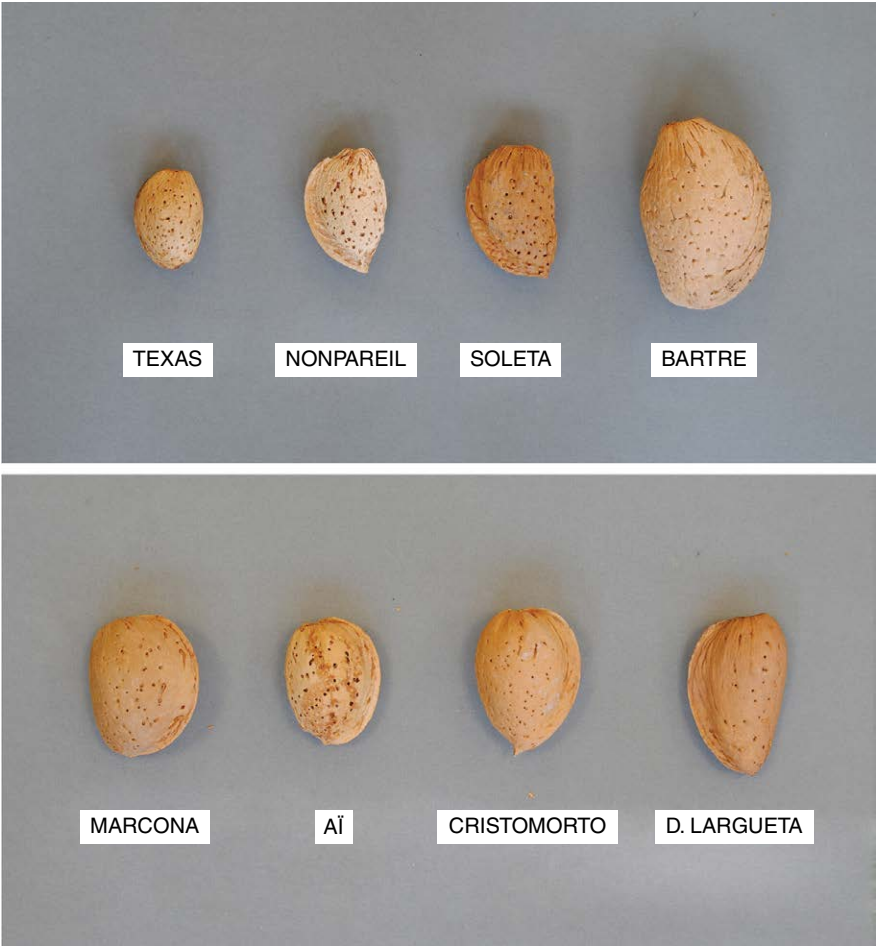


Fig. 1.24. Different sizes and shapes of almond nuts.



Fig. 1.25. Different types of almond shell.



Fig. 1.26. Different types of pores on the almond shell.



Fig. 1.27. Different types of ventral suture on the almond shell.

excessively reducing the shoot length, forces new shoots from low positions more prone to juvenile traits, and extends the unproductive period. Consequently, new systems of tree formation are being adopted with different planting densities.

There may be a positive correlation between the unproductive period of a cultivar and the juvenile period of the seedlings obtained from its seeds. As a consequence, all measures directed towards shortening these periods are extremely important to both the grower and the breeder.

Flower initiation takes place once the initial period has been overcome and the trees are in good physiological conditions, defined as a 'luxury stage', with an adequate C/N relationship

(Carrera Morales, 1975). Consequently, under conditions of drought or deficient nutrition, flower initiation is low, thus reducing the possibilities of obtaining a reasonable crop in the following year. Similarly, when the crop level is excessive, the effort of the tree in raising this crop reduces the possibility of allocating nutrient reserves to flower initiation, giving rise to a period of possible alternance of production. For a good nutrient status, the presence of a significant and active leaf surface is required (Polito *et al.*, 2002). Flower initiation studies have been undertaken by defoliating whole branches (Agabbio and Ortu, 1974).

There are no essential structural differences between the meristems producing a shoot

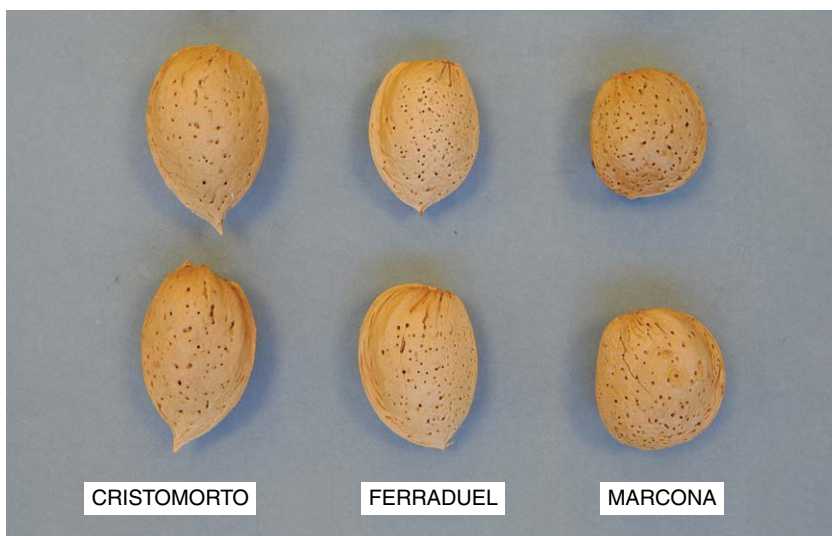


Fig. 1.28. Presence of an apex sting on the almond shell.



Fig. 1.29. Different shapes and colours of almond kernels.

or those producing a flower, although the first are of indeterminate growth and the latter are of determinate growth. Flower initiation takes place on a meristem by differentiating the different flower elements. The right moment for flower initiation is related to the crop of the previous year and to the temperature, as a thermal integral is required. Depending on the different cultivars and the region, flower differentiation starts

in the northern hemisphere from mid-August to early September and is simultaneous in all the fruiting shoots: brindles, spurs and mixed shoots (Agabbio and Ortu, 1974).

The requirement for a good C/N equilibrium for flower initiation may easily be observed in almond: in the shaded parts of the trees reduced formation of flower buds may be seen; this is due to the lower levels of photosynthesis in



Fig. 1.30. Double kernels in almond.

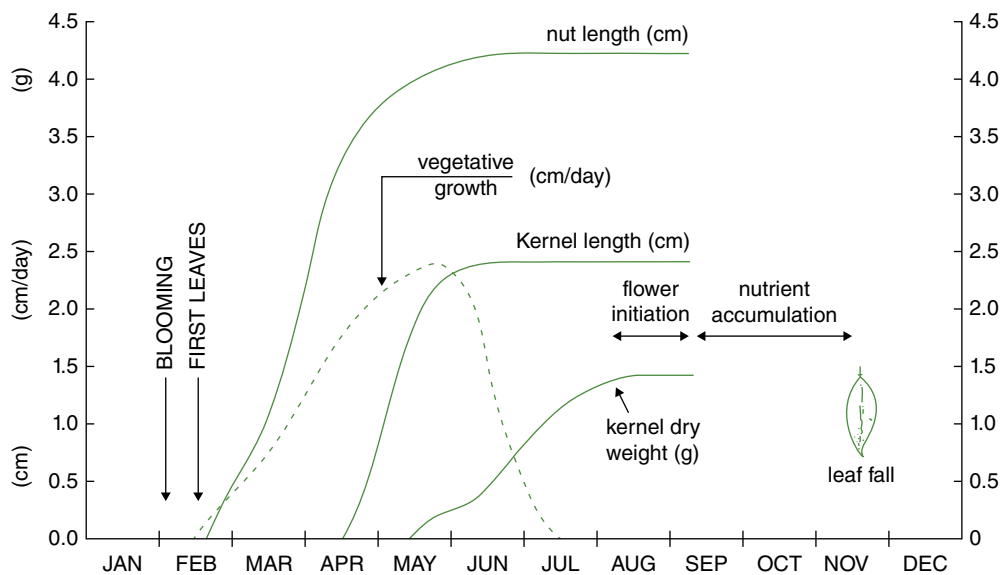


Fig. 1.31. Annual growth cycle of almond. (Modified from Ross and Catlin, 1978; Grasselly and Crossa-Raynaud, 1980; Felipe, 2000.)

these parts, leading to lower carbohydrate production. Similarly, excessive N fertilization decreases flower initiation while at the same time stimulating excessive vegetative growth, in the

same way that heavy pruning does. Any decrease in the leaf surface has a negative effect on flower initiation, and may happen with green pruning that is done too early, from leaf damage

by pests and diseases or from defoliation due to hail, chemical treatments and other causes. These deficiencies are not only reflected in reduced flower initiation, but also in reduced flower quality. Any reduction in the availability of nutrients as a consequence of drought or a decrease in the leaf surface increases the percentage of flowers with malformed or sterile pistils.

Bud development takes place once flower initiation has been established in the meristem and leads to the formation of the different flower organs (Fig. 1.32). This process begins relatively quickly with differentiation of the different primordia of sepals, petals, stamens and pistils. During winter this process is extremely slow but it does not completely stop, and suddenly accelerates at the end of winter, some weeks before blooming.

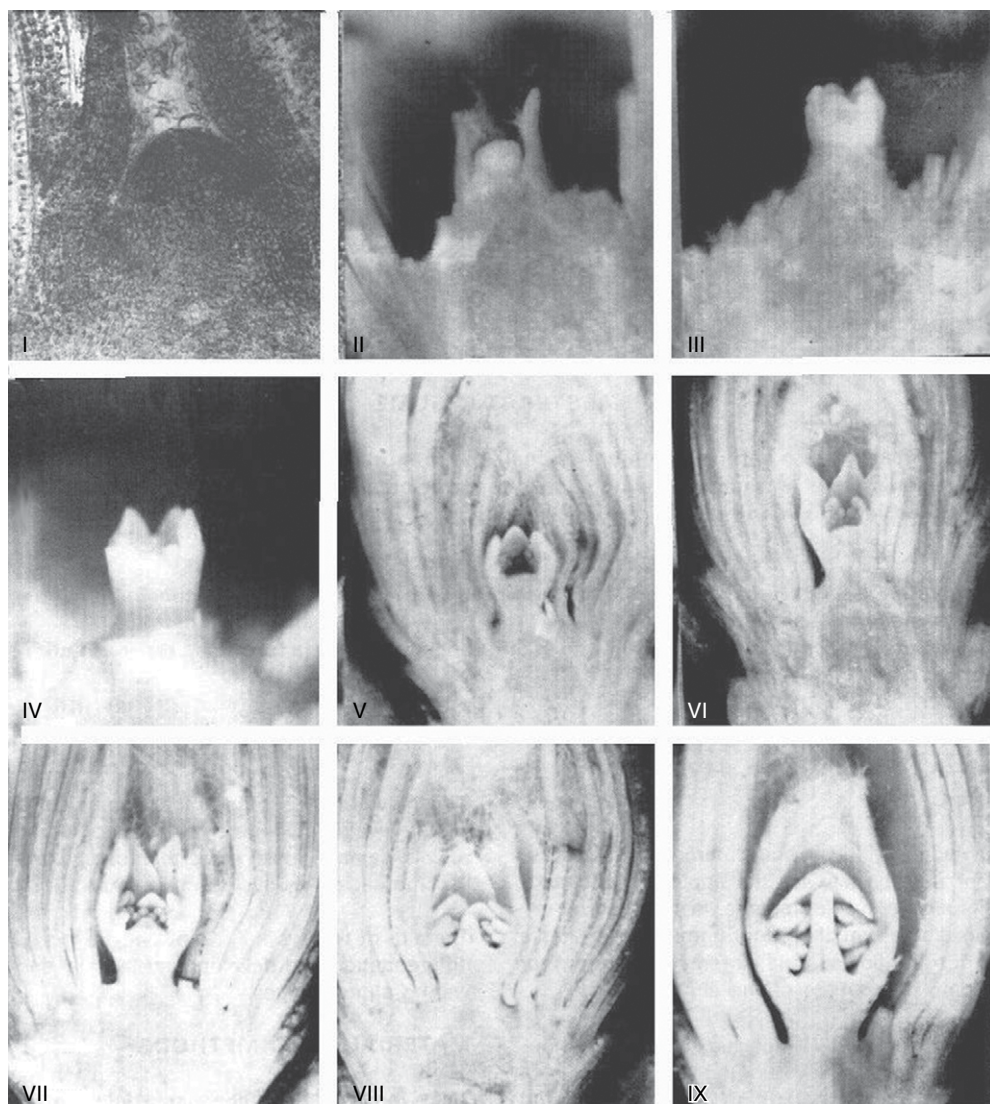


Fig. 1.32. Sequential evolution of the almond flower bud. Stage I shows the first evidence of the differentiation. Stages II–VI indicate the development of the sepals, petals and stamens. In stage VII, pistils start to develop and in stage IX development of anthers and pistil is advanced. (From Ünal *et al.*, 1981.)

Normally, when vegetation stops growing in the autumn, all flower primordia are already formed.

The slow winter development primarily affects the structure and biochemical pathways of the flower buds rather than the growth of the primordia, which are already differentiated. The content of growth regulators changes, with a decrease in abscisic acid and an increase in gibberellins during the winter. Gametogenesis also proceeds during this period, as the development of pollen grains or microsporogenesis takes place during late autumn and early winter, at the same time as chilling accumulation occurs. Meiotic cell division seems to coincide with the fulfilment of the chilling requirements and the end of endodormancy (Lang *et al.*, 1987). After this division bud development depends on air temperature in order to accumulate the heat requirements (thermal integral) to bloom (Alonso *et al.*, 2005), because the bud stage at this time is one of exodormancy (Lang *et al.*, 1987). Pollen grains are quickly formed once growth resumes at the end of winter or early spring, when buds enlarge.

The seminal primordia are differentiated in the carpel wall of the ovary, but the ovule only starts its development when growth is resumed at the end of winter. Pollen grain development is a relatively continuous and regular process, but that of the ovule is very slow at first and becomes quicker at the end. Temperature plays an important role in the length of time taken, as higher temperatures accelerate the process.

Almond flowers have a single carpel with two ovules, as in other stone fruits. The secondary ovule often degenerates (Hawker and Butrose, 1980; Pimienta and Polito, 1982), mostly due to differences in size between the two ovules (Egea and Burgos, 2000), and a single kernel is produced. If the two ovules reach full development and are fertilized, two deformed kernels (double kernels) are produced. Since the degeneration of the secondary ovule seems to be genetically programmed (Egea and Burgos, 2000), the presence of double kernels follows a genotype-dependent pattern and, as already mentioned, is a cultivar trait.

1.4.2 Blooming

Blooming takes place when the petals appear, showing the different elements of the flower,

and takes place when the chilling and heat requirements are fulfilled (see Chapter 12). Most almond cultivars have relatively slow chilling requirements, but there is high variability among them (Tabuenca, 1972; Alonso *et al.*, 2005). Similarly, the almond heat requirements are quite low and very variable (Tabuenca *et al.*, 1972; Alonso *et al.*, 2005). Blooming time is a cultivar trait, although very variable depending on yearly climatic conditions (Rattigan and Hill, 1986). Despite this variability, the order of blooming of the different cultivars is maintained over the years with very small oscillations depending on the location and the temperatures in each season (Felipe, 1977a; Dicenta *et al.*, 1993a). The duration of bloom also depends on the cultivar and the weather, since bloom proceeds more slowly in low temperatures (Hill *et al.*, 1985; Bernad and Socias i Company, 1995). Consequently, bloom duration may oscillate from several days to more than 1 month.

To compare blooming dates a homogeneous scale must be adopted, such as that of the different phenological stages defined in almond by Felipe (1977b) from the dormant winter bud to the ripe fruit (Fig. 1.33). Observation of the phenological stages allows the determination of the blooming date in relation to five points established according to the different percentages of open flowers:

- Beginning of bloom First open flowers.
- Beginning of full bloom 5% of open flowers.
- Full bloom 50% of open flowers.
- End of full bloom 95% of open flowers.
- End of bloom 100% of open flowers.

Flower opening follows a distribution close to normal, with a higher number of flowers opening in the middle of the blooming period. Normally the first flowers to open are those of higher quality and from single buds, whereas the last flowers to open are mostly twin in cultivars showing this trait, and sterile (Socias i Company, 1983; Socias i Company and Felipe, 1992a; Bernad and Socias i Company, 1995).

Since the order of blooming is quite constant for the different cultivars, a scale has been established to allow their comparison, from 1 for the very early blooming 'Cavaliera' to 9 for 'Tardy Nonpareil' (Gülcan, 1985). However, after the definition of this scale some new releases from



Fig. 1.33. Phenological stages of almond buds, flowers and nuts. A: resting bud. B: swelling bud. C: sepal appearance. D: petal appearance. E: stamen appearance. F: open flower. G: petal fall. H: set fruit. I: young fruit. J: developed fruit. K: suture opening. L: ripe fruit.

different breeding programmes were found to bloom later or much later than ‘Tardy Nonpareil’, making the scale inoperable. Accordingly, the rating of 6 was adopted for ‘Tardy Nonpareil’ at the X GREMPA Colloquium at Meknès in October 1996, leaving the higher ratings of 7, 8 and 9 for later blooming cultivars (Socias i Company and Felipe, 1999; Socias i Company *et al.*, 2008; Vargas *et al.*, 2008; Dicenta *et al.*, 2009; Socias i Company *et al.*, 2015).

Blooming time is very important in almond for two different reasons: one is the coincidence of blooming of two cross-pollinating

self-incompatible cultivars; and the other is the weather, mainly the possibility of rain and frosts during bloom, since climatic conditions affect bud/flower/fruitlet survival, pollination and fertilization. Frosts are a persistent problem in many – mainly inland – almond-growing regions. However, in some coastal locations frosts may also take place at bloom or just after, and substantially reduce or even destroy the crop (Egea Caballero *et al.*, 1980). Some almond cultivars show an intrinsic resistance to frosts (Felipe, 1988), but the best way to overcome their effect is by planting late-blooming cultivars.

Consequently, late bloom is an objective of most breeding programmes (Socias i Company *et al.*, 1998; Chapter 7). However, in locations with no frost problems, early-blooming cultivars may be advisable if grown under non-irrigated conditions, because fruit development may be completed before the hot and dry summer.

Anthesis is the stage when the flower is mature and ready for efficient pollination. At anthesis the stigma has already produced an exudate rich in carbohydrates, allowing the retention of pollen grains and their germination. At this stage pollen grains are normally available because the anthers have already dehisced. However, in very humid climates pollination may be hindered because anther dehiscence may be retarded by an excessive ambient humidity.

1.4.3 Pollination

Pollination may be defined as the transport of pollen from the anthers to the stigma. At present full differentiation must be established in almond between the self-incompatible cultivars (most of the traditional ones) and the self-compatible cultivars (most of them recent releases of the different breeding programmes) (Socias i Company, 2002; Socias i Company *et al.*, 2009).

Self-incompatible cultivars are characterized by the fact that, even though they produce viable pollen and ovules, their pollen is unable to fertilize the ovules of the same cultivar and, consequently, to produce a crop. Therefore, the presence of two or more cultivars is required in the same orchard to ensure the transfer of pollen from the anthers of the flowers of a cultivar to the stigma of the flowers of the other cultivar. However, the presence of two or more cultivars in the same orchard is not enough to obtain a commercial crop because efficient pollination also involves other requirements. A deficient pollination is a real loss of yield because the maximum number of flowers must be pollinated to ensure a good level of crop (Kester and Griggs, 1959a). Reductions in the numbers of fruit as a result of any pollination problem is seldom compensated by an increase in the size and weight of the remaining fruits, as happens in other fruit trees (Godini, 2002).

The productivity potential of an orchard will be determined by several conditions which can be summarized as:

- The presence of simultaneously blooming and cross-compatible cultivars in the same orchard;
- Adequate distribution of cultivars in the orchard;
- The presence of pollinating insects;
- Appropriate weather conditions; and
- Effective fertilization of the ovules of each flower.

The presence of simultaneously blooming and cross-compatible cultivars in the same orchard is essential for their pollen interchange. The different pollinating trials have shown the cross-compatibility of most traditional cultivars (Socias i Company, 1990) and that the cases of cross-incompatibility are few, between very close cultivars, often coming from the same breeding programme or between budsports. Tufts (1919) reported the first cases of cross-incompatibility in California, those of 'Nonpareil' with 'I.X.L.' and of 'Texas' with 'Languedoc'; Wood and Tuft (1938) that of 'Harpareil' with 'Jordanolo'; Almeida (1949) that of the Portuguese 'Côco Grado' with 'Côco Miúdo'; Kester (1966) that of 'Nonpareil' with its budsports 'Tardy Nonpareil' and 'Cressey' and that of 'Texas' with 'Ballico'; Herrero *et al.* (1977) that of 'Primorskij' with 'Yaltinskij', although their breeder, Rikhter (1972), recommended 'Yaltinskij' as one of the best pollenizers of 'Primorskij'; and Crossa-Raynaud and Grasselly (1985) that of 'Ferragnès' and 'Ferralise', both coming from the French breeding programme. Most cases were reported in California, where the first cross-incompatibility groups were identified as well as the *S* incompatibility alleles (Kester *et al.*, 1994), although the establishment of incompatibility groups has been advancing continuously (Barkley *et al.*, 2006; López *et al.*, 2006; Ortega *et al.*, 2006; Kodad and Socias i Company, 2009b).

Full-bloom coincidence of the cultivars present in an orchard is required to ensure the maximum possibilities of a flower being pollinated and, consequently, fertilized. The blooming time must be recorded carefully in any location in order to select the best simultaneously blooming cultivars. If full coincidence is not possible, three cultivars can be selected, one a little earlier than the

main cultivar and the other a little later, since in this way the main cultivar may be well pollinated. Coincidence in one year or in one location is not enough to ensure the requirement of full overlapping, as small changes may take place over the years or the locations. This has been observed repeatedly in Spain with 'Marcona' and 'Desmayo Largueta', with frequent losses in production because of lack of bloom coincidence despite their recommendation and good overlap in some years, mainly in high altitudes (Socias i Company *et al.*, 1994b). This requirement is completely removed by growing self-compatible cultivars, as they ensure the presence of compatible pollen for the whole blooming period.

Effective pollen transfer may be accomplished with adequate pollinating insects (Fig. 1.34). As in all rosaceous fruits, wind does not have any practical effect on almond pollination (Free, 1970; Williams, 1970). Several insects have been reported as almond pollinators, but honeybees are probably the most effective. Other pollinators are probably fewer in number and their efficacy is still under verification. Solitary bees, such as *Osmia cornuta*, for example, have promising possibilities.

Honeybees (*Apis mellifera*) show their highest activity at temperatures ranging from 15°C to 26°C (Free, 1970; Meith *et al.*, 1974). Their activity decreases at lower temperatures, stopping

under 10–12°C. Bees do not fly during rainfall or in winds faster than 24 km/h (Meith *et al.*, 1974). Placing beehives in blooming almond orchards is normal practice to increase the possibility of flower visits and, consequently, flower pollination. Placing between 2.5 and 5 beehives per hectare is recommended (Free, 1970; Rikhter, 1972; Meith *et al.*, 1974). These should preferably be protected from the predominant winds and facing east, so the hives receive the first sun-rays in the morning and become heated, favouring bee activity.

Other aspects involved in effective pollen interchange are the proportion and distribution of pollinizers. The ideal distribution is 50% of two cultivars with full-bloom coincidence, and it is recommended that the proportion should not fall below 33%. Their distribution must be decided at planting, noting that every tree must be close to a possible pollinizer, and having complete rows of a cultivar to facilitate harvest operations. More than two rows of the same cultivar must be avoided as bees do not cross between rows very much (Jackson and Clarke, 1991), and a decrease in production throughout the rows can result if solid blocks of several rows have been erroneously designed (Socias i Company *et al.*, 1994a).

The absence of compatible pollen in an orchard is a mistake that can only be corrected in



Fig. 1.34. Honeybee pollinating almond flowers. (Photo by J.M. Alonso.)

the medium term by grafting trees or complete rows with a cross-compatible cultivar. However, an immediate action may be to collect compatible pollen from another orchard and to spray it, once diluted with chalk or lycopodium powder (Thorp *et al.*, 1967). Artificial pollination by aircraft or ground blower has not been proved effective (Thorp, 1996). Pollen may also be distributed by bees if a pollen dispenser is placed at the exit of the beehive to spray the bees when they go out (Erickson *et al.*, 1977).

The requirement for pollen transfer is also solved by planting autogamous cultivars (Socias i Company and Felipe, 1993), as they are able to be self-pollinated without any foreign intervention, thus eliminating the need for pollen vectors.

Adequate temperatures for pollination are the same as for activity of the pollinating insects. Pollen grains germinate on the stigma surface giving rise to the pollen tubes. At low temperatures bees do not fly, but pollen germination is also very low, not enough to initiate effective pollen tube growth down the style. DeGrandi-Hoffman *et al.* (1989) reported that yield in almond is determined by the number of flowers per tree and the effective pollination period, and includes factors related to pollination efficiency, such as stigma receptivity, pollen tube kinetics and ovule longevity, as well as temperature. The concept of effective pollination period was defined as the period during which pollination was effective at producing fruit (Williams, 1965). This period is determined by the longevity of the ovules minus the time lag between pollination and fertilization, provided that this resulting value does not exceed the length of stigma receptivity. The most adequate times for effective pollination are the 2–3 days after flower opening (Griggs and Iwakiri, 1964; Ortega *et al.*, 2004; Kodad and Socias i Company, 2009a; Kodad and Socias i Company, 2013).

Once the pollen grains are deposited on the stigma they stick to the stigma surface thanks to the stigmata exudates, and pollen tube growth begins rapidly (Fig. 1.35). Pollen tube growth rate is temperature dependent (Socias i Company *et al.*, 1976), being sufficiently effective when temperatures are higher than 10–12°C (Griggs and Iwakiri, 1975), although pollen tube activity is maintained at very low temperatures (Socias i Company, 1982). Pollen tube growth is normal at temperatures between 18°C

and 27°C, being optimal at around 25°C (Socias i Company *et al.*, 1976; Socias i Company, 1982). At higher temperatures the process of ovule degeneration accelerates, giving rise to problems of infertility.

Not all cultivars have the same temperature thresholds for pollen germination and pollen tube growth. In cold regions it may be advisable to grow cultivars with acceptable pollen activity at low temperatures (García García and Egea Ibáñez, 1979).

1.4.4 Fertilization

For effective fertilization pollen tubes must reach the embryo sac of the ovule after growing the whole length of the style, and double fusion must take place. Effective pollen tube growth only takes place when pollination is compatible (Fig. 1.36). If pollination is incompatible, pollen tubes normally stop in the middle third of the style, normally giving rise to abnormal pollen tubes (Fig. 1.37) and enlargement of the growth end (Fig. 1.38).

Almond pollen is binucleate, with a vegetative nucleus and a generative nucleus. The latter divides during pollen tube growth giving rise to two generative nuclei. When the pollen tube reaches the embryo sac the two generative nuclei are discharged into the sac. One nucleus merges with the oosphere, the central nucleus of the embryo sac, giving rise to the zygote, whose development finishes with the formation of the embryo and the whole kernel. The second generative nucleus merges with the two secondary nuclei of the embryo sac to give rise to the triploid endosperm, a jelly-like structure which becomes the nutritious source of embryo development until its disappearance when the kernel is fully formed.

In normal field conditions ovule longevity is 6–8 days following flower opening. The pollen tube takes 3–5 days to reach the ovule. Consequently, as already pointed out, pollination must take place in the first days after flower opening to ensure correct fertilization. Some factors may affect ovule viability, shortening or lengthening its longevity or even causing its sterility, such as temperature or the nutrient status of the tree. Consequently, differences in productivity may be

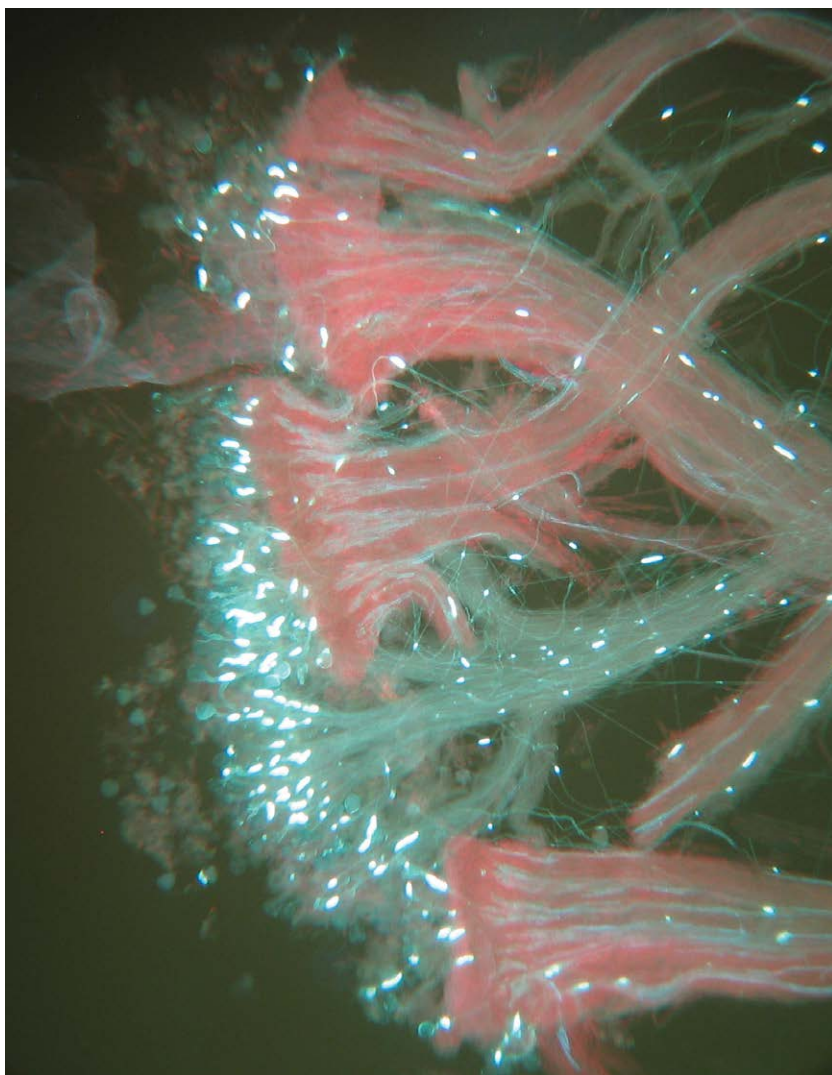


Fig. 1.35. Germinated pollen grains on the stigma surface giving rise to pollen tubes. (Photo by J.M. Alonso.)

observed in the same cultivar depending on these different circumstances. Another aspect to be considered in flower fertilization is the presence of sterile flowers (Fig. 1.39), a cultivar trait (Socias i Company, 1983), but very variable depending on the year (Bernad and Socias i Company, 1995) mainly because of drought and nutrient deficiencies.

The ovaries of pollinated but unfertilized flowers have an initial development until they reach the size of a pea or a little more, but fall 2–3 weeks after the end of bloom. Pollen tube

growth provides enough stimulus for this initial ovary growth (Kester and Griggs, 1959b; Meith *et al.*, 1974).

1.4.5 Fruit development

After fertilization the ovary enlarges and becomes a fruit (Pimienta and Polito, 1983). All the ovary structures become the different elements of the almond fruit. Thus, the ovary epidermis

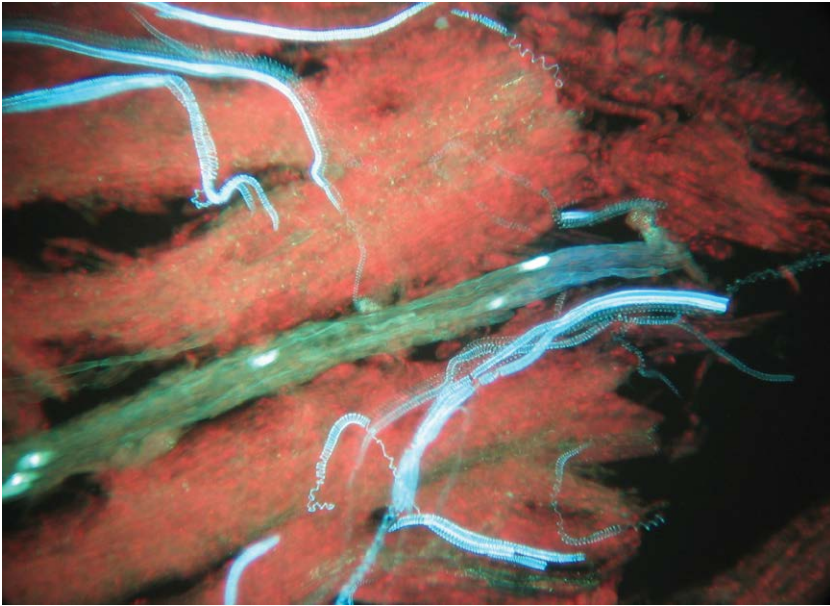


Fig. 1.36. Compatible pollen tubes reaching the style base. (Photo by J.M. Alonso.)



Fig. 1.37. Incompatible pollen tubes ceasing growth in the middle third of the style.

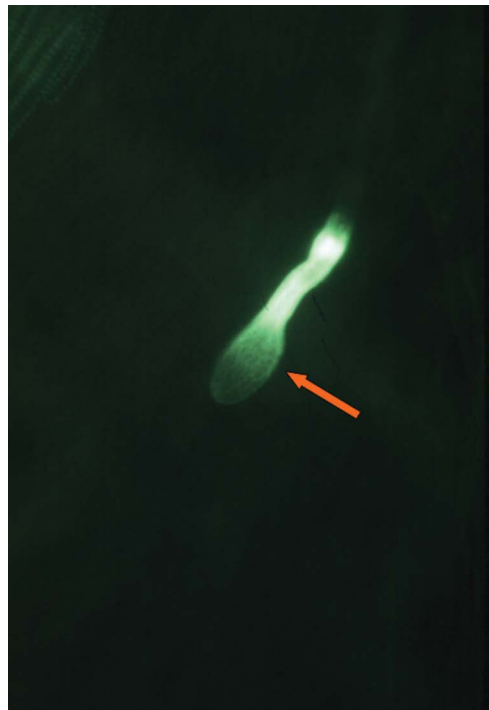


Fig. 1.38. Enlargement of the end of an incompatible pollen tube ceasing growth.

becomes the exocarp, which is normally very pubescent. The ovary wall becomes the mesocarp, the fleshy but corky part of the almond fruit, known as the hull. The inner part of the ovary wall is lignified and becomes the endocarp or shell. The line of ovary suture remains marked and is the line where the hull splits at maturity, showing the shell. The kernel is the embryo with two well-developed cotyledons, and the seed coat is the ovule tegument.

Stone fruit growth is characterized by three growth phases which form the sigmoid of fruit

development, although not all fruits follow the same type of growth:

- 1. Active cell division until the total cell number as found at maturity has almost been reached, with an important fruit enlargement.
- 2. Arrest of both cell division and enlargement, when the endocarp is normally lignified.
- 3. Final cell enlargement, with very few cell divisions, until reaching the final size at harvest (not present in almond).

These three phases have mostly been studied in peach, a species showing a very variable second phase depending on the cultivar, since the very early ripening cultivars have a very short or non-existent second phase. In almond the first phase takes approximately 12 weeks from flowering (Hawker and Buttrose, 1980), when the final size of the nut is reached. After this first phase no more growth is observed, and only the development of the kernel takes place (Fig. 1.40). At the beginning of endocarp lignification the embryo occupies a lower position in the tegument, and takes up limited space; the remaining space is filled by the endosperm. During the first weeks of the second phase, and without any visible variation in fruit size, the embryo completes its growth



Fig. 1.39. Aborted pistil giving rise to a sterile flower.

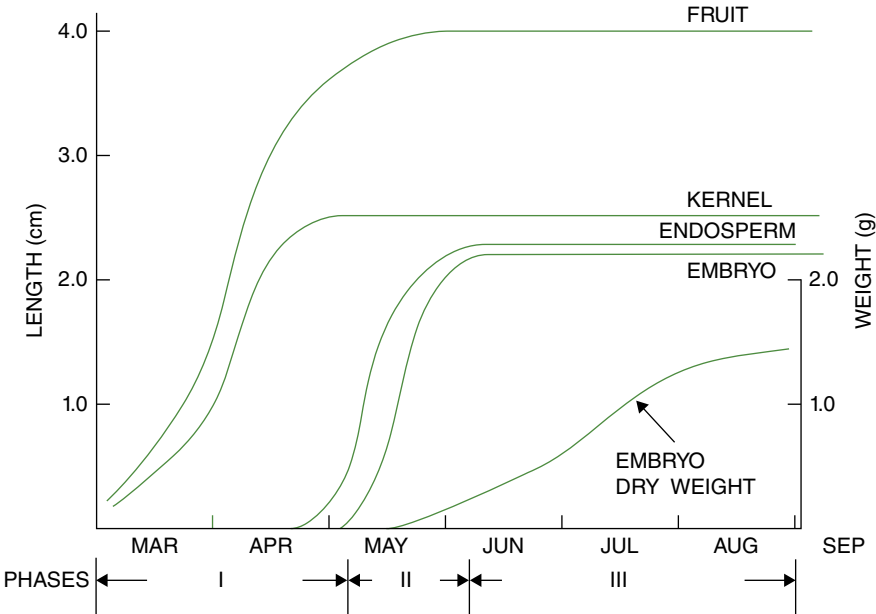


Fig. 1.40. Growth of the different almond nut components. (From Felipe, 2000.)

until it occupies all the space inside the tegument, whereas the endosperm disappears. Taking into account that the commercial part of the almond fruit is the kernel – the ripe embryo – this period of embryo enlargement is critical and any problem appearing at this stage, such as water stress, results in irregular kernel development such as wrinkled kernels, half-filled kernels or even empty seed coats (Fig. 1.41).

Consequently, irrigation must be very well programmed or, in non-irrigated conditions, cultivars must be selected for good adaptation to the possible lack of water. The water reserves in rainfed orchards may be too reduced to allow good kernel development and the final kernel size may be too small. The first phase of fruit growth in almond lasts about 2.5 months, and embryo development starts about 10 weeks after fertilization. This first phase is characterized by cell division of the pericarp until the final size is reached. At the same time the cells of the nucella surrounding the embryo sac also divide actively, producing a translucent tissue of large and fragile cells. Throughout this phase

the embryo remains a very small structure inside the nucella.

Cell divisions in the embryo sac proceed at a slow pace at the beginning, especially in the embryo. For the triploid endosperm, the rate of cell division accelerates after the sixth week until reaching the maximum size at a transition point from the first to the second phases of growth. From this point the endosperm only serves as a source of nutrition for embryo development. This development is very slow at the beginning, so that embryo size 50 days after blooming is between 100 and 200 μm (Grigorian, 1972). At this stage the embryo is a globular mass joined with the tegument by some cell rows which constitute the suspensor; after that, the radicle develops thanks to very active cell divisions. The plumule stays very small, but has a marked apex where differentiation of foliar primordia can be observed at 60–70 days. Despite the small plumule, at maturity the number of foliar primordia has increased to 14–16, so the embryo has a very well-differentiated structure which allows for quick development of the plant once the seed germinates.



Fig. 1.41. Aborted and semi-developed almond kernels.

Thus, embryo development mostly takes place in the second phase of fruit development.

The embryo is found between the two cotyledons that form the kernel, which fills completely the tegument in the 15th week after pollination. Not only water stress negatively affects kernel development, but also the nutrient status of the tree, mainly if there is nutrient competition among fruits after high fruit sets. An excessive crop reduces the size of the almond nut, although the size decrease is not as important as in other fruit trees (Felipe, 2000).

At maturity the mesocarp or hull dries out, provoking the rupture of the suture line, thus leaving the endocarp or shell partially visible. Some factors, such as an excessive drought or some diseases, may prevent the mesocarp splitting despite its dryness. The presence of sticky hulls is a problem for harvest processing as they are difficult to dehull. Ripening is not homogeneous for all fruits on the tree, depending on the cultivar, although simultaneous ripening is an interesting trait for harvest management. It may also be variable among trees of the same cultivar depending on age, nutrient and water status, and crop level. Nut fall before harvest also depends on the cultivar and may be a problem when harvest is delayed. Thus, nut retention must be as high as possible before harvest, but nut fall must be maximal at harvest to avoid leaving nuts on the tree. The nuts left are mostly those with some problem, such as sticky hulls. Ripening time is completely independent of blooming time, as almond cultivars show a large variability in the length of the reproductive cycle. The Spanish cultivars 'Desmayo Langueta' and 'Guara' are examples of this independence, as the first is early blooming and very late ripening, whereas the latter is late blooming and very early ripening.

Nut size and shell hardness are variable according to growing conditions such as temperature, water status and light, so no clear criteria on nut size can be established for cultivar identification. The percentage of double kernels is also variable. Nut and kernel shape, in contrast, are quite constant, as well as the ratios between the different dimensions, thus maintaining the typology of the cultivar (Kodad and Socias i Company, 2006).

In the fruiting process two aspects are particularly important: flower/fruit fall and production alternance. Flower and fruit fall may

occur for different reasons because in each reproductive stage the fall may affect flowers, ovaries or developing fruits. Taking into account that not all flowers will become fruits, a certain number of flowers must fail in their development. It is difficult to establish a normal fruit set for a good crop level, although Kester and Griggs (1959b) considered that a fruit set of 30–35% was an average for good conditions of cross-pollination in 'Nonpareil' and 'Texas' in California. However, lower fruit sets have also been considered acceptable (Socias i Company and Felipe, 1992a) because the level of fruit set needed for an acceptable crop would depend on the flower density of each cultivar (Socias i Company, 1988a), as well as on the percentage of flower sterility (Socias i Company, 1983). Consequently, a cultivar with very high flower density may attain an acceptable crop with low fruit set, whereas in other cultivars fruit set must be very high to compensate for a low flower density (Bernad and Socias i Company, 1998).

Flower and fruit drop take place at three different stages and in each case the fall occurs for diverse reasons (Kester *et al.*, 1959b; García García *et al.*, 1980). The first drop is of malformed flowers, mainly of sterile flowers due to deficient pistil development, and takes place just after bloom or even in the last stages of bloom. Normally these flowers have short pistils, often lacking ovary enlargement (Fig. 1.39). This morphological sterility is confirmed by deficient development of the ovule and often by its absence (Socias i Company *et al.*, 1976). Probably these flowers come from a deficient flower initiation due to poor water and nutrient conditions in the previous year. These flowers normally open during the last stages of the bloom period (Socias i Company, 1983; Bernad and Socias i Company, 1995). Even if these flowers were well pollinated, they would be unable to produce a fruit.

The second drop takes place at 3–4 weeks after bloom, and consists mostly of unfertilized flowers. They are well formed and start their development by cell enlargement and not by cell division (Bradley, 1962). These flowers could also have been pollinated but not fertilized because the pollination stimulus brings on initial development until they are pea sized. Some fertilized flowers may also drop at this stage because of improper embryo development, due mainly to cytological defects. These are also malformed

flowers, but with less pronounced defects than the sterile flowers of the first drop.

The third drop is basically physiological and takes place 6 or 7 weeks after bloom. It is often referred to as 'June drop', as in other fruit trees, but in almond the number of fruits is already stabilized in June and this physiological drop mostly takes place in April–May. At this stage nuts have practically reached their final size, but the nuts that fall are characterized by having a smaller size, yellowish epiderm and very light retention, as they fall due to simple physical contact. These fruits come from fertilized embryos, but at this stage, when the embryos have to drive their development, their size remains static. This is probably due to competition among fruits for nutrients. As a consequence of this competition, all fruits showing any deficiency cannot efficiently compete for available nutrients, especially if the fruit number is high, and even if the water and nutrient conditions are satisfactory.

Alternance has already been mentioned in connection with flower initiation. This phenomenon has important repercussions for production, because crop levels become very variable and commercial channels cannot be maintained. Alternance affects not only the production of an affected cultivar, but also that of the whole orchard, if self-incompatible cultivars are grown. This has been indicated in the very alternant cultivar 'Cristomorto' because in the years

when the reduction in the flowers is very marked, the resultant low level of flowers may be insufficient to produce pollen for pollinating the remaining cultivars in a mixed orchard.

1.5 Vegetative Growth

Vegetative growth in almond is the consequence of vegetative bud bursting and further shoot elongation. These simple processes are determined both by the intrinsic characteristics of the tree and by the environmental conditions, mainly the water status. Each cultivar has a characteristic growth habit, affecting tree vigour and tree habit. Some cultivars are very vigorous, with strong growth, whereas others of reduced growth are characterized by short shoots, often related to the formation of lower buds on spurs (Negrón *et al.*, 2013). Similarly, the tree habit may be very variable, sometimes with a willowy aspect giving name to a group of cultivars such as 'Desmayo Langueta'; although some other cultivars, such as 'Primorskij', may have an even more drooping growth habit. Growth habit is similar in related cultivars, as happens with other morphological traits (Sarvisé and Socias i Company, 2005).

Vegetative bud bursting develops from the dormant meristems (Fig. 1.42) and normally

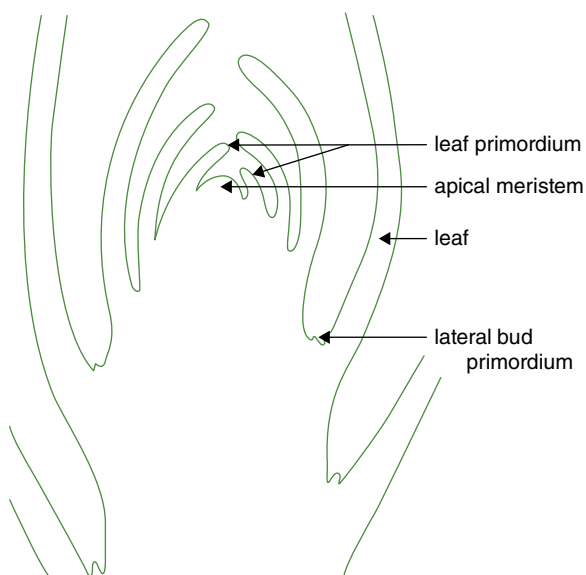


Fig. 1.42. Section of a vegetative almond meristem. (From Felipe, 2000.)

takes place just after blooming, but the time interval between the two phenomena may vary depending on the cultivar (Fig. 1.43), with cases of bud bursting before blooming (Büyükyilmaz and Kester, 1976). The initial bursting gives rise to a shoot with leaves along its length. Another vegetative bud is formed at the axilla of each leaf and usually remains dormant. This dormancy may easily be overcome by the removal of the apical bud, but in some cultivars these lateral buds burst easily during the same vegetative season, giving rise to the formation of anticipated shoots or feathers (Fig. 1.44).

Feathers are more developed at the shoot base than near the apex.

The different types of growth may require different pruning systems as seen in Chapter 11. Thus, summer or winter pruning may be recommended for some cultivars or growing systems. There has been recent interest in a type of growth defined as compensated (Socias i Company *et al.*, 1998), characterized by some growth for a gradual renewal of the canopy but not requiring too much pruning.

At the beginning of summer, with depletion of soil water reserves and temperature increase,



Fig. 1.43. Differential time relationship of vegetative bursting and blooming in two almond cultivars.

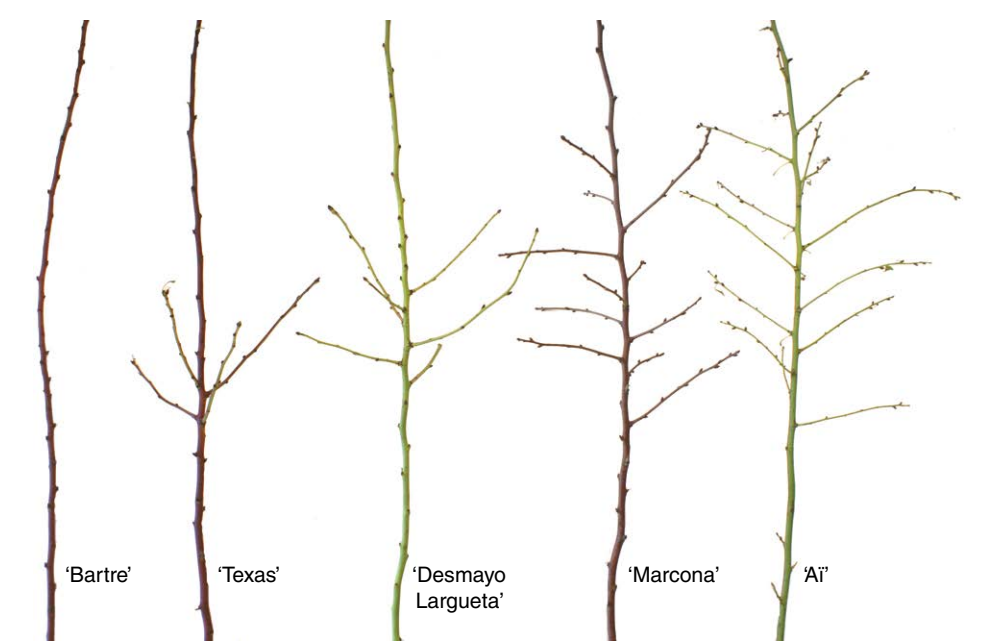


Fig. 1.44. Differential branching pattern in almond resulting in production of different feathers.

growth frequently stops in non-irrigated orchards. Under irrigation or in good water conditions only growth diminution takes place. In the autumn, after the normal rains of that season, growth is resumed and the shoots will be more or less thick, according to the growing conditions. The possibilities of growth and of bursting of the lateral buds are maintained during the whole vegetative cycle. Only at the end of autumn, with the onset of winter dormancy, does all vegetative growth cease, although the development of the flower primordia goes on inside the bud.

The internal regulation of growth and winter rest is controlled by the equilibrium of the different growth regulators. The study of hormone regulation and action has not been undertaken in almond as deeply as in other fruit species, although their metabolism and effects are probably similar. There has been some work on tree growth control by the application of growth inhibitors, as well as on watershoot control by applying auxin emulsions at the pruning cuts (also aiming to cicatrize the wounds), but they were not fully effective (Blanco, 1987).

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2 History of Cultivation

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2.1 Prehistoric Evidence

The kernels of a geographically wide range of Asian almond species represent a nutritious, compact and relatively non-perishable food source that is appetizing even when eaten in quantity or over a period of time. These qualities, combined with its presence throughout the range of early plant domestication by humans, ensured that it was among the first tree crops to be cultivated, probably during the third millennium BCE (Spiegel-Roy, 1976). The almond differs from the other *Prunus* crops both botanically and horticulturally, and the combinations of these differences have had widespread consequences for its role in human history and the role of humans in its dissemination. Botanically, the consumed part is a seed rather than a fruit, representing a durable propagation source for expanding plantings as well as a concentrated, desirable and relatively non-perishable food item, which made it a desirable commodity for trade even in ancient times. The wild almonds traded and consumed by early human communities were represented by over 30 species of diverse quality, morphology and geographic origin (see species list in Chapter 1). Early dissemination of this genetically diverse commodity followed the trade routes of emerging civilizations from central Asia westward to the Mediterranean.

Almond's widespread desirability and easy transportability appear to have made it an important commodity in prehistoric trade in Asia, North Africa and Europe, apparently leading to the establishment of an evolving market standard as well as a novel species: the cultivated sweet almond, or 'Greek nut' (*Prunus dulcis* (Mill.) D.A. Webb, syn. *Prunus amygdalus* Batsch., *Amygdalus communis* L., *Amygdalus dulcis* Mill.).

The subsequent, rapid reverse dissemination of these Greek nuts from the early Greek and Persian civilizations eastward into centres of almond's origin and beyond, including India and China, is an indication of the extent of global cultivation at this time. Also disseminated were a rich folklore and associated culinary practices based on almond's unique horticultural characteristics of very early flowering and associated traits, allowing it to thrive under harsh arid environments yet to produce a sweet and symmetrical amygdaloidal-shaped kernel. The natural range of these early almond species from north-western China to the northern Indus Valley in the east, to Mesopotamia and southern Europe in the west (Fig. 2.1), overlapped areas important in the transition of humans from hunter-gatherers to more permanent settlements. These cradles of civilization were also inherently cradles of plant cultivation and domestication, which undoubtedly involved selection

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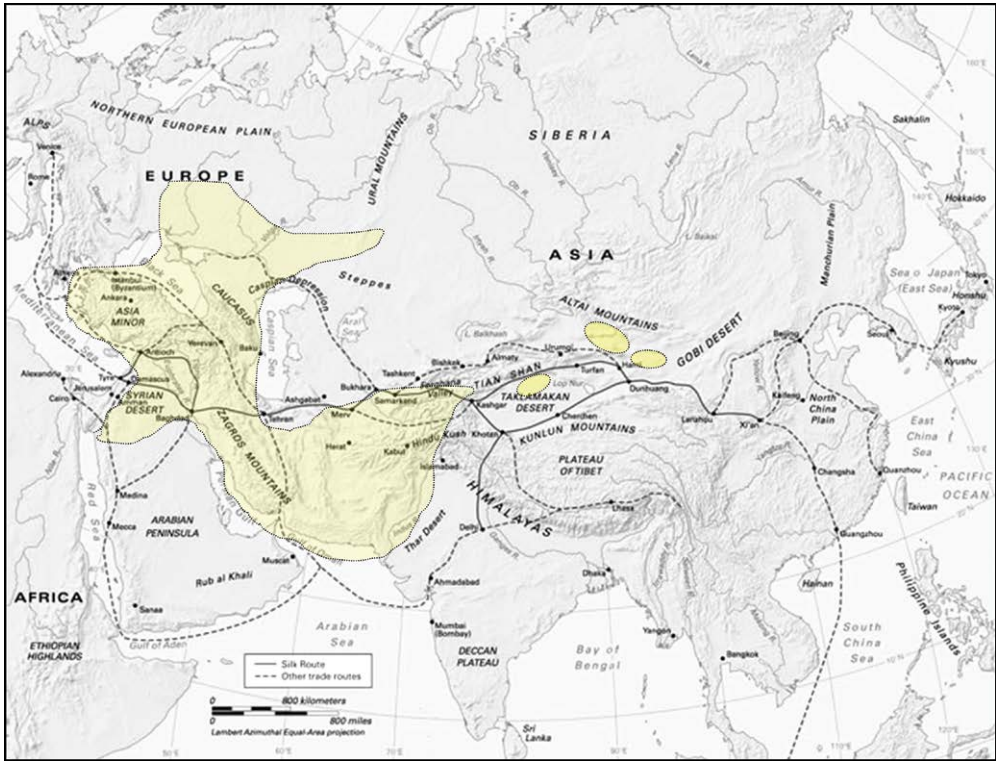


Fig. 2.1. Physical map of Eurasia showing distribution of wild almond species of the subgenus *Euamgdalus* (dashed outlines) and major ancient trade routes.

within the numerous wild almonds. The edible kernels of wild almonds and related species were thus important food staples from ancient times.

2.2 Wild Badam

Stone tools used for the apparent cracking of almond shells support the harvesting of wild almonds in northern Israel by our human ancestors as early as 780,000 years ago (Goren-Inbar *et al.*, 2002; Martinoli and Jacomet, 2004; Weiss *et al.*, 2004). Around 11,000 BCE, almonds, pistachios and lentils were being used at Franchthi cave in southern Greece, indicating that the farming of legumes and nuts preceded that of grain in Greece and possibly the rest of Asia Minor (Hansen and Renfrew, 1978; Farrand, 1999; William, 1999). In addition to wild almonds, kernels of wild apricots, plums and possibly peaches – also present in various western and central Asian ecosystems – would have been

consumed regardless of fruit quality, as they are to this day. The term *badam*, which when used alone refers to almond in a wide range of Asian languages (Turkish, Persian, Arabic (either *badam* or *loz*), Urdu, Hindi, Punjabi, Telugu Kashmiri, Kannada, Marathi, Gujarati, Tamil and Chinese (either *badam*, *bwa-dam* or *xing rén*, the latter also referring to apricot seed)) can also refer to the edible kernels of other *Prunus* species. For example, *tao'ze badam* refers to peach kernel in western China and *khasta badam* to apricot kernel (sometimes called 'poor man's almond') in India. Thus, in the absence of well-preserved endocarp remnants for species identification, it can be difficult to know which *Prunus* species was present in archaeological reports. Other distinguishing characteristics of almond species, however, are the symmetrical amygdaloidal (from amygdala, or αμυγδαλή) kernel shape (versus the more ovate peach and apricot kernel) (Fig. 2.2) and a flowering time among the earliest of all temperate trees and in which the flowers

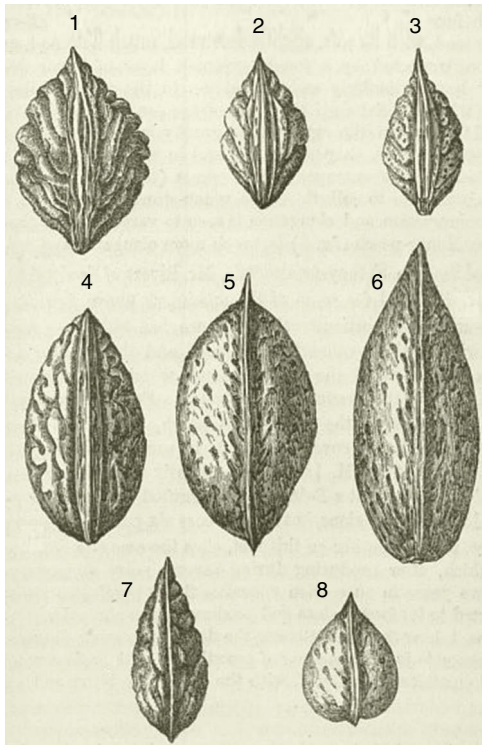


Fig. 2.2. Peach and almond stones, of natural size, viewed edgeways. 1. Common English peach. 2. Double, crimson-flowered Chinese peach. 3. Chinese honey peach. 4. English almond. 5. Barcelona almond. 6. Malaga almond. 7. Soft-shelled French almond. 8. Smyrna. (Darwin, 1868.)

emerge well before the leaves. This explosion of life towards the end of an otherwise barren winter apparently captured the imagination of ancient observers as it does now, contributing to a rich and varied folklore.

2.3 Historical and Cultural References

Almonds are mentioned in the earliest Sumerian culinary texts in a list of banquet menu items (Rosengarten, 1984). Biblical references to the almond show it was common in Palestine (where it can bloom as early as January) by at least 1700 BCE (Goor and Nurock, 1968; Janick, 2007). A reference to almonds in the book of Genesis 43:11 documents its high value: 'their father Israel said unto them, if it must be so now,

do this; take the best foods in the land in your vessels, and carry down to the man a present, a little balm, and a little honey, spices, and myrrh, nuts and almonds'. The man in this case was the governor of Egypt, from whom they were soliciting food aid in their time of famine, suggesting that almonds were also valued in Egypt but possibly not cultivated there. The Hebrew name for almond is *shaqed* or *shaqed*, which has its roots in an ancient Semitic term, as seen in the Akkadian *šiqdu* and Ugaritic *thaqid* as well as in old Ethiopic language. *Shaqed* may also be translated as 'watchful', symbolizing God's watchfulness over his people; as in Jeremiah 1:11–12, 'And the word of the Lord came to me, saying "Jeremiah, what do you see?" And I said, "I see an almond branch."' Then the Lord said to me, "You have seen well, for I am watching over my word to perform it.'" An early biblical reference, Numbers 17:8, describes how the staffs of the 12 princes of Israel were placed into the Tabernacle after the Exodus. Only the staff of Aaron of the house of Levy, which was almond, flowered (Fig. 2.3). This was interpreted as a sign of divine favour to Aaron, of God's watchfulness over him and his descendants (Rosengarten, 1984). According to tradition, the staff of Aaron bore sweet kernels on one side and bitter kernels on the other, symbolizing sustenance if the Israelites followed the Lord but bitterness if they were to forsake the Lord. The almond blossom also supplied a symbolic model for the menorah or ark that stood in the Holy Temple: 'Three cups, shaped like almond blossoms, were on one branch, with a knob and a flower; and three cups, shaped like almond blossoms, were on the other ... on the candlestick itself were four cups, shaped like almond blossoms, with its knobs and flowers' (Exodus 25:33–34; 37:19–20). That the golden candlesticks for the Tabernacle should have almond-shaped bowls may explain why ornamental pieces of crystal attached to candlesticks sometimes are still called almonds. Interestingly, in areas of Pakistan, western China and India, the prayer cap, or *topi*, is often adorned with paisley-like patterns said to represent almond blossoms (Fig. 2.4). In Arabia and other Muslim areas, almond's early flowering on leafless branches is seen as a symbol of hope (Rosengarten, 1984). The early-flowering habit of almond appears also to have made it symbolic for watchfulness or insight in ancient Greek mythology



Fig. 2.3. Flowering of the almond staff of Aaron at the Jewish Tabernacle during the Exodus. (Rosengarten, 1984.)

(Fig. 2.5). The symbolism derived from the Mycenaean Bronze Age (c.1200 BCE) myth of Phyllis of Thrace, who grieved so much when her lover Demophon did not return from Troy that the gods transformed her into an almond tree, thereafter called Phylla by the Greeks. Upon Demophon's return, he embraced the tree, which burst into blossom. A more ominous interpretation of almond bloom (perhaps alluding to the rather sudden whitening of a man's hair as he approaches old age and death), is given in Ecclesiastes 12:5: 'when men are afraid of heights and of dangers in the streets; when the almond tree blossoms and the grasshopper drags himself along and desire no longer is stirred. Then man goes to his eternal home and mourners go about the streets.' A similarly ominous significance appeared to be held by the Bronze Age Phrygian culture of Anatolia, in present-day Turkey, whose rites of worship to their main goddess Cybele were so savage that they were eventually banned. According to legend, the almond tree sprang from the blood of Cybele and played prominent roles in the creation of lesser gods. Besides symbolizing Cybele for her devotees, the almond was also regarded as the Father of Everything, according to Rawlinson (1917).

The symbolic importance of almond in these early cultures may have referred to its dramatic, life-reaffirming early bloom or to the

amygdaloidal kernel shape. The almond shape symbolized the female genitalia in the east, while in Europe it often represented the womb. In early Christian art, Christ is sometimes shown surrounded by almonds or an almond-shaped mandorla (from the Italian *mandorlo*, for 'almond') representing the womb (Fig. 2.6). In other parts of Europe, particularly central Italy, the almond symbolized the Virgin Mary. Consequently, almond nuts often have been associated with fertility. Romans showered newlyweds with almonds as a fertility charm. An old Romanian contraceptive measure was to carry roasted almonds on the person, perhaps because the roasting counteracted the nuts' traditional powers of fertility. It is still a modern European custom to give female guests at weddings a bag of five sugared almonds representing children, happiness, romance, good health and fortune. In Greece, almond cookies remain a popular wedding food. In Britain, their traditional Mothering Sunday Simnel cake is covered with almond paste in a possible reference to motherhood. The traditional almond paste and royal icing of British wedding cakes symbolizes the intermingled sweetness and bitter sweetness of the couple's new life together. Even in ancient China, almond's amygdaloidal shape was considered a symbol of female beauty as well as enduring sadness (perhaps because the symmetrical amygdalus or



Fig. 2.4. A prayer cap, or topi, adorned on top with paisley-like patterns said to represent single almond blossoms.



Fig. 2.5. Greek vase from 450 BCE showing the Oracle at Delphi holding almond branch in right hand. (From: www.coastal.edu/intranet/ashes2art/delphi2/misc-essays/oracle_of_delphi.html, accessed 27 January 2017.)

mandorla is the product of overlapping circles or because it refers to the inevitable bitterness present in the occasional bitter kernels).

In the wild, almond species usually produce cyanogenic and bitter seed; however, individual trees producing sweet and edible nuts have been reported in native populations of many almond and related species (Vavilov, 1930; Denisov, 1988; Werner and Creller, 1997). Although relatively rare, these individuals can readily be identified by consumption of their seed by native rodents and birds. Human gatherers from early times to the present would mark such trees for recurrent annual harvest, as has been documented in present-day hunter-gatherer groups



Fig. 2.6. A medieval Christian artistic convention in which an almond-shaped area, or mandorla, surrounds Jesus. (From: <http://en.wikipedia.org/wiki/Aureola>.)

in Asia and Africa (Alexander-Essers, 1994). The mutation for sweet kernel is expressed in all seed of the parent tree and – unlike in most other cyanogenic plants, including apricot and peach – is dominant in almond (Werner and Creller, 1997; Dicenta *et al.*, 2007; Negri *et al.*, 2008). Consequently, not only will all seed in a selected tree have sweet kernels, but the majority of seedlings derived from those seeds will also have sweet kernels. Domestication of sweet kernel genotypes would have been advanced by the propagation of these selected individuals, either through the germination and growth of harvested seed or by relatively simple propagation techniques, such as rooted cuttings developed during these prehistoric times, or through the weeding out of bitter almonds within a wild grove and the promotion of greater growth of the selected sweet types (Zohary and Spiegel-Roy, 1975; Grasselly, 1977; Gradziel, 2011). In addition, birds of certain species, such as the western scrub-jay, *Aphelocoma californica*, will systematically bury or cache single sweet almond seeds at several thousand distinct sites for later

Table 2.1. Nutrient composition of the almond kernel per 100 g fresh weight of edible portion. (Adapted from Socias i Company *et al.*, 2008.)

Nutrient	Value
Energy	578 kcal
Protein	21.26 g
Carbohydrate	19.74 g
Fibre, total dietary	11.8 g
Glucose	4.54 g
Starch	0.73 g
Calcium	248 mg
Magnesium	275 mg
Phosphorus	474 mg
Potassium	728 mg
Sodium	1 mg
Folate, total	29 mcg
Vitamin E	25.87 mg
Saturated fatty acids	3.88 g
Monounsaturated fatty acids	32.16 g
Polyunsaturated fatty acid	12.21 g

retrieval and consumption (Pravosudova and de Kortb, 2006). It is not unusual for many of these seeds to germinate and, if the site is appropriate, to grow into productive trees.

Toxins in bitter seeds can be removed through various basic processing methods. Bitter almonds possess the almond’s nutritional quality and long storage life as well as a natural protection against undesired feeding by mammals, birds and insects. Nutritionally, almond represents a compact, readily stored and high-energy food (Table 2.1). Both the edible, immature fruit and the mature kernel contain the amino acid linolenic acid, which is essential but not naturally synthesized in humans. Recent studies have also indicated that moderate consumption of almond kernels can suppress hunger. An exceptional post-harvest stability of almond kernels of over 2 years, when stored dry and in shell, further contributes to the value of almonds as an easily transportable, high-quality food source in early human transigrations and commerce. The same traits would also facilitate the establishment of widely dispersed almond stands through the accidental or deliberate planting of seed.

2.4 Greek Nut Cultivation

Although the kernel of native almond and even apricot and peach species continue to be harvested

today much as they were in antiquity, evolving market factors, particularly during the great flourishing of trade associated with the Achaemenian Dynasty of Persia (559–334 BCE), resulted in a market standard that appears to have become widely known in ancient times as the Greek nut and in more modern times as the cultivated sweet almond. Several hypotheses have been advanced concerning its origin. Russian scientists Kovalyov and Kostina (1935) suggested that the cultivated almond emerged by selection from within the species listed initially as *Amygdalus communis* L., whose range may have extended across Iran and eastern Turkey into Syria, Lebanon and Jordan (Browicz, 1974). Two natural sweet-kernelled *A. communis* populations have been reported on the slopes of the Kopet Dagh Mountains in central Asia between present-day Iran and Tadjikistan, and on the slopes of the Tian Shan Mountains between Uzbekistan and western China (Vavilov, 1930; Denisov, 1988) (Fig. 2.7). This species was reported to be adapted to mild winter and dry hot summer conditions by traits of low chilling, early bloom, rapid early shoot growth, deep penetrating root systems and high tolerance to summer heat and drought. Its phenotypic range closely resembles that of present-day cultivated almonds.

Another hypothesis originated by Evreinoff (1958) is that the cultivated almond arose by hybridization among *P. fenziiana* and possibly *P. bucharica*, *P. kuramica* and other species. This view, which has recently been supported by molecular analysis (Zeinalabedini *et al.*, 2009), holds that the cultivated almond originated through human intervention and is not a natural species. Grasselly (1976b) reported that *P. kuramica*, whose range includes Afghanistan and northern Pakistan, somewhat resembles cultivated almonds and coexists with it in many farming areas. *P. kuramica* grows on the more xerophytic sites, and *P. dulcis* is a mesophytic species. Different almond species, particularly those of the same section, cross readily, and considerable natural hybridization between cultivated almond and nearby wild species takes place (Grasselly, 1976a; Denisov, 1988). Introgressive hybridization and exchange of genes thus can take place whenever ranges overlap. Many of the more than 30 named almond species may not be true species but products of

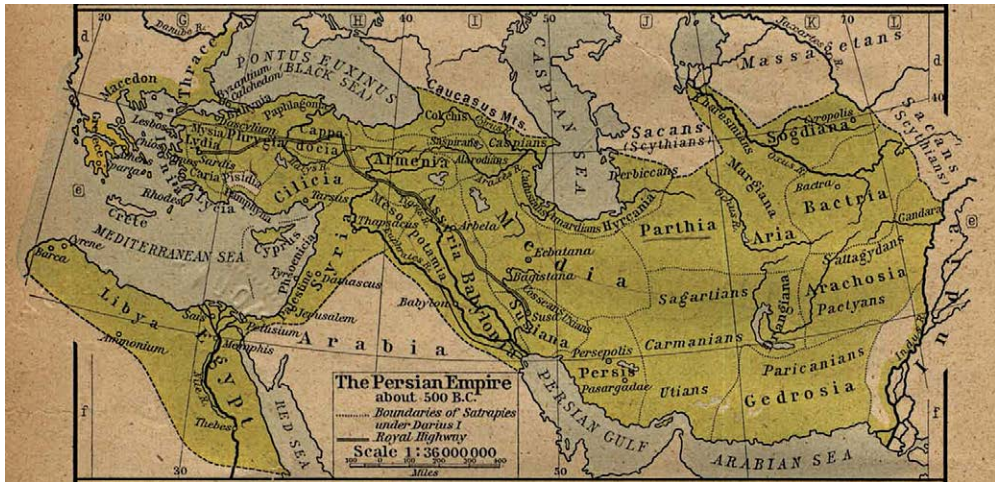


Fig. 2.7. Map of the Achaemenian Empire at about 500 BCE showing overlap with both time and locations of documented early almond plantings (see text).

such interspecific hybridization events (see Chapter 1). The previously described Kopet Dag and Tian Shan sweet-kernelled populations are proposed to be more recent feral populations of cultivated almonds or their natural hybrids (Browicz and Zohary, 1996). *A. communis* thus represents feral populations of *P. dulcis* (*A. dulcis*) rather than a native species. Furthermore, as almond cultivation expanded, new hybridizations/introgression would have occurred, as with the wild species *P. webbii* (Spach) Vierh. (Godini, 1977; Socias i Company, 1990; Socias i Company, 2002) and cultivated almond populations found along the northern shore of the Mediterranean sea from Greece and the Balkans to Spain and Portugal. Godini (2000) showed that *P. webbii* from the Italian region of Puglia was self-compatible, and that it probably contributed this trait to local cultivated almond landraces, since many almond orchards were made up of seedling plantings during prehistoric times to the present. Molecular analysis recently has shown this proposed interspecific introgression of self-compatibility from wild *P. webbii* to cultivated *P. dulcis* to be correct (Cortal *et al.*, 2002; Martínez-Gómez *et al.*, 2003; Martínez-Gómez *et al.*, 2007). Zohary and Hopf (1993) proposed that the area of initial domestication was the eastern part of the Mediterranean basin. However, the wild populations and species found in

the eastern Mediterranean appear genetically more distant from the cultivated almond than the wild populations and species of the Caucasus and Zagros Mountains of eastern Asia Minor and Persia (Sorkheh *et al.*, 2007). Both regions, however, were then part of the Achaemenian Dynasty of Persia (559–334 BCE), which actively encouraged both commercial and cultural exchange among its diverse regions (Fig. 2.7). The Achaemenian kings, including both Cyrus the Great (c.600–530 BCE) and Darius the Great (c.549–486 BCE), took a special interest in plant collection and cultivation. The Spartan mercenary Lysander, who joined the Achaemenian Persian king Cyrus the Younger in 401 BCE, reported to Xenophon of Athens how the Persian kings excelled not only in war but also in creating protected gardens, or *pairidaeza*, of plants, especially fruit-bearing trees, collected during their foreign expeditions. Xenophon (who was a supporter and chronicler of Socrates) went on to write in his *Oeconomicus* (Economics (399 BCE)) ‘The Great King ... in all the districts he resides and visits ... takes great care that there are “paradises” (from Greek form *paradeisos*) as they call them, full of all the beautiful things the soil will produce’ (Eduljee, 2009). One of the earliest *pairidaeza* may have been the mythical Hanging Gardens of Babylon, purportedly built by King Nebuchadnezzar (605–562 BCE)

to placate his homesick Median wife, Amytis, by copying the lush gardens from her childhood home on the slopes of the Zagros Mountains. Several wild almonds are common species in the Zagros Mountains, suggesting their possible inclusion in the Hanging Gardens. One of these species, *P. fenzliana*, from which cultivated almond was probably derived, also may have been present at an even earlier *para-deisos*; recent archaeological research has identified an area within the native habitat of *P. fenzliana* in the Caucasus Mountains as a possible site of the mythical Garden of Eden (Edujlee, 2009).

2.5 Old World Dissemination

It is known that the introduction of cultivated almond in the eastern Mediterranean area took place by the second millennium BCE, because cultivated almond remains have been found in the tomb of Tutankhamen, who was buried in 1323 BCE (Zohary and Hopf, 1993). Almond cultivation appears to have existed in Greece long before the creation of the Greek myths, to explain its incorporation into them (Graves, 1955), and there is evidence of extensive almond trade in the eastern Mediterranean in the 4th century BCE (Cerdá Juan, 1973) and possibly much earlier (Hansen and Renfrew, 1978; Farrand, 1999). The wide dissemination of modern almond and its cultivation have been separated into four phases: Asiatic, Mediterranean, Californian and southern hemisphere (Kester *et al.*, 1990). Often concurrent with the spread of cultivated almond is dissemination of a surprisingly similar folklore, including medical and culinary uses, suggesting that the spread was through well-established and interconnected trade routes (Albala, 2009).

2.5.1 Asiatic stage

The Asiatic stage included the initial domestication and the subsequent spread throughout central and south-western Asia. The Greek naturalist Theophrastus described almond, which he called *amygdalai* in his treatise on the history of plants in about 300 BCE. During the early Roman

expansion, Marcus Porcius Cato (c.236–149 BCE) referred to almond as the ‘Greek nut’, suggesting its dissemination via Greece. Pliny (23–79 CE), in his *Natural History*, also listed almond as *prima omnium*, or ‘first of all’. Within a few hundred years, the range of known almond cultivation included the regions now known as Turkey (Ayfer, 1975; Dokuzoğuz, 1975), Iran (Grigorian, 1976), Syria and Israel (Spiegel-Roy, 1976); and east to the Xinjiang Province of China (Gustafson *et al.*, 1989), northern Pakistan and north-west India (Singh *et al.*, 1977). In Kashgar, Xinjiang Province, cultivated almonds were reported to originate from central Asia across the Tian Shan Mountains to the west (Gustafson *et al.*, 1989). Kashgar is on the old Silk Road connecting China to India and the west, as were most other sites of eventual domestication. The extent and sophistication of this prehistoric trade was recently documented with the discovery of the wreck of the *Kyrnenia*, dating from around 350 BCE, in which both vessel and cargo remain remarkable intact (Fig. 2.8). In addition to cultivated almonds, the ship was carrying amphorae of wine and olive oil, grain millstones, coins and iron blooms. Using presumed and sometimes known origins for this cargo, the vessel’s probable trading route was deduced, showing that an advanced commerce had already been well established by this time (Albala, 2009). Such archaeological finds, as well as parallels in the associated folklore, support a central role of prehistoric Greco-Persian culture and commerce in advancing the cultivation, utilization and dissemination of the modern almond. As a traded commodity, the range of almond extended to the edge of the known world (Figs 2.1 and 2.7).

In far eastern China, the almond was described initially in the *Yu yan tsa tsu* as a flat peach from Persia. ‘The meat is bitter and acrid, and cannot be chewed; the interior of the kernel, however, is sweet, and is highly prized in the Western Regions and all other countries’ (Albala, 2009). The Persian name for almonds, *badam*, or in Old Persian, *vadam*, became the Old Chinese *p’o-tam* or *bwa-dam*. It also entered Tibetan as *ba-dam*. A later document by Li Si-cen, *Pen ts’ap kan mu*, identifies the origin of almonds as the land of the Mohammedans and states that they occur everywhere west of Gansu, the province bordering Xinjiang (literally, ‘New Frontier’). Because



Fig. 2.8. The *Kyrenia II* replica of the almond-carrying Greek cargo vessel sunk off Cyprus about 350 BCE (top). Proposed last route of the *Kyrenia* suggesting extensive commerce in the region (lower left). Samples of the 10,000 almonds found with the *Kyrenia* wreckage (lower right).

the more temperate and humid climate of eastern China limits almond cultivation, it was imported, especially in the T'ang Dynasty (618–907 CE), a period of rich cultural exchange between east and west. Fragments of Chinese pharmacopoeias survive, including 7th-century works by Meng Shen, which mention almonds and a number of western foods grown in imperial gardens (Simoons, 1991). Later, the *Hsin T'angshu* (new T'ang history) describes plants grown in western Asia, including almonds, grapes and figs from Persia (Albala, 2009). In the 16th century, Li Shizhen in his *Bencao Gangmu* (a classification of materia medica) reports: 'It comes from the lands of Hui people and is now in all the lands of the west... The tree is like an apricot but its leaves are smaller; the fruit is pointed and small, the flesh thin. Its kernel is like a plum-stone, the skin is thin and the almond is sweet and nice. It is eaten for tea, its taste is similar to that of the hazelnut. The people in the west consider it a local specialty' (Métailié, 2001).

In India, where almonds can be cultivated in the more Mediterranean climates of the northern provinces of Jammu and Himachal Pradesh,

descriptions of almond and its often medical and culinary uses appeared during the same period and also suggest a Greco-Persian origin (Peregrine and Melvin, 2003). Almonds in Hindi are called *badam*, which comes from the Sanskrit *vâtâma*, in turn from Persian *badam* or Old Persian *vadam*. As early as the 3rd century BCE (around the time of the voyages of the *Kyrenia*), the classic Indian medical texts *Caraka Samhitâ* and *Susruta Samhitâ* characterized sweet almond (*vâtâma* or *bâdâma*) as heavy, hot in potency, unctuous, sweet, strength promoting, alleviator of *Vata* (wind/spirit/air), nourishing, aphrodisiac and aggravator of *Kapha* (phlegm) as well as *Pitta* (bile). Although the *Caraka Samhitâ* and *Susruta Samhitâ*, ancient Indian Ayurvedic texts on internal medicine, are an early source of medical understanding believed to be independent of ancient Greece, Albala (2009) has recently pointed out the strong similarities between medical (and culinary) uses of almond as initially described in Greece with subsequent uses in Persia and its trading partners, including India and China. He proposes the apparent Greek origin of much of the traditional folklore associated with cultivated

almond is evidence for a Greek origin or major role in the dissemination of the cultivated form of almond (*P. dulcis*).

The ancient Greek physician Hippocrates (c.460–370 BCE), considered the father of western medicine, founded the humoral, or Unani, doctrine of medicine. Unani medicine is based on the concepts of the four humours: phlegm (*balgham*), blood (*dam*), yellow bile (*safrā*) and black bile (*saudā*). The word ‘Unani’ refers to Ionian Greek, meaning the Greek-populated west coast of Asia Minor, in what is now Turkey. Thus the term ‘Greek’ during this period of history refers not only to the Greek islands but to much of the eastern Mediterranean, which was then also strongly networked with Persian culture (Fig. 2.7). Hippocrates was among the first to record the medical uses of almonds. He reports: ‘Almonds are burning but nutritious; burning because they are oily, and nutritious because they are fleshy’ (Jones, 1967). In the Greek system of humoral physiology, this means that almonds would have been categorized as a hot and dry food, one that stimulates choler or energy in the body. Diocles of Carystus, a follower of Hippocrates, adds: ‘almonds are nourishing and good for the bowels, and are, moreover, calorific because they contain some of the properties of millet. The green are less unwholesome than the dry, the soaked than the unsoaked, the roasted than the raw’ (Bottero, 2004). Soaking almonds would activate seed digestive enzymes and facilitate removal of the bitter brown seed-coat. Roasting makes them more easily digested. Although green almonds would have been unknown outside their area of cultivation, soaking or blanching was a common practice in both Indian and Chinese medicine (Achaya, 1998). Medical philosophies differed, but the goal appears to be the same for each: to reduce their tendency to heat the body (Albala, 2009). Because of its classification as a hot food, which scours the body’s passages, almond was recommended by Hippocrates and his followers to relieve coughs, for weight gain and as an aphrodisiac (Albala, 2009). The Persian *Al-Qanun fi’l-tibb* (Canon of Avicenna) (980–1037 CE) became the standard medical text in medieval Europe and was the primary means of Greek humoral medicine reaching Arabia and Asia, particularly in Indian Unani medicine where the Canon is still the principal authority (Achaya, 1998; Bottero,

2004). Almond’s medical values were described in a manner very consistent with the earlier view of Hippocrates: ‘Almonds are more slowly digested and thus less likely to convert to choler’, and ‘sweet almonds comfort coughs and spitting of blood ... they open clogs of the liver and spleen on account of their bitterness. They even open clogs occurring in the extremities of the veins and if eaten fresh with the peel, clean humidity in the stomach’ (Albala, 2009).

The *Yin-Shan Cheng-Yao* dietary by Szu-Hui, of the Chinese Mongol era, dating from 25 CE also describes almonds in a similar fashion: ‘Almonds control coughing and bring down ch’i. They disperse impeded pressing of the chest and abdomen’ (Buell *et al.*, 2000). The Greco-Persian view of health as a balance of humours thus finds its parallel in the Chinese idea of *ch’i*, or the balancing yin and yang forces, which can also be described as cold and hot. In the Indian Ayurvedic medical system, almonds, in addition to the previously listed attributes of cough relief and aphrodisiac, are also classified as a hot food. In Ayurvedic medicine, as in Greek medicine, health consists of a balance of elemental forces or *doshas* (which are not exactly humours but energy principles that regulate physiological functions). Almonds are said to enhance the *kapha dosha*, which maintains the structural integrity of the body, but they also suppress an excess of the *vata dosha*, which is the principle of movement and transportation in the body. Thus, low weight gain in a thin overactive body in which nourishment dissipates quickly can be treated with a regimen including almonds (Albala, 2009). In the associated *Rasayana* approach to health and longevity, almonds provide vital energy, or *ojas*, a belief comparable to the Chinese concept of *ch’i* and the Greek concept of *pneuma* (Albala, 2009).

Almond kernels, in addition to being a high-quality food source, are also a source of high-quality oil. The oil, which can constitute more than 50% of the kernel by weight, is primarily composed of oleic acid (approximately 65%) and linoleic acid (approximately 30%), which results in good flavour and nutritional value as well as stability in storage. These qualities encouraged its use as a base for various ointments in both ancient and modern cultures. The most extensive use of almonds in Ayurveda is in the form of oil, used for various skin ailments

and to warm the body in massage and other therapies. Almond oil is used in various *vata* disorders, chronic constipation, dry cough, semen disorders, leucorrhoea and dysmenorrhoea. It is a good aphrodisiac, galactagogue and health tonic (Albala, 2009). In addition to the similar medicinal uses of almond in disparate Asian cultures, there were also a number of culinary dishes that appear to have a Greco-Persian origin. An ancient Baghdad cookbook, the *Kitab al-Tabikh*, records a number of sweet recipes, among which are *lauzinaj*, *faludhaj* and *samal wa-aqras* (Bottero, 2004; Albala, 2009). The Arabic word for almond is *lauz* or *loz*, whose occasional use in the Old Testament documents its use in antiquity. The *lauzinaj* recipe begins with finely pounded sugar and pounded almonds, kneaded together with rosewater. This is essentially marzipan, and it also comes with various flavourings, such as camphor or musk. These are ancestors of a number of dishes of India, such as *badam barfi*, *halwa* and similar almond sweets found throughout central Asia and as far as China. The term 'lozenge' is also derived from the Arabic *lauz*, perhaps in reference to its almond shape (Albala, 2009).

2.5.2 Mediterranean stage

The westward dissemination of almond cultivation into the Mediterranean had two stages. Almonds were first brought into the Peloponnesian peninsula and Greek isles (Stylianides, 1976), becoming well established by 300 to 400 BCE (Figs 2.1 and 2.7). Gradually almonds were introduced to all adapted areas of the Mediterranean, including Italy, southern France, Spain, Portugal, North Africa and the Madeira Islands. These introductions may have come from the early ocean trading Phoenicians of Asia Minor (Egea and García, 1975) and/or from the Greeks while establishing colonies in Sicily, Europe and North Africa, and from other groups as extensive trade routes were well established by this time. Cultivation typically was limited to within 50 miles of the Mediterranean coast, extending onto the slopes of river valleys and the interior plateau of Spain. Further introductions came from about 500 to 600 with the conquest of North Africa by the Arabs; they brought almonds

into Tunisia (Jaouani, 1976) and Morocco (Laghezali, 1985) and then into Spain and Portugal (Egea and García, 1975). One of the ancient Silk Road caravan routes also crossed north-central Africa, through Timbuktu into Morocco, thus representing an even earlier route of possible dissemination to North Africa and western Europe (Evreinoff, 1952; Evreinoff, 1958). Remnants of such pre-Arabic introductions may exist today in the diverse germplasm only now being documented in the geographically isolated Atlas Mountains in Morocco and Tunisia (Laghezali, 1985; Lansari *et al.*, 1994).

Archaeological studies at the site of the Mount Vesuvius eruption, which occurred in the year 79, indicate that almond was a common food of the Campanians of southern Italy by the 1st century. Almonds appear to be well known much earlier in Rome's history, though, as they were described by Marcus Porcius Cato as the 'Greek nut' as early as 200 BCE. In Latin, almond was called *amandola*, derived from the Greek *αμυγδαλη* (*amigdola*) (cf. *amygdala*). The Latin *amandola* appears to be the root for the term used for almond in Italian (*mandorla*), German (*mandel*), Swedish (*mandel*), Russian (*mindal*), Croatian (*mandula*), French (*amande*), Spanish (*almendra*) and Portuguese (*amendoa*). Interestingly, in the etymologically more isolated Romanian language, almond is *migdala* and so probably derived from the Greek. It has been widely postulated that the 'al' prefix, as in the Spanish *almendra*, and English *almond*, resulted from its fusion of the Arabic article 'al' with the Latin *amandola* (and dropping the initial 'a,' as done with the Italian *mandorla*), which represents an etymological and cultural legacy of the 800-year control of Spain by the Arabic-speaking Moors.

There is no doubt that Roman knowledge of almond was strongly influenced by the Greeks. Pliny the Elder (23–79 CE), the ancient Roman nobleman and historian, reiterated earlier Greek ideas that almonds act as a diuretic and emmenagogue. They provoke sleep and sharpen the appetite and are also useful against headache and fever, the latter presumably from their purging qualities to drive out the fever rather than to counteract or cool it (Albala, 2009). Galen of Pergamum (129–200 CE), a highly influential Greek physician serving several Roman emperors, believed that although bitter almonds were not very astringent, they cleansed and attenuated

and thus purged, and 'act towards the expectoration of moist matter from the lungs and chest. The very bitter cut through thick and viscous matter. But they are also oily, so not as useful for purging the stomach, and bitter almonds unlike the sweet afford little nourishment for the body' (Grant, 2000).

Rosengarten (1984) described an almond-based cold cream used by fashionable French women in the 17th century. This 'Ninon de Lenclos' ointment contained 4 ounces (112 g) of almond oil, 3 ounces (74 g) of hog's lard and 1 ounce (28 g) of spermaceti (a waxy component of sperm whale oil). These ingredients were melted, lemon juice was added, the mixture was stirred until cool and then scented with rose water. Almond oil continues to be an important oilbase for cosmetics and other pharmaceuticals to the present day (see Chapter 19). Additional medicinal uses of almond ointment included the treatment of pattern baldness and as a soothing ointment for burns. The continuing practice in parts of Asia is the application of almond oil to the heads of babies, as it is believed to promote their subsequent intelligence. Salves containing concentrations of oleic and related fatty acids may also help control of head lice (Socias i Company *et al.*, 2008), which might further explain the application of almond oil to the scalps of children.

While bitter almonds and almond oil were used primarily as medicine, sweet almonds and oil of bitter almond (an alcohol-based extract of bitter almond essence, primarily benzaldehyde) were used as both food and medicine. Both also found culinary uses. One of the oldest surviving Roman cookbooks, *Apicius* (4th or 5th century CE), describes a dish called *apothermum*, which is boiled wheat with pine nuts and almonds that have been soaked, skinned and whitened with silver chalk (*creta argentaria*) to which is added raisins and raisin wine, with pepper over the top (Bottero, 2004). As pointed out by Albala (2009), this recipe bears a striking resemblance to a rice dish in Persian cookery. In turn it has found its way to India and China and ultimately to England and the West as rice pudding. A similar soup made with ground almond, rice and sugar was used for sore throats in the Ch'ing court in China (1644–1911) (Simoons, 1991). In modern China, a popular dish to combat colds and sore throats is made with apricot kernels

powdered and mixed with rice congee (Simoons, 1991). A traditional Christmas dessert in Sweden and parts of northern Germany is cinnamon-flavoured rice pudding into which a single almond has been hidden; the one who finds the almond will have good fortune for a year.

A renaissance in European cuisine that involved spices as well as almond and other foods from Asia appears to have occurred from the 10th through the 13th centuries following the spread of Islam. Evidence for even earlier almond plantings in France can be found in the charter granted by King Chilperic II (716) and in an edict of Charlemagne in 812 (Rosengarten, 1984). An inventory of the household goods of the Queen of France in 1372 listed only 20 pounds of sugar but included 500 pounds of almonds. Almonds appear to have been relatively inexpensive and generally available to the emerging middle class even in countries such as England, where the climate would make local production more difficult. Rosengarten (1984) estimates an average price of approximately 2–3 pence per pound between the years 1259 and 1400. The almond trade appeared substantial by the 14th century, with Venice becoming an important centre of commerce. To capitalize on this extensive commerce, the Knights Templar levied tithes on almonds, honey and sesame seed in 1441.

The Arab conquest of North Africa and Spain introduced Middle Eastern cuisine, which included almond and sugar-based confections similar to the previously described marzipan, as well as more staple fare such as *harisa*, a popular dish of 10th-century Baghdad. This consisted of meat and vegetables served with a sauce thickened with powdered almonds (Rosengarten, 1984). Similarly, almond milk, which could be used as a milk substitute for direct consumption or in cooking, was made by soaking in water pulverized almonds from which the outer seed coat had first been removed. Almond milk became a more frequent ingredient in Mediterranean cuisine than in the Middle East. For example, the grand medieval European dish *blancmanger* was typically chicken stewed in almond milk. The comparable and popular Persian dish *isfidhabaj* (white stew) used almond milk in some recipes though many recipes contained no reference to almonds at all. Religious and medical views have played roles in the emergence of almond in European cuisine.

In addition, physicians from the 12th to the 16th century continued the belief that the nature of the food consumed was a critical component of health (Fig. 2.9). Echoing ancient Greek beliefs, foods such as almonds, chicken and rice were viewed as moderately warm and moist in their characteristics and so compatible with the healthy human body. The prevailing view among the upper class was that such moderate foods were more ideally suited for consumption and had the added value of moderating foods perceived as being too hot (ginger), too cold (many vegetables), too dry (turnip) and too wet (watermelon) (Albala, 2009). Even more extensive culinary uses of almond were eventually brought to Europe by the returning Crusaders, many of whom had developed a preference for more exotic Middle Eastern cuisine compared to the blander European diet of the time. As an example, a recipe from the *Forme of Cury*, dating back to 1390 (<http://homecooking.about.com/od/foodhistory/a/almondhistory.htm>), uses ground almonds in a gravy for oysters that is still fashionable. A rich folklore, including extensive medical and culinary uses of almond, can also be found in Spain, particularly southern Iberia, which was under Moorish control the longest. The origin of this folklore is often uncertain, though, since during the Dark Ages the Muslim Arab kingdoms were the main

repository and disseminator of the earlier Greek and Roman knowledge.

A bit of mythology often credited to Aristotle (but probably not disseminated by the abstinent Arab Muslims) was this proposal of Roman author Pliny the Elder: 'It is said that if five bitter almonds are taken by a person before sitting down to drink, he will be proof against inebriation'. Plutarch (c.42 BCE–37 CE) likewise says that Drusus, brother of Tiberius, who was a prodigious drinker, used almonds this way. The logic here is that the almond, due to its bitterness and diuretic properties, speeds the alcohol through the system before it has a chance to send vapours up to the head (which was believed to cause inebriation). The *Herbal* of John Gerard (1597) states that 'five or six (almonds) being taken fasting do keepe a man from being drunke' and may have been the forerunner of the cocktail nut of today (Rosengarten, 1984). Purported protection from inebriation may have also contributed to the previously cited popularity of almonds at weddings. In traditional Greek weddings, slightly bitter sugar-coated almonds called *koufeta* are placed in little bags in odd numbers and served on a silver tray. Odd numbers are indivisible, symbolizing how the newlyweds will share everything and remain undivided. Tradition holds that if an unmarried woman puts the almonds under her pillow, she

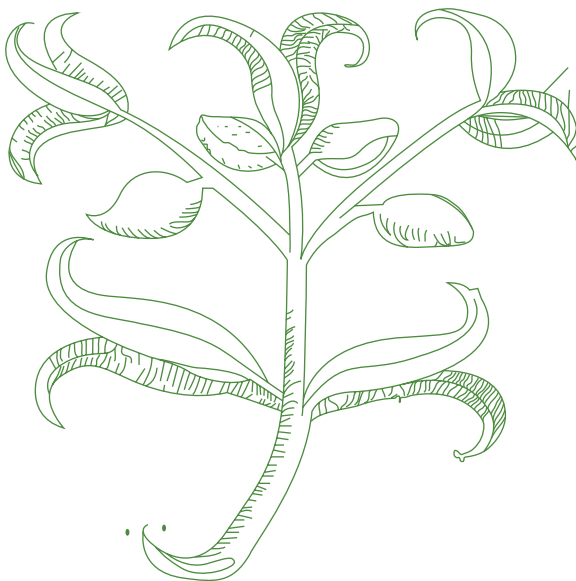


Fig. 2.9. Lithographic reproduction of an almond twig from the 1517 *Herbal* by Johannes Niger (Avanzato and Vassallo, 2006).

will dream of her future husband. Five (often sugar-coated or 'Jordan') almonds signifying five wishes (health, wealth, happiness, fertility and longevity) are common in traditional Italian weddings and other special occasions. These almonds decorate each place setting as favours, tucked into pretty boxes or tulle bags called *bomboniere* that are often personalized with the couple's names and date. A different and new type of favour is the favour cake or *Torta Bomboniera*, which is made using little boxes forming one or more tier of a cake. Inside each box are the sugared almonds and a card printed with the date of the ceremony. Sugar-coated almonds are also traditional in Middle Eastern weddings and are considered by some to be aphrodisiacs.

In southern Europe, almonds also symbolized good luck as well as long life and happiness. The heartening capability of a dormant almond shoot in winter to quickly flower, when cut and brought into warmer temperatures, is frequently alluded to in European art and literature from medieval times to the present. In the legend of Tannhauser, made famous by Richard Wagner's 1845 opera, the knight was informed by the pope that he was as likely to have his sins forgiven as the pope's staff was likely to bloom (Rosengarten, 1984). The staff, which was made of almond, did indeed bloom but, tragically, Tannhauser died before his pardon arrived. The almond's seemingly magical flowering ability also may have contributed to the preference for a wand made from almond by professional magicians during the Middle Ages, as well as to the use of almonds in Tuscany for making dowsing rods for the divination of underground water or other hidden items of value (reminiscent of the almond branch of the Oracle at Delphi; Fig. 2.5).

Almond was also well suited for cultivation in the Mediterranean climate with its traditional low-input moderate-return dry-land cropping systems. Easy propagation by seed, rapid development of the tree and adaptation to marginal soil, summer drought and heat, combined with its food value, made the almond well adapted to the subsistence form of agriculture predominating in early Mediterranean culture. High root tolerance to drought but high susceptibility to excessive soil moisture placed almonds in a mixed culture system with olives, carob and other drought-tolerant crops. Almonds were usually found on well-drained slopes at higher

elevations of valleys to escape shoot damage from frosts and root damage from excessive soil water. A traditional culture system evolved that minimized inputs of labour, fertilizers and use of supplementary water. Some trees producing bitter nuts were tolerated in local seedling orchards since bitter seed were mixed in small quantities with sweet kernel to produce the distinctive amaretto flavour. Because sweetness is the dominant trait, genes for bitterness may still be present in sweet-kernel trees, allowing the occasional recombination of bitter seedling trees. The Italian naturalist Scribonius Largus documented this distinction in the 1st century CE by naming bitter almonds *Amygdali amari*. Bitter forms of the cultivated almond sometimes are classified as *Prunus dulcis* (Mill.) D.A. Webb var. *amara* (DC) Buchheim (Fig. 2.10). Bitter almonds are also commercially cultivated for their oil, which is used to extract benzaldehyde for the amaretto essence, known to bakers and chefs as almond extract or oil of bitter almond but known to many consumers as the cherry flavour in cherry colas, drinks and confectionaries. The presence of excessive trees producing bitter or otherwise undesirable seeds in segregating seedling populations would be an incentive to use the undesired bitter trees as rootstocks for topworking with scions from the more desirable trees. Grafting was widely used in antiquity and is mentioned in writings of Hippocrates, Theophrastus, Cato, Varro, Columella and Pliny. Two thousand years of continuous almond culture in the Mediterranean basin concentrated production to specific regions where well-defined seedling ecotypes and local cultivars evolved (Grasselly, 1972; Grasselly, 1976b; Grasselly and Crossa-Raynaud, 1980). These highly selected regional cultivars and landraces represent a rich genetic, horticultural and culinary diversity (Table 2.2). Dry-land, seedling orchards remained the major almond culture system in the Mediterranean region for centuries, persisting to recent times in such areas as Sicily, Sardinia, Mallorca, the Madeira Islands, Greece, Turkey and Morocco. By the middle of the 19th century, cultivars of specific Mediterranean countries were recognized by nurseries by name (Fig. 2.11) and were introduced into California as early as 1843. By the turn of the 20th century, most of the almond countries of the Mediterranean region and the USA had identified 'local cultivars'



Fig. 2.10. Bitter almond (*Amygdalus communis* L. var. *amara* (syn. *P. dulcis* var. *amara*)) from 1926 *Textbook of Pharmacognosy* by T.C. Denston showing similar morphologies to cultivated sweet almond despite distinct botanical classification.

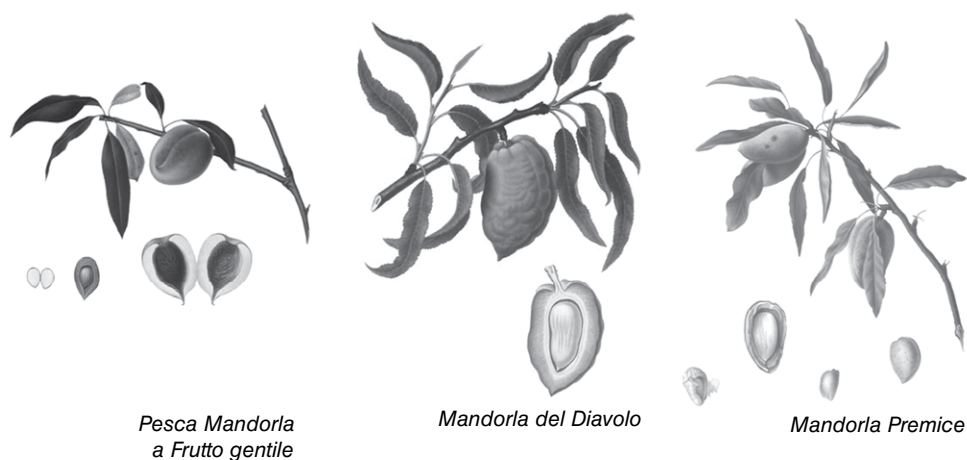


Fig. 2.11. Illustrations representing different heirloom almond varieties from the Genoa region of Italy. (wikipedia.org/wiki/Mandorla (accessed 27 January 2013).)

Table 2.2. Characteristics of important new almond cultivars from selected breeding programmes.

Cultivar	Description ^a
SPAIN	
CITA of Aragón (Zaragoza)	
Blanquerna	'Genco' OP, SC, mid-blooming, hard shell, large kernel of excellent quality, early ripening
Cambra	'Ferragnès' × 'Tuono', SC, late blooming, hard shell, medium ripening
Felisia	'Titan' × 'Tuono', SC, very late blooming, medium-hard shell, small kernel, very low alternance, early–medium ripening
Belona	'Blanquerna' × 'Belle d'Aurons', SC, late blooming, hard shell, large kernel with an outstanding composition, medium ripening
Soleta	'Blanquerna' × 'Belle d'Aurons', SC, late blooming, large kernel with an outstanding performance when roasted, medium–late ripening
Mardía	'Felisia' × 'Bertina', SC, extremely late blooming, disease tolerant, early–medium ripening
Vialfas	'Felisia' × 'Bertina', SC, very late blooming, vigorous, early ripening, hard shell, average kernel with a high oleic acid content
CEBAS – CSIC (Murcia)	
Antoñeta	'Ferragnès' × 'Tuono', SC, late blooming, hard shell, high vigour, spreading with dense branching, very early ripening
Marta	'Ferragnès' × 'Tuono', SC, hard shell, high vigour, upright, late blooming, early ripening
Penta	S5133 × 'Lauranne', SC, extremely late blooming, hard shell, intermediate vigour and branching, early ripening
Tardona	S5133 × R1000, SC, extremely late blooming, hard shell, small kernel, intermediate vigour with dense branching, medium ripening
IRTA–Mas de Bover (Reus)	
Constantí	('Ferragnès' × 'Ferraduel') OP, SC, late blooming, mid-ripening, vigorous, mid-branching
Marinada	'Lauranne' × 'Glorieta', SC, very late blooming, mid-ripening, mid-vigour, mid-branching
Tarraco	('Ferralise' × 'Tuono') × Anxaneta, SI, very late blooming, mid-ripening, mid-vigour, large kernel, mid-branching
Vairo	('Primorskij' × 'Cristomorto') × 'Lauranne', SC, late blooming, early ripening, high vigour, mid-branching
FRANCE	
INRA (Avignon)	
Lauranne	'Ferragnès' × 'Tuono', SC, medium-hard shell, medium vigour, late blooming, early–medium ripening, some double kernels
Steliette	'Ferragnès' × 'Tuono', SC, semi-hard shell, medium vigour, late blooming, early ripening, some double kernels
Mandaline	'Ferralise' × 'Tuono', SC, late blooming, medium ripening, hard shell, medium to upright growth
ISRAEL	
Shefa	'Tuono' × local cross, SI, vigorous, early blooming, highly adapted to Israeli conditions, soft shell, large kernel, early ripening
Matan	'Lauranne' × 'Um El Fahem', SC, medium blooming, vigorous, moderately spreading, early ripening, semi-hard shell, large kernel
USA	
University of California (Davis)	
Avalon	Probably 'Nonpareil' OP, SI, medium kernel, early blooming, paper shell, harvest approx. 8 days after 'Nonpareil'
Kahl	Chance seedling in a 'Nonpareil', 'Davey' and 'Mission' planting, SI, mid-blooming, large kernel, semi-soft shell, harvest 14 days after 'Nonpareil'

Continued

Table 2.2. Continued.

Cultivar	Description ^a
Morley	'Mission' × late-blooming almond seedling, SI, late blooming, medium kernel, semi-soft shell
Savanna	'Nonpareil' × late-blooming almond seedling, SI, late blooming (2 weeks after 'Nonpareil'), large kernel, semi-soft shell, harvest 14 days after 'Nonpareil'
Sweetheart	SB3,54–39E (('Lukens Honey' peach × 'Mission') 'Nonpareil') × Sel 25–26. SC, mid-blooming, large 'Marcona'-type kernel, harvest approx. 10 days after 'Nonpareil', semi-soft shell, high kernel oil and roasting quality, resistant to post-harvest worm damage
Winters	'3-1' ('Peerless' × 'Harpareil') × '6-27' ('Nonpareil' × 'Jordanollo'), SI, early blooming, large Carmel-type kernel, paper shell, good bloom overlap with early 'Nonpareil' bloom, harvest 3 weeks after 'Nonpareil'

^aOP: open pollinated; SC: self-compatible; SI: self-incompatible

that were seedling selections which, while representing the germplasm of the region, were often of unknown origin. Italy became a major almond-growing area, and many cultivars have been described in Puglia (Fanelli, 1939; Grasselly and Crossa-Raynaud, 1980), Sicily (Bianca, 1872; Spina, 1958) and Sardinia (Milella, 1959; Agabbio *et al.*, 1984; Chessa and Pala, 1985). Puglian cultivars were predominantly late blooming, had hard shells with a kernel/nut ratio of 25–30% and had a high percentages of double kernels. Sicilian cultivars tended to be early blooming, very hard shelled and more or less round in shape. Several Italian cultivars were also self-compatible.

Spain has a long history of almond culture, with almonds being grown in most provinces (Gardner and Lee, 1979). Principal concentrations are in coastal provinces (Tarragona, Valencia) along the Mediterranean coast (Murcia) and the Ebro valley (Lérida), although the range extends to the south-eastern border with Portugal and into the interior (Murua *et al.*, 1993). 'Marcona' and 'Desmayo Largueta', both of unknown origin, have been the most widely grown cultivars. Both have very hard shells, are highly productive and have well-recognized eating and cooking qualities such that they are marketed by name. Many other named cultivars of local origin (Vargas, 1975; García *et al.*, 1985; García *et al.*, 1988) have evolved from localized ecological niches that occur in different valleys extending inland from the Mediterranean coast.

France is an old almond-growing country with areas in the southern Rhône valley and the

surrounding foothills (Grasselly and Crossa-Raynaud, 1980). Cultivars originating in Provence and the foothills of the Alps tend to be late blooming. Cultivars associated with the Languedoc region nearer the centre of the Rhône valley ('Aï', 'Princesse', 'Languedoc', 'Pistache', 'Ardéchoise', 'Fournat de Brézénau', 'Rabasse') show a range of tree and nut characteristics from hard to soft shelled and from large to small kernels.

Portugal has two principal areas of production, the Algarve region in the south and the Alto Duoro in the north. Each region has cultivars specific to the region (Grasselly and Crossa-Raynaud, 1980). Similar situations exist in other traditional almond-growing countries such as Tunisia, Greece and the Canary Islands.

The traditional almond production system of the Mediterranean and Asian regions, however, began to fail in the 1940s, and the French almond industry essentially went out of production by the 1950s (Grasselly and Crossa-Raynaud, 1980). By the 1970s, production could not keep pace with the world demand for almond products because the most productive land was used for high-value crops such as peach and grape. Italy, historically the leading almond producer of the world, experienced a sharp decline in production (Federazione Italiana dei Consorzi Agrari, Ufficio Tecnico Agrario, 1973; Godini, 1977; Bacarella, 1994) and began to import almonds. Spain expanded its area and production but the scarcity of productive irrigated land has made production increases relatively modest (Murua *et al.*, 1993; Murua, 1994). In addition, supplemental honeybee pollination is not widely practised (Godini, 1977; Godini *et al.*, 1992;

Socias i Company and Felipe, 1992), significantly reducing final yield potential. Declines in production relative to other higher-value tree crops also took place in the Asiatic regions of cultivation, where in many areas almonds continue to be grown under conditions similar to those used thousands of years ago. The genetic, horticultural and culinary diversity that initially made these selections highly adapted to their production regions proved inconsistent with the new kernel ideotype of an increasingly global and so increasingly standardized market.

Similarly, attempts to extend the range of cultivated almond into continental areas, such as Yugoslavia (Ristevski, 1992), southern Russia (Rikhter, 1972), Romania (Cociu, 1985), Bulgaria (Serafimov, 1975; Serafimov, 1976) and Hungary, have met with only partial success. This has been due to almond's susceptibility to winter cold and spring frosts as well as to flower and foliar diseases, which is exacerbated by summer rains in these regions.

2.6 New World Dissemination

Almond dissemination to the New World followed early colonization by European and Asian settlers, eventually resulting in commercial plantings in North and South America, Australia and South Africa. Successful cultivation typically occurred only after a series of failures as early settlers, not recognizing the degree of almond's vulnerability to winter cold, spring frosts and summer rains, tested different growing regions and germplasm until suitable combinations were found. Almond's introduction into California, which began as an extension of the Mediterranean culture utilizing a limited range of European germplasm (Wood, 1925), is representative of the New World stage from 1850 to the present. California production, however, inevitably broke from the 'traditional' methods of almond growing utilized in the rest of the world. Key adaptations included: (i) selection of specific vegetatively propagated cultivars and rootstocks to maximize production; (ii) standardization of markets based on cultivar; (iii) selection and optimization of growing sites; and (iv) development of new cultural and management techniques, including increased mechanization, agrochemical

inputs and supplemental pollination. The impact of these changes has been to maximize yield and to promote modern industrial and marketing techniques (Kester *et al.*, 1990; Micke, 1994). Commercial cultivars introduced to California from the Languedoc area of southern France from 1850 to 1900 included 'Princess', 'Languedoc', 'Gros Tendre', 'Sultana' and others that ultimately provided the germplasm from which the California almond industry evolved (Wood, 1925). Originally these were grown primarily in solid plantings and considered to be 'shy-bearing' (because almond is self-incompatible and needs pollenizers), 'non-adapted' and 'susceptible to frost and disease' (Chappelow, 1893; Dargitz, 1910; Wickson, 1910). The eventual combination of highly adapted scions and rootstocks and multiple interplanted cultivars, the use of honeybee hives to maximize cross-pollination at flowering (Thorp and Roper, 1994), favourable soil and climate, abundant water and effective management has resulted in the highest productivity in the world and the domination of world markets (Kester *et al.*, 1990; Micke, 1994). Yields per hectare continue to show upward trends with yield as high as 4500 kg/ha not unusual.

An important initial step towards maximizing output was the (1900–1925) selection of four cross-compatible cultivars ('Nonpareil', 'Mission', 'Ne Plus Ultra' and 'Peerless') that established the basic industry cultivar pattern in the orchard and the marketplace. Shifts to more productive soil areas, changes in management and the change to peach rootstocks brought about changes (1925–1955) that resulted in a shift from what was initially a subsistence enterprise to a major, dedicated agricultural industry. This change reflected the contributions from many sources, including research and extension from the University of California, Davis, a progressive nursery industry, innovative growers and industry leaders and government policies that promoted the development of irrigation and marketing. In the period 1955–1965, the management patterns and world economic and marketing trends began to change and the industry entered an explosive growth period. Expansion occurred into all areas of the central valleys of California, increasing fivefold in area and tenfold in overall production. As a result, California production came to dominate world production.

Almond cultivars and cultural management methods were introduced to Australia, Chile and Argentina from California and different Mediterranean areas in the early to mid-19th century. In Australia, chance seedlings from this material resulted in selection of cultivars that have become more or less standard, such as 'Chellaston', 'Johnston', 'White Brandis', 'Bruce' and 'Boxendale' (Quinn, 1941). More recently, the list has been supplemented by new introductions from California. South Africa grows limited almonds following introduction about the turn of the 20th century. The principal cultivars are from California, but a local selection, known as 'Britz', was important in establishing the early industry (Davis, 1928).

2.7 Modern Cultivation

Recognizing the genetic, environmental and management deficiencies of the traditional almond industries, most almond-producing countries are pursuing almond research with an emphasis on germplasm evaluation and cultivar and rootstock improvement. These activities followed a pattern that included: (i) surveys of local cultivars and seedling populations within the traditional almond-growing areas; (ii) establishment of cultivar collections and test planting to evaluate local and introduced almond cultivars; (iii) establishment of germplasm collections for maintenance of local and introduced cultivated almond as well as related species; and (iv) development of controlled breeding methods to incorporate desirable new traits into locally adapted material and so generate new cultivars and rootstocks to optimize regional performance (see Chapters 5 and 9). Some traits, such as self-compatibility and certain disease resistances, were not readily available in cultivated almond and required introgression from related species. Crosses of *P. dulcis* with other almond species in Sections *Euamygdalus* and *Spartiodendron* are generally easily accomplished (Denisov, 1982; Denisov, 1988; Denisov, 1989; Kester *et al.*, 1990; Gradziel *et al.*, 2001). Hybridization with Section *Lycioides* is somewhat more difficult and even more so with *Chameamydalus* and *Leptopus* (Denisov *et al.*, 1983; Denisov, 1988; Chepinoga, 1990). Crosses with peach (*P. persica*) can be made easily, although

some types of sterility may occur in the progeny (Ryabov, 1969; Kester and Gradziel, 1996). Crosses with plum are possible but difficult. Crosses with apricot are very difficult but have been reported (Jones, 1968). The extent of new germplasm incorporation, as well as general breeding approach, however, varies by region as summarized in Table 2.3 (see also Chapter 5, Table 5.4).

2.7.1 Mediterranean

Modern cultivar improvement had its start with the beginning of cultivar collections at Bordeaux in 1951, and in 1961 at Nîmes, under the direction of Dr Charles Grasselly. A survey of the germplasm potential throughout the Mediterranean region was followed by collecting trips to Iran, the Soviet Union and Afghanistan (Grasselly and Crossa-Raynaud, 1980), and 450 accessions from ten different countries were collected, evaluated and described. This study provided the basis of the concept of local ecotypes in different almond-growing districts (Crossa-Raynaud, 1977; Crossa-Raynaud, 1981), and the collection is the base of an almond Germplasm Repository at Montfavet, France. Controlled crosses begun in France in 1961 produced valuable inheritance data (Grasselly, 1972), and resulted in new cultivars and rootstocks. Initial objectives were to combine late bloom, high production and improved nut and kernel quality. The first releases (Grasselly, 1975) from this programme, 'Ferragnès' and 'Ferraduel', were cross-compatible and when planted in combination shifted the blooming date approximately 2 weeks later than prevailing cultivars. This combination quickly became the basis for new orchard plantings in France and the rest of the Mediterranean. These cultivars arose from crossing representatives of two ecotypes: 'Cristomorto', an Italian cultivar from Puglia (which was late blooming due to a high chilling requirement for bloom), and 'Ai', a cultivar from France (which was late blooming due to a high heat requirement) (Egea *et al.*, 2003; Alonso *et al.*, 2005). Later disease resistance from the French cultivars 'Ardècheoise' and 'Ai' were incorporated into the programme, as well as late bloom from 'Tardy Nonpareil' (Grasselly, 1978; Duval, 1999). Almond improvement

Table 2.3. Objectives of modern almond improvement programmes.

Problem	Trait	Objective	Country
Spring frosts	Late bloom; blossom resistance	Avoid early and late spring frosts	USA, Ukraine, France, Greece, Spain, Italy, Romania, Turkey, Bulgaria
Winter freezing	Hardy buds and wood	Avoid loss of dormant flower buds; avoid tree damage	Ukraine, Romania, Bulgaria
Low winter chilling	Low chilling requirements	Grow in subtropical area	Israel, Tunisia, Australia, Morocco
Moisture stress	Drought tolerance	Grow with deficient irrigation	Spain, Italy
Lack of or reduced bee populations	Self-compatibility	Eliminate or reduce need for bee pollinizers	France, Greece, Tunisia, Italy, Spain, USA
Disease and pests	Resistance to fungus and bacterial diseases and insects	Eliminate or reduce need for chemical sprays	USA, France, Spain, Italy, Turkey
	Virus and virus-like organisms	'Clean' propagation sources	USA, France, Spain, Italy
High management costs	Modified tree size, shape, branching, growth habit	Efficient orchard management; adjust orchard density; pruning; shaking	USA, Spain
Difficult harvest and handling	Optimize time of maturity, ease and completeness of nut removal, hulling	Extend harvest period; efficient and complete harvest	All programmes
	Shell character – very hard shell	Kernel protection and storage; prevents worm infestation	Spain, France, Italy
	- semi-soft to soft - well sealed	Higher shelling percentages Kernel protection	USA, Ukraine, France USA, all programmes
Inconsistent yields	Improve tree: precocity, productivity, regularity of bearing	Early production; high yield, no alternate bearing	All programmes
	Nut quality	Increased kernel yield and reduced damage	All programmes

programmes were developed at Zaragoza, Spain, by A. Felipe (Felipe and Socias i Company, 1977; Felipe and Socias i Company, 1985; Felipe, 2000); at Reus, Spain, by F. Vargas (1975) and Vargas and Romero (1994); and at Murcia, Spain, by Egea and García (1975), García *et al.* (1988) and Dicenta *et al.* (1993). Genetic improvement programmes were initiated in Tunisia by Jaouani (1976) and El Gharbi (1977), and in 1970 at Rome, Italy, by F. Monastera (Monastera and Fideghelli, 1977; Monastera *et al.*, 1982; Monastera *et al.*, 1985). Almond improvement programmes were initiated in Greece in 1960 by D. Stylianides (1977).

Israel began a crossing programme in 1966 which involved early bloom, low chilling and

improved nut and kernel quality (Spiegel-Roy and Kochba, 1977). Parental material included 'Marcona' crossed with local cultivars 'Greek' and 'Poria'. Rootstock surveys of local seedling populations resulted in discovery of nematode-resistant almond clones, known as the 'Alnem' series (Kochba and Spiegel-Roy, 1975).

Turkey began a programme of selection among seedling populations for late bloom and frost resistance for interior Turkey (Ayfer, 1975) and in south-west Turkey for late bloom, adaptation and improved quality (Dokuzoğuz and Gülcan, 1973; Dokuzoğuz, 1975; Dokuzoğuz and Gülcan, 1977; Gülcan, 1977). Crossing programmes were begun in 1990 (Gülcan *et al.*, 1992).

Surveys of local germplasm also occurred in Turkey (Dokuzoğuz, 1975; Kuden *et al.*, 1994), Sicily (Barbera *et al.*, 1988) and Sardinia (Agabbio *et al.*, 1984; Chessa and Pala, 1985). Evaluations of unique North African germplasm in the local seedling populations were carried out in Morocco (Barbeau and El Baduami, 1981; Laghezali, 1985; Loussert *et al.*, 1989). These populations are considered to have good potential for germplasm exploration (Lansari *et al.*, 1994). In virtually all of these areas, destruction of many local groves and so loss of traditional germplasm has already occurred.

The Groupe de Recherche et d'Études Méditerranéen pour l'Amandier (GREMPA) was formed in 1974. This group organized almond researchers into an informal association whose purposes were to provide a forum to exchange information and plant materials and to coordinate breeding and testing efforts (Crossa-Raynaud, 1975; Socias i Company, 1998).

2.7.2 California

Beginning in the mid-1950s, the major source of new cultivars was the population of almond seedlings found throughout California, either along roadsides and near commercial orchards, or in orchards from unbudded almond seedling rootstock 'escapes'. Since 1957, many selections from this source were patented and introduced by individual growers as commercial cultivars through commercial nurseries (Brooks and Olmo, 1997). Most, including 'Merced', 'Price', 'Carmel' and 'Fritz', were used to provide cross-pollination to 'Nonpareil'. Others, such as 'Thompson' and 'Livingston', were later blooming and were combined with 'Texas'. A cooperative breeding programme initiated in 1923 at Davis, California, between the US Department of Agriculture (USDA) and the University of California, was an outgrowth of early pollination and cultivar evaluation studies (Wood, 1938). This programme was divided in 1948. The USDA programme was continued until 1975. The University of California programme has continued and resulted in the release of a number of cultivars and rootstocks (Kester and Gradziel, 1996).

2.7.3 Eastern Europe and Asia

After California, the second oldest continuous breeding programme for almond has been at the Nikitskij Botanical Garden at Yalta, Crimea, in the former Soviet Union, under the direction of A.A. Rikhter (Rikhter, 1972) and Yadrov (1994). These began with the research of N. Vavilov (1930) and were based on extensive species and cultivar collections. Other programmes have been in progress in the former Soviet Asiatic republics (Denisov, 1988). The primary objectives have been to develop hardiness to winter cold and spring frosts and to investigate the breeding potential of wild almond species.

Hardy, late-blooming cultivars were introduced for commercial production in the southern Soviet Union during the 1950s (Denisov, 1988), into Bulgaria in 1956 (Serafimov, 1975; Serafimov, 1976), and later into other Mediterranean countries (Guerriero *et al.*, 1974; Grasselly and Crossa-Raynaud, 1980; García *et al.*, 1988; Vargas and Romero, 1988). Additional almond cultivars have been introduced by Yadrov as a continuation of this programme.

Research has been carried out by other Asiatic republics including Turkmenistan, Tadjikistan (Mizgireva, 1973) and Uzbekistan (Isachkin *et al.*, 2001). This research involves populations of the Kopet Dag populations of *P. communis* (Denisov, 1977; Saparov, 1978) and many wild species (Denisov, 1982; Eremin and Denisov, 1984; Chepinoga, 1990). In Romania, selection of local cultivars has resulted in improved commercial plantings, and a breeding programme has introduced new cultivars (Cociu, 1981).

The foothill regions of northern India, including the Kashmir and the provinces of Jammu and Himachal Pradesh (Singh and Uppal, 1977; Singh *et al.*, 1977; Uppal, 1977), as well as north-eastern Pakistan, have historically had many seedling almond orchards. Studies of promising seedlings and introduced almond cultivars have been undertaken (Kumar *et al.*, 1989; Kumar and Uppal, 1990).

Several almond species and commercial cultivars exist in the Xinjiang Province of western China (Gustafson *et al.*, 1989) near the ancient cities of Kashgar and Sache on the ancient Silk Route. A programme of almond improvement has been carried out since 1970 by Professor

Zhu Jing Lin at the Xinjiang Academy of Forestry with the objectives of cold hardiness and dwarfing rootstocks.

2.8 Conclusions

The wide cultivation of almond in the often severe environments from Central Asia to the Mediterranean was possible largely because of the genetic and associated developmental/physiological diversity promoted by this typically self-incompatible yet interspecies fruitful genus. Even today, evidence of species mixing of the traditional Asian badam is evident in many rural bazaars by the presence of shell patterning characteristic of other species (Fig. 2.2). The cultivated Greek nut, which appears to have gained wide prominence in prehistoric Greco-Persian commerce, may well have originated from an interspecies cross with *P. fenzliana* and owes its existence and eventual commercial ascendancy to human selection. Gene transfer between wild and cultivated almond continued to be important in the early cultivation and dissemination of this crop, as evidenced in the transfer of self-compatibility from wild *P. webbii* to the old cultivated landraces in southern Italy. 'Tuono', an old Italian cultivar selected from these landraces, is currently the most important source for self-compatibility in Mediterranean and Asian breeding programmes (Table 2.3). However, despite its historical wide acceptance throughout the Mediterranean, 'Tuono' and many of the

regional heirloom cultivars are largely being replaced by new orchards designed to maximize production of a more globally standardized market quality. Regionally adapted (to both local culture and cultivation) cultivars are also being lost as traditional plantings are converted to other crops or to other consequences of modern development. Similar 'progress' is leading to the loss of native germplasm throughout the Asian and Mediterranean centres of origin and diversity (Ledig, 1992). The fragile nature of many of these wild almond ecosystems (e.g. the dependency on a minimum spring snowmelt for summer drought survival) makes them particularly vulnerable to a range of environmental perturbations from human enterprises to global warming. Concurrent with and contributing to germplasm loss is the rapid expansion of modern almond cultivation to satisfy an escalating global demand. The extensive commodity standardization associated with these complex, international markets has been largely achieved by dependence on a few standard cultivar types, as with the 'Nonpareil'/'Carmel' type in California, which is also increasingly grown in many international plantings. Recent history has demonstrated the dangers of too great a dependence on a limited germplasm, including significant economic losses from disease, pest and climate change (Tanksley and McCouch, 1997). A similar danger also exists in the loss of the extensive medical and culinary legacy of the current diversity of almond germplasm at a time when we are just beginning to understand its unique contributions.

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3 Production and Growing Regions

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3.1 Introduction

Almond is the most important tree nut crop in terms of commercial production. An adaptation to harsh climates combined with an ability to develop a deep and extensive root system has allowed almond to exploit a wide range of ecological niches. Almond is well adapted to mild winter and dry, hot summer conditions due to its low chilling requirement for early bloom, rapid early shoot growth and high tolerance to summer heat and drought. It is the earliest temperate tree crop to bloom, which limits production to areas relatively free from spring frosts, since late winter and early spring frosts can damage and even completely destroy the crop. Because almond is naturally self-incompatible, it often requires cross-pollination, which further acts to promote genetic variability and adaptability to diverse environments (Socias i Company and Felipe, 1992).

Despite its wide adaptation, almond production is usually in areas characterized by a Mediterranean climate (Kester and Asay, 1975), including regions in the Mediterranean countries, the Central Valley of California, the Middle East, central Asia, the Himalayan slopes and some equivalent areas in the southern hemisphere including Chile, Argentina, South Africa and Australia. The Mediterranean climate is also characterized by very low rainfall during late

winter, summer and early autumn, which is required because of almond's high susceptibility to foliar diseases (Ogawa and English, 1991). World production is variable from year to year, depending primarily on climatic conditions which affect pollination success as well as disease and insect damage. In the Mediterranean and Asiatic regions, most almond orchards are not irrigated, and rainfall, which mainly occurs in the winter and spring, is essential to ensure an acceptable crop. Rains during the autumn can disrupt harvesting operations, while rains during bloom interfere with pollination by reducing the activity of pollinating insects and also increasing the potential for disease damage (Thorp and Roper, 1994). This helps explain the strong negative correlation in California between total rainfall in February, the month of bloom, and final crop level (Alston *et al.*, 1995).

3.2 Almond Production

General production statistics for the past 60 years are shown in Table 3.1 with more detailed data from 2001 to 2010 presented in Table 3.2. In the New World production regions (USA, Chile, Australia) most cultivars are soft shell with a high shelling percentage, whereas in more traditional growing areas (Spain, Italy, central Asia) most

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Table 3.1. World almond production (metric tonnes (tm) in-shell) in the years 1947 to 1996 (FAO). (<http://www.fao.org/statistics/en/>)

Years	Italy		Spain		Portugal		Morocco		Iran		USA		Others		Total
	tm	%	tm	%	tm	%	tm	%	tm	%	tm	%	tm	%	tm
1947–51	31.0	35.3	22.6	25.7	3.8	4.3	3.2	3.6	6.7	7.6	18.5	21.1	2.0	2.3	87.8
1952–56	27.6	34.7	17.6	22.1	4.3	5.4	2.7	3.4	5.7	7.2	19.2	24.2	2.4	3.0	79.5
1957–61	36.4	32.7	29.4	26.4	4.1	3.7	4.3	3.9	8.4	7.5	24.0	21.5	4.8	4.3	111.4
1962–66	32.5	28.0	28.8	24.8	2.6	2.2	5.0	4.3	6.8	5.9	34.3	29.6	6.0	5.2	116.0
1967–71	30.4	27.1	31.2	22.3	4.8	3.4	4.4	3.1	7.2	5.1	55.3	39.4	6.9	4.9	140.2
1972–76	16.8	9.2	49.6	27.0	4.7	2.6	3.7	2.0	10.8	5.9	90.8	49.5	7.0	3.8	183.4
1977–81	21.0	8.7	47.6	19.8	2.8	1.2	4.6	1.9	6.4	2.7	145.0	60.2	13.6	5.6	241.0
1982–86	17.4	6.5	50.4	18.7	4.1	1.5	4.4	1.6	4.4	1.6	164.0	60.9	24.6	9.1	269.3
1987–91	15.6	4.3	55.2	15.3	4.2	1.2	6.1	1.7	6.1	1.7	245.0	67.9	28.6	7.9	360.8
1992–96	14.4	4.3	52.4	15.7	3.6	1.1	5.6	1.7	6.0	1.8	228.0	68.4	23.6	7.1	334.2

Table 3.2. World almond production (metric tonnes in-shell), 2001–2010 (FAO). (<http://www.fao.org/statistics/en/>)

Country	Year										Average
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	
USA	609,178	800,051	786,262	785,985	703,431	846,131	1,212,900	1,410,000	1,162,200	1,413,800	972,994
Spain	254,600	279,396	214,448	86,622	217,869	312,702	187,656	173,700	282,100	221,000	223,009
Syria	49,487	139,010	140,300	123,000	229,035	107,117	76,093	82,616	97,002	73,100	111,676
Italy	104,000	104,891	91,382	105,245	118,344	112,796	112,644	118,723	106,660	108,160	108,285
Iran	97,144	107,000	38,231	69,989	108,677	110,000	120,000	126,679	130,000	158,050	106,577
Morocco	81,820	82,400	70,808	60,200	70,629	83,000	81,437	86,902	114,700	102,170	83,407
Tunisia	32,000	18,500	40,000	44,000	43,000	56,000	58,000	51,500	60,000	63,000	46,600
Turkey	42,000	41,000	41,000	37,000	45,000	43,285	50,753	52,774	54,844	55,398	46,305
Greece	55,115	38,130	36,480	48,177	48,194	50,708	45,652	34,500	39,996	32,900	42,985
Algeria	25,199	32,287	33,234	37,985	45,379	53,673	34,110	39,521	47,393	44,300	39,308
China	20,000	22,000	23,000	24,000	25,000	28,000	30,000	32,000	35,000	37,900	27,690
Lebanon	23,900	23,000	27,400	27,500	28,300	28,000	29,400	29,400	30,500	28,500	27,590
Afghanistan	15,000	15,000	14,000	14,700	15,630	20,000	31,481	42,000	43,183	56,000	26,699
Libya	26,000	26,000	26,000	25,000	25,000	25,000	25,000	25,000	29,000	29,500	26,150
Pakistan	33,236	26,414	23,688	23,924	23,129	23,344	23,382	26,614	26,400	21,900	25,203
Uzbekistan	12,000	14,000	16,000	19,000	20,000	23,545	18,500	18,500	18,610	18,000	17,816
Australia	10,056	10,056	10,056	9430	11,755	12,420	18,024	19,000	30,000	30,000	16,080
Portugal	15,743	30,850	23,829	13,953	13,823	12,400	11,100	9796	12,454	7000	15,095
Chile	8600	9100	8800	9000	9500	9600	10,400	10,500	20,000	22,000	11,750
Israel	4418	9142	4900	4210	9233	9426	6478	6276	11,762	7806	7365
Palestine	4355	4832	4478	5979	5489	4862	5257	6233	7468	7200	5615
Tajikistan	3800	3500	3000	3000	3000	3300	3200	3200	3219	3200	3242
France	6931	6800	6800	2000	2137	1780	1591	1042	1100	947	3113
Jordan	1005	2484	2118	2094	2391	3144	1341	1344	1003	2419	1934
Kyrgyzstan	1000	1500	1500	1500	1500	1500	1500	1500	1447	1500	1445
Croatia	2574	2616	1567	1600	1368	1086	500	905	1835	319	1437
Burkina Faso	1250	1250	1250	1250	1250	1250	1300	1300	1450	1275	1283
Turkmenistan	1100	1000	1000	1000	1000	1200	1000	1000	1006	1000	1031
Moldova	1000	1000	1000	1000	1000	1000	1000	1000	500	500	900
Macedonia	400	208	1478	1175	511	685	1376	622	819	1006	828
Swaziland	1700	700	500	480	480	750	750	750	769	1000	788

Georgia	800	800	800	800	800	650	770	800	700	810	773
Côte d'Ivoire	500	600	600	700	700	800	800	800	1050	1100	765
Cyprus	500	2000	650	693	909	660	668	432	550	460	752
United Arab Emirates	188	182	722	650	700	780	850	850	850	1000	677
Argentina	470	480	480	480	480	490	500	500	626	626	513
Bulgaria	960	1371	572	531	243	161	245	300	50	368	480
Iraq	500	440	430	430	400	400	390	390	488	470	434
Azerbaijan	178	255	197	267	252	527	502	500	505	521	370
Kazakhstan	200	300	300	300	300	320	300	300	311	250	288
Hungary	207	225	257	347	323	279	182	161	144	105	223
Yemen	130	130	130	130	130	130	130	130	150	110	130
Bosnia and Herzegovina	66	74	64	64	64	57	55	48	49	80	62
Mexico	57	76	82	60	60	60	60	60	48	48	61
Ukraine	12	14	31	20	30	10	20	10	20	10	18
Nepal	11	11	11	11	11	11	11	11	9	8	11
Total	1,549,390	1,861,075	1,699,835	1,595,481	1,836,456	1,993,039	2,207,308	2,420,189	2,377,970	2,556,816	2,009,756

cultivars are hard-shell, with a lower shelling percentage. Consequently, data do not indicate actual kernel-meat production for each country, making comparisons between them difficult. Additionally, some production errors are apparent, such as the reported production in Burkina Faso and Côte d'Ivoire, as well as in Swaziland, (Table 3.2), which further caution against a strict reading of the data. Usually, these inconsistencies arise from the inclusion of data concerning production of local 'almond'-type fruit which often have no relation to the commercial almond of global trade. Important commercial almond-growing regions include Asia, Mediterranean countries and New World regions in North and South America and in Australia.

3.2.1 Asia and eastern Europe

Major production regions in Asia include Iran and Turkey (Tables 3.2 and 3.3). Turkey has important producing areas located in Izmir and in the south-eastern Anatolia region near the Atatürk dam, which provides irrigation water for extensive new plantings (Dokuzoğuz, 1975; Kuden *et al.*, 1994). Iran is widely considered a centre of origin for domesticated almond (Grasselly, 1976a; Sorkheh *et al.*, 2009) and historically has been an important producer of almonds for both domestic consumption and export. Modern Iranian commercial production varies between 8000 and 15,000 tonnes (t) of shelled product (Tables 3.2 and 3.3), obtained from both modern plantings and more scattered seedling orchards. The production area is mainly located in north-west Iran, in the Azerbaijan (Tabriz) region. In the past, most cultivated almond trees have been obtained from seed and thus display wide variability in kernel characteristics. This can be desirable in some of the domestic market, but presents challenges for export markets (Grigorian, 1976). Turkish production has averaged over 46,000 metric tonnes (tm) (in-shell) for the last 10 years that records are available (Table 3.2). As with other Asian countries, total production is difficult to quantify since a large portion is used in local markets.

Other important Asian production regions from ancient times until the present include Afghanistan, Pakistan and Uzbekistan (Table 3.2). In India almonds are cultivated in the more Mediterranean climates of the northern provinces

Table 3.3. Average almond production for 2001–2010 in the main producing countries (elaborated from FAO data). (<http://www.fao.org/statistics/en/>)

Country	Average production (tm)	Average surface (ha)	Average production (tm/ha)
USA	972,994	252,929	3.85
Spain	223,009	603,730	0.37
Syria	111,676	39,666	2.82
Italy	108,285	82,491	1.31
Iran	106,577	106,294	1.00
Morocco	83,407	139,665	0.60
Tunisia	46,600	174,062	0.27
Turkey	46,305	17,680	2.62
Greece	42,985	18,942	2.27
Algeria	39,308	41,097	0.96
China	27,690	11,836	2.34
Lebanon	27,590	6108	4.52
Afghanistan	26,699	10,652	2.51
Libya	26,150	50,861	0.51
Pakistan	25,203	10,467	2.41
Uzbekistan	17,816	6077	2.93
Australia	16,080	15,968	1.01
Portugal	15,095	33,497	0.45
Chile	11,750	6412	1.83
Israel	7365	2363	3.12
Palestine	5615	4526	1.24
Tajikistan	3242	4994	0.65
France	3113	1414	2.20
Jordan	1934	404	4.79
Kyrgyzstan	1445	871	1.66
Croatia	1437	326	4.41
Burkina Faso	1283	2410	0.53
Turkmenistan	1031	1475	0.70
Others	8073	12,065	0.67
Total	2,009,756	1,659,282	1.21

of Jammu and Himachal Pradesh (Singh and Uppal, 1977; Singh *et al.*, 1977; Kumar and Uppal, 1990). However, attempts to extend the range of cultivated almond into other continental areas of Asia and Europe, including former Yugoslavia (Ristevski, 1992), southern Russia (Rikhter, 1972), western China (Gustafson *et al.*, 1988), northern Pakistan (Thompson *et al.*, 1989), Romania (Cociu, 1985), Bulgaria (Serafimov, 1975; Serafimov, 1976) and Hungary have met with only limited success. This has been due in part to almond's susceptibility to cold temperatures in winter including spring frosts, as well as to flower and foliar diseases, with disease development further exacerbated by summer

rains in many of these regions. Very productive orchards using intensive cultural practices and improved cultivars have been developed in Syria (Thompson, 1983), Palestine and Israel (Spiegel-Roy, 1976), though intensifying civil strife in these regions challenges continued development.

3.2.2 Mediterranean

In the Mediterranean region, almond was also well suited to the low-input agriculture predominating in early Mediterranean tree culture. A high root tolerance to drought but high susceptibility to excessive soil moisture placed almonds in a mixed culture system with olives, carob and other drought-tolerant crops. Almonds were usually found on well-drained slopes at higher elevations of valleys to escape shoot damage from frosts and root damage from excessive soil water. A traditional culture system evolved that minimized inputs of labour, fertilizers and use of supplementary water. Some trees producing bitter nuts were tolerated in local seedling orchards since bitter seed were mixed in small quantities with sweet kernel to produce the distinctive amaretto flavour. Cultivation typically was limited to within 50 miles of the Mediterranean coast, but extending onto the slopes of river valleys and the interior plateau of Spain. Two thousand years of continuous almond culture in the Mediterranean concentrated production to specific regions where well-defined seedling ecotypes and local cultivars evolved (Grasselly, 1972; Grasselly, 1976b; Grasselly and Crossa-Raynaud, 1980). These highly selected regional cultivars and landraces represent a rich genetic, horticultural and culinary diversity (Socias i Company and Felipe, 1992; Gradziel, 2011). Dryland, seedling orchards remained the major almond culture system in the Mediterranean region for centuries, persisting to recent times in areas such as Sicily, Sardinia, Mallorca, the Madeira Islands, Greece and Morocco. By the turn of the 20th century, most of the almond countries of the Mediterranean region had identified 'local cultivars' that were seedling selections which, while representing the germplasm of the region, were often of unknown origin. The most important modern growing regions include Spain, Italy, Morocco, Tunisia, Greece and Portugal.

3.2.2.1 Spanish production

Spanish production currently dominates the region, with production of over 200,000 tm (in-shell) over the last 15 years (Table 3.4). Almond has been growing in Spain for more than 2000 years, probably introduced by the Phoenicians and later expanded by the Romans (Felipe, 2000). Both shipped almonds throughout the Mediterranean by sea, as documented by archaeological studies of several ancient shipwrecks (Colls *et al.*, 1985; Socias i Company, 1988).

Commercial almond production in Spain is very large in terms of acreage, with 530,200 ha in 2012, of which 503,100 were in production (Table 3.5). In addition, the total number of trees in production has been estimated to be as high as 732,000. Most (92.2%) of the almond orchards are not irrigated, resulting in very low productivity. The average kernel production for 2001–2014 has been 39,682 tm, but with large oscillations from year to year, as well as from region to region (Table 3.6). The most important producing regions continue to be those close to the Mediterranean Sea, such as Andalusia, Murcia and Valencia, but also including inland region such as Aragón and to a lesser extent Catalonia and Castilla-La Mancha. The Balearic Islands maintain a modest production and La Rioja dominates among the remaining production regions (Fig. 3.1).

An EU project targeting the renewal and structuring of this sector has resulted in improved production within the Spanish industry over the last 20 years. This has led to better organization within industry sectors, leading to increased productivity. Old orchards were re-grafted or renewed with better orchard practices, improved cultivars and, in many cases, supplemental irrigation. Results have varied by region, but comparing the average production of the first 7 years with that of the following 7 years, an increase of nearly 10% has been achieved (Table 3.6). Production changes in Andalusia, Catalonia and the Balearic Islands, however, were not significant. A significant decrease was observed in Murcia and Valencia while the increase in Aragón and Castilla-La Mancha was highly significant. Results indicate a trend towards more modern orchard practices, primarily in the coastal areas but gradually moving inland.

Table 3.4. Spanish almond production (metric tonnes (tm) of kernel) by region in 2001–2014 (Cooperativas Agroalimentarias de España).

Region	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Andalusia	7300	10,500	7350	2100	12,000	14,600	7300	5440	11,850	7000	9800	12,000	6290	13,000
Murcia	8900	11,300	8000	2000	8300	11,500	6900	6289	11,202	6200	8698	7900	5750	3128
Valencia	8000	11,200	4500	1600	6400	8900	6010	4450	6720	5630	4543	5100	3933	5100
Aragón	6600	6700	3600	800	5000	6700	5700	5116	8386	7940	9497	9189	4264	12,800
Catalonia	3600	5100	3460	1400	3693	4955	3413	3071	4999	4023	4365	3571	2339	4206
Castilla-La Mancha	3600	5000	1680	1284	3245	4500	1700	3206	8000	4560	4501	4545	2000	7192
Balearic Islands	1400	1800	570	2450	2000	2400	2500	2200	880	1400	1975	2150	2375	1850
Others	700	1050	500	500	833	910	605	898	1556	1765	1880	1648	862	1613
Total	40,100	52,650	29,660	12,134	41,571	54,465	34,128	30,670	53,593	38,518	45,259	46,103	27,813	48,889

Table 3.5. Almond surface in 2012 (ha) in the different Spanish growing regions.

Region	Almond surface			Disseminated trees
	Dry land	Irrigated	Total	
Andalusia	144,217	5435	149,652	88,726
Murcia	65,146	6353	71,499	0
Valencia	92,531	9368	101,899	27,500
Aragón	60,062	6914	66,976	0
Catalonia	36,237	3623	39,860	412
Castilla-La Mancha	50,302	7054	57,356	217,864
Balearic Islands	24,123	320	24,443	117,400
Others	16,154	2384	18,538	280,417
Total	488,772	41,451	530,223	732,319

Table 3.6. Average production of almond kernel (tm) by Spanish growing regions in the years 2001–2007 and 2008–2014.

Region	Production 2001–2007		Production 2008–2014		Difference (%)
	Average	%	Average	%	
Andalusia	8736	23.11	9340	22.48	6.91
Murcia	8129	21.50	7024	16.90	–13.59
Valencia	6659	17.61	5068	12.19	–23.90
Aragón	5014	13.26	8170	19.66	62.94
Catalonia	3660	9.68	3796	9.13	3.72
Castilla-La Mancha	3001	7.94	4858	11.69	61.88
Balearic Islands	1874	4.96	1833	4.41	–2.19
Others	728	1.92	1460	3.51	100.55
Total	37,815		41,549		9.87

Most Spanish cultivars are hard-shell (Table 3.7), allowing nut storage without excessive risk of seed becoming rancid. This allows extension of the cracking period for several months. In addition, the extensive planting of very early ripening cultivars such as ‘Guara’ has also allowed earlier harvesting, processing and marketing than previously possible, which is being further facilitated by utilizing production areas promoting earlier ripening. In-shell nuts are not extensively marketed, with most of this market type being made up of soft-shell cultivars. Following blanching, almond kernels are processed in many different ways, as detailed in Chapter 19.

Only two cultivars are marketed by name, ‘Desmayo Largueta’, which is used mainly for appetizers or roasted almonds, and ‘Marcona’, which is used mainly for nougat thanks to its high oil composition and tocopherol content. All others cultivars are included in generic denominations, such as ‘Comunas’, ‘Valencias’ or

‘Mallorcas’. The present trend is towards specific uses for the different cultivars, including small-sized nuts for chocolate bars and large sizes for slicing and processing. Depending on their composition, some cultivars such as ‘Belona’ may be a substitute for ‘Marcona’ in certain uses.

‘Marcona’ and ‘Desmayo Largueta’ had been the two dominant traditional cultivars grown in Spain, being found in most growing regions. Additionally, many local cultivars were also grown in each region and, while many are currently still being recommended as pollinizers, their bloom overlap is not sufficient to ensure their reciprocal pollination with ‘Marcona’ and ‘Desmayo Largueta’ (Socias i Company *et al.*, 1994). In the early 1970s some foreign cultivars were introduced in Spain, being extensively planted in all growing regions. These were mostly late-blooming cultivars, including two traditional Italian cultivars from the Puglia region, ‘Tuono’ and ‘Cristomorto’, and two more recent releases, ‘Ferragnès’ and

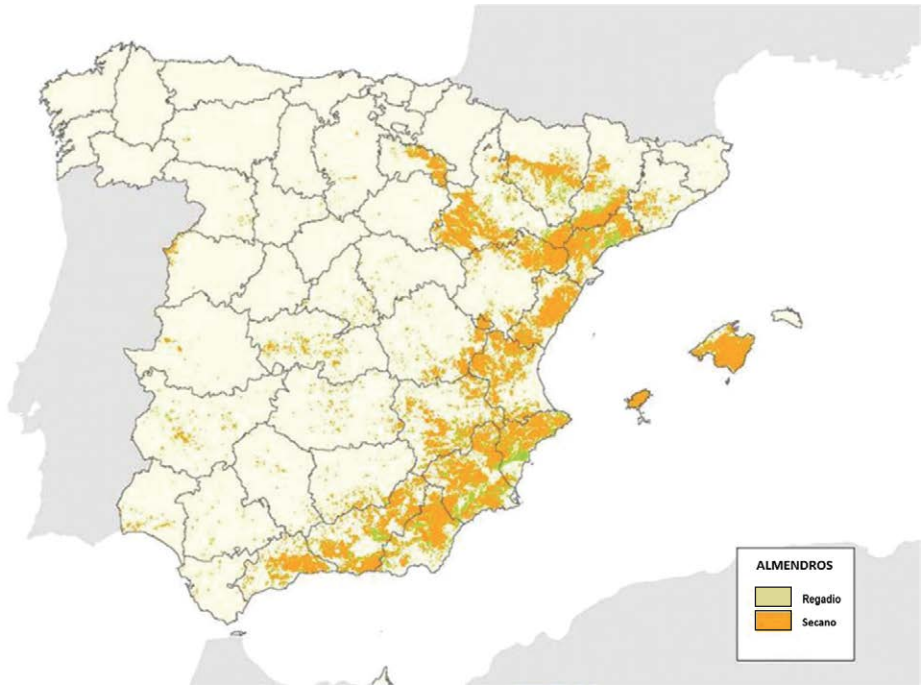


Fig. 3.1. Geographical distribution of almond growing in Spain.

Table 3.7. Characteristics of the main almond cultivars in Spain (Felipe, 2000; Arquero, 2013).

Cultivar	Blooming time	Harvesting time	Productivity	Self-compatibility	Shelling percentage	Kernel weight (g)
Marcona	Early	Middle	High	No	25	1.3
Desmayo	Very early	Late	Middle	No	26	1.3
Largueta						
Ferragnès	Middle–late	Middle	High	No	40	1.4
Ferraduel	Middle–late	Early–middle	High	No	28	1.3
Guara	Middle–late	Early	Very high	Yes	35	1.3
Belona	Middle–late	Middle	High	Yes	33	1.3
Soleta	Middle–late	Late	Very high	Yes	33	1.3
Mardía	Very late	Middle	High	Yes	24	1.2
Vialfas	Very late	Early–middle	Very high	Yes	25	1.3
Tarraco	Late	Early	High	No	31	1.6
Marinada	Late	Middle	Very high	Yes	32	1.3
Constantí	Middle–late	Middle	High	Yes	28	1.2
Vayro	Middle–late	Middle–late	Very high	Yes	29	1.2
Antoñeta	Late	Early	High	Yes	35	1.4
Marta	Late	Early–middle	High	Yes	34	1.3
Penta	Very late	Early–middle	Very high	Yes	29	1.0
Tardona	Extremely late	Middle	High	Yes	25	0.8

‘Ferraduel’ from the French breeding programme. These were subsequently supplemented by releases of the different Spanish breeding programmes, with ‘Guara’, developed by the CITA of Aragón

being the most popular, owing to its self-compatibility, late blooming and frost tolerance. Spain has three active breeding programmes, releasing cultivars becoming more or less planted

in all growing regions, and also in some foreign countries such as Argentina, Morocco and Tunisia (see Chapters 4 and 5). The oldest programme is that of CITA of Aragón, with releases such as 'Guara', representing 36.38% of the plants produced by the Spanish nurseries in the period 1995–2013 according to official figures of the Spanish Ministry of Agriculture. Although its importance has decreased due to the release of other cultivars, it still represented 23.56% in recent plantings. The most recent releases from the same programme, 'Belona', 'Soleta' and 'Mardía', are also being introduced. The total amount of the CITA cultivars has been 39.42% for the whole period and 29.27% for the last season.

The next programme was that of IRTA (Institut de Recerca i Tecnologia Agroalimentàries) at Mas de Bover, with releases such as 'Masbovera', 'Glorieta' and 'Francolí' in the first years and more recently 'Constantí', 'Marinada', 'Tarraco' and 'Vayro'. The total percentage of IRTA cultivars produced during the period 1995–2013 has been 8.68%, but this percentage has significantly increased with the latest releases, with 23.8% for the last season. The third programme is that of CEBAS-CSIC at Murcia, with more recent releases: a total percentage of 1.24% for the whole period and 0.77% for the last season. The traditional Spanish cultivars still represent an important proportion (23.92%) of new orchards, mainly consisting of 'Marcona' (8.77%) and 'Desmayo Langueta' (8.15%). Their importance has decreased recently, however, since these traditional Spanish cultivars only amounted to 15.47% of new plantings. Among the local cultivars, the most planted were 'Garrigues', 'Ramillete', 'Atocha' and 'Carreró'.

The French cultivars were particularly important following their early introductions, accounting for 22.77% of all trees propagated during that period. Their commercial importance has slowly decreased over time, and at present account for approximately 17.44% of all propagated trees. The most important cultivars are 'Ferragnès' (11.65% for the early period and 5.77% for the later season), 'Ferraduel' (9.82% and 5.27%, respectively), with an increase in importance for the self-compatible cultivar 'Lauranne' (1.19% and 6.38%, respectively).

The Italian cultivars, mainly consisting of the traditional 'Tuono' and 'Cristomorto' from Puglia, were important in the 1960s and 1970s

but their importance has subsequently decreased. For the whole period, their share has averaged about 2.92%, mostly with 'Tuono' at 2.58%. Few Californian cultivars are grown (0.41% for the period), and they are mostly for exporting propagated nursery plants.

Despite the introduction of many new cultivars, local cultivars are still grown in many production regions, though rarely outside their region of origin. The restriction of local cultivars and so germplasm diversity has been more important in the two archipelagos, the Balearic and the Canary Islands. Germplasm demonstrating useful variability for augmenting Spanish genetic resources has been collected by Dr Antonio J. Felipe since the 1960s, and has been used to establish the present collection of the CITA of Aragón, which belongs to the National Network of the Spanish Genetic Resources (Espiau *et al.*, 2002). This collection was subsequently designated as the reference collection for excellence by the international crop improvement organization GREMPA, as well as by the European and Spanish Plant Variety Offices.

The traditional rootstocks have usually been bitter almond seedlings, to protect the nursery rows from rodent and bird damage. Seed from cultivars such as 'Desmayo Langueta', 'Garrigues' and 'Atocha' were selected later because they produced well-adapted and homogeneous rootstocks. Peach and plum rootstocks are rarely used, as there are some graft-incompatibility problems present for plums. Currently, peach × almond hybrids are the most common rootstocks, primarily the French INRA GF-677 and increasingly the red-leaved and nematode-resistant selections 'Monegro', 'Garnem' and 'Felinem' from the CITA of Aragón (Felipe, 2009). More recently, less vigorous rootstocks are being tested for high-density planting, as described in Chapter 9.

Along with cultivars, production systems have also evolved in Spain. Since almond is a typical Mediterranean crop, it was initially grown near the Mediterranean shores. Vulnerability to frost somewhat restricted its expansion inland and represents a major factor affecting yield variability. Soils used initially were mostly poor, often with high lime content, but with the good drainage required by the almond susceptibility to asphyxia. Modern orchards, however, are predominately drip irrigated and located on more fertile soils. Traditional non-irrigated orchards

are characterized by low-density planting distances of 8×8 m or even wider to make more efficient use of the limited rainwater. Under irrigation, the planting distances have been reduced to densities of approximately 6×5 m. At present there are also several trials of high-density plantings, with distances as low as $1\text{--}1.5 \times 3$ m, as summarized in Chapter 9.

Traditional orchard practices have used mixed cropping systems, but this practice has almost disappeared. The trees were trained to three main scaffolds located 1.5 m or higher on the trunk, to allow sheep grazing under the canopy. At present, trees are usually trained to an open vase with a trunk area only high enough to allow mechanical harvesting. The new high-density plantings are mostly on a single central axis, with height and width limited by mechanical pruning.

More than 90% of Spanish orchards remain non-irrigated. Most new orchards, however, are drip irrigated, with some under some level of deficit irrigation. Fertilization has traditionally been reduced in rainfed orchards and is mainly applied in winter, though with a portion of the nitrogen applied during the growing period. Modern orchards usually have a fertilization calendar similar to other fruit trees under fertigation.

Old orchards were generally made up of a cultivar mixture, where pollination deficiencies were not noticeable. With a high number of cultivars present, there were always some trees in bloom, and wild populations of bees were available for pollination. New plantings are with improved, mostly self-compatible cultivars. The percentage of nursery plants of cultivars from breeding programmes for the period 1995–2013 was 72.06% (with 71.27% for 2013), whereas the percentage of self-compatible cultivars during this period was 49.19% (with 62.31% for 2013).

Almond was traditionally considered as quite tolerant to pests and diseases since their incidence was not much noticed because of the marginal condition of many traditional orchards. The most important pest is the green peach aphid, followed by the green leafhopper and several beetles; and, in some years and regions, San Jose scale, peach lace bug, almond leaf skeletonizer moth, peach twig borer and in the roots the wood-boring beetle *Capnodis tenebrionis*. The most important diseases have been leaf curl, shot hole and red leaf blotch. Under favourable climatic

conditions some other fungi may have importance, such as fusaricocum, brown rot blossom blight, rust and, in problematic soils, *Armillaria* and *Rosellinia*.

3.2.2.2 Other Mediterranean countries

Almond also continues to be important both culturally and economically in many other Mediterranean regions. Almond cultivation in Morocco dates back to the Punic-Roman period and it has been an important producer since the early 1900s (Table 3.1). Most traditional production has been under low-input, dryland culture with consequent low yields, though recent plantings have incorporated more intensive cultural practices as well as newer cultivars, often from Spain, France and California (Mahhou and Dennis, 1992; Lansari *et al.*, 1994). Current commercial almond production in Morocco is estimated to be almost 100,000 tm of kernel (Table 3.3), marketed both locally and increasingly to Europe due to its early production season. It is difficult to quantify the total cultivated area in Morocco as the species is also present in the Atlantic forest area where production is not homogeneous but is mixed with bitter almond, and used primarily for processing. Recently, important new plantations have been developed with international collaborators producing more traditional cultivars such as 'Marcona', 'Fournat de Brézenaud', 'Desmayo Largueta', 'Abiod', 'Ai', 'Ne Plus Ultra', 'Ferragnès' and 'Ferraduel', as well as newer cultivars primarily from Spain (Mahhou and Dennis, 1992).

In Tunisia, almond cultivation dates back to the Punic-Roman period as well. The Tunisian production in the past 10 years has been estimated to average about 6000 t of shelled product, most of which is exported. Most cultivation is in the region of Sfax, around Bon Cape, in the north of the country and also in the pre-desertic areas of Kairuan, Kasserine and Feriana (Jaouani, 1976). The region of Sfax is characterized by mild winters and low rainfall (200 mm/year). Historically, the most important cultivars have been 'Achaak' and 'Ksontin', as well as 'Mazzetto' (probably 'Tuono') in the newer orchards. In the area of Cape Bon, with a rainfall of 500 mm, planting is more intensive (200 trees/ha). The local cultivars present are 'Heuch Ben Smail', 'Blanco Khooukhi' and 'Abiof de Ras Djebel'.

In the valley areas and in the hills of the northern part of the country the rainfall is about 400–600 mm/year, the winters are colder and summers are hotter and the best cultivars have been ‘Tuono’, ‘Ferragnès’, ‘Monaco’, ‘Peerless’, ‘Fournat de Brézénau’ and ‘Desmayo Largueta’. In the north-west area, with a rainfall of about 350–550 mm/year, ‘Ferragnès’, ‘Ferra-duel’ and ‘Tuono’ are cultivated. Previously, most production was primarily marketed locally, making almond a low-cash crop owing to the very low production per hectare. However, Tunisian production is becoming more attractive to the European market, because of its early harvest potential. Now some of the newer Spanish cultivars are being planted, often under more intensive cultural conditions.

Portugal has two principal areas of production: the Algarve region in the south and the Alto Duoro in the north. Each region has its specific cultivars (Grasselly and Crossa-Raynaud, 1980). Total production is not well qualified because it is represented by mixed local and traditional cultivars and seedlings for which accurate records are not kept. While these regions have been important to Mediterranean production throughout the 1900s (Table 3.1), production decreased in the 2000s to approximately 15,000 tm in-shell on average (Table 3.2).

A similar production trend has been observed in Greece (Stylianides, 1976). Greek production has decreased in the last few years and at present is around 43,000 tm in-shell (Table 3.2). In the new areas, the most important cultivars have been ‘Texas’, ‘Troito’ (probably ‘Tuono’), ‘Retsou’ and ‘Ferragnès’. Crete remains an important production area, with new irrigated orchards also being planted in Tessalia.

Italy is historically a major almond-growing region, and many cultivars have been described in Puglia (Fanelli, 1939; Grasselly and Crossa-Raynaud, 1980), Sicily (Bianca, 1872; Spina, 1958) and Sardinia (Milella, 1959; Agabbio *et al.*, 1984; Chessa and Pala, 1985). Puglian cultivars were predominantly late blooming and had hard shells with a kernel/nut ratio of 25–30%, with high percentages of double kernels. Sicilian cultivars tended to be early blooming, very hard shelled and more or less round in shape. Several Italian cultivars, such as ‘Tuono’, are also self-compatible. As in Portugal, Italian production has decreased in recent years. It is presently

about 72,600 tm kernel meats (Table 3.3) and represents less than 5% of the world crop. Italy, historically the leading almond producer of the world, experienced a sharp decline in production in the 1900s (Federazione Italiana dei Consorzi Agrari, Ufficio Tecnico Agrario, 1973; Godini, 1977; Bacarella, 1994) and began to import almonds (Bacarella *et al.*, 1991). Many remaining orchards are fairly old, though more recently new orchards have been planted with selected cultivars such as ‘Tuono’, ‘Ferragnès’, ‘Filippo Ceo’ and ‘Falsa Barese’ (Monastra *et al.*, 1982). Production in other historically important regions is, however, decreasing.

France is also an old almond-growing country with areas in the southern Rhône valley and the surrounding foothills (Grasselly and Crossa-Raynaud, 1980). Cultivars originating in Provence and the foothills of the Alps tend to be late blooming. Cultivars associated with the Languedoc region nearer the centre of the Rhône valley (‘Aï’, ‘Princesse’, ‘Languedoc’, ‘Pistache’, ‘Ardèchoise’, ‘Fournat de Brézénau’, ‘Rabasse’) show a range of tree and nut characteristics from hard- to soft-shelled and large to small kernels. As in other Mediterranean and Asian regions, the traditional almond production system began to fail in the 1940s (Table 3.1), and the French almond industry essentially went out of production by the 1950s (Grasselly and Crossa-Raynaud, 1980). By the 1970s, production could not keep pace with the world demand for almond products because the most productive land was used for higher-value crops such as peach and grape. In addition, supplemental honeybee pollination is not widely practised in this region, significantly reducing final yield potential.

3.2.3 New World

In the New World, almond dissemination followed early colonization by European and Asian settlers, eventually resulting in commercial plantings in North and South America, Australia and South Africa. Successful cultivation typically occurred only after a series of failures, as early settlers – not knowing the degree of almond’s vulnerability to winter cold, spring frosts and summer rains – tested different germplasm in different growing regions until suitable combinations were

found (Kester and Gradziel, 1996). Production in the southern hemisphere is covered in Chapter 4, and so this section will cover almond production in California.

In California, successful production began in coastal valleys as an extension of the Mediterranean culture, using a limited range of germplasm (Wood, 1925). In particular, soft-shell almonds brought in from the Languedoc area of southern France from 1850 to the early 1900s provided the germplasm from which much of the commercial almond industry developed. These included the soft-shelled cultivars ‘Nonpareil’, ‘I.X.L.’ and ‘Ne Plus Ultra’, which, when planted together, resulted in high and consistent yields (Wood, 1925). ‘Texas Prolific’, later known as ‘Mission’, was another important cultivar originating in Dallas, Texas, and later brought to California (Wickson, 1910). ‘Nonpareil’ became the dominant cultivar because of its good kernel quality and dependable production (Taylor, 1916). Early rootstocks were almond seedlings because initial plantings were under non-irrigated conditions. As production moved inland to the Central Valley, a shift was made to peach and plum rootstocks, which were more tolerant to irrigated and sometimes flooded soils (Gradziel, 2009). Tremendous expansion occurred from the 1960s to the present in all areas of the central valleys of California (Fig. 3.2), starting in the northern Sacramento Valley and eastern San Joaquin Valley where irrigation water was plentiful, subsequently

progressing to the west side of the San Joaquin Valley as new irrigation projects made irrigation water available there (Fig. 3.3). Currently the main production areas include Colusa, Stanislaus, Madera, Merced, Fresno and Kern counties (Fig. 3.3), though the increasing costs of irrigation water is revitalizing plantings in the northern Sacramento Valley. Production appears to be levelling off at approximately 1.8 million tm (Fig. 3.2) following a more than doubling of acreage over the past decade (Table 3.2) combined with an industry-leading average productivity of 3.85 tm/ha (Table 3.3). The very high productivity is obtained from a combination of favourable soils, climate and intensive management systems, including the proper combination of cross-compatible cultivars, optimum use of honeybees for cross-pollination (Thorp and Roper, 1994), extensive mechanization, and high water and fertilizer inputs (Kester and Gradziel, 1996). More than 70% of the world’s almond crop is produced in California for distribution by a highly developed marketing system (See Chapter 18).

Navel orangeworm and peach twig borer are the principal insect pests in California, resulting in worm damage of kernels. Reject levels generally reflect kernel damage, although other conditions such as gummy nuts are also included in rejects. With its soft shell, ‘Nonpareil’ is susceptible to worm damage, while a number of hard-shell cultivars sustain less damage. Other cultivars such as ‘Sonora’ and ‘Winters’ have somewhat

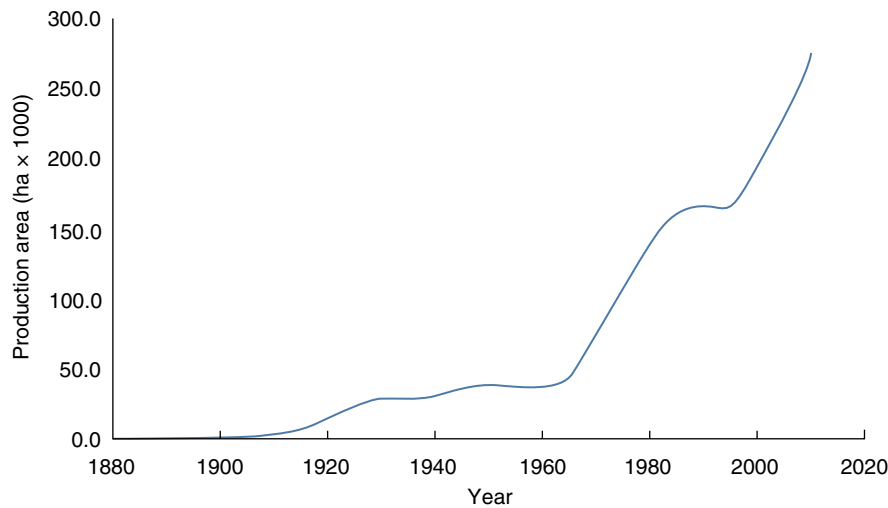


Fig. 3.2. Historical trends in California production (kernel meats).

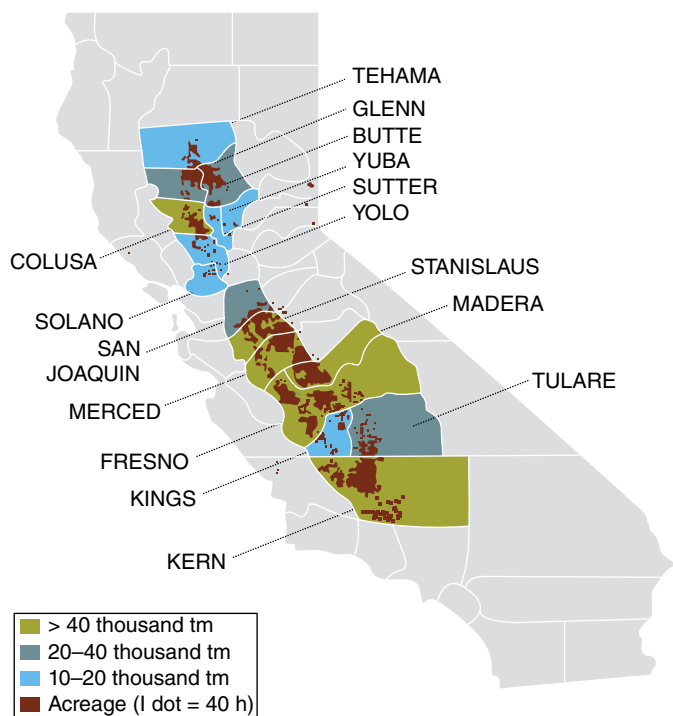


Fig. 3.3. Principal growing regions in California.

more damage than 'Nonpareil'. Worm pressure – particularly navel orangeworm and, secondarily, peach twig borer – varies by region, but the relative tolerance or susceptibility of different cultivars is mostly dependent on shell seal, time of hull split and harvest timing in relation to insect flights. Whether or not double kernels are a problem depends on the handler and market requirements.

Several diseases, including leaf blight, scab, anthracnose, alternaria and *Rhizopus* hull rot, can be problems in wet spring weather. *Alternaria* and *Rhizopus* hull rot are ongoing problems in Kern County. Noninfectious bud failure, a genetic disorder, has been observed in a number of California almond cultivars, though its severity varies among cultivars. Most nurseries have dealt with this disorder in their nursery stock at one time or another. All major nurseries have management practices in place to minimize the bud failure potential of the trees they produce, though it may be impossible to eliminate the problem in some commercially important cultivars such as 'Carmel'.

'Nonpareil' remains the dominant commercial cultivar since kernels have a paper shell

with a high shelling percentage (Table 3.8), are medium sized, uniform, fairly flat in shape and light (blond) in colour, resulting in the widest range of market uses. 'Nonpareil' is often used where the nut is visible and/or in manufactured products because the kernels can readily be blanched and mechanically cut to produce a variety of forms. Furthermore, the thin shell allows maximum yield of undamaged kernels after cracking. 'Sonora' is also considered a premium almond because of its similar use and acceptability as a substitute for the 'Nonpareil' cultivar for whole natural kernels. 'Mission', 'Butte' and 'Padre' are medium hard-shelled cultivars with shelling percentages typically of 50% or less. Kernels tend to be small and rounded and, while market prices are generally lower, these cultivars are still widely planted because of their good productivity and good resistance to insect pests provided by the harder shells. Remaining cultivars, including 'Monterey', 'Carmel', 'Fritz' and 'Price', are generally intermediate in market value but are often used as pollenizers for 'Nonpareil'. Most California almond cultivars, including 'Nonpareil', 'Monterey', 'Carmel', 'Fritz', 'Mission' and 'Price',

originated as chance grower seedling selections of uncertain origins. ‘Sonora’ and ‘Padre’ were early releases of the breeding programme at the University of California at Davis, with more recent releases including ‘Winters’, ‘Sweetheart’ and ‘Kester’. ‘Butte’ was bred by private breeder F.W. Anderson in the early 1960s. More recently, Zaiger Genetics has released the self-fruitful cultivar ‘Independence’, which is being widely planted.

Major almond rootstocks in California include ‘Lovell’ peach and Marianna plum in northern regions where rain-saturated soils can be a problem, and ‘Nemaguard’ in southern regions where nematodes are more problematic on the sandy soils. The recent loss of soil chemical fumigants has resulted in the proliferation of a large number of new rootstocks of diverse and often interspecific origins, with large-scale regional testing of these rootstocks currently underway.

3.3 Production Trends

Global commercial production was approximately 2.2 million tm (kernel meats) in 2013. California accounted for approximately 83%, with a 2013 production of 1,814,372 tm kernel meats (Table 3.9) from approximately 300,000 ha (bearing). Spain, historically the second leading region, produced approximately 7% of world production but used a cultivated area of over 436,500 ha. Australian production has recently surpassed that of Spain, being slightly more than 7% of total. The remaining world production comes from about 20 countries including Morocco, Iran and Turkey (~4% each), Italy (3%), Tunisia (2%) and Chile (1%) (ABC, 2014).

Limited almond production for both local and export markets also occurs in other areas of the Mediterranean, including Portugal, Morocco, Tunisia and Algeria; in areas of the Balkan Peninsula, including Bulgaria, Romania and

Table 3.8. Characteristics of the main almond cultivars in California.

Cultivar	Blooming time	Harvesting time	Productivity	Self-compatibility	Shelling percentage	Kernel weight (g)	Proportion of total 2014 production (%)
Nonpareil	Middle	Early	High	No	65.0	1.4	40
Monterey	Middle	Late	High	No	40.7	1.3	13
Butte	Middle-late	Middle-late	High	No	47.7	1.0	18
Carmel	Middle	Middle-late	High	No	53.4	1.2	8
Fritz	Middle	Late	High	No	53.7	1.2	6
Padre	Late	Middle-late	High	No	52.4	1.1	3
Sonora	Early	Early	Medium	No	71.1	1.4	2
Mission	Late	Late	Medium	No	43.7	1.2	1
Price	Middle	Middle	Medium	No	56.5	0.9	1

Table 3.9. Recent almond production (tm kernel) trends (ABC, 2014).

Country	Year			
	2010	2011	2012	2013
USA	1,413,800	1,841,587	1,814,372	1,814,372
Australia	90,000	90,000	115,000	160,000
Spain	222,518	208,800	211,700	149,000
Morocco	87,104	96,231	99,067	96,523
Iran	158,050	92,491	1,000,000	87,281
Turkey	55,398	69,838	80,261	82,850
Italy	108,160	104,790	89,865	72,633
Tunisia	52,000	61,000	70,000	52,000
Chile	22,000	24,000	24,500	28,560

Hungary; and in central and south-western Asia, including Syria, Iraq, Israel, Iran, Ukraine, Tajikistan, Uzbekistan, Afghanistan, Pakistan and China. Almond growing in much of this area is in a relatively traditional state, although modern production areas exist in Portugal and in some parts of Ukraine and Iran. Despite falling

production in most traditional growing regions, global production has more than doubled in the past 20 years from a 1994 crop of only 354,259.3 tm. Most of the increase in production has occurred in California, where a doubling in crop area combined with a tripling of per hectare productivity has occurred.

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4 Almond in the Southern Hemisphere

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4.1 The Australian Almond Story

4.1.1 History

The first recorded instance of almonds arriving in Australia occurred in the early 1800s, as food in the form of kernels or in-shell. An early advertisement in the *Hobart Town Gazette and Van Diemen's Land Advertiser* of 27 July 1822 shows that 'Jordan almonds' were imported to Tasmania and available for sale, presumably only for eating. Other newspaper advertisements from *The Sydney Gazette and New South Wales Advertiser* (1804) tell of almond trees 'of a particularly fine sort' for sale by a nursery, coming from the 'Cape', presumably meaning Cape Town, South Africa. Five years after the colony of South Australia was founded, Thomas Shepherd reported in the *South Australian Magazine* (1841) that several cultivars of almond were growing in the colony and included both hard- and soft-shelled cultivars. Later, George Quinn from the South Australian Department of Agriculture (1905) wrote that several cultivars were established in Adelaide, including 'Nonpareil' and 'Ne Plus Ultra' (imported from California by 1882), 'The Brandis' (a chance seedling also known as 'Brandis Jordan') and 'White Nonpareil' (a chance seedling). By 1908, 46 almond cultivars had been planted at the Department of Agriculture's

Adelaide and Blackwood experimental orchards in the foothills of Adelaide (Fowler, 1927). The idea of the experimental orchards was to observe all types of fruit trees under non-irrigated conditions and determine the best types suited to the Adelaide climate.

The fledgling Australian almond industry began in earnest in the late 1920s with the first commercial plantings of around 809 ha (2000 acres) in South Australia (Quinn, 1941). These early plantings were located close to the capital, Adelaide; however, orchards were soon developed further north and south of Adelaide at the rate of 50–100 acres per year. The cultivars in use at that time were made up of two types: locally raised seedlings, which became well established, such as 'Chellaston', 'Johnston's Prolific' and 'White Brandis'; and cultivars originating from California and Europe, such as 'Nonpareil', 'Ne Plus Ultra' and 'Jordan'. The locally named cultivars are of unknown parentage, but recent work using molecular markers has shown that they share incompatibility alleles with 'Nonpareil', 'Carmel' and 'Jordan' (Wirthensohn *et al.*, 2011; Fernández i Martí *et al.*, 2015) and hence are likely to be offspring of these overseas introductions. Table 4.1 shows some of the characteristics of many Australian cultivars including origin, self-incompatibility alleles and bloom time.

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Table 4.1. Australian cultivar characteristics.

Cultivar	Region of origin	Incompatibility alleles	Shell hardness	Bloom time
Atkinson's hard shell	Willunga	S ₅ S ₂₂	Hard	Early
Baxendale	Willunga	S ₅ S ₇	Soft	Early/mid
Biggs hard shell	Willunga	—	Hard	Early
Brandis (Brande's Jordan)	Enfield	—	Soft	Very early
Brown Brandis	Marion	—	Soft	Very early
Bruce	Langhorne Creek	—	Soft	Early
Chellaston	Edwardstown	S ₇ S ₂₃	Soft	Very early
Frenzy	Willunga	—	Soft	Early/mid
Johnston's Prolific	McLaren Vale	S ₇ S ₂₃	Soft	Early
Jose's B1	Edwardstown	—	Soft	Early
Keane's Seedling	Munno Para	S ₇ S ₇	Paper	Early/mid
McKinlay's Magnificent	Willunga	S ₇ S ₈	Paper	Early/mid
Parkinson's Pride	Willunga	S ₁₁ S ₂₃	Soft	Early
Pearce	Willunga	S ₈ S ₂₃	Soft	Early
Pethick's Wonder	Marion	S ₇ S ₂₃	Soft	Early/mid
Riverside Peerless	Felixstowe	—	Soft	Mid
Somerton	Somerton	S ₁ S ₂₃	Soft	Early
Stockham's paper shell	Fullarton	—	Paper	Very early
Strout's paper shell	Willunga	—	Paper	Early
White Brandis	Edwardstown/Marion	S ₇ S ₂₃	Soft	Very early
White Nonpareil	Payneham	—	Soft	Early

4.1.2 Production in Australia

As productive, irrigated land became available along the Murray River (Australia's longest), and urban encroachment occurred, almond growers moved from the suburbs of Adelaide to this new region. Today the current area planted to almonds in Australia is 31,115 ha predominantly along the Murray River in South Australia (SA), Victoria (VIC) and New South Wales (NSW) (Fig. 4.1). The major regions are: Sunraysia (VIC) 64%, Adelaide (SA) 2%, Riverina (NSW) 12% and Riverland (SA) 20% (Fig. 4.2). The climate in these almond-growing regions is classed as temperate, with wet cold winters, no severe spring frosts and hot dry summers, but with occasional summer rains which can hamper harvest.

Greater than 40% of the industry's plantings are fully mature (8 years and over) while 4% is non-bearing (ABA, 2014). The almond industry is composed of a mixture of family orchards, corporate ventures and investment company projects. The main commercial cultivars and the area currently grown in Australia are 'Nonpareil' (51%), 'Carmel' (32%), 'Price' (12%) and 'Peerless' (1%). Table 4.2 shows the production across Australia by cultivar from 2002 to 2013.

Australian almonds are graded as: fancy, extra supreme, supreme or manufacturing, and can be supplied in various blanched forms such as natural sliced, slivered and meal as well as kernel and in-shell. Australian producers supply domestic consumption and an expanding export market. Domestic consumption has increased by 6.5% in the past 12 months (2015/2016); per capita consumption of almonds in Australia is 0.97 kg. This increase is partly attributed to the credible health claims of almonds, which has helped drive the consumption in Australia to 20,802 tonnes (t).

Almonds are now Australia's largest horticultural export industry. Australian almonds are exported to over 40 countries with the major markets being India (A\$100m), United Arab Emirates (A\$43m), Germany, Italy, Spain, The Netherlands, New Zealand, the UK, the USA and Hong Kong. Export earnings were in excess of A\$739m in 2015 and it is expected that future exports will represent greater than 70% of total sales, with an associated value of A\$750m. Almonds are in high demand in many developing countries and Australia is well positioned to supply these markets. The market in India is mostly for in-shell almonds; however, the wider export



Fig. 4.1. The four major almond-growing regions in Australia.



Fig. 4.2. A large commercial almond orchard in the Sunraysia region, Victoria, Australia.

market is made up of 37% in-shell and 63% shelled. In 2015 there was a seven-fold growth in the export market to the Middle East. New market opportunities are also arising due to new

free trade agreements and improved living standards in countries such as China.

Almond production has increased steadily over the past decade from less than 10,000 t

Table 4.2. Australian almond production by cultivar (kernel tonnes).

Cultivar	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Baxendale	250	221	189	210	163	230	168	173	160	105	21	118
Carmel	1577	1840	2483	3807	4246	7383	7996	11,681	10,561	14,091	15,718	26,922
Chellaston	51	43	43	42	59	62	23	33	15	24	4	18
Fritz	284	309	288	321	249	331	240	151	198	85	108	99
Johnston	23	24	20	16	30	36	35	47	24	36	13	31
Keane	46	58	62	79	112	132	70	69	79	65	71	112
Mission	322	325	293	333	273	314	251	196	156	146	23	133
Monterey	—	—	—	—	5	24	42	97	181	328	429	689
Ne Plus Ultra	585	635	593	616	594	823	527	443	425	290	276	409
Nonpareil	5446	5572	6296	8944	7989	13,751	13,376	18,686	21,219	17,154	25,766	36,305
Peerless	340	384	558	466	576	936	597	693	747	765	715	949
Price	318	399	474	936	903	2037	2338	4023	3936	4196	5796	7212
Other	300	429	442	660	802	824	342	112	1,379	340	645	364
Total	9542	10,238	11,743	16,431	16,001	26,882	26,006	36,403	39,081	37,626	49,585	73,361

of kernel in 2000 to 73,361 t of kernel in 2013. Kernel production is expected to reach 83,000 t by 2017. The current farm gate value is A\$640m with an expected value of A\$780m by 2020. This growth will see Australia's contribution to global almond production increase from 3% in 2008 to an expected 8% in 2017, to become the world's second largest almond-producing nation (ABA, 2014) (Fig. 4.3).

Australia has a distinct advantage over other almond-growing regions. The nut-producing areas are quite isolated, giving them a pollution-free environment with plenty of solar radiation for photosynthesis and kernel nutrient accumulation. Australian almonds are also counter-seasonal to all northern hemisphere-grown almonds and are therefore fresh in the northern hemisphere 'off-season'.

Almond consumption worldwide has increased to 1.1 million t and this trend is expected to continue, helped by successful campaigns promoting the health benefits of almonds. Australian producers are well placed to serve this export demand for quality almond kernel. Crop yields have consistently been at or above the benchmark level of 3.5 t/ha, which puts Australia in a good position to fill growing Asian markets and also niche market requirements.

4.1.3 Cultivation

Historically almonds were planted using seedling or bitter almond as rootstock; however, modern plantings use a mixture of peach or peach–almond hybrid rootstock such as 'Nemaguard', 'Bright's hybrid' or GF677. All budded trees are dipped in NOGALL™ (BASE, NC, USA) for protection from crown gall. In the 1990s a budwood repository block was established by the Almond Board of Australia (ABA) at Monash in the Riverland, SA, to supply the industry with virus-tested plant material.

Almonds are generally planted on soil with good drainage, and orchard sites are generally selected for low frost risk. As mentioned earlier, 'Nonpareil' is the major cultivar and is planted at 50% with an early and late pollinator making up the other 50% of orchard space. Planting distances are 7 m × 5.5 m (260 trees/ha) or 6.5 m × 5.5 m (280 trees/ha). Row spacing is generally fixed to allow access for orchard machinery. The orchard floor is kept level and cover crops are closely mown to aid off-ground mechanical harvesting. With an appreciation that water is a major limiting factor, Australian orchards use sophisticated irrigation systems with greater than 90% of plantings under drip irrigation (above ground and sub-surface) and less than 9% using micro sprinklers. The almond industry has led the way with pulse drip – a technology

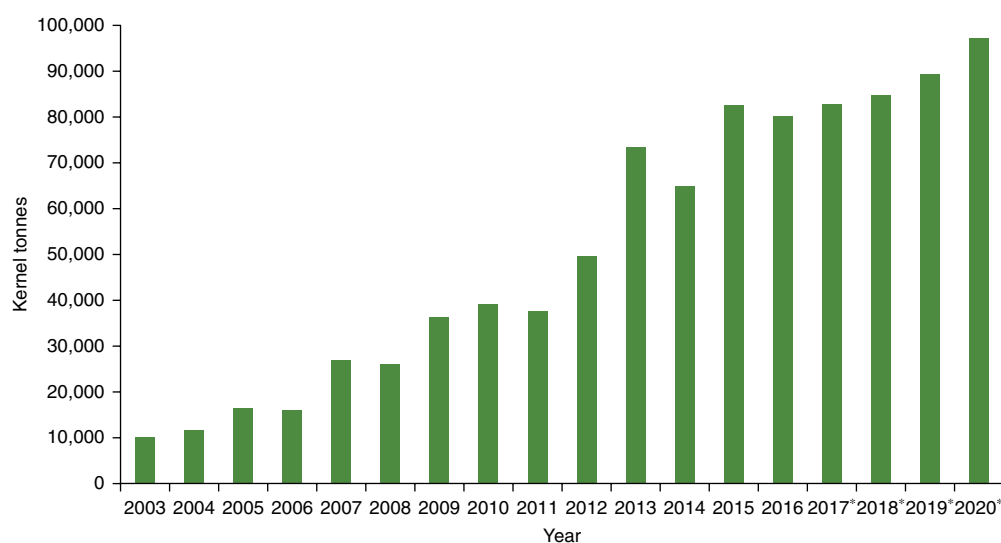


Fig. 4.3. Australian almond industry production: past, present and future. (*= estimated production.)

that further increases the return per unit of water applied (Phogat *et al.*, 2013). Sustained deficit irrigation (SDI) is another technique which has been trialled in some orchards to improve water use efficiency. The use of SDI has been shown to have no effect on the kernel quality of almonds (Zhu *et al.*, 2015). Along the Murray Valley, application of between 12 and 14 ML/ha is required for good production, with lesser quantities required in cooler and higher rainfall areas. Fertilizer is usually applied through the irrigation system and commonly applied elements include nitrogen, zinc and potassium. Under these conditions the trees are ready to harvest in year three. Australian almond growers have become proficient at managing the interaction between water and nutrients to achieve consistently higher yields and lower per unit production costs than competitors. The use of continuous soil moisture/salinity monitors, an Australian development in its own right, has been a critical element in this success.

Almond harvesting in Australia generally begins in February and continues until April. In most areas this is a dry time of the year, although occasional wet summers are experienced. During processing the waste products (hull and shell) are most commonly used as stock feed, compost or mulch. The ABA is investing in a number of projects to add value to this byproduct, including biofuel.

Early-flowering cultivars such as the Australian cultivar 'Chellaston' begin flowering in late July, with later cultivars finishing by mid- to late September. European honeybee hives are brought into the orchards at flowering time for effective pollination and fruit set. Adequate numbers are required and the general recommendation is for six to eight hives/ha.

4.1.4 Industry support and research

In response to the need for a coordinated approach to market their produce locally and overseas, almond growers formed the Almond Growers' Cooperative in the early 1930s. This was the indirect forerunner to Almondco Australia, currently one of the most successful horticultural cooperatives in Australia, with 85% of almond growers supplying produce. The three other major almond marketers are Nut Producers

Australia, Olam Orchards and Select Harvests. The peak almond industry body in Australia is the ABA. This is a non-profit, membership-based organization which represents the interests of almond growers, processors and marketers. It began as the Australian Almond Growers Association in 1985 and after restructuring in 2002 became the ABA. In conjunction with the Australian Federal Government and through the funding body, Horticulture Innovation Australia Limited (HIA, formerly Horticulture Australia Limited), the ABA supports a number of research and development projects. HIA's role is to manage the expenditure of levy funds collected by the government on behalf of horticulture industries.

One of the major projects funded by the levy is the Australian almond breeding programme, run by the University of Adelaide in the School of Agriculture, Food and Wine, which began in 1997. Its aim is to produce new cultivars with higher kernel yield and quality, self-compatibility, drought and disease tolerance, economic gain and diversity of products/markets for the Australian almond industry (Wirthensohn and Sedgley, 2002). Recent developments include the final-stage trialling of five selections suited to Australian conditions, three of which are self-compatible (Fig. 4.4). Alongside the breeding programme, new cultivars are tested from overseas breeding programmes in Spain and the USA. After a 2-year quarantine period, these cultivars are trialled in the field on growers' properties.

Early groundbreaking research in Australia on almond pollination and compatibility was reviewed by Bowman (1939). Thirteen old Australian cultivars were tested for cross- and self-compatibility. The study showed that most were cross-fertile but none was self-compatible. The work of Hill *et al.* (1985) greatly increased the knowledge of flowering times and compatibility of various cultivars growing in Australia at that time. Vezvaei and Jackson (1995) showed that flowers are more fertile on the day of opening than at any other stage of development. Other researchers, Jackson and Clarke (1991), used isozyme analysis to show that gene flow in a commercial orchard is generally restricted to neighbouring trees. The research led to the discovery that bees tended to only move along a row or across one row. This has implications for



Fig. 4.4. One of the selections from the Australian almond breeding programme ('Maxima'). (Photo by B. Rosenzweig.)

hive placement in orchards. Vezvaei *et al.* (1994) also used isozyme analysis to compare Californian and Australian almonds and showed that 'Nonpareil' was a major genetic contributor to Australian cultivars, as were cultivars imported from Europe.

Other recent research projects funded by the ABA and HIA include optimizing water use through deficit irrigation strategies (Sommer *et al.*, 2011), screening several almond rootstocks for resistance to *Meloidogyne* spp. (root-knot) nematodes; development of molecular diagnostic

tools to detect endemic and exotic pathogens of *Prunus* species for Australia (Constable and Rodoni, 2012); effective storage, aeration and dehydration of almonds (Gouk *et al.*, 2013); honeybee pollination for maximum yield; understanding factors influencing microbial colonization of almonds (Gouk and Kreidl, 2013); research and education of health professionals relating to the health benefits of almond consumption (Yates, 2012); improving the management of almond and prune rust (Magarey *et al.*, 2009); evaluation of pheromone-mediated mating disruption for managing carob moth in almonds and many more. The ABA also supports marketing and promotion of almonds through the media and export market development.

4.1.5 Pests and diseases in Australian almonds

Australia is fortunate in that being an island, and having strict quarantine controls, there are relatively few pests and diseases affecting almonds. However there are some diseases of concern including bacterial spot caused by *Xanthomonas arboricola* pv. *pruni*, bacterial canker (*Pseudomonas syringae* pv. *syringae*) and crown gall (*Agrobacterium tumefaciens*). There is no specific chemical control available for these bacterial diseases, although copper sprays are beneficial.

Root-knot nematode species found in almond-growing regions include *Meloidogyne javanica*, *M. arenaria* and *M. incognita*; these are generally controlled by the use of resistant rootstocks such as 'Nemaguard' and new introductions from Spain including 'Felinem' and 'Garnem'.

Fungal diseases are more common and include anthracnose (*Colletotrichum acutatum*), blossom blight (*Botrytis cinerea*), brown rot (*Monolinia laxa*), hull rot (*Rhizopus* sp.), rust (*Tranzschelia discolor*), shot hole (*Wilsonomyces carpophilus*) and verticillium wilt (*Verticillium dahliae*). These are currently monitored and then controlled by the use of fungicides from around green tip stage through to early summer.

Invertebrate pests include carob moth (*Ectomyelois ceratoniae*), bryobia mite (*Bryobia rubrioculus*), two-spotted spider mite (*Tetranychus urticae*), San Jose scale (*Diaspidiotus perniciosus*)

and Indian meal moth (*Plodia interpunctella*) (ABA, 2010). Biological control plays an important role in the management of some pests, such as two-spotted mites, aphids and light brown apple moth, in conjunction with softer pesticides. Good control of mites is also achieved with dormant oil.

Birds are often a problem in ripening crops, particularly paper shell cultivars, with galahs (*Eolophus roseicapilla*), crows (*Corvus* spp.) and corellas (*Cacatua* spp.) being the most troublesome species. Growers use a wide variety of measures to deter birds including gas guns, live shot, light aircraft, electronic devices and predatory bird kites in the shape of peregrine falcons.

Rabbits and hares often damage young saplings and chew irrigation lines, so tree guards are used to protect newly planted trees.

4.1.6 Future prospects

The prospects for the Australian almond industry look very good, with expanding production able to supply an increasing worldwide demand. This is due to increased consumer awareness of the health benefits of almonds and the increase in world population, along with greater prosperity in developing countries. There is little reason to doubt that the increases in almond consumption will continue into the future. Continuing research indicates possible expansion into food security and the health benefits of Australian almonds.

4.2 Almonds in Argentina

4.2.1 History

Although not a native fruit species in the country and in the South American continent, almonds are a very popular food and they are widely spread throughout Argentina. Knowledge of this species, and first almond consumption, dates back centuries as the first Spanish colonists and, especially, the Franciscan and Jesuit missionaries (Prataviera, 2006) introduced this species in the 16th and 17th centuries and established the first plantations in

the provinces of north-western Argentina. These plantations were managed in a 'traditional' way: orchards occupied a very small land area and most used plants originating from seeds, so had great genetic variability. Southern Spain was the major source of this material and, therefore, the almonds came from early-flowering plants. This is why the plants adapted well to the warm climate of Argentina and was also the reason for the great risk of crop failure due to spring frosts.

Some plant material was introduced in the form of cuttings bearing buds, which then were grafted onto rootstocks (seedlings). This took place from the late 19th to the early 20th century with the influx of large numbers of European immigrants, mainly Spanish and Italian, who are today the population base in this country. Farmers were interested in the remunerative prices for the product and then had to manage its commercial exploitation, and the use of plants grafted onto bitter almond rootstocks became generalized.

The special conditions of the western and north-western provinces of the country, with high summer temperatures, dry conditions with water scarcity and absence of pests and diseases, provide an excellent framework for the development of the almond tree. Moreover, the gastronomic traditions of these new settlers continued and, even today, remain the basis of national food. Almonds are eaten as dessert with other nuts (walnuts and hazelnuts) directly or 'fresh'. The product is consumed all year round, but the peak period is during the months of October and November, because bakeries require it for making Christmas breads, with great consumption for Christmas and New Year holidays. The confectionery factories manufacture sweets and chocolates which are sold in bags of 100 g and 200 g during these holidays. Most almonds appear in the form of processed confectionery products (pralines and caramel, hard and soft nougat), baked goods (pies, cakes and biscuits), pasta and marzipan, ice cream, chocolates and – more recently – oils for cosmetic as well as for culinary use. Traditionally, as a continuum of European customs, the greatest national consumption occurs in coincidence with Christmas holidays, as it is traditional to serve them at the table during the festivities of Christmas and New Year.

4.2.2 Production in Argentina

In Argentina, the main producing provinces are Mendoza, San Juan and La Rioja, with smaller areas in the provinces of Catamarca, San Luis, Salta, Buenos Aires and Rio Negro, and minor crops in the provinces of Entre Ríos, Córdoba and Neuquén (Fig. 4.5). In 2000 the planted area totalled almost 5000 ha. These cultures consisted mostly of early-flowering, soft-shell cultivars. In a short period the loss of productivity and profitability caused by spring frost, and damage by birds in the months before harvest, resulted in a decline in the planted area to 2500 ha in the early 2000s. In the province of Mendoza, this situation led to the eradication of almost two-thirds of the planted area, which declined from nearly 2000 ha in 1990 to less than 700 ha in 2002.

The appearance and knowledge of very late-flowering cultivars, mainly from Spain, contributed to the increase in prices in the national market and reawakened the interest of growers and investors. A new period began increasing the planted surface in Argentina to over 4000 ha (Table 4.3). Almost 40% of that area is still being developed and has not reached its full production inasmuch as plantations are less than 6–7 years old.

Since 2012 Mendoza Province is the main almond producer (Censo Frutícola Provincial, 2012). According to the 2012 Provincial Fruit Census and adjusted data provided by references on the subject, the total area of Mendoza with almond trees was c.2450 ha at that time, concentrated in the northern region, with 55% (1200 ha), followed by the east region representing 46% of the planted area. The Uco valley and the southern region, colder and with a higher risk of crop loss due to frost, contributed 40 ha of almond trees to the total. In 2008 in Mendoza Province, 95% of almond production was concentrated in three areas: San Martín (1150 ha), Lavalle (860 ha) and Maipú (400 ha) (Carmona Torres and Alonso, 2008).

The census indicated that 'Guara' was the most widespread cultivar at the provincial level, followed by 'Felisia' and 'Nonpareil'. The distribution of planted area by cultivar does not mean a proportional production, since 49% of the area was aged 5 years or less (i.e. not in production) and 43% of the planted area was

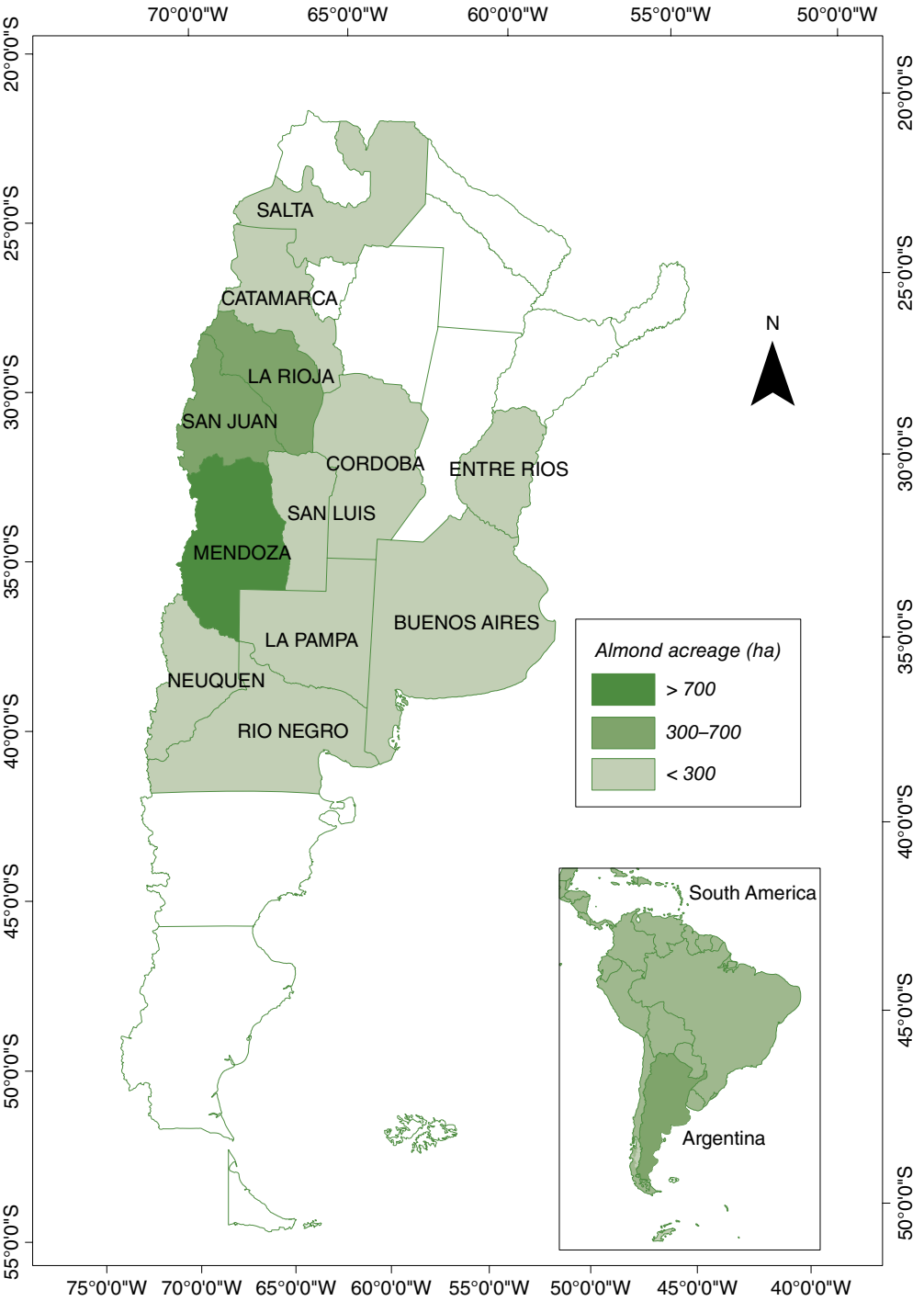


Fig. 4.5. Almond acreage in Argentina.

Table 4.3. Argentinian provinces and area planted with almonds.

Province	Surface (ha)
Mendoza	2580
San Juan	572
La Rioja	498
Salta	189
Rio Negro	147
Others	200
Total	4186

between 6 and 10 years. This meant that 51% of the surface is at a full production age.

In general, plantations are in the hands of small producers, although some companies have planted large areas. One example is Frusi Argentina, which in 2006 invested in 800 ha of almond trees in Divisadero (San Martín), using cultivars such as 'Guara', 'Ferraduel' and others characterized by a very good productivity, hard shell and late flowering. Another is Aceitera General Deheza (AGD), with 800 ha of almond trees in Jocolí, planted in late 2006 (Censo Frutícola Provincial, 2012).

The almond plantations in San Juan Province have increased since the 1970s, with modern plantations in medium densities and with Californian cultivars like 'Nonpareil', 'I.X.L.', 'Ne Plus Ultra', 'Peerless' and 'Paper Shell', and some years later 'Emilito INTA', 'Thompson' and 'Merced' being incorporated (Raigón, 2014). This province has generally favourable conditions for crop development, although the frequent and extremely warm and dry Zonda wind (26–30°C; 0–5% relative humidity) is a climatic factor that causes serious damage to the crop, mainly during the flowering period. As in Mendoza, frosts in late August and even until early September can occur, coinciding with blooming and fruit set, causing partial or total losses in early-flowering cultivars. Currently most of the plantations are located in the departments of Pocito and Zonda (Raigón, 2014). These plantations present higher plant densities (333–400 plants/ha, with distances of 6 × 5 and 5 × 5 m). 'Nemared' and 'Nemaguard' rootstocks are used and late-flowering cultivars of French and Spanish origin have been incorporated. Yields in these young plantations are still low, and are estimated at between 400 and 600 kg shelled almonds/ha.

Almond plants have been found in Patagonia, in the south of the country, since the arrival of the first settlers in 1779 (Pratavia, 2006), where they were part of orchards or small plantations subjected to very heavy spring frosts that damaged their production. In the 1970s the IDEVI (Instituto de Desarrollo del Valle Inferior del Río Negro) experimental station began important research and experimentation into nuts, in which almond was one of the three species considered in addition to hazelnut and walnut. Cultivars from other areas of Argentina were introduced, but fundamentally they were introduced from abroad: from Italy through an agreement with the ISF (Istituto Sperimentale di Frutticoltura di Roma), from INRA in France and from CITA of Aragón in Spain. 'Tuono', 'Cristomorto', 'Ferragnès', 'Ferraduel' and 'Guara' were the most important cultivars, first incorporated as study material and then grown in plantations. Since the 1990s research only into late- and very late-blooming cultivars has been carried out at the INTA Alto Valle experimental station. These cultivars include 'Ferraduel' and 'Ferragnès' from INRA; 'Moncayo', 'Guara', 'Felisia' and 'Mardía' from CITA of Aragón; and 'Tarraco' and 'Marinada' from IRTA in Catalonia.

The Patagonian region is characterized by a cold climate, so almond production is limited to special zones, though according to its relative location, the continent provides extreme temperatures favouring – to a greater or lesser extent – the possibility of almond culture. The behaviour of late-flowering cultivars is very satisfactory in almond yield and quality but, in all cases, in Patagonia and many parts of Cuyo Region, a secure harvest can be obtained only if the plantations have a frost defence system (Iannamico, 2010). Of the available technologies for this purpose, the most widely used, economical and safe is sprinkler irrigation (Figs 4.6 and 4.7), allowing Patagonia to position itself as the southernmost region of the world (latitude 38–40°S) to produce almonds.

4.2.3 Development of the industry

The profile of the Argentine productive sector is characterized by being composed of 80% of small- to medium-sized individual producers and small societies with areas below 10 ha.



Fig. 4.6. Ice on almond trees during sprinkler frost protection. (Photo by L. Iannamico.)



Fig. 4.7. Detail of a fruiting bud protected by ice. (Photo by L. Iannamico.)

The rest are represented by large companies with areas of orchard greater than 100 ha. The technology of almond production has evolved around the changes that have occurred in recent

decades (1990–2015) in the use of different forms of irrigation, planting densities, plant material and, lately, harvest and post-harvest technology.

In relation to irrigation, in the first cultures gravity irrigation techniques were used, usually with grooves on both sides of the plant. This was because water was scarce in the producing provinces of western Argentina and it was necessary to use it with care. From the 1980s, and especially in plantations established in the past decade (2006–2016), the predominant irrigation system has been pressurized drip, which has allowed the optimization of available water for each farm, with the consequent expansion of the planted area.

Associated with the irrigation system and intensification of modern training systems, planting distances were evolving to more intensive forms: from distances of 7 m and 8 m between rows to 5 m and 6 m; and the distance between plants from 6–7 m to 4–5 m, so that densities evolved from 230–250 plants/ha to 400–500 plants/ha (Iannamico, 2015a). Today, the latter constitutes almost 80% of plantations (Fig. 4.8). Harvesting is still manual in most of the orchards of small and medium producers, although each year the use of hand vibrators increases on small farms, and mechanized harvesting on large properties.

A current analysis of the almond industry taking into account the advantages and disadvantages suggests:

Disadvantages

- Limited production in Argentina, requiring increasing efforts to supply the domestic market and compete with larger volumes, but mostly with improving quality.
- Low level of technology in production issues: uncertified plant material; outdated harvesting technology (still manual); and belated post-harvest technology (drying, conditioning, sizing and packaging).
- An increasing global supply of almonds, primarily led by hi-tech countries (the USA, Australia, etc.) and, in some cases, subsidies for this industry.
- The lack of sector organization, mainly in post-harvest and marketing.
- The limited availability of nurseries producing high-quality plants, properly distributed in different regions to supply specific markets.



Fig. 4.8. Almonds growing at high density in Rio Negro (1000 trees/ha). (Photo by L. Iannamico.)

Advantages

- A large potentially productive area with excellent soil and climate conditions in many regions of the country for production of high-quality almonds (Iannamico *et al.*, 2009).
- Perishable fruit (stone and prune) growers' interest in entering into production and commercialization has increased.
- Growing national and – above all – international consumption based on knowledge and dissemination of high-quality nuts as food.
- The high value of the product, in contrast to conditions of declining profitability for fresh fruits, promotes production restructuring in various regions.
- Lower production costs and competitiveness in relation to the major producing countries (Marginet Campos, 2003; Iannamico, 2015b).

4.2.4 Cultivars in use

Studies of almond cultivars in Argentina have mainly been conducted in five locations, represented by the experimental stations of the National Institute of Agricultural Technology (INTA):

- EEA INTA Junín (Mendoza Province), in the central-east region: studies since the 1950s by Silvia Carra, Emilio Touza and Oscar Wouters. New cultivars such as 'Javier INTA' and 'Emilito INTA' have been developed (Carra de Tolosa and Herrera, 2006a).
- Catamarca Experimental Station (Catamarca Province): studies on breeding since 1957 by Antonio Prataviera, and three cultivars of local interest obtained (Prataviera, 1984).
- San Juan Experimental Station: studies by Juan Raigón, Alejandro Oviedo, Daniela Pacheco, Alberto Pacheco and Viviana Castro since 2000, and a new collection of cultivars adapted to San Juan Province (Raigón, 2014).
- IDEVI Experimental Station (now Valle Inferior Experimental Station of INTA): studies by Rolka, Rossi and Iannamico, begun in 1974, and the first introduction of Spanish, French, Russian and American

late-flowering cultivars (Iannamico and Villegas, 1998).

- Alto Valle Experimental Station (Rio Negro Province): studies since the 1990s by Luis Iannamico (Iannamico and Massari, 1992; Iannamico, 2006) related to cultivars of Spanish and French origin, late and extra-late flowering, to evaluate their behaviour in very cold climates.

In the early years of the development of major commercial cultivars in Argentina, self-incompatible cultivars prevailed. As these were unable to self-pollinate they required the presence of pollinating cultivars in the orchard to ensure fruit set of flowers. Limited knowledge of the self-incompatibility of the species led to errors in combining the cultivars, and the inclusion of non-compatible cultivars or the placing of blocks with only one self-incompatible cultivar, causing major failures (Felipe, 2000).

During early crop development (1950–2000) the almond orchards in Mendoza, San Juan, La Rioja and Catamarca, the main producing provinces, were composed of cultivars known as the 'Nonpareil' group. They are: 'Nonpareil', 'Thompson', 'Ruby', 'Merced', 'Javier INTA' and 'Emilito INTA', disseminated by the National Institute of Agricultural Technology, after being evaluated in the collection of the EEA INTA Junín, Mendoza (Carra de Tolosa and Herrera, 2006b).

Argentina began its commercial production around 1950, based on the cultivation of 'paper shell' cultivars, mainly because of the absence of machines required to remove the hull. This process was performed exclusively by hand until 1990. Up to that date, the widest cultivated cultivar in Mendoza, the first national producer province of almonds in Argentina, was 'Martinelli L', greatly appreciated for its high productivity and long flowering period.

'Martinelli L' is cross-compatible with 'Martinelli C' and 'Nonpareil'. These cultivars bound cultivation of the species to the warm and dry areas of the Argentinean middle west (the provinces of Mendoza and San Juan). Since then, attempts to cultivate the same cultivars in less dry areas (such as parts of the provinces of Córdoba, San Luis and Santa Fe) failed because of their sensitivity to diverse diseases.

It is relevant to mention that, until the end of the 20th century, all cultivars grown in

Argentina (Fig. 4.9) were self-incompatible and required cross-pollination. At present, INTA is extending the 'hard shell' group represented by the French cultivars 'Ferragnès' and 'Ferraduel' and the Spanish 'Guara', 'Mardia' and others (Felipe and Socias i Company, 1986) and 'Marinada', 'Tarraco' and others (Vargas, 2007) mainly into the areas considered 'marginal' for these cultivars (because of the incidence of delayed frosts and rain occurrence in flowering and harvest time).

The cultivar panorama in Argentina is varied and includes materials from different countries. Over past decades selections and genetic crossings have been made by the National Institute of Agricultural Technology-INTA, with the aim of disseminating better-adapted cultivars, mostly to the main producing areas such as the regions of Mendoza-San Juan and Catamarca-La Rioja (Iannamico *et al.*, 2009). Achievements include:

- 'Emilito INTA'. Origin: 'Peerless' × 'Nonpareil', created in the 1970s by Emilio Touza and Oscar Wouters at Junín Mendoza Experimental Station INTA. This is a widely spread cultivar in Argentina, always accompanying 'Nonpareil'. The fruit is very attractive, creamy and suitable for commercialization in-shell as its closure is perfect. The kernel is pleasant tasting and similar to Nonpareil ('golden') in appearance, but is larger and without doubles. Flowering is early to medium and it is a very productive cultivar. The tree is easy to train and prune. It requires cross-pollination, and proper fruit set is obtained with pollen from 'Nonpareil', 'Peerless', 'Ne Plus Ultra', 'Martinelli L', 'Martinelli C', 'Thompson', 'Ruby', 'Merced' and 'Javier INTA'.
- 'Javier INTA': Origin: created in 1985 from the cross 'Texas Prolific' × 'Nonpareil', by Silvia Carra de Toloza in the Experimental Station in Junín Mendoza INTA. Dissemination: beginning in the early 1990s, and achieving widespread acceptance by the average producer for its marked late frost tolerance and high productivity. Integrated in virtually all modern plantations, being part of the 'Nonpareil' group ('Nonpareil', 'Merced', 'Thompson', 'Ruby' and 'Emilito INTA'). This cultivar is mainly grown in areas of frequent rain during harvest, when

moisture ruins the kernels of soft-shelled cultivars. It is well pollinated with 'Cáceres Roja Grande', 'Emilito INTA' and 'Peerless' (Carra, 2002).

- 'Cáceres Clara Chica' and 'Cáceres Roja Grande': Origin: unknown. Dissemination: almond-producing areas in Mendoza. These cultivars are grown mostly in areas of frequent rain during harvest, when moisture ruins the kernels of soft-shelled cultivars. It pollinates well with 'Emilito INTA' and 'Peerless'. The name 'Cáceres' was given by INTA, using the surname of the owner of the farm where the original plants giving rise to these cultivars were selected.
- 'Martinelli C' and 'Martinelli L': Origin: unknown. They are believed to have been introduced to the country to serve as pollinators for 'Nonpareil' and other cultivars. The names of these two cultivars were given by INTA, using the surname of the owner of the farm where they were individualized for the first time. The 'L' identifies the principal cultivar that flowers for 'longer' and 'C' identifies the pollinator, with a 'shorter' flowering period. Dissemination: they are relatively widespread in the province of Mendoza. Although they were the most important cultivars in past years, they are no longer planted today because they are outdated in productivity and quality as compared with other cultivars.

4.2.5 Statistics

Based on official data from national and provincial surveys conducted in 2011 and 2012, and information provided by nursery suppliers of almond plants in 2014 and 2015, it can be said that the area planted with this species in Argentina is 4186 ha (Table 4.3). It is important to note that much of this area (50%) is composed of young plantations, under 6 years. This is the reason for the low average national production (400 peeled almond kg/ha).

Climatic contingencies, mainly frosts in spring, greatly influence the fluctuating annual production and this is the reason for cultivar replacement in different regions, substituting the early- and mid-flowering cultivars with



late-flowering ones, mainly of Spanish origin (Iannamico, 2007). Production in 'normal' years was estimated in 2012 at 1800 t, therefore national consumption requires the import of almonds from other countries, with volumes in the period 2011/2013 of approx. 2000 t (Garañiz, 2012). For more information see Table 4.4.

Currently, national consumption forces the import of large quantities of almonds, which is summarized for the last 10 years, according to information provided by the Ministry of Agriculture (P. Doreste, Buenos Aires, 2014, personal communication) in Table 4.4. Argentinian exports, on the other hand, have little significance in offsetting these (Table 4.5).

It is expected that during the decade 2015–2025 production levels will be considerably higher, projecting an annual increase of 10% from the current surface of 500 ha/year and turning to an area of 10,000 ha. This, bearing in mind that the orchards are still very young, would bring the volume of production to about

7000 t of kernel, which would supply the local market and provide exportable surpluses of 3000 t/year.

4.3 Almonds in Chile

4.3.1 History

The almond tree has been a traditional crop in Chile since the colonial period, when it was introduced by the Spanish conquerors. The trees succeeded, thanks to the favourable ecological conditions such as suitable soil and climate. The crop adapted perfectly and prospered as a nut producer, being intended to feed the colonists, who already had incorporated it into their original diets. However, the crops did not develop much as economic enterprises, as other activities – such as mining, ranching or, within agriculture, other important crops like wheat and grapes – were more important and almond was a very secondary crop.

From the 16th century Spanish colonists managed to adapt European plants to Chilean soils and climates, and began the process of production. The good natural conditions favoured the expansion of different fruit crops. However, some problems appeared which were difficult to solve, especially the preserving of harvested fruit. Most European fruit grown in Chile, such as stone fruit (peaches, plums, apricots) and pome fruit (apple, pear, quince) contained large amounts of water and sugar, which made them vulnerable to rapid deterioration. With no cooling technology or rapid transport in the early centuries, cultivation of these fruits had limited spread. In this context, nuts (especially walnuts and almonds) avoided the problem of other fruits, because they were dry seeds and preservation was not a problem. This advantage offset their other disadvantages. In the case of almonds, the main problem was the early blooming: those almond cultivars bloomed mostly during July, in the Chilean winter. The exposure to late frosts caused significant damage or total harvest loss. In these conditions, almond cultivation was considered a highly risky activity in Chile.

However, despite the risk of frost for its early blooming, almond was the most important nut species. Records indicated there were 22,832 almond

Table 4.4. Argentinian almond imports.

Year	Tonnes	Thousands US\$
2004	949.40	4836.44
2005	563.10	4295.21
2006	709.22	4285.56
2007	1068.66	5714.44
2008	1179.23	5969.20
2009	1120.88	4232.36
2010	1897.90	10,548.92
2011	2025.79	10,972.59
2012	1864.72	11,188.61
2013	1919.21	15,387.38

Table 4.5. Argentinian almond exports.

Year	Tonnes	Thousands US\$
2004	0.03	0.19
2005	0.10	1.14
2006	0.20	0.14
2007	0.00	0.00
2008	7.00	32.32
2009	0.02	0.28
2010	0.00	0.00
2011	0.00	0.00
2012	25.98	126.60
2013	33.39	257.72

trees, equivalent to 83% of all nut plants during the period of the Kingdom of Chile (Lacoste, 2009). Almond was followed in importance by walnut (4482 plants; 16% of all nut plants) and, far behind, by hazelnut (86 plants; 0.3% of all nut plants). The almond trees were concentrated mainly in Santiago (more than 20,000 plants), followed in importance by San Felipe (1300 trees) and La Serena (600 plants).

According to Professor Pablo Lacoste (2001), the Chilean population developed an early tendency to value almonds in cuisine and pastry. Convents of nuns led this trend, which then spread to other social groups and led to a strong demand for almonds. This incentive made fruit growers challenge climatic problems and take the risk of cultivating almond, despite its early blooming and the threat of frosts. In this context of high risk and high demand, the market value of almonds remained high, and well above that of walnuts.

The cultivation of almond and, secondly, walnut trees, helped to strengthen intensive agricultural practices in Chile. With these plants, Chilean growers managed to accumulate experience in handling fruit trees. They also managed to lay the foundations for intensive agriculture, familiarity with small orchards and a work culture. In this way the foundations were prepared for later phases, when the Chilean fruit-growing industry adopted modern methods of storage and transport (Lacoste, 2011).

4.3.2 Almond-producing areas

According to ODEPA (Oficina de Estudios y Políticas Agrarias), the planted area has increased in recent years, mainly in the period 2006–2011, registering an increase in area of 47% since 2006 (Table 4.6). Bravo (2014) notes that the area planted with almond represents only 2.8% of national fruit plantings, a slight lowering seen in 2012 and 2013 after a period of growth between 2007 and 2011. Data from Intercatastro CIREN (Centro de Información de Recursos Naturales) indicate that the planted

area in 2013 was 8593.6 ha, mainly distributed in six regions of Chile's central zone (Table 4.7 and Fig. 4.10).

According to information provided at the International Almond Seminar (Castro, 2013), the last update of the cadastral ODEPA-CIREN showed that the total number of orchards between the region of Coquimbo and Maule was 1822. The Metropolitana region accounted for 48.4% of the total and was followed by the O'Higgins region with 26.7% (Fig. 4.11). In addition, the total number of trees planted in various regions showed that 26.8% were young trees, 20.3% were increasing in production, 46.4% were in full production and only 6.5% were declining in production. It should be noted that 'full production' indicates trees between 13 and 35 years of age. The percentages show that the numbers of 'young trees' and 'increasing in production' were close to 50%, showing that there was still huge potential for increase in production.

4.3.3 Cultivars in use

Table 4.8 shows that 'Nonpareil', a cultivar obtained in Suisun, California by A.T. Hatch in 1879, is the most important in Chile and represents 55% of the total planted area. It has not yet been replaced because of its good quality and market demand. It is followed by another Californian cultivar, 'Carmel', which has a planted area that has shown strong growth, exceeding 1500 ha and with 20.4% of the total; it is also widely used as a pollinator for 'Nonpareil'. In recent years other cultivars, also originating in California – such as 'Price', 'Solano' and 'Fritz' – have been added, and these five cultivars represent 85% of Chilean almond trees (ODEPA, 2014).

Table 4.8 shows that the almond industry in Chile is based on soft-shelled cultivars because of their high kernel yield and ease of removal. In recent years there has been increased interest in planting some Spanish hard-shell and self-compatible cultivars. This may lead to an interesting niche development, especially for colder

Table 4.6. Evolution of the area planted with almond trees in Chile. (Source: ODEPA.)

Year	2006	2007	2008	2009	2010	2011	2012	2013	2014
Hectares	5820	5827	6192	6924	7617	8545	8621	8548	8569



Fig. 4.10. Almond-producing regions of Chile.

areas, given their late blooming, which can help to avoid crop losses caused by spring frosts.

‘Guara’ appears to be a new alternative, averaging 11% of total sales of almond by nurseries in 2013–2016. This cultivar of Spanish origin, obtained by A. Felipe and R. Socias i

Table 4.7. Distribution of the Chilean almond area planted by region. (www.odepa.cl/superficie-de-frutales-por-region-2/)

Region	Hectares	(%)
Metropolitana	3412.1	39.7
O’Higgins	2804.6	32.6
Coquimbo	1292.8	15.0
Valparaíso	1043.3	12.1
Maule	33.0	0.4
BioBío	7.8	0.1

Company in Aragón (Spain), has a number of features that make it very attractive for Chilean conditions. These include self-compatibility, late blooming, high kernel production, early fruit set and good adaptability in both dry and irrigated areas. New cultivars from the breeding programmes at IRTA of Catalonia and CITA of Aragón, both in Spain, have also been introduced in 2013–2016.

The ‘Pomona’ peach rootstock is traditionally used in Chile, but ‘Nemaguard’ still prevails for almonds. Several nurseries are offering some hybrid rootstocks which are still hardly known by growers, including interspecific hybrids between peach and almond such as GF677 or ‘Garnem’. These rootstocks will be useful in overcoming some of the limiting conditions of soil and replanting.



Fig. 4.11. Almond orchard in Rancagua, Chile. (Photo by L. Iannamico.)

Table 4.8. Main almond cultivars planted by region in Chile (ha). (Source: ODEPA.)

Cultivar	Coquimbo	Valparaíso	Metropolitana	O’Higgins	Maule	TOTAL	%
Nonpareil	702.11	546.71	1603.20	1263.70	21.59	4137.29	55.62
Carmel	219.85	211.77	549.30	533.40	3.99	1518.31	20.41
Solano	45.98	46.22	127.50	120.04	0.51	340.25	4.57
Price	59.27	83.10	82.70	123.75	0	348.84	4.69
Mission	0	0.75	0	74.44	0.84	76.03	1.02
Fritz	83.27	18.97	0	148.47	0.67	251.38	3.38
Butte	27.92	0	0	77.17	0	105.09	1.41
Ruby	23.12	1.40	0	66.60	0	91.12	1.23
Others						570.00	7.66
Total	1291.68	935.24	2517.70	2662.34	31.21	7438.21	100

4.3.4 Development of the industry

Reyes has written that:

expert estimates indicate that about 300 to 700 ha (average 500 ha) are planted per year in the country, of which it is estimated that near 300 ha would be new crops and 200 ha would replace old plantations. In fact, we should expect significant increases in the production of almonds in the coming seasons, excluding weather problems.

(Reyes, 2008)

The average yields are still low and in 2008 stood at around 800–1000 kg of kernel/ha. This was considered by specialists to be a low yield, since other Chilean growers in appropriate conditions are able to obtain yields close to 2000 kg/ha and, in good years and with good cultural management, yields have been even higher. The main causes of low average yield are:

- the location of orchards in areas where weather conditions are not ideal for almond, which is particularly sensitive to low temperatures; growers continue to plant cultivars with early or semi-early blooming, such those that bloom mostly in August, and have important crop losses in some years (such as spring 2013);
- late spring rains, which affect the recently set fruit, producing losses at harvest; and
- pollination problems, since the cultivars used in Chile require cross-pollination.

While cultural management is important, it is crucial to start development in a suitable area where the topography protects the crop from frost, but does not prevent the completion of cultural practices. This is the reason why an expansion into the IV region of Coquimbo has been observed, where climatic conditions and land suitable for cultivation are available, as well as into the frost-protected areas in the V region, in Valparaíso. The best yields in the orchards of the central region come from orchards in the foothills of the mountains, rather than in the flat valley area, where the risk of frost is greater. Bees are used to support pollination, but sometimes the winter rains do not allow the bees to fly, and low yields result.

The most commonly used cultivar in Chile is 'Nonpareil' (with the pollinators 'Carmel',

'Merced' or 'Solano'), since it has the right organoleptic characteristics for the European and Latin American markets. However, growers are increasingly using self-compatible and late-blooming cultivars derived from Spanish cultivars that do not have these pollination problems. The adoption of new cultivars can reduce management constraints and lead to increasing yields, since they do not require the use of pollinators. The market has begun to demand sweeter cultivars of almonds derived from the Spanish cultivars, and this will help to diversify crop production, reducing risks in years when the earliest production could be affected by frost or rain. A third factor is to promote the mechanization of crops, which reduces labour costs. This implies that plant density should facilitate the access of machinery (planting distances of 6×4 m or 6×2.5 m are being used with good results).

There are no accurate official data on total domestic production of almonds. However, according to the International Nut Council (INC), Chile's production was 22,000 tons (base shell equivalent) in 2012. Almond yield has increased significantly, according to the production reported in CIREN cadastral. Thus, the yield reported in the fruit CIREN cadastral for the Region Metropolitana in July 2014 has increased substantially, from 2.4 tons/ha of almond with shell in 2010 to 4.3 tons in 2013. The survey of the Valparaíso region recorded a production of 3.6 tons/ha in 2008, and the yield increased to 4.9 tons/ha according to the 2013 cadastral. The planting density also increased, from 380 to 400 plants/ha in Metropolitana and Valparaíso; and the Coquimbo region recorded 540 plants/ha in 2011, a rise from 490 plants/ha in 2005.

According to a study conducted by IDEA (Instituto de Estudios Avanzados) and the National University of Chile, which examined the evolution and current status of the Chilean almond industry, a strengths, weaknesses, opportunities and threats (SWOT) analysis indicated the following characteristics:

Strengths

- Product recognized abroad; of good size and quality.
- Product similar to that marketed by USA.
- Easy production of the product.
- Product not perishable.

- Low production cost.
- Appropriate technical management.
- Existence of a Mediterranean climate which favours production.
- Existence of water resources.
- Production and marketing in counter season in relation to the northern hemisphere.

Weaknesses

- Frost incidence during bloom.
- Excessive moisture at harvest time in some regions.
- Marketing handled by few companies.
- Low quality of contractor labour for harvesting (opportunity and qualification).
- Lack of a commercial organization to concentrate high volumes.
- Low added value.
- Many producers, but few are associated.
- Small-scale theft by pickers.
- Average yields and production volumes.
- Low-skilled labour force.
- Few quality specialists and advisors.

Opportunities

- Expanding international markets.
- Possibility of adding value.
- Agroindustry, product processing (domestic market).
- USA is decreasing production.
- Prospect of increasing planting area.
- Possibility of increasing domestic consumption market.

Threats

- Export barriers (tariff and non-tariff, phytosanitary regulations, etc.).
- Uncertainty about international prices.
- New producing countries entering the market.
- Uncertainty about adverse weather events (rain and frost at blooming).
- Market saturation.

4.3.5 Statistics

According to information from ODEPA (Bravo, 2014) there are no official data on domestic

production of almonds. However, as previously mentioned, the INC stated that Chilean production reached 22,000 tonnes (base shell equivalent) in 2012 (Calcagni, 2015).

Most of the country's production is exported. Chilean almond exports are concentrated in the shelled format, which in 2012–2014 increased its share from 97.3% to 98.7% of total exports, reflecting the lack of participation in exports of in-shell almonds (Bravo, 2014).

The exported volume experienced a downward trend in 2012–13 after reaching almost 9000 tons in 2011. Climatic difficulties have influenced export volumes in this period, with low temperatures during spring and unusual rainfall during blooming. The value of Chilean almond exports has ranged between US\$34 and US\$60 million in recent years, showing a continuous upward trend, with the exception of 2012, when there was a slight decline compared to the previous year (Bravo, 2014).

Domestic exports of almonds are mainly concentrated in Latin America, with Brazil, Argentina, Venezuela and Mexico as the most important markets. The Brazilian market suffered a significant drop of 21.7% in its volume imported from Chile in 2013, after experiencing continued growth in the previous 4 years. Argentina is recovering from the low demand in 2012, after 3 years of growth that led to nearly double imports of Chilean almonds in the period. The Venezuelan market, despite the difficulties faced by the country's economy, has maintained relatively stable levels of imports over the past 3 years, with about 1500 tons of Chilean almonds. The Mexican market showed a sharp drop in 2012–13, reducing its imports by two-thirds from the level recorded in 2009–2011 (Bravo, 2014).

4.4 Almonds in South Africa

The almond industry in South Africa is very small compared to Australia, Argentina and Chile, and not well documented. Approximately 125 ha have been established in the Western Cape by fewer than ten growers, and one of these orchards extends to 110 ha. Essentially only one commercially successful farm is in operation, producing approximately 81 tonnes (t) of kernel per year (D. van Rooyen, Montagu, Western

Cape, 2014, pers. comm.). A large company is expected to plant 200 ha in the near future. Horticultural nurseries offer several cultivars including 'Butte', 'Carmel', 'El Fahem', 'Ferragnès', 'I.X.L.', 'Ne Plus Ultra', 'Nonpareil', 'Paper shell', 'Peerless', 'Price', 'Texas' ('Mission') and 'Volcani 5'. Demand in South Africa for local almonds, and nuts in general, currently outstrips production, and packaging companies are urging farmers

to grow almonds. Imported almonds are mainly sourced from the USA (1859 t) and Australia (207 t kernel, 10 t in-shell). Areas such as the Western Cape are ideal for almond production; however, the lack of processing plants and low volume of nuts to supply the critical volume needed to warrant investing in processing facilities has resulted in a failure to expand the nut industry.

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5 Classical Genetics and Breeding

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5.1 Introduction

The cultivated almond was, along with olive, grape, fig and date, one of the first fruit trees to be domesticated in the Old World (Zohary and Hopf, 2000). Almond is well adapted to mild winter and dry, hot summer conditions, because of its low chilling requirements allowing early bloom and early shoot growth as well as a high tolerance to summer heat, drought and calcareous soils. These conditions are typical of the Mediterranean environment where the crop has been commercially developed. Almonds, whether wild, in cultivated orchards or as feral seedlings, are frequently found in Asia and the Mediterranean areas and have been an important food source for thousands of years. Within each region, the best local seedlings were selected by growers for propagation. Because almond, like the ancestor of all *Prunus*, is originally self-incompatible, it requires cross-pollination, which further acts to promote genetic variability, and so increases adaptability to new environments. The self-incompatible nature of almond also encouraged a continuous genetic exchange between cultivated and wild germplasm (Arús *et al.*, 2009; Fernández i Martí *et al.*, 2015). Clonal propagation in almond by grafting has been known since early Greek and

Roman times dating back to more than 2000 years ago (Columella, 1st century CE/1988).

Early domestication efforts involved selection for sweet and large kernel types. Subsequent selection concerned greater productivity, including tolerance to adverse growing conditions (frost, drought and diseases, etc.). Since wild almonds were harvested for food, the genotypes showing superior performance in each growing area would be identified and propagated. As a food commodity, almond nuts are easily handled and stored, allowing transport over long distances. More than 2000 years of continuous culture in the Mediterranean basin concentrated almond into specific regions where unique seedling ecotypes and local cultivars evolved (Grasselly, 1972). Most traditional cultivars in Asia, the Mediterranean and even California and Australia originated as seedlings selected for superior performance at their particular geographical region. Locally adapted seedling populations led to divergence within the traditional producing regions. Currently, a number of indigenous selections uniquely adapted to local climatic and cultural conditions can be found in the different regions of Spain (Ebro Valley, Levant, Andalusia and Balearic Islands), France (Provence and Languedoc), Italy (Apulia, Sicily and Sardinia),

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Greece, Portugal (Algarve and Trás os Montes), Morocco, Tunisia, Turkey, etc. The practice of grafting over bitter seedlings within these populations eventually led to selection of local graftwood sources resulting in unique native cultivars. These locally well-adapted genotypes have come to define the market quality standards for each individual region. New and modern cultivars thus have to demonstrate superior performance, such as late flowering, self-compatibility, disease resistance or productivity, while retaining the desired nut characteristics of the standard cultivars to be acceptable to the local market.

By the 19th century, the differentiation of regional Mediterranean selections had advanced to the point that many were recognized as named cultivars by regional nurseries. In Spain and Italy several cultivars were selected within the different production regions, with many still commercially cultivated including 'Marcona' and 'Desmayo Largaeta' in Spain or 'Pizzuta d'Avola' and 'Tuono' in Italy. Growers in Portugal and Greece also developed their own native cultivars. In North Africa, farmers in Tunisia selected locally adapted types, while in Morocco most cultivars grown came from Spain and France. Similarly, most almond cultivars currently grown in Turkey are foreign while almond production in Iran, north-western India and western China use native selections extensively. Many of the Mediterranean cultivars were introduced into California as early as 1843 (Kester and Gradziel, 1996; Gradziel, 2011). Californian almond production initially planted the hard-shelled Spanish genotypes brought by Franciscans to the Missions, though soft-shell types from the Languedoc area in south-eastern France later predominated (Kester and Gradziel, 1996). Almond orchards in Australia were initially planted with seedling selections from nuts brought by early settlers, though Californian cultivars were subsequently adopted (Wirthensohn and Sedgley, 2002). Chile, Argentina and South Africa followed this pattern, ultimately emphasizing soft-shell cultivars in orchard plantings. However, Argentina has recently shifted towards hard-shelled, late-blooming cultivars, primarily from Spain (see Chapter 4).

Research advances in genotyping and phenotyping promise to improve breeding efficiency for desired traits, including productivity, sustainability and nut quality. However, to fully exploit the available or newly created genetic potential will require an integrated genetic, horticultural,

molecular, biochemical and sensory strategy for cultivar improvement.

5.2 Germplasm

Breeders charged with developing improved cultivars strive for access to as wide a gene pool as possible, often including wild relatives. Typically, early breeding programmes were based on local selections as well as germplasm from other growing regions having similar breeding goals. A summary of cultivated almond germplasm available worldwide was published by Monastra and Rapparelli (1997) and recently reviewed by Socias i Company *et al.* (2012). In addition to the considerable genetic variability documented within cultivated almond, a much more extensive diversity has been demonstrated within closely related species (Grasselly, 1976a; Grasselly, 1977; Kester *et al.*, 1990; Socias i Company and Felipe, 1992a; Browicz and Zohary, 1996; Fernández i Martí *et al.*, 2009a; Potter, 2011; Chin *et al.*, 2014; Zaurov *et al.*, 2015). In most European producing regions, the exchange of genetic material among different growing areas has been extensive until recently. Presently, there are major almond germplasm collections in California, France, Iran, Italy, Spain and some countries of the former USSR. In the 1900s, breeding programmes were established in several major producing countries to develop improved cultivars through controlled crosses and subsequent progeny selection.

5.3 Breeding Programmes: Objectives and Achievements

Recent breeding progress has been summarized by Kester *et al.* (1990), Kester and Gradziel (1996), Gradziel (2008) and more recently by Socias i Company *et al.* (2012), Gradziel and Martínez-Gómez (2013) and in the former USSR by Zaurov *et al.* (2015). Worldwide, important almond scion breeding programmes have been active over the past 100 years. In California, breeding started in the 1920s. Breeding is occurring at both public institutions (USDA Parlier and UC Davis, mainly by M. Wood, R. Jones, D. Kester and T. Gradziel) and in private breeding programmes (F. Anderson, N. Bradford, Burchell Nurseries and

Zaiger Genetics). Major objectives include local adaptation, nut quality, self-compatibility, productivity, improved pollinizers for 'Nonpareil' (particularly its early bloom) and resistance to the noninfectious bud failure genetic disorder. Novel clonal selection procedures first developed by D.E. Kester in the 1980s proved successful in developing a foundation source for the commercially important 'Carmel' cultivar which has allowed continued plantings of this otherwise noninfectious-bud-failure-prone pollinizer for late 'Nonpareil' bloom (Kester *et al.*, 2004). Cross-compatible cultivars with good bloom overlap with the early 'Nonpareil' bloom include the cultivars 'Avalon', released in 1999, and 'Winters' released in 2006 (Gradziel *et al.*, 2007). 'Kahl' was released in 1995 as a pollinizer for the later 'Nonpareil' bloom. 'Morley' and 'Savanna' were previously released in 1993 as late- and very late-blooming cultivars, respectively. In 2007, the partially self-compatible 'Sweetheart' cultivar was released as a 'Marcona'-like, premium roasting quality almond with improved resistance to post-harvest pests (Gradziel *et al.*, 2013), while in 2012 the fully self-compatible cultivar 'Independence' was released by Zaiger Genetics.

In the former Soviet Union breeding started in the 1930s at the Nikita Botanical Garden (Yalta, Crimea) by A. Rikhter and A. Yadrov targeting tree hardiness and frost resistance, as well as late bloom and productivity. Many cultivars were released from this programme, which were mainly grown in the different Soviet republics (Rikhter, 1972). Some, such as 'Yaltinskij', 'Primorskij' and 'Myagkoskolupij', were also of interest in Mediterranean regions due to their very late bloom time, and several were used as parents in regional breeding programmes. Other breeding efforts in the former USSR were made at the Schroeder Research Institute in Uzbekistan, and large *Prunus* germplasm collections were made at Tashkent, Turkmenistan, as summarized by Zaurov *et al.* (2015).

In the 1960s breeding work started at INRA Bordeaux, followed later at Montfavet, France, by Ch. Grasselly and subsequently by H. Duval in the 1990s, aiming at late bloom, self-compatibility, high production, disease resistance and good kernel quality. The French breeding programme was the most successful programme in Europe for many years, with the successful introduction of 'Ferragnès' and 'Ferraduel' in 1967, and the late-blooming self-incompatible cultivars 'Ferralise'

and 'Ferrastar' in the late 1970s (Grasselly and Crossa-Raynaud, 1980). The 1989 releases 'Lauranne' and 'Steliette' were self-compatible (Grasselly *et al.*, 1992), with 'Lauranne' being particularly successful in France (Duval and Grasselly, 1994) and also in Spain. A more recent release from this programme has been 'Mandoline' (Duval, 1999).

At about the same time breeding work began in Israel at the Volcani Center, ARO, Bet Dagan by P. Spiegel-Roy and J. Kochba and subsequently continued by D. Holland, targeting improved kernel quality and local adaptation. In 1985, self-incompatible 'Kochba', derived from 'Nonpareil', was released (Spiegel-Roy, 1985). A later release from this programme was 'Shefa', which is a self-incompatible seedling of 'Tuono' characterized by a very large nut (Holland *et al.*, 2006). The most recent release has been the self-compatible 'Matan', from the cross 'Lauranne' × 'Um El Fahem', characterized by a large nut (Holland *et al.*, 2016).

In the 1970s a large and successful breeding effort started in Spain involving different public institutions in three regions: CITA of Aragón (A. Felipe and R. Socias i Company), IRTA (Institut de Recerca i Tecnologia Agroalimentàries) Catalonia (F. Vargas, M. Romero and I. Batlle) and CEBAS-CSIC Murcia (E. García, J. Egea and F. Dicenta). The main Spanish breeding objectives were similar: late blooming, self-compatibility, disease resistance and nut quality. The three programmes utilized mainly Spanish, French, Italian and Russian cultivars.

The first self-compatible releases from a breeding programme were 'Guara', 'Aylés' and 'Moncayo' (Felipe and Socias i Company, 1987) from the Zaragoza breeding programme. 'Moncayo', while showing self-compatibility in laboratory conditions, was subsequently shown by Kodad and Socias i Company (2008c) to be self-incompatible in the field. Consequently, most new releases from Zaragoza are self-compatible cultivars including 'Blanquerna', 'Cambra' and 'Felisia' (Socias i Company and Felipe, 1999). 'Felisia' also incorporated the late-blooming trait for avoiding spring frost damage. In 2006, 'Belona' and 'Soleta' were released as self-compatible cultivars with improved kernel quality (Socias i Company and Felipe, 2007). In 2008, 'Mardía' (Socias i Company *et al.*, 2008b) and in 2015 'Vialfas' (Socias i Company *et al.*, 2015) were released as extra-late-blooming cultivars.

The breeding programme from IRTA (Constantí, Spain) placed greater emphasis on improved kernel quality and late bloom, though self-compatibility remained an important objective. The first late-flowering cultivars released were 'Masbovera', 'Glorieta' and 'Francolí' (Vargas and Romero, 1994). 'Masbovera' was the most successful of the three despite being self-incompatible, unlike the self-compatible 'Francolí' (López *et al.*, 2005a). Two of the four 2006 releases ('Marinada' and 'Tarraco') further delayed bloom time, with the self-compatible cultivars 'Constantí', 'Marinada' and 'Vairo', and the self-incompatible 'Tarraco' (Vargas *et al.*, 2008). 'Vairo' is the most widely and successfully planted.

The breeding programme from CEBAS-CSIC (Murcia, Spain) has its main objectives based on self-compatibility and late bloom time. The first releases in the late 1990s were the self-compatible 'Antoñeta' and 'Marta' (Egea *et al.*, 2000), which resulted in increased plantings in Spanish orchards. Two more recent cultivars, 'Penta' and 'Tardona', are characterized by their self-compatibility and extremely late blooming time (Dicenta *et al.*, 2009). 'Tardona' seems to be the latest flowering cultivar ever released.

Scion breeding activity was also initiated in the 1970s, lasting 20 years, in Italy (E. Monastra in ISF Rome), in Greece (D.K. Stylianides and G. Syrgianidis at Naooussa) and in Tunisia (A. El Gharbi and B. Jraidi at INRAT Ariana), all with similar objectives of late flowering, self-compatibility and good kernel quality, but having no releases as far as we know, apart from 'Supernova' (syn. 'Tuono') (Marchese *et al.*, 2008). In Australia (University of Adelaide-Almond Board of Australia, M. Wirthensohn) a mixed public-private breeding programme started in the early 2000s aiming at high productivity, self-compatibility, kernel quality and drought tolerance (Wirthensohn and Sedgley, 2002). This programme is using both hard-shell cultivars from Mediterranean countries as well as soft-shell cultivars from the USA and Australia. Some advanced promising selections are currently under final assessment in trials. More recently (Acar *et al.*, 2014), a breeding programme in Turkey aiming at late flowering and self-compatibility, crossing local and Mediterranean cultivars, has been initiated jointly at the Gaziantep Research Station

and the universities of Harran and Kahramanmaraş. A large number of cultivar and germplasm evaluations took place in other countries, including: Bulgaria, Romania, Tunisia, Morocco, Iran and India, but most were discontinued before having significant cultivar releases. Advances in research, such as pioneering work on self-compatibility by Almeida in Portugal in the 1940s and A. Godini at the University of Bari, Italy, in the 1980s were, however, accomplished. Some recent breeding achievements are summarized in Table 5.1.

Almond cultivars can be characterized for different qualities: agronomic, commercial, industrial, sensory and nutritional (Socias i Company *et al.*, 1998). Thus, from a breeding perspective, cultivars have to fulfil many requirements to be successful, even when they are targeted for a single purpose. Quality in almonds is largely established through market acceptance (Socias i Company *et al.*, 2008a; Romero, 2014). Differences in shell and kernel characteristics can create problems because of the need for mass handling and product uniformity. Marketing categories can affect the price received by growers for their crop. An objective of future breeding efforts is combining good nut and kernel quality with good agronomic characteristics.

The breeding objectives in each region have evolved and vary according to their specific environmental and industrial needs (Table 5.2). In Russia the breeding goals are hardiness and frost resistance, late bloom and productivity. In the Mediterranean countries that are based on hard-shell cultivars, breeding efforts are focused on late flowering, self-compatibility, producing capacity, tolerance to fungal diseases and (more recently) on nut quality. In California, which is based on soft-shell cultivars, the goals mainly focus on good agronomic features combined with nut characteristics similar to the main grown cultivar, 'Nonpareil'.

Over recent decades, the release of improved cultivars has been reported in various countries, including Russia and the former Soviet republics ('Primorskij', 'Nikitskij 62', 'Yaltinskij' and others), France ('Ferragnès', 'Ferraduel', 'Lauranne' and 'Mandaline'), Greece ('Retsou', 'Phyllis' and others), Spain ('Guara', 'Masbovera', 'Glorieta', 'Francolí', 'Vairo', 'Marinada', 'Constantí', 'Antoñeta', 'Marta', 'Penta', 'Soleta',

Table 5.1. Origin and main characteristics of new almond cultivars (released in the past 10 years).

Cultivar	Origin	Some characteristics
Israel. Agricultural Research Organization (Bet Dagan and Newe Ya'ar)		
'Shefa'	'Tuono' × '73' (a local cross)	SI, early blooming, vigorous, highly adapted to Israel conditions, early ripening, soft shell, very large kernel
'Matan'	'Lauranne' × 'Um El Fahem'	SC, medium blooming, vigorous, moderately spreading, early ripening, semi-hard shell, large kernel
Spain. CEBAS-CSIC (Murcia)		
'Penta'	'S5133' × 'Lauranne'	SC, extremely late blooming, hard shell, intermediate vigour, early ripening, very high yield potential, hard shell, medium kernel, outstanding kernel composition
'Tardona'	'S5133' × 'R1000'	SC. The latest blooming cultivar, hard shell, intermediate vigour, intermediate ripening, small kernel
Spain. CITA (Zaragoza)		
'Belona'	'Blanquerna' × 'Belle d'Aurons'	SC, late blooming, medium vigour, early ripening, hard shell, large round kernel with an outstanding composition
'Mardía'	'Felsia' × 'Bertina'	SC, extremely late blooming, vigorous, early-medium ripening, hard shell, medium kernel
'Soleta'	'Blanquerna' × 'Belle d'Aurons'	SC, late blooming, vigorous, late ripening, hard shell, large kernel with an outstanding performance when roasted
'Vialfas'	'Felsia' × 'Bertina'	SC, very late blooming, vigorous, early ripening, hard shell, average kernel with a high oleic acid content
Spain. IRTA Mas de Bover (Constantí, Tarragona)		
'Constantí'	'FGFD2' (('Ferragnès' × 'Ferraduel') × OP	SC, late blooming, vigorous, very easy training and pruning, mid-ripening, hard shell, medium kernel
'Marinada'	'Lauranne' × 'Glorieta'	SC, very late blooming, mid-low vigour, very easy training and pruning, very early bearing, high yield potential, mid-late ripening, hard shell, medium kernel
'Tarraco'	'FLTU18' ('Ferralise' × 'Tuono') × 'Anxaneta'	SI, very late blooming, mid-low vigour, very easy training and pruning, mid-late ripening, hard shell, large kernel
'Vairo'	'4-665' ('Primorskiy' × 'Cristomorto') × 'Lauranne'	SC, late blooming, high vigour, very easy training and pruning, very high yield potential, early ripening, hard shell, medium kernel
USA. University of California (Davis)		
'Sweetheart'	'SB3, 54-39E' (('Lukens Honey' peach × 'Mission') × 'Nonpareil') × Sel 25-26	Partially SC (moderate levels of SC), mid-blooming, moderate vigour, harvest 10–14 days after 'Nonpareil', resistance to post-harvest worm damage, semi-soft shell, well-sealed shell, medium-large 'Marcona'-type kernel, high kernel oil and roasting quality
'Winters'	'3-1' ('Peerless' × 'Harpareil') × '6-27' ('Nonpareil' × 'Jordanolo')	SI (moderate levels of SC), early blooming, good bloom overlap with early 'Nonpareil' bloom, vigorous, upright, harvest 3–4 weeks after 'Nonpareil', paper shell, smaller kernels than 'Nonpareil'
USA. Zaiger's INC Genetics (Modesto)		
'Independence'	'All in one' × Almond selection 2168	SC, mid-blooming (with 'Nonpareil'), upright to moderately spreading, a prolific bloomer and excellent producer, harvest 2–3 days before Nonpareil, large kernels of light colour and high quality

OP: open pollinated; SC: self-compatible; SI: self-incompatible

Table 5.2. Classification of almond cultivars by shelling percentage (SP) and shell types. (Adapted from Socias i Company *et al.*, 2008b.)

Country	Very hard SP: < 30%	Hard SP: 30–40%	Semi-soft SP: 40–50%	Soft SP: 50–60%	Paper SP: > 60%
Australia			'Chellaston', 'Johnson's Prolific' 'Parkinson', 'Somerton'		
France	'Bartre', 'Ferraduel', 'Ferrastar', 'Flour en Bas', 'Tardive de la Verdière'	'Belle d'Aurons', 'Ferragnès', 'Mandoline', 'Lauranne'	'Aï', 'Fournat de Brézénau', 'Steliette'	'Princesse'	'Ardècheoise', 'Languedoc'
Greece	'Drepanoto'			'Distomou', 'Retsou'	
Israel		'Dagan'	'Greek' 'Kochba'	'Solo'	'Hanadiv' 'Shefa'
Italy	'Cristomorto', 'Fascionello', 'Montrone', 'Pizzuta d'Avola'	'Falsa Barese', 'Filippo Ceo', 'Fragiulio', 'Genco', 'Rana', 'Tuono'		'Cavaliera'	
Spain	'Atocha', 'Bertina', 'Constantí', 'Cambra', 'Desmayo Langueta', 'Esperanza', 'Garrigues', 'Glorieta', 'Marcona', 'Mardía', 'Masbovera', 'Moncayo', 'Planeta', 'Ramillete', 'Tardona', 'Vairo', 'Vialfas'	'Antoñeta', 'Aylés', 'Belona', 'Blanquerna', 'Colora- da', 'Felisia', 'Francolí', 'Tuono', 'Marinada', 'Marta', 'Penta', 'Rumbe- ta', 'Soleta', 'Tarraco'	'Rof'	'Mollar de Tarragona'	'Mollar de la Princesa'
Portugal	'Casanova', 'José Dias', 'Parada'				'Haf'
Tunisia			'Achaak', 'Blanco', 'Khoukhi', 'Ksonini', 'Zaaf'	'Heuch'	
Ukraine and former USSR		'Nikitskiy 62'	'Krimskiy', 'Sovietskiy', 'Yaltenskiy'	'Desertniy', 'Miagkoskorloupiy', 'Picantilli', 'Prianiy', 'Primorskiy'	
USA		'Peerless'	'Butte', 'Drake', 'Mission', 'Padre', 'Wawona'	'Carmel', 'I.X.L.', 'Ne Plus Ultra', 'Titan', 'Vesta'	'Jordanolo', 'Nonpareil', 'Sonora', 'Tardy', 'Nonpareil', 'Winters', 'Sweetheart'

'Belona', 'Mardía', 'Vialfas' and others), USA ('Avalon', 'Butte', 'Carmel', 'Padre', 'Price', 'Sonora', 'Sweetheart', 'Winters', 'Independence', 'Kester' and others) and Israel ('Shefa', 'Um El Fahem' and 'Matan'). In addition, scion breeding activities have been ongoing in Italy and Tunisia and, more recently, in Iran and Turkey.

5.4 Genetics

5.4.1 Ploidy and genome size

Almond is a diploid species. The chromosome number of the cultivated almond *Prunus dulcis* is $2n = 16$ (Darlington, 1930; Grasselly, 1977; Corredor *et al.*, 2004), which is the same as in many other *Prunus* species including peach (*P. persica*), and its wild relatives *P. davidiana* and *P. mira*. Almond genome has a small size (~300 Mbp), which is approximately twice that of *Arabidopsis* (Arumuganathan and Earle, 1991). Almond cultivars with reduced fertility associated with chromosome abnormalities have been described (Almeida, 1945). Recently, studies have shown several types of changes in chromosomes, both genetic (deletions, point mutations, etc.) and chromosomal, including aneuploidy (Martínez-Gómez and Gradziel, 2003), translocations (Jáuregui *et al.*, 2001) and epigenetic (gene modification/activation/silencing, etc.) (Martínez-Gómez *et al.*, 2005; Fernández i Martí *et al.*, 2014).

5.4.2 Qualitative characters and their inheritance

The cultivated almond is a variable, heterozygous group of both genetically non-related and related cultivars. The resultant genotypic and phenotypic variability represents large opportunities for future cultivar improvement. Only a few important horticultural traits such as kernel bitterness have so far been identified as controlled through major single genes. Self-compatibility, late bloom and shell hardness are under control of major genes but quantitative genes also appear to be involved. Well-documented and extensive reviews of almond characteristics have been reported (Kester and Gradziel, 1996;

Socias i Company, 1998; Gradziel, 2008; Socias i Company *et al.*, 2008a; Socias i Company *et al.*, 2012). In this discussion, only major characters like self-incompatibility and kernel bitterness are considered. Self-incompatibility is only weakly summarized as it is more extensively presented in Chapter 8. Flowering is considered both a qualitative and quantitative trait and it is also included in Chapter 7.

Self-incompatibility

Almond, in common with most *Prunus* species, shows gametophytic self-incompatibility (GSI) (Socias i Company, 1984; Socias i Company, 1990; Dicenta and García, 1993b). The compatibility of a cross is determined by the haploid genome of the pollen and the diploid genome of the pistil. A pollen grain carrying an *S* allele that is also present in the style is recognized as 'self' pollen and its pollen tube growth is inhibited. The specificity of the GSI response is determined by the *S*-locus, which encodes for at least two genes expressed in the pistil (*S*-RNase) and in the pollen (*F*-Box protein) (Kao and Tsukamoto, 2004). Almond cultivars are largely self-incompatible. However, some self-compatible cultivars exist. Some self-compatible cultivars are considered to have originated from interspecific hybrids with self-compatible species, mainly *P. webbii* and *P. persica*. Breeding for self-compatibility is a major aim in most scion improvement programmes. More than 30 *S* alleles in almond have been identified in the *S*-locus and cross-incompatibility groups have been established (Kester and Asay, 1975; Crossa-Raynaud and Grasselly, 1985; Kester *et al.*, 1994; Bošković *et al.*, 2003; Ortega *et al.*, 2005; López *et al.*, 2006; Kodad and Socias i Company, 2009). Self-incompatibility in almond is a monogenic character with a multi-allelic *S* series, involving expression of the self-compatible allele (*S_i*) dominant over the others (Socias i Company, 1984; Bošković *et al.*, 1997; Tao *et al.*, 1997; Bošković *et al.*, 1998; Bošković *et al.*, 2003; Kodad *et al.*, 2015). The use of a few self-compatible cultivars from Apulia, Italy, (mainly 'Tuono' but also 'Genco', 'Falsa Barese', etc.) to breed this trait into new European cultivars has caused some loss of genetic variability in most Mediterranean breeding programmes (Lansari *et al.*, 1994b; Socias i Company, 2002), leading to inbreeding depression and reduced expression and

transmission of self-compatibility (Alonso and Socias i Company, 2005; Alonso and Socias i Company, 2007). Transmission of self-compatibility is reported to be monogenic (Socias i Company, 1984) although, in some cases, modifier genes have been proposed (Fernández i Martí *et al.*, 2009b). A full account of this important breeding trait is provided in Chapter 8.

Kernel bitterness

Wild almond kernels (and leaves) tend to be bitter and the sweet flavour appears to be a mutation from an originally bitter almond (Grasselly, 1976a, b). Bitterness in almond was identified as a single recessive gene designated S_k (Heppner, 1923; Heppner, 1926; Dicenta and García, 1993a). Thus, a bitter almond has a genotype $s_k s_k$. Several almond cultivars are heterozygous for bitter taste $S_k s_k$, but a number of homozygous sweet $S_k S_k$ have been identified through breeding tests (Grasselly and Crossa-Raynaud, 1980; Dicenta and García, 1993a; Vargas *et al.*, 2001; Dicenta *et al.*, 2007). When two heterozygous cultivars $S_k s_k$ are crossed, 25% of the seedlings are expected to be bitter (Dicenta *et al.*, 1993a; Sánchez-Pérez *et al.*, 2007a).

The main trend under almond domestication was selection of types with sweet, non-poisonous seed. Most cultivated almonds produce sweet kernels, but some have a slightly bitter flavour. A mild bitter flavour can be detected in some cultivars and can be considered pleasing in some special confectioneries (see Chapter 19). Producing sweet kernels is essential for commercial cultivars. Characterization of almond flavour phenotypes through sensory analysis has been done (Wirthensohn *et al.*, 2008) and is routinely carried out in breeding programmes.

The bitter taste is due to the presence of the glucoside amygdaline, which is the end product of a metabolic pathway including prunasin and mandelonitrile as precursors (Sánchez-Pérez *et al.*, 2008). Monoglucoside prunasin is present in most vegetative organs of all almonds (sweet or bitter kernelled), but only those that are bitter kernelled are able to accumulate it in the mature seed, in the form of the diglucoside amygdalin (Dicenta *et al.*, 2002).

The sweet or bitter kernel taste depends on the cultivar, so all the fruits of each cultivar will

be sweet or bitter, regardless of the genotype of the pollen parent. The genetic influence of both parents will be shown in the following generation, so pollination with sweet or bitter pollen does not modify the sweetness or bitterness of the seeds of each cultivar (Dicenta *et al.*, 2000; Sánchez-Pérez *et al.*, 2012). Although this trait was located in linkage group 5 (Joobeur *et al.*, 1998; Sánchez-Pérez *et al.*, 2007b), the metabolic process that regulates this gene remains unknown. Sánchez-Pérez *et al.* (2010) determined the haplotype of 16 cultivars for four molecular markers very close to the bitterness gene allowing marker-assisted selection (MAS) for this trait. Later Sánchez-Pérez *et al.* (2008) and Sánchez-Pérez *et al.* (2012) showed the role of the prunasin hydrolases and of their location in tissues of the seed tegument, and their probable relationship with the sweet or bitter flavour of kernels. Finally Koepeke *et al.* (2013), by comparing sweet and bitter almond sequences, identified a set of candidate genes associated with bitterness in almond.

5.4.3 Quantitative characters

Most important horticultural traits including yield, blooming time, ripening time, nut size, shell hardness, shelling percentage, kernel size, kernel shape, double kernel, kernel quality, time of leafing, tree size and growth habit are largely quantitatively controlled. Early research (Kester, 1965a, b; Grasselly, 1972; Kester and Asay, 1975; Gülcan, 1977; Kester *et al.*, 1977a, b; Spiegel-Roy and Kochba, 1977; Spiegel-Roy and Kochba, 1981; Vargas and Romero, 1988; Socias i Company, 1998; Sánchez-Pérez *et al.*, 2007b) indicates that genetic control is largely additive. Phenotypic and genotypic heritabilities of some of these traits are relatively high (Kester *et al.*, 1977b; Dicenta *et al.*, 1993a, b; Arteaga and Socias i Company, 2002; Sarvisé and Socias i Company, 2005; Font i Forcada *et al.*, 2011). The considerable environmental effects that occur on phenotypic measurements of these traits, both among the different seasons and among different locations, can be minimized by the appropriate use of statistical techniques. Reported heritabilities for some important quantitative traits are summarized in Table 5.3.

Table 5.3. Heritability of some quantitative traits in almond. (From Socias and Company *et al.*, 2012.)

Trait	Heritability	Source
Physiological traits		
Blooming time	0.804	Kester <i>et al.</i> (1973)
	0.67	Dicenta <i>et al.</i> (1993b)
Leafing time	0.829	Kester <i>et al.</i> (1973)
Blooming duration	0.20	Dicenta <i>et al.</i> (1993b)
Blooming intensity	0.54	Dicenta <i>et al.</i> (1993b)
Ripening season	0.69	Dicenta <i>et al.</i> (1993b)
Production intensity	0.45	Dicenta <i>et al.</i> (1993b)
Morphological traits		
Bud density	0.30	Sarvisé and Socias i Company (2005)
Branching habit	0.19	Sarvisé and Socias i Company (2005)
Nut and shell traits		
Weight	0.81	Kester <i>et al.</i> (1973)
Length	0.50	Arteaga and Socias i Company (2002)
Width	0.37	Arteaga and Socias i Company (2002)
Thickness	0.28	Arteaga and Socias i Company (2002)
Width/length ratio	0.46	Arteaga and Socias i Company (2002)
Thickness/length ratio	0.53	Arteaga and Socias i Company (2002)
Thickness/width ratio	0.30	Arteaga and Socias i Company (2002)
Shell hardness	0.55	Kester <i>et al.</i> (1977b)
Shell thickness	0.51	Arteaga and Socias i Company (2002)
Kernel traits		
Weight	0.64	Kester <i>et al.</i> (1977b)
Length	0.77	Kester <i>et al.</i> (1977b)
Width	0.62	Kester <i>et al.</i> (1977b)
Thickness	0.71	Kester <i>et al.</i> (1977b)
Width/length ratio	0.46	Arteaga and Socias i Company (2002)
Thickness/length ratio	0.43	Arteaga and Socias i Company (2002)
Thickness/width ratio	0.21	Arteaga and Socias i Company (2002)
Double kernels	0.51	Kester <i>et al.</i> (1977b)
Kernel composition		
Oil	0.57	Font i Forcada <i>et al.</i> (2011)
Palmitic acid	0.15	Font i Forcada <i>et al.</i> (2011)
Palmitoleic acid	0.12	Font i Forcada <i>et al.</i> (2011)
Stearic acid	0.11	Font i Forcada <i>et al.</i> (2011)
Oleic acid	0.11	Font i Forcada <i>et al.</i> (2011)
Linoleic acid	0.25	Font i Forcada <i>et al.</i> (2011)
α -Tocopherol	0.21	Font i Forcada <i>et al.</i> (2011)
γ -Tocopherol	0.60	Font i Forcada <i>et al.</i> (2011)

Characters related to the nut

The almond fruit is a drupe similar to peach and other stone fruits, where the outer carpel layers (exocarp and mesocarp) develop into the skin and the fleshy hull, respectively, while the inner carpel or endocarp develops into the stony shell, which encloses the kernel or seed. Differences in these fruit parts can strongly influence final processing. Immature fruit and kernels can also be consumed fresh before shell

hardening, as is common in several Arab countries (Martínez-Gómez *et al.*, 2008).

Nut hull

The thickness and dry weight of the mature hull can differ significantly among cultivars. Hard-shell types, such as most Mediterranean cultivars, show a more extensive shell lignification than the soft-shelled ones. Greater lignification of the shell results generally in thin and dry hulls

at maturity (Godini, 1984a, b; Socias i Company *et al.*, 2008a; Alonso *et al.*, 2012). In California and other regions the hull serves as an important animal feed, particularly for dairy cattle, and so hull mass is of economic importance (Aguilar *et al.*, 1984).

Shell hardness and percentage of kernel

Hardness is the main genetic trait of the almond shell and is related to the total amount of lignin deposited in the endocarp during nut development and with its final structure. Shell hardness refers to the resistance of shell breaking by hand or using a hammer (subjectively scored) or with a texturometer (objectively scored). Percentage of kernel is a complex trait calculated as the ratio between the dry kernel weight and the whole nut dry weight (kernel plus shell). Both traits, related to the almond nut, are very important for industrial processing. Shell hardness and percentage of kernel are usually related but they are different traits (e.g. there are hard, thin-shelled cultivars with a high percentage of kernel). Largely, as the percentage of kernel becomes higher, shell hardness becomes lower, and vice versa. Due to their high correlation, shell hardness has often been expressed as kernel percentage.

Almond shells are generally characterized by their hardness (from extremely hard to paper soft) and percentage of kernel, which shows wide variation from 15% in 'Bartre' to 65% in 'Nonpareil'. Five classes were established to designate shell hardness (Kester and Asay, 1975; Gülcan, 1985; Kester and Gradziel, 1996). We have also considered five classes according to shelling percentage (SP): very hard (SP: < 30%), hard (SP: 30–40%), semi-soft (SP: 40–50%), soft (SP: 50–60%) and paper (SP: > 60%) (Table 5.2). Both traits have an important genetic determinism, present continuous variation and are affected by environment (growing conditions, year, location, etc.), a usual characteristic of quantitative traits.

Grasselly (1972) reported that shell hardness (measured as percentage of kernel) was controlled by one major gene with two alleles: *D* = very hard shell (SP of 15–35%), and *d* = soft (shelling 35–65%). Allele *D* ('dure' is French for hard) is dominant over *d*. Considerable phenotypic variation occurs among individuals of the *dd* genotype, where the SP typically ranges from

about 40% to as high as 80%. Later, Grasselly and Crossa-Raynaud (1980) suggested that the inheritance of the shell type seems to be qualitative for very hard-shell and paper-shell cultivars, but it is more complex when crossing semi-soft cultivars. Also, results in progenies from crosses of 'Nonpareil' (paper) × 'Mission' (syn. 'Texas') (semi-soft) suggested that the trait tends to be intermediate to their parents, typical of quantitative traits controlled by additive effects (Kester *et al.*, 1977b). Kester and Gradziel (1996) reported that both the hard (*D*) and soft (*dd*) genotypes are affected by a range of modifier genes, which are associated with different degrees of hardness.

Ballester (1998) reported two quantitative trait loci (QTLs) associated with SP: one major on LG2 (responsible for c.80% of the observed phenotypic variation) and another minor on LG8 (responsible for c.20% of the observed variation). Shell hardness, measured as SP or by shell hardness, respectively, is considered a putative gene and was reported to be located on the almond linkage group 2 (Arús *et al.*, 1998; Sánchez-Pérez *et al.*, 2007b).

Grasselly (1972) and Ballester (1998) analysed data of kernel percentages and obtained nice frequency histograms with variable distributions between both parents, typical of quantitative traits. They also showed distributions that could fit a bimodal curve. This may be due to the low number of seedlings analysed or to the presence of some major gene. Dicenta *et al.* (1993a), studying the kernel percentage in 53 families over 2 years, observed that this trait is polygenic, and thus it shows a quantitative behaviour with continuous variation between both genitors. In fact, heritability of shell type was estimated at 0.55 (Kester *et al.*, 1977b), 0.82 (Spiegel-Roy and Kochba, 1981) and 0.56, but only 0.19 using variance calculations, indicating a sizable environmental effect (Dicenta *et al.*, 1993a).

The shell consists of an outer layer and an inner layer separated by channels through which vascular vessels develop. The shell is thus not a uniform structure. As the hull dehisces and separates from the nut, the outer layer may remain attached to the hull and separate from the inner layer. This can happen in both types of shell cultivars, but is more common in paper-shell cultivars where it can be associated with poor seal. Within the soft-shell group, the heritability of

retention of the outer shell estimate is 0.34 (Kester *et al.*, 1977b), indicating considerable environmental effect specific of quantitative traits. The heritability of the double layer shell is also low (0.37) (Arteaga and Socias i Company, 2002).

Commercial shelling is different for hard-shelled and soft-shelled cultivars, since shelling ease is different. In the Mediterranean region, where hard-shelled cultivars largely predominate, when semi-soft-shell cultivars are shelled with the sheller adapted to hard-shell cultivars problems often arise (similarly in California or Australia when shelling hard-shelled nuts). For example, in Mediterranean cracking plants when mechanically shelling nuts from 'Ferragnès', or from semi-soft cultivars, the outer layer is often broken, but not the inner layer, requiring repeated shelling with a smaller nut gap. Re-adjusting the sheller for resultant smaller nut sizes, however, results in a higher proportion of kernels being broken, which leads to a lower value for the product (Grasselly and Crossa-Raynaud, 1980; Felipe, 2000; Socias i Company *et al.*, 2008a). Hard shells can reduce the proportion of broken kernels recovered after shelling if adequate equipment is used. Also, in some hard shell types, when trees are young and growing well the outer layer can separate from the inner layer. Similarly, in California, the standard soft-shell shoulders are not effective on hard-shelled European-type nuts.

Poorly sealed shells and poorly retained outer corky layers are also more common in high SP soft, or paper shells. In addition, distinct industries have developed different shellers and machinery based on the nut shell types produced in each growing region. In the Mediterranean basin, most cultivars are hard-shelled and the shelling plants are designed for cracking these types. In California and southern hemisphere growing regions (Australia, Argentina, Chile and South Africa), plants process mainly soft-shelled almonds.

The almond fruit basically includes kernel, shell and hull. When comparing hard and soft-shelled almonds, the total dry weight of kernel, shell and hull is rather similar. Thus, the plant resources invested in developing the nuts are also similar (Godini, 1984a, b; Alonso *et al.*, 2012). Commercial crack-out refers to the percentage of edible kernels in a sample of given weight and is particularly useful in determining yield of

marketable nuts. Hard-shelled cultivars are an advantage where insect or bird attacks in the orchard are a risk (see Chapter 15).

The shell type can strongly influence the occurrence of post-harvest infestation by navel orangeworm (*Amyelois transitella*), and thus to aflatoxin contamination, which is potentially more dangerous than direct damage by the worm. Infection of nuts by moulds (mainly *Aspergillus*) is associated with worm damage and this is directly related to orchard management and to nut shell hardness, shell sealing and nut handling after harvest. Thus well-sealed soft-shell nuts are less affected by navel orangeworm than unsealed ones. Hard-shelled nuts are less prone to navel orangeworm damage and thus to aflatoxin contamination. The recent California release 'Sweetheart' is a soft-shell cultivar but has well-sealed nuts (Gradziel *et al.*, 2013), which aids in reducing this insect and associated mould damage.

Kernel (shape, size and weight)

Nut shape is consistent among different genotypes and so is characteristic of different cultivars (Kodad and Socias i Company, 2006). There are a number of different kernel shapes. Five nut shape classes are generally considered: round, ovate, oblong, cordate and extremely narrow (Gülcan, 1985). Kernel size can be expressed by linear dimensions of length, width and thickness (Kester, 1965a). These parameters are established during the first growth phase of fruit development, in the spring, and are completed by late spring and early summer in Mediterranean countries (late May in California) (Martínez-Gómez *et al.*, 2008). There is a strong environmental and seasonal effect on size, including crop load, tree vigour, soil moisture and weather patterns. Crop load has a strong inverse relationship to average size, which needs to be taken into account in evaluations, although much less than in other fruit species (Godini, 2002). Genetic analyses (Kester *et al.*, 1977b) have produced heritability estimates of 0.77 for kernel length, 0.62 for kernel width and 0.71 for kernel thickness.

Kernel size and weight have an inverse relationship to the number of nuts on the tree (Hill *et al.*, 1987). Average kernel weight is not a major parameter of yield. Cultivar 'Butte' is very

productive and has a small kernel size; the opposite happens with 'Bartre', which has a large kernel. Individual kernel size is established during the first stage of fruit growth. Weight is determined in the last accumulative phase (during spring) of almond nut growth and increases continuously until maturity (Labavitch, 1978). Despite these sources of variation, kernel weight has a high heritability and appears additive. Kester *et al.* (1977b) calculated heritability of 0.64, while Yadrov (1993) reported 0.78. Dicenta *et al.* (1993a) calculated heritabilities of 0.71 (regression) and 0.44 (variance). Spiegel-Roy and Kochba (1977) reported similar results.

Industrially, kernels are screened through round-holed screens of different dimensions, to segregate them into different size categories, which can be correlated within cultivars to give kernel weights (Kester, 1965a; Grasselly and Crossa-Raynaud, 1980). Additional screening through slot hole (thickness) screens can separate nuts of different thickness if further refinement is desired. Commercial size grades are obtained by mechanical screens that range from 16/18 (17–18 mm) to 45/50 (8–9 mm) kernels/per ounce, respectively. Different almond sizes have different uses. Almond kernels of different sizes also vary in their roasting and processing requirements. Very large-sized kernels often bring increased market prices but appear associated with lower final tree yields (Gradziel and Lampinen, 2011). These authors also considered that at very high yields of 5–6 t/ha of kernel, cultivars showed kernel sizes of c.1 g, indicating a possible optimum size for maximizing tree productivity. Different almond cultivars can be grouped in relation to their average kernel size. At IRTA's almond germplasm collection, kernel weight varies from 0.84 g to 2.37 g. Most commercial cultivars in California had kernel sizes between 0.8 and 1.6 g (Gradziel and Lampinen, 2011). Kernels of more than 1.5 g are considered large, while those weighing less than 1.0 g are considered small. Almond quality is related to industrial purposes. Almond breeding programmes should include industrial aptitude (physical and chemical traits) testing before releasing (Kodad *et al.*, 2005; Kodad *et al.*, 2006; Socias i Company *et al.*, 2008a; Socias i Company *et al.*, 2009; Kodad *et al.*, 2011; Romero *et al.*, 2011a, b; Socias i Company *et al.*, 2010b; Romero, 2014).

Shell and kernel colour

Shell colour intensity has been classified by Gülcán (1985) into four classes: extremely light, light, intermediate and dark. A light-coloured shell that can easily be bleached is important for nuts sold in-shell. Kernel colour is an important characteristic, with light colours preferred to darker colours. In the almond industry, 'Non-pareil' is used as the standard for light pellicle skin colour. Almond kernels of different cultivars vary in colour from extremely light to dark. Pigment develops in the pellicle or seed coats during late maturation, so relative lightness or darkness may depend to some extent on maturity stage at harvest. The kernel becomes darker on drying and with age and light exposure (Kester and Asay, 1975). The heritability for seed coat colour is moderately low (0.42), partly because it is strongly influenced by growing environments and also depends on the stage of maturity and the length and type of storage (Kester *et al.*, 1977b; Dicenta *et al.*, 1993a).

Markings on the outer shell are expressed more or less uniquely for individual genotypes, and identify specific cultivars as well as related *Prunus* species, particularly those with hard shells (Kester and Gradziel, 1996). Within almond, the markings or openings (pores) tend to be mostly circular, sometimes elongated and sometimes a mixture of both. For identification, pores may be large or small, many or few with five distinct classes described by Gülcán (1985).

Kernel pellicle and texture

Generally, pellicles or seed coats with a smooth surface texture are preferred. Seed coat mass varies within and particularly between cultivars, showing some level of genetic control but also a large environmental effect (Kodad, 2006). In addition, the seed coat appears to contain a diversity of secondary compounds contributing to both sensory and phytonutrient value and thus its contribution to kernel quality may prove important at a biochemical level (Socias i Company *et al.*, 2008a). The seed coat represents from 5% to 10% of total kernel weight, with a lower ratio desirable for blanching to limit weight loss. However, for other processes such as roasting, a thicker or rougher seed coat facilitates coating with salts and flavourings

(Socias i Company *et al.*, 2008a). In cultivars such as 'Desmayo Langueta', a thick tegument eases peeling after roasting and confers special market recognition (Gou *et al.*, 2000).

Kernel surface texture is determined primarily by the seed coat and is affected by both macroscopic and microscopic features. Kernel wrinkling (shrivelling) describes the unevenness of the seed surface and is often associated with differences in vascular bundle sizes within the seed coat (Kester and Gradziel, 1996). It can be assessed by cutting the kernels crosswise and viewing the cut end. Wrinkling is characteristic of certain cultivars but can be affected by environmental and management factors such as water stress. Also, some cultivars produce a crease or deep depression on the flat side of the kernel. This seed 'creasing' has a high heritability (0.79) (Kester *et al.*, 1977b) and is usually undesirable. The seed coat of many almonds appears pubescent, which refers to waxy compounds on the surface of the pellicle (smoothness), and is not true pubescence or hairiness (Kester and Gradziel, 1996; Socias i Company *et al.*, 2008a). It can be detected visually or by feel. Four categories have been described by Gülcan (1985) for kernel pubescence. This characteristic is highly specific to cultivar and selection can be highly effective (Kester and Asay, 1975). Cultivars showing darker kernel colour and greater seed pubescence are less fitting for nuts to be consumed raw. A desirable almond kernel texture is difficult to define. Sterling and Simone (1954) showed that significant differences existed among cultivars in 'fragmentability' (ability to break into small pieces) and 'hardness' or 'crispness' (tendency to break sharply into larger pieces). Aydin (2003) found that the rupture strength of the kernel from 'Taşbaden' decreased with moisture content. Using a texturometer, a range of hardness has been characterized from 'Nonpareil' (hard) to 'Desmayo Langueta' (soft) using a similar approach, the relation between fragmentability during handling and soft texture has been observed (A. Romero, pers. comm., 2015, Constanti).

Callus and gumming are two common defects associated with certain cultivars. Callus appears as a scabby white to brown layer that sticks to the seedcoat, resulting from callus growth. Gum sometimes develops on the surface of the

kernel and is associated with abortive embryos or seeds with callus. It is common in certain cultivars such as 'Thompson', usually under vigorous growing conditions and reduced crop. Kester *et al.* (1977b) reported a low heritability value of 0.15 for gumming with a 65% genetic $\times$ environmental interaction.

Double kernel

Each almond flower usually has a single ovary which contains two ovules. One or both can develop into a seed. If both develop, two kernels are produced which are usually misshapen and undesirable because of difficulties in the process of shelling, sizing and blanching. Double kernel is therefore considered to be a negative trait, lowering crop value, even though organoleptic quality does not appear to be affected. The tendency to produce only a single, well-formed kernel is thus a highly desirable cultivar trait.

The development of double kernels appears to be a quantitative trait (Grasselly, 1972; Socias i Company and Felipe, 1994). Grasselly and Crossa-Raynaud (1980) indicated a possible dominance effect with strong seasonal differences. Low temperatures before blooming (Egea and Burgos, 1994) or at flowering time (Rikhter, 1969; Spiegel-Roy and Kochba, 1974) have been mentioned as promoting higher percentages of double kernels. Spiegel-Roy and Kochba (1974) demonstrated that heritability was unpredictable because of environmental influence. Kester *et al.* (1977b) estimated a heritability of 0.51, but with a very high standard deviation, and low estimates were reported by Vargas and Romero (1988) and Dicenta *et al.* (1993a). The use of parents with very low doubling seems to be the most effective strategy for minimizing this problem in the progeny. Recent research by Martínez-García *et al.* (2014) using several parental combinations confirmed the complex nature of this quantitatively transmitted trait and concluded that additive, non-additive and genotype $\times$ environment interactions effects are important.

Twin kernel

Twin kernels occur when two or more embryos develop within a single ovary and so are enclosed within the same seed coat. The outlines of

the multiple embryos can be detected through the seed coat and will not affect eating quality but are undesirable in blanching, when removal of the skin can cause the embryos to separate. Seedlings from the same polyembryonic seed are frequently viable, though one of the seedlings often shows weak growth and develops poorly. These dwarf seedlings have previously been considered as haploid but results were inconclusive (Gülcan, 1975). While the trait is more commonly associated with certain genotypes, heritability appears to be very low, probably due to strong environmental influence (Martínez-Gómez *et al.*, 2003a; Socías i Company *et al.*, 2008a). Martínez-Gómez and Gradziel (2003) reported in 'Nonpareil' the percentage of twin embryos showing aberrant phenotypes was approximately 30%, with mortality rates of about 90%. They also observed that many of these aberrant seedling phenotypes appear to be aneuploids, based upon chromosome counts. Most secondary embryos appear to be derived by division from the primary embryo following normal fertilization.

Characters related to the tree

Trees of different almond cultivars vary considerably in vigour, shape, branching pattern and growth habit. These characteristics affect productivity, training and pruning needs, and can affect harvesting operations and orchard management (Kester and Asay, 1975). The combination of all the traits listed above, along with foliage traits, provide characteristic tree phenotypes. Observations by breeders indicate that such phenotypes are heritable and both parental and intermediate types may often be recovered in the progeny. 'Marcona', 'Cristomorto' and 'Primorskij' produced desirable progenies for some of these traits (Vargas *et al.*, 1984; Vargas and Romero, 1988). 'Tuono' shows a distinct tree structure, which in its progeny has been considered undesirable (Grasselly *et al.*, 1992). 'Nonpareil', 'Mission' and 'Ne Plus Ultra' can easily be distinguished by these characteristics (Kester and Gradziel, 1996). However, as breeders, we should consider that for each cropping model (e.g. rainfed or irrigation) as well as each planting density, training, input conditions or harvesting system, the ideal almond tree will differ.

Vigour

Tree size is a relative term that depends not only on the individual almond genotype but also on orchard age, site (climate and soil), management (irrigation, fertilization and pruning) and, in particular, on the type of rootstock used. Sometimes vigour can be influenced by early bearing and productivity. 'Merced' or 'Tarraco' trees slow down in growth with age, partly as a consequence of precocious production, while 'Nonpareil' or 'Vairo' trees tend to maintain vigour, resulting in larger and more productive trees (Kester and Gradziel, 1996, Vargas *et al.*, 2008). Medium to low vigorous, early cropping and productive cultivars like 'Lauranne', 'Belona', 'Soleta' and 'Marinada' can be suitable for high-density orchards. Studies of tree size heritability have not been reported, but vigour is likely to be controlled by quantitative genes (Kester and Gradziel, 1996). Tree size can be assessed by visual observation or by trunk diameter compared to a reference cultivar. More precisely tree size can also be expressed by height and width measurements from which canopy volume can also be calculated. Canopy area is particularly useful in determining optimal orchard spacing and tree density (Kester *et al.*, 1987). According to Gradziel and Lampinen (2011), effective canopy light interception was strongly associated with final almond productivity, as in many other trees. This work showed the importance of both tree architecture and fruit-bearing habit in determining cultivar efficiency for light interception and energy conversion to final kernel yield. From an economic perspective it may be desirable to have either solid blocks of self-compatible cultivars or smaller pollenizer trees interplanted with the highest value cultivar, which will allow more sunlight and so have the crop on a high-value cultivar. Large almond trees with open branch architecture have a clear yield advantage, particularly in young trees (Gradziel and Lampinen, 2011).

Tree growth and bearing habit

Almond trees have distinguishable shapes determined primarily by angle and amount of branching. Gülcan (1985) established five categories for tree habit: extremely upright, upright, spreading, drooping and weeping. Tree vigour and size may

sometimes be related to branching habit, and vigorous trees are often spreading. Tree habit of modern European cultivars is largely spreading, with some exceptions, including 'Constanti', 'Marta', 'Soleta' and 'Tarraco', which are upright and 'Guara', which is weeping. 'Desmayo Langueta' also shows a distinct weeping habit.

Tree growth and branching habit are both important for orchard management, and almond breeders routinely consider them. Branch architecture in seedling almonds was characterized by the degree of lateral branching in current and previous season's growth (Gradziel *et al.*, 2002). In their work progeny distributions demonstrated that targeted and sometimes unique tree

architectures could be readily achieved through appropriate selection of parents.

Tree growth and bearing habit are both related to vegetative growth and flower bud development (Sarvisé and Socias i Company, 2005). As in all *Prunus* species, almond initiates flower buds laterally on the current season's growth, which then bloom and fruit the following year (Fig. 5.1). Almond trees develop fruit laterally on extension shoots and short shoots called spurs on 2-year-old or older wood (Kester and Gradziel, 1996). Spurs are the main fruit-bearing shoot type in mature almond trees (Kester and Gradziel, 1996) and generally remain viable for 3–5 years (Weinbaum and Spiegel-Roy, 1985).

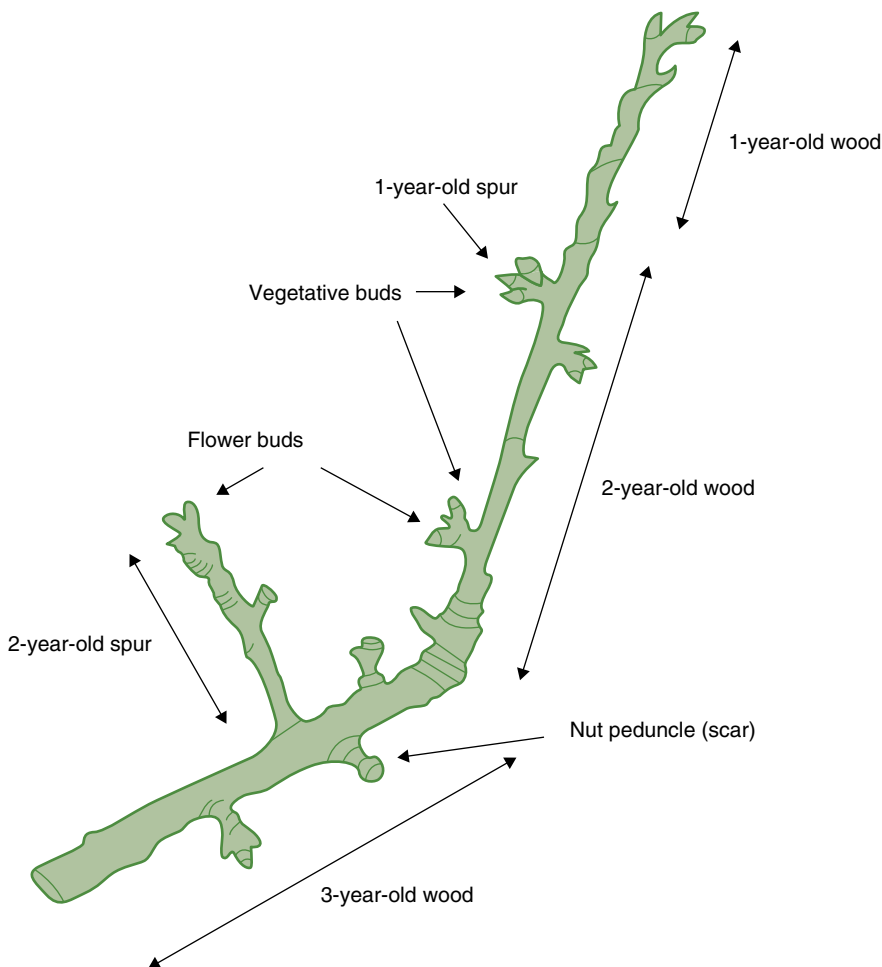


Fig. 5.1. Bearing habit typical of almond, showing flower and spur development on second year and older wood, and woody flowering spurs on third year and older wood. (From Gradziel, 2006.)

The pattern of shoots and spurs along branches is of major importance because this determines tree architecture and cropping potential (Bernad and Socias i Company, 1998). In general, three basic classes of bearing habits can be considered in almond: (i) most flower buds on 1-year-old shoots as in the cultivar 'Ai'; (ii) most flower buds on spurs as in 'Tuono'; and (iii) flowers buds mixed as in 'Nonpareil' and 'Mission' (syn. 'Texas'). However, when cumulative yield is evaluated, cultivars such as 'Mission' – which produce predominantly spur-borne fruit – appear to be at a disadvantage when compared to cultivars such as 'Nonpareil' which primarily produce on spurs but maintain the capacity for shoot production. Additional classes of growth habits can be distinguished based on variations in primary, secondary and tertiary shoot development (Gradziel *et al.*, 2002). Most almond cultivars produce both terminal and lateral bearing units but vary in the proportion of each depending upon genotype, age and growing conditions (Gradziel, 2008). A combination of both bearing habits (mixed, a and b) is considered advantageous. Shoot-bearing habits are associated with precocious production while spur habit increases the bearing surface exponentially. In mature bearing trees, spur production accounted for the majority of effective nut-bearing units contributing to yield, as spurs are more efficient than shoots in balancing fruit renewal with carbohydrate-costly vegetative growth. In addition to tree architecture, the nature of the fruit-bearing unit (shoot versus spur) appears to be a crucial component of final yield potential (Gradziel and Lampinen, 2011). Foliage density is in turn determined by the branching habit and the size and distribution of leaves.

The importance of genetically determined desirable tree architectures will increase in the future as costly cultural management activities such as training and pruning decrease with their reduction and with the trend towards more mechanization and high-input growing conditions. Cultivars which are densely branched are not suitable because of their large pruning costs. In addition, minimal pruning can favour diseases and lack of light inside the canopy, which can reduce production. Cultivars vary in the extent of tree branching, ranging from cultivars such as 'Cristomorto', which are sparsely branched to 'Ai', which is densely branched. Most cultivars

released in Europe tend to be intermediate in tree branching. Spur development and spur renewal, as well as regenerative shoot growth, tree size and architecture represent promising targets for almond breeding.

Foliage

Almond cultivars have characteristic differences in foliage density, leaf size and leaf distribution. Leaf characteristics are also affected by environmental conditions and the general health and vigour of the plant. Foliage density differences can be characterized visually among cultivars or, for more accuracy, the amount of photosynthetically active radiation under the canopy can be measured and compared for different cultivars. Density of foliage is related to leaf size and number, distribution and node closeness, and cultivars are often rated according to a three-grade scale (low, intermediate and dense) (Gülcan, 1985). Leaf size varies with position, with shoot leaves tending to be large and spur leaves small (Gradziel, 2008).

Many wild almond species have very small leaves, a probable adaptation to the xerophytic conditions under which the species evolved (Grasselly, 1976a). Hybrid progenies tend to be intermediate between the parents. Lansari *et al.* (1994a) found that phenotypes collected from native seedling populations in Morocco tended to have smaller leaves than introduced cultivars. Large, dark-green leaves characteristics of 'Harriott' and its offspring 'Jordanolo' are heritable (Kester and Gradziel, 1996). Individuals coming from the same cross tend to have similar leaf characteristics (Bernad and Socias i Company, 1998). Grasselly (1972) reported that in almond × almond and almond × peach progeny, the average leaf size was correlated to the mean of the parents.

Time of maturity

Time of maturity is an important feature when choosing cultivars in order to optimize harvesting operations. The biological complexity of the maturation process and large environmental and management effects makes it difficult to clarify its genetic basis. Ripening of the almond fruit involves hull (mesocarp) dehiscence and loss of moisture to dryness. The pellicle of the

kernel changes colour from white to brown. Ripening begins in the nuts on the periphery of the tree canopy and proceeds to the centre. The whole process of hull and nut maturation may require 4–6 weeks to complete.

Usually, maturity is most reliably discerned by the initiation and progress of dehiscence ('hull-split'). The dates of the splitting initiation (5–10% split) and the completion of splitting (95%) are useful criteria when comparison is made to standard cultivars. Moisture stress can initiate hull dehiscence, but moisture in the hull is required for proper splitting. If splitting begins prematurely the nut dries too rapidly and the hull may close tightly to the shell, making it difficult to remove (Goldhamer, 1996). These characteristics are more critical with soft-shelled cultivars (in California and Australia) than with hard-shelled cultivars (in Spain and other Mediterranean countries). The pattern of maturation across the range of almond genotypes extends from mid-July to early November in Spain. Cultivar classification of maturity time has been established (Gülcan, 1985; Kester and Gradziel, 1996), providing a means of classification. Some almond species (*P. webbii* and *P. bucharica*) mature earlier than almond and can be a source of early maturity traits.

Time of ripening is characteristic of each cultivar and the sequence among cultivars is typically maintained from year to year. Time of maturity is highly heritable and follows a quantitative pattern (Dicenta *et al.*, 1993b). On the basis of data over 2 years from 32 families, heritability was estimated as 0.90 based on variance components and 0.69 based on regression. The parental mean was slightly higher than the progeny mean in most of the families, suggesting a slight non-additive dominance effect of early maturity. Heritability based on variance and regression for time between bloom and maturation was 0.77 and 0.61, respectively, with a small family by year interaction.

Flowering characteristics

Flowering is a major phenological trait of the almond and usually a main concern for breeders. In almonds, flower bud differentiation coincides with the current season's hull-split to post-harvest period. The final yield of the tree (Kester, 1989) is a function of the total number of viable flowers

(density), the proportion of these flowers that are pollinated and fertilized, and the proportion of flowers and young fruits that are damaged by biotic or abiotic factors. In addition, there is a strong inverse relationship between blossom density of one year and blossom density of the next (tendency to alternate bearing). This is largely dependent on cultivars and growing conditions (rainfed or irrigation). Fruit number and kernel weight are the two most important parameters that determine yield.

Flower type

Flowers of different cultivars and species may differ in petal size, shape and colour (see Chapter 1), number and length of stamens (Grasselly and Crossa-Raynaud, 1980), as well as position of anthers in relation to stigmas (Weinbaum *et al.*, 1986; Godini *et al.*, 1992; de Palma and Godini, 1993; Godini and de Palma, 1994; Bernad and Socias i Company, 1995; Rovira and Miarnau, 2016). The number of stamens may vary from 20 to about 40, with the common number being 30–33. The distribution of stamen number within seedling populations from parents of different stamen numbers indicates quantitative inheritance with a tendency towards dominance of larger numbers (Grasselly and Crossa-Raynaud, 1980).

Two flowers can also be produced within the same flower bud, defined as twin flowers (Socias i Company, 1983). Italian cultivars from Apulia such as 'Cristomorto' and 'Tuono', largely used in European breeding programmes, often produce two flowers per bud, as this represents an opportunity to increase yield. Although the usual pistil number per flower is one, some genotypes tend to produce two or more. Cultivars like 'Marta', 'Tarraco' and others have most flowers with double pistils but one of them drops soon after set. In other cases, such as 'Eureka' (Kester and Gradziel, 1996), two pistils are produced, which may result in a double fruit. In other genotypes, particularly in older flowers, the pistils are bent, twisted and modified in various shapes often depending on the flower stage (Kester and Gradziel, 1996).

The structure of the pistil also varies with genotype and with age. Some pistils are straight and elongated, with stigmas extending above the anthers, a trait that has been associated

with enhanced cross-pollination by bees (Weinbaum *et al.*, 1986). In other individuals, the stigmas and the anthers are about the same level, a position associated with increased chances for self-pollination (Godini *et al.*, 1992; de Palma and Godini, 1993). On self-compatible genotypes, work by Rovira and Miarnau (2016) reported no relation between fruit set and relative anther–stigma position. Male sterility in the almond cultivar ‘Rof’ was first observed by Vargas and Romero (1978), although its genetic transmission has not been shown, suggesting a physiological origin (Alonso and Socias i Company, 2002).

Flower density

Almond trees initiate and develop flower buds laterally in leaf axils on extension shoots and on short shoots (spurs) during one growing season. These continue to develop during the following dormant period and, with adequate chilling and heat, flower early the next spring. Most flower buds of most cultivars are produced on spurs. These spurs can be as short as 2 cm or less but could be 14 cm long. The number of buds per spur, the number of spurs per branch and the distribution of these spurs within the tree vary with cultivar and environment (Kester and Asay, 1975; Kodad and Socias i Company, 2008a).

Bud density on individual plants in seedling populations can be referred to a subjective scale of 1 (lowest) to 9 (highest) for both shoots and spurs. Location of flower buds was scaled as 1 (most flower buds on 1-year-old shoots), 2 (most flower buds on spurs) and 3 (mixed) (Gülcan, 1985). The absolute density of buds gives an indication of production potential (Socias i Company *et al.*, 2004), and the relative density of buds produced on shoots as compared to spur (shoot/spur density) characterizes the fruiting habit of many *Prunus* species. The estimation of bloom density and fruit set of any almond genotype is important in the evaluation of its productive potential and its value as a commercial cultivar (Socias i Company *et al.*, 1998).

Considerable variation exists among individual cultivars in shoot/spur density ratios, flower bud density and bearing precocity. These traits appear to be heritable but inheritance seems polygenic. If seedlings are properly handled in the selection plots with a minimum of

pruning, flowering buds may appear by the beginning of the second/third year, enabling early seedling selection.

Grasselly (1972) observed that the precocious high-bud-density cultivar ‘Marcona’ transmitted higher density and precocity to its offspring than did the less dense, less precocious cultivar ‘Bartre’. ‘Marinada’ also shows the features of ‘Marcona’. Results indicated that reasonable reliable selection for these characters can be made by at least the fourth and fifth years.

Flowering time

Time of bloom is one of the most important single adaptive traits in almond selection. It indicates flower bud chilling and heat requirements, determines frost vulnerability and also effective bloom overlap when self-incompatible cultivars are combined in orchards (Egea *et al.*, 2003; Martínez-García *et al.*, 2011a; Sánchez-Pérez *et al.*, 2012). Flowering time can vary from year to year depending on the temperature patterns before and during bloom. The sequence of bloom among different genotypes tends to be relatively constant over the years (Felipe, 1977a), but this can be modified by different chilling requirements to overcome rest as well as different heat requirements to initiate bloom (Alonso *et al.*, 2005). Thus, this trait is the result of the interaction between winter chilling and heat conditions. Season of flowering is often referred to days before/after an indicator cultivar. In the Mediterranean countries, ‘Cavaliera’ and sometimes ‘Desmayo Largueta’ have been considered the reference, whereas in California and Australia ‘Nonpareil’ is commonly used as standard. The Almond Descriptor List defines nine different times of bloom classes with reference cultivars for each class (Gülcan, 1985).

Time of bloom is quantitatively inherited (Kester, 1965b; Grasselly and Gall, 1967; García *et al.*, 1985; Dicenta *et al.*, 1993b). A genetic heritability of 0.83 was obtained by Kester *et al.*, (1973), while Dicenta *et al.* (1993b) calculated a heritability of 0.67 to 0.78 for first flowering and full bloom, respectively, with lower heritabilities for end of bloom. Full bloom showed heritabilities of 0.78 (estimated by regression) and 0.98 (calculated by variance components). Molecular studies (Sánchez-Pérez *et al.*, 2014) at the DNA level confirmed this polygenic nature,

identifying several genome regions (QTL) which appeared to be involved.

Almond typically leafs out after blooming, but in some cultivars – including ‘Garrigues’, ‘Marta’ and ‘Marinada’ – leafing before flowering can occur. Strong correlations exist between leafing out and flowering time in the individual seedling (Kester *et al.*, 1976; Vargas and Romero, 1984). In addition, a high correlation occurs between the mean of the leafing time of the parents and that of the seedlings. However, the correlation is weaker when crossing late cultivars, and it has been observed that a lessening of the correlation occurs when one of the parents flowers before leafing.

Relationships occur among time of bloom, time of leafing and seed chilling requirements (stratification). Genotypes pollinated by cultivars with different blooming times produced separate seed populations with chilling requirements correlated with the bud chilling requirement of the pollen parent (Kester and Gradziel, 1996). A high correlation appears between the mean of the leafing time of the parents and that of their seedlings.

A major gene mutation is present in ‘Tardy Nonpareil’, a natural sport of ‘Nonpareil’, which flowers 10–15 days after ‘Nonpareil’ at Constantí, Tarragona, Spain, with a phenotype otherwise identical to the original ‘Nonpareil’ (Kester, 1965b; Grasselly, 1985) and having a heritability value of 0.83 (Kester *et al.*, 1973). The mutation was attributed to a dominant major gene controlling time of bloom named *Lb* (late bloom) (Socias i Company *et al.*, 1999). This gene was later mapped (Ballester *et al.*, 2001) and can be used in selection. Crosses with ‘Tardy Nonpareil’ give segregating populations with an early-blooming group and a late-blooming group, but a linkage between late bloom and low productivity also exists (Kester, 1965b; Grasselly, 1978). This unwanted linkage can be broken in the next generation of crossing (Grasselly and Olivier, 1984; Grasselly and Olivier, 1985) resulting in individuals with very late bloom and good productivity. Flowering time is described in more detail in Chapter 7.

Fruit set

Fertilization is essential in almond as the edible part is the kernel, a seed. A high percentage of

fruit set is a desirable horticultural trait. Many environmental factors affect set, but a number of genetically controlled traits are involved. Some almond cultivars appear to set more heavily than others even in the same environment.

Studies to evaluate self-fertilization after self- and cross-pollination in self-compatible genotypes have been developed, as this trait is important in breeding programmes (Kodad and Socias i Company, 2008c). Dicenta *et al.* (2002) indicated that the pollination type does not affect fruit set. However, Socias i Company *et al.* (2004) revealed that some self-compatible selections had higher fruit sets following cross-pollination than after self-pollination. Vargas *et al.* (2005) and Kodad and Socias i Company (2008b) indicated that fruit set from open pollination of self-compatible genotypes had higher values of weight in-shell, kernel weight, nut volume and kernel volume than those from self-pollination after bagging. However, Martínez-García *et al.* (2011b) reported no significant differences between self-pollination and cross-pollination for fruit set and fruit characteristics (weight in-shell, kernel weight, percentage of kernel, percentage of defective kernels, double kernels and split kernels) in six self-pollinated almond genotypes including ‘Antoñeta’, ‘Felsia’, ‘Lauranne’ and ‘Tuono’. Kodad and Socias i Company (2008b) also reported an increase of kernel quality after self-pollination as compared to cross-pollination. These findings suggested an absence of negative effects of self-pollination on these agronomic traits, supporting previous reports (Dicenta *et al.*, 2002). In commercial orchards, based on the two self-compatible cultivars ‘Francolí’ and ‘Guara’, and using different self-incompatible cultivars as pollinators, and isozymes and SSRs as markers to identify pollen origin, Rovira and Martín (2014) observed that more nuts were coming from selfing than from outcrossing.

Cold hardiness

Cold hardiness is the resistance to freeze damage during late autumn, winter and early spring. During the winter when trees are fully dormant and in their deep rest, trees and buds are reported to withstand temperatures of -25°C (Grasselly and Crossa-Raynaud, 1980). When flowers buds are close to open (stage D or ‘pink bud’) frost resistance hardiness is reduced.

Differences in cultivar resistance at 'pink bud' stage have been determined: 'Nonpareil' and 'Sonora' > 'Price' > 'Carmel' > 'Padre', 'Mission', 'Butte' and 'Peerless' (Snyder and Connell, 1995). Flower damage due to frost is largely dependent on several factors such as flowering, tree nutrition stage and environmental conditions (temperature and humidity). In Spain, 'Desmayo Largueta' and 'Guara' are known to show some flower frost tolerance in the field (Felipe, 1988).

Disease and pest resistance

Information on the inheritance and genetics of disease resistance is limited in almond (see Chapter 14). Blossom and twig blight ('brown rot'), one of the major crop-limiting fungal diseases worldwide, is caused by *Monilinia laxa* and *M. cinerea*. The most serious foliage disease is 'shothole', caused by *Stigmata carpophila* (syn. *Coryneum beijerinckii*). Other fungal diseases important in the Mediterranean region (depending on relative humidity and temperatures) include 'polystigma' (*Polystigma ochraceum* (Whalenb. Sacc.)), 'fusisocum' (*Phomopsis amygdali* (Delacr.) or *Fusicoccum amygdali* as imperfect form, 'tavelure' (*Fusicladium amygdali*) and 'anthracnose' (*Gloeosporium amygdalinum* and *Colletotrichum acutatum*). More recently, bacterial diseases like *Xanthomonas campestris* pv. *pruni* are also becoming important in Europe. Most of these diseases can be controlled by chemical sprays; however, natural resistance has become more important with the continued loss of agrochemicals due to regulations restricting use. For many diseases, relative susceptibilities of important cultivars in different countries have been determined and potential sources of resistance have been identified (Romero and Vargas, 1981; Kester *et al.*, 1990; Vargas and Miarnau, 2011). Grasselly (1972) and Grasselly (1981) identified 'Ardéchoise' as having resistance to most fungal diseases tested and suggested that broad resistance might be controlled by genetic factors.

Research on resistance to fusisocum, an important fungal disease in humid Mediterranean areas, has shown recent success. This disease mainly affects twigs of the lower part of the tree, causing cankers on shoots and necrosis on leaves, and thus reduces nut production. The diffusion of the disease is very slow but its eradication from an orchard is difficult and

expensive (Romero and Vargas, 1981). Some classification of cultivars according to field susceptibility to fusisocum has been made (Vargas and Miarnau, 2011) and segregating progenies assessed (Luque *et al.*, 2006). The use of MAS for breeding can be useful in screening for disease resistance, particularly when natural disease development is slow, requiring several years before seedlings/cultivars can be classified. Martins *et al.* (2002) and Martins *et al.* (2005) have identified three RAPD fragments related to tolerance/susceptibility to fusisocum; however, to date they have not been used for selection.

Almonds can be infected by the same range of viruses as other *Prunus*, including the ILAR viruses (ringspot, prune dwarf, line pattern, calico, apple mosaic), and NEPO viruses (tomato black ring, tomato ring spot, yellow bud mosaic). However, many almond cultivars appear to be immune to plum pox virus, which is a serious problem for most other stone fruits (Rubio *et al.*, 2004). Indeed, induced resistance to sharka (PPV) from almond 'Garrigues' to peach by grafting has been reported (Rubio *et al.*, 2013).

There is no known resistance to the almond wasp pest *Eurytoma amygdali* End. However hard-shell, thick hull and early ripening cultivars are less prone to be attacked because the shells of their nuts rapidly become too hard for the female ovipositor to pierce (Talhouk, 1977) or too thick for laying eggs inside the kernel.

Aflatoxins produced by *Aspergillus flavus* infections of the kernel can be a major problem, particularly in paper-shell or soft-shelled and poorly sealed nuts where insect damage is more common ('Nonpareil', 'Ne Plus Ultra' and 'Merced'). However, resistance through better-sealed shells is possible, as in 'Carmel', 'Mission' and 'Butte', or through hard-shell and well-sealed nut types, and in Mediterranean cultivars (Gradziel, 2008). Susceptibility of 21 Californian almond cultivars to aflatoxigenic *A. flavus* was assessed on seeds (intact and injured) after inoculation, finding two barriers to fungal development (seedcoat and cotyledon) (Gradziel and Wang, 1994). Dicenta *et al.* (2003) evaluated the susceptibility of 40 almond genotypes to *A. flavus* in controlled inoculation conditions. All the almonds assessed showed different levels of susceptibility to *A. flavus* unrelated to their genetic origin.

5.5 Breeding

The three basic objectives of scion almond breeding are to increase yield (self-compatibility, late blooming, high productivity, early bearing, etc.), to improve kernel quality (size, good appearance and colour, absence of double kernel, texture, taste, chemical composition, etc.) and to decrease orchard production costs (reduce training and pruning, increase disease, pest and drought tolerance, etc.) (Kester and Gradziel, 1996; Socias i Company, 1998; Socias i Company *et al.*, 1998; Vargas *et al.*, 2011). The efficiency of breeding programmes depends on the information available about the heritability of those traits to be improved. The main aim of almond breeding programmes is the development of cultivars highly adapted to local environments as well as to market and industry demands (fresh, roasted, blanched, fried, milk, nougat, etc.). A number of other objectives have also been considered, depending on the environmental conditions in different growing regions (Table 5.4). Modern breeding goals must consider not only the agronomic and commercial requirements of the current situation but also look at long-term trends that will affect future production and marketing. In addition, almond species show a large polymorphic variability (Byrne, 1990; Kester *et al.*, 1990; Socias i Company and Felipe, 1992a; Potter, 2011; Gradziel, 2012), which indicates considerable breeding potential.

Most Mediterranean almond breeding programmes have focused on late blooming to avoid spring frost; self-compatibility to prevent cross-pollination failures and favour nut set; and a hard shell, high productivity, tolerance to dryness and diseases, good nut quality and improved tree architecture to reduce training and pruning costs. In California, the market dominance of the soft-shelled ‘Nonpareil’ cultivar has focused breeding towards developing cross-compatible pollenizers as well as self-compatible replacement types. Recently some ‘Marcona’-like types such as ‘Sweetheart’, as well as self-compatible cultivars such as ‘Independence’, have been released.

Modern almond-growing systems worldwide use vegetatively propagated clones. Clonal selection has probably always been a part of almond culture, particularly for topworking undesirable trees in seedling plantings, such as

eliminating bitter kernels. Improvement of specific agronomic and commercial traits requires that new genes be introduced through controlled crosses using germplasm from varied sources. To be effective, cultivar breeding requires gathering genes controlling desired traits, recombination by hybridization, followed by appropriate selection and testing.

Traditional orchards in the Mediterranean region are largely under rainfed conditions and thus resilient scion/rootstock combinations are required. As almond orchards evolve, new production systems such as super-high-density plantings under irrigated conditions are implemented. In Europe, northern Africa, California and Australia, orchard lifespan is being reduced (in some cases to less than 20 years) because of this more intensive production, and thus the need to breed and release new cultivars is increasing. New cultivars need to be better adapted than the traditional cultivars to continue to meet new production models and market requirements. Cultivar tree architecture and bearing habits, and well-tested scion/dwarfing rootstock combinations, are being considered for these higher-density systems.

Broadly considered, almond quality (organoleptic and phytonutrient) is already a primary breeding objective. This goal is further complicated because nut quality is genetically controlled but also strongly influenced by a range of environmental parameters including plant age, soil, climate, orchard management and post-harvest treatment. In addition, improved nut quality must be combined with the range of tree and crop characteristics demanded by the grower, processor and consumer (Socias i Company *et al.*, 2008a). Improved efficiencies in almond breeding can be achieved through innovative breeding methods using genomic-based technologies such as genome-wide selection (GWS) and statistical approaches like multivariate statistical analysis.

By the middle of the 19th century, cultivars in most Mediterranean countries were recognized by name in nurseries. In California, both seeds and budwood were introduced from Europe and Asia at this time (Kester and Gradziel, 1996). By the turn of the 20th century, most of the almond-producing countries of the Mediterranean region and the USA had identified local cultivars, which were often selected seedlings of

Table 5.4. Objectives of modern almond improvement programmes. (Adapted from Gradziel, 2008.)

Problem	Trait	Objective	Country
Spring frosts	Late bloom; blossom resistance	Avoid early and late spring frosts	USA, Ukraine and former USSR, France, Greece, Spain, Italy, Romania, Turkey, Bulgaria
Winter freezing	Hardy buds and wood	Avoid loss of dormant flower buds; avoid tree damage	Ukraine, Romania, Bulgaria
Low winter chilling	Low chilling requirements	Grow in subtropical area	Israel, Tunisia, Australia, Morocco
Moisture stress	Drought tolerance	Grow with deficient irrigation	Spain, Italy, Iran, Turkey, Morocco, Australia
Lack of or reduced bee populations	Self-compatibility	Eliminate or reduce need for bee pollinators	France, Greece, Tunisia, Italy, Portugal, Spain, USA
Diseases and pests	Resistance to fungal and bacterial diseases and insect pests	Eliminate or reduce need for chemical sprays	USA, France, Spain, Italy, Turkey
	Free of virus and virus-like organisms	'Clean' propagation sources	USA, France, Spain, Italy
High management costs	Modified tree size, shape, branching, growth habit	Efficient orchard management; adjust orchard density; pruning; shaking	USA, Australia, Spain
Difficult harvest and handling	Optimize time of maturity, ease and completeness of nut removal, hulling	Extend harvest period; efficient and complete harvest	All programmes
	Shell character: very hard shell	Kernel protection and storage; prevent worm infestation	Spain, France, Italy
	Shell character: semi-soft to soft	Higher shelling percentages	USA, Ukraine
	Shell character: well sealed	Kernel protection	USA, all programmes
Inconsistent yields	Improve tree: precocity, productivity, regularity of bearing	Early production; high yield, no alternate bearing	All programmes
		Increased kernel yield and reduced damage and need for control measures	All programmes
Quality drawbacks	Nut quality	Improving	Italy, Spain, USA, Australia, Iran

unknown origin but represented the adapted germplasm of the region.

Traditional breeding approaches have predominated in almond breeding. These are based on designing controlled crosses between parents selected for desired traits. The breeding techniques are well established (Kester and Asay, 1975; Kester and Gradziel, 1996) involving

cross-pollination (pollen collection, female tree preparation, flower emasculation and pollen application), followed by nut collection, seed stratification, germination, planting, seedling raising, field evaluation, selection and regional testing.

Almond is characterized by a short juvenile period relative to other nut species, being similar

to peach. Most seedlings in the selection plots, if well managed, should start flowering during the third year after planting. In some crosses between early cropping cultivars such as 'Marcona' and 'Marinada', a number of seedlings already develop flower buds during the first year after germination.

Intraspecific crosses, followed by seedling evaluation and selection, have been the main method used for traditional clonal improvement. Conventional breeding within the species can provide significant genetic advances both to increase adaptation and to improve nut quality. Similarly, observed variation in disease and pest tolerance should allow breeding progress through classical breeding. Interspecific hybridization with several *Prunus* species, and also backcrossing to the cultivated almond, was made with varied results in California and the former Soviet Union (Kester *et al.*, 1990; Denisov, 1998; Gradziel *et al.*, 2001; Gradziel, 2012). The use of cultivated almond and peach as bridge species has facilitated the introgression from wild accessions to commercially acceptable types (Gradziel, 2012).

When planning an almond breeding programme, the biological characteristics that most directly control the targeted horticultural and industrial traits should first be identified. The relative genetic and environmental components controlling these characters must then be determined, obtaining information about their inheritance patterns. With this information it should be possible to devise strategies (parental cross combination, family size and selection methods) by which the required characteristics can be combined to increase the likelihood that a cultivar superior in horticultural qualities can be obtained and successfully commercialized. For instance, RNase markers to determine *S* alleles were used successfully to design crosses which yielded only self-compatible seedlings, by having an *S* allele of self-incompatibility common to both parents (Batlle *et al.*, 1997; Batlle *et al.*, 2001; Ortega and Dicenta, 2003). More recently, genotyping of parents using DNA-based markers can also be used routinely for seedling selection before planting in the field (Gradziel *et al.*, 2001; Ortega and Dicenta, 2004; Sánchez-Pérez *et al.*, 2004; Ortega *et al.*, 2005; Martínez-García *et al.*, 2011a).

5.5.1 Objectives

Major breeding objectives include productivity, self-compatibility, late blooming time, kernel quality, tree architecture, and disease and pest resistance (Table 5.4). Except for kernel taste (sweet or bitter), self-compatibility and late flowering from 'Tardy Nonpareil', which are controlled primarily by single major genes, the most important agronomic and commercial traits in almond are quantitatively inherited and still poorly characterized.

5.5.2 Floral biology and selection cycles

Almond is mainly heterogamous and intraspecific crossing is the main breeding strategy used by breeders worldwide, although some interspecific hybridization targeting self-compatibility and disease resistance from peach and related species was made at UC Davis. A large number of cultivars and selections has been developed, which are also available for further use in crossing. Depending on soil, climatic and growing conditions, additional crossing can be achieved from about 3 years after germinating the seeds, particularly if the selected seedlings are used as pollen donors. Thus progression is based on a 3–5-year breeding cycle.

Interspecific hybridization

The range of closely related almond species is extensive (see Chapter 1), showing a wide diversity of traits. It is advisable and sometimes even necessary to look to other related *Prunus* species for novel traits, such as climatic adaptation, self-fruitfulness and disease and pest resistance. Potential sources of resistance have been identified (Kester *et al.*, 1990). The wide genetic diversity present among almond and related *Prunus* species provides an enlarged pool of available germplasm that has not been sufficiently exploited (Grasselly, 1977; Kester *et al.*, 1990; Socias i Company and Felipe, 1992a). Improvement of specific genetic traits can benefit from the introduction of genes from species other than almond. Crosses of almond with other almond species in sections *Euamygdalus* and *Spartoides* have been readily carried out and have fertile

progeny. The occurrence of natural hybridization between cultivated almond and nearby wild species is well documented. Thus *P. webbii* grows throughout the Mediterranean region and its range overlaps with cultivated almond in Italy, Sicily, Spain and Greece where hybridizations occur naturally. Gradziel (2003) demonstrated that interspecific hybridization and backcrossing can provide an effective means of introgressing desirable genes from wild species into both almond and peach cultivars. Crosses with peach (*P. persica*), wild peach (*P. mira*), other *Prunus* species and hybrids can be made quite easily, although some types of sterility may sometimes occur in the progeny. Crosses with plum are possible but difficult. Crosses with apricot are very difficult but have been reported (Gradziel, 2006). In addition, the existence of several almond $\times$ peach genetic maps (Dirlewanger *et al.*, 2004; Howad *et al.*, 2005) provides a useful starting point for inheritance studies of these characters as well as introgression of valuable genes into almond or peach.

5.5.3 Methodology

Almond scion breeding efforts utilizing controlled crosses in the field, followed by seedling selection and testing, is the most common approach to develop new cultivars. To be effective, these programmes require the availability of a wide gene pool having the desired traits. Almond breeding techniques applied are well established (Kester and Asay, 1975; Kester and Gradziel, 1996; Vargas *et al.*, 2011; Socias i Company *et al.*, 2012; Gradziel and Martínez-Gómez, 2013) involving controlled crosses (following pollen collection, flower preparation and pollen application), fruit collection, seed stratification and germination, seedling raising and selection.

Parental choice and cross design

Parental selection based on phenotype and genotype is the most efficient and effective method of achieving improvement in almond. When choosing parents the flower setting performance of the female genitor should be also considered as it can vary largely. Fruit set after flower emasculation varied widely between parents (Vargas *et al.*, 2011). Thus some cultivars

are good as seed parents and some behave poorly. It is also convenient that the female parent blooms later than the male genitor (usually less risk of frost, better weather conditions and better opportunities to collect fresh pollen). If it is not possible, it will be necessary to use pollen collected and kept from the previous year (Martínez-Gómez *et al.*, 2000; Martínez-Gómez *et al.*, 2002) though earlier flower development to favour pollen dehiscence can sometimes be promoted on cut branches brought into warmer environments. Intervarietal reciprocal crosses have been performed in almond; these largely found no differences for most of the evaluated traits (Dicenta and García, 1993c). Combining ability has also been considered when parental choice is being made for some traits, and reports indicate that most showed additive effects on their genetic control (García *et al.*, 1994). In addition, if one of the parents is self-incompatible and the other is self-compatible, it is preferable to use the self-incompatible parent as female. The possibility of selfing will thus be negligible and the damage to flowers from emasculation will be eliminated. S allele markers can be used to genotype cultivars and selections to be used as parents to design suitable crosses which should produce only self-compatible progenies. This approach is being used successfully (Batlle *et al.*, 1997; Batlle *et al.*, 2001; Gradziel *et al.*, 2001; Mnejja *et al.*, 2002; Ortega and Dicenta, 2003) by appropriate parental choice.

Crossing and growing seedlings

Pollen is collected from flowers harvested just before opening, at the balloon stage or stage D of Felipe (1977b). Anthers are removed from the flowers with scissors or tweezers, or by brushing over a fine-meshed wire sieve. The anthers are dried on a sheet of paper or in a Petri dish by exposure under light for about 24–48 h or placed in an incubator at 20°C for 48 h until anther dehiscence occurs. Pollen can be extracted from the dried anthers by filtering through a fine screen. Filtered pollen can be then kept in vials stoppered with cotton. Dry pollen will remain viable for several weeks at room temperature, especially if stored inside a desiccator with self-indicating silica gel, to maintain low humidity. For longer storage, pollen kept at –20°C inside a desiccator will remain viable for several years (Parfitt and

Almehdhi, 1984; Martínez-Gómez *et al.*, 2000; Martínez-Gómez *et al.*, 2002). The viability of the collected pollen can be tested by microscopy, observing the percentage of germination on media containing 10% sucrose in 5% agar and 50 ppm boric acid (Brown, 1975). Pollen can be transferred with the fingertip or brush into this medium on Petri dishes and kept in an incubator at 20°C for pollen tube formation, being examined after 24 h. Pollen grains in which the pollen tube emerges at least the length of the grain itself are considered to be germinated and viable. Samples showing less than an estimated 75% germination are less desirable for crossing. For routine pollination there is no need to extract the pollen from the anthers; it can be transferred with the fingertip after shaking the vial containing the anthers covered by the finger.

For controlled crosses flower emasculation or branch bagging is usually practised. Emasculation is easily achieved on flowers just before anthesis by removing the midportion of the floral cup (petals and stamens) using the fingernails of two or three fingers and thumb, or notched tweezers. Early opening flowers are usually more fertile when crossing than later opening flowers (Socias i Company, 1983; Socias i Company and Felipe, 1992b; Socias i Company and Felipe, 1994; Bernad and Socias i Company, 1995). Pollen can be applied to the receptive stigma with a hairbrush, glass rod, rubber or fingertip, which should be sterilized with 70% v:v ethanol between different crosses. Flowering shoots and spurs closely placed to the emasculated and pollinated flowers should be removed to increase fruit set. It is also necessary to remove late opening flowers after controlled crossing to avoid open pollination of these flowers. Branch bagging to prevent unwanted pollen contamination is possible but it is usually not necessary as pollen-collecting bees are not attracted by apetalous flowers; additionally it is more time consuming and is not usually practised. The modified environments inside bags (light, temperature air movement and humidity) could also affect fruit set (Socias i Company *et al.*, 2005).

Variable seed sets of 10–40% are typically obtained in pollination trials, when crosses are made with untouched flowers in enclosed branches and lower percentages are common in crosses of emasculated flowers, presumably due to flower damage during handling or fewer flower

reserves available after emasculation (Kester and Gradziel, 1996; Vargas *et al.*, 2002; Socias i Company *et al.*, 2005). As mentioned before, genotype response to emasculation is highly variable (Vargas and Romero, 2001) and should be considered when planning crosses.

Fruits are collected when the hull dehiscence and splits. The nuts are then extracted and dried for storage. Almond seeds require stratification for about one month at 4°C. Seed can be stratified either within the shell or the seed can be stratified alone after cracking. Before stratification and to wet the kernels, both in-shell seeds or seeds alone should be soaked in tap water (12–36 h, depending on kernel type or shell hardness). Stratification is carried out in moist perlite or sphagnum moss using bags or containers at temperatures around 4°C. Fungicide treatment is desirable since almonds are susceptible to fungal development. Once germination starts, sprouted seeds can be planted directly in the field (around 3.5–4 m × 0.3–0.4 m) or at a density of up to 6000–10,000 seedlings/ha). In the northern hemisphere stratification is made in November or December and sprouted seeds are planted outdoors directly in selecting plots in January or February and fitted with drip irrigation. Some breeding programmes stratify the almonds and then germinate and grow the seedlings in pots in the greenhouse using irrigation, before transplanting to the field.

Seedling evaluation and selection

Genetic gain from the crossing–cloning breeding approach is limited only by the genetic quality and diversity of the parents used and the size of the progenies developed. As larger populations are evaluated, the chances of recovering outstanding cultivars increase. In addition to kernel quality, good performance is required for a number of traits including tree structure, easy training, precocity, productivity, disease and insect resistance, desirable harvest time, uniformity and ease of harvest, and freedom from alternate bearing; as well as good post-harvest performance, market type and consumer preference.

Early selection

Strategies for shortening the breeding cycle through early field screening of almond seedlings

have been presented by Kester and Gradziel (1996), Vargas *et al.* (1997) and Vargas *et al.* (2005). Several important agronomic and commercial traits can be detected at an early seedling stage, and this allows early removal of unwanted individuals (Vargas and Romero, 1984; Vargas *et al.*, 1997; Vargas *et al.*, 1998). Kester *et al.* (1977b) reported high heritabilities of time of flowering and time of leafing in almond progenies. Vargas and Romero (1984) assessed 602 seedlings derived from four open-pollinated cultivars, and observed that there were correlations between flowering and leafing time: these were higher when using early-blooming cultivars. However, Dicenta *et al.* (2005) studied 502 seedlings belonging to 13 families over 4 years and showed that, although some correlations were observed between germination or leafing time and flowering time, these correlations were not strong enough to be used as efficient criteria for early selection of late-flowering seedlings. Leafing time could be used for early selection when parents have very different flowering times (very early and very late), since the expected variability within families will be large. Later, García-Gusano *et al.* (2010) studied 136 seedlings from six families and observed some relationship between germination times of the seeds and seedling flowering times, but this was not sufficient to be used as an early selection criterion. According to the results these authors concluded that, to date, to breed late-flowering cultivars the best method is to cross genitors that are as late flowering as possible, and then to select seedlings based on their blooming time.

The following sequence of operations for growing seedlings to an early evaluation stage was developed and successfully utilized at IRTA Mas de Bover, Spain. Early selection makes it possible to raise and handle a large number of seedlings using limited resources (Table 5.5). Kester and Gradziel (1996) also developed a selection scheme, based on 5 years, which was used successfully in California. Its basic purpose is to allow seedlings to develop naturally in plots from as young an age as possible to allow them to show their potential vigour, bearing habit and tree architecture, and so allow early selection.

Following the early selection process carried out at IRTA by the end of the third year, less than 20% of the initial seedling number is kept in the selection plots, and by the end of the fifth year only about 0.6% of the seedlings are left in the field (Vargas *et al.*, 2010). In some programmes, such as that at CEBAS (Centro de Edafología y Biología del Seguro), CSIC (Consejo Superior de Investigaciones Científicas) Murcia, Spain, early seedling selection is made testing them for self-compatibility using PCR-derived molecular markers, with varied results depending on cross combinations (Ortega and Dicenta, 2004).

The main tree and fruit observations at IRTA follow. In evaluating the tree, seven features are recorded: (i) self-compatibility (by bagging flowering branches and recording nut set); (ii) flowering date (F stage); (iii) flowering intensity; (iv) production intensity (scale: 0–9); (v) vigour (measured as trunk diameter at 30 cm

Table 5.5. Main steps of the almond early field seedling selection process followed at IRTA from controlled crosses.

Year	Station	Activity/observation
0	Winter	Making controlled crosses in the field
0	Autumn–winter	Seed stratification, germination and sowing in the field
1	Spring–autumn	Seedling growing in the field at selection plot
2	Winter	Leafing date
2	Autumn–winter	Amount of vigour, growth and branching habit
3	Winter–spring	Flowering date, early bearing
3	Autumn–winter	Fruit characteristics, tree architecture
4	Winter–spring	Self-compatibility, flowering date, production
4	Summer–winter	Fruit characteristics, tree architecture
5	Winter–spring	Self-fertility, flowering date, production
5	Summer–winter	Fruit characteristics, tree architecture, drought and disease susceptibility

from the ground); (vi) disease incidence; and (vii) tree architecture. Observations with large subjective components such as production intensity, tree architecture and disease incidence are usually made several times, and by different observers. Six fruit traits are measured using a sample of 25–50 nuts, depending on the selection phase: early (25 nuts), intermediate (50 nuts) or advanced (at least 100 nuts). Records taken include: (i) average weight of in-shell nuts; (ii) kernel weight; (iii) shelling percentage; (iv) double kernels (%); (v) kernel aspect score (from 1–9 by three observers); and (vi) kernel taste (sweet or bitter).

Several methods have been applied for evaluating self-fruitfulness in self-compatible genotypes, since self-fruitfulness requires both self-compatibility and autogamy (i.e. the structural capacity for self-pollination) (Socias i Company *et al.*, 2010c). Often unopened blossoms are enclosed within mesh bags during 40 days of flowering and subsequent fruit set recorded (Socias i Company *et al.*, 2005). Complex traits to assess, such as self-compatibility, can now be screened using molecular MAS during initial seedling growth (Gradziel *et al.*, 2001; Ma and Oliveira, 2001; Ortega and Dicenta, 2003; Dicenta *et al.*, 2005; López *et al.*, 2005b).

The final aim of breeders is to commercially release new cultivars only after they have performed well in trials for some years and when the propagating material to be distributed has been ascertained to be free of known viruses. A new cultivar should provide an improvement over the cultivar to be replaced. This is particularly relevant in almond orchards where cultivars are expected to be productive for 20 years or more to be commercially viable. Recovering part of the investment via 'royalties' can allow further cultivar breeding and development.

Marker-assisted selection

The effective use of molecular markers allows greater efficiency in cross design and controlled hybridizations, as well as seedling selection, as explained further in Chapter 6. All major types of markers have been developed for almond for use in variability assessment, gene mapping and other applications (Hauagge *et al.*, 1987a, b; Cerezo *et al.*, 1989; Viruel *et al.*, 1995; Bošković

et al., 1997; Bartolozzi *et al.*, 1998; Joobeur *et al.*, 1998; Joobeur *et al.*, 2000; Bliss *et al.*, 2002; Testolin *et al.*, 2004; Dangl *et al.*, 2009). In almond, both major gene and QTL mapping has been reported (Martínez-Gómez *et al.*, 2007; Sánchez-Pérez *et al.*, 2007a; Arús *et al.*, 2009). With the development of DNA-based approaches capable of detecting large numbers of markers, whole-genome linkage maps were made possible (Viruel *et al.*, 1995; Sánchez-Pérez *et al.*, 2007b). The first breakthrough in developing MAS in almond was the identification of stylar RNases for self-incompatibility *S* allele identification (Bošković *et al.*, 1997; Bošković *et al.*, 2003) and later the amplification of *S* alleles using designed primers for PCR (Tamura *et al.*, 2000; Channuntapipat *et al.*, 2001; Channuntapipat *et al.*, 2002; Channuntapipat *et al.*, 2003; Martínez-Gómez *et al.*, 2003b; López *et al.*, 2004; Ortega *et al.*, 2005). Identification of *S* genotypes including the *S_j* allele is useful for both cross design and seedling selection. Consequently, this technique is being used routinely for the identification of cross-incompatibility groups for cultivars and breeding selections, and to efficiently introgress self-compatibility from donor species (Gradziel *et al.*, 2001; Ortega and Dicenta, 2003; Kodad *et al.*, 2010). More recently a multiplex PCR strategy was developed for identification of *S* alleles using different sets of primers (Sánchez-Pérez *et al.*, 2004). When breeding for late flowering using 'Tardy Nonpareil' or derivatives, rapid seedling selection is feasible using known markers linked to the late blooming (*L_b*) gene (Ballester *et al.*, 2001). The sweet gene *S_k* (*S_w*) was also found to be linked to a marker (Sánchez-Pérez *et al.*, 2010). In addition, Campalans *et al.* (2001) showed that the levels of leaf expression of genes associated with drought tolerance provided valuable information for drought stress breeding. In the future, it is anticipated that new nut traits may be selected because of the greater importance given to characters related to industrial quality, taste and nutritional or functional compounds (Font i Focada *et al.*, 2012; Fernández i Martí *et al.*, 2013). Most of these traits are quantitatively inherited and thus their effective selection will reinforce the role of MAS. To promote this, an initiative is currently trying to develop the whole almond genome sequence, because of its utility for breeding.

5.6 Breeding Prospects

There is a long-standing dilemma affecting tree crops, which partially affects almond breeding: despite extensive genetic improvement efforts, commercial production remains dominated by a few elite and usually old cultivars (Janick, 2006). This can be seen in California where the main cultivar 'Nonpareil' was selected in the late 1800s. In Spain, however, the two major cultivars, 'Marcona' and 'Desmayo Largueta', possibly selected in the early 1900s, are now being replaced by newer Spanish releases (Socias i Company *et al.*, 2011). In other Mediterranean countries the current trend is similar to Spain, where breeding is impacting production (Socias i Company *et al.*, 2010a).

The availability of useful genetic variation, as well as genomic tools allowing their efficient selection, remains a key determinant of future breeding success. A better integration of classical breeding and modern genomic tools will be a step forward for these achievements (Arús, 2010; Koepke *et al.*, 2013). In addition, germplasm enhancement through interspecific gene introgression using marker-based selection shows promise for cultivar improvement.

Future prospects in almond as part of larger *Rosaceae* research are described in detail by Martínez-Gómez *et al.* (2007) and Arús *et al.* (2009), as well as in Chapter 6. Eight major genes, as well as various QTLs for characters of agronomic interest, are currently mapped in almond. Hundreds of markers, most of them SSRs, are available and have been used for diversity analysis, genetic fingerprinting and for the construction of high-density maps. Almond, in common with all *Prunus* tree crops, shares a common genome (Dirlewanger *et al.*, 2004), allowing synergy with other crop breeding programmes (Arús *et al.*, 2005).

An initiative for sequencing the almond genome through the use of the next generation of high-throughput sequencers is underway. This will allow rapid and relatively inexpensive sequencing of large amounts of DNA, which, combined with knowledge of the full peach genome sequence already provided, will greatly facilitate almond breeding efforts.

In future almond cultivar breeding, several evolving objectives need to be considered carefully.

Improvements in kernel quality should be explored further, particularly novel uses and markets from 'candy bar type' to fresh consumption. Quality improvements ranging from physical to chemical modifications are described by Socias i Company *et al.* (2008a). Related issues like functional compounds and heart-healthy nutrition should be explored further (Fernández-Cuesta *et al.*, 2012). Recent work on almond quality traits via association mapping opens new methods for gene identification and molecular marker-assisted breeding (Font i Forcada *et al.*, 2015a, b). In addition, greater efforts to incorporate disease and pest resistance into new cultivars will be required by increasing regulatory restrictions and consumers' preferences. Important diseases like bacterial spot (*Xanthomonas arboricola* pv. *pruni*) should also be considered when future breeding takes place, as different varietal susceptibility has been observed. Almond relatives include a large number of inter-compatible species available for breeding. Little use has been made of this variability due to slow progress when using classical breeding methods. However, genomic methodologies make possible quick detection and incorporation of genes of interest from wild species to the cultivated almond. Candidate gene approaches to identify biotic and abiotic stresses have also been suggested (Barros *et al.*, 2010). The use of new methodologies like GWS is already offering opportunities to improve breeding efficiency. Adaptation of new cultivars to new production models such as high-density planting in irrigated areas will require compact trees of reduced size as well as dwarfing rootstocks. Prospects of developing even later-blooming cultivars seem less promising, because some extra-late blooming selections appear associated with lower productivity. However, this may also reflect breeding progress since, for many Spanish selections, a flowering delay of more than 2 months has been already achieved through breeding.

Additional advantages encouraging the use of new technologies in almond breeding include a small genome size, high level of synteny among *Rosaceae* genomes and well-established international cooperation. Finally, both public and private funding will be needed to fully integrate these new approaches and technologies to develop better cultivars adapted to each orchard system.

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6 Molecular Breeding and Genomics

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6.1 Introduction

Molecular breeding is the use of genetic manipulation at the DNA molecular level to improve traits of interest in plants and to shorten the time for developing new crop varieties to be brought to the market.

In traditional plant breeding, genetic variation and characterization are usually identified by visual selection of morphological traits. However, with the development of molecular biology, the problems related to environmental factors and, in some cases, the slow development process, might be solved by the use of molecular markers. 'DNA marker' is a term used to refer to a specific DNA variation between individuals that has been found to be associated with a certain characteristic. These different DNA or genetic variants are known as alleles. Thus, the use of DNA markers to define the genotype and predict the performance of a plant is a powerful aid to plant breeding. Molecular breeding implies molecular marker-assisted selection (MAS), which is a strategy to facilitate the exploitation of existing genetic diversity in breeding populations and therefore, in combination with linkage maps and genomics, can be used to improve a whole range of desirable traits.

6.2 Genetic Markers in Plant Breeding

Genetic markers are used to identify different features in the DNA sequence that can be used to differentiate between individuals in a population, or to classify individuals between different varieties or cultivars within a species. They can be transmitted from one generation to another, and thus they can be used as a tag to keep track of a plant, tissue, chromosome or a gene.

Genetic markers can be classified mainly in two categories to be used in genetics and plant breeding: classical markers (morphological, cytological and biochemical) and DNA markers, which are classified into two sub-categories, hybridization-based markers and polymerase chain reaction (PCR)-based markers. In this chapter we describe several types of DNA markers, each of which has different advantages/disadvantages which make them more or less desirable to use in certain applications (highly discriminative and environmentally stable, inexpensive, repeatable across different tissues and co-dominant):

- *RFLP (Restriction Fragment Length Polymorphism) markers* are the first generation of DNA markers and are important tools in

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plant genome mapping. A first linkage map in a crop plant based on RFLPs was constructed in 1986 for tomato (Bernatzky and Tanksley, 1986). Thus, RFLP markers are powerful tools for comparative and synteny mapping. Most RFLP markers are co-dominant and locus-specific. RFLP genotyping is highly reproducible, the methodology is simple and no special equipment is required.

- **RAPD (Random Amplified Polymorphic DNA) markers** are predominantly dominant markers. These markers do not need either DNA probes or sequence information for the design of specific primers. In addition, RAPD analysis requires relatively small amounts of DNA and the procedure can be automated. The primers can be universal and one set of primers can be used for any species. Also, they can be converted into simple and robust PCR markers known as SCARs (Sequence Characterized Amplified Regions). However, RAPD also has some limitations, such as low reproducibility, and it is incapable of detecting allelic differences in heterozygotes.
- **AFLP (Amplified Fragment Length Polymorphism) markers** combine elements of RFLPs and RAPDs. They are based on the selective PCR amplification of restriction fragments. This kind of marker uses selective amplification of a subset of restriction enzyme-digested DNA fragments to generate a unique fingerprinting for a particular genome.
- **SSR (Simple Sequence Repeat microsatellites) markers** are the preferred technique for the study of genetic relationship among species and for the assessment of genetic diversity within crop species (Gupta *et al.*, 1996), due to their high polymorphism, abundance and co-dominant inheritance. In *Prunus*, most SSRs used for fingerprinting have been developed in peach (*P. persica* (L.) Batsch) and sweet cherry (*P. avium* L.) (Cipriani *et al.*, 1999) and have been used successfully for molecular characterization and genetic similarity of genotypes in several *Prunus* species, mainly peach (Aranzana *et al.*, 2002).
- **Single Nucleotide Polymorphism (SNP) markers** are the most abundant forms of genetic

variation among individuals of the same species. Even if they are less polymorphic than SSR markers because of their biallelic nature, they are abundant, and amenable to high- and ultra-high-throughput automation. However, despite these obvious advantages, to date such automation is only available in few plant species.

6.3 Introduction to Marker-assisted Breeding

In recent years the concept of marker-assisted breeding (MAB) has expanded and includes all the activities of a breeding programme that uses molecular markers. These activities include mainly: (i) germplasm characterization; (ii) MAS; (iii) marker-assisted introgression (MAI); and (iv) genetic profiling to protect the plant variety intellectual property rights. In this section we will focus on the state of the art of MAS and MAI in almond.

MAS refers to the selection of plants based on genotypic information that can be obtained in the very early stages of plant development. This is, in fact, the main advantage in respect of conventional selection especially in tree species like almond with long intergenerational periods. Fruit traits cannot be phenotyped until trees have reached the adult stage, between 3–5 years after germination in the case of almond. The convenience of using MAS will depend basically on the genetics of the traits under analysis and on the methodology used to measure. For the genetics we can differentiate between qualitative and quantitative traits, and for the methodology used to measure we can differentiate between easy traits or complex traits. The more complex and difficult a trait is to evaluate, the more convenient it is to use MAS. It could save resources even for qualitative traits that are easy to phenotype (Edge-Garza and Peace, 2010) and these resources could be dedicated to screening more seedlings, better evaluating the remaining seedlings or to decreasing the funding necessary to run the breeding programme.

Since the first application of isozyme markers in MAS in the 1980s (Tanksley, 1983), the development of molecular markers and genotypic technologies has evolved quickly, increasing the

number of markers available and decreasing the costs and time needed to generate them. Although marker development is not a limiting step any more, the implementation of MAS is still limited, especially for species with limited economic impact and for breeding programmes with low budgets. Even in cereal species such as wheat, the application of MAS is still limited to some monogenic traits (Gupta *et al.*, 2010). In species closer to almond, such as peach, the situation is not very different and MAS is only used for monogenic traits including fruit shape and acidity (Picañol *et al.*, 2013; Eduardo *et al.*, 2014). Several factors have been identified that could explain why this is the most common situation (Xu and Crouch, 2008) and, of these, marker validation is the most difficult one. Mostly molecular markers are described in biparental populations and, when the marker must be validated in a wider germplasm of a particular breeding programme, validation often fails. Another important factor is that most important agronomic traits such as productivity are very complex, where association between genotypes and phenotypes is more difficult to determine. Last but not least, high-throughput protocols for DNA extraction and genotyping with a very robust sample and data tracking system are still lacking.

6.4 Almond Breeding and Molecular Markers

6.4.1 MAS in almond: state of the art

Almond belongs to the *Prunus* genus, for which several genomic tools are available. One of the most remarkable tools has been the *Prunus* reference map, constructed in an interspecific population of a cross between almond and peach (Joobeur *et al.*, 1998). In 2004 the *Prunus* reference map was further saturated with transferable markers, allowing comparison of the genetic maps of seven diploid *Prunus* species that were found to be highly colinear, and the integration of mapping information of 28 major genes segregating in different *Prunus* species including almond (Dirlewanger *et al.*, 2004a). Since then the *Prunus* reference map has been used for different purposes: to generate new markers to further saturate regions of interest in the genome; to add

more traits mapped in different populations to a common map; to efficiently map candidate genes, especially in combination with the bin mapping strategy; and to anchor the sequenced genome (Verde *et al.*, 2013). Before this map was available only gametophytic self-incompatibility was used in breeding programmes for MAS in almond, apricot and cherry. In species such as peach, other monogenic traits such as the flat fruit shape or subacid fruit are being incorporated into breeding programmes (Picañol *et al.*, 2013; Eduardo *et al.*, 2014). The major genes mapped to date in interspecific almond progenies include self-incompatibility (*S*; Ballester *et al.*, 1998; Fernández i Martí *et al.*, 2011), shell hardness (*D*; Arús *et al.*, 1999), late blooming (*Lb*; Ballester *et al.*, 2001) and kernel taste (*Sw*; Bliss *et al.*, 2002; Sánchez-Pérez *et al.*, 2010). Almond breeders could use this information to implement MAS for these traits. Information for other major genes mapped in interspecific populations is also available; most are not among the main traits used by almond breeders although could be of interest for other species such as peach. These traits include anther colour (*Ag*; Joobeur, 1998), flower colour (*B*; Jáuregui, 1998) and root-knot nematode resistance (Dirlewanger *et al.*, 2004b; Duval *et al.*, 2014).

Mapping information for some quantitative traits is also available, including several agronomics traits such as productivity, maturity date or double kernels (Sánchez-Pérez *et al.*, 2007), kernel composition (Font i Forcada *et al.*, 2012) and physical nut traits (Fernández i Martí *et al.*, 2013). In this case further validations using more cultivars would be necessary before MAS could be directly implemented in almond breeding programmes. Most of these traits have been mapped using biparental populations representing a small amount of the allelic variation present in the almond germplasm, therefore more populations using different parents could be analysed or different approaches taking into account a major number of cultivars, as association mapping (AM) or genomic selection, could be explored.

6.4.2 MAI

Originally the concept of MAI consisted of the identification of a small chromosomal region

defined by two molecular markers that contains a favourable allele from an inferior cultivar, and the introduction of only this fragment into an elite cultivar (Visscher *et al.*, 1996). We will include in this concept the development of introgression line (IL) or near-isogenic line (NIL) collections. In these types of populations each individual contains a unique chromosome fragment of a donor cultivar in the genetic background of a recipient cultivar, and all the fragments from each individual represent all the genome from the donor cultivar (Eshed and Zamir, 1994). Generally donor cultivars are exotic cultivars or sexually compatible species and the recipient cultivars are commercial cultivars. IL collections can simplify quantitative trait loci (QTLs) mapping and could be considered as pre-breeding material to efficiently introgress exotic favourable alleles in commercial cultivars. The first IL population was developed in tomato (Eshed and Zamir, 1994) and more populations have been developed in other species.

Interspecific crosses involving almond have been obtained with several other species. For example, in California self-compatibility has been introduced in almond cultivars from species such as *P. persica*, *P. mira*, *P. davidiana* and *P. webbii* (Gradziel, 2009). Interspecific crosses have also been used to introduce root-knot nematode resistance genes into peach using MAI (Dirlewanger *et al.*, 2004b; Duval *et al.*, 2014). An IL population derived from the cross between the almond cultivar 'Texas' as a donor and the peach cultivar 'Earlygold' as the recipient has been started recently (Donoso, 2014). Using this population as proof of concept, an efficient breeding scheme for introgressing traits into peach from other *Prunus* species using MAI has been proposed (Donoso, 2014). This scheme is based on MAS of individuals having three or less genomic fragments of the donor genome in the BC1 generation. To do this a large BC1 population having between 800 and 1500 individuals must be generated.

6.4.3 Molecular tools in breeding (QTL versus AM)

Linkage maps have been used for identifying chromosomal regions that contain genes controlling simple traits (single gene) and quantitative

traits using QTL analysis (QTL mapping) (Mohan *et al.*, 1997; Collard *et al.*, 2005). By using the presence or absence of molecular markers in place of or in addition to phenotypic selection, crop improvements are more effective, efficient, reliable and cost effective than with conventional breeding alone (Collard *et al.*, 2005). In QTL mapping, once tight linkages between markers and QTL are established, these can be used as tools for trait diagnosis. Similarly, for mapping simply inherited traits, establishing tight linkage between the marker and the trait can aid in trait diagnosis. MAB provides breeders several advantages: (i) selection is performed more efficiently by eliminating variable phenotypic evaluations that vary due to environmental effects; (ii) the technique saves time by exchanging multiple-year replicated field trials for molecular tests in the early stages of evaluation and selection at the seedling stage; and (iii) the technique reduces linkage drag by minimizing transfer of undesirable alleles in backcrossing practices. Saving time is not only a crucial factor in herbaceous crop improvement, but is even more important in woody perennials due to their long juvenile phases (Dirlewanger *et al.*, 2004a). Theoretically, selection for a single QTL can be beneficial in plant breeding when such a QTL accounts for the largest proportion of phenotypic variance (Tanksley, 1993; Ribaut and Betrán, 1999). This is supported by reports that polygenic traits can be selected using qualitative traits as selectable markers (Tanksley, 1993). Therefore, a single QTL with a relatively large proportion of variance can be treated as a qualitative trait and can have a positive effect when marker selection is applied. Although, theoretically, all tightly linked QTLs can be used in MAB, no more than three closely linked QTLs are typically used because of the cost of using such technologies (Ribaut and Betrán, 1999; Collard *et al.*, 2005).

QTLs in almond

Traditionally, QTL mapping has been used as a methodology for gaining understanding of the genetic control of polygenic traits and has been useful for identifying QTLs in different species, including almond (Sánchez-Pérez *et al.*, 2007; Fernández i Martí *et al.*, 2011; Font i Forcada *et al.*, 2012; Fernández i Martí *et al.*, 2012). However, linkage mapping strategies are limited

in detecting loci underlying QTLs because, commonly, only two extreme parents are used for generating the segregating population, and only a few recombination events are studied (Flint-Garcia *et al.*, 2003). The study carried out by Sánchez-Pérez *et al.* (2007) involved 11 QTLs controlling agronomic traits in almond, in an F_1 almond progeny of 167 seedlings from a cross between the French selection 'R1000' and the Spanish cultivar 'Desmayo Largueta'. The main traits were blooming date, blooming density, productivity, leafing date, in-shell weight, double kernels and shell hardness. This map allowed the detection of 10 QTLs and two major genes, most of them being main traits studied in breeding programmes. More recently, the availability of the almond cross 'Vivot' $\times$ 'Blanquerna' ('V $\times$ B') made it possible to generate a linkage map of this population (Fernández i Martí *et al.*, 2011; Font i Forcada *et al.*, 2012; Fernández i Martí *et al.*, 2013), as well as to determine 20 physical parameters of nuts and kernels. The traits analysed were width (W), thickness (T), length (L), weight, geometric diameter (GD, $(L \times W \times T)^{1/3}$), spherical index (SI, GD/L), size ($L \times W \times T$), and the L/W, T/L and W/L ratios, for both nut and kernel. Eight QTLs controlling primary dimension in nuts (width, thickness and length) were detected in LG (linkage group) 1, LG2, LG3, LG5, LG6 and LG7. In addition, eight QTLs were detected for the same kernel traits in LG1, LG3, LG5, LG6 and LG7. Another three QTLs were detected for nut weight and size at the beginning of LG1, LG2 and LG7. For kernel weight and size, not only was the same QTL detected in LG7, but a new QTL for kernel weight was also detected in LG7. A total of ten QTLs were detected for derived dimensions such as spherical index, geometric diameter, and the L/W, T/L and W/L ratios, both in the nut and the kernel (Fig. 6.1).

Font i Forcada *et al.* (2012) carried out the first attempt to identify QTLs controlling nut quality traits. Two QTLs controlling the total protein content were detected in LG6 and LG7. For fatty acid composition, seven QTLs were located in the first seven LG. The QTLs for oleic acid concentration (C18:1) were mapped on LG2. For linoleic acid (C18:2), the QTLs were found in the same position as those detected for oleic acid. For palmitic acid (C16:0), once again two QTLs were detected: one in LG7 and another at the beginning of LG3. For stearic acid (C18:0), four QTLs

were identified in LG1, LG5, LG6 and LG7, with the first three not yet identified for the other fatty acids. It is significant that a QTL in LG2 affects the two main fatty acids, oleic and linoleic, and that another in LG7 affects all five fatty acids studied, thus giving a genetic basis for the correlation between the different fatty acids inside the lipid pool. Additionally, a total of five different QTLs were detected using the interval mapping approach for the α -, γ - and δ -tocopherol homologues. Two QTLs for α -tocopherol were located in LG1 and LG4 and a second QTL was detected at the end of LG4. For γ -tocopherol, four QTLs were detected; two in the same position as for α -tocopherol, and two newly identified also in LG1 and LG4. For the third tocopherol homologue, δ , three QTLs were located. Two coincided with those found for α and γ -tocopherol, although an additional QTL was located in LG7 (Fig. 6.2).

AM in almond

As an alternative to analysis in controlled crosses, AM in unstructured and complex populations is now being applied to many crops. This approach relies on the strength of association between genetic markers and phenotype. Genetic resources consist of a large number of accessions with different histories, mutations and recombination events, and may represent a large reservoir of phenotypic and molecular diversity. Thus, AM detects and locates genes relative to an existing map of genetic markers. While linkage analysis searches for associations within populations developed from biparental crosses, AM uses unstructured or loosely structured populations (e.g. a collection of varieties or landraces), case-control designs and transmission disequilibrium tests (Oraguzie and Wilcox, 2007). Consequently, this method detects relationships between phenotypic variation and gene polymorphism in existing germplasm and in unrelated individuals. The effectiveness of this approach is greater when information on population structure and LD is available (Cao *et al.*, 2012). Since many important crops have complex population structures which arose from a long history of domestication and breeding (Flint-Garcia *et al.*, 2003), understanding these structures is important to avoid identifying spurious associations in AM. In addition, AM complements and enhances previous QTL

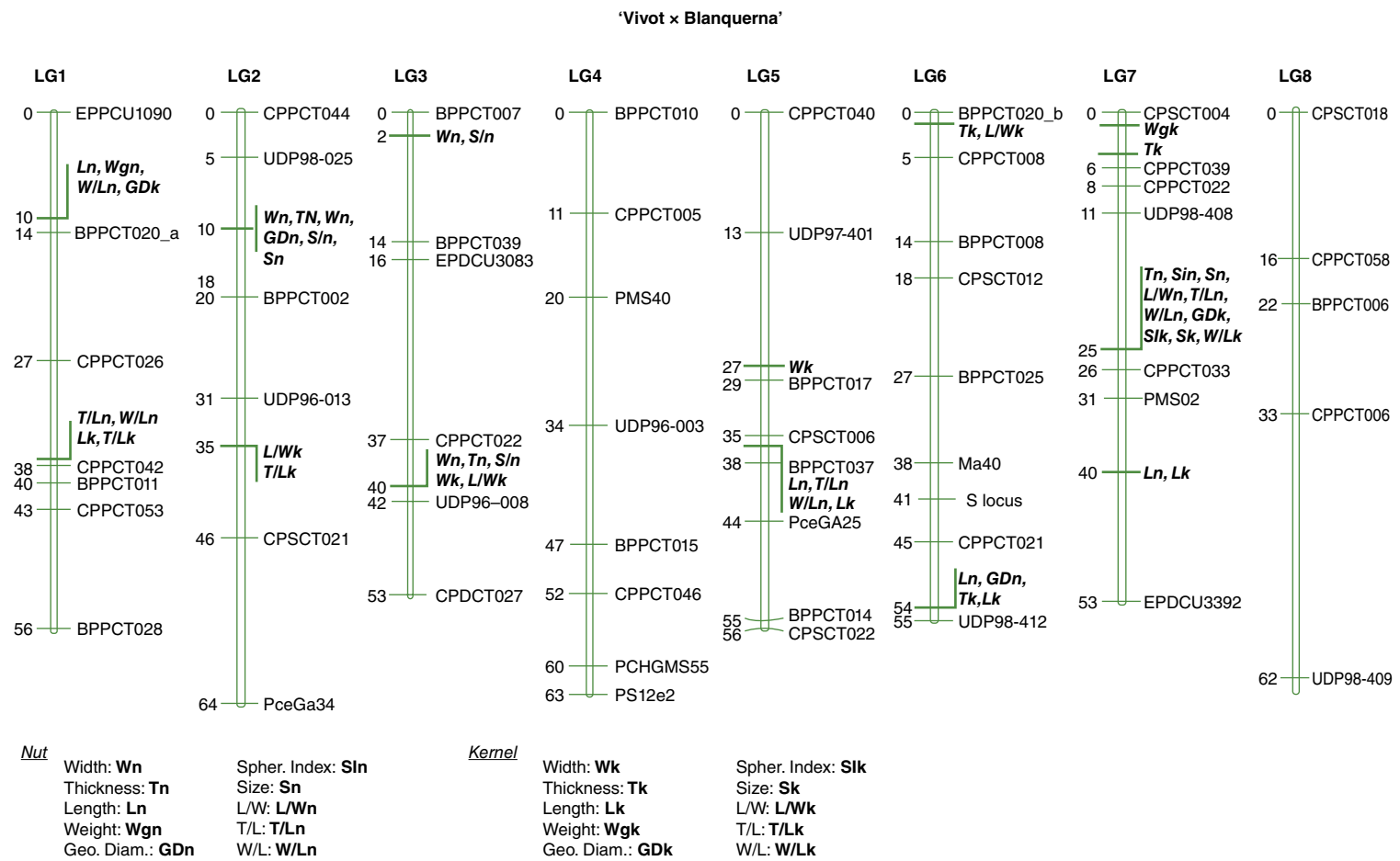


Fig. 6.1. Combined linkage map of 'Vivot' × 'Blanquerna' population constructed using MAPCHART V. 2.1 (Voorrips, 2000) showing putative quantitative trait loci (QTLs) associated with physical nut and kernel traits in almond (Fernández i Martí *et al.*, 2013).

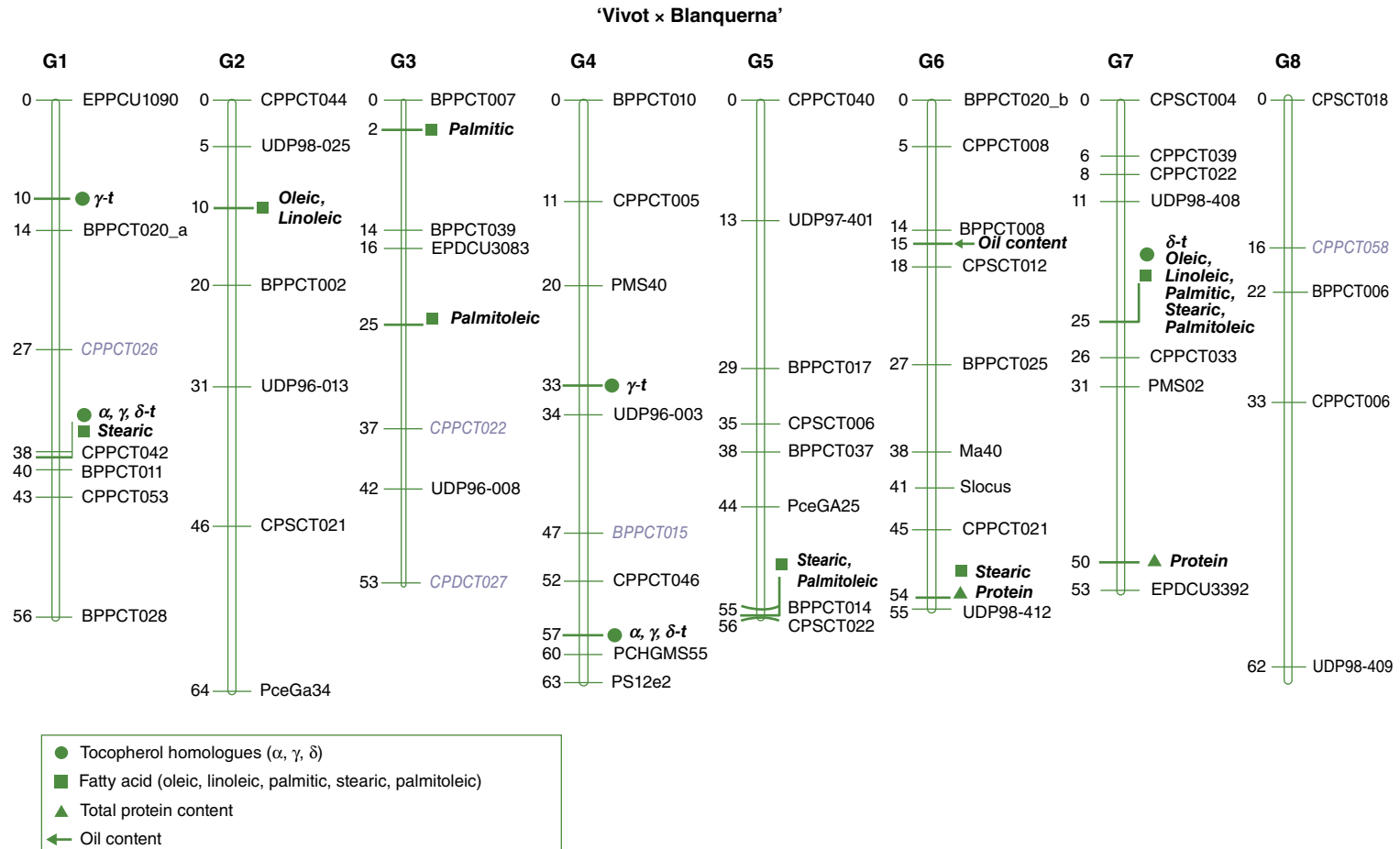


Fig. 6.2. Combined linkage map showing the location of quantitative trait loci (QTLs) for chemical composition of the almond kernel. Combined linkage map of the 'Vivot' × 'Blanquerna' population constructed using MAPCHART V. 2.1 (Voorrips, 2002) with the location of the QTLs related to the chemical composition of the almond kernel. The newly incorporated markers are in blue italics (Font i Forcada *et al.*, 2012).

information by incorporating the effects of recombination occurring in many past generations into a single analysis (Jorde, 2000).

AM has been used successfully to identify genes involved in several traits in different plant species (maize, sunflower, lettuce, potato and wheat), but only a few studies have been carried out in fruit tree crops such as peach (Cao *et al.*, 2012; Font i Forcada *et al.*, 2013), sweet cherry (Ganopoulos *et al.*, 2011) and apple (Khan and Korban, 2012). In general, AM is more suited to organisms with little or no pedigree information and to populations with rich allelic diversity, moderate to high nucleotide diversity and traits with little or no selection history, and which are controlled by many loci with small effects and lower frequencies of older alleles (Oraguzie *et al.*, 2007). All these conditions are common in vegetatively propagated fruit trees, such as almond, with a high level of heterosis and a short breeding history (Socias i Company *et al.*, 2012).

In almond, two unique studies (Font i Forcada *et al.*, 2015a, b) have been carried out to determine physical and chemical traits in a germplasm collection of 98 almond cultivars encompassing a wide range of geographic origins (Europe, USA, Argentina, Australia and North Africa). To determine population structure and/or identify the number of subgroups in the almond germplasm, different methodologies and software packages were employed and compared. For quantitative assessment of the number of groups in the panel, a Bayesian clustering analysis was performed using a model-based approach implemented in the software package STRUCTURE v 2.3 (Pritchard *et al.*, 2000). In a second approach, principal component analysis (PCA) based on the dissimilarity matrix was performed using TASSEL (Yu and Buckler, 2006). The hypothesis for associating each molecular marker with the trait of interest was tested using the software TASSEL. Different statistical models were used to calculate *P*-values, along with accounting for population structure to avoid spurious associations. Five models comprising both general linear models (GLM) and mixed linear models (MLM) were selected to test for marker–trait associations. The following models were tested: (i) naïve model: GLM without any correction for population structure; (ii) Q-model: GLM with Q-matrix as correction for population structure; (iii) P-model: GLM

with PCs as correction for population structure; (iv) QK-model: MLM with Q-matrix and K-matrix as correction for population structure and kinship relationships; and (v) K-model: MLM with K-matrix as correction for kinship relationships structure. The mean value of the markers at $P < 0.005$ was used for determining the significance of marker–trait associations. The AM was focused on all LGs on the *Prunus* reference map. Significant markers were identified using the Bonferroni procedure at the $P < 0.00125$ experimental-wide threshold. Alleles with a frequency (MAF) lower than 5% were removed before data analysis.

The variation observed in the chemical and physical traits used in these studies was high, thus confirming the diversity in the almond germplasm and the representativeness of the gene pool. The traits analysed were nut width, nut thickness, nut length, nut weight, nut T/L ratio, double kernels, kernel width, protein content, oil content, percentages of the main five fatty acids (oleic, linoleic, linoleic, stearic, palmitic and palmitoleic), content of the main three tocopherol homologues (α , δ and γ) and content of the main phytosterol compounds (campesterol, stigmasterol, Δ^7 -campesterol, cleros-terol, β -sitosterol, Δ^5 -avenasterol, Δ^7 -stigmasterol and Δ^7 -avenasterol).

For the phytosterol content, seven markers revealed that several putative genomic regions are involved in the expression of these phenotypes. To confirm the candidate genes and the marker location for the phytosterol traits, the genes belonging to the plant sterol biosynthesis pathway and close to the associated markers were screened using the browser *P. persica* genome sequence data (<http://www.rosaceae.org>). Interestingly, all the associations found in that work and located on LG1, LG2, LG4, LG6 and LG7 appeared to map within the interval where many candidate genes involved in the sterol biosynthesis pathway are predicted in the peach genome. As an example, the candidate gene ppa1027202m was strongly correlated with all the sterol traits analysed in that study (Font i Forcada *et al.*, 2015b). This gene spans the region where PMS40, CPDCT045 and UDP96-003 map on LG4 and has been associated with cytochrome P450, which encodes the sterol C-22 sterol desaturase.

Regarding the other chemical traits, a total of 16 associations were found between four DNA

markers and nine chemical traits phenotyped (Font i Forcada *et al.*, 2015a). For example, the SSR BPPCT011 located on LG1 was associated with α -tocopherol, δ -tocopherol, γ -tocopherol and stearic acid. The map positions for these marker–trait associations are in agreement with a previous study (Font i Forcada *et al.*, 2012), which reported a QTL mapped close to BP-PCT011, and are also associated with three tocopherol compounds and stearic acid. Additional associations were found between γ -tocopherol and UDP96-003 on LG4. This association is also in agreement with the QTL identified for the same traits by Font i Forcada *et al.* (2012). Similarly, protein content showed association with the locus EPDCT3392 located on LG7, which was in agreement with the QTL found at the same position by Font i Forcada *et al.* (2012). In addition, Font i Forcada *et al.* (2015b) identified other loci associated with δ -tocopherol, γ -tocopherol, oleic, linoleic, stearic and palmitic acids, and protein content on LG3 (CPDCT025); and oleic, linoleic and oil content on LG4 (UDP96-003) that were not reported by Font i Forcada *et al.* (2012). The discrepancies in marker–locus trait associations between the two studies could be attributed to the number of marker loci used. Font i Forcada *et al.* (2012) proposed candidate genes including Acyl-CoA (controlling the synthesis of long-chain saturated fatty acids), Enoyl-CoA hydratase (controlling the two main fatty acids such as oleic and linoleic) or ACP (controlling stearic acid) for the fine mapping of almond quality components. In agreement with that study, the association found between α -tocopherol, δ -tocopherol, γ -tocopherol and stearic acid and the marker BPPCT011 on LG1 (Font i Forcada *et al.*, 2015a) also appears to map within the interval of the Acyl-CoA gene (ppa002255m).

A total of 12 associations related to physical nut and kernel traits have been identified by Font i Forcada *et al.* (2015b). One was between the marker CPDCT006 and nut length, nut T/L ratio and kernel length, and has been corroborated by the QTL identified on LG5 (CPDCT006) by Fernández i Martí *et al.* (2013). The other association was between the marker BPPCT011 (LG1) and the nut T/L ratio and kernel length. This association is located close to the QTL (CPPCT042) for the same trait found on LG1 by Fernández i Martí *et al.* (2013).

Locus CPPCT021 (LG6), associated with nut length and thickness and kernel length, agrees with the QTL found in the same position by Fernández i Martí *et al.* (2013).

The results found by Font i Forcada *et al.* (2015a, b) will allow for improved efficiency in breeding for quality in almond and, in particular, the simultaneous selection for physical and chemical traits. These studies also identified many new marker–locus trait associations, which may be useful for predictions of genetic progress in a breeding programme. These associations would provide an efficient platform for classical and MAS breeding in almond and these results demonstrated that AM using a reasonable number of markers, distributed across the genome, could be very useful in detecting significant complex traits of interest.

6.4.4 Important traits well studied at the molecular level: bitterness

The original almond tasted bitter. The first studies about the genetic control of bitterness are from the 1920s (Heppner, 1923; Heppner, 1926). These studies suggested that bitterness was a monogenic trait, that sweetness was dominant over bitterness and that the most frequent genotype is heterozygous. Moreover, Heppner defended the view that sweetness appeared as a consequence of a mutation in the original bitter almond.

Human domestication has resulted in low numbers of bitter varieties, and most cultivated almonds are sweet. However, when sweet heterozygous varieties are crossed in the breeding programmes, 25% of the offspring are bitter and so eliminated from the selection process. This fact, which greatly reduces the efficiency of breeding programmes, is especially predominant when obtaining self-compatible varieties, since most self-compatible progenitors are heterozygous for this trait (Grasselly and Crossa-Raynaud, 1980; Dicenta, 1991). An extreme example occurred in American breeding programmes using the self-compatible species *P. webbii* as genitor, as this led to 50% of progeny having bitter seeds when the other parent was heterozygous (Ledbetter and Pyntea, 2000). Over the years, these crosses have allowed classification of numerous varieties

as homozygous (sweet or bitter) or heterozygous (sweet or slightly bitter) (Grasselly and Crossa-Raynaud, 1980; Dicenta, 1991; Vargas *et al.*, 2001; Dicenta *et al.*, 2007).

In almond the sweet kernel taste versus the bitter taste has been the first trait considered in breeding, from the days of the traditional growers to the present breeding programmes (Socias i Company, 1998). These bitter seedlings are generally discarded, although occasionally they have been selected for their high productivity (Spiegel-Roy and Weinbaum, 1985). From an ecological point of view, the bitter taste of the kernel assures the embryo protection from insects and animals, guaranteeing the permanence of the species (Jones, 1979; Kakes, 1990; Vetter, 2000). On the other hand, some varieties contain a slightly bitter taste that is really appreciated (Kester and Asay, 1975), and the Spanish cultivar 'Garrigues' is a good example of this.

Bitterness in almond is due to the accumulation of the cyanogenic diglucoside amygdalin (McCarty *et al.*, 1952). When tissue containing amygdalin or its precursor prunasin is disrupted by herbivores or pathogens, the cyanogenic glucosides are degraded by the action of three different enzymes: the β -glucosidases amygdalin hydrolase and prunasin hydrolase, and mandelonitrile lyase. This is followed by the release of glucose, hydrogen cyanide (toxic) and benzaldehyde (which is responsible for the bitter flavour) (Evreinoff, 1952). In a study to characterize the flavour phenotypes of almond, two compounds were found to be involved in bitterness: benzyl alcohol and 2,3-butanediol (Wirthensohn *et al.*, 2008).

During ripening prunasin is first detected in the tegument/seed coat (Sánchez-Pérez *et al.*, 2008) and, with amygdalin, in the nucella and endosperm and finally in the cotyledon. Amygdalin is present in very low amounts in sweet and slightly bitter cultivars and up to 1000-fold times higher in bitter cultivars (Frehner *et al.*, 1990; Dicenta *et al.*, 2002; Franks *et al.*, 2008; Sánchez-Pérez *et al.*, 2008). Some studies have suggested that the bitterness *Sk* (*Sweet kernel*) gene could be related to the degradation pathway. This has been studied before (Sánchez-Pérez *et al.*, 2008; Sánchez-Pérez *et al.*, 2012a), showing that if prunasin hydrolases were localized differently in the tegument of sweet compared to bitter genotypes, different concentrations of prunasin would

be available in the seed for amygdalin synthesis, determining whether the mature almond will be bitter or sweet. Further studies are needed to confirm if prunasin hydrolase is the gene responsible for bitterness in almond.

Moreover, the determination of sweetness or bitterness depends on the genetic background of the sporophyte. In other words, all fruits of an almond tree will have either sweet or bitter kernels (Kester and Asay, 1975; Spiegel-Roy, 1979; Grasselly and Crossa-Raynaud, 1980); thus, the influence of both genitors can only be shown in the following generation (Dicenta *et al.*, 2000; Sánchez-Pérez *et al.*, 2010). However, in some years, the slightly bitter taste in slightly bitter varieties is not detectable (Kester *et al.*, 1977; Arrázola, 2002), even though this trait is maternally inherited, and is not influenced by the environment (Spiegel-Roy and Kochba, 1974; Kester *et al.*, 1977; Sánchez-Pérez *et al.*, 2012b). This slight variation can be even noticed among almonds belonging to the same tree in 1 year, suggesting that the slightly bitter taste is controlled by other genes not related to bitterness in almond. In addition, Dicenta and García (1993) observed that the slightly bitter seedlings were more abundant when one of the parents had a slightly bitter taste.

Almond and other fruit crops are woody perennial species with a long intergeneration period because of the long juvenile phase (Socias i Company, 1998), so breeders must wait at least 3–5 years to evaluate fruit traits in the field. For this reason, it is important to develop molecular markers to distinguish between sweet and bitter genotypes in the nursery. Kernel bitterness is determined by the *Sk* locus that has been located in linkage group five (LG5) in an almond genetic linkage map (Joobeur *et al.*, 1998; Sánchez-Pérez *et al.*, 2007). However, the *Sk* function remains unknown. Two RFLP molecular markers (FG202 and AG46) were identified close to the *Sk* locus (Joobeur *et al.*, 1998). However, RFLPs are not adequate for routine plant-breeding applications due to the high-quality amounts of DNA (μ g) that are needed and the requirement for a hybridization step using probes against genomic DNA. Instead, SSRs require smaller amounts of DNA (ng) and a simple PCR reaction more appropriate to MAB. Several SSRs were mapped in the *Prunus* reference linkage map of the interspecific cross between an almond

and a peach ('Texas' × 'Earlygold', 'T×E'), in the region of LG5 where *Sk* is located (Dirlewanger *et al.*, 2004a; Howad *et al.*, 2005). These markers were studied in the almond population 'R1000' × 'Desmayo Largueta' ('R×D') segregating for the bitterness trait (Sánchez-Pérez *et al.*, 2007). Later, six more markers (UDA-045, EPDCU2584, CPDCT028, BPPCT037, PceGA025 and CPDCT016) were added to saturate the area surrounding the *Sk* locus (Sánchez-Pérez *et al.*, 2010). When they measured the efficiency of MAS in the 'R×D' population by the EC coefficient (EC = the number of correctly classified plants per total; maximum efficiency is EC = 1), they found that selecting against the alleles associated with the *Sk* allele in only one marker, the EC ranged from 93.5% to 96%. However, when they chose two markers, one above and one below the *Sk* locus (BPPCT037 and PceGA025), the EC was 1. Furthermore, four of the six SSRs flanking the *Sk* locus were analysed in a number of parents from different almond breeding programmes, including 'Nonpareil' (USA) and 'Lauranne' (France), and in a few seedlings of each cross. This helped to estimate the haplotypes of the most-used parents of the CEBAS-CSIC (Murcia, Spain) almond breeding programme (e.g. 'Desmayo Largueta', 'R1000', 'Tuono', 'Garrigues', 'Marta' and 'Antoñeta'), allowing the use of these SSRs for selection against bitter individuals at the seedling stage, without having to wait for 3 years for this phenotype to be identified in the almond kernel. Nevertheless, the *Sk* locus remains to be identified for its use in any other crosses in which a pre-study of these six markers is not available.

Candidate genes involved in the amygdalin pathway have been also mapped, one AH at the top of LG1, one PH at the bottom of LG1, two UDP-glucosyltransferases (one in LG3 and the other in LG8) and CYP79D16 at top of LG6 (Wu *et al.*, 2010), so none of them was located on LG5 (Sánchez-Pérez *et al.*, 2010).

Recently, a reference mapping approach using the peach genome v1.0 as the reference genome and high-throughput sequencing from four almond accessions (two sweets and two bitters) were used to identify the *Sk* locus, among other interesting traits in almond (Koepke *et al.*, 2013). With this study, more than 300,000 polymorphisms were found in chromosome 5, from which 56,000 were localized in a 3.6-Mb

region where *Sk* is localized. Interestingly, only 228 SNP candidates had a codon change in the two sweets versus the two bitter cultivars. Further studies should be focused on the identification of which SNP, if any, is responsible for bitterness in almond.

6.5 SNP Markers and HRM as Promising New Tools for Molecular Breeding

SNP comprises DNA sequence variations in a population or species that involves the polymorphism of only a single nucleotide in the genome. The mark system has increasingly been applied in a variety of research areas including association studies (Ohnishi *et al.*, 2001), biodiversity assessment (van Tienderen *et al.*, 2002), genetic map construction (Batley and Edwards, 2007) and molecular breeding (Chen *et al.*, 2011). The SNPs are valuable and efficient molecular markers as these have been demonstrated to be the most abundant of all the classes of molecular markers (Gupta *et al.*, 2002; Hayashi *et al.*, 2004), and the mutations in the coding regions may be valuable for the identification of genes related to important traits such as diseases (Thomas and Kejariwal, 2004; Liao and Lee, 2010). Furthermore, advances in SNP detection techniques allow high-throughput assays and consequently high-density genome-wide scans by SNP microarray and deep sequencing (Gunderson *et al.*, 2005; Davey *et al.*, 2011). High resolution melting (HRM) analysis has been developed to detect SNPs in small PCR amplicons in the past decade because it is simple to operate and low-cost compared to other methods (Hoffmann *et al.*, 2007; Liew *et al.*, 2007; Hung *et al.*, 2008). In *Prunus*, a few studies reported the conversion of RFLP markers to SNPs in *P. mume* (Fang *et al.*, 2006) and the use of six candidate gene SNPs to map blooming time in almonds (Silva *et al.*, 2005). Wu *et al.* (2008) developed a set of SNPs in almond using HRM based on predicated SNPs from assembled ESTs, and validated in a population of 25 cultivars. A few SNPs associated with complex haplotypes were also analysed with the same method by the same group (Wu *et al.*, 2010). Later, the SNPs were used for the construction of a linkage map in a cross

between 'Nonpareil' and 'Lauranne' (Wu *et al.*, 2009; Tavassolian *et al.*, 2010). With the technological progress of next-generation sequencing (NGS) large-scale identification of SNPs in the *Prunus* genome has become possible (Koepeke *et al.*, 2013). There is no doubt that increasing numbers of SNPs will be developed through NGS in almond and the genus *Prunus*, and that different genotyping technologies will be used for the analysis of SNPs in the taxa depending on the purpose of the analysis.

HRM analysis is a powerful tool for the analysis of DNA polymorphisms including single nucleotide mutations (Chou *et al.*, 2005), INDEL markers (Wu *et al.*, 2008) and microsatellite polymorphisms (Mackay *et al.*, 2008; Distefano *et al.*, 2012). It can also be used for the detection of epigenetic differences, mainly DNA methylations (Wojdacz *et al.*, 2008a, b). This technique was first reported in 2003 with the successful identification of all four possible SNP base changes in the engineered plasmid amplicons and clinically important genes (Gundry *et al.*, 2003), and has since been applied in a variety of areas with substantial improvement of the technology. It has advantages over other technologies for the genotyping of SNPs and other variations in the genome, as HRM is more cost effective, more efficient and easier to operate. In almonds, Wu *et al.* (2008) assembled 3864 almond EST sequences obtained from the NCBI Genebank EST database, and 17 EST contigs were predicted to have SNPs out of 276 contigs assembled. Additionally, ten EST contigs with putative SNPs were selected from a *Prunus* SNP database at ESTree (<http://www.itb.cnr.it/estree/>), and all 27 EST contig sequences were used for validation and/or identification of SNPs by HRM analysis. From the above 27 EST contigs, 15 were demonstrated to have polymorphic melting curves when assayed against 25 almond cultivars. Based on subsequent sequencing analysis, the polymorphisms identified by HRM analysis were revealed to be SNPs, INDELs and microsatellite variations. Altogether, 17 PCR amplicons were polymorphic in the population assessed, and 109 SNPs, INDELs or microsatellites were reported in the study and deposited in the NCBI dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP/>) with the RefSNP(rs#) IDs of rs118204549–rs118204657.

Among the identified SNPs, all four classes of base changes were present and resolved by

HRM analysis. In the HRM assay, the curves of heterozygous genotypes are distinctive from those of homozygous genotypes, and the homozygous G/C, G/A or C/A genotypes are also relatively easy to differentiate from each other. However, the homozygous individuals of A/T variation were considered the most difficult SNP to detect (Liew *et al.*, 2004). In the above study, HRM analysis was able to separate all the homozygous genotypes (including A/T homozygous cultivars) from each other as well as from heterozygous genotypes (Wu *et al.*, 2008).

In subsequent studies the SNPs and other markers identified were applied in cultivar clustering and linkage mapping constructions. Within the 25 cultivars involved in the clustering study, markers in the 17 genic fragments were able to resolve the genetic differences between all the cultivars but two: 'Price' and 'Pethick Wonder'. The almond populations were clustered into six groups, with most of the Australian cultivars and most of the French cultivars clustered in the same group, while the Californian cultivars were present in five of the six groups. In a mapping study, 12 of 15 ESTs containing these polymorphic markers in a crossed population of 'Nonpareil' × 'Lauranne' were blasted against known proteins in the public databases, and these genes were then mapped to the almond genome (Wu *et al.*, 2009). HRM genotyping of the population resulted in the alleles segregating following Mendelian segregations as 1:1 or 1:2:1 depending on the heterozygosity of the parents. These genes were located in six of the eight linkage groups with two functionally similar genes, a stress-responsive gene *pdDhn3* and a detoxification gene *pdMT2*, mapped closely on linkage group 6 at a distance of 0.4 cM. Together with other molecular markers (mainly microsatellites and RAPDs) these markers were used to construct a moderately saturated almond linkage map (Tavassolian *et al.*, 2010).

In addition to the analyses of SNPs in different species, HRM has also been used as an alternative to electrophoresis-based assay of microsatellites (Muleo *et al.*, 2009; Costa *et al.*, 2010; Distefano *et al.*, 2012). The merit of using HRM to analyse microsatellites is that it can detect unknown SNPs present in the microsatellite flanking sequences; these are otherwise undetectable with any electrophoresis-based approaches including capillary electrophoresis. In citrus, for

example, it has been proved that HRM can not only produce distinct polymorphic melting curves among the cultivars tested, due to microsatellite variations, but also show more genotypes than the capillary electrophoresis analysis results owing to the presence of SNPs in the amplicon sequences flanking microsatellites (Distefano *et al.*, 2012). Hence, this technique could be applied to the genotyping of almond populations.

Since the release of the peach draft genome in 2010 (Verde *et al.*, 2013), attempts to sequence almond genomes have been made aiming at the comparisons between *Prunus* genomes and the identification of intraspecies variations. Koepke *et al.* (2013) partially sequenced four almond accessions. They detected 260,000 polymorphisms in the coding sequence for the almond accessions and 52.1% and 43.3% of sense and mis-sense mutations, respectively, were predicted in the exons of cDNA. This sequencing result is a valuable resource for SNP analysis in other almond populations and will greatly assist the molecular breeding process and biodiversity studies in the species. Further to this first almond genome sequencing effort, whole-genome resequencing of more genotypes representative of cultivars grown in different regions worldwide has been proposed and the work is under way (Almond Genome Consortium, P. Arús and Á. Fernández i Martí, pers. comm., July 2014). It is expected that more variations will be revealed, especially SNPs, following such resequencing efforts, as more genotypes of the species will be involved. Additionally, the sequencing outcomes produced from transcriptomic analysis (Mousavi *et al.*, 2014) can also be potentially useful in identifying SNPs in almonds. Combinations of these SNP detection methods (via the SNP database) through deep sequencing, and more useful applications of the markers, will markedly facilitate the development of new varieties and efficiently use the germplasm resources in almond.

6.6 Impact of New Technologies

Large-scale sequencing methods have been developed rapidly and applied widely in genomic and transcriptomic analyses, not only in model species, but also in many economically and environmentally important species. The invention

of NGS pioneered by Roche (or 454, Pyrosequencing) (Margulies *et al.*, 2005), Illumina (synthesis sequencing) (Balasubramanian *et al.*, 2004) and Applied Biosystems (SOLiD sequencing) (Valouev *et al.*, 2008) has led to enormous advances in *de novo* sequencing and resequencing of genomes in many species because of cost savings and greater bioinformatics capability. Although widely used in recent years, NGS platforms have been shown to have amplification biases produced by PCR and dephasing (or loss of synchronicity in DNA synthesis). This may produce sequencing errors, causing substantial difficulties for studies focusing on SNP discoveries, despite the many SNPs identified through this generation of sequencing platforms. A third-generation sequencing (TGS) platform using single-molecule sequencing without the need for prior amplification of a template, and so eliminating the bias imposed by NGS, was launched some years ago (Eid *et al.*, 2009). This newer generation of sequencing approaches will undoubtedly accelerate the identification of SNPs in species of interest such as almond, assisting breeders more effectively with their breeding programmes. Furthermore, the development of 4th-generation sequencing (FGS) is in progress and it is clear that this will become available in the future (Gut, 2013; Mignardi and Nilsson, 2014) in spite of some challenges (e.g. in image processing and resolution) (Niedringhaus *et al.*, 2011; Gut, 2013). Although FGS may not benefit the SNP discovery in that its advantage is to sequence DNA or RNA *in situ* in the tissue, mutation detection in tissues and potential development of biomarkers may indirectly assist plant-breeding progress, so may have longer-term potential.

SNP microarray is another relatively new approach, which can be used to assist studies in plant and animal breeding programmes. Microarray-based high-throughput SNP genotyping systems including Illumina, Affymetrix and Backman Coulter are available (Gupta *et al.*, 2008). Researchers can also produce custom-made array chips according to their study requirements and the availability of the markers in the species. The discovery of vast numbers of SNPs is expected in the future following genomic and transcriptomic sequencing, and we believe that commercially available or homemade chips will more often be developed and applied in the almond research community. The cost at

a commercial scale of a genome-wide array of a sample is now low enough to be affordable for the majority of research groups. These costs will presumably continue to decline in line with advances in the related technologies such as more efficient automation (Nagaraja and Manjunath, 2013), improvement of the reagents (Chakravarty and Chen, 2013) and enhanced ability to use pooled samples (Chiang *et al.*, 2011). Therefore, it can be expected that SNP-assisted molecular breeding, genetic mapping and biodiversity analysis in almonds will be widely employed following the rapid advances in modern molecular biological techniques such as high-throughput sequencing and microarray systems.

6.7 Conclusion

In recent decades considerable effort has been made to develop genetic and genomic tools to allow the implementation of MAS in almond breeding programmes and so improve their efficiency. One of the next steps will be publication of the genome of the cultivar 'Texas' and the resequencing of 50 other representative cultivars

from Spain, the USA, Australia and France (Almond Genome Consortium, P. Arús and Á. Fernández i Martí, pers. comm., July 2014). Although the available resources generated in peach such as the peach genome (Verde *et al.*, 2013) and the 9K IPSC peach SNP array v1 (Verde *et al.*, 2012) might be useful for almond, the availability of the almond sequence could accelerate development of resequencing projects based on NGS or TGS techniques to increase the number of markers available in almond, so allowing the exploration of more recent strategies to improve MAS for quantitative traits (e.g. association-mapping studies) (Mackay and Powell, 2007; Font i Forcada *et al.*, 2015a, b) or genomic selection (Heffner *et al.*, 2009). These strategies are already being explored in other fruit tree species such as peach (<http://www.rosbreed.org/>; <http://www.fruitbreedomics.com/>) or apple (Chagné *et al.*, 2012). NGS also allows exploration of the possibility to construct genetic maps based on the resequencing of each individual of a population, so increasing mapping resolution to the biological limits of the recombination process, as is being done in the BC1 population of the 'T×E' cross between almond and peach (Donoso *et al.*, 2015).

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7 Late-blooming Cultivar Development

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7.1 The Importance of Flowering Time in Wild and Cultivated Almonds

7.1.1 Flowering time of wild almonds

Flowering is one of the determinant processes in the adaptation of plants to the environment, most likely because of the impact of this trait on the survival of the species. During the time flowers are open, which is typically ephemeral, an interaction between the flowering plant and other plants and, in many cases, insects, occurs. The sole aim is successful pollination and seed formation. This process involves numerous factors and is the first step in ensuring the survival and expansion of the species in each environment.

In stone fruits like almond, blooming occurs once a year after the winter rest period, marking the beginning of a new cycle. Flowering is usually brief, although it is shorter in high temperatures and longer in colder climates. However, the process begins many months before with the formation of buds and the induction and breaking of dormancy. It finally culminates with the opening of flowers (Felipe, 2000; see Chapter 1).

The flowering season of almonds is not a curiosity of nature but in fact responds to specific adaptations to the area in which the variety

originated. Wild almond flowers in response to an established pattern of low and high temperatures in each area, ensuring that flowering takes place in a favourable environment for pollination and seed production. Plants that do not genetically fit the requirements in a particular area are either not pollinated efficiently or lose their flowers to frost. Such plants do not produce seeds and thus become extinct.

Moreover, most of the wild almonds are self-incompatible, so they need different almond varieties for pollination and fruit set. In fact, flowering time is largely determined genetically so that all nearby trees should flower at similar times to promote inter-pollination, produce seeds and thus propagate the species in the same area.

7.1.2 Flowering time of cultivated almonds

In the cultivated almond, flowering has a different meaning in terms of the survival of the species. In this case, the growers want trees to flower when the weather is most favourable to ensure that pollination is as effective as possible, the flowers avoid freezing and to obtain the highest number of fruits.

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The flowering time of each cultivar thus determines the environmental conditions in which flowering will occur and its impact on production. In general the earliest blooms are accompanied by less favourable weather (low temperatures, rain, wind and fog) and late blooms by more favourable weather. With respect to environmental conditions, the following aspects of flowering should be noted:

- Almond pollen is transferred through pollinating insects, so the weather should be good enough to allow bee activity (absence of rain or fog, suitable temperatures).
- Rain and high humidity impede anther dehiscence and hinder pollen movement (in the same flower and between different flowers of the same tree or from different trees).
- Due to their genetic origin, most almond cultivars are early flowering, and in many areas the flowers are lost in late frosts. In such areas it is therefore of interest to grow late-flowering cultivars that bloom when the frost risk is minimal.
- In addition, many almond cultivars are self-incompatible and need to be grown with one or more different cultivars that are genetically inter-compatible and have the same flowering season so that insects can carry pollen between them. In other words, the time of flowering determines the design of plantations with self-incompatible cultivars.

The flowering time of cultivars is therefore very important and largely determines the success of commercial exploitation.

7.2 Variability of Flowering Time in Almond

Due to the importance of the flowering season in the adaptation of the almond, the variability of this trait is a reflection of the diversity of different habitats this species has occupied throughout its expansion. This variability was originally favoured by sexual reproduction by seeds. Through natural selection, populations in colder areas have delayed flowering times to avoid frost and survive while, in warmer areas, this has not been necessary, and almond populations here have earlier flowering times.

The environment has selected the ideal flowering time in each area depending on the temperature and other factors like availability of water. Nature has performed slow but steady work from the first genotypes with similar flowering times living in the same environment. As a result of this process of adaptation, significant inter- and intraspecific variability can be found in the flowering season of wild almond trees.

Different studies performed on wild almond confirm such variability. Grasselly and Crossa-Raynaud (1980), for example, indicated that *A. bucharica* and *A. fenzliana* have been used by breeders to delay the flowering time. Felipe (2000) indicated that *Prunus communis* is early flowering, whereas *P. fenzliana* and *P. bucharica* are middle–late flowering. Gradziel *et al.* (2001) pointed out the high chilling requirements of *P. fenzliana*, *P. bucharica* and *P. argentea*. Furthermore, Sorkheh *et al.* (2009) showed the enormous variability of this trait in 18 wild almond varieties, from very early to very late.

The first contribution of man to the variability of flowering time in almond was performed by growers for a long time, by selecting the genotypes by function with respect to their adaptation to each area, thus resulting in the broad variability observed among our local genotypes.

Yet, despite the influence of the environment, what has contributed most to the variability of flowering time in almond has been breeding by humans. Man has been able to shape natural processes by crossing almond varieties of very different geographic origins and with very different flowering times. Such crosses would never have happened in nature.

Breeders have released cultivars adapted to the climates of each area where man has established almond culture. Due to the importance of the flowering time for the profitability of farms, many studies on the genetic control of this trait have been carried out, and new cultivars with flowering times suitable for each zone have been released.

Breeders have focused their efforts on delaying flowering time to extend cultivation to extensive but cold inland areas or to high-altitude zones, where early-flowering cultivars inevitably freeze. We can consider the following three groups of cultivars in terms of flowering time (Table 7.1):

- Early-flowering cultivars: these flower in the Mediterranean area in late January or early February. They are traditional cultivars of unknown genetic origin resulting from the selection carried out by farmers in each area for centuries.
- Late-flowering cultivars: these flower 20–30 days after early cultivars and usually come from breeding programmes, from crosses between other late-flowering cultivars with local cultivars adapted to each area. Some traditional cultivars from Italy or the former Soviet Union are also late flowering.
- Extra-late-flowering cultivars: these flower 50–60 days after early cultivars. They have generally been obtained in breeding programmes by crossing previously obtained selections.

We will see later that this variability among almond varieties is an essential tool that breeders have used in breeding programmes to release late-flowering cultivars.

7.3 Genetic Control of Flowering Time

As we will see throughout this section, flowering time is a very complex trait that is tightly controlled at the genetic level but is also modulated by environmental factors.

7.3.1 The flowering process

The almond, like the rest of the *Prunus* stone fruits (peach, plum, apricot, cherry), is deciduous. Each year almond trees undergo a cyclical process of flowering, fruiting, leafing, development and winter dormancy. The purpose of the winter rest period is to prepare the tree to avoid possible damage from low temperatures during winter. These plants have thus developed an environmentally dependent mechanism that activates when their climatic requirements have been fulfilled and a new growth cycle can begin.

Table 7.1. Full flowering time of almond cultivars in a warm area of Murcia (south-east Spain) expressed as Julian days.

Group	Cultivar	Full flowering time (Julian day)
Early	Achaak	27
	Desmayo Largueta	29
	Garrigues	37
	Ramillete	38
	Atocha	41
	Chellaston	44
	Marcona	45
	I.X.L.	47
	Ardècheoise	48
	Price	49
	Del Cid	51
Late	Marta, Genco, Carmel, Vairo, Soleta, Belona	55
	Antoñeta, Constantí, Nonpareil, Tuono, Guara	56
	Morley, Ferraduel, Ferragnès	57
	Mono, Mandaline	58
	Primorskii	60
	Texas, Lauranne	61
	Butte, Savana, Titan	62
	Marinada, Tarraco	67
	Felisia	68
	Wawona	69
	Tioga	70
Extra-late	Penta, Mardía	74
	Tardona	88

This mechanism is based on the accumulation of chilling during the autumn and the beginning of winter, which breaks the dormancy of the plant. Heat then favours the development of floral and vegetative buds. Most studies indicate that cold is the major determinant factor in breaking dormancy and is therefore responsible for the flowering time (Tabuenca, 1972; Tabuenca *et al.*, 1972; Egea *et al.*, 2003; Sánchez-Pérez *et al.*, 2012).

Many models have attempted to quantify the chilling requirements of the different almond cultivars. The method most used involves periodically collecting branches with flower buds in the field (once or twice a week) in plots where temperatures are recorded hourly until each cultivar has overcome dormancy. Finally, the date of flowering is noted. The initial date for recording chill hours is determined according to when temperatures have no negative effect on the accumulation of cold (Erez *et al.*, 1979).

Branches collected in the field are placed in jars with a solution of 5% sucrose in a chamber at 25°C with a photoperiod of 16 h and a relative humidity of 70%. After 10 days, the development of flower buds is evaluated. Dormancy is considered to have been broken when at least 50% of the flower buds reach the 'B'-'C' stages (Felipe, 1977; Egea *et al.*, 2003).

Chilling requirements are calculated as chill units (CU), which accumulate as a function of the different temperature ranges (Richardson *et al.*, 1974). The chilling requirements of each cultivar are those necessary to break the dormancy of this cultivar. Heat requirements, on the other hand, are calculated as growing degree hours (GDH) by subtracting 4.5 points from the average hourly temperature in degrees Celsius. The heat requirements of each cultivar are calculated as the number of GDH accumulated between the end of dormancy breaking and the time of full flowering (50% open flowers).

While the influence of chilling requirements on breaking dormancy is a complex and well-studied phenomenon (Couvillon and Erez, 1985; Scalabrelli and Couvillon, 1986; Erez and Couvillon, 1987), studies on the specific heat requirements of each cultivar are more scarce, and the effect of heat requirements on flowering time is less clear (Rom and Arrington, 1966; Couvillon and Erez, 1985; Citadin *et al.*, 2001).

Tabuenca (1972) estimated the chilling requirements of almond cultivars as hours below 7°C. Depending on the climatic conditions of each area, chilling requirements calculated as hours below 7°C and CU calculated according to the model of Richardson *et al.* (1974) may be different for each cultivar. Tabuenca *et al.* (1972) also described the heat requirements of almond cultivars for flowering.

Other authors have studied the chill (Rattigan and Hill, 1986) and heat (Rattigan and Hill, 1986; DeGrandi-Hoffman *et al.*, 1996) requirements of several almond cultivars for flowering in field conditions in Australia. Rattigan and Hill (1986) found a narrow range of chilling requirements in a group of almonds with different flowering times using a method differing from that of Richardson *et al.* (1974).

Egea *et al.* (2003), using the method of Richardson *et al.* (1974), studied the chilling and heat requirements in ten cultivars representing the whole range of flowering time in almond species. The authors observed significant variability between the chilling requirements related to flowering time in these cultivars, from the Tunisian cultivar Achaak (266 CU) to the French selection R1000 (996 CU). Lower variability was found, however, in terms of heat requirements (from 5942 GDH to 7577 GDH). The authors concluded that flowering time was mainly determined by chilling requirements (Table 7.2).

Sánchez-Pérez *et al.* (2012), studying the chilling and heat requirements in 167 seedlings of the R1000 × 'Desmayo Largueta' family for 2 years, concluded that flowering time was mainly determined by chilling requirements (with a high correlation with flowering time). Heat requirements, on the other hand, were found to have less influence on this trait (no significant correlation). These authors found large differences between the early cultivar Desmayo Largueta (158–148 CU) and the French selection R1000 (750 and 540 CU), which flowered more than 40 days later. The heat requirements were 7600–8500 GDH for 'Desmayo Largueta' and 9500–9800 for R1000.

The annual differences in the estimation of CU and GDH confirm the environmental influence on the measurement of these traits, which has also been described in apricot (Ruiz *et al.*, 2007). Differences in the estimates of CU and

Table 7.2. Chilling (chill units, CU) and heat requirements (growing degree hours, GDH) for breaking dormancy and full flowering time (50% flowers open F⁵⁰) for ten cultivars studied by Egea *et al.* (2003).

Cultivar	Chilling requirements			Heat requirements		
	Breaking dormancy	Days ^a	CU	Flowering (F50)	Days ^b	GDH
Achaak	30 Nov	21	266	24 Jan	55	6444
Desmayo	6 Dec	27	309	28 Jan	53	5942
Ramillete	7 Dec	28	326	3 Feb	58	6538
S2332	12 Dec	33	417	6 Feb	56	6481
Marcona	13 Dec	34	435	8 Feb	57	6681
Marta	16 Dec	37	478	15 Feb	61	7577
Antoñeta	18 Dec	39	514	15 Feb	59	7512
Ferragnès	20 Dec	41	558	15 Feb	57	7309
S5133	13 Jan	65	973	27 Feb	45	7003
R1000	15 Jan	67	996	3 Mar	47	7438

^aDays after the start of chill accumulation (10 November)
^bDays after breaking dormancy

GDH of each cultivar for flowering can also be observed when comparing different publications. Such discrepancies are due to the precision used in applying this methodology and the shortcomings of the Utah model in the warmer years. In fact, Harrington *et al.* (2010) suggested that chilling and heat requirements are not independent processes, occurring one after the other, but that they work together. As a result, these authors argued that different combinations of both processes may produce different estimates of CU and GDH.

Okie and Blackburn (2011) considered that heat requirements are subordinate to chilling requirements in peach (very close to the almond species), and Ruiz *et al.* (2007) estimated that chilling requirements contributed 70% to breaking dormancy in apricot versus 30% for heat requirements.

Alonso *et al.* (2005) claimed that in almond the heat requirements (ranging between 5500 and 9300 GDH) had a greater influence than the chilling requirements (between 400 and 600) in the cold area where they carried out their study. These authors did not evaluate the breaking of dormancy by collecting branches in the field, but instead by studying the significance of the correlation coefficient between temperatures during dormancy and the flowering time, thus not breaking the internal development of the physiological processes of the branch by cutting the sticks and placing them in an artificial environment.

7.3.2 Heritability and environmental influence

Heritability represents the probability of transmitting a particular characteristic to offspring. It is a reflection of the genetic determinism of the trait against environmental influences.

The accumulation of the chill and heat units necessary for flowering of each cultivar is a genetically determined trait. In each environment, the amount of cold and heat that a tree receives is different and, consequently, its flowering date is also different. Nevertheless, the different cultivars usually maintain the same order of flowering.

Due to the importance of the flowering time in breeding programmes, many works have studied the transmission of the trait using data recorded during the selection of seedlings (Kester, 1965; Grasselly and Gall, 1967; Grasselly, 1972; Vargas *et al.*, 1984; Sánchez-Pérez *et al.*, 2007a; Sánchez-Pérez *et al.*, 2012). In a few cases, the heritability of the trait has been calculated (Kester and Asay, 1973; Kester *et al.*, 1977; Dicenta *et al.*, 1993).

Kester and Asay (1973) and Kester *et al.* (1977) obtained, by regression, heritability values close to 0.8 under the conditions found in the almond-growing regions of California. These authors also determined the control of flowering by additive genes. Vargas *et al.* (1984) obtained a correlation coefficient of $r = 0.88$ between the

mean of the offspring and that of the parents, although these authors neither associated this value with heritability nor presented its standard error.

Dicenta *et al.* (1993) estimated the heritability of flowering time by regression and variance components in 2483 individuals belonging to 51 families for a period of 3 years. The values of h^2 varied depending on the method and year, but were usually very high (between 0.8 and 1.0) (Fig. 7.1).

This high heritability shows that the most efficient way to obtain late-flowering progenies is by crossing progenitors as late as possible. The greater influence of the general combining ability (GCA = 85–93%) versus the specific combining ability (SCA = 7–15%) in the transmission of flowering time supports this statement (García *et al.*, 1994).

Flowering time is controlled by nuclear DNA, and no maternal effect has been observed. Dicenta and García (1993) studied the flowering time of 826 individuals from nine families and their reciprocal crosses over 3 years

and found no significant differences between the direct and reciprocal crosses. These authors therefore concluded that the direction of crossing does not affect the flowering time of descendants.

Some authors have studied the flowering time of descendants of the ‘Tardy Nonpareil’ almond (extra-late flowering, presumably from a mutation of ‘Nonpareil’) and have observed a bimodal distribution of this trait (Kester, 1965; Grasselly, 1978; Socias i Company *et al.*, 1999; Ballester *et al.*, 2001; Sánchez-Pérez *et al.*, 2007b). This fact has been explained by the presence of a major gene which could be quantitatively modified by other minor genes (Fig. 7.2).

Sánchez-Pérez *et al.* (2012), studying the flowering time as a function of chilling and heat requirements in 167 seedlings of R1000 × ‘Desmayo Largueta’, found that the segregation of flowering time was similar to that of chilling requirements. Furthermore, descendants generally segregated between both parents (Fig. 7.3).

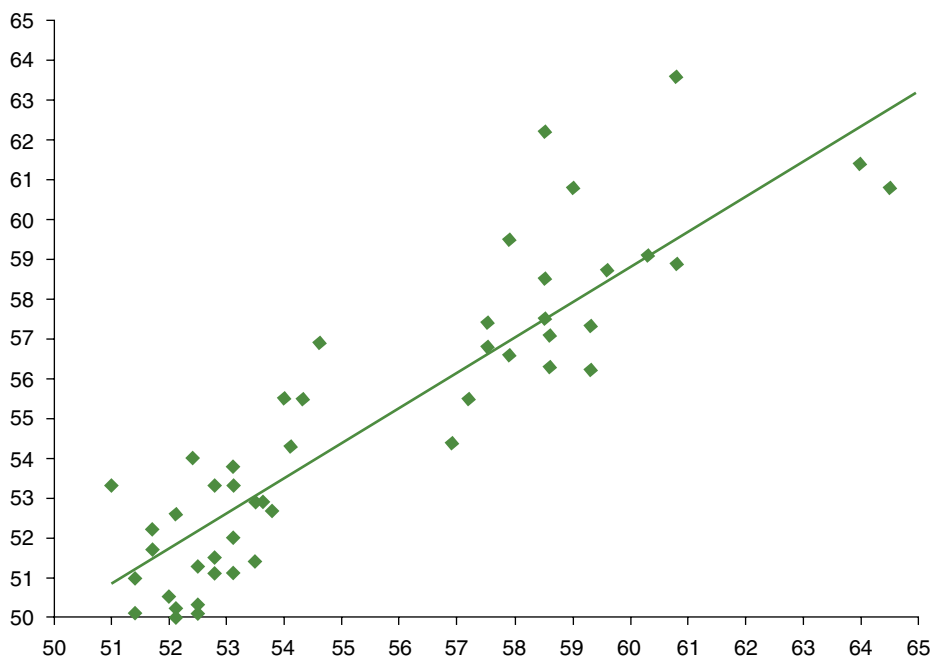


Fig. 7.1. Regression of the mean flowering time of descendants on the mean of parents in 51 almond families evaluated for 3 years. The slope of the regression line represents heritability in the narrow sense (h^2). (From data in Dicenta, 1991.)

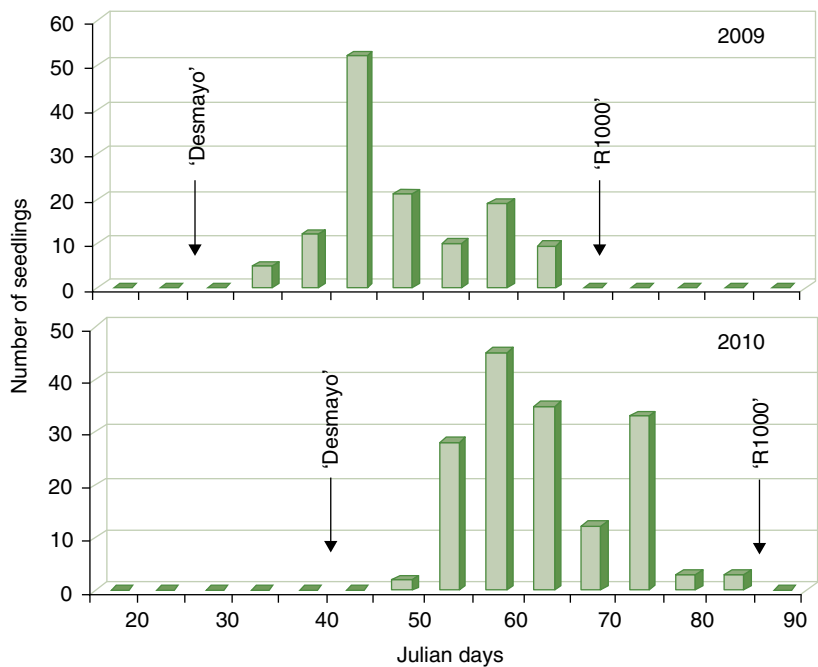


Fig. 7.2. Distribution of the flowering time of 167 seedlings of the R1000 x 'Desmayo Langueta' population for 2 years. The flowering time for each progenitor is indicated by solid arrows.

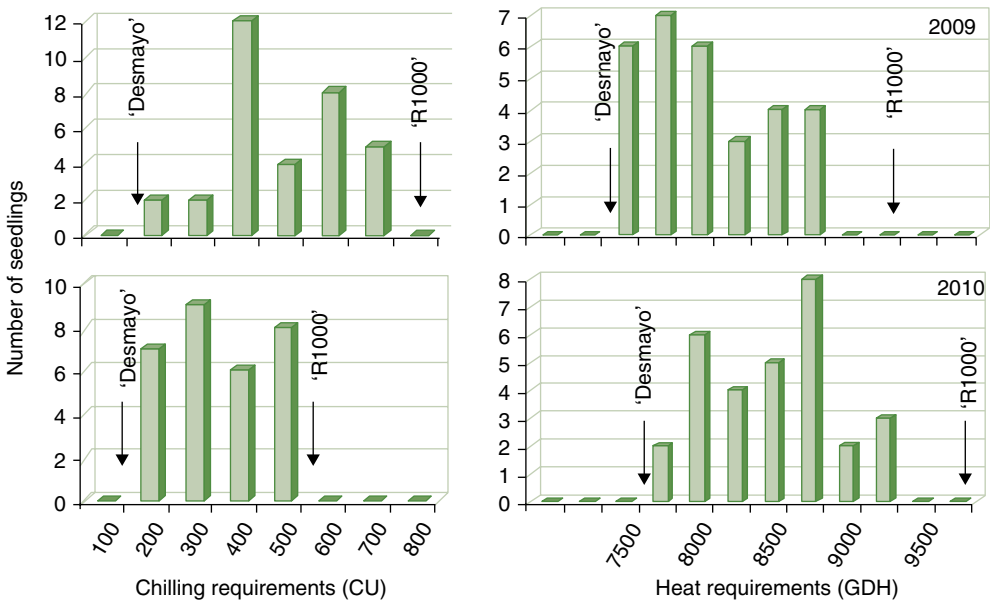


Fig. 7.3. Distribution of the chilling requirements (chill units, CU) and heat requirements (growing degree hours, GDH) of 30 seedlings of the R1000 x Desmayo Langueta population for 2 years. The CU and GDH for each progenitor are indicated by solid arrows.

7.3.3 Correlation with other characteristics

The importance of being aware of the correlations between traits is well known by breeders. Within this field of knowledge, the search for correlation between the characteristics that appear early in seeds or young plants and flowering time, to be used as criteria for early selection, represents a special case. Due to its particular importance in breeding, this point will be further explained later.

There are not many publications that link the flowering time of almonds with other traits. Some authors have observed a relationship between flowering time and density of flowering and production. Kester (1965), studying descendants of 'Tardy Nonpareil', found a significant negative correlation (-0.28 to -0.72) between the flowering time and density of production but not between flowering time and density of flowering. This lack of fertility of the offspring of 'Tardy Nonpareil' was also observed by Grasselly (1978), who subsequently managed to break the link between late flowering and low production by crossing descendants of 'Tardy Nonpareil' (Grasselly and Olivier, 1985). Dicenta and García (1992) (studying 51 families from 12 progenitors for 3 years) and Sánchez-Pérez *et al.* (2007a) (studying the R1000 × 'Desmayo Langueta' family) found no significant correlations between flowering time and density of flowering and production. R1000 is a descendant of 'Tardy Nonpareil'. Based on these studies we can conclude that, in general, these traits are independent and therefore that late flowering can be accompanied by high productivity.

More recently, in obtaining extra-late-flowering cultivars, breeders have observed low productivity in some of the latest descendants. As Kester (1965) and Grasselly (1978) noted, such seedlings are floriferous but unproductive. This phenomenon is probably associated, at least in part, with the high temperatures during flowering at this late time to which almond has not adapted. Such weather conditions produce very short flowerings with short effective pollination periods and are also likely to affect the development of flower buds or the newly formed embryos. Furthermore, high temperatures during late-flowering dates favour the sprouting of vegetative buds, which compete with flower buds

for nutrients. Some extra-late cultivars such as Penta do not show these problems, but others ('Tardona', 'Mardía') do to varying degrees, depending on the growing areas. This topic is discussed further in section 7.4.4.

The link between flowering and ripening times is another interesting relationship between characteristics involved in almond breeding. Although in general there is a positive correlation between the two characteristics in other fruits (apricot, peach, plum), this is not true for almond.

Dicenta and García (1992) found positive (albeit low) correlation coefficients between flowering and ripening times, which shows a slight tendency for late-flowering almonds to ripen later. Sánchez-Pérez *et al.* (2007a) found no significant correlations between these characteristics.

In fact, we know there are very-early-flowering cultivars that ripen late and late-flowering cultivars that mature early (Martínez-Gómez *et al.*, 2008). Therefore, the flowering time may affect the ripening time to some extent, but it is not a very reliable indicator of ripening time. This is a huge advantage for breeders who can obtain extra-late flowering and early-ripening cultivars, such as Penta, the cultivar with the shortest cycle obtained to date.

7.3.4 Genomics, transcriptomics and metabolomics of flowering time

Flowering time is a complex trait, and different regions of the genome have been identified as being involved in the control of this trait (Sánchez-Pérez *et al.*, 2014).

The first studies in which quantitative trait loci (QTLs) were found to be related to flowering time in almond were done with isozymes (Asins *et al.*, 1994). In this study, a 123-seedling population was analysed for 3 years. The authors found a relationship between some isozymes and flowering time, suggesting that these isozymes could be used to perform early assisted selection in the new seedlings from breeding programmes.

Subsequently, DNA-level studies with Random Amplified Polymorphic DNAs (RAPDs), were performed in a 'Felisia' × 'Bertina' population ('Felisia' is a seedling of 'Titan' × 'Tuono') using

bulk segregant analysis (BSA). It was found that flowering time was also controlled by a major gene called *Lb* or Late blooming. It was suggested that the *Lb* gene had probably been inherited from 'Tardy Nonpareil', a late-blooming cultivar. In total, three RAPDs and *Lb* gene related to flowering time were found in LG4 (Ballester *et al.*, 2001). Moreover, Silva *et al.* (2005) described other QTLs linked to flowering time in an inter-specific family between almond $\times$ peach in G1, G2, G3, G5, G6 and G7 (Fig. 7.4).

More recently, the localization of the *Lb* gene in G4 has been confirmed in other studies using Simple Sequence Repeat Markers (SSRs) in the population R1000 $\times$ 'Desmayo Langueta' (R1000 is a French selection resulting from 'Tardy Nonpareil' $\times$ 'Tuono'). In this study, other QTLs linked to flowering time were found in G1, G6 and G7 (Sánchez-Pérez *et al.*, 2007b; Sánchez-Pérez *et al.*, 2012) (Fig. 7.4). In the first study mentioned here, from 2007, the authors found that the SSR UDP-96003 was localized very close to the *Lb* gene in G4, explaining between 56–86% of the variance in R1000 and between 54–67% of the variance in the R1000 $\times$ 'Desmayo Langueta' family.

Furthermore, Silva *et al.* (2005) and Sánchez-Pérez *et al.* (2012) described different QTLs in G1, G3, G4, G5, G6 and G7 linked to chilling requirements (which, as we have mentioned, are critical in determining flowering time) and in G2 and G7 linked to heat requirements, confirming the polygenic control of this trait (Fig. 7.4).

Moreover, homologous genes previously characterized in *Arabidopsis* have been used to study ten candidate genes (CGs) from peach and almond (PrdLFY, PrdMADS1, PrpAP1, PrpFT, PrpAGL2, PrpFAR1, PrdTFL, PrdGA20, PrpAP2 and PrpCO) (Silva *et al.*, 2005). These ten genes were localized in the *Prunus* reference map ('Texas' $\times$ 'Earlygold', T $\times$ E) in G1, G2, G3 G5, G6 and G7 (Fig. 7.4). However, none of these genes co-localized with the *Lb* gene in G4. One of the reasons for this could be that the mechanisms to control flowering in annual plants are different from those in perennial plants (Sánchez-Pérez *et al.*, 2014). Recently, 429 CGs have been found in a region of 3.7 Mbp around the *Lb* gene in the sequenced genome of the peach cultivar Lovell (Castède *et al.*, 2014). These genes could be used in a study in almond.

There are fewer transcriptome-level analyses performed to determine flowering time in almond. Nevertheless, some transcription factors (TFs) such as leafy (LFY) and MADS have been described as being related to flowering time (Silva *et al.*, 2005). MADS genes such as DAM6 (dormancy associated MADS genes) have been found to be involved in different stages of flower development like flowering time (Riechmann and Meyerowitz, 1997). Thus far, more than 50 MADS-type TFs have been identified in peach. Further studies in almond should thus be performed within this MADS-type gene family to identify the MADS genes related to flowering time. RNA-seq analysis should help in this identification process, as RNA-Seq represents a powerful tool for characterizing transcriptomes in different physiological stages (Wang *et al.*, 2009; Martínez-Gómez *et al.*, 2012).

Recently, a programme has been developed to identify genetic pathways and potential epigenetic mechanisms involved in the control of chilling requirements and flowering time in peach (Zhebentyayeva *et al.*, 2014). For example, these authors described the transcription factor TFL1, which regulates the vegetative to reproductive transition, and the PcG (polycomb group) genes, which are involved in the epigenetic regulation of flowering in *Arabidopsis*. However, the authors were not able to find other famous genes such as an FLC gene orthologue and its regulator FRI, suggesting that the control of flowering time in *Prunus* species has a complex genetic architecture.

Moreover, metabolomic analyses to identify the control of flowering time have been based on using hydrogen cyanamide to bring forward flowering time in apple, apricot, peach and cherry (Faust *et al.*, 1997), among other fruit species. Recently, a correlation has been found in *Prunus* spp. between the mechanisms involved in breaking flower bud dormancy, which influences flowering time, and the breaking of seed dormancy (Leida *et al.*, 2012). This relationship has been found in the secondary metabolites called cyanogens, involved in the germination of cocklebur (*Xanthium pennsylvanicum* Wallr.) (Esashi *et al.*, 1996). This correlation has also been identified in the cyanogenic glucoside prunasin described in the flower buds of eucalyptus (*Eucalyptus cladocalyx* F. Muell.) (Gleadow and Woodrow, 2000).

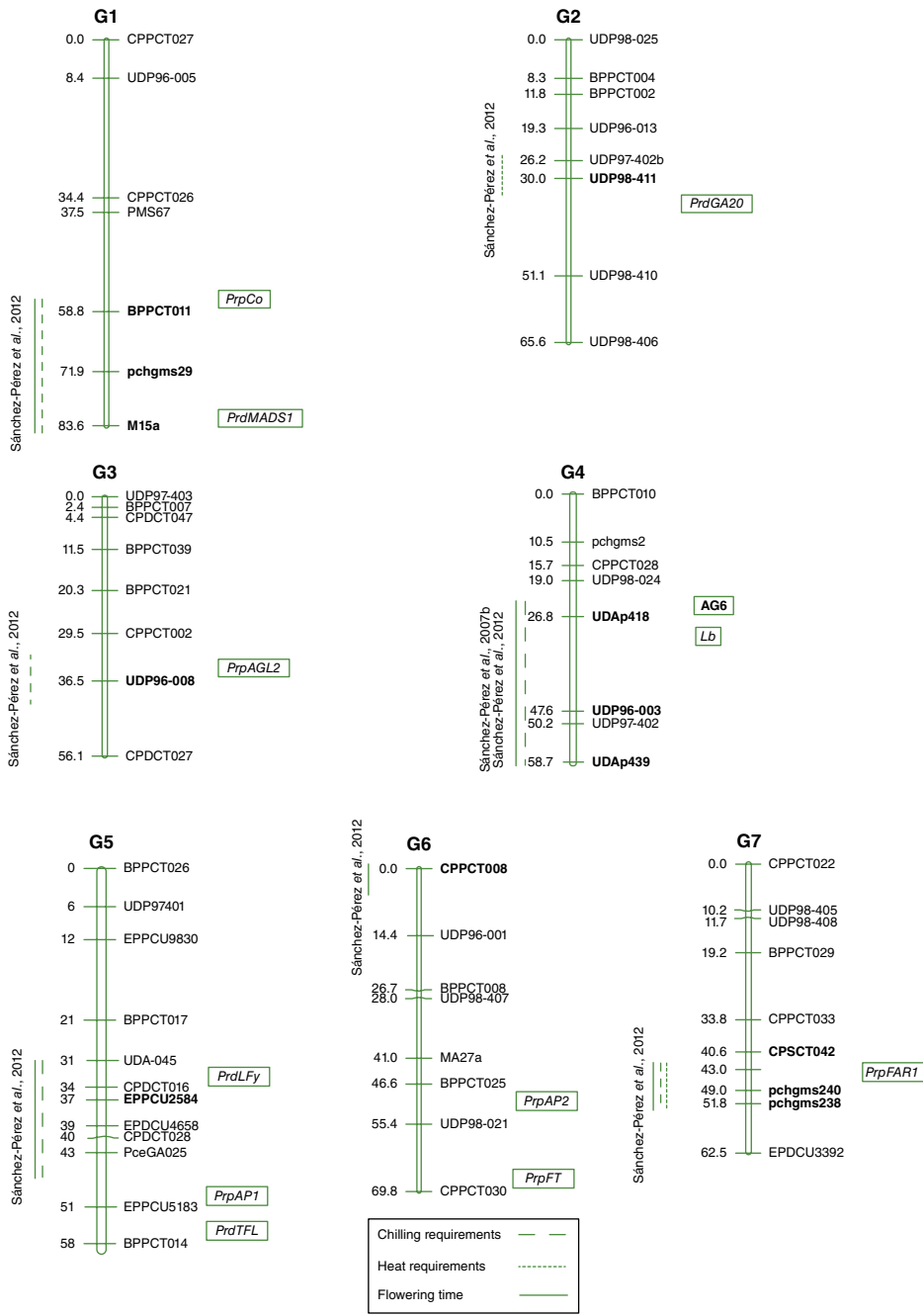


Fig. 7.4. Location of QTLs linked to flowering time and chilling and heat requirements in the almond map from the population R1000 × 'Desmayo Largueta'. The closest SSR marker linked to the QTL is indicated in bold letters in this map. The approximate location of RAPD markers (in bold) and candidate genes (in italics) linked to flowering time in other almond populations is indicated inside the boxes. The integration of this information from different genetic maps has been performed using the centimorgan (cM) distances indicated by the different authors in each linkage group and each map. QTL, quantitative trait locus; SSR, Simple Sequence Repeat Marker; RAPD, Random Amplified Polymorphic DNA.

The study of the accumulation of cyanogenic glucosides and the regulation of flowering time through TFs opens up new possibilities for discovering the molecular mechanisms involved in flowering time in almond and other *Prunus* species.

7.4 Breeding for Flowering Time

Obtaining late-flowering cultivars to avoid spring frosts is one of the main objectives of the researchers in different European almond breeding programmes.

Like other temperate trees, the almond tree is at rest during the winter and is usually without leaves. In these conditions, it is very hardy in its ability to endure very low temperatures. Nevertheless, the almond is very susceptible to cold during flowering and early fruit development. At this time, depending on numerous factors, particularly phenological stage, temperature and exposure time, temperatures below -1°C or -2°C can cause crop loss. Therefore, breeders are interested in delaying the flowering time of new cultivars so that they flower when the risk of frost is minimal.

7.4.1 Breeding methods

The method most often used to obtain late-flowering almonds has traditionally been to cross progenitors adapted to each area with late-flowering cultivars. Breeders have then used their own selections in subsequent crosses. Given the quantitative nature of this trait, this strategy has led to the accumulation of late-flowering alleles in the new selections. This traditional method involves the extraction of pollen to be used as the male parent. When the flowers of the female parent are about to open they are emasculated and the pistils are pollinated with a brush.

Since late-flowering progenitors are being used more and more often, if we work in warm areas, it is advisable to use the earliest-flowering cultivars as female progenitors. To do this, the pollen must be collected the previous year and kept frozen until used (Martínez-Gómez *et al.*, 2000; Martínez-Gómez *et al.*, 2002). If the area is cold, we should use the late progenitors

as female, to prevent losing the crosses to frost. Since differences between reciprocal crosses have not been observed (Dicenta and García, 1993), similar offspring are expected in both cases.

The ripened fruits are picked in the summer, and in winter the nuts are stratified into an inert, moist and aerated substrate (vermiculite, perlite) until the emergence of the root (García-Gusano *et al.*, 2004). When germination starts, the seeds are placed in pots and a few months later the plants are taken to the final plots where they are to be evaluated 3 years later.

Phenotypic evaluation of the flowering time is an important aspect of breeding, and there are various ways to do it. The most traditional method has been to observe every tree once or twice a week, estimating the percentage of flowers in each phenological stage (Felipe, 2000). This is a reliable but complicated method of scoring and analysing when working with a large number of offspring.

Other breeders use the dates of initial flowering (5% open flowers), full flowering (50% open flowers) and final flowering (95% of flowers open). This method is simpler and makes it possible to observe a large number of offspring. Due to the high correlation between these three traits (Dicenta and García, 1992), if we are not interested in the duration of flowering, annotation of full bloom is enough. If these flowering dates are recorded as Julian days (days from 1 January), calculating the flowering time with respect to the reference cultivars ('Cavallera' for GREMPA (Mediterranean Research Group for Almond and Pistachio) countries or 'Nonpareil' in USA and Australia) is very simple. To minimize differences in relative flowering time to the reference cultivars due to climatic variations during the flowering period, it is very useful to use early, late and extra-late reference cultivars ('Desmayo Largueta', 'Ferragnès' and 'Penta', for example).

Other researchers have used radiation as a breeding method for late flowering. According to Monastera *et al.* (1988), 'Supernova' was obtained by irradiating buds of 'Fascionello' (self-incompatible) with Co^{60} gamma rays, delaying flowering for about 15 days and making this cultivar self-compatible. Subsequently, Marchese *et al.* (2008), using molecular markers, showed that 'Supernova' really came from 'Tuono', from which it inherited self-compatibility.

Legave and García (1992), using 'Ferragnès', initiated a breeding programme by irradiating vegetative buds with gamma rays. The goal was to delay the flowering time and incorporate self-compatibility, but results were inconclusive.

7.4.2 The juvenile period and early selection methods

While the capacity of breeders to generate numerous seedlings is almost unlimited, the capacity to evaluate and select these descendants remains a limiting factor. The selection of new cultivars of almond is a long and expensive process. Trees do not have their first flowering until the third year after planting, which means we have to wait at least 3 years to begin selecting late-flowering seedlings.

As a result, breeders have studied strategies for early selection for flowering time, either based on the observation of correlated traits that are expressed early, or involving the search for molecular markers, as we saw in section 7.3.4. Such strategies would allow for very early selection (during the first year) of the late-flowering genotypes, which would be the only ones evaluated for other characteristics. Some of these early selection strategies are based on the similarity of the mechanisms of dormancy of seeds, vegetative buds and flower buds, and the possible relationships between these mechanisms to be used in the early selection process (Fig. 7.5). Seed and bud dormancy show some common characteristics, such as the optimum temperature at which dormancy breaks (Powell, 1987).

Early selection of flowering time as a function of chilling requirements of the seeds obtained from crosses

A first solution would be to correlate the chilling requirements of the seeds for germination with the flowering time of trees obtained from these seeds. If this relationship exists, the late-flowering descendants could be selected early during germination in the nursery.

Grigorian (1972) observed a relationship between the time of flowering of almond cultivars and the chill requirements for germination of seeds they produced in open pollination, as well as the inverse relationship. Despite this generality, some late cultivars (Cristomorto, Tardive de la Verdère) produced seeds with low chilling requirements. Conversely, some early cultivars (Desmayo Largueta and Ardècheoise) produced seeds with high chilling requirements.

Kester *et al.* (1977) determined that the chilling requirements of almond seeds for germination and the flowering time of the resulting plants were highly correlated (0.8) only if the means of the families were correlated. However, if this correlation was calculated from individual values (each seed with the resulting tree), the correlation was very low (0.1). For early selection, only individual correlation is valid, allowing us to select the latest-flowering seedlings within each family.

These results confirm that on average the seeds of late-flowering cultivars have higher chilling requirements for germination, although the variability is enormous (García-Gusano *et al.*, 2004), and that the descendants of these

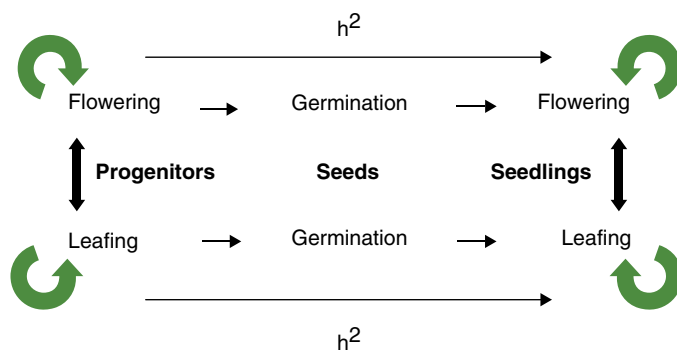


Fig. 7.5. Relationships between germination, leafing and flowering of the parents and descendants in an almond breeding programme, potentially related to the chilling requirements of almond.

cultivars will also flower later. However, at the individual level – which is the only level of interest for the early selection of late-flowering trees – such correlations are low.

Mehlenbacher and Voordeckers (1991) showed in apple that selection based on the time of seed germination could be used to select progeny for late leafing when the variability for the two traits was high but not when it was low.

Dicenta *et al.* (2005), studying 502 individuals belonging to 13 families for 4 years, determined the relationship between the chilling requirements of seeds for germination and of the descendants obtained for sprouting and flowering (Fig. 7.6). These authors noted that the late-flowering progenitors produced seeds that on average took longer to germinate, which demonstrates a relationship between the chilling requirements of the vegetative or floral buds with the chilling requirements of the seeds produced by these cultivars. Such a relationship has also been observed in apple (Mehlenbacher and Voordeckers, 1991) and almond (Grigorian, 1972; Kester *et al.*, 1977; García-Gusano *et al.*, 2004). When studying family means, these authors also found high correlations between time for seed germination and the leafing and flowering times of the trees that grew from the same seeds, which *a priori* demonstrates the possibility of selecting late-flowering trees in the nursery by choosing those trees that take longer to germinate. However, when they studied the correlations between the individual values of germination and flowering (Fig. 7.6), these authors observed that the correlations were not significant within each family. These results indicate that there is not a close relationship between the time each seed requires for germination and the flowering time of the tree obtained, unlike the results found using family means.

García-Gusano *et al.* (2010) studied the relationship between germination and flowering in 136 descendants belonging to six families for 2 years and found a correlation coefficient of 0.50, which showed some relationship, but not enough to be used as a criterion for early selection.

Selecting early-flowering time depending on the time of leafing

On the other hand, the simple observation of leafing and flowering of almonds seems to indicate

that these two traits are related. Although in general almond trees flower and then sprout, depending on the cultivar, these processes can coincide and even the opposite can occur. This fact may affect the study of the correlation between these traits in the offspring.

Kester *et al.* (1977) obtained a low correlation (0.34) between leafing and flowering in the same year studying numerous almond descendants. As a result, these authors questioned the usefulness of leafing as a criterion for early selection of flowering in almond.

Furthermore, if these authors had studied the relationship between the first leafing and flowering a few years later (which is the most useful in terms of early selection criteria), this correlation would have been even lower (Dicenta *et al.*, 2005). These results could be due to various factors, such as the different ages of the trees and the environment $\times$ genotype interaction, which may affect the relationship between the first leafing and the first flowering years later. In this regard, it is known that in a given genotype, the lack of flowers can make trees leaf earlier and vice versa. During the first years, descendants have no flowers and thus tend to sprout sooner than they eventually do when they flower as adults.

Vargas and Romero (1984) found a correlation of 0.56 between flowering and leafing when studying 602 descendants resulting from the open pollination of 'Cristomorto', 'Tardive de la Verdrière', 'Primorskij' and 'Marcona', although these results were based on data from the same year. These authors also observed that this correlation was greater when the parents had very different flowering times. Subsequently Vargas *et al.* (1997) and Vargas *et al.* (2005) used the leafing of 1-year-old descendants as a criterion to select for late flowering among the offspring they obtained between 1991 and 2000, totalling over 18,000 descendants, eliminating the earliest-flowering seedlings at the beginning of the second year.

Dicenta *et al.* (2005) obtained a very high correlation (0.9) between these traits using the average of families with progenitors with very different flowering times, even from year to year. Such a high correlation would demonstrate the usefulness of the first leafing as criterion for early selection for flowering time. However, as with germination, when these authors used individual

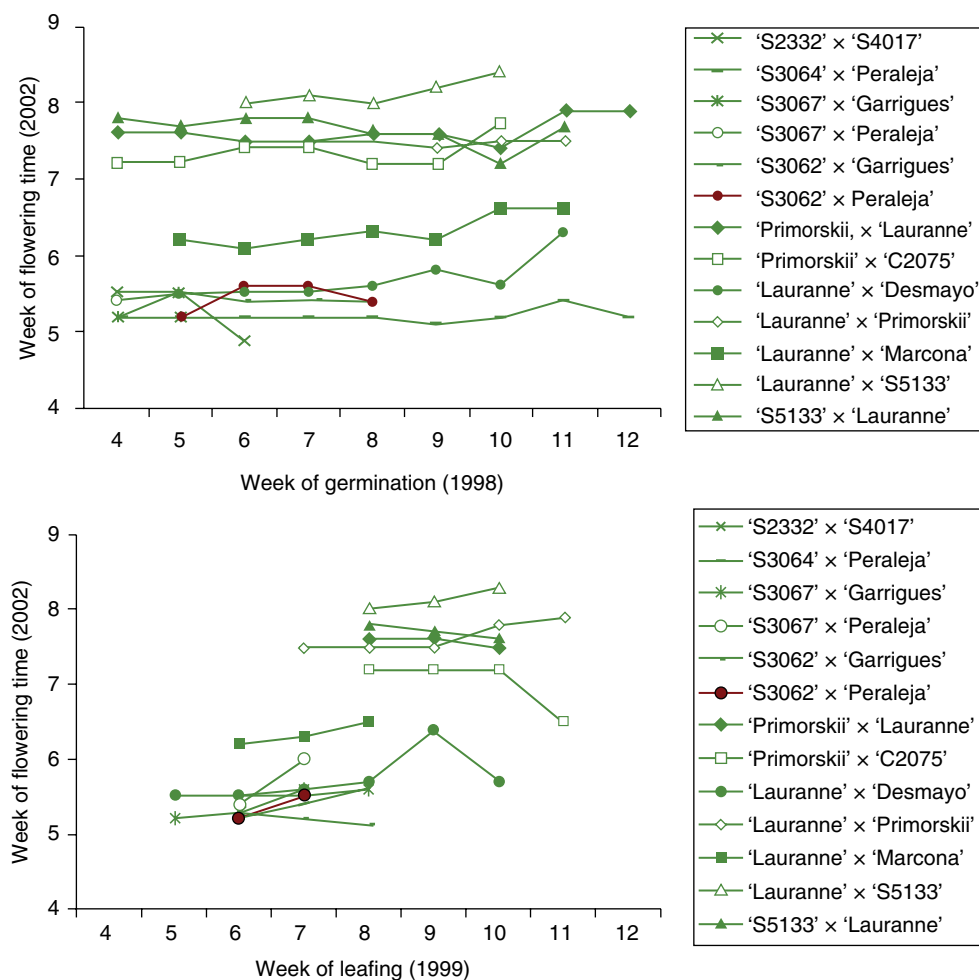


Fig. 7.6. Simulation of early selection based on the time of seed germination (1999) (above) and the time of leafing (2002) (below). Each value represents the mean flowering time of seedlings and their germination (above) or leafing time (below). In both figures, the families with two late-flowering progenitors ('Primorskii', 'Lauranne', 'C2075' and 'S5133') are above (later-flowering time) the other families, with one or two early-flowering progenitors.

values within each family, the relationship between leafing and flowering was less important. Only when they considered all individuals, when the range was high, were the correlations high (0.7–0.8) and significant. These results suggest that although there is some relationship between leafing and flowering, it is not close enough to be used within each family when there is little variability. We can select late-flowering individuals by choosing the late-leaving seedlings within each family, but we must assume a significant margin of error. Considering

all of the families (late and early), correlations were high; that is, in general, the later the leafing, the later the flowering. Nevertheless, to obtain late-flowering seedlings, the best option is simply to cross the latest-flowering progenitors, given that this trait is known to have high heritability (Dicenta *et al.*, 1993). From a practical point of view, selecting individuals based on the time of leafing during the first year would help us select late seedlings, but only if we select the descendants of the latest families, eliminating all seedlings of early families (Fig. 7.6).

We should note that the only valid correlation that would allow us to select the latest seedlings within each family is that at the individual level. Family means are predictable based on the flowering time of the parents. Furthermore, the only useful correlation for early selection would be that between the first budding and first flowering 2 or 3 years later, conditioned by all of the effects of the environment and plant juvenility that have an impact on this correlation.

Despite such caveats, it is important to emphasize that this type of early screening could be applied with success when we want to select late seedlings from crosses between a very late and a very early progenitor. In this case, the significant variability expected could compensate for the differences between the leafing and flowering of the descendants.

7.4.3 New late-flowering cultivars

Since Chapter 5 is devoted to cultivar breeding, we will not go into detail here.

Flowering time has generally been taken into account in most almond breeding programmes. In some cases, like in the programme of the University of California, Davis (USA), this has entailed the search for pollinators with the same flowering time as the self-incompatible 'Nonpareil'. More generally, however, programmes have sought to obtain late-flowering cultivars to prevent frost damage.

In 1925, Vavilov had a collection of 12 almond cultivars in the Nikita Botanical Garden in the former Soviet Union; but Rikhter was in charge of the almond breeding programme in this country, which was highly oriented towards obtaining late-flowering cultivars. Rikhter had 900 almond genotypes in the Nikita Botanical Garden and released late-flowering cultivars that would later be cultivated in Russia ('Desertnyj', 'Bumazhnoskorlupij', 'Krymskij', 'Nikitskij 2240', 'Yaltinskij', etc.).

In 1960, Dr Charles Grasselly began a cross-breeding programme using late-flowering progenitors ('Cristomorto', 'Ai', 'Tardy Nonpareil') at the Domaine de la Grande-Ferrade (Bordeaux, France). Grasselly's efforts would produce the currently very widespread late-flowering cultivars Ferraduel, Ferragnès and Lauranne.

In Italy, breeding programmes have included late flowering among their objectives since 1957 in Bari and since 1970 in Rome. Turkey and Greece also developed almond breeding programmes with the same objective.

Nevertheless, the greatest results in this regard have probably been those obtained by the following Spanish breeding programmes: Institut de Recerca i Teconologia Agroalimentàries (IRTA) of Reus, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) of Zaragoza and Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC) of Murcia. Late-flowering time is among the main objectives of these three teams. Indeed, these programmes have released a number of late-flowering cultivars, some of which are widely cultivated in Spain and abroad with great success (Masbovera, Glorieta, Marinada, Vairo, Constantí, Guara, Soleta, Belona, Marta, Antoñeta, Penta, etc.).

We can identify two groups of new cultivars according to their time of flowering: late and extra-late (Table 7.1).

The late-flowering cultivars include Ferragnès, Ferraduel, Lauranne, Aylés, Guara, Moncayo, Masbovera, Glorieta, Francolí, Cambra, Felisia, Antoñeta, Marta, Soleta, Belona, Vairo, Constantí, Marinada and Tarraco. All bloom a few days before or after Ferragnès.

The extra-late-flowering cultivars include Mardía, Penta and Tardona. Depending on the areas, Penta and Mardía bloom 15 days after Ferragnès, while Tardona blooms about 30 days later. Tardona is the latest-flowering cultivar released to date.

7.4.4 Limitations in the delay of flowering time

As we have seen in this chapter, the delay in flowering time in almond achieved by breeding has been spectacular. Nevertheless, the results obtained, in addition to common sense, make us guess that the potential amount of delay is not in fact unlimited and that the limit is quite possibly linked to the natural cycles of almond, conditioned by temperature.

Delayed flowering is usually associated with a very long period of rest, during which time the plant accumulates enough cold to

break dormancy. But as the season progresses, daytime temperatures are higher and higher, which may affect the proper development of flowers, fruit set and fruit development.

Traditional early-flowering almonds are designed to flower in a cool climate (the origin of almond is central Asia) in which the development of floral organs can proceed normally. High temperatures can cause alterations in flower and young fruit development. In addition, the flowering period is shorter under high temperatures and flowers evolve very quickly, shortening the effective pollination period and negatively affecting fruit set. We know that some extra-late-flowering cultivars show fruit set problems, despite being very floriferous. Such problems can be also the result of the coincidence of flowering with leafing and the resulting competition for water and nutrients. However, some exceptions, such as the extra-late 'Penta', are very productive. Around 20 days after the flowering of late cultivars (such as Ferragnès) may possibly be the limit for efficient flowering in almond trees.

7.5 The Impact of Climate Change on the Flowering of Almonds

The increase in greenhouse gases caused by human activities is producing a significant increase in global temperatures, known as climate change. According to the fourth assessment report of the Intergovernmental Panel on Climate Change (IPCC), the average global temperature has increased by 0.74°C over the past century and is expected to rise between 1.1°C and 6.0°C in this century (IPCC, 2007).

This climate change is affecting all life processes on earth, and obviously crops as well. Increases in temperature are modifying the growth stages of plants, especially those in temperate zones that are adapted to seasonal changes in solar radiation, temperature and humidity. This phenomenon has thus far been undervalued (Wolkovich *et al.*, 2012).

In fact, climate warming in winter and spring is the main cause of alterations in temperate fruits (Hanninen and Tanino, 2011; Luedeling, 2012). The almond is no exception, and its flowering time may be affected as

a result of the interaction of cultivars with temperature changes.

Almond cultivars grown in each zone are adapted to seasonal weather conditions to meet their chilling requirements, avoid frost damage and flower in the best conditions for pollination. Climate change may cause current cultivars to be unsuitable in the new climatic conditions (Campoy *et al.*, 2011).

The first aspect that would be affected by increases in temperature is flowering time, which would undergo uncertain modifications in each zone as a result of changes in the accumulation of cold and heat. Knowledge of the genetic control of these characteristics linked to flowering time is very important to anticipate the effects that the new climatic conditions may produce on temperate fruit (Castède *et al.*, 2014).

In general, we can acknowledge that rising temperatures would advance the flowering time of the cultivars in each zone if they had satisfied their chilling requirements. Peñuelas *et al.* (2002) observed that between 1952 and 2000 rising temperatures (1.4°C) strongly affected many species, including almond. The comparison of flowering time of 31 cultivars between the periods 1981–1990 and 2001–2010 in the IRTA of Mas de Bover showed a mean flowering time of 4 days earlier in the second period.

In the current warm growing areas, rising temperatures could have a negative impact on dormancy breaking in many cultivars whose chilling requirements would not be satisfied (Luedeling, 2012). In turn, this would delay and lengthen the flowering period and produce problems of bud drop and fruit set (Erez, 2000).

Atkinson *et al.* (2013) warned of the importance of the effects of climate change on all processes related to the time of flowering of temperate fruit. These authors proposed solutions to mitigate the lack of cold by exploiting genotypic variability through breeding to develop cultivars with low chilling requirements where necessary.

On the other hand, higher temperatures in current cold areas would provide an opportunity to extend almond cultivation to these areas, which have not been considered suitable due to the high risk of frost.

In general terms, considering the varietal panorama today, global warming would produce a lack of adaptation of cultivars to their current crop areas and would promote a general shift of

almond-growing areas to the north in the northern hemisphere and to the south in the southern hemisphere.

7.6 Conclusions

Despite having a different meaning in nature and in commercial orchards, flowering time is a key characteristic in the adaptation of a tree to its environment, ensuring that the tree shows efficient behaviour in either case. Due to the relationship between weather, time of flowering and almond production, flowering time has become one of the main traits to consider in almond plantations.

Almond presents enormous variability for many characteristics as a result of its origins, both by multiplication by seed and, more recently, by breeding. Flowering time is the most notable example of a characteristic displaying such variability. This is an advantage for almond cultivation, which can thus be established in a wide range of environmental conditions.

Flowering time is a complex trait that is largely determined genetically and thus has high

heritability. Nevertheless, temperatures have an appreciable effect on this trait. We hope that scientific advances at the molecular level will make it possible to develop molecular markers in the medium term to facilitate breeding for this trait.

Delaying the flowering time has been one of the main objectives of almond breeding programmes, and great achievements have been obtained. Through classical breeding, late- and extra-late-flowering cultivars have been released that can be grown in very cold areas. Yet this delay in flowering time has its limits and will be conditioned by the climatic requirements necessary for the proper development of flower buds and the processes of pollination, fruit set and fruit development. Although efforts have been made in the search for early selection methods, we must await the development of molecular markers that can be used efficiently on a routine basis.

The progressive warming of the planet is a documented fact that is affecting many biological processes, including the flowering of the almond. Increasing temperatures will have important future implications for the distribution of almond cultivation, and problems could be alleviated by exploring variability and genetic breeding.

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8 Pollen–Style (In)compatibility: Development of Autogamous Cultivars

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8.1 Almond Self-incompatibility

Almond, with very few exceptions, is a self-incompatible species (Socias i Company, 1990), thus requiring cross-pollination for commercial production (see Chapter 1). Traditional almond growing primarily utilized open-pollinated seedlings, not only found in orchards, but also as single trees at the borders of fields and roads (Grasselly, 1972; Rikhter, 1972). Occasional bitter or non-productive almonds were noticed and used as rootstocks for grafting using scions of productive trees, since almond grafting was already known before the current era (Columella, 1st century CE/1988). Thus, from the seedling almond population, cultivars began to be selected and became more or less established, often in very large numbers in the same orchard. With this situation, problems associated with self-incompatibility were not apparent. Different trees were intermixed promoting cross-pollination via wild bees. If rainy, cold or windy weather disturbed insect pollination and the crop was poor, the situation was not considered disastrous because of the marginal position of this crop in the economy. However, the requirement of bees for almond production was probably already suspected in the Middle Ages (Socias i Company, 1992). Vallés y Vallés (1902) did recommend placing beehives

inside almond orchards at blooming time, even without recognizing the real effect of the bees. Campbell (1915) observed that isolated trees had poor or no production, although he incorrectly attributed it to the floral morphology preventing self-pollination.

Gradually, commercial orchards were established using grafting to perpetuate desirable clones, and problems of poor crop yields not only became more frequent but also were more noticeable. This system started in California in the latter part of the 19th century, where the first pollination problems were detected and studied. Only later were similar trends in eliminating unsatisfactory cultivars followed in the traditional areas of almond growing around the Mediterranean basin and the Middle East, although a few regions still plant seed-propagated almonds, such as the Middle East (Grasselly, 1972), Morocco (Mahhou and Dennis, 1992) and the Canary islands (Padilla *et al.*, 2014).

Almond self-incompatibility was first described in California by Tufts (1919) after examining the problems of poor production in commercial orchards of a single cultivar. The first study of 17 cultivars was further expanded (Tufts and Philp, 1922), with the conclusion that all 21 cultivars studied were self-incompatible, although all produced viable pollen. For the first time a

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small set was observed in four cultivars ('Harriott', 'Big White Flag', 'Sellers' and 'Reams') when self-pollinated, although these sets were so small (0.2–2.5%) that they did not reach the level of a commercial crop and these cultivars were also considered self-incompatible. These studies quickly spread to other almond-growing countries, including the first mention in Spain (Salom, 1922), and also in countries where almond was not so important, such as Germany (Zimmerman, 1928) and the former Soviet Union (Ryabov, 1930).

There was significant, active research into almond in the former Soviet Union, mainly in the Nikita Botanical Garden of Yalta, Crimea, where a very important almond collection was established and an active breeding programme was being developed (Rikhter, 1972). Early studies on almond pollination by Pashkevich (1930) only included 1 year of work with five cultivars, suggesting that full self-incompatibility was not present in them: three gave similar seed sets when self- or open-pollinated, one gave higher seed set when selfed than when open-pollinated, and the other gave a practically null set when self-pollinated. These results were later questioned by Ryabov and Rikhter (1934), who attributed Pashkevich's results to error. They demonstrated self-incompatibility in all 30 types they examined, independently of their origin, as they included cultivars from France, accessions from local prospection and six bitter types. In some genotypes, however, they obtained low levels of selfed seed, with a maximum of 0.8% in 'Grosse Sultane'. Rikhter (1953) confirmed these conclusions again with three Russian, 12 Californian and two Italian cultivars, as well as with selections of his breeding programme. He also found that self-incompatibility was independent of the floral morphology of each genotype, contrary to Campbell's (1915) observations.

In the southern hemisphere, where mostly Californian cultivars had been introduced, the results were similar. Thus, Reinecke (1930) studied four Californian cultivars in South Africa and confirmed their self-incompatibility, though a small set was obtained in bagged branches. Bowman (1939) in Australia reported that among the 13 cultivars studied, some were fully self-incompatible whereas others had partial self-compatibility, but self set was far below the level needed for commercial production. Welkerling (1954) in Argentina studied 12 cultivars of

Californian and European origin and found a very low self set (0.09%) in three of them, considering that these few fruits were due to the introduction of foreign pollen and concluding that all cultivars were self-incompatible.

As Italy was the leading almond producer at the beginning of the 20th century, unsurprisingly many pollination studies were carried out in the different Italian growing regions. Lutri (1935) tested seven cultivars from the island of Sicily and found three cases of weak self-compatibility: 'Albicoccara grossa' (2.59%), 'Albicoccara piccola' (4.09%) and 'Romana' (4.92%), concluding incorrectly that these cultivars were fully self-compatible despite these set levels. However, Spina (1954) later concluded that all the Sicilian cultivars were self-incompatible. On the same island, Crescimanno (1960) tested six soft-shell cultivars and found weak selfing in all: about 1% in 'Cavaliera', 'Mollese da tavola' and 'Mollese pizzuta'; between 2% and 3% in 'Mollisetta' and 'Pistacchio'; and about 6% in 'Mollisona'. These sets, however, are too low for a commercial production and only 'Mollisona' could be considered as partially self-compatible.

On another Italian island, Sardinia, Milella (1959) studied 14 cultivars and found low levels of selfing in four: 'Schina de porcu' (1.97%), 'Arrubia' (4.18%), 'Nocciolara' (4.98%) and 'Francese' (12.96%). Despite the high set obtained in 'Francese', much higher than that obtained by Lutri (1935), he considered that the rate of selfing was not high enough to prove self-compatibility. It is noteworthy that 'Romana', which had low levels of selfing in the tests by Lutri (1935), was found to be completely self-incompatible.

The main growing region in continental Italy is the Puglia. There, the Stazione Agraria Sperimentale de Bari (Anonymous, 1951) conducted self-pollination of 40 cultivars of this region in 1949 and concluded that 'without doubt, the almond cultivars grown in Puglia are self-compatible'. Seed sets were as high as 85% in 'Cazzolecchia', suggesting that weather conditions greatly influenced fruit set. However, this conclusion was probably hazardous and later studies (Anonymous, 1957) reduced the number of self-compatible cultivars to five, when sets were reasonably based on the number of ripe fruits instead of on the initial number of fruitlets, when the very improbable set of 85% was obtained. Most likely the pollination effect (see Chapter 1)

retained the ovaries for some time on the peduncles, but fruitlet fall was important until a very low final set. Only five cultivars (and not in all localities where the tests were carried out) were weakly self-compatible: 'Rachele' (0.1%), 'Barese' (0.5%), 'Tuono' (3%), 'Genco' (3%) and 'Occhiorosso' (1%). Probably these studies were not well conducted since self-compatibility was later found in this population, precisely in some of these cultivars.

Pollination tests were also conducted in countries where almond was not so important. Rudloff and Schanderl (1935) found complete self-incompatibility in four selections in Germany. Gebauer (1952) in Austria also indicated that poor fruit production by isolated almond trees was due to self-incompatibility. Gagnard (1954) studied the most important cultivars in Algeria, all of foreign origin. Three of them, 'Avola', 'Fournat de Brézénaud' and 'Marcona', had some fruits, but self-incompatibility was confirmed again, like in Bulgaria by Vasilev (1972), with an average self fruit of 1.6% for both local and foreign cultivars. Self-incompatibility was also confirmed for several clonal selections in Turkey (Gülcan and Dokuzoguz, 1981).

In Portugal Almeida (1945, 1949), concerned about low production in almond, carried out a thorough study on the different causes of sterility, especially self-incompatibility. These studies included the main cultivars grown in Portugal (23 in 1945 and three more in 1949, mostly Portuguese, but some Spanish). Cultivars were divided into four groups according to degree of self-compatibility: (i) fully self-incompatible (16 cultivars); (ii) partially self-compatible, but economically self-incompatible (six cultivars with self sets ranging from 0.2 to 2.2%); (iii) self-compatible (a single cultivar, 'Pestaneta', with a self set of 15.1%); and (iv) highly self-compatible: 'José Dias' (33.9%) and 'Duro Italiano' (34.5%). Although 'Pestaneta' had a high self set, the conclusion was that only 'José Dias' and 'Duro Italiano' could be considered practically self-compatible. This was the first study to clearly describe self-compatible cultivars.

In Spain, the first report by Salom (1922) proved that orchards of a single cultivar were unproductive, and that the presence of a single tree of another cultivar raised the production of the neighbouring trees. Fruit production decreased as the distance to that single tree increased.

Reporting the study of Tufts (1919) incorrectly, Salom concluded that floral morphology (especially the number of stamens) and tree vigour were responsible for low production. Later on, Sala Roqueta (1941) and Cambra (1954) proved the self-incompatibility of 'Desmayo Largueta', one of the most important almond cultivars in Spain. Self-incompatibility was also confirmed for the main cultivars of the island of Mallorca (Anonymous, 1958–1972) and of other regions (Herrero *et al.*, 1977).

All these studies on almond self-incompatibility were done by pollination trials involving artificial hand pollination and bagging flowering branches. These studies also allowed the establishment of the first cross-incompatible groups of cultivars, as seen in Chapter 1, and the identification of the first *S* alleles of incompatibility, identified by letters in the Californian studies and by numbers in the European ones (Crossa-Raynaud and Grasselly, 1985; Kester *et al.*, 1994a), although the number identification was later adopted. The identification of *S* alleles advanced significantly after the correlation of ribonuclease zymograms and incompatibility alleles was found in almond (Bošković *et al.*, 1997), and the new molecular approaches of allele identification by PCR and gene sequencing were applied to almond.

The first identification of *S* alleles allowed the numbering of six alleles (Crossa-Raynaud and Grasselly, 1985). Kester *et al.* (1994a) reported four alleles, and assigned 31 Californian cultivars to seven incompatibility groups. Later, 35 more cultivars were genotyped and new alleles were suggested (Bošković *et al.*, 2003). The utilization of genomic DNA for PCR analysis allowed the amplification of new *S* alleles and more than 160 cultivars were genotyped (Tamura *et al.*, 2000; Ma and Oliveira, 2001; Certal *et al.*, 2002; Channuntapipat *et al.*, 2003; López *et al.*, 2004; Ortega *et al.*, 2005; Barckley *et al.*, 2006; Ortega *et al.*, 2006; Kodad *et al.*, 2008; Kodad *et al.*, 2010). The determination of the *S* genotype of new almond populations is still in progress as little studied germplasm is being included, such as genotypes from Iran (Hafizi *et al.*, 2013), Morocco (El Hamzaoui *et al.*, 2015), Sicily (Currò *et al.*, 2015) and Hungary (Halász *et al.*, 2016), involving also the wild almond species (Sánchez and Oliveira, 2005; Rahemi *et al.*, 2010). The sequences of the *S* alleles have been deposited in international databases.

8.2 Almond Self-compatibility

Despite the overwhelming evidence of the predominance of self-incompatibility in almond, some self-compatible genotypes were discovered during the different pollination tests described in the previous section. Although some authors erroneously concluded that some genotypes were self-compatible, the first unequivocal description of self-compatibility in almond was by Almeida (1945). However, no attention was paid to the issue, nor to other scattered references in the following years. Thus, Costetchi (1967) described 'Exinograd' as a self-compatible cultivar in the comprehensive *Pomologia Republicii Socialiste Romania*. This cultivar is of Bulgarian origin and spread all over the Balkans, reaching the island of Cyprus, where its self-compatibility was also noted (Anonymous, 1973). Almond self-compatibility was also assumed when recommending a self-compatible cultivar such as 'Mazzetto' for planting in Tunisia (Anonymous, 1972).

The considerable possibilities of hybridization between peach and almond (Bernhard, 1949; Cambra, 1979) have led to some confusion over the presence of self-compatibility in almond. Thus, Schanderl (1960) reported that four of 23 German cultivars were self-compatible, but they showed many hybrid traits, pointing to a peach $\times$ almond hybrid origin. The same consideration may apply to some indigenous selections from India (Singh *et al.*, 1969; Kumar and Kumar, 2000). Self-compatibility was widespread among these forms previously selected within seedlings growing in groups close to a wild or feral situation. Selection was based on their good productivity, a trait that has been closely related to self-compatibility (Godini *et al.*, 1977), thus suggesting that the prevalence of this trait could be due to an indirect selection. Different wild peach species can be found in the Himalaya region where almond is grown in India, as well as in natural hybrids among them (Kishore and Randhawa, 1993), thus raising the possibility of self-compatibility transmission from peach to almond as suggested by Kester (1970).

In the early 1970s different approaches were directed towards the identification of almond self-compatibility, since the work by Almeida (1945) and the other references to almond self-compatibility had not been taken into account. In France Charles Grasselly assembled a large

collection of almond cultivars from all over the world and reported that several self-compatible cultivars were found among the almond population of Puglia (Grasselly and Olivier, 1976). These cultivars included 'Tuono', 'Genco', 'Filippo Ceo', 'Occhiorosso' and 'Cristomorto', although the latter name was probably an incorrect identification, as later reports showed that 'Cristomorto' is self-incompatible. These authors pointed out that there were probably some name inaccuracies due to the collecting methodology.

At the same time in California self-compatibility was researched in several almond selections derived from a peach $\times$ almond hybridization, applying for the first time the examination of pollen tube growth in almond (Socias i Company *et al.*, 1976). This work confirmed the transmission of self-compatibility from another species through successive backcrosses, as suggested by Kester (1970), although these selections showed different degrees of sterility. Additionally, this work sustained the probable hybrid origin of the selections studied by Schanderl (1960) and Kumar and Kumar (2000), as well as some cultivars reported by Crane (1961) as grown to a limited extent as backyard trees in eastern and central USA, such as 'Ridenhover', 'Pioneer' and 'Hall's Hardy', although these names identify identical or similar cultivars (D.E. Kester, 1990, unpublished results).

As a consequence of the work developed in California, pollination trials were also conducted in Zaragoza (Spain), with the identification of two self-compatible cultivars based on their pollen tube growth, 'Tuono' from the Italian region of Puglia and 'AS-1', a local Spanish selection (Herrero and Felipe, 1975). Both had received attention because of their consistent production. Although not very late in blooming, 'AS-1' blooms much later than the usual Spanish cultivars, which allowed its identification. Later studies, however, showed that 'AS-1' is self-incompatible, as confirmed by pollen tube growth, fruit set and allele identification (Kodad *et al.*, 2011). On the other hand, a very high self set was obtained in 'Tuono', with 27.6% by natural self-pollination in a large cage without pollinating insects, and 41.8% with hand self-pollination (Herrero *et al.*, 1977).

Another important event in the early 1970s affecting almond self-compatibility was the formation in 1974 in Zaragoza (Spain) of the

Group de Recherches et d'Études Méditerranéen pour l'Amandier (Mediterranean Group for Almond Research and Study, GREMPA), whose colloquia have been the meeting point of most almond researchers, and where most advances in almond research, including self-compatibility and the development of new self-compatible cultivars, have been discussed. The report by Hererro and Felipe (1975) was presented at its second colloquium, and the transmission of almond self-compatibility was already being assessed at the third meeting (Socias i Company and Felipe, 1977).

Excluding the self-compatible forms derived from peach and some inaccurate observations, most self-compatible cultivars belong to the Puglia population identified by Grasselly and Olivier (1976), who suggested that these forms were maintained and propagated through selection for late blooming and productivity among the original seedling population. Many other self-compatible genotypes have later been reported in this population (Godini, 1977; Godini *et al.*, 1977; Reina *et al.*, 1985; Godini *et al.*, 1992). To this Puglia population also belong 'Duro Italiano', reported in Portugal; and 'Mazzetto', reported in Tunisia, where it was probably introduced through Libya when Italian settlers planted almond orchards there. Both 'Duro Italiano' and 'Mazzetto' are probable synonyms of 'Tuono' (C. Grasselly, 1990, unpublished results). Outside the Italian region of Puglia, another self-compatible cultivar, 'Truuito', was reported in Greece (Moukhthouri-Stylianidou, 1977; Vasilakakis and Porlingis, 1985).

8.3 Genetics of Self-compatibility

8.3.1 Historical

The behaviour of pollen tube growth and the studies in some related species of the genus *Prunus*, such as cherry (Crane and Brown, 1937), suggested that almond self-incompatibility is of a single locus gametophytic type (Socias i Company *et al.*, 1976). This self-incompatibility system is ruled by the recognition of the pollen tube by the pistil through which it grows. The compatible or incompatible reaction is controlled by a single locus, the locus *S*, with a series of alleles,

the S_i alleles. The pollen, as a haploid cell, has a single *S* allele. If this allele coincides with one of the two alleles of the pistil, a diploid maternal tissue, recognition takes place and pollen tube growth stops. Any almond cultivar, as a diploid plant (i.e. S_iS_j), produces two different kinds of pollen: genotypes S_i and S_j . Both kinds of pollen are unable to grow through the pistils of the same plant due to the identity between the allele of the pollen and one of the alleles of the pistil. This system explains the inter-compatibility of a parent plant with its offspring, as the offspring of an S_iS_j plant must be either S_iS_i or S_jS_j , $i \neq j$. Consequently, a parent plant produces half of its pollen compatible with any of its offspring in a semi-compatible pollination, as well as its offspring in relation to its parents. This system additionally explains the existence of cross-compatible and inter-incompatible groups, formed by genotypes possessing the same *S* alleles.

Studies of the genetic basis of almond self-compatibility examined this trait in the first crosses of almond breeding programmes. The expectation was that self-compatibility would be transmitted to the offspring, and this was soon confirmed (Socias i Company and Felipe, 1977). Transmission data suggested that self-compatibility expression was dominant over self-incompatibility, since self-incompatible forms were found both among offspring arising from matings of two self-compatible cultivars and from matings of a self-compatible and a self-incompatible cultivar. Consequently, the self-compatible cultivars used in these crosses were deemed heterozygous for self-compatibility (Socias i Company and Felipe, 1982). It was later concluded that self-compatibility was not only dominant over self-incompatibility, but that it was due to the presence of an allele (named S_p) allelic to the *S* alleles of incompatibility (Socias i Company, 1984).

Early work was based on pollen tube growth and fruit set and was mostly conducted in France and Spain. Grasselly *et al.* (1981) studied the offspring from crosses between the self-compatible cultivar 'Filippo Ceo' and several self-incompatible cultivars, as well as the seedlings resulting from self-pollination of self-compatible cultivars, reporting fruit sets obtained by bagging branches. The threshold of 6% was decided for self-compatibility recording, although this set is too

low from a horticultural viewpoint. However, not only must self-compatibility be accounted for in obtaining fruit sets by bagging, but floral morphology for anther to stigma contact (Weinbaum, 1985) and environmental conditions (Socias i Company *et al.*, 2005) must also be considered. Consequently, hand selfing could raise these levels of self set (Socias i Company and Felipe, 1987). As a result of their trials, Grasselly *et al.* (1981) hypothesized that the self-compatible cultivars were homozygous for self-compatibility, and that the null or low sets in some seedlings were not due to self-incompatibility but mostly to inbreeding effects. Although inbreeding has been shown to affect self-compatibility expression (Alonso and Socias i Company, 2005a) and transmission (Alonso Segura and Socias i Company, 2007), the re-examination of the results of the French (Grasselly and Olivier, 1984; Grasselly *et al.*, 1985) and of the Zaragoza (Socias i Company and Felipe, 1988) breeding programmes confirmed the dominance of the S_f allele over the other alleles of the S locus, as well as the heterozygous genotype of the self-compatible cultivars (Socias i Company, 1990). The same conclusion was later confirmed by Dicenta and García (1993).

8.3.2 Inbreeding effects on self-compatibility

Grasselly *et al.* (1981) suggested that inbreeding could reduce set in the offspring of some crosses. Inbreeding effects were shown by reduced vigour, low sets from open pollination, leaf abnormalities and low number of flowers. Low seed germination and survival of seedlings coming from inbred populations have also been observed (Grasselly and Olivier, 1981), as well as growth abnormalities and noninfectious bark necrosis (Grasselly and Olivier, 1988). The reduced fruit set and low seed germination could be related to the anomalous embryo sac development in inbred genotypes (Martínez-García *et al.*, 2012). In Spain, three levels of inbreeding depression were observed in crosses involving ‘Tuono’ (Socias i Company and Felipe, 1988): (i) no inbreeding depression when crossed with unrelated cultivars; (ii) some inbreeding depression when crossed with other cultivars of the same Puglia region; and (iii) high inbreeding

depression when selfed, evidenced by reduced vigour in growth and by a longer juvenile period.

Inbreeding may also affect self-compatibility transmission, as shown by deviations from the expected 1:1 ratio in some crosses. Grasselly *et al.* (1985) presented a hypothesis to explain these deviations in crosses involving ‘Tuono’ and another parent of ‘Cristomorto’ lineage, such as ‘Ferragnès’ and ‘Ferralise’. They suggested that ‘Tuono’ and ‘Cristomorto’, both from the Puglia region, had an S allele in common, as deduced from studies of cross-incompatibility among several seedlings coming from crosses involving these two cultivars, and as later confirmed by allele identification by RNase activity and PCR analysis. Consequently, they assigned the genotype $S_f S_f$ to ‘Tuono’, S_f being the common allele with ‘Cristomorto’, and the genotype $S_f S_3$ to ‘Ferragnès’, a seedling from the cross ‘Cristomorto’ × ‘Ai’ having inherited the S_f allele from ‘Cristomorto’. In the cross ‘Ferragnès’ × ‘Tuono’, only the ‘Tuono’ pollen grains carrying the S_f allele would be able to grow along the ‘Ferragnès’ pistils, whereas those carrying the S_f allele would not be able to grow in pistils carrying the same allele. If this might explain the low number of self-incompatible seedlings coming from the cross ‘Ferragnès’ × ‘Tuono’, the same argument would apply to the cross ‘Ferralise’ × ‘Tuono’, because ‘Ferralise’ has the same genotype as ‘Ferragnès’ and both are cross-incompatible (Cossa-Raynaud and Grasselly, 1985), but in the cross ‘Ferralise’ × ‘Tuono’ the deviation from a theoretical 1:1 ratio was not very large. Consequently, the different behaviour of ‘Ferragnès’ and ‘Ferralise’ in the transmission of self-compatibility was attributed to genetic factors other than the S alleles of incompatibility (Socias i Company and Alonso, 2004).

The explanation for this different behaviour was attributed to the inbred condition of ‘Ferralise’, as coming from the cross of two full siblings, ‘Ferragnès’ and ‘Ferraduel’ (Grasselly and Cossa-Raynaud, 1980). Inbreeding not only negatively affects pollen tube growth (Alonso and Socias i Company, 2005b), but also the expression of self-compatibility (Alonso and Socias i Company, 2005a) and its transmission (Alonso Segura and Socias i Company, 2007). Unsurprisingly, deviations from the expected ratios of self-compatible and self-incompatible seedlings were observed in offspring involving the inbred cultivar ‘Ferralise’.

8.3.3 RNase activity

The first incompatibility gene product was identified by McClure *et al.* (1989) as a ribonuclease in *Nicotiana glauca*. The same product was also identified in other species with a gametophytic incompatibility system such as Japanese pear (Sassa *et al.*, 1992) and cherry (Bošković and Tobutt, 1996). These RNases can be detected by separation of stylar proteins by non-equilibrium pH gradient electrophoresis (NEPHGE) and subsequent staining for activity, allowing the identification of the *S* alleles (Bošković *et al.*, 1997). However, when this approach was applied to self-compatible genotypes, no RNase activity was detected for the *S_f* allele (Bošković *et al.*, 1999). Consequently, they concluded that genotypes showing only one band for RNase activity were self-compatible. However, the presence of one band is not enough to assess the presence of the *S_f* allele. The absence of RNase activity may not only be due to the lack of transcription of the *S*-RNase in the pistil, but also to the very low level of this transcription. This low transcription level, reported in Japanese plum by Watari *et al.* (2007), has not been clearly noticed so far in almond (Hanada *et al.*, 2009). As mentioned above, inbreeding may also produce an incompatible expression of self-compatible genotypes with a single RNase band (Alonso and Socias i Company, 2005a).

Some problems, however, have arisen when RNase detection has been applied to different genotypes. Two different RNase bands may coincide after electrophoresis separation, thus giving a wrong 'one band' result when a real superposition of two bands is occurring. Consequently, this technique is only fully reliable for seedling identification when the genotypes of the two parents are already known (Bošković *et al.*, 2003).

8.3.4 Mapping

Since self-compatibility was supposed to be allelic to the *S* locus of self-incompatibility (Socias i Company, 1984), the mapping efforts were directed towards identifying the locus for self-incompatibility because the first attempts to locate self-compatibility were not successful.

Self-incompatibility was located at the end of linkage group 6 of the 'Ferragnès' map' (Ballester *et al.*, 1998) using the results of the RNases of the 'Ferragnès' × 'Tuono' population. However, as only one band was found in 'Tuono' and in its progeny, presumably self-compatible, no segregation was found of the RNase isozymes with any of the loci of the 'Tuono' map. The absence of an identified band for *S_f* in the RNase electrophoresis and the impossibility of locating its locus reinforce the allelic nature of *S_f* and the *S* alleles, revealing in self-incompatibility as a lack of action of an *S* allele, as seen later.

8.3.5 Allele identification by PCR

More recent advances in genetic analysis at the gene level have allowed a closer examination of the *S_f* allele in almond, both of the stylar and the pollen components. First, *S* alleles including *S_f* were identified by PCR analysis using conserved and allele-specific primers, and the partial sequence of the *S_f* allele gene associated with *S_f*-RNase was obtained (Channuntapipat *et al.*, 2001; Ma and Oliveira, 2001).

Various consensus primer sets have been designed to determine *S*-genotypes in almond. They were designed from conserved regions of *S* genes to amplify across the second intron (Tamura *et al.*, 2000; Channuntapipat *et al.*, 2003), the first intron (Ortega *et al.*, 2005) or both (Sutherland *et al.*, 2004). However, PCR primers designed from conserved regions do not always distinguish between alleles with a similar number of nucleotides (López *et al.*, 2004). This fact must be carefully taken into account, as occurs with RNase identification. As a result, this technique is only fully reliable for seedling genotyping when the genotypes of the two parents are already known. In addition, the detection of some alleles is masked by the presence of another allele, thus giving a wrong single band. This confusion was first detected by Channuntapipat *et al.* (2003) when the presence of either *S_i* or *S_j* masked the amplification of *S_g* by PCR when using conserved primers. The same masking has also been observed with other alleles (Alonso and Socias i Company, 2005c; Fernández i Martí *et al.*, 2009). As a consequence, other primer sets have been specifically

designed to amplify some S genes, including S_f (Channuntapipat *et al.*, 2001; Ma and Oliveira, 2001). PCR-based markers of almond S alleles have been employed to facilitate the integration of self-compatible S alleles from related species (Gradziel *et al.*, 2001). Screening efficiency and flexibility have also been greatly improved with the development of successful multiplex PCR techniques by Sánchez Pérez *et al.* (2004). This technique prevents one allele being masked by the expression of another.

8.3.6 Allele sequencing

Once the S_f allele was identified, the amino acid sequences of the RNases could be determined. From the beginning, several amino acid sequences for the S_f -RNase have been deposited in databases by different authors. When these sequences have been compared, several differences were observed between them. The diversity of the S_f -RNase sequences was closely examined by Hanada *et al.* (2009) to solve previous confusion over their identity. As a result of this examination, the sequences could be contrasted because most of them had been determined in ‘Tuono’ and genotypes derived from it, consequently for the same S_f -RNase. This identity allowed different sources of self-compatibility for the genotypes studied to be discarded. The first sequences reported by Channuntapipat *et al.* (2001) and Ma and Oliveira (2001) were already different. Further sequencings suggest that the sequence by Channuntapipat *et al.* (2001) was correct and must be taken as the consensus sequence.

Figure 8.1 shows the alignment of some of the sequences published for the S_f -RNase, as well

as some other S -RNases for comparison. This alignment mostly agrees with the results of Hanada *et al.* (2009), Kodad *et al.* (2009), Fernández i Martí *et al.* (2010) and Kodad *et al.* (2010). The first is considered the consensus sequence and was amplified in ‘Lauranne’ and selection IRTA12-2 (Channuntapipat *et al.*, 2001). These two self-compatible genotypes are derived from ‘Tuono’, but despite this origin their sequence is different from several published sequences for the ‘Tuono’ S_f -RNase (Table 8.1). It is however identical to the S_f sequence of ‘Cambra’, another cultivar derived from ‘Tuono’; to the S_{β} sequence of ‘Blanquerna’, a cultivar derived from ‘Genco’, not from ‘Tuono’; and to five S alleles reportedly conferring self-incompatibility in almond (Table 8.1).

These results show that some mis-sequencings and misinterpretations must have occurred during allele analysis. Ma and Oliveira (2001) showed valine instead of isoleucine and histidine instead of arginine in the C2 region, probably as a result of a mistake in sequencing (Fig. 8.1). Bošković *et al.* (2007) had to recognize a mis-sequencing in a note added in proof, thus invalidating most of the reasoning of their conclusions. Their ‘Tuono’ S_f did not really show the supposed histidine substitution instead of arginine in its sequence. Barckley *et al.* (2006) gave an amino acid sequence for ‘Tuono’ S_f identical to S_{β} , probably due to mis-sampling. Consequently, the ‘Tuono’ genotype studied by Barckley *et al.* (2006) in California must be the same as that utilized for the other analysis, as opposed to their original suggestion.

These mistakes led Bošković *et al.* (2007) to incorrectly name a new allele, $S_{30'}$, which is identical to S_{β} , but showing a different activity (Kodad *et al.*, 2009). This new name may create

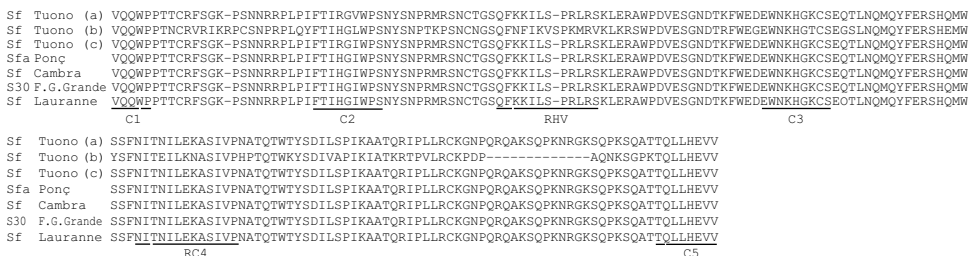


Fig. 8.1. Multiple alignment of the deduced amino acid sequence of different S almond alleles. Accession numbers and references are given in Table 8.1. (Socias i Company *et al.*, 2010.)

Table 8.1. Similarity of different almond S-RNases with the consensus S_f -RNase. (From Socias i Company *et al.*, 2010.)

Allele	Genotype	Database code	Coincidence with the S_f consensus allele (%)	Reference
S_f consensus	'Lauranne' Selection IRTA12-2	AY291117	100	Channuntapipat <i>et al.</i> (2001)
S_f	'Tuono' a	AF157009	98	Ma and Oliveira (2001)
S_f	'Tuono' b	DQ156217	64	Barckley <i>et al.</i> (2006)
S_f	'Tuono' c	AM690356	99.3	Bošković <i>et al.</i> (2007)
S_{fa}	'Ponç'	EU293146	100	Kodad <i>et al.</i> (2009)
S_f	'Cambra'	EU684318	100	Kodad <i>et al.</i> (2009)
S_{30}	'Fra Giulio Grande'	AM690361	100	Bošković <i>et al.</i> (2007)
S_{fi}	'Blanquerna'	AB467371	100	Fernández i Martí <i>et al.</i> (2010)
S_{fa}	'Alzina'	FJ887784	100	Kodad <i>et al.</i> (2010)
S_{fa}	'Garondès'	FJ887783	100	Kodad <i>et al.</i> (2010)
S_{fa}	'Vivot'	AB467370	100	Fernández i Martí <i>et al.</i> (2010)

new confusion in almond S-allele research because the identity of the S_f allele must be preserved, in spite of showing two different phenotypic expressions. This identity is not only restricted to the coding region (C1–C5), as deduced from their sequences (Fig. 8.1), but also to the alignment of their 5'-flanking regions as shown by the construction of a fosmid library (Fernández i Martí *et al.*, 2010).

8.3.7 The pollen component of self-incompatibility

The pollen component of self-incompatibility was identified later than that for the pistil. Ushijima *et al.* (2003) sequenced the pollen S haplotype termed F-Box (SFB), finding that this could be a good candidate for the pollen S product. This candidacy is based on its specific expression in the pollen tube, to be physically linked to the S-RNase gene, to show allele sequence diversity, and dysfunction in the self-compatible S haplotype (Entani *et al.*, 2003; Ushijima *et al.*, 2003; Yamane *et al.*, 2003; Tao and Iezzoni, 2010). Although identified later, the SFB gene was quickly sequenced, without the discrepancies described for the RNase gene. Thus, the sequences for 'Tuono' (AM711126) by Bošković *et al.* (2007) and for 'Lauranne' (AB361036) by Hanada *et al.* (2009) are identical.

8.3.8 The double expression of the S_f allele

The problems related to inbreeding in the expression and transmission of self-compatibility lead to the search for sources of self-compatibility other than the cultivars from the Italian region of Puglia as an alternative to avoid inbreeding. The survey of the almond germplasm collection of the CITA in Zaragoza (Espiau *et al.*, 2002) allowed identification of the S_f allele in several cultivars from the island of Mallorca (Fernández i Martí *et al.*, 2009; Kodad *et al.*, 2008; Kodad *et al.*, 2010) which had always been described as self-incompatible (Rubí, 1980). Furthermore, several almond cultivars from other geographical origins, although possessing the S_f allele, were also self-incompatible (Bošković *et al.*, 2007; Martínez-García *et al.*, 2011). This discrepancy imposes a new perspective for the identification of self-compatibility in almond and compels a revision of previous results based solely on the molecular characterization of self-compatibility (Socias i Company *et al.*, 2010).

The self-incompatible phenotype of these genotypes possessing the S_f allele has been confirmed by pollen tube growth, fruit set after field pollinations and stylar RNase activity, since two bands were detected after NEPHGE as in any self-incompatible genotype. Additionally, the presence of the S_f allele was confirmed after PCR

amplification. Thus, it was concluded that the S_f allele has two different expressions, one self-compatible and the other self-incompatible (Kodad *et al.*, 2009). The results confirmed that in the self-incompatible cultivars S_f is an active allele, which may be considered as another allele of the S series of self-incompatibility (Socias i Company *et al.*, 2012a). As a consequence, it has been designed as S_{fa} (Kodad *et al.*, 2009), in opposition to the inactive form of the same allele, S_{fi} (Fernández i Martí *et al.*, 2009). Thus, S_{fi} does not produce RNase and is linked to self-compatibility, whereas S_{fa} is distinguished by producing RNase, as the other SI alleles, and is linked to self-incompatibility.

The two forms of the S_f allele, however, show a full genetic identity. Sequencing of the S_f -RNase from both self-compatible and self-incompatible genotypes showed their full genetic identity, not only in the coding region of the S_f -RNase (Kodad *et al.*, 2009), but also in the 5' regulatory sequence of the S_f -RNase (Fernández i Martí *et al.*, 2010). The sequence of the SFB is also the same for the self-incompatible SFB_f allele sequenced in 'Ponç' (EU310402) by Kodad *et al.* (2009) and in 'Fra Giulio Grande' (AM711127) by Bošković *et al.* (2007). These coincidences indicate that the SFB_f allele, as well as the S_f -RNase allele, show two different phenotypic expressions. In addition, both the pistil and the pollen components of the S_f allele show the same compatible or incompatible behaviour simultaneously. These results suggest that the coding region of the S_f gene may not be the exclusive origin of self-compatibility in almond (Kodad *et al.*, 2009). Thus, some genetic modifications outside this coding region are affecting that expression (Fernández i Martí *et al.*, 2009).

The full genetic identity of the two forms of the S_f allele with different expression was further confirmed when the reciprocal recognition of the two forms was examined. This recognition was established after studying several offspring from genotypes showing different S -allele combinations, examining the S genotype of the offspring and how the alleles were transmitted. S_{fi} pollen, characterized by self-compatibility, is able to grow in S_{fi} pistils, but not in S_{fa} pistils, where the RNase produced by the S_{fa} pistils is able to recognize the S_{fi} pollen, thus stopping its growth and resulting in an incompatible pollination (Fernández i Martí *et al.*, 2010). In contrast S_{fa}

pollen, characterized by self-incompatibility, is not able to grow in S_{fa} pistils, but can in S_{fi} pistils, where no S_f -RNase is produced. Consequently, S_{fi} functions as a null allele in the style, not producing any S -RNase. Due to the interaction of both forms of the S_f allele, S_{fa} S_{fi} homo/heterozygotes behave as fully self-incompatible genotypes because the presence of the S_{fi} allele does not ensure self-compatibility when the S_{fa} allele is also present (Kodad *et al.*, 2015).

The double phenotypic expression of the same genotype has also been observed in other *Prunus* species, such as Japanese plum (Watari *et al.*, 2007; Guerra *et al.*, 2009) and sweet cherry (Wünsch *et al.*, 2010), and may explain the cases of unilateral incompatibility (Bošković *et al.*, 2007; Fernández i Martí *et al.*, 2009; Socias i Company *et al.*, 2012a). These pollination trials have resulted in fruit set in only one direction whereas in the other the pollination was incompatible. This phenomenon is not the same as that observed in the almond 'Jeffries' (Kester *et al.*, 1994b), attributed to a dysfunctional S -allele haplotype in both pistil and pollen (Ushijima *et al.*, 2001).

The incompatible reaction has not yet been fully explained. It seems that the S -RNase exerts its cytotoxic effects in the pollen tube through RNase activity leading to cessation of pollen tube growth. Pollen S seems to be involved in the release of the S -RNase cytotoxicity. Thus, in self-pollination, pollen and pistil interaction leads to an incompatible reaction. A compatible reaction would require a pollen tube to have a mechanism to avoid S -RNase cytotoxicity (Tao and Iezzoni, 2010).

8.3.9 Origin of self-compatibility

Grasselly and Olivier (1976) first suggested that self-compatibility could have arisen by a mutation in the almond population of the Puglia, where selection for productivity and late blooming indirectly maintained and expanded this trait. With respect to the Puglia cultivars, Godini (1979) suggested that self-compatibility could be acquired by hybridization with *P. webbii* (Spach) Vierh., a self-compatible species growing wild in southern Italy and the Balkans (Grasselly, 1976), where most self-compatible almond cultivars

have been found, often in some locations close to the places where *P. webbii* grows wild (Reina *et al.*, 1985). This hypothesis was supported by the distribution of the different self-compatible almond cultivars and the transmission of self-compatibility in hybrids between almond and *P. webbii* (Socias i Company, 2004), as well as by the unequivocal self-compatibility of *P. webbii* (Godini, 2000), despite the misinformation of Ladizinsky (1999). However, the attempts to sequence S_f from *P. webbii* have not led to any conclusion on the identity of the S_f allele from almond (Sánchez and Oliveira, 2005).

The double expression of the S_f allele has offered a new possibility of explaining the origin of self-compatibility in almond. This double expression confirms the hypothesis that S_f is allelic to the S alleles of self-incompatibility (Socias i Company, 1984) and suggests that S_{fa} is probably the original allele, being another allele of the S locus in a predominantly self-incompatible species such as almond. Consequently, S_{fi} expression may have resulted from a mutation, as first suggested by Grasselly and Olivier (1976), although outside the coding region of the S_f allele (Kodad *et al.*, 2009).

The elucidation of which kind of mutation could have taken place and in which species may still require further efforts, but recent research could explain this mutation by an epigenetic change in the 5'-flanking region of the S_f -RNase (Fernández i Martí *et al.*, 2014). Even if the S locus encodes genes determining the S -specificity, an epigenetic change in this region of the S_f -RNase could be involved in the activation/inactivation of the S_f haplotype. DNA methylation has been suggested to suppress gene expression as a regulatory factor (Jones and Takai, 2001), indicating that these changes are not 'anomalies', but rather different faces of the same underlying system. This regulatory factor may thus bring about the double expression of the S_f allele. Consequently, the qualitative shift from self-incompatibility to self-compatibility could be due to the epigenetic changes in this region.

The original S_f haplotype would have been in its active form, thus conferring self-incompatibility, as with the other almond S haplotypes. This haplotype might have undergone an epigenetic change due to DNA methylation, appearing in another form of expression that gave rise to self-compatibility, a new physiological behaviour

in a self-incompatible species such as almond. All the S_f alleles examined belong to different Mediterranean regions and could be identical by descent from the original pool of Mediterranean almonds. The epigenetic change could only have taken place in the Italian region of Puglia, where most SC cultivars have been described. Additionally, all SC almond genotypes identified so far have shown an identical S_f allele (Socias i Company *et al.*, 2010) and are of Mediterranean origin, thus probably related to the Puglia almond population (Socias i Company *et al.*, 2012a). The identity of the S_f allele in all self-compatible cultivars so far identified may also sustain the hypothesis that this mutation is relatively recent in almond evolutionary history (Halász *et al.*, 2016).

8.3.10 Partial self-compatibility

Many almond cultivars are weakly self-compatible, as shown above with the results of self-pollinating different genotypes. There is no clear-cut separation between self-compatible and self-incompatible forms; and almond has gradations of self-compatibility, as do many other species (Fryxell, 1957), as already seen in the first work by Almeida (1945). This weak self-compatibility was first named pseudo-self-fertility by East and Park (1917), but it later became known as pseudo-self-compatibility. The presence of genetic factors that influence self-incompatibility can be hypothesized because null and small self sets are reported from different cultivars in the same study. These differences could be due, as suggested by Pandey (1959), to chance combinations of modifier genes weakening the incompatibility reaction. Partial self-compatibility also appears in almond selections derived from crosses with self-compatible cultivars, based on self set in bagged branches (Grasselly *et al.*, 1985) and pollen tube growth (Socias i Company *et al.*, 2013).

Socias i Company (1990) had already suggested that almond is a self-incompatible species with a genetic background of pseudo-self-compatibility as indicated by the small self set observed in some cultivars. With this background, only one S_f allele could break the self-incompatibility system, probably by interacting with this background of pseudo-self-compatibility. Additionally, Fernández i Martí *et al.* (2011) identified two

QTLs affecting almond self-compatibility, sustaining the quantitative nature of self-compatibility in almond. A similar condition could affect the Sicilian genotypes identified by Currò *et al.* (2015) as self-compatible, but without the presence of the S_f allele, although the given level of fruit sets may not allow a clear classification of these genotypes. From the agronomical point of view fruit set has been considered the main evaluation criterion for self-compatibility selection in almond (Kodad and Socias i Company, 2008). However, the joint effect of the S_f allele and other genes, such as the QTLs recently identified by Fernández i Martí *et al.* (2011), might explain this wide range of variation for both pollen tube growth and fruit set (Socias i Company *et al.*, 2013).

Hybridization may be also involved in this gradation of self-compatibility expression. Since Kester (1970) suggested transferring self-compatibility from peach to almond, many attempts have been directed in California towards obtaining self-compatible almond cultivars originating in peach × almond hybridizations. The most recent release from this origin has been ‘Sweetheart’, of S_7S_{14} genotype, which does not have the S_f allele and probably does not reach a sufficient self-compatibility level for commercial production (Gradziel *et al.*, 2013).

8.4 Breeding Autogamous Cultivars

Self-compatibility has the highest priority among the desirable traits for any almond cultivar (Socias i Company *et al.*, 1998), having become a requirement to establish more efficient orchards (Socias i Company, 1990). The importance of self-compatibility in almond is reflected by the very different level of importance given to it in two distant chapters of almond breeding: the first review (Kester and Asay, 1975) only mentioned self-compatibility as one of the three aspects of blooming, which appeared together among 15 modern breeding objectives. The last review (Socias i Company *et al.*, 2012b) expressed special emphasis on self-compatibility and the breeding programmes including this trait as an objective.

The importance of self-compatibility for almond breeding was recognized early (Socias i Company, 1978) and the breeding programmes were initiated with the expectation that

self-compatibility would be transmitted to the offspring, as was the case, even from another species. As knowledge of the transmission and regulation of self-compatibility increased, new strategies were developed to obtain offspring of only self-compatible genotypes (Ortega and Dicenta, 2003). Different methods of assessing self-compatibility have been considered (Ortega and Dicenta, 2004), although fruit set remains the main criterion as the plant material must be always checked for its behaviour in commercial conditions (Kodad and Socias i Company, 2008). Additionally, a successful cultivar must be autogamous, thus being able to be naturally self-pollinated without the presence of pollinating insects (Socias i Company, 1996).

The first attempts, when self-compatibility was not yet recognized in almond, included transmission from another species, since the S locus seems to be allelic in closely related species (Pandey, 1968), and hybridization is easy among the different species of the subgenus *Amygdalus* (Kovalyov and Kostina, 1935). Kester (1970) suggested peach because it is easy to cross with almond and there are natural hybrids, as seen in reviewing self-compatibility in almond. The University of California obtained several self-compatible selections from peach × almond hybridization after successive backcrosses to almond (Socias i Company *et al.*, 1976), from which ‘Sweetheart’ has been released. However, this cultivar was mostly recommended as a pollenizer of ‘Nonpareil’ with high-quality kernels since its self-compatibility level may not be complete, probably because its self-compatibility derived originally from peach (Gradziel *et al.*, 2013). Some private breeders from California have also utilized peach as a source of self-compatibility, including F.W. Anderson with ‘LeGrand’, although this cultivar cannot be considered self-compatible enough to be horticulturally useful (Weinbaum, 1985). Similarly, the Burchell Nursery introduced ‘Madera’ and ‘Self-set’, but little information is available (Kester, 1979). C.F. Zaiger also introduced ‘Garden Princess’ of peach origin (Gradziel, 2000), as well as ‘All-in-One’, soon discarded because of a large proportion of double kernels, and ‘Independence’, a seedling of ‘All-in-One’ (Eddy, 2011). Several attempts in India also utilized a peach × almond hybrid in crosses for obtaining self-compatible almond selections (Uppal *et al.*, 1984).

The University of California has pursued the use of other species for introducing self-compatibility in almond, including peach, *P. mira*, *P. webbi*, *P. feniziana* and *P. argentea* (Gradziel and Kester, 1998). The use of these different species aims at the introgression of self-compatibility alleles from multiple sources (Gradziel, 2003; Gradziel, 2016). The use of these wild species, usually with bitter kernels, stresses the importance of using homozygous sweet kernel cultivars to avoid the presence of bitter-kernelled cultivars in the breeding progenies (Ledbetter and Pyntea, 2000). *P. mira* was also included in the Yalta breeding programme because of its easy hybridization with almond (Kostina and Ryabov, 1959), and some F_1 plants were close to almond in appearance, with sweet kernels (Rikhter, 1969) as well as self-compatible (Ryabov, 1969).

Induced mutations have also been suggested for self-compatibility incorporation in almond, as has been done in cherry (Matthews and Lapins, 1967). Monastrá *et al.* (1988) introduced 'Supernova' as a self-compatible mutation of 'Fascionello' obtained by budwood irradiation, although this origin has been questioned (Marchese *et al.*, 2008).

At present most breeding programmes include self-compatible cultivars and selections as a source of self-compatibility. The most active programmes are developed in Spain, although important efforts are underway in other countries such as in Australia (Wirthensohn and Sedgley, 2002), and some selections were under evaluation in Italy (Palasciano *et al.*, 1998).

The first self-compatible releases from a breeding programme were 'Guara', 'Aylés' and 'Moncayo' (Felipe and Socias i Company, 1987) from the Zaragoza (Spain) breeding programme. 'Moncayo', while showing self-compatibility in laboratory conditions, was subsequently shown by Kodad *et al.* (2008) to be self-incompatible in the field. The success of 'Guara' has led to an important renewal of almond plantings in Spain, representing nearly 50% of new plantings (Socias i Company *et al.*, 2011), distinguished by its autogamy (Socias i Company and Felipe, 1992) and productive efficiency (Alonso *et al.*, 2012). Subsequently all new releases from Zaragoza are self-compatible cultivars including 'Blanquerna', 'Cambra' and 'Felisia' (Socias i Company and Felipe, 1999). 'Felisia' also incorporated the

late-blooming trait for avoiding spring frost damage. In 2006, 'Belona' and 'Soleta' were released as self-compatible cultivars with improved kernel quality (Socias i Company and Felipe, 2007). The two last releases, 'Mardía' (Socias i Company *et al.*, 2008) and 'Vialfas' (Socias i Company *et al.*, 2015) have been released as extra-late-blooming cultivars.

The breeding programme from IRTA (Reus, Spain) placed greater emphasis on fruit quality and late-blooming traits, with self-compatibility as a secondary objective. The first cultivars released (Vargas and Romero, 1994) were not considered self-compatible, although 'Francolí' was later shown to be so (López *et al.*, 2005). The 2006 releases further delay bloom time, including three self-compatible cultivars, 'Constantí', 'Marinada' and 'Vairo' (Vargas *et al.*, 2008).

The breeding programme from CEBAS-CSIC (Murcia, Spain) has as its main objectives self-compatibility and late-blooming time. The first releases in the late 1990s were 'Antoñeta' and 'Marta' (Egea *et al.*, 1999). Two more recent cultivars, 'Penta' and 'Tardona', are characterized by their extremely late-blooming time (Dicenta *et al.*, 2009).

The French breeding programme was the most successful programme in Europe for many years. The 1989 releases 'Lauranne' and 'Steliette' were self-compatible (Grasselly *et al.*, 1992), with 'Lauranne' being particularly successful in France (Duval and Grasselly, 1994). A more recent release from this programme has been 'Mandaline' (Duval, 1999).

Most recently the breeding programme of Israel has released its first self-compatible cultivar, 'Matan', characterized by a large nut (Holland *et al.*, 2016).

8.5 Conclusion

Almond breeding programmes for self-compatibility were initiated with the expectation that self-compatibility would be transmitted to the offspring, as was the case. These programmes have been really successful and more than 20 cultivars have been released from the different breeding programmes (Table 8.2). The Californian programmes have mostly introduced

Table 8.2. Self-compatible almond cultivars released from breeding programmes.

Cultivar	Origin
Spain	
CITA de Aragón (Zaragoza)	
‘Guara’	Clonal selection
‘Aylés’	‘Tuono’ OP
‘Blanquerna’	‘Genco’ × AS-1
‘Cambra’	‘Ferragnès’ × ‘Tuono’
‘Felisia’	‘Titan’ × ‘Tuono’
‘Belona’	‘Blanquerna’ × ‘Belle d’Aurons’
‘Soleta’	‘Blanquerna’ × ‘Belle d’Aurons’
‘Mardía’	‘Felisia’ × ‘Bertina’
‘Vialfas’	‘Felisia’ × ‘Bertina’
CEBAS-CSIC (Murcia)	
‘Antoñeta’	‘Ferragnès’ × ‘Tuono’
‘Marta’	‘Ferragnès’ × ‘Tuono’
‘Penta’	S5133 × ‘Lauranne’
‘Tardona’	S5133 × R1000 (‘Tardy Nonpareil’ × ‘Tuono’)
IRTA – ‘Mas de Bover’ (Reus)	
‘Francolí’	‘Cristomorto’ × ‘Tuono’
‘Constantí’	(‘Ferragnès’ × ‘Ferraduel’) OP
‘Marinada’	‘Lauranne’ × ‘Glorieta’
‘Vairo’	(‘Primorskij’ × ‘Cristomorto’) × ‘Lauranne’
France	
INRA (Avignon)	
‘Lauranne’	‘Ferragnès’ × ‘Tuono’
‘Steliette’	‘Ferragnès’ × ‘Tuono’
‘Mandaline’	‘Ferralise’ × ‘Tuono’
USA	
University of California (Davis)	
‘Sweetheart’	SB3, 54-39E ((‘Lukens Honey’ peach × ‘Mission’) × ‘Nonpareil’) × Sel 25-26
Zaiger Genetics	
‘Garden Princess’	(‘Merced’ × dwarf peach)
‘All-in-One’	F ₂ from peach
‘Independence’	‘All-in-One’ × 21G8 almond seedling
Israel	
Agricultural Research Center (Newe-Ya’ar)	
‘Matan’	‘Lauranne’ × ‘Um El Fahem’

self-compatibility from peach, whereas the European programmes have used the Puglia cultivars as a source for self-compatibility. With the exception of ‘Blanquerna’ and its two seedlings ‘Belona’ and ‘Soleta’, all the other European releases have inherited self-compatibility from ‘Tuono’ or selections derived from it. In addition, other cultivars descending from the Puglia genepool such as ‘Ferragnès’ and its seedlings ‘Ferralise’ and ‘Lauranne’, have been included as parents in many crosses. As a

consequence, inbreeding problems have appeared in some of their offspring, requiring special caution to avoid negative consequences in the new releases (Alonso and Socias i Company, 2005a). Self-compatibility maintains its importance in most almond breeding programmes (Socias i Company *et al.*, 2012b) and some of the new releases are being planted on a large scale (Eddy, 2011; Socias i Company *et al.*, 2011), showing the real need for autogamous cultivars for efficient almond growing.

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9 Rootstock Development

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9.1 Introduction

Almond rootstock utilization differs between the two main almond-growing regions of the Mediterranean basin and California, with each region developing in a different direction, and the changes are much more notable in the Mediterranean basin due largely to the different almond-growing system of that region (Fig. 9.1).

In the Mediterranean basin, almond production has transitioned in recent decades from traditional cultural practices with marginal inputs, to new orchards aiming to obtain high yields, including the challenges of planting intensive and semi-intensive orchards. Almond had been grown in the past under endemic rainfall, and seedling almonds had been used for centuries as rootstocks because their roots were apparently more efficient in the extraction of water and nutrients from the soil. Initially, seedlings were produced with seeds of unselected plants, primarily from bitter almonds. Eventually, some cultivars were selected because they produced more homogeneous plants among the seedling populations (Felipe, 1989), or because they showed some root-knot nematode (RKN) resistance (Kochba and Spiegel-Roy, 1976).

In the 1970s almond × peach hybrids like ‘GF-677’ were introduced as almond rootstocks. Although they were originally selected for peach,

they showed excellent performance with almond and eventually became viable substitutes for almond seedlings. Due to their good performance under natural rainfall conditions in arid regions and also to their vigorous root system, these hybrids competed directly with almond seedlings as preferred rootstocks. Under Mediterranean conditions, the hybrid ‘GF-677’ has been the most-used rootstock in the past 10 years. In southern Spain, where seedling almonds are widely used, ‘GF-677’ represents 15% of the rootstocks used for almond (Arquero *et al.*, 2002). However, the present trend in the Mediterranean region is towards the utilization of other interspecific hybrids, mainly ‘Garnem’ (Rubio-Cabetas *et al.*, 2005; Felipe, 2009). At present the interest in using the new red-leaved ‘Garnem’ hybrid for almond has increased exponentially (Felipe *et al.*, 1997; Felipe *et al.*, 1998b; Felipe, 2009), mainly for replant conditions as well as traditional planting distances where vigour is desirable. Both hybrids, ‘GF-677’ and ‘Garnem’, have shown good performance for years with several cultivars (Bielsa *et al.*, 2015; Rubio-Cabetas, 2015) (Fig. 9.1). For the most recent orchards, where increased yields are expected, these rootstocks are less productive under natural rainfall conditions, because they are very vigorous and thus require more water than almond seedlings. Despite this need for water, the use of these clonal hybrid rootstocks usually improves tree growth

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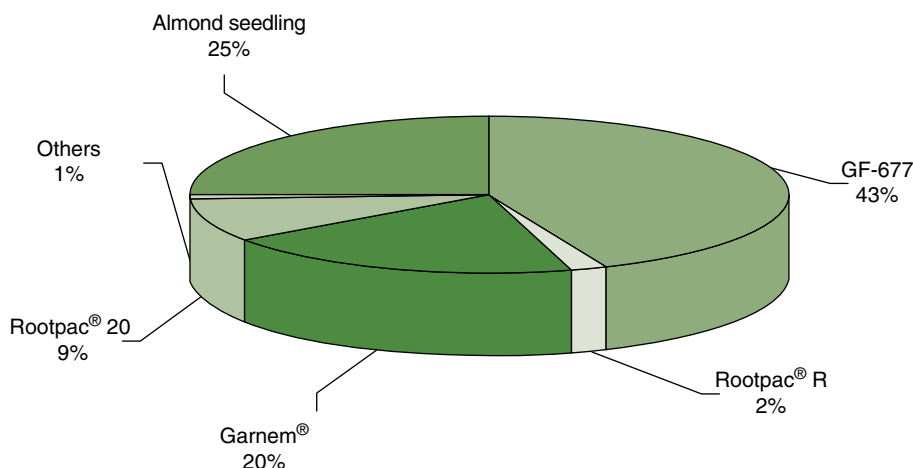


Fig 9.1. Almond rootstock genotypes planted in Spain during 2014–2015 based on survey data from estimates of the main nursery companies.

and plant yields. Moreover, with irrigation the production and yield from these vigorous rootstocks is significantly greater, even in the early years of the orchard.

In California and Australia the most commonly used rootstock is the peach seedling 'Nemaguard', though this may be primarily due to its availability for these rapidly expanding industries. Currently, important trials are under way to select better rootstocks for almond growing in both California and Australia. 'Nemaguard' had been used extensively because of its resistance to RKNs in sandy soils, where the seedling peach rootstock 'Lovell' is susceptible. More recently, almonds on 'Guardian®' instead of 'Nemaguard' are being planted on sandy, bacterial canker-prone sites, and 'Viking' is being planted extensively on similar sites and saline soils (Figs 9.2 and 9.3). The American red-leaf 'Nemared', derived from 'Nemaguard', was not well adapted to the calcareous soils of most of the Mediterranean region, but it was used as a parent in crosses to produce red-leafed peach × almond hybrids with RKN resistance and tolerance to calcareous soils (Felipe, 1984).

9.2 Selection Criteria for Almond Rootstocks

The most important traits of an almond rootstock are categorized into five groups: (i) nursery characteristics; (ii) graft compatibility; (iii) or-

chard characteristics; (iv) resistance to biotic and abiotic factors; and (v) good sanitary status (Felipe *et al.*, 1998a). The nursery production system determines if the rootstocks are to be seed or vegetative (asexually) propagated. In any case, any rootstock must fulfil all or most of the requirements from each group.

9.2.1 Nursery characteristics

Easy propagation is the first requirement for any rootstock. In the case of seedling rootstocks, this implies mother plants with very high fruit set and seeds with a high germination rate. Although seedlings are always heterogeneous, some seed sources produce seedlings with a good level of homogeneity and are consequently preferred.

For clonal rootstocks, propagation by cuttings is usually preferred because it is less expensive than other propagation methods. However, today micropropagation is now considered as the best alternative method by progressive nursery and fruit industries and also the only alternative for clones difficult to propagate by cuttings. In any case, the mother plants must have a growth habit allowing the production of many propagules. For cuttings, an erect and vigorous growth habit with minimal production of lateral shoots (feathers) is preferred. In any propagation method, rooting must be easy and the resulting root system vigorous.

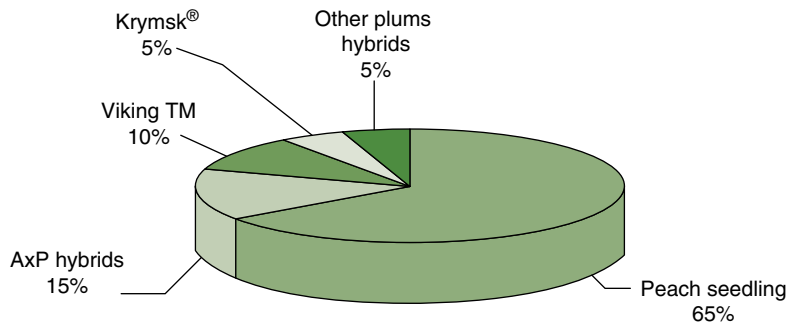


Fig 9.2. Almond rootstock genotypes planted in USA in 2013–2014 and estimated percentage of the rootstock makeup of the 8.33+ million trees sold.

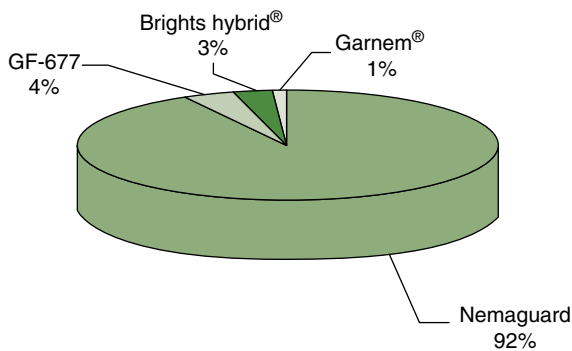


Fig 9.3. Almond rootstock genotypes planted in Australian almond orchards in 2013. (Almond Board of Australia: survey response equating to 88% of planted hectares, and own data.)

A good root system is always a requirement for any rootstock. Seedling rootstocks frequently have the undesirable trait of a taproot with little branching, but some seedling rootstocks produce enough lateral roots to allow good plant establishment and growth.

Growth habit in the nursery must facilitate the nursery operations. Consequently, an erect growth habit with no production of feathers near the budding point is a positive trait to facilitate budding. Also a morphological marker differentiating the scion from the rootstock, such as the case of red-leaved rootstocks, simplifies rootstock regrowth suppression during nursery management.

9.2.2 Graft compatibility

A commercial rootstock must be compatible with all or most cultivars of the species for which it has been selected. In almond, graft compatibility is successful with rootstocks of almond and peach (*Prunus persica* (L.) Batsch)) and their

hybrids. So far there has been no reported incompatibility between almond and peach. With hexaploid plums (*P. domestica* L. and *P. insititia* L.) there are frequent cases of localized incompatibility, and with diploid plums (myrobolan and Marianna), there is often translocated incompatibility. Results with some diploid plums show that there is an increased frequency of translocated incompatibility with 'Desmayo Langueta'. However, 'Ardècheoise' exhibits no translocated incompatibility and only a few cases of localized incompatibility (Felipe, 1989). These differences indicate the importance of the choice of the scion/rootstock combination when testing a new rootstock for compatibility.

The scion or aerial part can be replaced throughout the life of the trees by topworking. Although this operation is expensive, it can be cost effective. On the other hand, if the rootstock choice is not compatible, the whole tree must be replaced, thereby requiring orchard removal and replanting, which exacerbates any existing replant problem. Progressively, the group of available rootstocks is larger now since new

selections resulting from existing breeding programmes in several countries are being quickly introduced to the marketplace. The diversity of soils and the need to overcome adverse edaphic factors have prompted the search for rootstocks that can adapt to and grow well in a wide range of soil conditions. Since stone fruit rootstocks can be used with different species, this genetic flexibility can be used to improve efficiency of nursery operations and new cultivar selections.

9.2.3 Orchard characteristics

Tree failure after transplanting stress results in heterogeneity in tree ages within the orchard and affects both orchard system development and economic returns. Consequently, a high rate of successful transplanting and a quick recovery is required to obtain a homogeneous orchard. Since almond seedlings are generally sensitive to improper transplant techniques, new interspecific rootstocks might be a better choice for replant conditions.

Fruit tree size generally depends on the rootstock. In some fruit species tree size is an important consideration for ease of pruning, harvest and phytopathological treatments. In almond it is not as important but some aspects must be taken into consideration. For xeric environments, vigorous rootstocks are preferred to induce good growth and cropping under seasonally unfavourable growing conditions. With irrigation, vigorous rootstocks are not as important, but they still induce rapid orchard development and increase the early productivity of the orchard.

The rootstock must promote high and consistent levels of production during the whole life of the orchard. Generally, orchard lifespan is long with almond seedlings and almond $\times$ peach hybrids. Peach rootstocks have a shorter life span. The effects of other rootstocks, especially hybrids with plums, are not as well known.

There are large differences among rootstocks in their ability to absorb water and nutrients from the soil. High absorption efficiency is desirable to sustain good productivity even under less favourable conditions. A well-developed root system, besides improving the nutrient uptake efficiency of the rootstock, is essential to ensure anchorage of the tree.

Several rootstocks, mainly plums, produce suckers. This trait must be selected against as much as possible because of possible orchard management constraints (weed control, labour access and irrigation) and the risk of disease through mechanical or chemical wounds to the suckers.

9.2.4 Resistance to biotic and abiotic factors

A good rootstock should tolerate and even thrive in most soils. Excessive soil requirements by a rootstock limit its utilization, thus restricting its commercial use. Consequently, a good adaptation to common soil problems, such as heavy and calcareous soils, is essential. Heavy soils frequently cause asphyxia. Almond and peach roots are very susceptible to this problem (Ama-dor *et al.*, 2012), whereas plum roots show the lowest degree of susceptibility. Since almond is grown largely on calcareous soils, resistance to lime-induced chlorosis is also an important requirement, as well as resistance to drought under non-irrigated conditions.

The soil has specific phytopathological problems unrelated to those of the aerial part. There are no available sources of resistance to soil diseases, such as crown gall caused by *Agrobacterium* and several root rots caused by different fungi, opening a wide field of research for possible sources of resistance. Work is ongoing in the search for resistance to the insect *Capnodis* (D'Hallewin *et al.*, 1992; Dicenta *et al.*, 1999). Important innovations and significant genetic progress in many programmes have been made in the development of broadly resistant rootstocks more specifically in RKN resistance; there have also been considerable advances in the PTSL syndrome caused by a ring nematode, but much less progress for lesion nematodes, where genetic resistance still remains unknown. Screening methodologies are somewhat laborious, involving either field tank or greenhouse assays. Improvements in methodology offer greater efficiency and reliability, as for RKN (Esmenjaud *et al.*, 1996), which is closely linked to the level of knowledge of the *Prunus* genome. This is one of the most advanced research areas, where several genes have been located in genetic maps.

However, fundamental knowledge still is lacking for some other soil-borne diseases caused by bacteria or fungi, which are a high priority. Additionally, RKN resistance could prevent some bacterial damage, and thus indirectly increase resistance to *Agrobacterium* (Rubio-Cabetas *et al.*, 2001) in stone fruit rootstocks. One of the current or impending demands in production areas with mild winters is resistance to *Capnodis* sp., flathead beetles whose larvae feed on roots. Some Spanish research centres are focusing on producing selected cultivars capable of producing resistance to *Capnodis*, but at present there has not been a significant positive result (Soler *et al.*, 2013).

9.2.5 Good sanitary status

A rootstock must be free from viruses, phytoplasmas and other graft-transmissible diseases because the vegetative propagation of clonal rootstocks can spread the disease to all trees. Thus, before releasing a rootstock, all the necessary tests must be performed to certify the rootstock as clean.

With these objectives, focused efforts have been successful in improving the nursery characteristics, such as the introduction of red-leafed rootstocks and *in vitro* propagation techniques. In relation to the phytosanitary status, the modern nursery industry has made considerable efforts in releasing commercial rootstocks with good phytosanitary status. However, for graft compatibility significant improvements still need to be made, especially when new genetic sources are used in the rootstock breeding programmes besides *P. persica*, *P. amygdalus* or *P. insititia*, which have proven good graft compatibility. Concerning orchard characteristics, a lot of work still remains to be done. Other adaptation traits need to be incorporated to have a productive rootstock for new areas where almond acreage is expanding that require tolerance to waterlogging, drought and soil diseases (fungal and bacterial), as well as cold hardiness and adequate vigour for modern almond orchard systems.

Rootstock evaluation requires long-term observations in the field to establish all the traits defining its behaviour. Only the addition of genomic tools, physiological assays and different approaches within distinct specialized studies can

reduce the time in the development and release of a clone possessing the traits just reviewed. At present there is no single rootstock fulfilling all these requirements. However, if this ideal rootstock does not exist, it is possible to select in each growing region the rootstocks best adapted to the conditions of that area, thereby improving almond production by using the best genetic material. The next section will describe the best rootstocks available at present for almond.

9.3 Characteristics of the Almond Rootstocks

9.3.1 Seedlings

Almond seedlings

Traditionally, seedlings from bitter almonds were used without paying attention to their origin, since bitter almond orchards were not established for production of seedling rootstocks. Seeds were often collected from wild almond populations that were cross-pollinated by the nearest orchards. This is the origin of seeds collected from their area of origin in East Asia and also in a population of *P. webbii* in Toledo, central Spain (Felipe, 1989). Later some cultivars producing more homogeneous seedlings were selected for producing seedling rootstocks. Classical Spanish cultivars described as the best for seedling rootstock production are 'Atocha', 'Desmayo Largueta', 'Garrigues', 'Marcona' and 'Ramillete' (Ramos, 1978). Other cultivars used for producing seedling rootstocks are 'Texas' in California, 'Chellaston' in Australia and 'Don Carlo' in Italy (Fideghelli and Loreti, 2009). Almond seedlings are generally sensitive to nematodes, fungi, bacteria and capnode worm (*Capnodis tenebrionis*).

The most remarkable features of almond seedlings are their hardiness and ability to grow in poor, high-limestone content soils, with little natural rainfall. In extreme situations, such austerity allows almond to survive better than other crops, though growing almonds under these conditions has little or no economic interest today. Another characteristic is a deep root system; however, observations on different soil types indicate that depth of root growth is limited by available oxygen in the soil pores. Therefore, in

porous soils roots reach deeper levels than in more compact soils. However, this deep root is lost when transplanting from the nursery to the field because the taproot is lost. A negative attribute of seedlings is that they are not homogeneous in growth development and behaviour and are sensitive to handling and transplanting, often with poor survival after planting. They are also susceptible to soil pathogens such as nematodes, *Agrobacterium* and *Armillaria*, and sensitive to root asphyxia by *Phytophthora* and waterlogging, so they are unsuitable for cultivation under irrigated conditions, except when the irrigation system is localized (drippers, microjet, etc.) and the soil has good drainage. As a general rule almond seedlings are suitable for growing in rainfed conditions (Felipe, 1989).

Today the almond cultivars most often used in Europe for seedling rootstock production are 'Garrigues' and 'Atocha'. In California, the typically used cultivar is 'Texas'. In Israel there are several bitter almond cultivars, 'Alnem 14' 'Alnem 88' and 'Alnem 201', resistant to RK nematodes and used as seedling rootstocks.

'GARRIGUES'. 'Garrigues' has been the most commonly used cultivar for seedling production because its seedlings are uniform with a strong and deep root system, although some feathers are produced at the graft union area. In contrast, 'Atocha' seedlings have fewer feathers. 'Garrigues' seedlings are used for rainfed conditions, with good performance in relation to drought. The roots are able to explore deeper soils and to maintain moisture. They are also highly adapted to calcareous soils, but are very sensitive to waterlogging. They are compatible with all almond cultivars, and induce excellent scion vigour and productivity. Their best performance is in fairly good soils that are permeable and well aerated in medium-latitude regions (Felipe, 1989). However, they are sensitive to *Agrobacterium*, *Phytophthora*, *Armillaria* and *Meloidogyne* sp. and *Pratylenchus vulnus* nematodes (Esmenjaud *et al.*, 1994). Despite these limitations, 'Garrigues' is still one of the most widely planted almond seedling rootstocks in Spain, according to information from the Spanish nurseries.

Peach seedlings

Peach seedlings were selected for irrigated almond production because they are less sensitive

to problems affecting almond seedlings, but they are not suitable for cultivation in rainfed environments. Peach has very good compatibility with almond, and almond cultivars on peach grow rapidly during the first years after planting. Peach seedlings have been selected explicitly for seed production as they typically give more homogeneous plants since peach is a self-compatible species with a high level of self-pollination. Some good seedling-producing cultivars are INRA's 'GF-305', 'Montclar' and 'Higama', and the USA cultivars 'Lovell', 'Nemaguard' and 'Nemared', which have been widely described (Felipe, 1989) and used for a long time both for peach and almond cultivars in different countries.

The positive features of peach seedlings are their better adaptation to cultivation in irrigated soils and the knowledge of their agronomic performance against some stresses, such as tolerance to certain species of nematodes. In general, peach seedlings cannot be regarded as a definitive solution for almond growing, but they represent an improvement for irrigated almond production. In contrast, they remain highly sensitive to some of the most common pathogens: *Agrobacterium*, *Armillaria*, *Phytophthora*, etc. Also they induce a shorter tree life than do almond seedlings. Plants produced from specific cultivars (e.g. 'Nemaguard' and 'Nemared') are resistant to RKN *Meloidogyne* spp.; and others (e.g. 'Rutgers Redleaf') are more tolerant to heavy soil conditions. Some cultivars have been used in crosses aimed to introduce resistance to RKN as well as other interesting characters like red leaf colour.

'NEMAGUARD'. 'Nemaguard' was selected among seedlings from a seedlot received in 1949 by the USDA, which was labelled *P. davidiana* and was eventually released in 1959. Thought to be a putative hybrid of *P. persica* × *P. davidiana*, field observations (Okie, 1998) and molecular studies (Lu *et al.*, 1998) indicate that it is primarily *P. persica*. A 'Nemaguard' seedling is uniform and vigorous, compatible with almond cultivars and imparts excellent scion vigour and productivity. It has good resistance to *Meloidogyne incognita*, *M. javanica* and *M. arenaria*, but recent research has confirmed that a new root-knot species (*M. floridensis*) can reproduce in the roots of 'Nemaguard' (Nyczepir and Beckman, 2000). 'Nemaguard' is fairly tolerant of crown gall, but is sensitive to *Pratylenchus vulnus*, fungal root rots, *Verticillium*, iron chlorosis and root

waterlogging, and may reduce winter hardiness of scion cultivars in cold climates. 'Nemaguard' suckers extensively and is very sensitive to ring nematode (*Mesocriconema xenoplax*) feeding, which leads to tree injury and death from bacterial canker (*Pseudomonas syringae* pv. *syringae* van Hall) and peach tree short life (Reighard and Loreti, 2008). Despite these limitations, 'Nemaguard' is one of the most widely planted stone fruit rootstocks in California and South America. For almond production in California, it performs best on fumigated ring nematode-infested soils, virgin stone fruit sites and soils that have RKN problems. 'Nonpareil' almond on 'Guardian[®]', an F₅ progeny descendant of 'Nemaguard', has produced slightly better on sandy, ring nematode sites (Duncan *et al.*, 2008; Paramount Farming Co., unpublished data) and is now being planted in place of 'Nemaguard' on a limited basis (Burchell Nursery, personal communication, 2015).

9.3.2 Clonal rootstocks

Clonal propagation, though more expensive than seedling production, offers the advantage that the behaviour of the material produced is very homogeneous and growth characteristics are more consistent and better known. In Spain the almond 'Garfi', a progeny of 'Garrigues', was selected because it was easier to propagate asexually. Research trials of propagation by rooting hardwood cuttings gave satisfactory results for 'Garfi' when compared with other almond cultivars (e.g. 'Garrigues'), peach and almond × peach hybrids (Felipe, 1984, 1992).

Plum as almond rootstock

Plum root systems tend to be shallow and generally roots are smaller in number and thickness than for peach or almond. Plums are usually more tolerant to certain pathogens and more resistant to the waterlogging conditions in heavy soils than almond and peach roots. Therefore, these rootstocks are often used if soils have a high bulk density, fine textured or are poorly aerated (Table 9.1). Graft compatibility with almond is highly variable, and so preliminary trials are required before a particular combination of almond on plum is utilized.

LOW-VIGOUR PLUMS. The plums belonging to *P. domestica* L. and *P. insititia* L. (i.e. European plums, 'Saint Julien', 'Damas', etc.) are included in this group. There is some experience with almond grafted on these rootstocks (Table 9.2). In many cases the compatibility is good, but some cultivars have shown localized incompatibility when grafted on some of these plums, and the graft union eventually deteriorates. The well-known 'pollizos' originating from Murcia are fully compatible if they are true 'pollizos' and are free of infectious agents like viruses. It is recommended to use selections currently in an advanced commercial stage such as 'Adesoto' and 'Montizo'. The compatibility tests conducted over several years with these rootstocks showed that very few of them had incompatibility problems when grafted with almond cultivars. The selected pollizo clones are plums that offer the best prospects for use as almond rootstocks for specific soil conditions or vigour reduction since they offer greater compatibility. Other European

Table 9.1. Rootstock choices depending on water availability and edaphic factors.

Type of cultivation	Type of soil	Rootstock			
		Almond	Peach	Hybrids	Plums ^a
Rainfed	Deep and aerated	xxx	xx	xxx	x
	Heavy or loam	x	x	xxx	x
Irrigated	Sandy	xx	xxx	xxx	xx
	Loam	x	xxx	xxx	xxx
	Clay-loam	x	xx	xxx	xxx
	Clay	x	x	xx	xxx
Replanting		Fair	Fair	Very good	Good
Graft compatibility		Very good	Very good	Very good	Variable

xxx, xx, x = best (xxx), good (xx) and least desirable (x) rootstock option

^agraft compatibility must be known

Table 9.2. Genetic and regional origin of plums and *Prunus* species hybrids used as almond rootstocks.

Rootstock	Breeder	Country	Genetic origin	Parents
Montizo	CITA-Zaragoza	Spain	<i>Prunus insititia</i>	Pollizo de Murcia seedling
Adesoto 101 (Empyrean® 101)	EEAD-Zaragoza	Spain	<i>P. insititia</i>	Pollizo de Murcia seedling
Nemaguard	USDA	USA	<i>P. persica</i>	
Guardian®	USDA-Clemson	USA	<i>P. persica</i>	
Controller™ 6 (HBOK 27)	UC Davis	USA	<i>P. persica</i> × <i>P. persica</i>	Harrow Blood × Okinawa
Myran®	INRA-Bordeaux	France	<i>P. cerasifera</i> × <i>P. persica</i>	Belsiana plum × Yunnan peach
Isthara®	INRA-Bordeaux	France	<i>P. cerasifera</i> × (<i>P. cerasifera</i> × <i>P. persica</i>)	Belsiana plum × (Myrobalan × peach)
Atlas	Zaiger Genetics	USA	<i>P. persica</i> × (<i>P. dulcis</i> × <i>P. blireana</i>)	Nemaguard × (Jordanolo almond × <i>P. blireana</i>)
GF-677 Paramount®	INRA-Bordeaux	France	<i>P. dulcis</i> × <i>P. persica</i>	Natural hybrid
Viking	Zaiger Genetics	USA	<i>P. persica</i> × (<i>P. dulcis</i> × <i>P. blireana</i>)	Nemaguard × (Jordanolo almond × <i>P. blireana</i>)
Cadaman	INRA-Bordeaux	France & Hungary	<i>P. persica</i> × <i>P. davidiana</i>	Peach × <i>P. davidiana</i>
Barrier 1 (Empyrean® 1)	CNR Rome	Italy	<i>P. persica</i> × <i>P. davidiana</i>	Peach × <i>P. davidiana</i>
Flordaguard	Univ. Florida, Gainesville	USA	(<i>P. davidiana</i> × <i>P. persica</i>) × <i>P. persica</i>	(<i>P. davidiana</i> × peach) × OP Okinawa peach
Hansen 536	UC Davis	USA	<i>P. dulcis</i> × <i>P. persica</i>	Almond B × (Okinawa × H91 peach)
Kuban 86 (Krymsk® 86)	Krymsk Breeding Station, Krasnodar	Russia	<i>P. cerasifera</i> × <i>P. persica</i>	Myrobalan × peach
Cornerstone	Burchell Nursery	USA	<i>P. dulcis</i> × <i>P. persica</i>	Almond × peach
Nickels	UC Davis	USA	<i>P. dulcis</i> × <i>P. persica</i>	UCD5-33 × Nemaguard
Bright's Hybrid® 5	Bright's Nursery	USA	<i>P. dulcis</i> × <i>P. persica</i>	Peach × almond
Monegro (POV)	CITA-Zaragoza	Spain	<i>P. dulcis</i> × <i>P. persica</i>	Garfi × Nemared
Felinem (POV)	CITA-Zaragoza	Spain	<i>P. dulcis</i> × <i>P. persica</i>	Garfi × Nemared
Garnem (POV)	CITA-Zaragoza	Spain	<i>P. dulcis</i> × <i>P. persica</i>	Garfi × Nemared
Rootpac® 40 (Nanopac)	Agromillora Ibérica	Spain	(<i>P. dulcis</i> × <i>P. persica</i>) × (<i>P. dulcis</i> × <i>P. persica</i>)	(Marcona × Nemaguard) × Felinem
Rootpac® 20 (Densipac)	Agromillora Ibérica	Spain	<i>P. besseyi</i> × <i>P. cerasifera</i>	Sand cherry × myrobalan
Rootpac® R (Replantpac)	Agromillora Ibérica	Spain	<i>P. dulcis</i> × <i>P. cerasifera</i>	Almond × myrobalan
Titan	USDA-Fresno	USA	<i>P. dulcis</i> × <i>P. persica</i>	Natural hybrid
AB/2 3	Sicily	Italy	<i>P. dulcis</i> × <i>P. persica</i>	Natural hybrid
Pedro	Univ. Chihuahua	Mexico	<i>P. dulcis</i> × (<i>P. davidiana</i> × <i>P. persica</i>)	Ne Plus Ultra × Flordaguard

plums such as 'Penta' and 'Tetra' are also being used in Italy with somewhat delayed blooming time as compared to 'GF 677'. The advantages of these slow-growing plums are that this group is more resistant than almond and peach seedlings to root asphyxia, as well as to root parasites such as nematodes, *Phytophthora*, *Armillaria*, *Agrobacterium*, etc. Trees are smaller, allowing for semi-intensive cultivation of irrigated almonds and also providing good anchorage. Because of their minimal resistance to drought, plum rootstocks should be considered only for irrigated conditions. The good compatibility of the clones in this group with almond is not universal, so there is a possible risk in some cases of localized graft incompatibility with certain almond cultivars. They usually have a tendency to produce suckers, which are a problem for orchard management.

VIGOROUS PLUMS. This group includes plums belonging to diploid species such as *P. cerasifera* Ehrh. (myrobalan), *P. salicina* Lindl. (Japanese plum), some hybrids like 'Marianna 2624' (*P. cerasifera* × *P. munsoniana*) and other diploid plums from different backgrounds (Table 9.2). This group of plums generally has poor compatibility with almond cultivars. Usually this incompatibility is of the translocated type, although in some combinations the incompatibility is localized at the graft union. Some clonal selections, being grafted with certain almond cultivars, show apparently good compatibility, as in the case of 'Marianna 2624' grafted with some California cultivars, and some clones of myrobalan with certain European cultivars. Some combinations of either almond/myrobalan or almond/Marianna have shown some compatibility in our trials. The advantages of these fast-growing plums are that they usually provide good vigour and canopy development when grafted with compatible cultivars of other species. In addition, they have a good adaptation to different types of soils, and are tolerant to higher humidity. They also have some tolerance to *Phytophthora* and *Agrobacterium*. Some selections are resistant to RKN *Meloidogyne* spp. In general, they are easily asexually propagated, provide good anchorage and have little tendency to sucker except for 'Marianna 2624'. These rootstocks should be used only with irrigation, since their behaviour on dry land is not satisfactory. Their compatibility problems with almond cultivars

make these plums virtually impossible to use without prior experience with each particular almond scion/rootstock combination.

'MONTIZO'. 'Montizo' was selected at the former Servicio de Investigación Agraria, now Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) in Zaragoza (Spain) from a population of open-pollinated seedlings of 'pollizo de Murcia' planted in 1975 (Felipe, 1989). This clonal rootstock is compatible with almond, apricot and peach (Felipe *et al.*, 1994; Felipe *et al.*, 1998b). It is a polyvalent rootstock, as are all the 'pollizos de Murcia'. It confers medium vigour with precocity and good productivity. It tolerates root asphyxia in heavy soils, as well as calcareous soils and those with fairly high salinity (Felipe, 1989). It propagates easily by hardwood cuttings during November and plants must be early grafted. Its root system is of medium depth. The main characteristic, as compared to other 'pollizos', is that it does not sucker extensively in the first few years. 'Montizo' is resistant to root pathogens such as *Agrobacterium*, *Phytophthora* and *Armillaria* (Felipe, 2000).

'ADESOTO 101'. 'Adesoto 101' ('Empyrean® 101' in the USA) was selected by the Experimental Station of Aula Dei (ESAD-CSIC) in Zaragoza (Spain) from a population of open-pollinated seedlings of 'pollizo de Murcia' (Moreno *et al.*, 1995). This clonal rootstock can be used for peach, apricot, plum and almond (M.A. Moreno, personal communication, 2002). It propagates easily by both hardwood cuttings and *in vitro* techniques (the preferred nursery method) and also adapts satisfactorily to calcareous (up to 10–11% limestone), fine-textured and waterlogged soils. 'Adesoto 101' is resistant or immune to *Meloidogyne arenaria*, *M. incognita* and *M. javanica* and is moderately tolerant to *Pratylenchus vulnus* (Pinchet *et al.*, 1999), but has been found to be highly susceptible to bacterial canker and peach tree short life in South Carolina (Reighard *et al.*, 2006).

Prunus interspecific hybrids

Almond growing has limitations in the Mediterranean region because of the prevalence of calcareous soils, where most rootstocks exhibit lime-induced chlorosis. In addition, the presence of RKNs (*Meloidogyne* spp.) in many sandy,

irrigated orchards renders almond growing extremely difficult. Thus, the need for rootstocks to overcome these limitations is essential for growing stone fruit, including almonds, in many regions of the Mediterranean basin (Felipe, 1989). For successful almond growing, the use of peach × almond hybrid rootstocks became a necessity in the Mediterranean region. Among the interspecific *Prunus* hybrids, some almond × peach selections have been better known and more disseminated, such as ‘Garnem’ (Felipe, 2009). In recent years other peach × almond hybrid clones have been studied, involving almond, peach and several plum species parentage, mainly those of interest because of their graft compatibility and satisfactory agronomical behaviour (Rubio-Cabetas *et al.*, 2005). After many years of attempted crosses there are now several clones commercially propagated, such as ‘Replantpac’, a myrobalan × almond hybrid with compatibility with almond (Pinochet, 2010). There are also several selections commercially propagated that could eventually replace ‘GF-677’, although their field behaviour with almond cultivars is still being evaluated (Table 9.2).

In the USA, the best-known and most commonly planted interspecific *Prunus* hybrids are the almond × peach hybrids, the most recent releases being ‘Nickels’ and ‘Cornerstone’. Most recently a peach × peach hybrid named ‘Controller™ 6’ has been also released. In Spain, the best-known new hybrids commercially propagated because of their high vigour are ‘Garnem’, ‘Felinem’ and ‘Monegro’. In recent years some progenies from a private Spanish breeding programme incorporating crosses between different *Prunus* species have been released as rootstocks for intensive orchard systems. Two of these are ‘Rootpac® 20’ (‘Densipac’) and ‘Rootpac® 40’ (‘Nanopac’). Though test data are not yet sufficient, these two rootstocks have exhibited medium vigour, equivalent to or less than peach seedlings (Table 9.2).

Clones of peach × almond hybrids in use

INRA ‘GF-677’. ‘GF-677’ (‘Paramount®’ or ‘Amandier®’) is a natural hybrid of peach and almond, selected between 1944 and 1950 at the French station of La Grande Ferrade (INRA, Bordeaux) from a seedling population (Bernhard and Grassely, 1981). The leaves are of intermediate

appearance and size between almond and peach. The appearance of the branches in winter is closer to peach because of the thick and downy buds. The flower is of rosaceous type, and pink. The growth habit is open and highly branched. The root system consists of numerous strong roots. As an almond rootstock it is very vigorous, inducing precocity and high yields. Its behaviour in rainfed regions is better than that of almond seedling rootstocks. Its tolerance to waterlogging is similar to peach seedlings, but it is more resistant to chlorosis. It is also relatively resistant to *Phytophthora*, but it is sensitive to *Agrobacterium* and nematodes.

The propagation of ‘GF-677’ was not easy initially, although progress has been made with both *in vitro* and *in vivo* propagation to obtain acceptable percentages of rooting, either by softwood or semi-hardwood cuttings in summer and autumn, respectively. The number of feathery leaves and branches produced in the nursery makes cutting preparation a slow and expensive operation. For the best grafting results in the nursery scions must be grafted later when vegetative growth begins to stop. ‘GF-677’ is a standard rootstock for difficult soils (calcareous, arid, nutrient poor). It also tolerates moderate salinity levels and shows good tree growth and higher productivity with the traditional cultivars. However, its behaviour in replant conditions is not optimal, and it is also very branched in the nursery, making grafting difficult. It is an excellent rootstock for growing almonds, under both rainfed and irrigated conditions, as long as the soil is nematode free and not compacted or of high bulk density. It is compatible with all almond cultivars. Due to its inherent induced plant vigour, it is essential to maintain a good training system using summer pruning and pinching, and less winter pruning in the early years.

‘GARNEM’, ‘MONEGRO’ AND ‘FELINEM’. The three clones were selected among the progeny obtained from the cross between the Spanish almond ‘Garfi’ as the female parent and the North American peach ‘Nemared’ as the pollen donor, giving rise to the G×N series. ‘Garfi’ had been previously selected because of its good morphological characteristics and ease of clonal propagation (Felipe, 1989; Felipe *et al.*, 1997). ‘Nemared’ was chosen mainly as a source for RKN resistance and red leaves (Ramming and Tanner, 1983). Selections

from among the progeny were made at the former Servicio de Investigación Agraria de la Diputación General de Aragón, now CITA.

Unbudded trees of 'Garnem', 'Felinem' and 'Monegro' are vigorous and there are no obvious size differences between them. The vigour induced in grafted cultivars is comparable to that induced by 'GF-677' or 'Hansen 536', with similar productivity. Non-grafted plants are vigorous and in the nursery exhibit an erect growth with little or no feathering during the first season (Feliipe, 1989). Leaves are big, red, lanceolate and intermediate between almond and peach. Active growing shoots exhibit an intense red-purple colour similar to the male parent, 'Nemared'. As they mature in the season, they turn brownish green to green. The first-year shoots are straight with little or no lateral branching. Shoots are green with red areas, with the red colour turning to an intense and continuous red-purple during winter dormancy. Internode length is medium to long. The three clones bloom early, showing similar low chilling requirements. Flowers are large, of the rosaceous type, with large pale-pink petals. The flowers have between 30 and 45 stamens and one pistil. Fruits are small, rounded, with a pubescent epidermis. Fruits are green with reddish tones. The mesocarp is thin and non-edible, leaving a free stone. Despite similar morphology and performance, these clones can be distinguished by molecular markers (Serrano *et al.*, 2002).

'Garnem', 'Felinem' and 'Monegro' were selected because of the important edaphic limitations that prevail in Mediterranean environments, such as iron chlorosis (Said *et al.*, 1993) due to alkaline soils (pH = 8.0–8.5) with high levels of active lime (10–12%). Their level of tolerance to iron chlorosis is similar to that of 'GF-677' and 'Adafuel' (Feliipe, 1989). They also tolerate drought conditions well, with higher resistance to water stress, especially in 'Monegro'. Adaptation to poor soils is good if the soils are well drained. Incorporation of RKN resistance was the primary breeding objective to replace several widespread nematode-susceptible peach × almond hybrids used for peach production in Spain, especially in replant situations (Pinochet, 1997). They possess a high level of resistance to the main RKN species attacking *Prunus*, including *Meloidogyne arenaria* (Neal) Chitwood, *M. hapla* Chitwood, *M. hispanica*

Hirschmann, *M. incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub.) Chitwood (Marull *et al.*, 1994; Pinochet *et al.*, 1996; Pinochet *et al.*, 1999). The three clonal rootstocks were evaluated over several years using up to 13 RKN populations from different geographical areas of the world, from which they demonstrated a broad spectrum of resistance (Marull *et al.*, 1994; Esmenjaud *et al.*, 1997). Resistance mechanisms to nematodes are determined by hypersensitive reactions (Marull *et al.*, 1994). Partial resistance breaking may occur under extreme heat conditions when mean soil temperatures exceed 35°C (Fernández *et al.*, 1993; Esmenjaud *et al.*, 1996). Like most almonds and almond × peach hybrids, they exhibit a low tolerance to root asphyxia caused by waterlogging. They are also susceptible to the root-lesion nematode *Pratylenchus vulnus* (Pinochet *et al.*, 1996) and to crown gall caused by *Agrobacterium tumefaciens* (unpublished).

The clones released so far from the GxN series show agronomic adaptation to different soil types and are better than almond seedlings for cultivation with irrigation (Table 9.3). These hybrid rootstocks have also been observed performing well under rainfed cultivation with good growth and production as compared to almond seedling rootstocks. Transplanting them is also easier than with almond seedlings.

'Garnem', 'Felinem' and 'Monegro' propagate well by hardwood and herbaceous cuttings in aerated and well-drained soils. Best results for hardwood cuttings are obtained in autumn. Cuttings are easily obtained thanks to the lesser degree of feathering and shoot branching. They also propagate very well *in vitro*. Nursery operations are facilitated by the low presence of feathers and the red leaves, as well as by the long vegetative period of the plants, allowing the production of nursery plants in a shorter period. The widespread opinion of all nurserymen is that the percentage of bud take is high for all known peach, nectarine and almond cultivars. These rootstocks have been selected primarily for almond and peach, showing good graft compatibility with numerous almond cultivars. Trials with different almond × peach hybrids have shown differences in their agronomic performance when grafted with different cultivars. They also provide good vigour and performance in replant conditions (Gómez-Aparisi *et al.*, 2000).

Table 9.3. Behaviour of almond rootstocks to biotic and abiotic stresses.

Rootstock	Vigour rating ^a	Drought hardiness	Calcareous soil tolerance	<i>Agrobacterium</i> ^b	<i>Phytophthora</i> ^c / <i>Armillaria</i> ^d	Root-knot nematodes ^e	Lesion nematodes
Almond seedlings							
Atocha	2	Yes	T				
Desmayo	2	Yes	T				
Garrigues	2	Yes	T				
Marcona	2	Yes	T				
Texas	2	Yes	T				
Chellaston	2	Yes	T				
Don Carlo	2	Yes	T				
Cichercia	2	Yes	T				
Peach seedlings							
Higama	2	No	S	–	S/S	T	T
Montclar	2	No	T	S	S	S	–
GF-305	2	No	–	S	S	S	S
Nemaguard	1	No	S	S	S	R	S
Nemared	2	No	S	S	S	R	S
PS A6	2	No	–	S	–/–	–	–
PS B2	2	No	–	–	–/–	R	T
Guardian ®	1	No	–	S	S/S	R	T
Controller™ 6 (HBOK 27)	4	No	S	S	S/S	R	–
Lovell	2	No	S	S	S/S	S	S
Plums							
Marianna 2624	3	No	S	S	T/R	I	S
Montizo	3	No	R	mR	–/–	I	S
Adesoto 101	3	No	R	mR	T/S	I	T
Hybrids							
Myran®	2	No	S	S	S/T	R	
Isthara®	4	No	T	S	mR/T	I	
GF-677	1	Yes	R	S	S/S	S	

Paramount®						
Atlas	1	No	T	mR	T/-	mR
Viking	1	No	T	mR	T/-	R
Flordaguard	1	No	S	-	-/-	R
Hansen 536	1	Yes	R	S	S/S	R
Krymsk® 86	2	Yes	mR	S	T/T	S
Cornerstone	1	Yes	R	S	S/S	R
Nickels	1	Yes	R	S	S/S	R
Bright's Hybrid® 5	1	Yes	R	S	-/-	mR
Monegro	1	Yes	R	-	-/-	R
Felinem	1	Yes	R	-	-/-	R
Garnem	1	Yes	R	S	-/T	R
Rootpac®40	4	-	T	-	-/-	mR
Rootpac® 20	4	-	T	-	-/T	mR
Rootpac® R	1	-	R	S	mR/T	mR
Titan	1	Yes	R	S	S/-	T
AB/2 3	3		R	S	S	S
Pedro	1	No?	-	S	S	R

^aVigour rating: 1: vigour similar to or greater than GF-677 or Nemaguard; 2: vigour similar to Lovell; 3: vigour 5–25% less than Lovell; 4: vigour 25–50% less than seedling. ^b*Agrobacterium tumefaciens*; ^c*Phytophthora* spp.; ^d*Armillaria* spp.; ^e*Armillaria* spp.; *Meloidogyne incognita*, *M. arenaria*, *M. javanica*; I, Immune; R, Resistant; mR, moderately resistant; T, tolerant; S, susceptible; -, unknown

Other *Prunus* hybrids in use

'REPLANTPAC'. 'Replantpac' ('Rootpac® R') is a new plum × almond hybrid selected by Agromillora Iberia, S.L., Barcelona, Spain, for use mainly as a rootstock for Japanese plum, peach and nectarine cultivars, but it can also be used for almond and some apricot (*P. armeniaca* L.) cultivars. Trees are green leaved, with a vigour comparable to 'Marianna 2624'. Its high productivity, broad range of compatibility with several *Prunus* species and outstanding adaptation to poor, heavy soils with high lime content that causes iron chlorosis offer a better production alternative for several *Prunus* species, especially in sites where soil limitations do not allow, for example, peach cultivation with traditional peach or peach × almond rootstocks (Felipec, 1989). The high survival rate of 'Replantpac' as compared with peach, plum and other peach × almond rootstocks makes this rootstock ideal for replant situations.

This rootstock originated from an open-pollinated hybrid of a myrobalan plum, probably the female parent; and almond, both of unknown origin. This new rootstock is probably a standard diploid genotype, like both parents. Several original and similar clones were found in a nursery in Zaragoza, Spain. They were observed and tested for root-knot and root-lesion nematode tolerance, salinity tolerance and ease of propagation by hardwood cuttings, resulting in the selection of this clone.

Unbudded trees of 'Replantpac' are vigorous with a slight weeping habit of growth with a spreading form. Its low propensity to produce root suckers offers significant savings in labour. Trunk bark is relatively thick, forming lenticels ranging in size from 4 to 6 mm in length and 1.5–2.3 mm in height, moderate in number and horizontally oriented. Branches are medium-sized, slightly smaller than those of the 'Marianna 2624' or 'Myrobalan 29 C' plums.

In an RKN trial conducted in 2008 with *M. javanica*, 'Replantpac' was found to be resistant. No galling was observed nor was any life stage of the nematode recovered at the end of the trial after initial inoculation with 20,000 eggs per plant (Pinochet, 2010). This clone was shown to be resistant to the root fungus *Rosellinia necatrix* Prill. (ana. *Dematophora necatrix* Hartig) under field conditions. This pathogen, known as the

white root rot fungus (Sztejnberg and Madar, 1980) is common in Spain (López Herrera, 2000). It is associated with a high mortality rate in young trees in the initial years of establishment in replant sites where peach, and peach × almond hybrids, are commonly used as rootstocks. The clone adapts well to heavy-textured soils where peach-based rootstocks grow poorly. Like many plum and plum hybrids, 'Replantpac' exhibits high tolerance to root asphyxia caused by water-logging. In relation to its susceptibilities, 'Replantpac' has proved to be a poor host to the root-lesion nematode *Pratylenchus vulnus* in Spain (J. Pinochet, personal communication, 2014). When challenged with two different *P. vulnus* isolates in California, the clone had a poor host response in one case and a good host response in the other and is likely to be susceptible (M.V. McKenry, personal communication, 2009). Differences in pathogenicity among populations of this pest in *Prunus* hosts tend to be common (Pinochet *et al.*, 2000).

'Replantpac' propagates with some difficulty by hardwood cuttings. In contrast, it propagates well *in vitro* (Pinochet, 2010). Initial stages of growth in the greenhouse have low to moderate feathering and an even rate of growth. It is also graft-compatible with the Spanish almond cultivars 'Desmayo Largueta' and 'Moncayo', as well as with 'Nonpareil', 'Monterey' and 'Butte' in California.

'DENSIPAC' (ROOTPAC® 20). 'Densipac' is a clonal plum hybrid rootstock for peach and nectarine from Agromillora Iberia, Barcelona, Spain (Gasic and Preece, 2014). It is a cross between *Prunus besseyi* and *P. cerasifera* (both unpatented and unnamed clones) and was selected in 1998 by J. Pinochet, tested as PAC 9801-02 and introduced in 2011. The plant is propagated easily via tissue culture. Unbudded trees are of low vigour with a semi-erect, compact form, medium chilling requirements (600–800 CU) and reproductively sterile. Its performance is characterized by low vigour, similar to 'GF655-2', advanced fruit maturity and increased fruit size when compared to peach rootstocks, with little or no suckering. It tolerates calcareous soils and asphyxia and it is moderately resistant to RKNs (*Meloidogyne* spp.). It is resistant to lesion nematode populations (*Pratylenchus vulnus*) and to *Rosellinia necatrix* root fungus. It shows good compatibility with peach and some almond cultivars.

'HBOK 27' OR 'CONTROLLER™ 6'. 'Controller™ 6' is a clonal peach rootstock for peach and nectarine from the University of California, Davis (Gasic and Preece, 2014). It is a *Prunus persica* F₂ peach hybrid from P248-139 OP ('Harrow Blood' × 'Okinawa') tested as 94-94-27 and introduced in 2012. The plant is propagated via leafy cuttings, hardwood cuttings or tissue culture. It has reniform leaf glands and showy flowers. The fruit is freestone with little, if any, red blush, and white flesh. It is rarely used in almond although it has been described as compatible for almond.

Its performance shows a low to moderate vigour, c.50% the size of trees on 'Nemaguard'. Yield efficiency in the first years is comparable to trees on 'Nemaguard' but becoming significantly better as trees age. Fruit size is increased as compared to trees on 'Nemaguard'. It produces very few root suckers and shows moderate resistance to RKNs (*Meloidogyne* spp.) with good compatibility with peach and some almond cultivars.

Hybrids between different species of *Prunus*

Recently, an increased emphasis has been placed on developing dwarfing or semi-dwarfing rootstocks adapted to different soil fertilities and allowing higher density in the orchard, as has occurred for peach or other fruit crops. Several size-controlling clonal rootstocks have been released recently. These include a complex almond-peach hybrid, 'Felinem' × ('Marcona' × 'Nemaguard') (Agromillora, personal communication, 2010), and the *P. besseyi* × *P. cerasifera* hybrid 'Rootpac® 20'. Traditionally, other *Prunus* interspecific hybrids have been developed and have been proved to be valuable rootstocks for a wide number of stone fruit species (Felipe, 1989; Rubio-Cabetas and Socias i Company, 1996). There are already some interspecific hybrids: *P. davidiana* × peach, almond × *P. davidiana*, *P. mira* × peach, etc., being produced commercially. Some have shown interesting agronomic performance as almond rootstocks (Felipe, 1989). In addition to the previously mentioned hybrids, other hybrids between almond and peach with myrobalan and other plums were created, and there were individuals that looked interesting and whose behaviour is being studied. Grasselly and Olivier (1987) also made several hybrids between almond and myrobalan for their potential use as new rootstocks in combination with

others, such as 'Myrandier 613' and 'Myrandier 617'. Grasselly and Duval (1997) reported good compatibility of almond with hybrids 'Myran' and 'Ishtara'. All these genotypes are currently under re-evaluation to be used as pollen donors in new breeding programmes.

9.4 Almond Rootstock Breeding Programmes

Work on genetic improvement of stone fruit rootstocks continues to produce innovations that expand the range of rootstocks available on the world market. Active rootstock breeding programmes are under way in both the Mediterranean basin and the USA.

9.4.1 Almond rootstock development in Europe

In Spain almond plantings will increase exponentially in coming years. From the almost 600,000 ha existing for many years, only less than 400,000 ha can be considered as productive. According to nursery information, approximately 6 million trees were planted in 2014–2015 alone. The demand for almond rootstocks has increased considerably in Spain and North Africa, where the Spanish nurseries are also selling all of their production. The actual number of hectares planted is difficult to calculate since, unlike the USA, some new orchards are being planted under intensive systems using dwarfing rootstocks at row and tree spacings of 3.5 m and 1.0–1.5 m, respectively (2222–2857 trees/ha). The demand for almond rootstocks has exceeded the supply in Spain, in such a way that essentially all 'Garnem' and 'GF-677' liners ready for peach grafting had been used for almond grafting in most nurseries (Rubio-Cabetas, 2015).

Agromillora began rootstock breeding in 1998 to develop new rootstocks more adapted to intensive fruit production. They have selected rootstocks of low vigour, good soil adaptation and high productivity. Two dwarfing rootstocks, R-20 and R-40, were released and were considered for almond, although without prior extensive field evaluation. Research on high-vigour almond × peach hybrids conducted in

Spain led to the selection of 'Monegro', 'Felinem' and 'Garnem' from the Spanish breeding programme of CITA. Other rootstocks with different levels of vigour were also developed, and tolerance to calcareous soils and resistance to RKN nematodes are under study. Recently, CITA in collaboration with Agromillora has moved towards developing rootstocks with greater tolerance to different soil and climate conditions. Several progenies are under evaluation for selecting dwarfing rootstocks, and other desirable traits are also being taken into consideration, such as tolerance to active limestone content, prolonged summer drought and poor soil fertility. Similarly, developing rootstocks with lower chilling requirements to be better adapted in a scenario of climate change are being considered. These circumstances will be important to expand almond plantings in North Africa. Root-knot and root-lesion nematode resistance will be of paramount importance for growing in replant conditions for many almond-growing areas. The order of importance of the breeding objectives in the programmes searching for better adapted rootstocks may be ranked as follows: nematode resistance, waterlogging tolerance, vigour control, calcareous soil adaptation, drought tolerance, soil disease (fungi and bacteria) resistance and cold hardiness (Rubio-Cabetas, 2010).

In France a myrobalan rootstock has recently been released by INRA, 'Myrotop®' with tolerance to *Armillaria* root rot, resistance to nematodes and waterlogging tolerance. Its graft compatibility with 'Ferragnès', 'Lauranne' and 'Nonpareil' is under study (Duval and Masse, 2013).

Three RKN resistance genes have been identified in three different *Prunus* species, *Ma* in the myrobalan plum clones P2980 and P2175, *RMia* in the peach rootstock 'Nemared' and *RMja* in the almond 'Alnem'. Thus, the pyramiding of these three genes by interspecific crosses of almond × peach × myrobalan is the main objective of the French rootstock breeding programme (Duval, 2015). However, to ensure the presence of the three genes in the same rootstock, it has been necessary to develop molecular markers to detect genes in the hybrids. The identification of intra-gene markers for the two nematode-resistance genes *Ma* and *RMia* has allowed the application of marker-assisted selection for these two genes (Claverie *et al.*, 2011; Duval *et al.*, 2014). A new F₂ almond

population of 'Lauranne' × 'Alnem', with more than 1000 seedlings, has been created to clone the *RMja* resistance gene of 'Alnem' and to find intragenic molecular markers for this gene. The *RMja* gene is localized on linkage group 7 of the *Prunus* genome in the same region as the *Ma* gene (Van Ghelder *et al.*, 2010). Therefore, it will be possible to select – in the hybrid population of ('Alnem' × 'Nemared') × myrobalan – the hybrids with the three pyramided nematode-resistance genes. Controlled interspecific crosses have been made with the purpose of bringing together the desirable traits of dwarfing rootstocks, as well as introducing commercial rootstocks from other areas to give valuable rootstock options for the modern almond industry.

9.4.2 Almond rootstock development in the USA

In the past 20 years, almond acreage in California has more than doubled from 200,000 ha in 1995 to 410,000 ha in 2014 with approximately 8.3 million trees planted in 2013–2014 (www.nass.usda.gov/Statistics_by_State/California/Publications/Fruits_and_Nuts/2016/201605_almac.pdf). The demand for almond rootstocks has exceeded supply such that many new cultivar releases other than historical peach seedling rootstocks (e.g. 'Nemaguard' and 'Lovell') have been planted without prior extensive field evaluation. In California, almond growers are using clonal hybrid *Prunus* spp. rootstocks previously used for peach including almond × peach ('Nickels', 'Brights Hybrid® 5', 'Cornerstone', 'Nanopac'), peach × *P. davidiana* ('Empyrean® 1' also known as 'Barrier 1'), plum × peach ('Krymsk® 86'), plum × almond ('Replantpac') and complex hybrids ('Viking', 'Atlas'). Most of these rootstocks require additional testing with almond to determine their optimal adaptation in California. In addition, the peach–almond hybrid 'Hansen 536' and the peach seedling 'Guardian®' rootstock are currently being planted on saline/high pH soils and bacterial canker sites, respectively. Potential almond rootstocks in initial testing include 'Sam 1' and 'Sam 2', which have shown tolerance to *Armillaria* root rot, and 'BB 106®', a peach × almond hybrid with increased tolerance to wet soil conditions.

9.5 Final Outlook

The need for consistent and high-quality rootstock production cannot be realized when using seeds of unknown origin or gathered from various sources, nor when using cuttings from root sprouts from established plantations, because of their unknown sanitary status. Rootstocks must be developed, propagated and cultivated in a technical and logical manner by an experienced team of scientists that includes multiple disciplines before a commercial release is made. This requires many years of observations and controls to complete their evaluation, which is necessary before selecting a new rootstock release. Modern almond cultivation requires rootstocks that are environmentally adapted, productive and resistant to diseases, etc. Thus, the horticultural traits of new rootstocks should be defined as precisely as they are for modern scion cultivars. The importance of increased orchard efficiency and profitability elevates the need to obtain, select and propagate preferentially clonal rootstocks. Since almonds will continue to be grown both under rainfed and irrigated conditions, two groups of rootstocks will be necessary, one for each of these cultural conditions. The use of hybrids in both situations has given the best results when there were no other limitations

or specific problems. Moreover, there remains a broad range of possibilities for recombination of positive features into future rootstocks. In any case, easy and cost-effective propagation by *in vitro* must be envisaged in the near future in the nursery industry.

Due to the large number of genetically diverse rootstock cultivars available for almond in California, public and private breeders have focused on almond seedling propagation (e.g. USDA-Parlier) and better tolerance to salinity, high pH, drought, soil diseases and increased anchorage (e.g. UC Davis, Zaiger's Inc. Genetics, and Progressive Genetics Group™). Some of the newer rootstocks being planted still require more efficient propagation techniques, compatibility evaluation with different scion cultivars and additional testing for adaptation to abiotic and biotic site factors. Furthermore, almost all new almond rootstocks are proprietary and require licensing agreements and patent royalties that will likely encourage a significant use of less expensive, peach seedling rootstocks in the future. However, as hybrid rootstocks become better tested and less expensive to produce, they could eventually exceed peach seedlings as the rootstock of choice for almond production in California because of inherent edaphic factors and water use regulations.

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10 Propagation Techniques

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10.1 Introduction

Tree quality depends largely on the right choice of plant material, correct propagation techniques and adequate cultural management in the nursery. Almond cultivars, like most fruit crops, are genetically heterozygous and are exclusively propagated in asexual ways. This type of propagation is a basic requirement in fruit crops since propagation from seed produces genetic recombination and, therefore, the uniqueness of each individual seedling, whereas vegetative propagation maintains the full identity of any cultivar. The quality of the plants coming from the nursery to establish the orchard also plays an important role in the future development and profitability of the orchard independently of its environment, soil and climate, and its correct management.

The different propagation methods normally used in almond nurseries have been seedling propagation, cutting propagation (either by hardwood or softwood cuttings) and grafting or budding. These techniques continue to be commonly used, although at present the modern almond industry is largely using *in vitro* propagation.

10.2 Seedling Propagation

Since almond is a very heterozygous species, seed propagation results in a very heterogeneous offspring not reproducing faithfully the characteristics of the mother cultivar. Consequently, seed propagation cannot be used for cultivar propagation, but only in nurseries to obtain seedling rootstocks or in breeding programmes (Ledbetter and Sisterson, 2008) to obtain breeding progenies from which new cultivars may be selected.

The seed is the element for sexual propagation of plants. To obtain a viable seed, the stigma has to be pollinated and the ovule has to be fertilized (see Chapter 1). Thus, botanically the seed is the fertilized and mature ovule. The two cotyledons form the largest part of the seed; and the endosperm finally is reduced to a thin layer inside the testa or seed coat, where the residual embryo (considered apart from the cotyledons) remains as a very small portion of the whole seed. The embryo, however, contains all the components to form the new plant. The testa and the residual embryo germ contain growth inhibitors, whose function is to prevent germination (Westwood, 1993).

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The production of seeds to be utilized in obtaining seedling rootstocks should be done in isolated plots in order to control the pollen parent as much as possible. Therefore, the orchard must be of a single cultivar when this is self-compatible (such as for peach seeds); or of two cross-pollinating cultivars if these are self-incompatible (such as for traditional almond cultivars), where the parents must be selected to produce seeds giving rise to good-quality seedlings, finally requiring the independent harvest and management of the seeds of both cultivars. In this way the variability of the progeny may be substantially reduced. Once harvested, the seeds should be stored properly in conditions of low humidity and temperature until the time of preparation for germination.

10.2.1 Seed germination

Once the fruit or the nut is collected, only the mesocarp containing the seed is stored. Then the activity and the metabolism processes of the seed decrease to a minimum level with no apparent activity. The seeds require a certain period of time before germination since they are in the stage of dormancy and do not germinate even with favourable external conditions. Germination occurs due to internal mechanisms once three requirements are simultaneously fulfilled: (i) viability of the seed (ability to germinate); (ii) fulfilment of the dormancy requirements; and (iii) favourable external conditions.

To overcome dormancy, almond seeds need cold stratification, which normally is satisfied in no more than 40–50 days, followed by heat requirements before germination. During germination, cell metabolism is reactivated and the embryo starts growing, the two protecting teguments open and the seedling emerges.

The environmental factors affecting germination are water absorption, temperature, gas exchange and light. Water availability affects germination and an adequate humidity must be maintained constantly. Temperature is perhaps the most important factor in germination regulation and in the first stage of plant growth. Each species has maximum and minimum limits of temperature for correct germination, depending on the season of the year and the daily cycle. After fulfilling the cold requirements, warmer

temperatures are needed to overcome dormancy and start germination.

Gas exchanges involve oxygen, CO₂ and ethylene. Oxygen is essential for correct seed respiration; therefore the soil or stratification substrate must be well drained and with good aeration. CO₂ released from respiration can accumulate, inhibiting germination. Also, ethylene can in certain cases break dormancy. Finally light can accelerate or inhibit germination according to the type of seed.

10.2.2 Seed stratification

To meet the requirements of breaking dormancy, the seed must be stratified on a wet, porous medium, exposed to ambient temperatures or in special chambers with controlled temperature. During this process of stratification seeds experience a series of changes such as water absorption and exposure to low temperatures, but always above freezing, for a variable period. The length of this chilling period differs depending on the cultivar, being generally longer in seeds from late-blooming cultivars. During this process the rhythm of respiration increases.

After this chilling period the seeds are ready to start germination, for which the seeds must be submitted to higher temperatures, between 15°C and 25°C, which must be raised gradually thereafter. A sudden increase of temperature can produce secondary dormancy and stop the germination process. The removal of the seed tegument improves the germination response and accelerates seed germination; however, this removal renders the seed more sensitive to possible infections by bacteria and fungi.

The germinating seeds must be sown in seedbeds or directly in the field using drills in the nursery. When the seeds are sown in seedbeds, the seedlings must be replanted in winter in the nursery to be grafted during the following summer. Otherwise, when they are directly sown in the nursery, which is usually practised in the production of almond seedlings, the planting distance is normally in rows 0.8–1 m apart with the seedlings at 8–12 cm apart in the row. To obtain an optimal germination of almond seeds, it is enough to stratify for 1 month at 4°C and plant by mid-February. The seeds can also be sown directly in January after being moistened

for 48 h. Peach seeds, however, need to be stratified for 3–4 months at 4°C and sown directly in the nursery, in November, after being immersed in water for 1–2 days.

10.3 Vegetative Propagation

Vegetative propagation is mostly done by cuttings. These can be made from the vegetative portions of the plant, and are classified according to the part of the plant from which they are obtained, such as stems, modified stems, leaves or roots (Hartman *et al.*, 2002). Stem cuttings can be hardwood, semi-hardwood, softwood and herbaceous depending on the season of propagation and the lignification stage of the stem. Leaves are retained on herbaceous and semi-hardwood cuttings, whereas hardwood cuttings are leafless. For almond rootstock propagation, only stem cuttings are produced. The ability of rooting depends on three groups of factors: (i) genetic; (ii) environmental; and (iii) internal, mainly related to the chemical equilibrium of both nutrients and hormones.

The mother trees for cutting production must be specially pruned in order to have long shoots. It is well known in nursery practice that the management techniques applied to the mother trees can exert positive effects on the rooting potential of cuttings, although no documented studies on this aspect are available. Thus, to promote rooting, metabolic activity must be shielded from the adverse effects of stress that could impair rhizogenesis.

The absence of feathers on the shoots selected for cutting production is favourable for preparing the cuttings. Only genotypes characterized by a naturally high rooting potential can be propagated by setting the cuttings to root directly in the nursery. Consequently, in most cases some root-induction hormones must be applied at the cutting base, such as indole butyric acid (IBA), naphthalene acetic acid (NAA), etc.

The rooting process takes place because many cells, under special conditions, can return to the meristematic condition (undifferentiated cells). It is a process of dedifferentiation, which offers the new possibility to form stems or roots or both. This process makes possible the propagation of plants starting from an incomplete

portion, such as the cutting. Treating with auxins, which are plant regulators, increases the percentage of cuttings that form roots, hastens root initiation and increases uniformity of rooting (Hartman *et al.*, 2002).

10.3.1 Semi-hardwood cuttings

Semi-hardwood cuttings are those made from woody, leafy, summer and early autumn shoots of deciduous plants with partially matured wood which is partially lignified. During that growing season the shoots are more or less woody and have leaves. Consequently, the cuttings obtained from them are considered as softwood when lignification is still light; or semi-hardwood when they have a half-woody consistency. These cuttings are made during the summer, when new shoots are more or less mature, usually from the time when the shoots reach a sufficient size (then herbaceous cuttings) until growth ends and the dormant period begins (then both semi-hardwood and herbaceous cuttings). Too many leaves make handling operations difficult and cause wilting, but also facilitates respiration.

Plants of good nutritional status and growing under favourable conditions of moisture, light and temperature have a high carbohydrate/nitrogen (HC/N) ratio. Cuttings taken from these plants produce more and better roots than those taken from plants with a low HC/N ratio. However, the greater rooting effect on rooting semi-hard cuttings is due to the presence of leaves and buds, probably owing to some factors directly related to their presence. Auxins are produced in leaves and buds, showing their effects directly below them due to movement of these hormones towards the base (Felipe, 2000).

Semi-hardwood cuttings are usually 8–30 cm long; leaves are removed from the lower part, which is introduced into the rooting medium. Although the shoot terminals are often used in making cuttings, the basal part of the shoots will usually root better whenever the diameter of the shoot is not excessive. The collection of branches for cutting production should be done early in the morning, when their water content is higher. They must be kept in a cool room and wrapped in plastic until preparation and planting in the rooting medium. The duration of this process should not last more than a few hours.

Cutting preparation should be in a shaded and cool room, to avoid dehydration damage. Semi-hardwood cuttings root more quickly and easily than hardwood cuttings, but they require more complex and expensive installations. Bottom heat, auxin treatment and sometimes wounding are also beneficial to increase rooting percentages.

This propagation method is especially useful when used to multiply clones of peach × almond hybrids and some similar rootstocks. This method allows a wider use of plant material produced by the mother plants. In that case the plant material can be taken:

- During the summer, in July, when the top of the apical shoots should have stopped growth to promote the development of lateral feathers.
- At the end of summer, to be able to utilize all shoot ends once they have stopped growing in early September. At this time the rooting percentages obtained can be very high.
- In autumn and winter the rest of the shoots, without leaves, can be used to obtain hardwood cuttings.

The leaves can be trimmed from one-third to one-half their original number to reduce the leaf surface area, which lowers water loss by transpiration and allows closer spacing in the cutting bed. The auxin treatment for this type of cuttings is IBA (indole-3butyric acid) at 1000 ppm (1 g/l). This auxin is prepared as a hydro-alcoholic solution (50% water and 50% commercial ethyl or methyl alcohol) or a hydro-acetone solution (50% water and 50% commercial acetone). If the potassium salt is used, it must be dissolved in distilled water.

The treatment should be by immersing the base of the cutting for 2–5 s in the solution mentioned above. After the treatment the cuttings are placed under mist or in double tunnels placed in pots of 9 × 9 cm with a peat substrate (40%) and washed sand or perlite (60%). The environmental conditions during rooting must be of very high humidity, as close as possible to 100%, a situation in which transpiration is minimal. Normally the presence of leaves is favourable for rooting, but the loss of water through evapotranspiration by the leaves is a negative effect which can induce wilting before achieving rooting, and finally cutting death, since before rooting the cuttings cannot receive their required water supply from the

roots whereas the leaves continue transpiring. Different methods can be used to maintain the high ambient humidity during rooting.

Mist propagation

This system, developed in 1940, was a breakthrough in plant propagation. It consists of applying a thin film of water on the leaves of the cuttings. Due to the constant evaporation of water there is a noticeable increase of humidity and a decrease in air and leaf temperature. This effect is so remarkable that when the system works properly, the plants can be placed in full sun without injury to the cuttings and still obtain the benefit of receiving high brightness to increase leaf photosynthesis.

In this system the conditions are favourable for rooting leafed cuttings: high humidity and low leaf temperature. In addition, the plant is able to synthesize a higher proportion of nutrients needed for respiration and required for rooting initiation. In mist propagation water provision is regulated so that, theoretically, there is neither excess nor defect. The temperature of the rooting medium is also maintained at a favourable level for the rapid development of the roots.

The fog system is a variant of the mist system. In the fog system, true fog is made by fog generators and atomizers that produce very fine water droplets from 2 microns (μm) to 40 μm (Hartman *et al.*, 2002). The fog greatly raises the environmental humidity, thus providing small amounts of water and additionally cooling the substrate temperature. It may be arranged continuously or discontinuously depending on the need for moisture supply.

Fertilizers and nutrients may be added to the nebulization water from the time that roots emerge, thus improving their quality and development. The fertilizer (containing N, P and K) must be added in a soluble form and in suitable proportions at very low concentration. The fertilizer solution is prepared in the injection tank providing the irrigation water. However, the contribution of fertilizers to enhance rooting is not essential in practice to attain acceptable levels of rooting.

After the mist stage, hardening and acclimatization of the rooted plants must be gradual. In the case of the different *Prunus* cultivars, the cuttings must be removed from mist immediately after rooting to avoid defoliation.

Double tunnel system

The double tunnel system is widely used for the propagation of ornamental plants, and it may also be used for the propagation of semi-hardwood cuttings for stone fruits during the summer. This system involves placing the cuttings under a small plastic tunnel which ensures the formation of an environment of very high humidity. This tunnel is placed inside another larger tunnel, in either a greenhouse or a shade house, which ensures protection against winds and provides good lighting but avoids direct sunlight. Soil watering can be done the day before planting the cuttings in order to maintain a favourable environment moisture in the rooting tunnel. The air space between the cuttings and the plastic should not be too great; a height of 15–20 cm is sufficient.

The control of pests (mainly aphids) and diseases (several fungi) is essential in these tunnels, although fungi rarely occur due to the high temperatures normally registered during the summer. For hardening, the cuttings rooted in the tunnel must follow the same process as described for the mist propagation system.

There is no universal or ideal media mix for rooting cuttings. The substrate of the rooting media has three functions: (i) to hold the cuttings in place during the rooting period; (ii) to provide moisture for the cuttings; and (iii) to allow exchange of oxygen at the base of the cuttings. Therefore, an ideal propagation medium shows sufficient porosity to allow good aeration and has a high water-holding capacity, but with good drainage to avoid waterlogging. The type of substrate influences the shape and consistency of the roots. Those obtained in a sandy environment are long, unbranched, thick and brittle. When a mixture media is used, such as sand and peat or peat and perlite, roots are branched, thin and flexible. This difference is due to the different ability of these substrates to contain air and moisture. Aeration is essential for roots to grow, since rooting is a process of rapid cell division requiring the presence of enough oxygen in the environment.

Those cuttings that root easily are insensitive to changes in substrate, but it is very important to provide a suitable substrate fulfilling all the basic requirements for those that root with difficulty. An appropriate substrate will not only affect the proportion of rooting cuttings, but

also the quality of the roots obtained. Mixtures of soil, peat, sand, perlite, vermiculite, etc., tend to perform better than either of them separately.

10.3.2 Hardwood leafless cuttings

Hardwood cuttings are those made of matured, dormant wood after leaves have abscised during the dormant winter season. The use of hardwood cuttings is one of the least expensive and easiest methods of vegetative propagation whenever the cultivar shows the ability to root this type of cutting. This is not the case in most almond genotypes, although some almond rootstocks of other origin may be easily propagated by hardwood cuttings.

Auxin and other plant regulators trigger the process of root induction in some cases and in others accelerate it, but each plant can give a maximum response when auxin is applied in a particular form and concentration. Therefore, at present the use of hormones is considered a routine practice, reinforcing their action with other complementary techniques.

Hardwood cuttings are prepared during the dormant season – late autumn, winter or early spring – usually from wood of the previous season. The traditional nursery practice has been to make cuttings during the whole dormant period, from leaf fall until the leaves sprout. However, the same rooting percentages are not achieved every year using this practice for different reasons, such as the time of cutting preparation, the substrate and the temperature of the rooting media, or rainfall during the season. Therefore, the results indicate that hardwood propagation could better be considered as ‘autumn cutting’ and not ‘winter cutting’ because the conditions in autumn, mainly concerning soil temperature, are more favourable for cutting propagation and provide the best natural conditions in terms of plant regulators, proper irrigation and soil selection. In Mediterranean conditions, the soil temperature at a depth of 20 cm does not usually drop below 12°C until the end of November. This temperature is considered the lower limit of favourable conditions for triggering the internal process of root differentiation. When temperatures are lower, rooting will be much more difficult; thus early autumn cutting propagation is recommended to benefit from the favourable temperature conditions of

the ground. The soils for planting the cuttings must be permeable and covered with black plastic, which prevents soil drying during the winter and reduces the output of competing weeds, especially in spring. The plastic cover avoids also the need to water frequently, thus reducing the excess moisture which is harmful to the young and tender rootlets.

It has been extensively documented for many species that there are distinct advantages in taking cuttings from trees reared exclusively for the production of propagation material. The propagation material for hardwood cuttings should be taken from healthy, moderately vigorous stock plants growing in full sunlight. These stock plants must be heavily pruned every year to induce strong shoots and to maintain the juvenility stage of the plant as long as possible, since juvenility is a favourable stage for inducing rooting and can be lost with time if the plant is not forced to produce vigorous new growth every year.

The shoots selected for cutting production should not have abnormally long internodes, or be from small, weakly growing shoots from the inside canopy. In general, hardwood cutting material is ready when the leaves may be removed without tearing the bark. Shoots of moderate size and vigour are the most desirable. Shoot tips are usually discarded because they usually have low carbohydrate levels and often contain unwanted flower buds (Hartman *et al.*, 2002). Those parts of the plant showing juvenility traits produce roots more easily.

Mother plants produce the best cuttings for rooting when 3–4 years old, and they continue to produce adequate cuttings for several years. In the case of very vigorous plants, pruning should be somewhat less severe to obtain cuttings with the most suitable diameter. Pruning should be adapted each year, benefiting from the experience of the previous year, in order to produce the maximum number of adequate shoots with the most convenient size.

Hardwood cutting length may range from 20 cm to 60 cm, although longer and shorter cuttings may also root. Long cuttings with roots, when used as rootstock for almond, allow the insertion of the cultivar bud on the original cutting rather than into smaller new shoots arising from the original cutting.

For better rooting, cuttings are preferably taken from the basal areas. The basal cut should

be just below a node and the top cut at 1.3–2.5 cm above a node. In some species, such as the almond × peach hybrids, the origin of the wood is not very relevant. The diameter of the cuttings, however, should not be too thick: a diameter of 1–2 cm is considered the most suitable, depending on the species.

As in the propagation of semi-hardwood cuttings, many auxin compounds are available to induce rooting, among which the most frequently used is indole-3-butyric acid (IBA). It can be applied at concentrations ranging from 10 ppm to 20 ppm (dilute solutions) up to 1000–3000 ppm (concentrated solutions). In the former case, the treatment consists of dipping the base of the cuttings into the solutions for 12–24 h, while a quick dip of just a few seconds is enough with concentrated solutions. The solutions generally used for propagation of hardwood cuttings of almond rootstocks are water–alcohol solutions with IBA concentrations from 1000 ppm to 2000 ppm (Felipe, 1989; Nicotra and Moser, 1997). Generally, if a cutting does not respond to IBA, other root-promoting compounds will not be effective (Hartman *et al.*, 2002).

Before planting a stratification period may be desirable to promote rooting of cuttings, although this treatment will depend on the clone. Stratification can be done in sand or soil, at room temperature, applying bottom heat at controlled temperatures and maintaining the cuttings in plastic bags at these favourable temperatures for a few days (Felipe, 2000). Temperature influences root induction both for the promptness of the onset of roots and their number.

Two processes take place in the cuttings for the appearance of roots: (i) their initiation; and (ii) their development. Root initiation occurs because active cell division takes place at the cambium cells. The number of dividing cells and their division rate are essential for the effectiveness of rooting. The activity of these processes increases with temperature, since the response to the action of auxin is greater at higher temperatures, reaching a maximum between 30°C and 35°C. Root growth depends on the division, differentiation and cell elongation involved in their formation. Root growth starts about 7.5°C and increases as temperature rises, with the optimum at temperatures not exceeding 20°C. For good production and quality of roots, cuttings must undergo two consecutive temperature

regimes: high for the initiation and medium–low for elongation and growth (Felipe, 2000).

Tests conducted with interspecific peach × almond hybrids confirm the existence of some clones whose cuttings show good ability to produce roots in autumn and early winter if they are submitted to very specific treatments with regard to hormone application and environmental conditions. Indeed, Pascual and Felipe (1988) conducted several trials with cuttings prepared in late October treated with IBA at 2000 ppm for 5 s. Some of these cuttings were planted directly outdoors in the field and the remainder placed in bottom heat at different temperatures (13.5, 17, 20 and 22.5°C) for various time periods (6, 9, 13, 16, 20 and 23 days) prior to planting in the field. At the end of the bottom heat exposure the rate of callus formation at the base of the cuttings was directly related to the temperature and time elapsed. Root formation similarly appeared from the 15th day when the bottom heat temperature was 22.5°C, being delayed as temperature decreased. In relation to survival in the field, the best results were obtained with a length of only 6 days of the bottom heat treatment, at any of the four temperatures. The cuttings planted directly in the field, with hormone treatment but without bottom heat, produced an intermediate percentage of plants, which was explained by the still moderate soil temperature at that time, close to the lowest bottom heat treatments. The cuttings with a small root development when planted once the bottom heat treatment was finished resulted in higher percentages of viable plants after emergence.

Humidity is also important, although the importance of the water status of the cutting has often not been sufficiently considered and needs to be taken into account. Howard and Harrison-Murray (1988) found that even small changes in the water status of the cuttings are detrimental for rooting and that the water content was modified by the weather, the environment and the time to produce them. When these changes occur in the first phase of the propagation period, before the appearance of roots, they are detrimental both to rooting and to establishment. This implies the need for careful handling of the cuttings from the cutting edge, during manipulation and when planting. Special attention must be paid in autumn since the propensity to lose moisture is greater due to the wounds of the falling leaves. Similarly, in late winter the

evolution of buds and shoots may also produce greater water loss.

The stratification in bags is a cheaper and easier method developed by Felipe and Pascual (1990) to improve rooting of hardwood cuttings. Most published results on plant propagation refer to trials being conducted using sophisticated techniques. These techniques are not always available to professional propagators, who need to know the approximate rooting percentage expected and that this would be acceptably consistent over the years. Hardwood cutting propagation during the dormant period can be achieved at a satisfactory level with most of the rootstocks for almond and other fruit trees using the following method:

1. Preparation of cuttings about 25 cm long at the end of October or beginning of November.
2. Treatment of AIB at 2500 ppm, either in the hydro-alcoholic or in the aqueous solution of the potassium salt; the immersion must last 5–10 s at a depth of 2 cm or less.
3. Immediate placement of the treated cuttings in plastic bags, later closed removing as much as possible of the inside air.
4. Placement in a chamber with ambient temperature close to 20°C for 8–10 days (not more, because rooting decreases).
5. Planting of cuttings in a well prepared and fluffy soil; in these conditions they should be rooting at the beginning of November.
6. Immediate irrigation to settle the soil, maintaining good moisture during the rest of the winter. When there are many cuttings, the bags may be replaced by other containers where the cuttings must be in some way wrapped in plastic and protected from moisture loss.

With this procedure better rooting was achieved as compared to the control (planting without the bag treatment) in all the years the trials were conducted. The position of cuttings during the stratification period, horizontal or vertical, did not affect the results (Felipe and Pascual, 1990).

Bottom heat

This method has been successful for difficult-to-root species such as plum rootstocks, or when the favourable season for cutting preparation in the autumn is over. Therefore, the cuttings may

be placed in such an environment stimulating a more favourable rooting, such as higher temperatures or longer duration of the treatment. Cuttings may be collected either in the autumn or late winter; the basal ends are treated with IBA at 2500–5000 ppm and placed upright for about 4 weeks on a 20-cm sand base with bottom heat. The temperature may be raised by electric resistance or circulation of fluids whose temperature is regulated by thermostats. Operating temperature and length of treatment must be determined experimentally for each clone; in some cases 20°C and 1 week may be enough. The medium is usually made of mixtures of peat, perlite, vermiculite or sand in different ratios. Taking into account that during the rooting process a rapid cell multiplication takes place, this implies a high respiration rate. Consequently, the cuttings require a high level of oxygen and the medium in which the cuttings are immersed must be capable of providing good air renewal. Therefore, the rooting substrate must be porous and not flooded by irrigation water. However, a good water content must also be maintained and the substrate must consist, at least in part, of some material having a good water storage capacity without losing a high degree of porosity. Some caution must be taken when planting the cuttings: (i) the exposure must avoid excessive sunlight and winds that may cause desiccation; and (ii) planting must be done on a plastic cover with holes previously perforated to avoid the risk of a piece of plastic stays attached to the lower part of the cutting, thus preventing its contact with the soil, and giving as a result a lack of rooting. They must be planted in such a way that about one-third of the cutting length is above ground, so that at least two buds are visible.

10.3.3 *In vitro* plant production

The methods and different phases from the initial step to the final micropropagated plants are described in this section.

Rootstock production by in vitro culture

ORIGIN OF THE STARTING MATERIAL. The genotype collection established from the plant material – category ‘base’ – is located in a plant reservoir under controlled sanitary conditions. This collection of

mother plants is preserved in a manner that prevents recontamination – containers inside an insect-proof greenhouse – and is regularly subjected to official inspection for genetic identity and sanitary status.

The buds of these plants are used as starting material for commercial *in vitro* propagation in the nursery. Depending on the origin and category of the starting materials in the certification programme, the micropropagated plants are commercialized as *Conformitas Agrarias Communitatis* or certified material in EU countries.

COLLECTION OF SHOOTS. Apical shoots from the almond mother plants are taken in the spring season (March–April), selecting the most appropriate type for the *in vitro* culture. For almond plants it might be helpful to collect branches in early spring before the growth has begun and maintain them for a time in a growth chamber or greenhouse to force growth.

RECEPTION OF THE PLANT MATERIAL IN THE LABORATORY. The collected cuttings are brought to the laboratory and used immediately or stored refrigerated at 4°C in a cooler until used in the multiplication process.

DISINFECTION OF EXPLANTS. At this stage the goal is reducing the potential contamination by fungi, viruses and other pathogens. The endogenous contaminants are not eliminated by surface disinfection unless ‘clean’ virus-free or pathogen indexed stock plants are used as starting material.

The disinfection is carried out in a laminar flow cabinet under aseptic conditions. This process consists of a pre-washing with alcohol, immersion in a solution of 0.15% sodium hypochlorite for 10 min, followed by three washes of 5 min, 10 min and 15 min with sterile distilled water. Antioxidants such ascorbic acid or citric acid in the preliminary washing might be helpful.

ESTABLISHMENT OF *IN VITRO* CULTURE. After disinfection the shoots are cultured in test tubes or jars containing agar-based nutrient media. The components of the media vary depending on the species or cultivar. Basal media include the ingredients described by Murashige and Skoog (1962) plus sucrose and occasionally other supplements. Growth development control can be

achieved with the balance auxin/cytokine. If axillary shoots are desired a moderate level of cytokine (BA, kinetin) is used (0.5–1 mg/l) and the auxin level is kept very low (0.01–0.1 mg/l). When the aim is the development of adventitious shoots onto the explants a higher level of cytokine is needed. Several woody species can take up to 1 year to complete stabilization in culture. An *in vitro* culture is considered stabilized when explants produce a consistent number of normal shoots in each subculture. Abnormal shoots usually stop developing and fail to elongate.

These cultures are maintained in the growth chamber under controlled conditions at a temperature of 22–24°C and a photoperiod of 16 h light/8 h darkness.

MULTIPLICATION PHASE. Every 15 days the explants are subcultured to fresh medium; the number of cycles after the adaptation phase does not exceed the maximum set (ten cycles, in Spanish legislation for certified plants). For flexibility in the planning of the commercial plant production stock, explants are stored in a cooler and are available to enter the multiplication cycle when needed.

ELONGATION PHASE. On the basis of plant production forecasts a number of jars are transferred to the next phase, in which the shoot elongation is promoted. This phase lasts 8–10 days, and explants are kept in the growth chamber under controlled conditions at 22–24°C and photoperiod of 16 h light/8 h darkness. At the end of this period the plants have reached a height of 6–8 cm.

ROOTING AND ACCLIMATIZATION PHASE. Well-developed plants are individualized by cutting, planted in containers and transferred to the greenhouse for acclimatization. These plants are grown under tunnels in conditions of high relative humidity by nebulization to prevent dehydration. Under these conditions the plants develop a root system *in vivo*, while becoming hardened for the growth phase. The duration of this phase is from 21 to 30 days depending on the season and the type of material.

GROWTH PHASE. From the acclimatization greenhouse the plants are moved to growth greenhouses, where they remain until plants

reach about 20 cm. At this stage, they can be sold directly as commercial rootstocks or transplanted into larger containers and maintained in culture until they can be budded and marketed as 'grafted plants'.

The different stages for the *in vitro* production of rootstocks are summarized in Fig. 10.1. Any 'out of type' plant is removed as soon as detected at any stage of the process.

Production of grafted plants

ORIGIN AND ESTABLISHMENT OF INITIAL SCION PLANT MATERIAL. A reservoir of almond cultivars is established from scions provided directly by

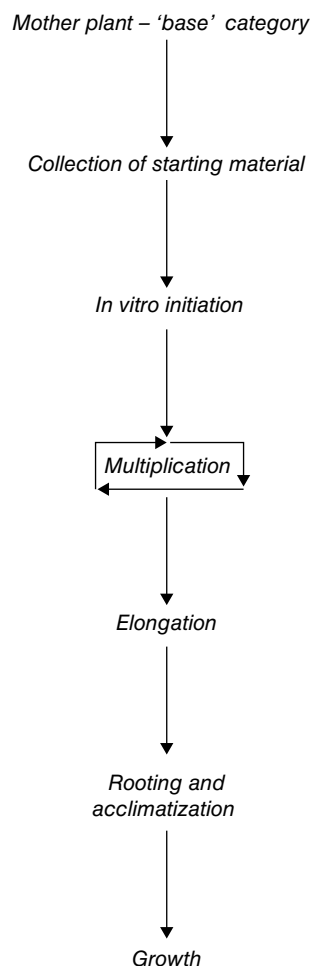


Fig. 10.1. Scheme of the production process of *in vitro* rootstocks.

breeders. This collection of plants is preserved in controlled conditions that ensure the maintenance of varietal identity and disease prevention. Plants are grown in containers inside greenhouses protected from insect vectors (insect-proof) to prevent recontamination. This collection is used as starting material in the certification programme for commercial propagation of fruit trees in the nursery. Visual inspections for pests and diseases are required frequently to ensure the sanitary quality of these materials, and pesticide treatments are scheduled when required. Analysis of the main viruses and phytoplasmas and adequate tracking are also undertaken according to legislation.

ESTABLISHMENT OF MOTHER PLANTS IN FIELDS. Axillary buds of the initial plants are taken and grafted on compatible TC (tissue culture)-propagated rootstocks. Grafted plants are planted in rows and properly identified. Also, to ensure the sanitary quality of these materials, regular visual inspections for pests and diseases are performed and the pesticide treatments are applied when needed. Analysis of the main viruses and phytoplasmas are also undertaken according to legislation.

GRAFTING. TC-propagated rootstocks are grafted with scions from mother plants or provided by the customers. These grafted plants are grown in containers inside greenhouses under management practices to maintain their sanitary

status. When these plants reach commercial size they are packed and delivered to the customers. Plants are labelled to allow easy identification of the combination scion/rootstock, and some nurseries use the barcode method for traceability.

The different stages for the *in vitro* production of grafted plants are summarized in Fig. 10.2. Plants are marketed differently depending on the target customers/destination.

10.4 Scion Propagation in Almond

Grafting is the art of joining two pieces of living plant tissue together in such a manner that they will unite and subsequently grow and develop as one composite plant (Hartman *et al.*, 2002). These two pieces are the rootstock (understock, stock), the lower portion of the graft, which develops into the root system of the grafted plant; and the scion, the new shoot system of the graft. The new shoot is composed of a short piece of a detached shoot containing several dormant buds which, when joined with the rootstock, comprise the upper portion of the grafted plant. Budding is a form of grafting in which the scion is reduced in size and usually contains only one bud.

Grafting and budding are the common techniques for producing almond plants to combine different cultivars into a composite plant, the rootstock being the part controlling

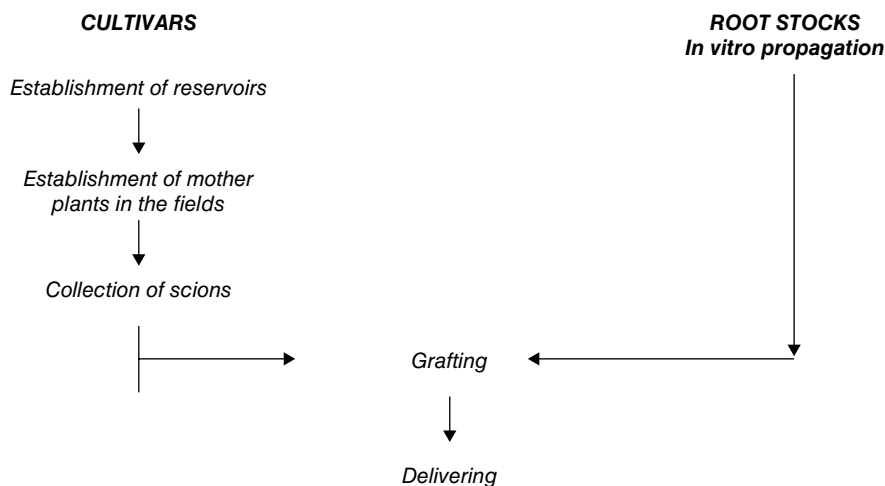


Fig. 10.2. Scheme of the production process of *in vitro* grafted plants.

size, giving adaptation to soil conditions, hastening reproductive maturity and plant growth rate.

Five important things need to be taken into account for grafting success (Felipe, 2000):

1. Scion and rootstock must be compatible.
2. Scion and rootstock cambium should stay in contact.
3. The grafting should be done at the right physiological stage of rootstock and scion.
4. Immediately after grafting, all the cut surfaces should be carefully protected to avoid tissue desiccation.
5. The grafted plant should be controlled until the scion has grown properly.

The formation of the graft union is typically the combination of three major events: (i) adhesion of the rootstock and scion; (ii) proliferation of callus cells at the graft interface or callus bridge; and (iii) vascular differentiation across the graft interface (Hartman *et al.*, 2002; Pina *et al.*, 2012).

10.4.1 Budding

The most popular budding methods in almond nursery are T-budding and chip budding.

T-budding

Widely used in the nursery industry, T-budding is a fast and cheap budding system. Being simple and easy to learn, it allows the development of expertise that results in 2000 buddings per day. It must be done when rootstocks are approximately 1 cm wide and actively growing, especially when bark easily loosens from the stem at the cambium layer. It consists of making a T-shaped cut on the rootstock plant, loosening the bark sides from the stem, sliding the scion bud into the T-cut and binding the bud to the rootstock by winding with a rubber band or grafting tape. After the grafted bud area has healed (often about 2–3 weeks) the binding should be removed.

Chip budding

Chip budding can be done when the rootstock and/or the scion are not actively growing. A trained grafter can do it as fast as T-budding and healed buds give rise to more uniform plants. Chip budding has gradually replaced T-budding

as the primary budding method for many woody ornamental trees, shrubs and fruit trees in many parts of the world (Kidd, 1987; Osborne, 1987).

When scion and rootstock have the same diameter, budding consists of replacing a piece of rootstock by a piece of scion with one bud in. This piece is obtained by two cutting movements: (i) with a gentle curve for one-third of the stock diameter depth; and (ii) downward until the cut is approximately 2 cm long. The scion piece, cut in the same manner, is slid into the stock, ensuring that at least one side of the scion and the rootstock is aligned to allow cambium contact. Binding should be done from the top to the bottom of the bud, keeping the bud out of the plastic but maintaining moisture and excluding air which would dry out the cut. When the shoot has grown 8–10 cm, the binding can be removed.

Season for budding

T-budding should be always performed when the rootstock is actively growing. This means that the cambium cells are in a multiplication process and the bark loosens easily from the stem. At the same time, scion buds should be well developed and mature when grafted. These conditions are only achieved at three stages of the vegetative development of the plants: spring, the beginning of summer and the end of summer.

Chip budding can be performed in any season. Nevertheless, to achieve a fast healing before the scion bud become desiccated, the rootstock should be in a vegetative growing season. Bud shoots can be taken from the tree during the dormant winter season and kept in a freezer, well protected against desiccation, or can be taken directly from the tree in the growing season.

10.4.2 Regrafting

It is sometimes convenient for the farmer to change the scion for another cultivar when the trees have already grown for several years. This technique can be used to introduce a pollinator cultivar into ill-designed orchards, or simply because of the wish to replace the cultivar or even the species. In these cases, the two grafting methods described above may be utilized, after

inducing the shooting of new branches through heavy pruning of the old aerial part.

In this case it is recommended not to eliminate the old cultivar completely to avoid an excess of shooting or an imbalance between the root and the aerial parts, a situation that can even produce the death of the tree or the loss of

the root system. It is convenient to maintain one-third of the old cultivar, to allow the production of enough new shoots

In the case of grafting on old – therefore thicker – branches, other grafting techniques can be used, such as wedge, splice-side and saddle types of grafting (Felipe, 2000).

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11 Orchard Management

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11.1 Introduction

An almond orchard is a dynamic structure made up of the trees over a fixed infrastructure defined by the planting distance. Consequently, the selection of the right plant distance and the operations of planting the trees are of the highest importance since they imply a high cost and determine the productivity along the entire commercial life of the orchard. Any mistake at this stage is carried through the whole life of the orchard and, even if it can be corrected, is at a very high cost.

The dynamic structure of the trees will be defined by the training and pruning systems adopted and will be additionally affected by the orchard management. Therefore this chapter will concentrate on the three main aspects of orchard planting: tree training and pruning, and soil management.

11.2 Orchard Planting

11.2.1 Previous steps

Before planting, a deep ploughing at more than 50 cm is recommended to facilitate root development and water infiltration in the soil. Two different pieces of ploughing equipment may be used in

this task: a deep plough turning over the soil, or a subsoiling to crack the lower soil profiles. In the first case the different soil profiles are partially changed in their disposition and a sole (or 'plough pan', a hardened profile at the limit of the plough work) may be formed at depth; therefore the second ploughing is recommended, being done in a cross direction when the soil is dry. Later, superficial ploughings may be done to eliminate weeds and crumble the soil to make it ready for planting. Different equipment may be used for this, such as disc or spur harrows, cultivators or rakes.

In orchards with potential swamping risk, certain measures such as the correct levelling of the land and planting in ridge should be taken, as well as the work required to ensure good drainage of any excess water, mainly in case of heavy rainfall. When the risk of root asphyxia is high, as happens in heavy soils and very rainy locations, it may be necessary to plan a drainage network or to plant on berms. This last option can also be considered when the soil is not sufficiently deep (e.g. high water table, minimal-quality topsoil). The value of improved drainage in the root zone must be weighed against the increased complexity of operations of soil management and harvest.

Other operations to be considered are the elimination of previous plant material and stones, soil amendments, irrigation facilities and access roads.

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11.2.2 Planting design

Planting density, which refers to the number of trees per surface unit, determines many aspects of orchard profitability including the precocity of production, yield in the mature stage of the orchard, the initial investment, the cost and type of orchard management and the commercial life of the orchard. An increase in planting density will result in earlier production, thus reducing the unproductive stage of the orchard. The maximum productive level will depend on the environmental conditions and the growing system, including planting density. The level of capture of the light radiation will be proportional to the canopy volume; consequently, productivity will increase according to planting density until reaching a maximum density after which productivity increases are minimal, or even nil and negative. The value of the optimal density will depend mostly on the environmental and growing conditions, and for traditional almond growing these were determined at about 300 trees/ha. On the other hand, at higher densities the planting costs will be higher, usually also with higher management costs, as well as a higher incidence of diseases and probably a shorter useful life of the orchard.

Three main determining factors need to be taken into account for establishing the most adequate planting design and density: (i) the environmental conditions; (ii) the potential vegetative development; and (iii) the type of machinery required for orchard management. A tree must have enough soil volume to extract all its water and nutrient needs. In soils of lower quality, larger soil volumes are required, thus requiring an increase in the planting distance. In non-irrigated orchards, with poor soils and probable drought conditions, it has been recommended not to exceed densities of 150 trees/ha (Arquero, 2013, p. 78).

At high and very high densities, once a certain canopy volume has been attained, the lower part of the canopy becomes shaded by the upper part, leading to decreasing yields and to orchard decline. At the same time the vegetation-free zone among tree lines is narrowing, making the machinery traffic for the different growing operations more difficult.

Vegetative growth depends on many different factors: the most important are the vigour of the plant material (scion and rootstock) and the

growing conditions. Plant material that is not excessively vigorous must be selected for orchards at very high density. Similarly, under optimal environmental (soil and climate) and management (irrigation) growing conditions, planting distances must be increased (mainly the distance between lines) in order to avoid the problems of overshadowing and of limitation in machinery transit. At present, taking into account the general traits of the plant material, the conditions of the environment and the growing conditions and, especially, the type of harvesting equipment, it is recommended that irrigated, commercial orchards have rows 7–8 m apart with tree distance inside the rows of 5–7 m, thus giving planting densities of between 179 and 286 trees/ha (Arquero, 2013, p. 78).

Recently orchards at very high density have been introduced, with more than 1000 trees/ha (Lovera *et al.*, 2016), but they must still be considered to be at an experimental stage because the behaviour of these orchards in the medium and long term, at the complete mature age of the trees, is not yet known. In high-density orchards, low vigour rootstocks (of plum tree origin), suitable training and pruning systems, and integral harvesting machinery, such as the overriding harvesters of olive hedges and grapevine trellis, are under trial. The results obtained in the first years of these orchards are promising, although it will be necessary to manage them and to monitor their behaviour in the future.

During the first unproductive years of the orchard it is not recommended to have associated crop (or 'inter-crop') between the rows, such as annual herbaceous crops, because they retard the onset of production and complicate management of the orchard. Another common but not suggested practice is the increase of tree density by interplanting young trees in a mature orchard. In this case, the young trees will require more time for developing enough canopy due to competition with the mature trees, making orchard management rather difficult.

Trees may be distributed in the orchard according to different frames. The most common are squared and rectangular. The squared planting frame maintains the same distance between rows as between trees, allowing all types of cultivation in perpendicular directions. In a rectangular planting frame the distance between trees is lower than between lines. The traffic of

machinery and cultivation only takes place between rows. This frame allows a better use of the soil space than the squared frame, and is the most utilized in fruit growing.

When establishing the row distribution aspects to be considered include the slope, geographical position of the plot, its dimensions and the predominant winds. In sloping plots, the loss of soil due to water erosion may be of great importance, resulting in heavy damage, thus requiring a plot design to avoid or minimize this damage. When the slope is small, the rows must be perpendicular to the direction of maximal slope. When the slope is 5–10% it is convenient to have the rows aligned according to the contour lines. For slopes of 10–20%, tree distribution must be in terraces, and if it is steeper than 20%, in firmer terraces.

When the slope is not a problem, and in flat soils without problems with erosion, the rows must be directed north–south to obtain the best lighting for the canopy. This fact will be the more determinant the higher the planting density.

The design of pollinizers is essential when self-incompatible cultivars are grown (see Chapters 1 and 8). It is advisable to have at least 30% pollinizers in order to have optimal pollination. It is also advisable to have no more than two contiguous lines of the same self-incompatible cultivar and to have two lines of the same cultivar to provide for an easier mechanical harvesting. If the cultivar is self-compatible, no other cultivar is required and the orchard may be of a single cultivar. It may, however, be advisable (mainly in large orchards) to have two cultivars, although in lower percentages than for self-incompatible cultivars. The main criterion in selecting a pollinizer must be the coincidence of blooming times. Ripening time must also be taken into account, considering the possibility for coincidence or not with the main cultivar. Since pollinizers occupy an important space in the orchard, they must also have value as cultivars from the agronomical and productive points of view.

11.2.3 Planting

Traditionally, the planting layout and the opening of holes were done by laying down a main line, over which perpendicular lines were laid. At present global positioning system (GPS) devices are used, allowing excellent precision and a very good speed of execution. Some planting devices

have incorporated these systems, being capable of marking and planting in the same pass. If the soil has been prepared correctly, the planting hole must only be of the size to loosely hold the root system of the plants. The holes were traditionally made by hand, but many mechanical devices can be used to make the holes in the trenches created by ploughing. It is not advisable to place fertilizers or organic matter at the bottom of the holes, since they may induce plant phytotoxicity or root burning.

At present almond orchards are established with grafted plants from nurseries and commercialized as plants with bare roots or surrounded with soil. Planting with bare roots must be done during winter dormancy, between December and February. From nursery pulling to soil planting the roots must remain moist. Potted plants (in containers, with soil around their roots) may theoretically be planted at any time. If frosts are not a problem, autumn may be an appropriate season. In spring it is better to plant before May–June, long before the high summer temperatures.

With plants having bare roots, any deteriorated piece of root must be removed and excessively long roots may be pruned. The plant must be buried 5–10 cm above the point it was in the nursery or the pot, taking care to not bury the grafting point. If the roots are placed too deep, bad root aeration may result, even leading to tree death. The soil to fill the hole after placing the plant must be soft and free of stones and clods. Once the hole is filled, the soil must be compacted to ensure good contact with the roots, thus avoiding desiccation.

11.2.4 Post-planting care

Immediately after planting three steps must follow: irrigation, pruning and staking. If the orchard does not have an irrigation system because it will not be irrigated, a pool must be filled around the tree after planting to moisten the soil and to remove the possible air pockets between the roots and the soil.

Pruning at planting is addressed in section 11.3.2. To make tree formation easier and to obtain a straight trunk for easier harvesting by shaking, the plant must be staked. The stake must be buried at about 50 cm depth and reach to the first branching point, being of suitable material and width to have the required strength.

The stake must be placed 5–10 cm from the plant on the side of the predominant winds. If the plant comes from the nursery with a bent trunk, it must be tied to the stake to correct this bending. An elastic and degradable material is preferable for tying to reduce the risk of trunk girdling.

In the case of the presence of rodents (hares, rabbits, etc.) or the use of herbicides, protective cylinders must be placed around the trees. Their height and consistency must be such as to avoid possible bites. If used only for rodent protection, it is good practice that these protective cylinders be perforated to allow good trunk aeration.

11.3 Training and Pruning Systems

The growth habits of almond trees did not evolve with farming in mind. An almond tree left to grow freely would be difficult to harvest and manage on a commercial scale. The primary goal of both training and pruning is to harness the tree's natural tendencies to create and maintain a habit that will produce optimal yields and facilitate cultural practices. In the first years the tree is trained to a structure that will support future crop weight and allow for cultural practices, while minimizing cuts which could decrease early yields.

After tree structure has been established, pruning primarily facilitates cultural practices like spraying and harvesting, and removes dead and diseased wood. In the past, pruning has been viewed as a way to invigorate tree growth. Numerous long-term trials have shown minimally pruned trees (pruned for cultural practices and removing diseased limbs) yield as well or better than more heavily pruned trees.

Though generalized pruning guidelines are discussed below, the pruning practices for any particular orchard will depend on several factors: planting density, vegetative growth habit of the cultivar, tree vigour, growing system (irrigated or not), orchard management practices, the harvesting system and the availability and cost of labour and equipment.

11.3.1 How pruning works

Pruning changes where growth occurs by changing the hormone and resource balance. The response to pruning will depend on whether

cuts are made during the dormant or growing season, and whether the cut is a thinning or a heading cut. However, *all pruning is dwarfing* because you are cutting nutrients and carbohydrates out of the tree and removing leaves that could supply carbohydrates for new growth.

Dormant pruning, which can be done as early as October (in the northern hemisphere), creates vigorous regrowth in the spring from the area where the pruning cut was made. This is because when wood is removed, carbohydrate and nutrient reserves in the trunk and roots are divided among fewer growing points. The heavier the pruning, the greater the localized regrowth. However, because this regrowth is the result of re-apportioning reserves from the previous season, *regrowth will not surpass the total growth that would have occurred if no cuts had been made.*

Summer pruning is more dwarfing than dormant pruning. Pruning in the summer instead of the dormant season will reduce the amount of regrowth that will occur at the pruning point. Summer pruning removes carbohydrate-producing leaves before they can send carbohydrates and nutrients to reserves. Total growth of all shoots will be less compared to total growth on unpruned trees.

The two different types of pruning cuts – thinning and heading cuts – produce different growth responses and should be done with different goals in mind.

Thinning cuts should be made with the goal of directing growth in a particular area, and/or removing dead or diseased wood. To make a thinning cut, remove the limb or branch entirely by severing it at the branch collar, at the point of origin from the parent limb (Fig. 11.1).

Heading cuts should be made with the goal of encouraging abundant vigorous growth immediately below where the cut is made. Heading cuts create several vigorous shoots where one large shoot or branch was headed back. To make a heading cut, remove the terminal portion of an existing branch. Multiple buds just below the cut will then grow, producing a concentrated area of vigorous branching (Fig. 11.2).

11.3.2 Training young trees

Training young trees needs to address attributes and risks of a given site including vigour, wind, rainstorms, tree spacing, cultivar and rootstock

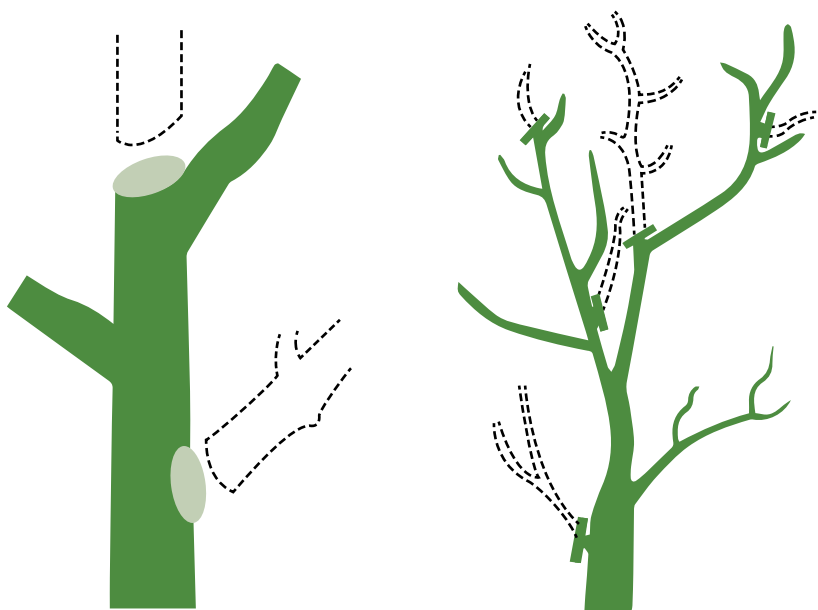


Fig. 11.1. Thinning cuts redirect growth or remove diseased wood. Remove the branch entirely by severing it at the branch collar.

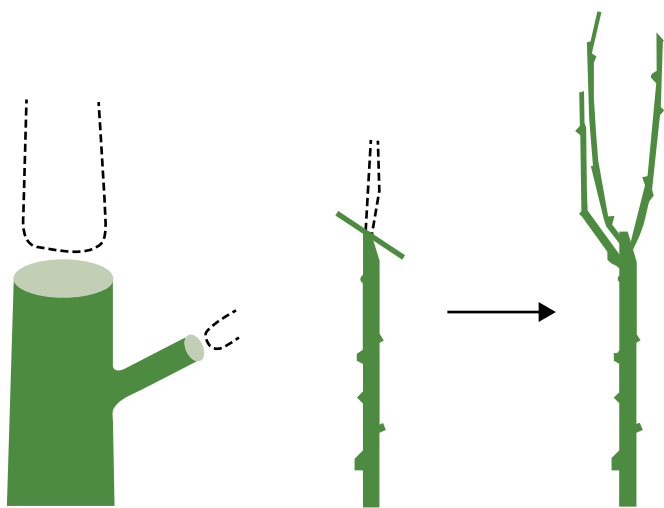


Fig. 11.2. Heading cuts encourage vigorous growth below where the cut is made. Remove the terminal portion of an existing branch. Multiple buds below the cut will then grow.

selection. Training becomes more important the larger the tree will be, because tree architecture needs to be able to support more crop per scaffold. Thus, training is more important the more vigorous the rootstock and/or cultivar, the wider the spacing and the better the growing conditions.

At planting and during the first growing season

Dormant, bare-root trees that have already grown for 1 year in the nursery should generally be topped at 1 m when first planted. This leaves enough trunk space for future shaking and to

vertically stagger future scaffolds. For minimal scaffold selection (see below), consider topping 15–30 cm higher.

For the rest of the season pruning is very minimal. Focus on growing leaf area to produce carbohydrates to maximize tree and root growth. During the first season, remove cross limbs, rootstock suckers (branches growing from the rootstock) and watersprouts (vigorous, vertical shoots) that are too low on the trunk to be good scaffolds. In very vigorous trees, preliminary midsummer scaffold selection can reduce large winter pruning wounds.

Some trees are now being sold as young 'potted' trees, either as just rootstocks which must then be grafted, or as grafted trees without significant scion growth. If significant scion growth has not occurred before planting, allow one season of scion growth, then cut the tree back to 1 m and proceed as discussed above.

FIRST DORMANT PRUNING

Scaffold selection. The primary task during the first dormant pruning is scaffold selection.

After scaffolds are selected, they will be pruned (next section). This is the most important decision time for shaping the tree. *The goal of scaffold selection is to encourage strong, well-anchored scaffolds that will not break or split from the trunk in future years.*

Branches selected to remain on the tree are called 'primary scaffolds' because they are the first branches that grow from the trunk. Branches picked next year arising from these primaries are 'secondary scaffolds'. Branches that grow from secondary scaffolds are called 'tertiary scaffolds'.

The first step in scaffold selection is generally to decide how many scaffolds each tree will have. The traditional and still most common almond tree structure has three or four scaffolds per tree. This is often referred to as the vase style, and sometimes labelled the open, level or French vase (Fig. 11.3).

Many growers in California have moved to four to five primary scaffolds. Trials in different areas of California compared yield of trees with three primary scaffolds to yield of trees with four to six primary scaffolds (Duncan and Lampinen,

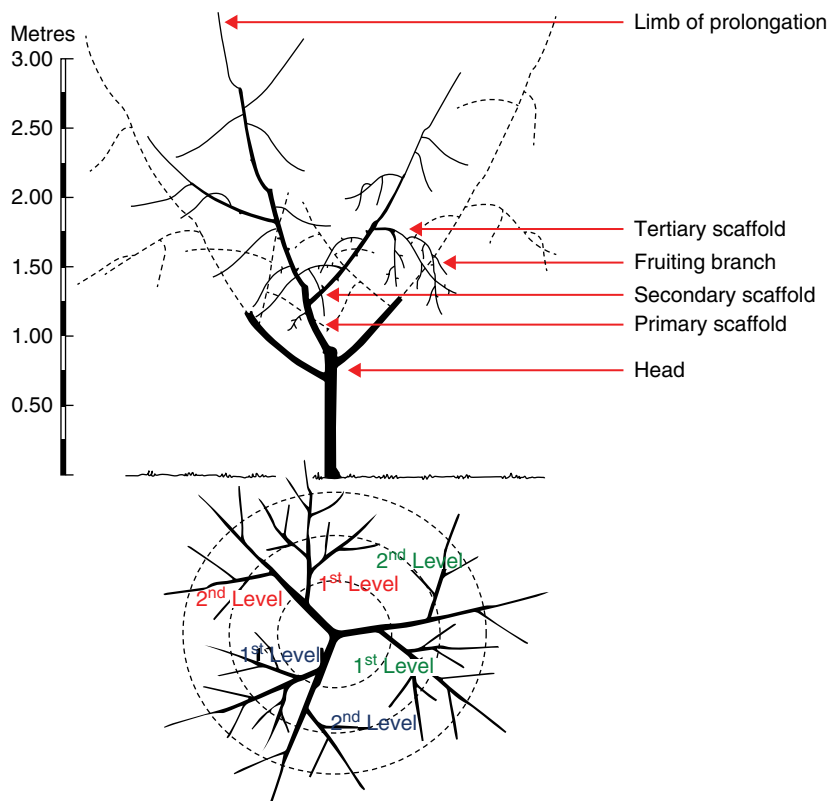


Fig. 11.3. Basic structure of the training system in level vase. (From Cambra and Cambra, 1991.)

2015). After 15 years, there has been no cumulative difference in yield. Where canopy fill was measured, three scaffolds with minimal pruning after the first 2 years filled in the canopy space just as quickly as four to six scaffolds. This higher number of scaffolds can also potentially (not always) make trees more vulnerable to blow-over and make scaffold shaking difficult later in the life of the orchard.

At present planting systems of high and very high density are under evaluation (Ruis *et al.*, 2013; Lovera *et al.*, 2016), with 500–2400 trees/ha, mostly of the hedge type. In this planting system not only are the different plant materials (rootstocks and scions) of low vigour evaluated, but also the different training systems and pruning criteria. Usually, the most appropriate formation system for the high-density orchards is the central axis, with pruning mechanics to maintain the dimensions of the hedge and eliminating manually the branches that grow towards the path to facilitate the movement of machinery.

Once the desired number of scaffolds has been decided, scaffolds are next selected on a tree-by-tree basis. First, remove branches that would obviously make bad scaffolds – those that cross the middle, or have very narrow or wide attachments. Then select scaffolds from the remaining branches.

Keep in mind the *three factors that affect scaffold anchorage and structure: angle, spacing and orientation* of the branches (Fig. 11.4).

For maximizing attachment strength and load-bearing capacity, the ideal angle where a scaffold attaches to the trunk is 45° from the vertical and the horizontal (Fig 11.4a). If branches are at too narrow an angle (less than 30° from the vertical) bark will grow like a wedge in the scaffold crotch, preventing strong attachment and forcing the branch out. Branches wider than 70° from the vertical may not support heavy crop weight.

Even spacing of scaffolds around the tree is critical to give scaffolds room to attach to the trunk and to balance the crop weight distribution. If the goal is three to four scaffolds, they should be spaced 90–120° apart when viewed from above, providing room for strong branch attachment. The scaffolds should be spaced evenly around the tree, 7–15 cm apart up and down the trunk (Fig 11.4b).

When selecting scaffolds, attempt to evenly distribute their orientation (Fig 11.4c). The goal is to balance future crop load around the tree. At sites where strong winds are present, attempt to select a strong branch in the direction of the pre-

vailing wind to help fill out that portion of the canopy. The above consideration become less important when trees are planted at closer spacing.

Once the best scaffolds are picked, remove all competing potential scaffold branches from the trunk. Leave short lateral branches coming off the scaffolds. These will be the first fruitwood. Anchorage and structure are more important than blindly sticking to a required number of scaffolds. In other words, if only two scaffolds can be found on a tree with appropriate angle, spacing and orientation, it is better to only have two scaffolds than to leave an inferior branch on the trunk that may split or break and damage the whole tree at some point in the future.

Scaffold pruning. Once primary scaffolds have been selected and other branches have been pruned out, it is time to prune the selected scaffolds. The goal of pruning growth from the primary scaffolds is to direct growth in the next season for secondary scaffold selection. There are two extremes when it comes to primary scaffold pruning: long pruning and short pruning. It is generally most appropriate to end up somewhere in the middle, with what is called intermediate pruning, but understanding the two extremes helps understand the middle ground (Fig. 11.5).

With long pruning, no cuts are made to the selected scaffolds. Branches are usually roped or tied to avoid severe bending or breakage in the second growing season. Early yields are higher compared to short pruning, but secondary scaffold growth is often concentrated near scaffold tips, making branches top-heavy and likely to bend.

Short pruning heads the scaffolds at 45–60 cm, stimulating vigorous regrowth from which to select secondary scaffolds. While this creates a uniform-looking orchard, such severe pruning sacrifices early yields, creates primary scaffolds that may not be long enough for limb shaking, and increases costs from thinning heavy vigorous regrowth and tying regrowth to avoid limb breakage.

Intermediate pruning *achieves more advantages than short or long pruning*. Heading cuts are made on scaffolds at 100–120 cm from the trunk. This approach keeps much of the canopy for future leaf growth, improves the distribution of secondary scaffolds, allows growth in girth for increased wood strength before heavy load bearing and leaves more lateral future fruitwood. There is less canopy to catch wind than long pruning and less vegetative growth removed

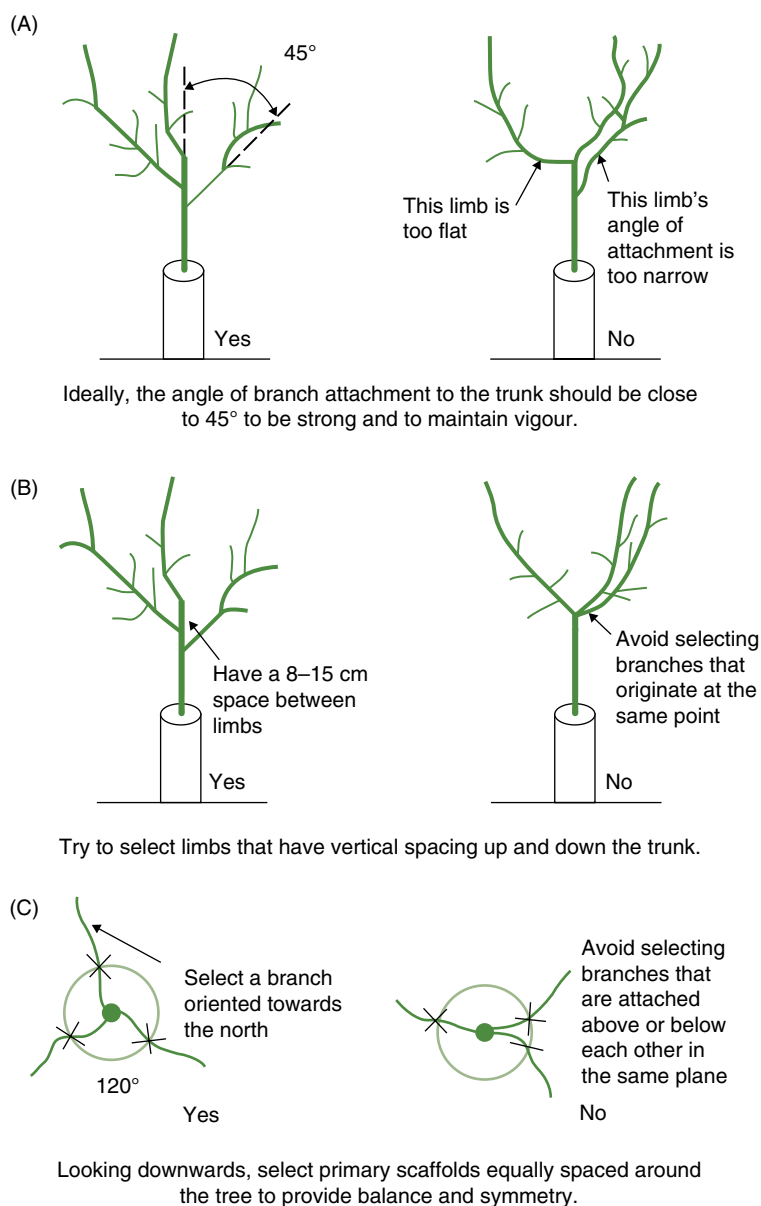


Fig. 11.4. The three considerations in selecting primary scaffolds are: limb angle of attachment (A), spacing (B) and orientation (C).

than short pruning. Roping may still be required with a vigorous rootstock, cultivar or soil, to support scaffold development while preparing the tree for heavy early production.

Pruning intensity will mainly depend on the vegetative habit characteristic of each cultivar (Arquero *et al.*, 2008). Cultivars possessing limbs with good mechanical strength and a high

level of branching may receive longer pruning, whereas those showing reduced branching and spreading tendency may benefit from shorter pruning during the first to second year to attain a good structure (Fig. 11.6). Shorter pruning may be appropriate at sites with strong winds to establish more root growth before extensive top growth leaves the trees vulnerable to blow-over.



Fig. 11.5. Tree with structure in level vase, practising short pruning (left); tree with structure in open vase, practising long pruning (right).

SECOND DORMANT PRUNING. The goal of the second dormant pruning is to *continue to shape the structure of the tree up and out* to create strong architecture and fill the available space with canopy as quickly as possible. First, remove crossing branches, central watersprouts and branches that would become an unwanted primary scaffold if not removed. Then select secondary scaffolds from the remaining branches.

Selection of secondary scaffolds and removal of overly vigorous watersprouts reduces corrective pruning needs later in the life of the orchard without significantly affecting yield. Select two to three secondary scaffolds from each primary scaffold, keeping in mind angle, spacing and orientation to encourage anchorage and balance. Secondary scaffolds should be vertically well spaced along the primary scaffold, ideally at least 30 cm apart and on different sides of the primary scaffold. Avoid narrow attachment angles with the primary scaffold.

With upright cultivars (e.g. 'Mission', 'Padre', 'Aldrich', 'Ferragnès'), select the most spreading branches. With spreading cultivars

(e.g. 'Monterey', 'Carmel', 'Guara'), select more upright branches. Selected secondary scaffolds are not normally headed, but if very long may need to be tied as high on the secondaries as possible during the dormant season. This will result in optimum production and support growth and crop in the third growing season.

If labour is available, some of the above secondary dormant pruning can be done during the growing season. This is referred to as 'green' or 'in-season' pruning. It has the benefit of directing growth earlier towards the desired structure. Excessive green pruning can increase the risk of sunburn and can slow growth overall by decreasing leaf cover. However, removing watersprouts, crossed limbs and other branches that would certainly be removed later avoids wasting the investment of carbohydrates and nutrients on wood that will be cut out. In these cases, thinning cuts should be made, removing the branch at its point of attachment.

THIRD DORMANT PRUNING. By the third dormant season, the architecture of the tree is largely established. Very minimal pruning is required at this

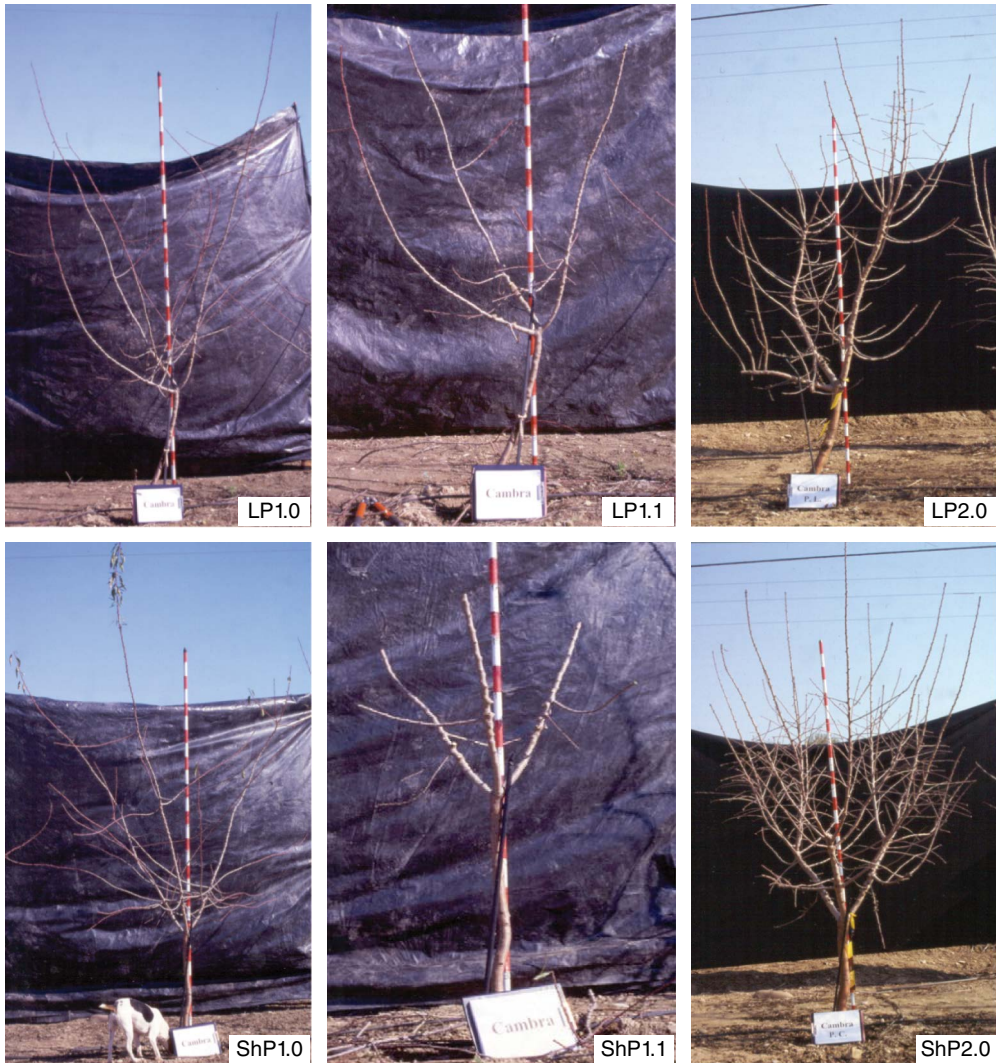


Fig. 11.6. Response to long pruning (LP) and short pruning (ShP) in a cultivar with reduced branching and leaning tendency. *Left:* one-year-old trees before their pruning (LP1.0; ShP1.0). *Centre:* the same trees after their first pruning (LP1.1; ShP1.1). *Right:* the same trees 2 years old, just before pruning (LP2.0; ShP2.0).

stage. *This is the time to transition from training to minimal maintenance pruning.* Remove limbs that are going to interfere with equipment, are broken or may threaten the shape you have already established (e.g. crossed limbs, watersprouts that may interfere with scaffold attachment).

11.3.3 Pruning mature trees

There are important reasons to prune but maintaining yield is not one of them. Traditionally,

almond trees were pruned annually to renew fruiting wood to maintain productivity. However, more than 30 years of research in multiple field trials in California has shown that little to no pruning is necessary to sustain yields in almond orchards. Trials throughout California have found that trees which were not pruned after the first or second year of scaffold selection showed no difference in cumulative yield, and often had higher yields early on, compared to traditionally pruned trees (Duncan and Lampinen, 2015).

The primary reason for pruning is to facilitate cultural practices, such as canopy spray coverage, orchard machinery entry, operator safety, scaffold shaking and drying on the orchard floor at harvest. Once the architecture of the tree has been established, transition to minimal maintenance pruning.

Pruning is also important for disease management. Removing limbs with *Ceratocystis* or *Botryosphaeria* cankers can prevent the disease from spreading into the trunk of a tree. Research in California orchards found no proof that pruning reduces foliar diseases such as *Alternaria*; however, these results may differ in more humid regions (Viveros *et al.*, 2003). In areas with high winds selective pruning to thin the top of the canopies of tall cultivars such as 'Aldrich' and 'Padre' may help avoid blow-overs during heavy storms.

Most commercial almond cultivars bear on spurs, very short shoots with a minimal annual growth. The useful life of these spurs generally does not exceed 7 years, depending on the vegetative and phytosanitary status of the tree as well as on the canopy lighting. Bearing shoots and spurs get exhausted with time. Additionally, flower initiation, and consequently bearing, is inhibited in the poorly lighted parts of the canopy. Because of both spur exhaustion and lower light penetration, the area of fruit bearing tends to move to the outer edge of the canopy.

In some regions pruning the inside limbs and the upper part of the canopy is practised to improve lighting within the canopy. This results in a burst of new shoot growth, which can then bear fruit. In California, pruning is minimal and the bearing surface is concentrated at the outer edges of the tree canopy.

Vegetative growth and bearing are two antagonistic processes. In the case of a strong vegetative growth, bearing decreases, and vice versa. Almond, like many other orchard trees, shows some alternate bearing, more or less pronounced depending on the cultivar and the growing conditions. If yearly pruning of intermediate-low intensity is applied, a certain level of constant vegetative growth is promoted, thus attaining a better regularity of cropping. However, overall (cumulative) yield over multiple years will be reduced.

In non-irrigated conditions, canopy volume would need to be adapted to the rainfall regime and availability of soil water and nutrients by means of the type of pruning practised. An excessive development of the trees in very dry periods would result in yield fall and poor-quality nuts.

The tree vegetative status would also be negatively affected, with premature leaf fall, limb drying and, eventually in extreme cases, tree death.

Do not expect or depend on pruning to reinvigorate an orchard. In the past, pruning has been used to attempt to reinvigorate trees which have little new growth and declining yields. However, research over the past three decades has shown such *pruning will not improve vigour or yields any more than minimal maintenance pruning* (Micke *et al.*, 1991). If trees have begun to decline in yield, resolve whatever may be stressing the trees (e.g. water, nutrition, disease).

11.3.4 Pruning equipment

A great diversity of tools may be used when pruning almond trees (Fig. 11.7). The most used are polesaws and long-handled loppers, which can be manual, electric or air-powered. Handsaws and chainsaws are also frequently used, both air-powered or mechanical, with an electric or combustion engine. These different tools, with many different models, are adapted to the different calipers of the almond limbs and to the different heights of the cuttings points. The equipment must also be adapted to the orchard area.

When using pruning equipment, make sure the tools are sharp, clean and do not touch the ground. To use pruning shears and loppers (most commonly used with small branches), place the hook of the shears on the top of the limb and cut upwards, with the blade close to the trunk or branch. The result is no branch stubs, and clean cuts without tears.

When using a chainsaw, cut at the branch collar to minimize the wound size and promote healing. Clean (not jagged) cuts are critical for wounds to heal, preventing infection. In most growing conditions, wound dressings on pruning cuts are not necessary. However, they may be beneficial in some humid climates.

Mechanical pruning is also applied in almond, utilizing multiple and rotating toothed discs mounted on a bar. Mechanical pruning is quick and cheap, but cuts are indiscriminate, thus often too many are made, provoking a burst of growth of too many vigorous shoots creating later problems of canopy light penetration. Mechanical pruning, if done, should be limited to pruning that facilitates cultural practices, such as opening the centres of orchard rows to improve nut drying at harvest.

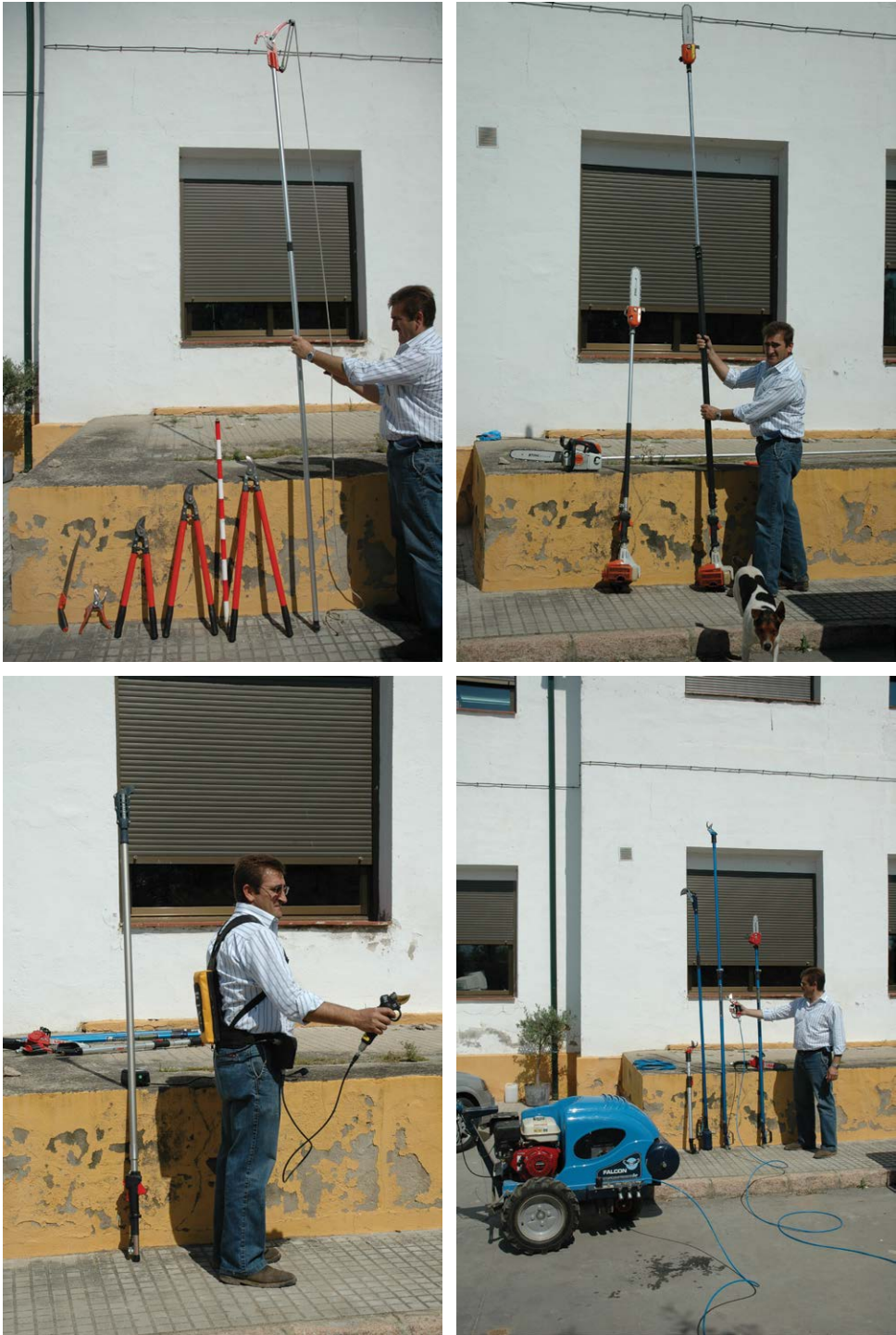


Fig. 11.7. Pruning equipment. Above, polesaws and handsaws (left) and power saws (right); below, electrical equipment (left) and air-powered equipment (right).

Mechanical pruning is sometimes used as an alternative or complement to hand pruning. A California trial comparing mechanical pruning and hand pruning found mechanically pruned trees yielded much lower than hand pruned trees (Viveros, 1991). The most severely pruned (topped and hedged treatments) had the lowest yields. Yields in most of the mechanically pruned treatments eventually recovered to pre-treatment levels, but never made up for the cumulative yield losses following mechanical pruning. However, in regions where hand labour is very costly, the loss in yields may have less value than the money saved on the cost of labour of hand pruning.

11.4 Soil Management

Soil management directly affects some very important orchard conditions, such as water availability in the soil, erosion control, soil fertility and structure, machinery transit, microclimate, and pest and disease control. In fruit growing three soil management systems are applied: (i) tillage; (ii) no tillage with bare soil; and (iii) vegetative cover.

The traditional soil management system is tillage, based on continuous passing of ploughing teams. The most common calendar has been: a deep plough in the autumn with the objective of favouring water infiltration during the winter; another plough at petal fall; in spring another plough to remove weeds; and a final plough around July to decrease water loss by evaporation. The use of ploughs working vertically, such as harrows, vibro-cultivators or cultivators, is preferable. Disc harrows are very effective in removing well-developed weeds. Turning the soil, however, has several negative impacts such as disrupting the soil profile; increasing water loss by evaporation; and creating a 'tillage sole' or 'plough pan' which reduces water infiltration and disturbs root penetration at depth. Among the advantages of tillage may be mentioned easy weed control; possibility of fertilizer application in the same passing; no technical difficulty for the grower; elimination of small pits in the soil; and breakage of the superficial crust formed after the rains.

Tillage is applied probably in 100% of the traditional almond orchards of the Mediterranean region. However, it is less and less applied in other fruit species due to its many inconveniences: it prevents root development in the superficial soil level, where ploughs work, thus rains of low intensity

cannot be exploited; it hurts roots and trunks, even with the death of young trees; it decreases the content of organic matter and hurts soil structure; and it makes machinery transit more difficult. In addition, of the three soil management systems, tillage shows the highest energy costs, induces the highest water loss by evaporation and fosters the most significant erosion.

All these reasons make tillage the least advisable system. However, if it is applied, the number (maximum two or three) and the depth of the ploughings must be reduced as much as possible, a practice known as 'minimal tillage'. In addition, in order to reduce soil losses by erosion the direction of the ploughs must be perpendicular to the maximum slope of the plot.

In the second system, no tillage with bare soil, weed control is ensured by herbicide application. Consequently, it cannot be applied in organic growing. This system is based on the application of pre-emergence residual herbicides in the autumn, before or immediately after the first rains. The weeds resistant to this treatment will be killed in the spring by post-emergence treatments, using systemic or contact herbicides. Herbicides must be applied with herbicide application bars (vertical application) with the right nozzles according to the type of treatment. Only herbicides allowed for almond will be applied. In addition, it is advisable to alternate different kinds of herbicides to avoid a flora inversion towards those species not controlled by the herbicide treatment.

The main advantages of no tillage are allowing root development in the superficial soil level and the low energy cost. Its main inconvenience is the decrease in the infiltration rate, thus reducing the possibilities of rainwater storage in the soil. This problem is especially serious in sloping orchards with loamy soils, where this system is not advisable. Another negative aspect is that it favours soil compaction.

The third system is vegetative cover. In this system the soil is maintained by being covered by vegetation. Normally the naturally spontaneous species are maintained, and the grass species may be selected by the application of selective herbicides for broadleaf species. If the presence of other species is low and their natural establishment is slow, they may be sown. Coverings with pulses have the advantage of fixing atmospheric nitrogen, but their value as covers is lower, due to their limited soil covering and their quick degradation when drying. For better erosion control,

cover establishment and machinery transit must be perpendicular to the maximum slope.

In non-irrigated orchards vegetative cover must be managed extremely carefully. It must be removed at the end of winter or beginning of spring before competing directly with the almond trees for the water stored in the soil. The elimination or control of the vegetative cover may be done by chemical treatments, applying herbicides; or mechanically, using weeding machines or mowers. Chemical control has the advantage of inducing the total death of the weeds, eradicating all possibility of water extraction by them, and additionally maintaining good protection of the soil surface when it dries out. Some grass species used as vegetative covers have a very short vegetative cycle and may dry before competing with the almond trees, thus not requiring to be controlled. If the vegetative cover is removed in the spring before going to seed, some plants must be left to have some seed production to regenerate the cover in the following year. Also the fertilizer needs of the cover crop must be taken into account when planning the fertilizer needs of the orchard.

Vegetative covers show many advantages over the two other systems: they lead to a better soil structure and a higher content of organic matter; improve the ratio of water infiltration into the soil and reduce water loss by evaporation;

facilitate machinery transit; and reduce erosion losses more than the other systems. On the other hand, this soil management system has the inconvenience of being very complex, requiring a certain degree of preparation and dedication from the grower. For such a purpose, the most appropriate plant species must be selected, planted in the orchard and maintained for several years. At the same time, it should have the right type and time of growth to avoid competition with almond trees for water, an extremely important aspect in non-irrigated orchards.

Inert covers from inorganic materials such as stones, as well as organic covers such as pruning wood and straw, can also be applied in almond orchards. However, in general, the organic covers are more difficult to establish and maintain.

There are also mixed systems, a common practice being to have a different system in the tree row and in the space between rows. The most frequent practice is maintaining bare soil under the tree canopy by herbicide application, whereas between rows a minimum tillage is applied or a vegetal cover is maintained. This last system is the most advisable and the most frequent in orchard production, both for conventional and integrated growing, but not in organic growing due to the ban on herbicide application in this last case.

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12 Environmental Requirements

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12.1 Almond and Environment

The important environmental factors for tree growth, including flower bud formation, flowering and fruit growth and maturation, are primarily photoperiod, light intensity, chilling and heat units, soil type, temperature and moisture content.

Almond is characterized by its adaptation to the Mediterranean climate; consequently its area of cultivation is restricted to those regions with this type of climate (see Chapter 3). The Mediterranean climate is characterized by mild winters, although with possible frost periods of variable length, as well as by dry and hot summers which can cause intense water stress to the trees. Rainfall is irregular, but generally it is low and mainly occurs at the end of winter, the beginning of summer and at the beginning of autumn. The effect of rains is particularly negative during two important developmental stages of almond: pollination and harvesting. In addition, rains directly affect the incidence of fungal diseases as these are favoured by high temperature and high humidity. On the other hand, lack of rain is an important limiting factor in non-irrigated orchards, which still represent a substantial amount of almond acreage worldwide.

Almond has been traditionally considered a species adapted to adverse environmental conditions, but yearly fluctuations in production

resulting from adverse effects of climatic conditions show that this adaptation is not real, although it has allowed the expansion of almond to many different ecological regions. Similarly, almond has been referred to as being well adapted to mild winters and dry and warm summers because of its low chilling requirement which is conducive to early blooming. However, in many countries almond growing is shifting from coastal to inland regions with a more continental climate (Socias i Company, 2001), where only high-chilling cultivars (Alonso *et al.*, 2005) can be grown successfully (see Chapter 7). In these conditions, as in any other crop, an economically feasible production can only be obtained by limiting the adverse environmental factors as much as possible. Consequently, such aspects as light, cold acclimation and dormancy, winter cold hardiness, spring frosts, rains, summer water stress and soil properties should be examined because of their direct effect on almond production.

12.2 Light

The importance of light to stone fruit crop productivity has been the subject of several recent studies. Solar radiation influences the energy balance of the plant and the photosynthetic capacity of the leaves, and may influence responses that

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are either photomorphogenic or photoperiodic. The amount of irradiation received by a plant is dependent upon the intensity and the duration of photosynthetic active radiation (PAR). These factors are determined by latitude, elevation and slope of the site. In addition, light interception and shading within and between trees will depend upon the degree of foliage development, tree shape and size, foliage density and the geometry of the orchard. For many crops, light interception has been positively related to dry matter production and yield (Palmer, 1988; Flore and Layne, 1990).

Light is the main driving force for photosynthesis (Flore, 1994). Other factors include temperature, carbon dioxide, nutrient and water supply, and leaf number and exposure (DeJong, 1996). Typical asymptotic photosynthetic response curves to increasing photosynthetic photon flux (PPF) have been reported for stone fruit, with saturation occurring between 400 $\mu\text{mol}/\text{m}^2/\text{s}$ and 800 $\mu\text{mol}/\text{m}^2/\text{s}$ (Sams and Flore, 1982; Kappel and Flore, 1983; Rom and Ferree, 1985).

Leaf gas exchange characteristics of container-grown 'Nonpareil' almond trees were unique among five *Prunus* species tested (DeJong, 1983). Net CO_2 assimilation (A) of almond leaves did not approach an asymptote at a PPF up to 1600 $\mu\text{mol}/\text{m}^2/\text{s}$, whereas maximum A (A_{max}) occurred at a PPF of 400–700 $\mu\text{mol}/\text{m}^2/\text{s}$ for leaves of peach (*Prunus persica* (L.) Batsch), plum (*P. cerasifera* Ehrh.) and cherry (*P. avium* L.). Significantly higher A_{max} values were recorded for almond (up to 18 $\mu\text{mol}/\text{m}^2/\text{s}$) compared to apricot (*P. armeniaca* L.) (7–8 $\mu\text{mol}/\text{m}^2/\text{s}$) and other *Prunus* species (13–14 $\mu\text{mol}/\text{m}^2/\text{s}$) (Andersen, 1994).

The photosynthetic apparatus of most almond leaves is 'light-saturated' at approximately one-half of full sunlight. When the light levels are approximately one-half the intensity found at solar noon, the rate of photosynthesis is already at maximum (DeJong, 1996). As a consequence, only leaves on the outer surface of a tree canopy are exposed to full sunlight and then only for a part of the day as the sun moves across the sky. Thus, most leaves on a mature tree function most of the day with relatively little light. Each leaf, located in different zones of the tree canopy, has its own ever-changing light environment. Light becomes increasingly limiting to photosynthesis from the outer edge towards the centre of the foliar canopy. Thus, if tree canopies are allowed to become too dense, the inner parts

will become so shaded that the leaves can no longer carry on an appreciable amount of photosynthesis. Those interior leaves will then contribute less energy than needed by the fruitwood to which they are attached. Eventually this fruitwood will die.

Photoperiod response is widespread in fruit crops and influences flowering as well as growth cessation in deciduous species prior to endodormancy. *Prunus* species initiate flower buds throughout the growing season with flower buds occurring along shoots produced during the year. Photoperiod is clearly important to trigger cessation of growth in autumn, the first step preceding leaf abscission, the initiation of cold hardiness and endodormancy.

Summer pruning of many fruit crops is done to promote large-diameter fruiting wood in the lower and inner parts of the canopy to increase not only the number of flower buds, but also their quality, since fruiting shoots of larger diameter promote stronger flower buds and larger fruit sizes. Tree crops with dense foliage develop most of their fruit in the top or on the outside of the tree because interior shading inhibits the flowering processes of induction, initiation and development. However, there is considerable genetic diversity in tree fruits for density of branches, leaf size and internode length, to allow selection of desirable tree structure to enhance adaptation to the light intensity in which the crop is grown.

12.3 Temperature

12.3.1 Cold hardiness and dormancy

Following dormancy induction, the decrease in average temperatures in autumn leads to an increase in cold hardiness and freezing tolerance, which reach maximum levels during winter (Rinne *et al.*, 1998). Injuries to fruit trees are associated with either low autumn or winter temperatures which kill whole trees, limbs or sometimes only flower buds, as well as with late spring frosts during bloom which affect flowers or young fruitlets. Cold hardiness of plants is a complex phenomenon which is affected by temperature, day length and plant condition such as maturity, water content, nutritional stage, physiological age and dormancy status (Stushnoff, 1972).

Ashworth and Wisniewski (1991) described how various tissues of deciduous fruit trees respond to low temperatures. Flower buds and xylem are the most frost-sensitive tissues in *Prunus* species (Quamme *et al.*, 1982). There was no correlation between flower bud and xylem hardiness, indicating that cold hardiness of these different tissues are independent traits. On average, shoot xylem was hardier than flower buds (Layne and Gadsby, 1995). In midwinter, it was observed that a temperature of -35.3°C caused a lethal effect in 50% of shoot xylem tissue samples (LT_{50}), whereas the LT_{50} of flower buds was -28.7°C . These temperature thresholds determine the area of survival of any species. Freezing injury to xylem and flower buds of *Prunus* species is avoided by deep supercooling (Quamme, 1974; Quamme, 1978; Ashworth, 1982; Quamme *et al.*, 1982; Ashworth *et al.*, 1983). Deep supercooling in *Prunus* flower buds is related to vascular development (Ashworth, 1982; Ashworth and Rowse, 1982; Ashworth, 1984; Callan, 1990). The lack of xylem continuity between the primordium and the adjacent tissues is the morphological feature that allows deep supercooling. When xylem continuity is established during dehardening, buds lose their ability to deep supercool.

In *Prunus*, the flower primordium in dormant buds has a lower osmotic potential than other parts, possibly as a consequence of high sucrose levels (Quamme and Gusta, 1987). Lower water potential of the flower primordium may be associated with the recovery of water content of flower buds after freezing. Callan (1990) associated flower primordium deep supercooling after deacclimation with flower bud endodormancy, because deacclimated flower primordia ceased to deep supercool earlier when chill unit accumulation was accelerated.

Cold hardiness is determined primarily by genotype, but may be enhanced by different methods of management. According to Andrews *et al.* (1983), a tolerance begins in *Prunus* when the ability to supercool is lost during early bud swell in spring and continues until petal tip emergence. During that period it is possible to reduce freezing injury by controlling the epiphytic population of ice nucleation active (INA) bacteria (Lindow and Connell, 1984). In almond these populations were reduced by applications of bactericides or non-INA antagonist bacteria starting at bud-break, and by applications of cupric hydroxide to

dormant tissues or after budbreak, obtaining a reduced number of INA bacteria at -5°C or warmer.

In *Prunus* species large numbers of flowers, a long bloom period and a late bloom all contribute to spring freeze injury avoidance (Byrne, 1986; Kodad and Socias i Company, 2008). On the other hand, there is a negative relationship between the percentage of live buds after a spring freeze and crop load in the previous season (Byers and Marini, 1994). Ethephon improves hardiness and prolongs dormancy of flower buds by increasing the chilling requirements, thus delaying late winter deacclimation and spring bloom (Durner and Gianfagna, 1991). According to Blanco (1990), paclobutrazol treatments advanced the bloom during the year after treatment and, at the same time, enhanced the flower frost hardiness, both of which resulted in almost a doubling of yield.

Cold acclimation is an important survival mechanism in the life cycle of plants. After an active growth period in summer, specific photoperiod and temperature conditions act as cues for bud set, growth cessation and dormancy (Nitsch, 1957; Tanino *et al.*, 2010). Dormancy involves a number of phenomena including cessation of active growth, formation of terminal buds, formation of abscission layers in leaves, development of cold resistance, development of winter rest and leaf fall. Dormancy also affects seed germination and later seedling growth (Lipe and Crane, 1966). Seed dormancy affects the distribution of germination in time and space, thus preventing ill-timed germination either in the fruit or on the ground. Bud dormancy inhibits buds from initiating new vegetative growth under both favourable as well as unfavourable environmental conditions (Chao *et al.*, 2015).

Dormancy is a phase of development that occurs annually and enables plants to survive cold winters (Perry, 1971). It is traditionally defined as the state in which visible growth is temporarily suspended, but it does not necessarily entail a cessation of biological development since it is known that important differentiation processes normally occur in dormant organs, which permit a slow but steady increase in bud weight (Saure, 1985).

Initiation as well as cessation of dormancy can be triggered by a number of environmental variables including photoperiod, nutrition and water status, but the most important and most heavily studied factor is temperature.

Almond was traditionally the first flowering species among cultivated *Prunus* species. However, since late blooming is a desirable trait in breeding programmes, significant progress has been made in the delay of the bloom time for new cultivars. As a consequence, almond cultivars probably show the widest range of blooming dates among all the fruit and nut species (Socias i Company and Felipe, 1992a). In Zaragoza (Spain) bloom starts in the middle of January for cultivars of North African origin, with very low chilling requirements, and continues until the end of March or early April for the extra-late-blooming almond breeding programme releases (see Chapter 7).

Bloom date is considered to be dependent on the progression of winter temperatures affecting the different stages of dormancy (Lang *et al.*, 1987). At dormancy onset, flower organogenesis is complete (Lamp *et al.*, 2001). During endodormancy, bud growth is constant but barely perceptible. Low temperatures are the most important factor affecting endodormancy completion, though other climatic factors such as heat, photoperiod, light intensity and mist humidity can also affect endodormancy development (Freeman and Martin, 1981). After accumulating sufficient chilling, endodormancy concludes, giving way to ecodormancy. At this later stage, flower bud growth mostly depends on heat accumulation. Accumulation of heat units is important in the active growth processes of most plants, including breaking of endodormancy (Sherman and Rodriguez-Alcazar, 1996). Once ecodormancy has started, bud growth rate depends on favourable growth temperatures and can increase considerably with warm temperatures during the final stages of winter in Mediterranean climates (Westwood, 1978; Kester and Gradziel, 1996). Ecodormancy is considered complete at F^{50} , or anthesis of 50% of the flowers (Tabuenca *et al.*, 1972).

In almond, only a few reports have examined chill and heat requirements. Brown (1952) and Crossa-Raynaud (1955) reported that almond had lower chilling requirements than the other *Prunus* species since it was well adapted to warm winters. Most almond cultivars show rather low chilling requirements, although high variability is observed among cultivars (Tabuenca, 1972; Alonso *et al.*, 2005). Similarly, almond heat requirements are quite low though very variable (Tabuenca *et al.*, 1972; Alonso *et al.*,

2005). The first estimates of chilling requirements for dormancy break in almond were quantified by Tabuenca (1972) as the accumulation of hours under 7°C (44.6°F) and 12°C (53.6°F) from dormancy onset until endodormancy break, a critical point determined by changes in the bud dry weight (Brown and Abi-Fadel, 1953), in 15 cultivars and local Spanish clones. The chilling requirements obtained ranged from 90 h to 427 h below 7°C and from 422 h to 940 h below 12°C. Tabuenca *et al.* (1972) also pioneered the estimation of heat requirements for blooming in 38 almond cultivars, which was quantified as the accumulation of maximum and minimum daily temperatures from dormancy break until full bloom. In this work, the endodormancy break dates were estimated through a statistical method based on correlations between temperatures and bloom dates using data accumulated over several years. Cultivars with lower heat requirements showed totals ranging between 470°C and 572°C for the maximum daily temperatures and 152°C and 202°C for the minimum daily temperatures. Cultivars with higher heat requirements showed totals of 1104–1331°C, and 373–513°C, respectively.

Several years after the studies of Tabuenca in almond, researchers from the University of Utah proposed what has become the most adopted method to measure the chilling and the heat requirements of fruit tree species (Richardson *et al.*, 1974; Richardson *et al.*, 1975). The Utah Model quantifies the chilling requirements as well as the relative effectiveness of various temperatures for releasing dormancy, and is supported by controlled environment studies on the influence of particular temperature ranges on dormancy (Erez and Lavee, 1971). However, the heat sum requirement for floral development is measured as growing degree hours (GDH), a linear accumulation of hourly temperatures above a threshold temperature.

As computer technology developed, Rattigan and Hill (1986) applied the systematic trial-and-error statistical approach of Ashcroft *et al.* (1977), and evaluated the chilling and heat requirements of 12 almond cultivars grown in the mild winter climate of Australia, where they also observed a significant location influence on the blooming behaviour of three cultivars (Rattigan and Hill, 1987). These researchers found early completion of chill requirements in almond and

low variability between cultivars (220–350 Utah Chill Units (CU)) and a larger variability for heat requirements (5300–9000 GDH), suggesting a major effect of the heat requirements on determining the final bloom date.

More recently, Egea *et al.* (2003) estimated the chilling and heat requirements of ten almond cultivars and breeding selections grown in the mild winter climate of south-eastern Spain by assessing the endodormancy breaking date using the traditional bud weight method. In this study, almond chilling requirements for flowering showed a very high variability, ranging from 266 to 996 CU, whereas the heat requirements showed less variability, ranging from 5942 to 7438 GDH.

A more recent quantification of chill and heat requirements for flowering in almond has been reported by Alonso *et al.* (2005). This work can be considered an extension of the work done by Tabuenca *et al.* (1972), which was carried out in the same geographical location, Zaragoza, but used improved statistical methods for determination of the end of endodormancy. In the cold winter climatic conditions of the Ebro central valley of Spain, the almond chilling requirements

ranged between 350 and 500 CU, and the heat requirements ranged between 5500 and 9300 GDH (Fig. 12.1).

The avoidance of cold injury is often accomplished by increasing either chilling requirement or post-chill heat requirement to delay bloom. It is evident that the cultivars ‘Felisia’, ‘Moncayo’, ‘Guara’, ‘Cambra’, ‘Aylés’ and ‘Masbovera’, and the French cultivars ‘Ferragnès’, ‘Ferralise’ and ‘Lauranne’, obtained in breeding programmes, show high heat requirements and occupy the upper-right section of Fig. 12.1. Traditional cultivars such as ‘Desmayo Largueta’, ‘Marcona’, ‘Ramillete’, ‘Constantini’ and ‘Pou de Felanitx’, show lower heat requirements and occupy the lower-left quadrant.

If a cultivar gets too much chilling, either because it has been planted in a colder climate than it is adapted to, or because of unusually cold winters in a traditional growing region, it may be subject to early bloom with possible damage by later spring frosts. If a cultivar gets too little chilling, flowering and fruiting are delayed, the tree may not have sufficient fruit set for a commercial crop, while leafing is delayed and erratic.

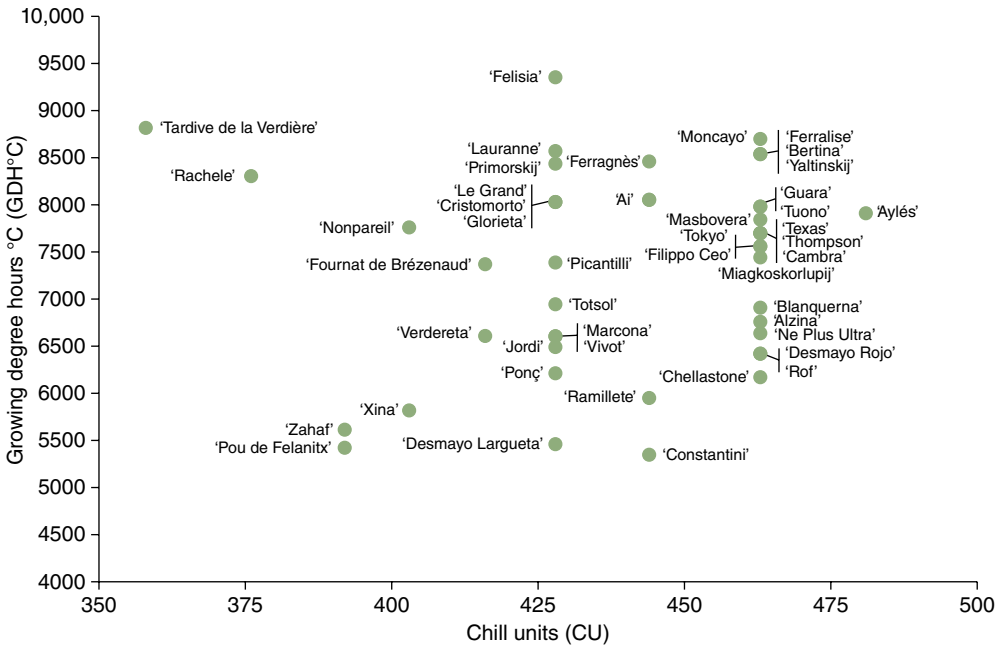


Fig. 12.1. Distribution of some almond cultivars based on their chill (CU, chilling units) and heat (GDH°C, growing degree hours in degrees Celsius) requirements. (Adapted from Alonso *et al.*, 2005.)

Since almond chill requirements are quite low, heat requirements are a greater determinant than chilling requirements in regulating the blooming date in climates characterized by cold temperatures during dormancy. Flower buds are thus exposed to an excess of chilling, with continuous low temperatures with high chilling effectiveness. Consequently, the endodormancy phase is shortened and the chilling requirement is accomplished in a very short period of days in most almond cultivars (Rattigan and Hill, 1986; Alonso *et al.*, 2005). On the other hand, completion of heat requirements is very slow due to the very scarce occurrence of favourable temperatures for vegetative growth, resulting in a low effectiveness in the completion of heat requirements during ecodormancy. Tabuenca *et al.* (1972) explained that almond genotypes growing in climates where they are saturated with CUs during winter start the accumulation of heat units at the same time, beginning with spring warming, and as a consequence, the final bloom date is strongly correlated with the heat requirements of each genotype. Consequently, a shortening of the blooming season to only 4 weeks is observed between the first- and the last-blooming cultivars. However, the pattern of completion of chill and heat requirements in climates characterized by mild temperatures during dormancy is just the opposite. The quality of temperatures to complete chill is very limiting, whereas the quality of warm temperatures to promote growth is very high, resulting in chilling requirements being the major determinant of bloom dates in these conditions (Egea *et al.*, 2003).

While the succession of bloom timing among the different almond cultivars is generally maintained over the years (Felipe, 1977a), small shifts may result from subtle cultivar differences in endodormancy and ecodormancy interactions (Girreath and Buchanan, 1981; Couvillon and Erez, 1985). As a consequence, unless two cultivars have identical heat and chilling requirements, their bloom overlap will depend mainly on the temperature accumulation during dormancy. The incidence of low temperatures following the start of bloom will result in a prolonged bloom, whereas warmer than normal temperatures results in the compression of the bloom season (Chandler and Brown, 1951), since flower development is dependent on the temperature (Rattigan and Hill, 1987; Bernad and Socias i Company, 1995).

Another cause of bloom variation is the base temperature required for flower bud development, which differs not only among species (Richardson *et al.*, 1975; Rattigan and Hill, 1987), but also among cultivars within a species, including almond (Hill *et al.*, 1985; Bernad and Socias i Company, 1995; DeGrandi-Hoffman *et al.*, 1996). There is genetic variability for the optimum temperature at which chilling takes place. It has been suggested that low chill cultivars have a higher optimum temperature for chill unit accumulation than high chill cultivars (Gurdian and Biggs, 1964). In addition, each cultivar can react in a different way to occasional extreme temperatures or to a sudden alternation of cold and warm temperatures, a typical phenomenon occurring during the spring in temperate climates (Couvillon and Erez, 1985; Erez and Couvillon, 1987).

Prunus species develop vegetative and flower buds separately, with flowering usually taking place before leafing. Thus, it has been speculated that flower buds have lower chilling requirements than leaf buds, or that leaf buds have higher heat requirements than flower buds. In contrast, at given locations, some genotypes with low chill requirements leaf out simultaneously with or just before flowering. It may be that leaf buds start accumulating heat units at a lower threshold than flower buds; consequently, during prolonged cool weather leaf buds get ahead of flower buds in heat accumulation and thus begin breaking dormancy earlier.

On the other hand, plants have developed mechanisms that delay seed germination to the most convenient season of the year for survival. One of the regulation mechanisms is seed dormancy. Seed dormancy can be defined as the temporary failure of an intact and viable seed to complete germination under favourable conditions (Bewley, 1997). Seed dormancy is nature's way of setting a time clock that allows seeds to initiate germination when conditions are normally favourable for germination and survival. If a seed is not exposed to sufficient moisture, proper temperature, oxygen and – for some species – light, the seed will not germinate. In *Prunus* species, seed dormancy is an adaptive mechanism to protect these temperate fruit trees from freeze damage during winter (García-Gusano *et al.*, 2004). A seed that is dormant will not germinate even when the environment is adequate for

germination (Martínez-Gómez and Dicenta, 2001). In *Prunus*, seed dormancy is caused by two mechanisms: (i) an external mechanism which inhibits seed germination and is controlled by the endocarp and the seed coat; and (ii) an internal mechanism controlled by the embryo which affects later growth of seedlings (Mehanna and Martin, 1985; Mehanna *et al.*, 1985; Seeley *et al.*, 1998). The latter mechanism is the most common type of dormancy in plant seeds of temperate regions and is essentially governed by antagonistic roles of abscisic acid (ABA) and gibberellic acid, which, in turn, are influenced by environmental factors such as temperature and light (Hazebroek *et al.*, 1993; Debeaujon and Koornneef, 2000; Koornneef *et al.*, 2002).

The breakage of dormancy is necessary for the completion of seed germination. All *Prunus* species show different germination requirements due to their different chilling (stratification) requirements, the thickness of the seed teguments, the concentration of germination inhibitors and the kernel percentage (Zeinalabedini *et al.*, 2009). A pretreatment of dormant seeds to force germination can avoid the prolonged duration of seeds in germination beds in nurseries. In this regard, several treatments are used to eliminate seed dormancy such as scarification; stratification; and gibberellic acid (GA_3) and hydrogen peroxide (H_2O_2) application, as well as the removal of the seed tegument.

Seeds of most *Prunus* species have a stony endocarp. This hard endocarp has a mechanical effect, offering physical resistance to germination while affecting the gas exchange and preventing seed imbibition and washing-out of the tegument hormones, thus determining the stratification period required for germination (Du Toit *et al.*, 1979). As a consequence, endocarp removal may hasten or increase germination in stone fruit species (Hartman and Kester, 1967). Therefore, seeds of many species of *Prunus* require different scarification treatments before cold stratification (Lawyer, 1978). Scarification is any process of breaking, scratching, mechanically altering or softening the endocarp to make it permeable to water and gases. Removal of the endocarp by hand increased seed germination in almond (Gaudio and Del Pedone, 1963). Nasir *et al.* (2001) suggested that almond nuts should be kept in boiling water for 10 min before sowing to obtain maximum germination. Acid scarification

is another procedure for breaking physical dormancy in seeds such as almond which have an intact stony endocarp.

Almond seeds also require a cold treatment under humid conditions to overcome dormancy and promote germination. Stratification has been the method traditionally used to break seed dormancy in almond (Grigorian, 1972). Germination has three requirements: (i) seed germination capacity; (ii) satisfaction of chilling requirements to overcome internal seed dormancy; and (iii) environmentally favourable conditions for germination and growth. Temperature is the main factor affecting seed dormancy completion during stratification. The most effective temperature regimes for moist-chilling are similar to those during the winter and early spring in the native environments. Temperatures between 2°C and 7°C are generally the most effective, with more time required at both higher and lower temperatures (Seeley and Damavandy, 1985). Abbott (1955) indicated 5°C as the least efficient temperature for seed germination, and temperatures higher than 17°C could reimpose seed dormancy.

The time required to stratify seeds results from the interaction among genetic characteristics, seed development, seed bed and seed handling (Hartman and Kester, 1967). In almond, a high quantitative correlation exists between the mean germination time for the progeny seedling populations and the mean of both seed and pollen parents (Kester, 1969), but only a low correlation was found between the individual seed and the buds of the new plant coming from the embryo (Kester *et al.*, 1977). On the other hand, Grigorian (1972) examined the relationship between the flowering time of a cultivar and the stratification requirements of seeds for germination, and reported a large variability in the stratification requirements of seeds for germination, though this may have been due to their open pollination origin. Similar conclusions were later reached by García-Gusano *et al.* (2005a) and García-Gusano *et al.* (2009).

A period of between 40 and 50 days of stratification at 4°C is usually sufficient to complete the chilling requirements to break almond seed dormancy. During stratification, as seeds are completing their chilling needs, they are absorbing more water from the moist substrate and increasing the respiration rate. Following

this period, the seed needs to stay in a moist substrate at favourable growth temperatures (15–25°C) during a variable period depending on the genotype for germination to occur (Felipe, 2000). However, further heat treatments did not induce germination in those seeds that had not yet fulfilled their chilling requirements.

Removal of the endocarp and the tegument in seeds that had not fulfilled their chilling requirements produced the same effect as stratification, and has been described as a mechanism to accelerate seed germination (Chao and Walker, 1966; Zigas and Coombe, 1977; García-Gusano *et al.*, 2005b). In addition, overcoming of seed dormancy may be mediated by the biosynthesis of GA₃ (Hilhorst and Karssen, 1992), or stimulated by exogenous application of hydrogen peroxide H₂O₂, which at the same time promotes seed sterilization (Chien and Lin, 1994; Fontaine *et al.*, 1994).

12.3.2 Spring frosts and protection

The flowers and young fruit of almost all deciduous fruit trees are vulnerable to frost damage (Andrews *et al.*, 1983). Consequently, spring frost injury is a major limiting factor in the production and distribution of horticultural crops (Ashworth, 1992). Damage to crops by freezing temperatures results in crop losses every year. The frequency and severity of freezing temperatures during bloom and early nut development determine if almonds can be produced at a particular site. In the present context of climate change, many studies have focused on forecasting frost risk, but these analyses have yielded conflicting results (Charrier *et al.*, 2015), since frost risks have been predicted either to increase (Augsburger, 2013) or decrease (Scheffinger *et al.*, 2003). A mean temperature increase, with constant variance, would be expected to lower frost exposure, even though the sensitive stages of plant development would occur earlier in the spring. An increase in both the mean and variance implies that freezing events could still occur and damage flushing buds.

The anatomical, physiological and biological aspects of frost damage have been reviewed in fruit trees (Rodrigo, 2000). In *Prunus*, fruit damage is detectable by observing ovule browning,

ice formation (Saunier, 1960) and browning of the petals and pistils.

Since almond is an early-blooming species, almond cultivation has been restricted to regions with low risk of spring frosts. However, the expansion of the culture into inland Mediterranean areas, where the occurrence of spring frost commonly overlaps with bloom of most almond cultivars, the risk of reducing or even nullifying yield has increased significantly (Socias i Company *et al.*, 1999). Thus, almond breeding has attempted the development of late-blooming cultivars (Kester and Assay, 1975). Nevertheless, this solution is not enough to overcome spring frost damage, and frost hardness is also considered a selection objective in European breeding programmes (Socias i Company *et al.*, 1998).

Almond flower buds will tolerate temperatures down to about –7°C (20°F). As the bud breaks and petals are exposed, their sensitivity increases markedly until at petal fall, when 1 h at –1°C (30°F) may result in significant damage (Brewer, 1978).

The evaluation of frost tolerance among cultivars has shown the presence of a high genetic variability in the cultivar response to very low temperatures, as well as to frost. This evaluation has been based on traditional methods, such as by visual assessment of frost damage on flowers and fruits (Büyükyılmaz and Kester, 1976; Kodad and Socias i Company, 2004), by evaluating the magnitude of damage on fruit set and yield (Felipe, 1988; Kodad and Socias i Company, 2005) and by the measurement of chlorophyll fluorescence variation (Kodad *et al.*, 2010).

However, little is known in almond about the minimum threshold temperature for causing damage in flowers and young fruits. Frost injury differences among several commercial almond cultivars (Felipe, 1988) and breeding selections (Kodad and Socias i Company, 2004; Imani *et al.*, 2011) at different critical low temperatures have been described, concluding that a temperature at –2.5°C causes heavy production losses in most genotypes. Damage differences were also found among genotypes at the same phenological stage (Proebsting and Mills, 1978; Kodad and Socias i Company, 2004; Imani and Khani, 2011), which could indicate differential resistance to frost damage, with the young developing fruit being the most vulnerable stage to frost.

On the other hand, the intensity and duration of the period of low temperature have a stronger effect on frost severity (Mazur, 1969) at all development stages: buds, flowers and young fruits. In this regard, Snyder and Connell (1996) indicated the degree of frost damage observed in several Californian almond cultivars following 30 min of artificial freezing of branches at different development stages (Table 12.1). They concluded that frost damage is influenced by the duration of low temperatures; flower stage and position on the shoot; tree vigour; and weather conditions preceding the frost.

Later, Miranda *et al.* (2005) studied the variability and relationship between frost temperature and injury level for the main cultivated *Prunus* species. Two almond cultivars, 'Marcona' and 'Ferragnès', were included in this study. Buds at different phenological stages, designated from 'B' to 'I' (Felipe, 1977b), were placed in seven frost treatments to assess final bud injury levels (Table 12.2) and to ascertain the confidence intervals for lethal temperatures of 10% (LT_{10}) and 90% (LT_{90}). They found that sweet cherry (*Prunus avium* L.) demonstrated the highest frost tenderness of the assayed species, followed by almond (*P. amygdalus* Batsch), peach (*P. persica* (L.) Batsch), Japanese plum (*P. salicina* Lindl.) and blackthorn (*P. spinosa* L.). At post-bloom stages, the difference between the LT_{10} and LT_{90} in almond was less than 1.5°C, much less than observed for the other *Prunus* species.

Other approaches have been applied for evaluating the tolerance of almond buds and flowers to spring frosts, such as differences of chlorophyll fluorescence (CF) in tissues before and after cold treatments (Khanizadeh and DeEll, 2001), the measurement of the increase of freeze-induced electrolytes in frost tissues (Lindén *et al.*, 2000) and proline accumulation in cold-treated tissues (Chu *et al.*, 1974). Kodad *et al.* (2010) used the chlorophyll fluorescence method to correlate the chilling and freezing tolerance with the CF in 12 commercial almond cultivars. They obtained the lower values for CF at temperatures between -2°C and -3°C, signifying that the flower buds of all genotypes are vulnerable at these temperatures, agreeing with previous results (Felipe, 1988; Connell and Snyder, 1989; Miranda *et al.*, 2005).

Other estimation of frost injury is the measurement of freeze-induced electrolytes. Frost

damage to cell membranes results in enhanced leakage of solutes into the apoplastic water solution. Thus, recording the amount of leakage after cold treatments provides an estimate of frost tissue injury (Lindén *et al.*, 2000). With this approach, Imani *et al.* (2011) found a direct correlation between frost injury and ion leakage in 60 almond selections, demonstrating that this method can be used as a cold hardiness assay. Finally, the accumulation of proline is often associated with whole-plant responses to chilling (Chu *et al.*, 1974) as a cold-protective reaction. Proline quantification in cold-treated tissues has been suggested as an indicator of chill hardiness (Van Swaau *et al.*, 1985). Imani *et al.* (2011) evaluated the resistance of different almond genotypes to frost, finding that the content of proline may serve as an indicator of frost tolerance in almond breeding selections.

The observation of frost damage indicates that only tolerant or resistant genotypes (cultivars or selections) can produce a crop when frost takes place at bloom time or somewhat later. However, the presence of a high number of flowers increases the probability of survival of some flowers after a frost, thus increasing the possibility of an acceptable crop, as seen in almond (Kodad and Socias i Company, 2004), and also in some peach cultivars from the USA and Canada (Werner *et al.*, 1988; Okie and Werner, 1996) and Hungary (Szabó *et al.*, 1998). Californian cultivars such as 'Nonpareil' show a lower flower bud density (Kester and Griggs, 1959) than traditional Spanish cultivars such as 'Marcona' and new releases from breeding programmes such as 'Guara' (Socias i Company, 1988). Frost risks are lower in California than in Spain, thus leading to the prevalence of cultivars with low or medium flower bud density, probably to reduce the problems related to flower quality (Socias i Company and Felipe, 1994). In Spain, where frost risks are important (Felipe, 1988), a high flower bud density has been a positive trait for cultivar evaluation to ensure a crop, as demonstrated in the new cultivar 'Soleta', which shows the highest value of flower bud density.

Despite all the studies carried out on the effect of spring frost damage in almond, its real effect on fruit set has not been sufficiently clarified because fruit set in almond depends on many different factors, including other weather conditions (such as rain), insect activity among

Table 12.1. Percentage of flower buds or fruits damaged by frost after 30 min at different temperatures for the main cultivars grown in California. (Adapted from Snyder and Connell, 1996.)

Cultivar	Phenological stage	Temperature										
		30°F –1.1°C	29°F –1.7°C	28°F –2.2°C	27°F –2.8°C	26°F –3.3°C	25°F –3.9°C	24°F –4.4°C	23°F –5°C	22°F –5.6°C	21°F –6.1°C	20°F –6.7°C
'NePlus Ultra'	Pink Tip (D)*						1	10		20		20
	Pink Bud (E)					0	70	90	90	90	90	
	Full Bloom (F)			5	70	90	100					
	Small Nuts (H–I)	1	5	20	50	100						
'Sonora'	Green Bud (C)						1			5		5
	Pink Bud (E)						20	10	30	10	5	10
	Full Bloom (F)					70	80	70	80	90		
	Small Nuts (H–I)		1	5	60	100						
'Peerless'	Green Bud (C)						5			5		10
	Pink Bud (E)			0	1	50	100					
	Full Bloom (F)		0	5	90	100						
	Small Nuts (H–I)		0	5	60	100						
'Nonpareil'	Pink Bud (E)						20	40	40	30	50	40
	Full Bloom (F)				50	70	90	90	90			
	Small Nuts (H–I)	1	1	40	90	100						
							30	30	30	40	40	20
'Price'	Pink Bud (E)						90	100	100			
	Full Bloom (F)				50	70						
	Small Nuts (H–I)		0	30	80	100						
							40	50	40	70	40	70
'Carmel'	Pink Bud (E)						100	100	100			
	Full Bloom (F)				60	90						
	Small Nuts (H–I)	1	10	30	70	100						
							80	70	80	90	90	
'Butte'	Pink Bud (E)					40	90	100				
	Full Bloom (F)		0	0	60	90						
	Small Nuts (H–I)		1	5	80	100						
							70	90	100	90		
'Padre'	Pink Bud (E)					100	100					
	Full Bloom (F)		0	1	50	100						
	Small Nuts (H–I)		1	5	30	100						
							90	70	80	100		
'Mission'	Pink Bud (E)					90		90	80			
	Full Bloom (F)		0	1	80	100	100					
	Small Nuts (H–I)		0	40	90	100						

*Letters in parentheses correspond to phenological stages as defined by Felipe (1977b) for almond.

Table 12.2. Mean lethal temperatures (°C) for the 10% (LT₁₀) and 90% (LT₉₀) of almond flower buds or fruits for each phenological stage (PS). Confidence intervals at 95% are also given. (Adapted from Miranda *et al.*, 2005.)

PS ^a	LT ₁₀	LT ₉₀	Confidence interval for LT ₁₀		Confidence interval for LT ₉₀	
			UL ^b	LL	UL	LL
B	−6.6	−15.4	−5.6	−7.6	−13.2	−17.6
C	−3.9	−10.7	−1.4	−6.4	−5.7	−15.7
D	−3.1	−10.3	−1.3	−4.9	−6.7	−13.8
E	−2.7	−4.8	−1.2	−4.2	−1.7	−8.0
G	−2.6	−4.0	−2.3	−2.9	−3.3	−4.6
H	−2.3	−3.6	−1.9	−2.7	−2.7	−4.4
I	−2.4	−3.0	−2.2	−2.5	−2.6	−3.3

^aPhenological stages as defined by Felipe (1977b) for almond
^bUL = upper limit, LL = lower limit

inter-compatible and simultaneously blooming cultivars and the capacity for natural autogamy in self-compatible cultivars (Socias i Company and Felipe, 1992b). However, breeding for frost resistance appears feasible for obtaining cultivars adapted to the harsh weather conditions of many almond-growing regions in the more continental climates (Socias i Company *et al.*, 2003).

Frosts are frequently classified as advective, radiative and evaporative (Felipe, 1968; Snyder and Connell, 1996; Snyder and Melo-Abreu, 2005), depending on the atmospheric conditions under which they occur. An advection frost occurs when cold air blows into a region and replaces warmer air. These frosts are characterized by moderate to strong winds, no temperature inversion and low humidity. Radiative frosts are characterized by clear skies, calm winds and temperature inversions. These frosts occur because of loss of heat in the form of radiant energy. Under clear night-time skies, more heat is radiated from an orchard than it receives, so the temperature drops. It drops faster near the ground, producing a temperature inversion (cooler near the ground than in the air above) (Snyder and Connell, 1996). Evaporation frosts are characterized by the extraction of heat from the plants due to the evaporation of the water covering the plant after rain (Felipe, 1968). This situation could occur if the relative air humidity decreases after a rainy, cold front, producing quick water evaporation and taking the heat of evaporation from the plants, which can freeze the more sensitive tissues. The intensity of these frosts depends on the amount of water evaporating, the air temperature and

the air relative humidity. If wind occurs the evaporation increases, removing more heat from the plant tissues and raising the freeze risk.

Frost losses can be reduced by different protection methods. These methods are classified as either active and passive and have been thoroughly reviewed by Felipe (1968), Snyder and Connell (1996) and Snyder and Melo-Abreu (2005), among others. However, frost control has become more complicated because of environmental laws, increased costs of labour and changing management practices used in high-density plantations, as well as new techniques for prevention of frost. Two main types of frost protection methods, however, are typically applied: passive and active.

Passive methods are those applied well in advance of the actual freeze danger and probably are the most economical and effective. They are based on the idea of preventing low-temperature damage in areas with a high probability of frost occurrence. For almond, planting cultivars of late- or ultra-late bloom time can help avoid the presence of open flowers during the periods of high frost probability. In this sense, planting orchards on north-facing slopes could also help to delay blooming until the danger of frost is over. Orchards should not be set in depressions. Thinning hedgerows or clearing forested areas can sometimes reduce the risk of frost in sloping terrains by allowing cold air to drain to lower areas. Soils should be maintained free of weeds, though it is advisable not to cultivate the soil just before the frost period and not to leave much straw or other material on the floor. Excessively wet soils

gain less heat energy during the day as more of the sun's energy goes into evaporating moisture. This can reduce the heat available to the orchard at night. Excessively dry soils are poorer heat conductors and are able to store less heat, and therefore a higher risk of frost results.

Active methods are those applied right at the start of the frost as well as during it. They are usually only effective under radiative frost conditions since advective freezes usually cannot be prevented by active means. Active freeze protection needs accurate weather forecasts. These methods attempt to reduce the heat loss from the tree surface, stirring the air to break up the temperature inversion, or adding heat to maintain the temperature above the danger point. There are several ways to cause air warming in radiation frosts. The use of large propellers, including helicopters, produces mechanical mixture of the cold air close to the ground with the warm air of the atmospheric higher layers (Rhoades *et al.*, 1955). This is known as a temperature inversion. This method can be effective when there are large temperature differences between air layers near the surface and those up higher. Equipment and operating costs are high. Effectiveness varies in the range of 1–4°C.

Fog and smoke clouds from smudge pots or burning tyres may reduce radiative heat loss from the surface. However, this method is not very effective because it is difficult to maintain the smoke over the sensitive crop area, and the environmental laws in most countries prohibit the use of frost protection methods where smoke is involved. Frosts can also be avoided by the use of heaters, mainly in the form of oil or gas burners uniformly spaced throughout the crop (Brooks and Schultz, 1958), to add enough heat to the layer of air surrounding the crop and, through radiant heat, to the crop to maintain the temperature above the freezing point. The best results occur when the air is calm, so that a steep temperature inversion exists. This method can provide protection from frost as low as –4°C.

Other protection methods are based on the release of heat during water freezing. A very low rate of water application through irrigation can be effective in preventing or reducing freeze damage. Water application through under and/or over tree sprinkling should begin with the onset of freezing (Rogers *et al.*, 1962; Connell and Snyder, 1989; Iannamico, 2016) and a film of water

should be continuously maintained until temperatures have risen above the freezing level (0°C). Trees must be able to support the added weight of ice that builds up on the branches, so the grower must avoid leaving excessively long branches after pruning. However, after the application of this method, the heating of the soil during daytime is reduced and the moisture may also change the critical temperature needed to cause freeze damage in the crop.

The return on investment of equipment for active freeze protection depends on many factors, including the degree of risk, the likely duration and severity of frosts, value of the crop and effectiveness of the method to be used.

12.3.3 Flower bud and fruit development

Stone fruit have shown a high vulnerability to bud drop, also referred to as bud failure and bud jump. Flower buds fail to swell in spring, and desiccate, abscising when other flowers on the tree are opening. Bud drop has been suggested to result from high autumn temperatures during flower bud formation (Brown, 1958), inadequate winter chilling or high temperatures during early endodormancy (Layne *et al.*, 1996), large fluctuations in winter temperatures (Nakasu *et al.*, 1995) and high night temperatures in December and January (Weinberger, 1967). On the other hand, Ristevski and Koleckevski (1998) in Macedonia reported massive flower bud drop in almond cultivars of warmer origin. Dropping flower buds showed completely healthy internal tissues, without frost symptoms. The flower drop was increased in cooler areas, at high altitude and with smaller summation of growing active temperatures. From these studies, it is possible to elucidate that from a certain threshold, an excess or a defect in the chill and heat requirements produces a lack of adaptation to the environmental growing conditions and the expression of physiological disorders, which results in flower drop at dormancy break.

The effective pollination period (EPP) is one of the most important factors determining successful fertilization. EPP is determined by the longevity of the ovule minus the time lag between pollination and fertilization, providing that this

value does not exceed the length of stigmatic receptivity. As reviewed by Sanzol and Herrero (2001), the EPP is limited by three main events during the reproductive process: (i) stigma receptivity; (ii) pollen tube kinetics; and (iii) ovule longevity. Incidence of high temperature during blooming affects the duration of these events and consequently shortens the EPP (Williams, 1966). The first studies of EPP in almond were done by Griggs and Iwakiri (1964), concluding that flowers are most receptive for 1–2 days after anthesis, remaining receptive for 3–4 days (DeGrandi-Hoffman *et al.*, 1989; Ortega *et al.*, 2004; Kodad *et al.*, 2011; Kodad and Socias i Company, 2013). Pollen tube growth along the style of almond flowers takes 4–5 days (Griggs and Iwakiri, 1975; Socias i Company *et al.*, 1976). Pollen tubes require from 2 to 4 days more to reach the ovule (Pimienta and Polito, 1983), once the embryo sac has fully matured (Pimienta and Polito, 1983), although a high variability among cultivars on the time of embryo sac maturation has been found (Egea and Burgos, 2000). In the case of orchards composed of a combination of two self-incompatible cultivars, not only is a similar flowering time needed, but EPP synchronization is also required to achieve a commercial crop. Kodad *et al.* (2011) found productive problems in ‘Marcona’–‘Fournat de Brézinaud’ orchards in Morocco, due to absence of full coincidence of flowering time of these cultivars and a much-reduced period of optimal stigmatic receptivity of ‘Marcona’. This problem is a consequence of the lack of adaptation to excessively warm temperatures during bloom in Morocco, a problem that could apply to other production areas in the future as a consequence of global warming.

Temperatures may affect the presence of double kernels, a trait that is commercially negative. In *Prunus* species, two ovules differentiate and develop in each carpel. One of these, the secondary ovule, usually aborts some time after pollination, leaving only the viable, primary ovule, to be fertilized (Pimienta and Polito, 1982). In some cases both ovules may be viable and double kernels are produced (Spiegel-Roy and Kochba, 1974). The percentage of double kernels seems to be dependent on the environmental conditions of the year, but with large variations (Spiegel-Roy and Kochba, 1974). In general, favourable conditions for pollination and fruit set lead to a higher percentage of double kernels (Grasselly

and Gall, 1967), as well as the presence of flowers of higher quality (Socias i Company and Felipe, 1994). Egea and Burgos (1995) found that pre-blossom temperatures influenced the viability of the secondary ovule, resulting in a negative relationship between high pre-blossom temperatures and double-kernelled seed production.

The fruit development period is from full bloom to fruit maturity. Temperature has an effect on the fruit development period length. It has been observed that the length of the period of fruit development from full bloom (FB) to the start of ‘hull-split’ (HS) was negatively correlated with the accumulation of degree-days between FB and 90 days after FB (Tombesi *et al.*, 2010). Also, during the last month of fruit development, high temperatures and water deficits can affect the timing of HS and harvest (Teviotdale *et al.*, 1995; Teviotdale *et al.*, 2001).

In almond, the harvest date is not always closely related to nut maturity date. Harvesting can be delayed until nuts are dry enough to harvest. Normally the date of HS signals the beginning of fruit maturity. A timely harvest is essential to maintain nut quality and, in the case of nuts picked up from the floor, to minimize post-harvest microbial contamination (Danyluk *et al.*, 2007). Harvest date is of important consideration when choosing cultivars for an orchard in order to allow the logistics of scheduling orchard management practices, including harvesting operations. Harvest of late cultivars can be more difficult since nut humidity is higher due to shorter days, cooler weather and more frequent rains. Delay in harvesting can also increase costs by extending operations over time. An early or timely harvest is the principal method of avoiding damage to soft-shelled almond cultivars (Connell *et al.*, 1989). A short fruit development period could also help to save irrigation water and reduce the negative effect of summer drought on nuts and the whole tree (Felipe, 2000).

In almond, the relationship of fruit growth and growing unit accumulation is not as clear as in peach (Weinberger, 1948), mainly due to the small mesocarp thickness in almond and its relative low weight percentage in relation to the whole fruit, as compared to the other *Prunus* species. Examination of the nut size of a series of almond cultivars and selections (Socias i Company *et al.*, 2014) has shown that there is no relationship between nut size and blooming time

in these genotypes, despite the hypothesis that delaying bloom time in almond could be linked to a decrease in nut size. As a consequence, it is possible to obtain selections of very late bloom time and early ripening time, as happens with 'Mardía', which retains an average nut size. The breeding of new cultivars with late bloom time and short fruit development period, as well as a large nut size, currently appears feasible.

12.4 Rains

Rains are essential to obtain acceptable crops in almond, especially in non-irrigated orchards. However, there are two particular periods when rains are undesirable: bloom and harvest.

12.4.1 Untimely rains

Long rainy periods at bloom time are not frequent in the traditional almond-growing areas, since these areas are characterized by a Mediterranean-type climate, where rains are less frequent during February and March. However, some years are especially rainy during bloom, and with the current climatic warming trend, the frequency and incidence of rains during bloom may increase and become a crop limiting factor (Dorfman *et al.*, 1988). Rains during bloom suppress both anther dehiscence (and so pollen release) and insect-mediated cross-pollination, crucial for self-incompatible almonds and still important for self-compatible almonds.

A strong negative correlation has been found in California between total rainfall in February (flowering time of most Californian cultivars) and final crop level (Alston *et al.*, 1995). Despite the problems caused by rains during the bloom period, the self-compatible cultivars are much less affected than the self-incompatible ones because they do not depend on bees for cross-pollination. In a combined orchard of 'Guara' (autogamous) and 'Ferragnès' (self-incompatible), whose blooming is coincident and where beehives were placed during the pollination period, persistent (although weak) rains during the blooming period caused an almost total crop loss in 'Ferragnès', whereas in 'Guara' the production was almost normal. These observations highlight

the importance of both compatibility and autogamy as important objectives in modern breeding programmes, such as for the cultivars registered by the Centro de Investigación y Tecnología Agroalimentaria (CITA) of Aragón. This efficiency has been proved by many monovarietal orchards of 'Guara' and of other self-compatible cultivars released subsequently.

Ortega *et al.* (2007) studied the possibility that pollen grains could be washed off by rain from the stigma surface and the effect of this hypothetical washing on final fertilization efficiency. They concluded that the adhesion of pollen grains to the stigma was very quick and strong, and that simulated rain was not able to completely wash off pollen grains from the stigma surface, although it did appear to affect adhesion in future pollinations.

Rains during flowering could also delay pollen dehiscence in the anthers due to the increase in environmental humidity. Rains during bloom can also result in favourable conditions for infection by several plant pathogenic fungi causing springtime diseases, including *Monilinia laxa*, *Botrytis cinerea* and *Sclerotinia sclerotiorum*.

Rains are not usually very frequent during the almond harvest periods of late summer and early autumn, though sporadic storms may occur. However, these rains can greatly hinder harvest efficiency, mainly since harvest cannot be carried out during rain, but also because additional or lengthier drying operations to dry rainsoaked nuts are required.

Summer rains can also negatively affect harvest operations because they may reactivate the tree vegetative activity, which is normally suppressed for better harvest efficiency and less harvest damage. Therefore, the water supply is dramatically reduced in irrigated orchards about 3 weeks before harvest, with the purpose of decreasing the sap flow and of reducing damage during the vibration of the trunk by the harvesting machine. Rains before harvest are uncontrolled risks because the sap flow is reactivated, making the trunk more vulnerable to vibration damage such as bark breaks.

The strategy of advancing harvest has also been considered to avoid the inconvenience caused by rains during later harvest periods, similar to the strategy of delaying bloom to avoid spring frosts. Thus, if the harvest date is earlier, it takes place on longer and warmer days, allowing the intensification of harvesting as well as of

nut drying, and where the risk of storm occurrence before harvest is diminished.

Earlier harvest has only been pursued recently in almond breeding programmes. In fact, one of the most important Spanish cultivars, 'Desmayo Largueta', which is very late ripening, was the favourite in some areas of joint vineyard and almond production because its harvest could be delayed until the grape harvest was over. Nevertheless, the introduction of 'Guara', which displays very early ripening, indicated the value of an early harvest; in addition, this should stimulate the commercial channels towards an earlier commercialization of the new harvest. 'Guara' is harvested during the first 2 weeks of August, although it can be advanced to July in some precocious zones. Other recent releases also ripen at the same time as 'Guara' (see Chapter 5).

The examples of 'Guara' and 'Desmayo Largueta' demonstrate that there is no strong relationship between blooming and maturation dates, since 'Guara' is late blooming with early maturation, and so opposite to 'Desmayo Largueta', which has a very long reproductive cycle. Cultivars with staggered maturation dates allow for successive harvests of different cultivars; this applies to the CITA releases 'Belona' and 'Soleta' (Socias i Company and Felipe, 2007). A short reproductive cycle and a thin fruit mesocarp or hull are desirable traits in cultivars, allowing efficient water and nutrient utilization and thus being more environmentally sustainable (Alonso *et al.*, 2012).

12.4.2 Deficiency and excess of rains

Almonds are more tolerant to drought than their close cultivated relatives, allowing most early commercial plantings to be placed in non-irrigated areas (Ross, 1981). The reputation of almond as a drought-tolerant crop which is also well adapted to poor soils results from the strong taproot of almond seedlings, which grow deeply in well-drained soils, contributing to their adaptation to arid summer climates. These seedling rootstocks were often not grafted or, in some cases, grafted *in situ* without transplanting (Felipe, 2000). The need to retain this deep taproot, which is lost and not recovered after transplanting, was essential to almond survival in the harsh conditions of drought common to many early growing regions.

Rootstocks continued to play a major role in determining drought tolerance in almond plantings (see Chapter 9). *Prunus* species and interspecific hybrids (Layne, 1987; Perry, 1987) vary greatly in their tolerance to soil moisture, and almond, peach and apricot are not tolerant of wet soils. In fact, almond is the most sensitive of the stone fruits even to vocational 'wet feet' (Kester and Gradziel, 1996), greatly limiting its use.

12.5 Soil Properties Limiting Almond Production

Almonds prefer fertile, deep, well-drained, non-stratified loamy soils, but can be grown on a wide variety of soils. While many of these soils are well suited for almond, others are marginal because of some limiting soil feature such as restrictive layers (claypans, hardpans, siltstone layers, sandy lenses), stratification, waterlogging, salt problems or other soil deficiencies (Perry, 1984). These soil limitations affect the health and development of roots, and ultimately the tree size and vigour. Under ideal soil conditions, seedling roots can be found to depths of 3 m.

The early identification of growth-limiting conditions is essential for correcting the problems before planting, or for selecting the rootstock most tolerant of the undesirable condition (Begg, 1978). The most common physical soil properties affecting root development and limiting growth of almond trees are: soil texture; soil depth and restrictive horizons; saturated soils including water tables and flooding; and soil erosion.

Almond roots deeper and grows more vigorously in non-stratified, medium- and moderately fine-textured loamy soils than in fine-textured clay or coarse-textured sandy soils. The permeability of these soils is good, available soil moisture is high and aeration is adequate to favour optimum development of root and tree growth. With fine-textured clay soils (silty clays and clays), the problems include slow drainage (slow permeability) and limited aeration (Ravina and Magier, 1984). Rooting depth is shallower in clay soils because aeration is reduced and oxygen becomes less available with depth. Sandy soils (sands and loamy sands) are rapidly drained and well aerated. The problems with sandy soils are those of

limited nutrient and water-holding ability (Bargioni, 1959; Gras, 1962). If the deficiency in moisture-holding ability is not compensated for by more frequent irrigation, tree growth is stunted and yields are reduced (Pisani, 1960). Because much of the rain and irrigation water percolates through sandy soils, nutrients are also leached from the soil. Therefore, heavier and more frequent applications of fertilizers are needed to maintain adequate levels for good tree growth.

Usually, the young alluvial fan and floodplain soils have stratified subsoil layers with different textures. Percolating waters from winter rains and irrigation can build up above the claypan, hardpan or siltstone horizons to form a temporary perched water table. If the perched water table persists for any length of time, roots in the waterlogged zone will be damaged. Hardpan and siltstone horizons, which are much more restrictive barriers than claypan, are cemented rock-like layers which stop root development and are essentially impervious to water movement. Thus, the depth of the hardpan layer determines the rooting depth of the tree. Deep subsoiling to improve the permeability of the claypan horizon is often not very effective since, following rewetting, the clays swell and seal up again. However, this method has been very effective in breaking up hardpan and siltstone, allowing roots to grow down the cracks to seek moisture and nutrients at lower levels. For loamy soils underlined by sandy materials, it has been found that the number of roots decreased sharply in the sandy substratum. The small number of roots in the substratum can be attributed to the limited amount of soil moisture and low nutrient status of these sands.

In soils having a permanently high water table, the depth of the rooting system of the tree is limited to the depth of soil above the water table. Roots will not grow into the water-table zone because of lack of aeration (oxygen). Thus, the water-table level determines the depth of the soil reservoir which the rooting system of the tree can tap for nutrients and moisture. In the case of an intermittent water table the potential hazard or injury to the rooting system is greater during the vegetative period than during dormancy, when it is minimal. The extent or degree of injury is a function of the duration of waterlogging, the amount of oxygen dissolved in the water and the soil temperature, with increased damage with longer waterlogging periods and

higher soil temperatures. These physical limitations in the soil profile can have devastating effects on tree longevity and productivity (Atkinson and Wilson, 1980).

Superficial flooding can cause waterlogging damage in fruit tree orchards, and their importance depends on the tree growth stage and its length. *Prunus* species are less tolerant to waterlogged and anaerobic soil conditions than are many other genera in the family Rosaceae (Schaffer *et al.*, 1992). Almond and apricot are among the least tolerant to waterlogged soil conditions of all *Prunus* fruit tree species (Norton *et al.*, 1963; Wicks and Lee, 1985; Schaffer *et al.*, 1992). After 2 days of flooding, root growth of 'Chellaston' almond and 'Nemaguard' peach were severely and moderately inhibited, respectively (Wicks, 1989). The susceptibility of almond rootstocks is exacerbated by a low resistance to fungal infection by *Phytophthora* spp. (Wicks and Lee, 1985; Wicks, 1989). Almonds, regardless of rootstock, should be grown in well-drained soils that are not prone to flooding for even a short period of time (Rowe and Catlin, 1971).

Floodwaters contain suspended sands, silts and clays, which produce superficial sediments in the orchard. The depth of material deposited may vary from a few millimetres to several centimetres in thickness (O'Connell *et al.*, 2007). If the sediments are not removed from around the base of the trees, crown rot and other diseases may develop. Another effect of flooding is scouring, where soil material is eroded away as the faster floodwaters pass over the land. If scouring is deep, tree roots may be exposed or, in extreme cases, a tree may be uprooted or dislodged. Relevelling is necessary to recover exposed roots and to carry on normal orchard operations (Bratkovich *et al.*, 1994).

Another soil problem is the erosion that can be produced in almond orchards planted on gently undulating to hilly slopes. The degree of erosion is generally related to the steepness and length of slope, influenced by the rainfall intensity and duration, nature of the soil, type of ground cover and management practices. Erosion reduces the nutrient level of soils by removing the more fertile upper soil horizons; larger amounts of fertilizer must then be applied to replace these losses. If erosion is severe, roots can be exposed. Erosion can be minimized by converting to non-cultivation and, where tillage is

necessary, the land should be cultivated across the slope (Elmore, 1989).

Almond production can also be influenced by the chemical limitations of the soil. Nutrient deficiencies, excess soluble salts, high exchangeable sodium and excess boron are the most common chemical problems of soils reducing growth of almonds (Brown and Uriu, 1996). Soil analysis is a useful tool to indicate the general level of a nutrient in the soil but, in many cases, plant tissue analysis is a better indicator of nutrient availability because it reflects the actual amount of a nutrient taken up by the plant. Once the current nutrient status of a soil has been established, it is often not too difficult to correct the deficiencies by the use of fertilizers or amendments.

Almonds are generally considered to be a low salt-tolerant crop (Ottman and Byrne, 1988). Reliable diagnosis of the salinity problem requires laboratory analysis because other conditions, such as herbicide damage and lack of moisture, can produce symptoms similar to salt damage. When soluble salts occur in excessive amounts they can stunt growth and severely damage plants. Shoot growth of almond is suppressed by relatively low concentrations (25 mM NaCl) of salt in the soil solution (Zrig *et al.*, 2011, 2016). Stunted growth is generally determined by the total salt concentration, although most fruit and nut crops are especially sensitive to chloride and sodium salts. Most salts are highly mobile and are easily translocated within the soil. Excess soluble salts in soils may originate from the parent material of the soil, or may accumulate from the use of saline irrigation waters or by capillary rise of dissolved salts from a high water table.

Soils containing enough exchangeable sodium (> 15 %) to interfere with the growth of most crops, either with or without appreciable quantities of soluble salts, are termed alkali or 'sodic' soils (Massai *et al.*, 2004). Excess exchangeable sodium has the dual effect of deterioration of soil structure and toxicity to sensitive

crops. Poor soil structure is caused by dispersion of clay particles by the excess sodium. This results in poor aeration, reduced permeability and sealing of surface soil. High exchangeable sodium produces a patchy chlorotic pattern of the leaves which eventually becomes necrotic. Sodic soil conditions can be corrected with the use of soil amendments, such as gypsum, and establishment of good soil drainage (Oster and Frenkel, 1980).

Almond is also sensitive to an excess of boron (El-Motaïum *et al.*, 1994), which can cause small corky areas to form at the midrib on the underside of the leaf. Progressive chlorotic yellowing of the leaf then develops, plus burning of the leaf tip and margins. Control or corrective measures depend upon the source or cause of the excess boron. If soils are inherently high in boron, use of sufficient water to leach the root zone is recommended. Should the soils be irrigated with water high in boron, the best solution is to find another source of water and/or more tolerant rootstocks.

Soil analysis can be useful in diagnosing whether the salt problem is one of excess soluble salts, exchangeable sodium or boron. Concentrations of the different chemicals limiting in soils vary widely within both regions and soil depth. In sampling soils to determine a potential or existing soil problem, samples should be taken to the full depth of the rooting system of the tree in order to diagnose the problem adequately.

The early identification of soil problems can also be helpful in selecting rootstocks or cultivars which are more tolerant of the hazardous condition. Stone fruit grafted on peach rootstocks usually show Fe and Zn deficiencies in soils above pH 7.5, but almond and peach × almond hybrids convey fewer deficiency symptoms, making these rootstocks much better adapted (Kester and Grasselly, 1987). Remedial measures also can be taken to correct or minimize the problems before planting the orchard.

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13 Almond Water Requirements

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13.1 Introduction

Water is one of the most important inputs to maximize almond production. For efficient water management the variables of weather, water availability and plant stress need to be considered. Each of these variables may change throughout the irrigation season, increasing the complexity in determining when irrigation should occur and the amount of water to apply. The amount of water used by the almond orchard is largely determined by the weather and thus is influenced by the humidity, temperature and time of day. The demand for water by the almond tree is called evapotranspiration (ET). ET accounts for the loss of water through soil surface evaporation and the loss of water through leaf stomata which allow the gas exchange required for photosynthesis.

A standard method for scheduling irrigations is to calculate the ET as this is a relatively simple, science-based approach to estimate irrigation requirements of an orchard. It is done by measuring water use of a reference crop (ET_o) and multiplying it by a research-determined crop coefficient (K_c). The K_c is a simplified way of representing the ratio of water use by the crop in question (ET_c) to the ET_o . Knowing this, the

following equation can be constructed to calculate almond water use:

$$ET_c = ET_o \times K_c \quad \text{Eqn 13.1}$$

Evapotranspiration of the reference crop (ET_o) is a weather-based index determined by year-long observation of weather conditions, taken at specified intervals (usually hourly). Traditionally, it was the measured water use of fully irrigated grass. However, estimates can also be made using a weather station that collects temperature, humidity, wind speed and solar radiation. These variables are utilized within the Penman–Monteith equation (Allan *et al.*, 1998). Many agricultural weather services currently provide these data. ET_o changes over the course of the year, reflecting the changing water use and loss relative to the index crop, as climatic conditions change.

The crop coefficient (K_c) is determined by field research and is used as a multiplier against ET_o to determine the total water use of the almond orchard. Since almond trees experience changes in physiological conditions and canopy coverage throughout the year, there are different K_c values for different periods of the year. In the case of almond, there have been several recent efforts to determine these values (Table 13.1). Various

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Table 13.1. Estimates of the monthly crop coefficient (K_c) values for mature almond trees using a grass reference crop (ET_0).

	Doorenbos and Pruitt (1977)	Fereres and Puech (1981)	Sanden (2007)	Goldhamer and Girona (2012)	Girona <i>et al.</i> (2005)	Doll and Shackel (2015)
January						0.40
February						0.41
March	0.50	0.60	0.59	0.20	0.40	0.62
April	0.75	0.71	0.78	0.67	0.65	0.80
May	0.90	0.84	0.92	0.95	0.80	0.94
June	0.95	0.92	1.01	1.09	0.92	1.05
July	0.95	0.96	1.08	1.15	0.96	1.11
August	0.95	0.96	1.08	1.17	1.05	1.11
September	0.85	0.91	1.02	1.12	0.85	1.06
October	0.80	0.79	0.89	0.85	0.60	0.92
November	0.70		0.69		0.40	0.69
December						0.43

methods have been used including the water balance method and eddy-covariance measurements. All of these have led to significantly higher calculated water needs, with one set suggesting that around 152 cm of water was needed in the conditions of California’s southern San Joaquin Valley.

To reduce plant stress, the amount of water calculated by determining ET_c should be applied by either irrigation or rainfall. This would also include any water reserves within the soil from winter rainfall. As an equation, the total amount of irrigation applied during a season would be:

$$\begin{aligned} \text{Water applied} &= ET_c - (\text{In-Season Rainfall}) \\ &\quad - (\text{Stored Soil Moisture}) \end{aligned}$$

Eqn 13.2

When K_c and ET_0 have been determined, almond water use can be calculated. As shown in Table 13.2, a mature almond orchard grown under the described conditions had 164.1 mm in ET_c .

The crop coefficient for young orchards or low canopy coverage orchards will be slightly lower than that of a mature, full canopy covered orchard. To calculate how much lower, estimate the canopy coverage of the orchard at midday by estimating the area of the orchard floor that is shaded by the tree. Multiple this percentage by 1.67 and then multiply by the corresponding crop coefficient (Ayers *et al.*, 2003). For example, a 3-year-old orchard covering 40% of the orchard floor will use 67% of the water that a mature

orchard would use. Once the canopy shades 60% of the orchard floor, assume that it will use full ET_c .

Tables of historical ET_c values for each week of the year for almonds may be available. The historical values are based on several years of ET calculations for each week. They cannot account for variations in weather that occur every year (unseasonably hot or cool weather). Nevertheless, they are simple to use and are much better than attempting to guess the orchard’s water demand.

Understanding the needs of the tree is only one part of efficient irrigation. The second component is understanding how much water can be held within the given soil of an orchard. Conceptually, soil is similar to a sponge, being filled with water upon irrigation and slowly being drained or ‘squeezed out’ over the course of the irrigation cycle. If too much water is applied to the soil, it either runs off the surface or drains out of the bottom of the soil column. If too little, there is not enough water for the tree to make it to the next irrigation without undergoing stress. The maximum amount of water that can be held within the soil after it has had time to drain is the soil water-holding capacity. When discussing soil water-holding capacity, a few terms must be defined. Soil saturation is when all of the soil pores in a given layer of soil are filled with water; this condition occurs immediately after irrigation and continues until gravity pulls the water through the pores of the soil. Once the soil has drained, usually 1–3 days after an irrigation/rain event, the soil is at field capacity. This water

is held against gravity within small pores by the soil particles, and is the largest volume of water that a given amount of soil can hold between irrigations. In contrast, the permanent wilting point of a soil is the point at which all the water available to plant roots is gone. The difference in water volume between the field capacity and wilting point is the available soil water-holding capacity – or the amount of water available to the plant.

It is important to note that, at field capacity, not all of the pore space is filled with water, and at permanent wilting point not all of the water is removed from the soil. The amount of water to refill the soil profile is related to the texture of the soil: coarser soils hold less water while fine, heavy soils hold more. This is due to the differing sizes of soil particles and pore space in these soils. In general, soils are about 50% solids, 50% pore space. The pore spaces hold varying proportions of water and air.

For efficient irrigation, water needs to be applied to refill the soil profile to field capacity but prevent the loss of water to deep percolation. Thus, determining how much water can be applied when irrigating depends on the soil within the orchard. The available soil water-holding capacity of various soils are in [Table 13.3](#). It is given in terms of cm of water in 1 m of soil depth. [Table 13.3](#) gives a rough approximation

of available water capacity by soil types, but more specific information about the soil type and texture should be determined using a soil analysis or soil maps. Soil sensors also can help determine when the soil profile is filled.

Soil is three-dimensional. Soil sampling at various depths can help determine if the soil texture throughout the root zone is uniform or made up of layers of textures. It is advisable to have on hand soil maps, if available, to help identify known texture changes. Root-zone depth is usually determined by soil being excavated by an auger/probe, texturing the soil and checking for roots. For most orchards, if the ground was prepared properly during establishment, there will be between 0.9 and 1.2 m of root zone. The presence of a clay lens, hardpan or gravel layer may reduce the root profile depth and water availability to the tree. Once a soil texture profile has been developed, the amount of water that the soil can hold can easily be calculated by using [Table 13.3](#). An example of the process is in [Table 13.4](#). With drip and micro-sprinkler systems, the wetted surface area of the soil should be taken into account to determine the water-holding capacity of the soil volume. This will vary by soil type, flow rate and emitter spacing.

In the example in [Table 13.4](#), there are 7.99 cm of water available for the tree to use. When scheduling irrigation, do not deplete the available

Table 13.2. Evapotranspiration calculations for a mature California almond orchard for May 2015. All units are in mm per ha.

Week	ET _o for the week (grass water use) provided by weather station	Almond K _c for the month of May (from Table 13.1)	ET _c for the week (water lost from the orchard)	Cumulative total of water use by the almond orchard with a cover crop
1–7	42	0.95	39.9	39.9
8–14	30.50	0.95	29.0	39.9 + 29.0 = 68.9
15–21	35.30	0.95	33.5	68.9 + 33.5 = 102.4
22–28	30.22	0.95	28.7	102.4 + 28.7 = 131.1
29–31	18.33	0.95	17.4	131.1 + 17.4 = 148.5

Table 13.3. Estimates of available water for various soil types.

Type of soil	Range (cm/m)	Average (cm/m)
Coarse (sand/loamy sand)	5–8.3	6.25
Sandy (loamy sand/sandy loam/loam)	8.3–12.5	10.4
Medium (loam/silty clay loam)	10.4–18.3	12.5
Fine (silty loam/silty clay loam/clay loam/silty clay)	14.2–20	16.6

Table 13.4. Example calculation of available water-holding capacity.

Soil surface	Soil texture	Depth (m)	Available water-holding capacity (from Table 13.3 in cm)	Available water in each soil layer (cm)
1–30 cm	Sand	0.30	5	1.5
31–60 cm	Loamy sand	0.30	8.3	2.49
61–100 cm	Sandy loam	0.40	10	4
Total:				7.99

water completely. Irrigation should occur after using approximately 50% of the available water. Letting the profile go completely dry will deplete all deep soil moisture, stress the tree and affect tree performance including yields. Irrigating at 50% depletion also provides some insurance, helping to avoid potential mistakes. In the example above, irrigation should be scheduled when 4–5 cm of water has been used by evapotranspiration.

Knowing the amount of water that can be held within the soil is also helpful in determining how much water is needed to refill the entire rooting profile. In the soil profile used in Table 13.4, irrigating more than 40 mm may lead to deep percolation. Deep percolation may be of benefit if leaching of salts from the rooting profile is desired, but it may also leach nutrients out of the root zone. This not only wastes fertilizer but also may result in nitrate contamination of groundwater.

Whenever water is applied to an orchard, loss of some amount of water should be expected, and must be considered when calculating the amount of water being applied. If not, an over-estimation of the amount of water reaching the trees will occur. This amount of loss will vary on the irrigation system used, soil and climatic conditions, and water management conditions. Water applied to a field can be lost by run-off, percolation below the root zone, sprinkler spray evaporation and off-target drift. Efforts to minimize these inefficiencies should be employed, and could include longer irrigation sets at lower rates, the construction of tailwater return systems, soil sensors to determine when the profile is filled or subsurface installed irrigation systems. Irrigation application efficiency (E_a) is defined as the percentage of applied water that is held in the root zone. Mathematically, it is:

$$E_a = \text{water stored/water applied} \quad \text{Eqn 13.3}$$

Irrigation methods commonly used within the almond orchard include surface irrigation

systems, such as flood and furrow; and pressurized systems, including sprinkler, drip and micro-sprinkler. Irrigation efficiency differs with each system (Table 13.5). With surface systems, a faster movement of the water from the head end to the tail end of the field usually results in higher irrigation efficiency. Within pressurized systems, distribution depends on design parameters including spacing, nozzle type and size, riser height and operating pressure. Keep in mind that maintenance and filtration affect pressurized systems more than surface-based systems. Pressurized systems, however, are usually more efficient due to the reduction of both run-off and water loss through deep percolation. When calculating orchard water needs, irrigation efficiency must be taken into account or under-irrigation will occur. Table 13.6 includes some examples that show the different water application needs due to differences in irrigation efficiency.

Over the course of the crop year, 350 mm of water or more may be needed to fully irrigate a flood-irrigated orchard in comparison to a drip-irrigated orchard. If unaccounted for, lack of this water will lead to water stress and loss of potential yield.

The area of wetted soil within an orchard can also vary by irrigation system. Drip systems have a relatively small area of wetted soil (Fig. 13.1), which reduces the overall volume of soil receiving irrigation. Full coverage sprinklers, however, may apply water to the entire orchard. This fraction of the orchard floor must be taken into account when determining the amount of water that can be applied. As an example, an irrigation system that only wets 20% of the orchard floor can hold two-thirds of the water of a system that wets 30% of an orchard floor. Over-irrigating this soil volume will lead to deep percolation and leaching of fertilizer, reducing water use efficiency.

Water deficits occur when climatic water demands and the amount of water absorbed by

Table 13.5. Application efficiency (E_a) typical of various irrigation systems.

System	E_a (%)
Basin/flood	65–80
Furrow	65–75
Solid set sprinkler	75–85
Micro-sprinkler	85–90
Drip	90–95

the roots exceed the amount of water available in the soil. This is the condition when there are limitations of available water or during drought. Prolonged deficits reduce the reserves of water held in the soil, while an increase of climatic demand through evaporation can cause tree stress. Severe water stress can have both in-season and subsequent season effects. Impacts of drought stress are described below. Most of the material

Table 13.6. Example of differences among system efficiencies (location: San Joaquin Valley; soil: sandy loam; rooting depth: 1.5 m; available water-holding capacity (AWC): 10 cm/m; depletion of AWC before irrigation: 50%; irrigate when AWC reaches 8 cm).

Flood irrigation	Micro-sprinkler	Drip irrigation
$E_a = 0.70$	$E_a = 0.85$	$E_a = 0.90$
Weekly $ET_c = 125$ mm	Weekly $ET_c = 125$ mm	Weekly $ET_c = 125$ mm
Water required to meet $ET_c = 178$ mm	Water required to meet $ET_c = 156$ mm	Water required to meet $ET_c = 139$ mm
Difference from drip: + 39 mm	Difference from drip: + 17 mm	

**Fig. 13.1.** Double line drip systems often wet a limited area of the orchard. In this young orchard, the wetted area is around 20% of the orchard, as evident by the lateral movement of the water.

in sections 13.2–13.7 was sourced from Doll and Shackel (2015).

13.2 Impacts on Almond Growth and Yield

Almond trees are generally tolerant to drought conditions, but respond positively to increasing water availability with increasing yields. Research has shown that trees are able to survive on as little as 19 cm of water (Shackel *et al.*, 2011), but will maximally produce with 137–147 cm in most areas of California (Sanden, 2007). This response in growth and yield is due to increased rates of photosynthesis and respiration due to minimization of water stress, leading to higher productivity.

Both water and carbon dioxide are required by plants for photosynthesis. Water is provided through the root system of the tree, while stomata or ‘windows’ on the lower leaf surface are responsible for allowing carbon dioxide to come into the leaf and oxygen to leave. As this gas exchange occurs, substantial amounts of water are also lost through the stomata via transpiration. When water loss from transpiration exceeds the amount of easily accessible soil water, the plant exhibits stress responses, including the beginnings of stomatal closure. If water applications through either irrigation or rainfall are not adequate to alleviate this stress, stress levels will increase, resulting in additional stomatal closure, further reducing gas exchange and so rates of photosynthesis and the production of carbohydrates. This will limit the amount of energy available for the many plant processes, impacting vegetative growth, and fruit and kernel development (Doll and Shackel, 2015).

The level of stress will determine its effect on the tree. Low to moderate levels of plant stress often occur within orchards and may be beneficial. Research has shown that an application of moderate stress at the onset of hull-split will help reduce hull rot and synchronize hull-split. Mild to moderate stress levels, if monitored, are useful for irrigation scheduling, as plant stress levels indicate the current soil water status. Severe plant stress, however, should be avoided when possible as it suppresses plant growth. Responses to severe water stress depend on when the stress

is imposed. Impacts on vegetative growth, fruit and kernel development, and floral bud development are summarized below.

13.3 Impacts on Vegetative Growth

The period after leaf-out is a time of rapid vegetative growth. This growth of shoots and leaves is necessary to establish fruiting positions and carbohydrate reserves for future yields. Water use requirements for the tree start off low, but increase as leaves fully expand and the canopy develops. Typically, the cooler temperatures, shorter day length and higher relative humidity during this period mean trees require less water, and water demand may be met by the stored soil water from winter rains. In these cases, trees may grow relatively stress-free until full leaf expansion is achieved at around 4–5 weeks after bloom (Doll and Shackel, 2015).

Moderate to severe water stress applied long enough after full leaf expansion or at any point in the growing season will reduce vegetative growth. Research has shown that nut load is directly related to canopy growth and size (Prichard *et al.*, 1996; Lampinen *et al.*, 2007). Therefore, lack of canopy growth due to pre-harvest irrigation deficits leads to a reduction in fruiting spurs, reducing future yield potential. One year of reduced spur production will not necessarily lead to a dramatic decrease in next year’s yield, but the effect can be cumulative as the total number of fruiting spurs will decrease (Doll and Shackel, 2015). If the viable spur population is already limited due to a previous year’s droughts or other issues, future yields will drop. This phenomenon has been observed in trials in Spain and California, where fruit loads were unaffected by applied water stress in the first 2 years of a trial, but were reduced in the final 2 years of a 4-year study.

13.4 Impacts on In-season Kernel Development

Fruit and kernel development follow a three-stage process (Kester *et al.*, 1996) (Fig. 13.2).

During stage I, rapid growth of the hull, shell and integuments occurs. The kernel begins to

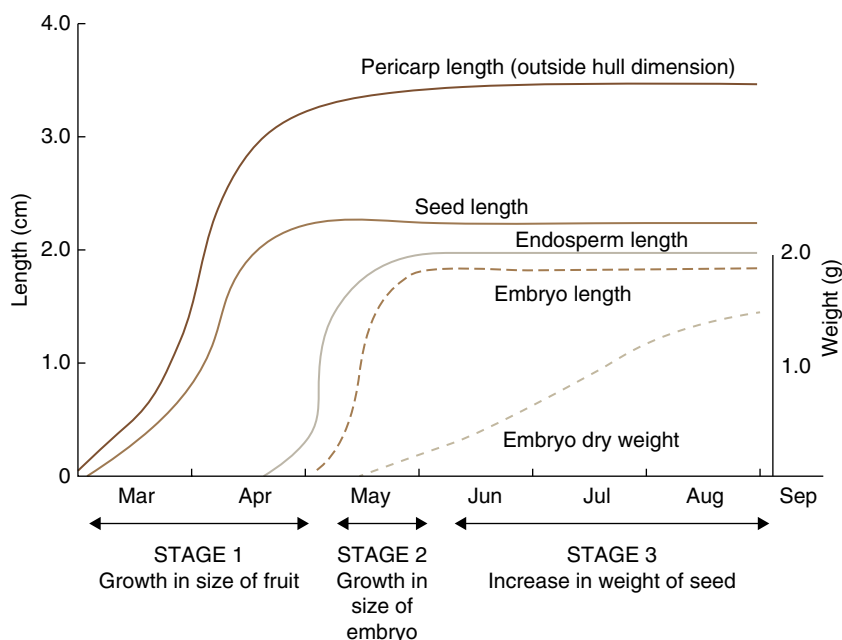


Fig. 13.2. The three stages of almond fruit development and the typical length and weight of the fruit at each stage. (From Kester *et al.*, 1996, with permission from the University of California Agriculture and Natural Resources.)

form as a white structure within a translucent jelly. Stage I ends once the maximum external dimensions of the hull, shell and kernel have been reached, which is about 2 months after bloom. Severe tree water stress rarely occurs during this stage of fruit growth (petal fall through late April/May) due to stored soil moisture and cooler temperatures but, if it does, it is thought that smaller fruit and kernel size will be observed because of reductions in cell division and subsequent expansion (Doll and Shackel, 2015).

Stage II is a period of rapid development following fruit expansion. The hull, shell and kernel have reached the maximum external size about 2 months after pollination, and this is followed by hardening of the shell and kernel expansion with a corresponding increase in the dry weight of the kernel. This period (late May or early June in California) has a high seasonal irrigation demand and is a time when almonds are very sensitive to water stress.

The final period of fruit growth is the pre-harvest period, or stage III. At this point, hull, shell and kernel differentiation are complete and the kernel begins to accumulate weight at a continuous rate until harvest (maturity). Harvest is

signalled by two events: (i) the onset of hull-split; and (ii) the formation of an abscission layer between the peduncle and nut. Both of these events are impacted by irrigation practices (Doll and Shackel, 2015). Too much water will increase the duration of the hull-split period and thus delay the onset of harvest. In contrast, too little water could decrease kernel weight, increase the number of dried hulls adhering tightly to the shell (i.e. hull-tights), or poor nut removal due to a hull being too dry (i.e. stick-tights). Determining the timing and potential impact of water stress during stage III of kernel development is complicated. A severe stress imposed during kernel fill through harvest will reduce kernel dry weights and produce textured or shrivelled almond kernels. This kernel weight loss during this stage is due to reduced carbohydrate production as well as a redirection of carbohydrate away from the kernel due to early hull-split. If a moderate, regulated stress level (-14 to 18 bars stem water potential (SWP)) is imposed from kernel fill through hull-split, however, the impacts on kernel weight and size are minimized. In a 4-year study, this regulated deficit did not impact kernel yields over the duration of the experiment,

but did reduce kernel weight by 2–3% compared to the full irrigation treatment (Stewart *et al.*, 2011). However, this reduction of applied water saved 10–15% in the total season's water budget.

It is critical to maintain proper irrigation through the post-hull-split/pre-harvest period because too great a water stress level will reduce kernel weight as well as quality. A study conducted in Kern County, California, determined that there is an indirect relationship between the length of pre-harvest irrigation cut-off and kernel quality (Goldhamer and Viveros, 2000). In this study, pre-harvest irrigation cut-offs occurred from 8 to 57 days prior to shaking. Kernel quality was found to be negatively impacted when pre-harvest cut-offs extended beyond 15 days. This cut-off period, however, will vary among soil types and irrigation management strategies. Monitoring should be done on an orchard by orchard basis to minimize the stress applied during the pre-harvest timing (post hull-split to harvest) to prevent negative impacts on kernel moisture content, weight and quality.

13.5 Impacts on Development of Fruit Buds

Severe stress from the lack of post-harvest irrigation has been found to decrease next year's crop yield more than pre-harvest water deficits did. Studies have shown that very severe post-harvest stress (–40 bars pre-dawn leaf water potential) caused a 52% reduction in 'Nonpareil' bloom density and a 94% reduction in fruit set, resulting in a 73.6% reduction in the following year's yield (Goldhamer and Viveros, 2000). Furthermore, yield loss was observed in all treatments across this trial that withheld post-harvest irrigations, regardless of the pre-harvest cut-off. This reduction was attributed to stress impacts on the late season floral bud development. In contrast, a study near Manteca, California, was successful in reducing post-harvest irrigations without any negative impact on crops (Prichard *et al.*, 1996). In this study, water was applied based on tree stress; and, due to low observed tree stress levels, water applications were withheld. This reduction was thought to be possible because of adequate

soil water reserves. Thus, tree stress levels should be monitored into the post-harvest season to determine the need for post-harvest irrigation.

Timing of bud development varies by cultivar and geographic region, and can occur before or after harvest for late and early harvested cultivars, respectively (Lamp *et al.*, 2001). Since multiple cultivars are typically planted within an orchard, it is important to minimize stress during the period between hull-split and harvest to maximize next year's production. If possible, water demands should be met to the end of September, and application amounts will vary depending on water reserves within the soil. Care should be taken during this period when using low-volume irrigation systems because the lower soil reserves of water mean that water deficits can develop rapidly.

13.6 Managing Orchard Irrigation in Water-limited Environments

Water deficits occur when the tree's water demand exceeds the amount of water available within the soil. These deficits increase water tension within the plant, and when this stress is high enough there will be a negative effect on many plant processes. As described above, almond trees have a varying tolerance of stress throughout the season. Ideally, a grower would achieve the most efficient use of irrigation water when they only irrigate when tolerance to water stress is low enough to cause a significant reduction in yield. The method of applying water during critical almond development periods, as well as limiting water application during less critical periods, will be referred to as strategic deficit irrigation (SDI) (Doll and Shackel, 2015). An explanation of the components of monitoring plant stress and imposing SDI follows.

Plant water stress is evaluated by measuring midday SWP using a pressure chamber or equivalent device. SWP is a direct measure of water tension within the plant, and is given in metric units of pressure, such as bars (one bar is about one atmosphere of pressure) or megapascals (MPa; 1 MPa is 10 bars). Even under fully irrigated conditions, SWP in almond trees at midday can be substantial (1 MPa) simply because this tension is required to pull water out of the soil

and through the tree (for reference, a value of 1 MPa corresponds to about 145 psi). Technically, SWP should always be shown as a negative value (e.g. -1 MPa), but in conversation it is common to omit mentioning the 'negative' before the value. More information about the operation of the pressure chamber can be found in Fulton *et al.* (2014).

Through the use of a pressure chamber, plant stress can readily be monitored. Water applications can be made once specified levels of stress are reached, reducing stress extremes and damage to the current crop and future yields. Further, the practice of SDI may be used to extend watering intervals and save water. Alternatively, if the pressure chamber is not available or if the anticipated water deficit is greater than 15% of the seasonal ET_c , then reduced water applications can be made by applying a fixed proportion of ET_c . In this method, the amount of water available for the season should be calculated as a percentage of full ET_c . This percentage should be applied to spread the deficit evenly across the season. In other words, if 55% of ET_c is determined to be available for the whole season, then each irrigation would match 55% of the determined ET_c for that irrigation period. An example is given in Table 13.2. Current season and future yield loss should be expected when using this strategy, but research has shown this to be the most effective strategy in minimizing yield losses (Goldhamer *et al.*, 2006). Imposing whole-season SDI or applying water as a percentage of ET_c will preserve kernel quality and future yields even though the current season's yield will drop. Stress imposed during stage I will reduce fruit load and size. This will lead to a reduced amount of photosynthate required to fill the remaining nuts during stage II, leading to more complete kernel fill and higher quality. This contrasts with the erroneous strategy of fully irrigating the almonds through stage I and deficit irrigating in stage II, which results in increased fruit set, but only partial kernel fill and shrivelling across a greater proportion of nuts. Both strategies will lead to similar field kernel yields/acre, but quality and marketability – and thus kernel price – will be reduced in the latter strategy. Future yield reduction will be dependent upon the severity of stress applied during the post-harvest period and the impact on in-season vegetative growth.

13.7 Additional Factors and Considerations of Water Management

13.7.1 Effects of crop load

Research in peaches suggests that crop removal has little, if any, impact on water use efficiency (Goldhamer and Holtz, 2009). Although there has not yet been equivalent research done on almonds, crop water use is thought to be similar to peaches. Speculation by almond researchers in California and Spain suggests that the crop can account for up to 10% of water usage. In periods of severe drought, however, artificially reducing crop load to save water is not recommended due to the redirection of resources into vegetative growth, which could require more water. Crop load will thin naturally, especially if stress is applied prior to the nut drop period experienced in May.

13.7.2 Negative effects of canopy reduction

Severe pruning, stumping, or 'dehorning' is not recommended as it will increase new growth, potentially leading to higher transpiration rates. Furthermore, a study on severe drought has found that pruning trees often removes more wood than would be killed by severe water stress. For instance, in a 1-year drought experiment that withheld all irrigation water from drip-irrigated almond trees on a shallow soil, the most severely stressed trees reached a SWP of -5 MPa (-50 bars) in July and only showed about 15% canopy dieback after 2 years. This is in contrast to test trees in which scaffolds were cut back to reduce the canopy by 50% during the drought treatment (Shackel *et al.*, 2011).

13.7.3 Increasing soil salinity from reduced water quantity or quality

Reduction of water applications or reliance on low-quality groundwater may increase soil salinity, leading to a reduction in yield (Hanson *et al.*, 1993; Grattan, 2002). Soil salinity should be monitored through annual sampling, especially

when using low-quality water, or deficit irrigation, or when winter rainfall is low. Under drought conditions, a leaching programme is best implemented in the dormant period to reduce root-zone salinity levels. Leaching during the dormant season will be more efficient when ET_c is lower, and there is also less potential to leach nitrate and other nutrients from the root zone, since they will have been applied and taken up by the trees relatively early in the growing season. Leaching salinity with additional water during the growing season is difficult due to the higher water demand of the trees and, in many cases, slow infiltration rates of orchard soils. Consequently, fertilizers or soil amendments containing chlorides should be minimized in periods of drought.

13.7.4 Cultivar influences on water requirements

Although different cultivars may exhibit differing stress levels when growing in the same soil water condition (e.g. 'Monterey' begins to wilt at -18 SWP while 'Nonpareil' begins to wilt at -22 SWP), research has shown that the thresholds and types of impacts of water stress on physiological processes are similar among cultivars (Shackel and Doll, 2012). These findings indicate that the differences in the field are not due to a cultivar being more sensitive to stress but rather that in-field differences among cultivars are due to different growth habits that may influence water uptake (i.e. root system depth, lateral length or architecture), increase water consumption (canopy architecture, or leaf count) or both. Hence, different cultivars will reach thresholds for implementing SDI at different rates and times. This highlights the importance of properly delivering water to all of the trees within the orchard to prevent the onset of severe water stress. Other factors that impact the effect of water stress on cultivars include the timing of maturation and harvest. Later-harvested cultivars will undergo floral bud differentiation prior to harvest. Thus, pre-harvest cut-offs for these cultivars may negatively impact fruiting bud differentiation (Lamp *et al.*, 2001). Timing to induce SDI, as well as the time required for harvest 'dry-down' (depletion of stored soil moisture) also

varies among cultivars and soil types. In an ideal setup this would require an irrigation system that could irrigate cultivars differently. Therefore, early and late harvesting cultivars will require more water to be applied in the post-harvest and pre-harvest period, respectively. Some researchers, however, believe these differences in practice are minor because water use is more directly dictated by canopy coverage. Thus trees should be fully irrigated if full water allocation is available prior to harvest and through post-harvest.

13.7.5 Micro-irrigation systems and timing of water applications

Micro-irrigation systems can maximize distribution of water by minimizing losses to evaporation. These systems can apply smaller amounts of water and irrigate more frequently than flood or furrow irrigated fields. Systems should be fully maintained to ensure uniformity of water distribution, and water application rates should not exceed soil intake rates (Schwankl *et al.*, 2007). Reduced applications of water are usually not possible with flood or furrow irrigation because these irrigation methods require a minimal amount to advance the water across the field. The only option with these systems is to wait longer between irrigations.

13.7.6 Reduction of water losses through evaporation

The run time for micro-sprinkler systems may need to be shortened to reduce water applications (Doll and Shackel, 2015). If the run time is too short, however, water will not infiltrate deep enough into the soil and this leads to a higher percentage of water being lost to evaporation. Therefore, run times should be no less than 6 h, with longer run times having reduced losses to evaporation. Run times, however, should not exceed soil intake rates or the water-holding capacity of the soil. Minimum run time is not as much of an issue with drip systems due to a reduced wetted area on the soil surface (L. Schwankl, Merced, California, 15 December 2014; interview with D. Doll). Timing of irrigation should also take into account the higher evaporative losses due

to increasing temperatures and wind speeds. Therefore, the evaporative losses will be greater during the day, and irrigation sets ideally should be started in the evening and completed before late morning.

13.7.7 Removal of the cover crop

Vegetation cover of the orchard floor should either be removed or managed carefully to eliminate water loss through cover crop transpiration. Depending upon the coverage, cover crops or residual vegetation can increase orchard water use by as much as 30% (Prichard *et al.*, 1989). In water-short years, the cover crop should be removed prior to leaf-out, but no earlier than mid-January. This will allow the cover crop to reduce run-off from any winter rains, but eliminates any water use after February.

13.7.8 Minimal impacts of anti-transpirants

Research within the University of California has not been able to document water savings or reduction of plant stress with the application of anti-transpirants or 'plant coolants', and so they are not recommended (Shackel *et al.*, 2011). Many new products enter the market annually, however, and there is always the possibility that some may prove to be of benefit. If applying these products, it is important to leave several untreated areas in the field to determine the product's actual effectiveness.

13.7.9 Reduction in nitrogen applications

Nitrogen applications should be reduced during periods of drought (Doll and Shackel, 2015). The reduction in applied nitrogen rate should be proportional to the expected reduction in yield from deficit irrigation. Nitrogen rates in the spring should be reduced to prevent growth, as excessive vegetative growth will increase tree water demand. Most data suggest that long-term yield reductions generally follow a 1:1 relationship with long-term water reductions, meaning that a 30% reduction in relative applied water will lead to a 30% reduction in relative yield.

13.7.10 Insect management

Periods of drought will influence insect pest populations. Mites will rapidly increase on stressed trees (Youngman and Barnes, 1986), and so an increase in miticide applications may be needed. Stressors include water stress and dust, both which are common in periods of low rainfall. Navel orangeworm (NOW) populations are impacted by drought as well (D. Goldhamer, Merced, California, 17 December 2014: interview with D. Doll). Reduced winter rains can make it difficult to remove mummies with winter shaking, leading to an increase in over-wintering populations (Doll and Shackel, 2015). Warmer temperatures common during drought years will lead to faster insect development. Furthermore, hull-split is generally accelerated in drought years, which will change the timing for application of a hull-split NOW insecticide spray.

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14 Almond Tree Nutrition

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14.1 Introduction

Almond requires 17 elements for growth and development and, if any of these elements are deficient, growth and production are impaired. These 17 elements are called essential elements and include carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), boron (B), zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), chloride (Cl), molybdenum (Mo) and nickel (Ni). C, H and O come from air and water while the other elements are either present in the soil or provided as synthetic fertilizers. N, P and K are required in large quantities and are called primary macro-elements, while S, Ca and Mg are required in smaller quantities and are called secondary macro-elements. B, Zn, Cu, Fe, Mn, Cl, Mo and Ni are required in much smaller amounts and are called micro-nutrients. Irrespective of the amount required, a deficiency of any of these elements will impair growth and production of the almond tree. The concentrations of elements that correspond with deficiency, adequacy and toxicity are provided below.

Nutrient availability to plants depends on the amount and solubility of the nutrient present in the soil. Some nutrients are mobile in the soil when soils are moist and move to the plant root with water; this is termed 'mass flow' and

includes nutrients such as nitrate nitrogen, Mg, S, B, Ca and K in some soils. Others, including Fe, Zn, P, Cu and Mn are immobile in the soil and roots must grow in very close proximity to the nutrients to obtain these elements. For certain elements soil pH strongly influences nutrient solubility, and strongly acidic or alkaline soil pH can result in nutrient deficiencies or toxicities. In almond in California, soils with neutral to alkaline pH values commonly limit the availability of Zn, Mn, Cu and Fe, and may result in deficiencies (Fig. 14.1).

Nutrient availability is also influenced by soil mineralogy and origin, and by historic management practices. Given the wide diversity of soil types and the complexities of root distribution and soil nutrient uptake it is not possible to generalize about the potential for a nutrient deficiency to occur in any particular orchard soil. Soil testing can provide important information about the potential for a deficiency to occur, while plant tissue testing provides insight into the availability of soil nutrients under the growing conditions currently in practice at that site.

Nutrient availability from soils is also dependent upon root health and root distribution in the soil profile. Any soil property or environmental condition that limits plant root growth such as soil compaction, waterlogging or presence of toxic ions (including Na and Cl) can also

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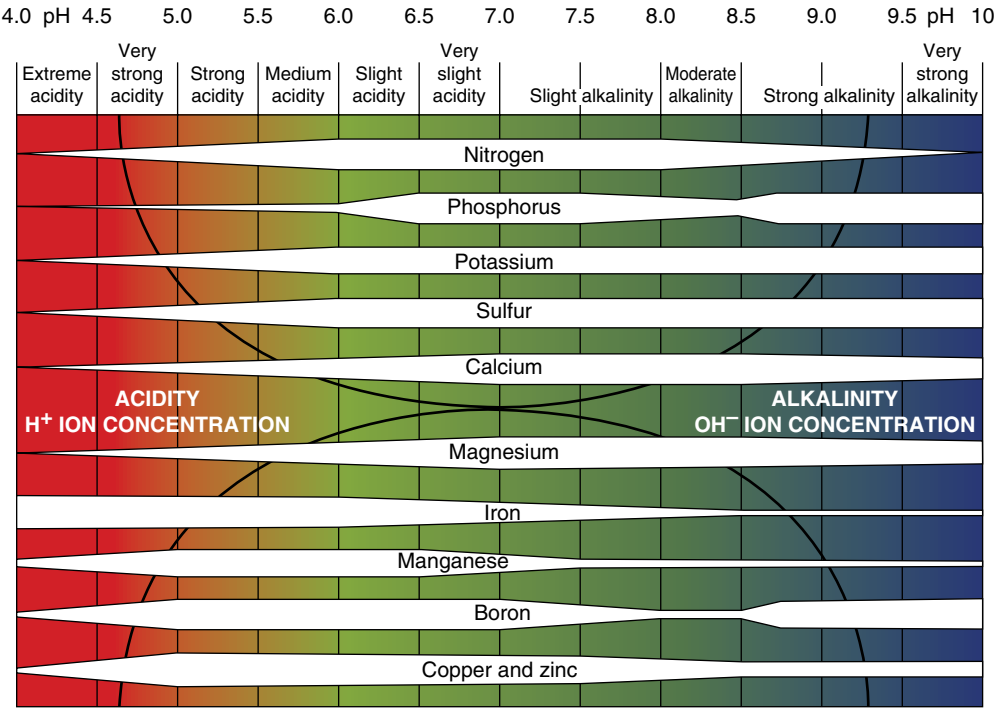


Fig. 14.1. Influence of soil pH on the availability of nutrient elements. The width of the white shaded areas indicates the relative nutrient availability to the plant root at a given pH value.

reduce the availability of essential nutrients by restricting root growth.

Once a nutrient has been taken up by the plant it is distributed to growing shoots, roots and fruits. Some nutrients remain mobile within the plant while others become immobile and cannot be redistributed. Plant immobile elements (Fe, Mn, Cu and Ca) can only move with water through the transpiration stream (xylem), while mobile elements (N, P, K, S, Cl and B) can move through the xylem and the phloem. Zn is intermediate in its mobility, with a small fraction of tissue Zn being mobile in the spring and autumn while the majority is immobile. Immobile elements accumulate at the sites of greatest transpiration and once deposited in a plant organ cannot be re-translocated to other parts of the plant. Immobile elements must therefore be continually supplied to the plant from the soil or by foliar fertilizers to avoid deficiencies. Deficiencies of the immobile elements always occur in the youngest parts of the tree, while toxicities are expressed in the oldest leaves.

Mobile elements (N, P, K, S, B, Mg and Cl), in contrast, can move freely within the plant and when present in mature leaves and tree organs can be remobilized to supply young growing organs as needed. This remobilization will continue until the nutrient is depleted from mature leaves. As a consequence of this mobility, deficiencies in these elements are expressed in the oldest leaves first.

Management of nutrients requires consideration of mobility in the soil and the plant, and knowledge of total plant demand and when during the season that demand is greatest.

14.2 Diagnostic Methods

Almond nutrient management should focus on ensuring optimal productivity while minimizing the biological, environmental and financial costs from the overuse of fertilizers. To supply and maintain a balanced concentration of essential nutrients in plants, prediction of nutrient

availability from the soil, and monitoring the nutrient status of the plants during the season, is necessary.

Different methods are used to predict and monitor adequacy, deficiency and toxicity of mineral nutrients in plants. This section will discuss three methods of monitoring the nutrient supply and diagnosing nutrition problems: (i) soil analysis; (ii) leaf analysis; and (iii) visual inspection (symptoms).

14.2.1 Soil analysis

The goal of soil analysis is to predict plant nutrient and toxic ion availability in the soil and to provide a description of the soil physical and chemical properties that may contribute to deficiency or toxicity. Soil chemical analysis provides information about soil conditions such as pH, cation exchange capacity (CEC), sodium (Na) adsorption ratio (SAR) and salinity, which can be used both to predict likely deficiencies and to determine the likely responsiveness of trees to particular fertilizer regimes. Soil physical analysis will identify constraining soil layers and can be used to plan soil preparation strategies and irrigation practice. Soil analysis performed prior to orchard establishment is a valuable tool for site assessment and identifies potential problems that may require correction. Ideally a soil analysis will help determine if a deficiency is the result of an absolute lack of an element or the result of unfavourable soil conditions, which either limit the solubility of nutrients or impede root growth and function. In established orchards, soil analysis can be useful in monitoring the efficacy of fertilizer treatments and soil amendments. It can indicate if salts such as Na, Cl or B are accumulating, or if there are imbalances in Ca, Mg and Na, which may influence water infiltration.

The purpose of a soil analysis is to predict how almond will respond to a given soil and to develop strategies to correct soil conditions that may negatively influence the plant growth. Given the depth and complexity of the almond root system and the difficulty in knowing how almond will respond to a particular soil, soil analysis is most valuable when conducted in combination with a visual symptom assessment of the tree and plant tissue analysis.

Sampling procedures

In general, soil samples can be taken at any time since the availability of most soil elements does not change seasonally. An exception to this is the levels of N, B, Cl and Na, which are more variable and will be influenced by nutrient applications, rainfall and irrigation conditions. The goal of any soil analysis is to provide an estimate of the nutrients that would be available for uptake by an almond tree and, as such, samples should be collected from the areas of active root growth. In regions with little in-season rainfall the area of active root is largely determined by the distribution of irrigation water. While almond roots can reach depths of greater than 1.8 m the vast majority of root activity occurs in the root zone wetted by the irrigation system. In most micro-irrigated systems roots are restricted to the top 0.08–0.5 m of soil for most of the year (P. Brown and A. Olivos, unpublished data). In flood-irrigated orchards roots occupy the entire wetted area, while in a drip-irrigated orchard roots are restricted to a smaller volume of soil. Sampling varies, therefore, between orchards irrigated with different irrigation systems. Compared to light-textured soils, roots in a clay soil are more concentrated near the surface, and sampling to a depth of 0.46 m can provide enough information on availability of nutrients. To determine the concentrations of toxic elements deeper sampling up to 1.2 m may be necessary, particularly if roots occasionally access water at that depth.

Taking representative soil samples in an orchard is a great challenge given the high spatial variability in soil type across an orchard, which may vary from point to point and also with depth. Nutrient deficiencies in a section of an orchard can result from the presence of an old riverbed, corral or pasture; from differences in topography; or from cuts or fills produced during field levelling. Depending upon the extent and degree of soil variability it may be necessary to conduct soil sampling and analyses of each soil type, and to differentially manage each zone. In orchards that are irrigated and fertigated from a single source with no capacity for within-orchard zone management, the presence of soil variability will compromise the efficiency of nutrient management. This occurs since it is not possible to satisfy the demands of trees in all soil

zones without simultaneously over- or under-fertilizing trees in different soil zones. For the management of N this can be particularly important since providing sufficient N to satisfy the most demanding trees will inevitably over-fertilize the remainder of the orchard. If variability in soils and productivity is significant then the installation of multiple, individually regulated zone-irrigation systems or targeted surface fertilization may be required.

As a general rule, soil samples should be collected independently from each major soil type in the orchard. At each site, soil samples should be collected from the area in which roots are active, which is determined by the availability of moisture in soil. In flood irrigation where water is applied uniformly to the entire field, soils can be sampled from anywhere under the tree canopy. Under drip and sprinkler irrigation the wetted zone is smaller and samples should be restricted to this zone. Three to ten samples can be collected from the wetted root zone (usually 0–0.5 m) at different positions around a single tree or trees growing in similar soils. Samples can be composited if the soil at that depth is uniform. In a flood-irrigated orchard, soil samples can be collected 1.5–1.8 m from the trunk of the tree, while in micro-sprinkler and drip-irrigated orchards, soil samples should be collected from the wetted area (avoiding the edges). Orchards under drip irrigation require sampling approximately halfway between the emitter source and the edge of the wetted area.

If salinity is an issue samples should be collected from both the middle of the wetted zone and at the margins, and analysed separately. Salt tends to accumulate at the margins of the wetted zone under micro-irrigation and the finding of high salinity can be indicative of a potential issue if rainfall or irrigation has the occasion to move those salts back into the active root zone.

Laboratory analysis

Samples should be submitted to a laboratory for analysis as soon as possible to avoid loss of volatile N compounds. The purpose of laboratory tests is to predict the fraction of soil nutrients that the almond tree may have access to; this is termed 'plant-available' nutrients in soil. Various chemical extraction methods have been developed, including extraction with strong or weak acids, chelating compounds (such as

diethylenetriaminepentaacetic acid (DTPA)) or organic extractants. Ideally these methods have been calibrated for almonds growing in similar regions. In areas where almond is not widely grown, care must be taken to ensure that the test recommended by the analytical service is appropriate for almond. For more information about nutrient extraction, consult the excellent publication by Page *et al.* (1982).

Interpreting the results

The analysis of physical and chemical soil properties is an important step to predict performance of almond in the given soil conditions and to guide fertilization and soil amendment measures. For example, a heavy clay soil may restrict root growth or water movement in the soil, which can be amended by improving soil structure through the addition of manures and organic matter. Similarly, a high-pH soil may reduce the availability of Fe, Mn and Cu, and steps can be taken to manage soil pH. Soil analysis can be of considerable help in identifying toxicities caused by excess of Na, Cl and B. The value of a soil analysis to guide fertilization practices is, however, limited by the difficulty in predicting the relationship between soil chemical analysis and plant nutrient uptake, and the difficulty in obtaining a representative soil sample in an orchard system. Frequently, the expected response of almond to a given soil is best predicted from experience with growing almond in similar soils and environments, and from determination of gross soil chemical properties such as pH, saturation percentage, CEC and salinity.

Soil pH

Soil pH is a measure of hydrogen ion concentration in the soil. It is expressed on a log scale and ranges from 0 to 14 with 0–4 being strongly acidic, 9–14 being strongly basic or alkaline and pH 6.5–7.5 being ideal for almond production. Excellent almond production can be achieved in native soil pH ranges outside of this ideal range (5.5–6.5 and 7.5–8.5); however, at these more limiting soil pH values greater levels of management intensity will be required to avoid the nutrient imbalances that occur in these soil pH ranges. Soil pH strongly affects nutrient availability for root uptake and can be used to predict likely

deficiencies and toxicities. Most nutrients are ideally available for almond at pH range 6.5–7.5, while nutrient availability is affected at higher or lower pH values. If the soil pH is > 7.5, nutrients such as Zn, Mn, Cu and Fe form insoluble complexes, become less available for plant uptake and deficiencies in these elements become more likely. The availability of P, Mg and Ca may also become compromised at pH values below 6. At very low pH (< 4.5) the availability of some nutrients such as Al, Fe and Mn becomes excessive and toxicity may occur.

Soil salinity

The amount of salts in a soil is determined by measuring the conductivity of the saturation extract (ECe). For almond the conductivity value at which yields begin to decline in sensitive rootstocks ('Lovell' and 'Nemaguard'), is about 2.5 deciSiemens per metre (ds/m), while for more tolerant rootstocks such as 'Empyrean 1' and 'Viking' this threshold may be significantly higher. Scion cultivars also differ in their salt tolerance, with 'Nonpareil' being significantly more tolerant of Na than either 'Fritz' or 'Mission' (B. Kutman and P. Brown, unpublished data). Exchangeable Na of greater than 15% will cause structural deterioration, soil compaction and surface scaling of soils, and can directly affect tree growth by limiting water penetration and soil O availability.

Na, Cl and B are the primary salts present in many almond-growing areas and affect plant growth and performance. These salts may be present in the soil or added to it from low-quality (high EC/B) irrigation water. Irrigation water is the most significant source of B in many almond-production regions, and long-term irrigation with water exceeding one part per million (ppm) B can result in the accumulation of B in almond tissue and the development of toxicity symptoms. B is a cumulative toxicant and symptoms will become increasingly worse as the duration of exposure to high-B irrigation water increases. The choice of rootstock can significantly impact the uptake of B from the soil. Peach rootstocks tend to uptake more B resulting in greater tissue B concentrations than in almond or peach almond hybrids. In regions with chronically high soil and water B levels the choice of the correct rootstock is essential for

long-term viability. B tends to accumulate in fruit tissue, and hull is a useful and sensitive indicator of B toxicity.

Both Na and Cl are toxic to almond trees and produce similar leaf symptoms including marginal chlorosis and ultimately necrosis. When Na is the predominant toxic ion, almond may also exhibit symptoms of water stress with flaccid, curled leaves, particularly in young trees and shoots. Some almond cultivars ('Nonpareil') accumulate Na in the perennial trunk tissues and thus limit Na transfer to the leaves. No similar effect is observed with Cl, which accumulates in the leaves in direct proportion to root uptake.

Salts accumulate over time as the trees transpire water and it is ultimately the accumulation of these elements in the plant and in the root zone that will damage productivity. Salt damage in almonds is therefore largely a consequence of the quality of the irrigation water and the duration of exposure to the poor-quality water. Availability of water of better quality can diminish and ultimately reverse salinity damage. Soil amendments with gypsum or organic matter can also improve plant performance under saline conditions by improving soil aeration and root growth, which will improve plant tolerance. If salts accumulate in the soil then a leaching treatment must be applied to the soil. Care should always be taken to minimize the application of leaching treatments when there is significant residual N in the profile. Leaching can be done in-season by adding a leaching fraction or in the dormant season with irrigation water and rainfall.

14.2.2 Plant tissue analysis

While soil analysis can be useful in predicting if a given nutrient may be problematic, plant tissue analysis is a measure of the current status of nutrients in a plant and hence is a more accurate indicator of nutrient availability. Because of uncertainty of soil sampling and lack of robust soil extraction protocols, tissue analysis is often more useful in diagnosing mineral deficiencies and toxicities than is soil analysis. The mineral composition of a plant tissue reflects many factors including stage of plant development, climatic conditions, availability of mineral elements in the soil, root distribution and activity,

and irrigation. The tree integrates all these factors and the composition of the plant tissue reflects that integration.

Plant tissue analysis is conducted by collecting defined tissues (typically leaves, though hulls are used for B analysis) at a specific time of year using established protocols, determining the elemental concentration of nutrients and toxic elements in that sample and then contrasting the resulting value with established standards. Tables of expected values for most nutrients and common toxic elements have been established for almond leaves and hulls (see ‘Critical values’, below). While this approach is effective in determining the nutrient status of a tree, to fully understand the causes of any reported deficiency or toxicity often requires additional information from soil and water analyses, fertilization practices and orchard productivity.

Critical values

Critical value (CV) curves have been developed for many crops and are widely used to define the optimum tissue concentrations for a given crop and element. The concentration of nutrients within plant tissues varies depending upon nutrient supply, the growth rate of the crop and phenological stage of plant growth. Correct sampling protocols are therefore essential to properly

interpret information from plant tissue analysis. The concentrations of nutrients in the sampled tissue are then compared with established CVs that have been developed by comparing plant yield (or leaf symptoms) with tissue nutrient concentration under controlled experimental conditions. The nutrient concentration in tissues at which yield (or growth) is 90–95% of maximum is termed the CV or critical level (Ulrich, 1952). The curve is composed of four zones (Fig. 14.2):

- The deficient zone, in which leaf symptoms are clearly evident and an increase of nutrient concentration in the tissue results in an increase in yield and a diminishment of symptoms.
- The transition or ‘marginal’ zone, in which deficiency symptoms may or may not be present but yield is compromised.
- The adequate zone, in which growth is maximal and no symptoms are evident and where an increase in nutrient concentration has no effect on relative yield.
- The toxic zone, in which additions to the nutrient supply result in yield loss due to toxicity.

Ideally, CVs are established under carefully controlled experimental conditions in which the relationship between yield and nutrient concentration is determined as nutrient supply is varied.

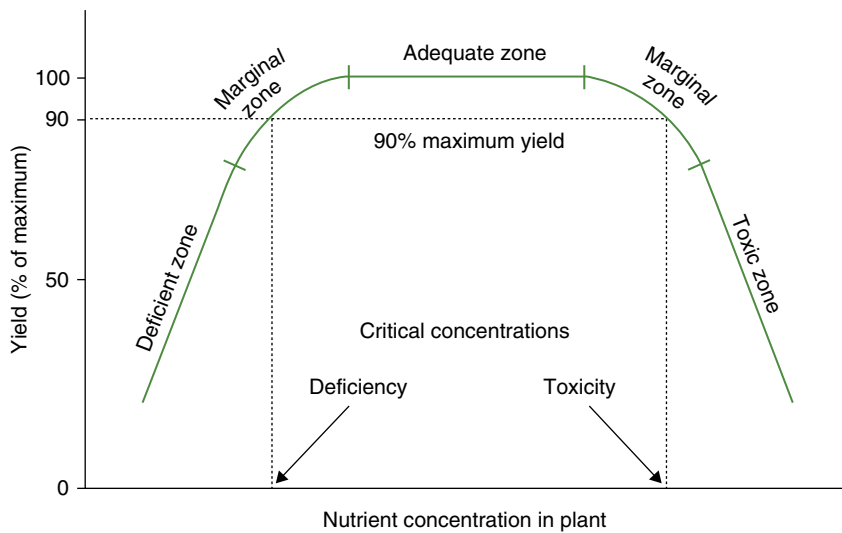


Fig. 14.2. Derivation of critical concentrations for diagnosing nutrient deficiency and toxicity in plants. (From Smith and Loneragan, 1997.)

In almond, yield-based CVs are only available for N, K and B and all other CVs are based on the expression of visual symptoms and not from yield-response experiments. CVs for almond, whether yield or symptom based, have only been established for samples collected during mid-late summer as the kernel reaches full maturity and the first fruit have commenced hull-split, and until the majority of fruits have reached 30% hull-split. In California this is typically in July to early August. With the exception of the early-season N analysis described in the previous section, CVs have not been established for samples collected at different times of plant development. The CVs for almond tissues at a mid-summer growth stage are presented in [Table 14.1](#).

Sampling procedure and seasonal changes in nutrient concentrations

While almost any plant organ can be sampled for nutrient concentration, guidelines for interpretation of tissue analysis only exist for leaf samples or hull samples (for B only). Concentrations of nutrients in leaves vary with season, leaf age, position in canopy and the presence or absence of fruit or sun exposure. This variability makes it essential to follow a standardized sampling so that results can be contrasted with established standards. Leaf tissue nutrient concentrations follow discrete patterns of change over time, with N, P and Cu concentrations declining over the season, while Ca and Mg increase ([Fig. 14.3](#)).

The patterns of change in these elements have been used to develop the early-season sampling protocols for N that are described below.

Field sampling strategies

The field sampling method can vary depending upon the purpose of the sampling. If its aim is to identify the cause of poor tree performance in an isolated tree or area of the orchard, then collecting samples from poor trees and comparing them with a sample from healthy trees should suffice in helping to identify the specific nutrient deficiency or toxicity present. Isolated areas of poor performance are frequently a consequence of differences in soil type, the presence of historic soil disturbances (cuts and fills during leveling, differences in prior farming operations at this location) or topographical changes that result in differential water distribution or drainage. To identify the cause of poor performance in a sub-region of an orchard it is generally worthwhile to collect both soil and plant samples and to note any specific differences in topography and current or historic management at that location. This will aid in interpretation of results and determining the remedial action required.

If the objective of a field sampling is to determine the nutrient status of an orchard so that fertilizers can be scheduled, then the following sampling strategy should be used.

Currently, only two periods of leaf sample collection and analysis have been validated in

Table 14.1. Critical nutrient levels (dry-weight basis) in almond sampled in July.

Nutrient	Unit	Critical value	Critical range	Toxicity threshold
Nitrogen	%	2.3	2.3–2.5	
Phosphorus	%		0.1–0.3	
Potassium	%	1.4	1.4–2.0	
Sulfur	ppm		1100–1400	
Calcium	%	2.0	2.0–5.0	
Magnesium	%	0.25	0.25–0.8	
Zinc	ppm	20	20–80	
Manganese	ppm	20	20–100	
Boron	ppm	80	80–160	> 300
(in hulls ^a)				
Copper	ppm	4.5	4.5–5.5	
Sodium	%			> 0.25
Chloride	%			> 0.3

^aBoron concentration in mature hull. Leaf analysis is not effective in determining boron deficiency, sufficiency and toxicity; hull boron analysis has been found to be a better indicator of tree boron status. Blank cell = value not known for almonds

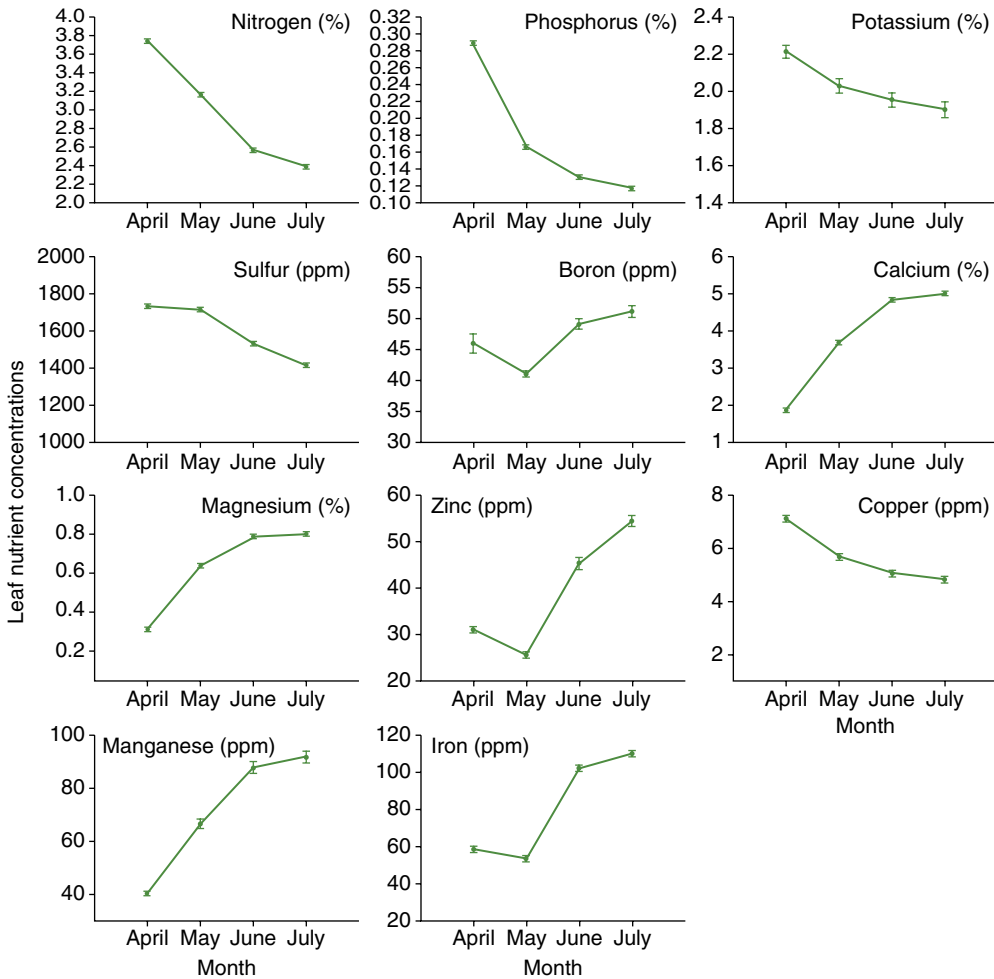


Fig. 14.3. Seasonal changes in nutrient concentrations of leaves collected from non-fruiting mature almond spurs. The trees were fertilized with 310 kg/ha nitrogen and 225 kg/ha potassium.

California for almond: (i) a summer sample collected in mid-July to early August; or (ii) an early spring sample collected towards the completion of spur leaf expansion (which is validated for N prediction only) (Saa *et al.*, 2014). To ensure that the collected sample adequately reflects the nutrient status of the management zone the following protocol must be followed for both ‘early spring’ and ‘summer’ samples. Leaves from five to eight well-exposed non-fruiting spurs are sampled from around the canopy of each of 15–30 trees, each tree being a minimum of 27 m apart. All leaves can be combined as a single sample for submission to a reputable laboratory using the protocol developed by UC Davis. If there are

marked differences in tree behaviour between zones in a single orchard, then ideally this protocol should be repeated in each zone and fertilizer application adjusted according to the needs of each zone.

‘Summer’ samples can be collected at any time from mid-July to mid-August, corresponding with full fruit maturity to full hull-split. ‘Early spring’ leaves are sampled at approximately 43 ± 6 days after full bloom when the majority of leaves on non-fruiting spurs have reached full size. In the majority of California orchards, this corresponds to mid-April. Careful sample labelling and record keeping, with the exact location of the samples, the condition of the orchard and

the management practices used in that year, are essential and will greatly enhance the value of the leaf sampling by allowing comparison across years and integration with soil information.

For 'summer' samples or for samples targeted to identify a specific nutrient deficiency, samples should be analysed for the element of interest and results contrasted with established standards (Table 14.1). Many testing laboratories routinely conduct analysis of relevant nutrients and toxic elements in all tissue samples; this can provide useful information since information on nutrient balance may be useful in diagnosing for poor tree performance. For 'early spring' predictive tissue sampling, leaves must be analysed for a full nutrient panel (N, P, K, S, Ca, Mg, B, Zn, Cu, Fe and Mn) and then analysed using the UCD-ESP program. Most Californian testing labs use this program. For those that do not, results can be entered manually for calculation into the UCD-ESP program using resources at the Almond Board of California website (Nitrogen Calculator, <https://www.sustainablealmondgrowing.org>) and the Fruit and Nut Center website (FNRI Nitrogen, fruitsandnuts.ucdavis.edu).

With the exception of the analysis of B in almond hulls, all of the CVs shown in Table 14.1 refer to leaf samples collected during the summer sampling period.

Sampling and analysing for boron

In almond, sampling hulls rather than leaves provides the best measure of B status. This is a consequence of the very rapid mobility of B in almond and the tendency of B to accumulate in fruits. In mature fruiting trees, hull B analysis is a more sensitive indicator of both B toxicity and deficiency than is leaf analysis. In very young trees, or trees with little fruit, leaf tissue can indicate severe B deficiency, but will not be very effective in identifying B toxicity (which can be best characterized by tree symptoms; see description below). The sampling strategy for hull analysis is identical to the leaf sampling strategy outlined previously. Hulls from five to eight fruit should be sampled from around the canopy of each of 15–30 trees with each tree being a minimum of 27 m apart, and all hulls can be combined as a single sample for submission to a reputable laboratory. If the aim of the sampling is to identify the cause of poor tree performance

in an isolated tree or area of the orchard, then collecting samples from poor trees and comparing them with a sample from healthy trees should suffice to help identify if B deficiency or toxicity is present. If fruits have been harvested, hull samples can be collected from the windrow or after pickup. The level of available soil B or the B concentration in irrigation water frequently determines B status in the orchard; analysis of both soil and water B concentrations is therefore critical.

Preparation and analysis in the laboratory

Leaf tissues can be degraded if samples are exposed to extreme heat (such as inside a closed vehicle), or to excessive moisture (such as when samples are stored in a plastic bag). Collected samples should be kept in cool, dry conditions and submitted promptly to a reputable laboratory. It is standard procedure in reputable laboratories to wash sample leaves in a weak detergent mixture, rinse them in distilled water and dry them at 60°C. The dried samples are then ground and a subsample is analysed to determine the content of the requested elements. Leaves that have been sprayed with a micro-nutrient cannot be reliably analysed for that nutrient, since surface contamination cannot be adequately removed. These samples can, however, be analysed for nutrients that have not been applied foliarly.

Interpretation of results

Leaf and hull analysis results are expressed as percentages or parts per million of each element in dried samples. Table 14.1 presents the CVs for almond leaves and hulls sampled in July according to the methods outlined previously. Three values are presented: (i) the CV, below which yield will be reduced; (ii) the critical range, within which crop productivity can be expected to be optimal; and, if relevant, (iii) the toxicity threshold, above which yield will be compromised. With the exception of N, K and B (in hulls) the CVs do not reflect yield responses; rather, they reflect the nutrient level at which deficiency symptoms first appear. Generally, if symptoms are visible on the tree then yield (or growth) will already be negatively impacted. For N, K and B the data provided in Table 14.1 are the result of extensive field experimentation.

14.2.3 Visual diagnosis of nutrient status

Deficiencies or toxicities of essential elements cause specific metabolic disturbances that will eventually result in characteristic visual symptoms. These may include an overall reduction in growth or be manifested as specific foliage disorders. Identification of nutritional disorders by symptomology can be extremely useful and, when performed by a trained individual, is the quickest method of deficiency diagnosis.

Visual diagnosis does, however, have important disadvantages and limitations. In general, by the time visual symptoms appear, tree productivity has already been affected by the deficiency or toxicity. Accurate visual assessment of foliar symptoms requires years of experience, and symptoms vary with time and plant species. Diagnosis can be complicated by the occurrence of multiple deficiencies in the same tree as well as by environmental conditions, and marginal deficiencies are particularly difficult to identify. The effects of low temperatures, waterlogging, root or crown diseases, toxic elements, and mechanical and spray damage can all be misinterpreted as nutrient deficiencies.

Often the pattern of symptom development, such as the site of deficiency within the tree, can be used to identify a nutrient deficiency. Because the mobility of different elements varies within the tree, symptoms of various nutrient deficiencies appear first in different portions of the canopy. In general, a lack of immobile elements first becomes apparent in the youngest tissues and a deficiency of a mobile element first becomes apparent in the oldest leaves.

14.3 Management Strategies for Specific Elements

While all nutrients are equally important for the production of almond and deficiencies of any element impair growth and production, N and K are required by almond in larger quantities than any other element. Because of their central importance to almond production, N and K fertilizer management will be discussed in more detail.

14.3.1 Nitrogen and potassium

N and K are the nutrients required in the largest amounts by an almond crop. N is an integral constituent of proteins, nucleic acids, chlorophyll, co-enzymes, phytohormones and secondary metabolites (Marschner, 2012). K has an important role in stomatal opening, plant growth and cell expansion. K is also required for phloem loading of carbohydrates from photosynthesis and their transport to fruit or roots. It functions as a counter-ion for the transport of anions in the xylem/phloem and is critical for various processes in plant–water relations including stomatal function and plant adaptation to water stress.

N deficiency reduces photosynthesis and plant growth, resulting in stunted trees with small, pale, narrow leaves. As N is mobile in the phloem the deficiency symptoms appear first in older leaves as a result of remobilization of N from older leaves to younger leaves. Severe N deficiency results in pale green or yellow leaves, and shoot growth and canopy height is reduced. In severe N deficiency fruit drop may occur and nut quality is affected by reducing protein content. In heavy cropping years, or in trees with limited N present in woody organs, N deficiency can occur rapidly and impact yield in the current year. In trees previously well fed with N, or when yield is low, a depletion of N may have little impact on yield for a couple of years. N fertilization in excess of tree demand is poorly utilized by the plant and may be lost to leaching or result in excessive vegetative growth and will increase susceptibility to diseases such as almond hull rot. Over-fertilization with N fertilizers is a leading cause of contamination of ground water with nitrate and the deterioration of drinking water quality. Proper management of N to ensure full productivity while reducing the potential for N excess or N loss to the environment is required, and is essential to the continued viability of the world almond industry.

Large amounts of K are present in the hull of almond, and K export in the harvested fruit generally exceeds N export. K deficiency reduces photosynthesis and carbohydrate transport and accelerates premature leaf senescence and premature leaf abscission. Leaf K concentration of less than 0.5–0.6% also limits leaf CO₂ exchange rates and reduces leaf carbon fixation by imposing biochemical limitations on photosynthesis

(Basile *et al.*, 2003). Because the almond tree can store significant amounts of K, deficiency in K may take several years to appear (Reidel *et al.*, 2004).

The nitrogen cycle

In order to protect groundwater resources, N requires to be managed in such a way as to minimize the possibility for its loss to leaching below the active root zone. To achieve this an understanding of the N cycle in the soil and plant is important.

N in soils exists in pools of different availability to plants. Ammonium, nitrate, urea and – to a limited extent – soluble organic nitrogenous compounds (including amino acids and phenolics) are water soluble and readily available for uptake by plants. These soluble forms of N may be derived from fertilizers, added soluble organic residues and the breakdown of soil organic matter, or may be present in irrigation waters. All soils also contain organic N, which is derived from the decomposition of plant, animal and microbial residues. Over time various bacteria and fungi act upon these organic residues, converting them into simpler water-soluble forms of N that may then be absorbed by roots. A significant proportion of soil organic matter is highly resistant to degradation and only very slowly (years or decades) provides N to plants. N may also be incorporated in soil mineral components. This fraction of soil N is poorly understood and is not thought to be a major source of N for commercial almond orchards.

Although the atmosphere consists of 78% gaseous nitrogen (by volume), N is unavailable for use by trees until it is reduced to ammonium or nitrate by biological nitrogen fixation. N fixation does not occur in almond orchards except in the presence of well-managed leguminous cover crops. Soil organic matter also contains N, which can be released by the action of micro-organisms; this N is readily available to plants. Where a grower does not add substantial amounts of organic matter amendments (composts, manures and cover crops), the levels of naturally occurring organic matter in most almond orchard soils is generally less than 1%. The amount of N that can be provided from mineralization (N_{min}) of native soil organic matter in an almond orchard is therefore very low and at most

represents only 5–10% of annual demand in a well-established orchard. The majority of N released from organic matter will be available in the spring as ammonium (NH_4^+) that will be rapidly converted to nitrate (NO_3^-) once soil temperatures rise above 13°C. The primary effect of N_{min} on nitrogen fertilization programmes in almond is to serve as a source for N in late spring once N reserves in the tree have been depleted. While N derived from soil organic matter can contribute a small amount of N to total orchard N demand, the depletion of N present in native soil organic matter without replacement will ultimately result in degradation of soil quality.

If well managed (to limit food safety issues, volatilization and leaching losses), organic matter amendments (composts, manures, cover crops, green wastes, etc.) can represent a valuable source of N for almond and often have the added benefit of improving soil structure and health, providing additional essential elements and improving the ability of soils to retain nutrients and water.

The management of N in orchards can be viewed as a balancing act with the goal of applying sufficient N to supply tree needs for growth and nut production, while maintaining or increasing soil organic N and minimizing potential loss through leaching or volatilization. Application of N in excess of the capacity of the almond tree to utilize that N increases the likelihood that nitrate will move beyond the root zone, where it is at risk of being lost to groundwater.

Nitrogen transformation into nitrate in soils

Soil N is present in three major forms: (i) organic N; (ii) ammonium N; and (iii) nitrate N (Fig. 14.4). Plants primarily take up ammonium and nitrate forms of N. Organic N must therefore be broken down by soil microbes into ammonium or nitrate in processes called mineralization or nitrification, respectively. The conversion of organic N into ammonium and nitrate may take from days to months and years depending on the stability and complexity of the organic matter.

The ammonium form of N is the first by-product of organic N breakdown. Ammonium has a positive charge and can bind to negatively charged soil particles and is not subject to leaching. By the action of soil microbes ammonium is then changed into nitrate in the process of nitrification. The rate of conversion of ammonium

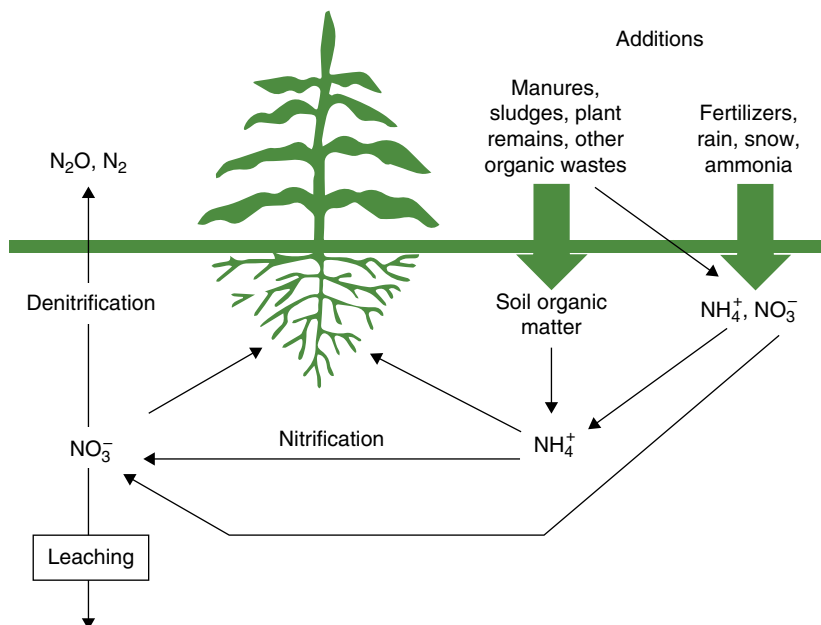


Fig. 14.4. Transformation of nitrogen into nitrate and other forms of nitrogen in the soil. (From <https://nevegetable.org/cultural-practices/nitrogen>.)

into nitrate is mainly influenced by soil temperature and under the warm soil temperatures prevalent in late spring, summer and early autumn, conversion from ammonium to nitrate is very rapid, with full conversion occurring in a matter of days. During the winter when soil temperature is low ammonium to nitrate conversion may take weeks to months.

Nitrate is negatively charged and is repelled by the negatively charged soil particles; thus nitrate can easily leach through the root zone with irrigation water or rainfall and is readily lost to groundwater. Under waterlogged conditions such as during flood irrigation, soil microbes use the O in the nitrate and release N₂ gas that is lost to the environment. The process is called denitrification, during which nitrous oxide (N₂O) gas is released to the environment.

Losses of nitrogen from an almond orchard

Managing orchard N to prevent losses requires an understanding of tree N demand and the losses of N from the orchard. In a mature almond orchard, the harvested fruit is by far the largest pool of N that is removed from the orchard on

an annual basis. N is also present in fallen leaves, pruning and in fine root turnover; however, this N is not lost from the orchard but rather enters the soil organic matter pool where it is eventually mineralized and made available for uptake. Microbial denitrification is a naturally occurring process in all soils that results in the formation of gaseous nitrous oxides that can be lost to the atmosphere. Denitrification is greatest under waterlogged conditions, in fine-textured soils such as loams and clays and under warm and saturated soil conditions. These conditions occur in flood-irrigated orchards and significant N loss through denitrification can occur if fertilization coincides with a flooding event. Under drip and micro-sprinkler conditions in California the loss of N as nitrous oxides is modest at 0.22–0.78 kg N per ha per year (Schellenberg *et al.*, 2012), and predominantly occurs immediately following fertigation events when soils are saturated and fertilizer N is abundant.

Application of N fertilizers, or organic matter amendments containing N, can also result in N loss either through direct volatilization or through leaching of nitrate N below the root zone. If urea is broadcasted, part of it may

convert into ammonia and volatilize; this can be reduced by ensuring soils are moist or incorporating the urea into the soil. High concentrations (such as those caused by banding) of ammonium-based fertilizers also favour volatilization losses. Because of its positive charge, ammonium (NH_4^+) binds to negatively charged sites in the soil and volatilization losses decrease as the CEC of soils increases. While N in the form of nitrate (NO_3^-) is not subject to volatilization, it can be lost through denitrification as described above, and through leaching.

Under warm and wet soil conditions, all forms of fertilizer N (urea-ammonium nitrate (UAN), calcium ammonium nitrate (CAN), ammonium sulfate, etc.) and soluble N derived from manures or composts will be very rapidly converted to NO_3^- . Because NO_3^- is negatively charged it does not bind to the soil and will move freely with water through the soil. Fertilizer N must therefore be managed on the assumption that it will rapidly move with irrigation and rain and will be lost from the soil if water moves below the active root zone (typically 0–0.46 m). The potential for leaching loss is greatest in sandy soils and if N is applied when plant N uptake is limited (autumn, winter and early spring). Leaching losses can be particularly high if fertilizer is broadcasted in young orchards that use a full-coverage irrigation system or if there are significant rainfall events following fertilization.

The use of well-managed fertigation to apply nitrogenous fertilizers can help in reducing the chances of volatilization and leaching loss by ensuring that the soluble N fertilizer is maintained in the active root zone.

Potassium cycle

Only a very small amount of the native soil K is available to plants, while the majority of soil K is present in the mineral form. Soil K can be divided into three groups based on availability to plants: (i) K contained in minerals (90–98%) and non-exchangeable K (1–10%), which are released as soil minerals are weathered at such a slow rate as to be unavailable to plants; (ii) K fixed or trapped between layers of certain soil clay minerals which may become slowly available (1–2%); and (iii) readily available K, including that present in the soil solution and the exchangeable K in organic matter and clay (0.1–0.2% of total) (Brady and Weil, 1996; Prabhu *et al.*, 2007).

The main K additions to an orchard are chemical fertilizers, crop residue and organic manures. The main K removal from an almond orchard is in the harvested crop, while leaching, erosion and run-off losses are trivial, especially in finer particle soils (clay and loam) where K mobility is limited. Almond crop removal accounts for the majority of the soil K depletion from the orchard. Almond hulls contain 2–3% K on a dry-weight average basis, while the kernel contains less than 0.8% K. An 1800-kg kernel crop can therefore remove 130 kg of K from the orchard. Leaching losses of K are very small except in very sandy soils, while erosion and run-off losses occur only in flood-irrigated sloped soils; micro-sprinkler and drip-irrigation technologies have helped in eliminating these losses.

Nitrogen and potassium uptake by plants

The primary forms of N present in fertilizers are ammonium (NH_4^+), nitrate (NO_3^-) or urea ($\text{NH}_2\text{CO-NH}_2$), and all three forms of N can be taken up readily by plant roots. N is taken up from the soil almost exclusively as nitrate, ammonium and – to a very small extent – amino acid. The uptake and assimilation of nitrate by the plant is a relatively energy-expensive process and results in the release of a hydroxyl ion, which may modestly increase pH around the root. Ammonium uptake is marginally less expensive energetically; however, there are no reports of a relative growth benefit to almond of one N source over another. The uptake of ammonium results in the efflux of H^+ from the root and results in soil acidification that can be significant in cooler growing regions and where N fertilization is localized to a restricted root zone such as under drip irrigation (Meyer, 1996).

Plants use multiple transport proteins to transport N into the cell and subsequently assimilate that N into amino acids, which are then used as substrate for all N-containing processes in the plant. The plant tightly regulates the processes of N uptake and assimilation, and this determines the rate at which N can be acquired from the soil.

There are three important characteristics of N uptake in almond:

- N uptake occurs through transport proteins at the root surface and these proteins

are limited in number and the rate at which they can transport N.

- The uptake and assimilation of N is energetically expensive for the plant and will be restricted if photosynthesis or plant growth is restricted for any reason.
- The intermediate products that are formed after N uptake and prior to amino acid generation are toxic to the plant; hence, N uptake is shut down if there is insufficient energy or carbon building blocks to complete the assimilation of N to amino acids.

These three characteristics of N uptake and assimilation have a profound effect on N uptake by plants. Notably, uptake is driven by plant demand for N and by the availability of energy and carbon skeletons from which amino acids are formed. During periods of high demand for N, such as when there is a large crop load and when fruit are actively growing, demand for N will be great and hence uptake of N will be high. Plants with limited demand due to low crop yield or poor growth will have decreased N uptake. Plants with restricted photosynthesis due to water stress, disease, nutrient deficiency, etc., will have decreased N uptake. The provision of N in excess of this uptake capacity will result in N accumulation in the soil with the resultant potential for N loss through leaching.

An important principle of N management is that almond cannot be 'forced' to utilize more N than the current crop load and plant growth demands. Plants that are growing poorly will have compromised N uptake that can only be remediated through correction of the factors that are limiting growth. The nutrient budget approach outlined at the end of this section is designed to provide growers with the means to apply the correct amount of N to optimize productivity, without risking the loss of N to the environment and the consequent waste of resources.

14.4 The 4Rs of Nutrient Management

The 4R (right rate, right time, right placement and right source) approach to fertilizer management has gained widespread acceptance and provides an ideal basis for managing nutrients in

crops (<http://www.ipni.net/4R>). In the nutrient budget and 4R approach, fertilizers are applied in proportion to the demand of the crop (right rate) and timed with periods of nutrient uptake (right time) so that plant demand is satisfied in a timely fashion, avoiding the application of fertilizers in excess of plant capacity for uptake. The choice of the correct fertilizer source (right source) and placement within the active root zone (right placement) is essential to maximize fertilizer use and avoid losses.

14.4.1 Sources of nitrogen and potassium fertilizer: right source

Many sources of N and K fertilizers are available in granular and fluid forms, and include urea, ammonium sulfate, calcium ammonium nitrate, calcium nitrate, urea ammonium nitrate for N; and potassium sulfate, potassium nitrate, potassium chloride and potassium thiosulfate for K. The N sources are popular because of their widespread availability, low cost and effectiveness; however, cost, convenience, potential soil acidification and susceptibility to leaching and other losses vary.

Nitrogen

Irrespective of fertilizer N source, nitrate leaching can occur since, in the warm, moist soils characteristic of many almond-production areas, urea will be hydrolysed within days to ammonium, and ammonium will be converted to nitrate within a matter of hours or days. Only under strongly acidic soil pH (soil with a pH less than 5.0) or cold temperatures (those less than 10°C), will ammonium to nitrate conversion be slow. These conditions, however, are atypical during the majority of the growing season in almond orchards, with the exception of cold and wet spring conditions. Nitrate and urea readily move in the soil with water and may be leached below the root zone if rainfall is heavy or irrigation excessive. In contrast, ammonium (unless applied in concentrated bands) is tied up near the soil surface and is not leachable until micro-organisms convert it to nitrate.

A soil solution pH of between 6.0 and 7.0 appears to be optimum for almond nutrient availability. Ammonium-containing fertilizers frequently

have an acidifying effect on soil. Ammonium sulfate is very acidifying; urea, ammonium nitrate and urea ammonium nitrate solution (UAN-32) are moderately acidifying. Calcium nitrate, on the other hand, creates a basic reaction in the soil. Each grower should determine which source of N works best with his or her specific management practices and orchard conditions.

Organic matter amendments (OMA) can also provide significant amounts of N to orchards; however, while these materials have benefits in terms of nutrient supply and improvements to soil health, there are concerns which must also be considered. The benefits of OMA include positive impacts on soil biology, tree nutrition and water-holding capacity. Concerns may include negative impact on food safety, negative short-term impacts on nutrient availability and the cost/logistics of use compared to conventional fertilizers. OMA are available in multiple forms including (in California) composted or raw manure from dairy cows or chickens, green waste compost from municipal sources and post-processing residues such as almond shells or rice chaff.

The N content of OMA varies significantly depending upon the source, age and production methods used. OMA are less predictable sources of N than are synthetic fertilizers because N content can vary from 1% to 3%, as does the rate of N release. While 90% of the N in chicken manure may be available to trees the first year, many other types of manure may release less than 50% of their N in the same period. Nitrate leaching occurs from cropland fertilized with manure, as well as from cropland fertilized with synthetic fertilizers. Factors other than the material, such as rate applied, soil characteristics and irrigation water management largely determine the amount of nitrate leaching.

Information on relative leaching potential is relevant only in the short term or when soils are cold and wet. Any form of N fertilizer is rapidly converted to nitrate in a matter of days during the growing season, and is at risk of leaching if moisture levels are high under heavy rain or irrigation.

Potassium

Selection of K fertilizer source is important in almond production. Some common considerations

are: fertilizer price, salt content, solubility and fertilization methods. If the soil or irrigation water is high in Cl content, avoid using potassium chloride as it may increase chloride toxicity in trees. In most California almond orchards potassium sulfate (sulfate of potash, SOP) is currently the major K source. SOP is frequently applied in winter so that the winter rain can assist with dissolution and movement into the root zone. SOP can also be applied followed by irrigation, or 'solubilized' using dedicated SOP injection machinery. In the case of surface application of SOP in drip-irrigated orchards application should be made immediately below or adjacent to the drip emitters. If soil or water salinity is not an issue, potassium chloride may be used provided the impact on tissue Cl is monitored. Fluid KCl is also available and can be fertigated with the irrigation water. Potassium nitrate is a water-soluble source of K which provides an excellent source of both N and K, is highly soluble and suitable for fertigation systems. Excellent results have also been obtained using a combination of low-cost granular SOP with soluble KNO_3 or potassium thiosulfate (KTS).

OMA can safely be used during orchard planting as well as at the non-bearing stage by either backfilling or incorporating in the tree berm, or by spreading and incorporating in the alleyway. Use of OMA during planting and non-bearing years has significant potential to improve soil health, root growth and water infiltration, all of which may contribute to greater tree performance. Application during non-bearing years has minimal risk to food safety.

The majority of OMA in bearing almond orchards are spread on the tree berm and left on the soil surface as a mulch under the irrigated zone of the orchard floor. For sources of OMA that have a potential food safety risk, timing of the placement of organic matter amendments in bearing orchards should be limited to up to 120 days before harvest to give adequate time for decomposition and to reduce any food safety risk. If the OMA have a high C:N ratio, placing them in the irrigated zone of the orchard floor may alter the availability of conventional synthetic fertilizers delivered through the irrigation system through greater N immobilization.

Raw or uncomposted OMA tend to have higher nutrient content but may harbour human pathogens including *Escherichia coli*,

Salmonella and *Listeria*. This is a particular problem for almonds harvested from the ground and coming into contact with soil. In contrast, composted OMA eliminate human pathogens when the process is conducted according to composting standards. From a nutrient management perspective, the integration of OMA may improve soil nutrient retention in the rooting zone by the initial and partial immobilization of applied nutrients, followed by remineralization during subsequent weeks or months.

In addition to N, OMA contain salts (such as Na and Cl). If allowed to accumulate in the soil, these salts can become toxic to almond. Different sources of OMA can have different levels of Na and Cl salt loading. Thus, the rate of OMA application should consider total salt loads and available irrigation water to leach excess salts.

14.4.2 Fertilizer application methods: right placement

The right placement of fertilizers in the root zone is important to optimize the availability of the nutrients to roots for uptake. The goal is to apply fertilizers to the active root zone to maximize uptake and avoid losses. The active root zone is predominantly determined by the distribution of water in the soil. Under micro-sprinkler and drip-irrigation systems in environments with limited in-season rainfall, the majority of active roots are restricted to the soil zone wetted during an irrigation cycle. In regions with substantial winter and spring rainfall, roots will be active to a greater depth, particularly early in the year when deep soil moisture is still present. The top 0–5 cm of soil usually has limited root activity due to the sharp moisture fluctuations and the high temperatures that can occur in that soil zone. If irrigation schedules are more frequent, so that the shallow soil surfaces do not dry out, then active roots will be present even in the top inches of soil. In studies conducted in Kern County, California, a region having minimal winter rain with irrigation cycles of 5–14 days, 90% of active roots were found between 0.1 m and 0.5 m (Brown *et al.*, 2014). A small percentage of roots were also present at depths of up to 1.5 m or greater.

N and K fertilizers can be broadcast, banded, drilled and applied through an irrigation system.

The historic practice of broadcast applications is becoming less prevalent as growers adopt fertigation systems. Broadcasted N fertilizers are at risk of substantial N loss through deep leaching or surface run-off if rains occur soon after application. Broadcasted K is at greater risk of fixation. Banding in a 30–45-cm wide strip or in the herbicide strip is still used in furrow, flood and solid-set sprinkler-irrigated fields in which fertigation may be impractical. Compared to broadcasting, banding limits grass and weed growth on the orchard floor and hence reduces competition for N and K. The availability of fluid nitrogenous and K fertilizers and the use of micro-irrigation have made the application of N and K fertilizers through the irrigation system easy, efficient and economical. Injection of N and K fertilizer through low-volume irrigation systems (such as drip or mini-sprinkler) results in a higher percentage of nutrient recovery and far greater capacity for integration of irrigation with fertilization than does application by traditional systems.

The majority of almond growers who use micro-irrigation for water management in orchards also apply nitrogenous fertilizers through fertigation. Low-volume irrigation systems encourage root proliferation in the wetted volume of soil and offer the potential for highly targeted fertilizer management. While fertigation offers the potential for greater fertilizer efficiency, poor fertigation or irrigation practices can also result in substantial losses of N from the orchard through deep leaching. Fertilizer injections during an irrigation event should be managed so that the highly soluble N remains within the root zone and is not pushed with the irrigation front to the bottom of the active root zone. The exact timing of a fertilizer injection to achieve this goal will be determined by soil type, irrigation volume and frequency, and the stage of growth of the tree. As a general guideline, fertilizers should be injected into the irrigation in the last one-third of the irrigation event and followed with just sufficient water to clean out the irrigation system and push fertilizer a small distance (perhaps 3 cm) below the soil surface. This will help to minimize deep leaching and volatilization. The exact strategy needed to achieve this goal, while maintaining uniformity of nutrient delivery across the orchard, will vary from site to site based upon system design, soil type, run-times and other factors. The assistance of an

irrigation expert in designing and testing an ideal fertigation scheme for the particular conditions present in the management unit is highly recommended. Even when perfectly mixed and carefully injected, the uniformity of nutrient distribution will always be limited by the uniformity of the irrigation system itself.

Since some soils (clay, calcareous soils) can fix applied K, and as tree roots tend to be restricted to the drip line or directly in the wetted zone of the irrigation system, broadcasting K fertilizer over the surface is generally not an efficient approach. When not injected or placed directly under the irrigation emitters, K fertilizer should be applied in winter in bands 30–45 cm wide on both side of the trees, allowing the winter rains and irrigation in the spring to dissolve the fertilizer and incorporate it into the soil. This method will create a concentrated zone of K where fixation will be reduced and plant roots can scavenge the K provided soil moisture is available. It has been demonstrated in a number of tree crops that fertigation of fluid K fertilizers through drip irrigation can be very effective, with drip-applied K_2SO_4 increasing leaf K concentration more effectively than broadcasted or band-applied K_2SO_4 . In prune, K_2SO_4 placed directly below the emitter at the beginning of the season responded in a way that was similar to drip-applied K_2SO_4 (Uriu *et al.*, 1980). Using this method, K in the soil under the dripper was maintained at a high concentration and defused out laterally about 0.9 m and down to about 0.75 m. In broadcast applications K was substantially increased only in the surface soil (0–15 cm) but was very low below that depth (Uriu *et al.*, 1980).

14.4.3 Fertilizer application methods: right rate and right time

Traditionally, nutrient management in almond has been based on the collection of leaf samples in July; analysis and comparison with the established CVs; and application of fertilizers when leaf analysis for a particular element falls below a specified CV (see 'Critical value' section). While this has been a useful tool for diagnosis of nutrient deficiency or excess, it is now recognized that this approach does not provide sufficient information to define the rate and timing of fertilizer applications.

Many different nutrient management tools have been used in annual and perennial plants to guide the quantity and time of fertilizer application and diagnose nutrient deficiency or excess. In recent years nutrient budget and the 4R (right rate, right time, right placement and right source) approaches to fertilizer management have been gaining widespread acceptance (<http://www.ipni.net/4R>). In the nutrient budget and 4R approach, fertilizers are applied in proportion to the demand of the crop (right rate) and timed with periods of nutrient uptake (right time) so that plant demand is satisfied in a timely fashion, avoiding the application of fertilizers in excess of plant capacity for uptake. The choice of the correct fertilizer source (right source) and placement within the active root zone (placement) is essential to maximize fertilizer use and avoid losses. This approach, integrated with the use of early-season leaf analysis, has been developed for almond.

The goal of any fertilizer management strategy is to ensure that adequate nutrients are available to supply the current demand of the plant. The demand for each nutrient varies, depending upon the role of that nutrient in specific plant processes, and on the development and size of the plant organ in which the nutrient will be used. While N and K are required for all plant processes and in every organ, it is the development of the fruit that represents by far the largest sink for N and K use in almond. N and K uptake is demand driven; that is, it is the size of the crop that will determine how much N or K will be taken up by plant roots, and when that uptake will occur. While a shortage of N and K can reduce yield by preventing full fruit growth, adding N and K in excess of plant demand will not increase yield and may result in loss of N to groundwater and a reduction in production efficiency.

In the most detailed analysis of the nutrient demand of almond conducted to date, the size of N storage pools and the pattern of N demand and uptake was determined in a high-yielding commercial orchard planted in 1999. Six 'Nonpareil' trees were excavated at the commencement of the 13th year (26 January 2012) and at the completion of the year (17 December 2012). The nutrient content in organs of an additional five trees was determined at seven intermediate growth stages by subsampling all above- and

below-ground tree parts at approximately monthly intervals (Fig. 14.5). The orchard used in this experiment was at 13th leaf, 85–90% full canopy orchard with a 3-year yield average of 1450 kg and a yield in 2012 of 2200 kg. Since N and K demand is directly proportional to yield, the specific quantities of N and K present in these trees are representative of these yield levels. Lower yields will use proportionately less nutrients; however, the timing of nutrient accumulation in trees will be similar, regardless of yield levels.

At the commencement of the 13th season of this orchard, 204 kg N and 77 kg K was present in all perennial tissue (above and below ground) in January (Fig. 14.5). This represents the total N and K accumulated in perennial organs in the 13 years preceding tree removal.

N and K in the tree in January represent both permanent structural N and K, and N and K present in storage forms available for remobilization during bloom, leaf out and fruit set. The difference between the size of the total tree N pool in January and the total tree N and K pool at its lowest seasonal point (10 April in this example) provides an estimate of the size of the storage N pool that can contribute to early-season N demand. In this example an estimated 40–45 kg N and 29 kg K per hectare of storage N or K was present.

The total N and K content of the trees did not increase from January to 12 March (14 days after full bloom (DAFB)), demonstrating that no measurable net uptake of N from the soil occurred during this period. The apparent lack of uptake was likely to have been a consequence of

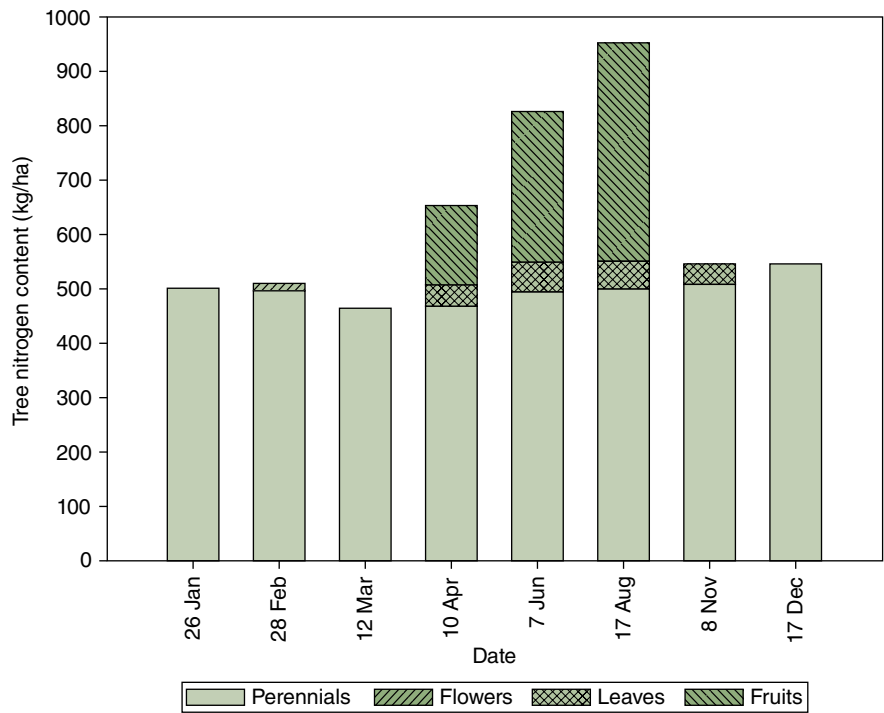


Fig. 14.5. Nitrogen partitioning in perennial and annual organs of mature almond trees (trees planted in 1999; 2012 data are shown). Nitrogen content at each of nine stages of development was determined by excavating six entire trees at the commencement of the year (26 January) and at the completion of the year (17 December). The nutrient content in organs at seven intermediate stages was determined by subsampling above- and below-ground tree parts from five trees. The nitrogen accumulation in annual tree parts (flowers, leaves and fruits) was predominantly (> 95%) associated with nitrogen in the fruit, which in the year of these excavations totalled 2200 kg kernel. The accumulation in perennial biomass at the commencement of the year (26 January) represented total nitrogen accumulated in perennial organs in the 13th leaf trees. (From Muhammad *et al.*, 2015.)

a lack of root growth at this time and a lack of N demand as a result of the abundant reserve N in the tree. The pattern of root growth in almond was also determined in this same orchard, using in-situ root observation chambers and root excavation methods (A. Olivos, unpublished). Root growth commenced with leaf out (7 days post-full bloom) and did not peak until late spring, 45–55 DAFB (Fig. 14.6). There was also a small flush of root growth post-harvest in 2013, but not in 2012 (Olivos, 2016).

These data demonstrate that flowering, early leaf growth and fruit set depend entirely upon the remobilization of nutrients stored in perennial organs, and that new root uptake does not commence until nutrient reserves have been depleted – which in this example did not occur until 15 DAFB. Net N and K uptake from soil increases rapidly between 15 and 50 DAFB as fruits develop and spur leaves expand to full size (Fig. 14.5). By sequentially analysing fruits, which represent greater than 90% of the total annual N demand in a high-yielding orchard, it is

possible to accurately estimate the total N uptake of the tree during the season (Fig. 14.7). Thirty-eight per cent (%) of the final N accumulation in fruits, and 30% of total annual N uptake from soil (with the remaining 11% being provided from stored N in perennial organs), was reached by 50 DAFB and this coincided with the full expansion of spur leaves (Fig. 14.7). Seventy per cent of total fruit N content was accumulated by 83 ± 3 DAFB and 90% by 123 ± 3 DAFB (Fig. 14.7). While the scale of N uptake will be directly proportional to yield present on the tree, the pattern of N uptake through the year did not differ significantly from year to year.

K demand in almond is also driven mainly by fruit development and nut fill (Fig. 14.8). Compared to N, K accumulation in fruits is slower early in the season and continues longer than N accumulation through full fruit maturity at a constant rate. Twenty-five per cent of the K accumulates in fruits by 43 ± 3 DAFB, 45% by 83 ± 3 DAFB, 74% by 123 ± 3 DAFB and 92% by 163 ± 3 DAFB (Fig. 14.8).

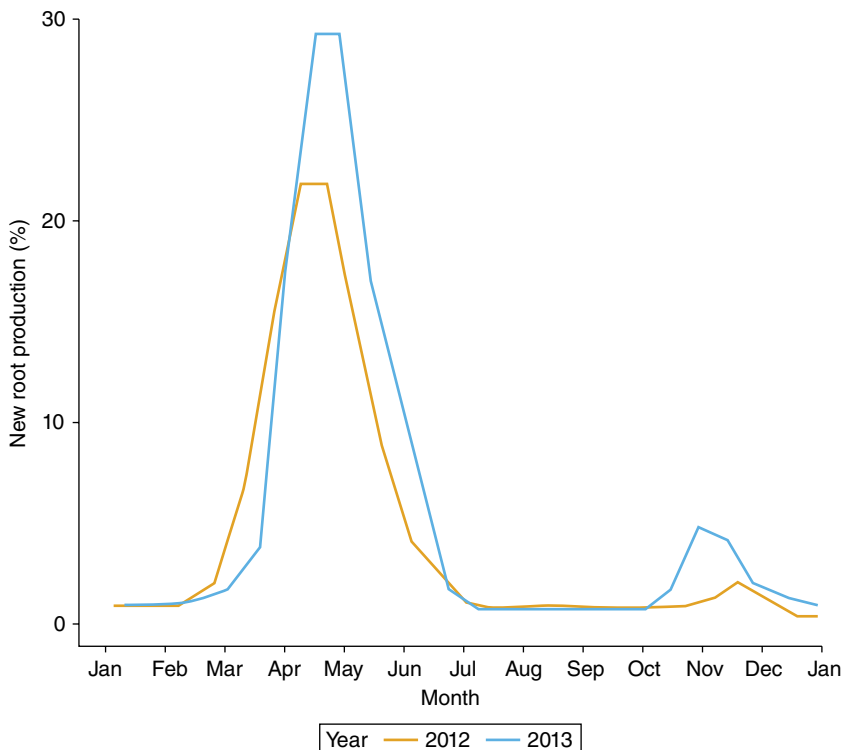


Fig. 14.6. Seasonal pattern of root growth in 2012–2013. (From Olivos, 2016.)

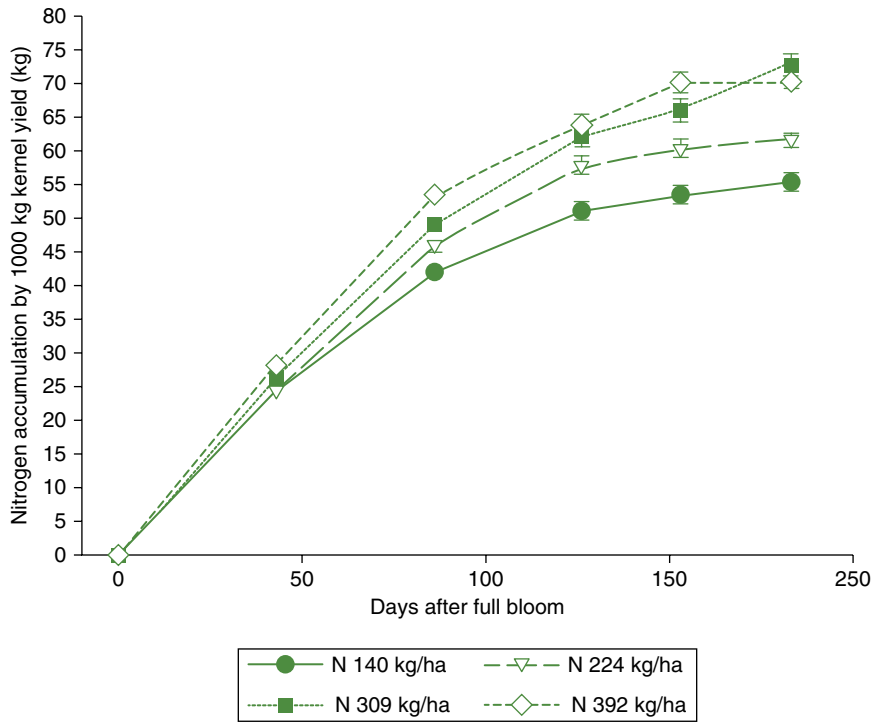


Fig. 14.7. Nitrogen accumulation in almond fruit by 1000 kg kernel yield throughout the season. More than 80% of the nitrogen accumulated in the fruit at 126 days after full bloom.

N and K export from orchards has been determined across a range of fertilizer N and K rates and locations (Saa *et al.*, 2013; Muhammad *et al.*, 2015). In mature almond trees (over 7 years old) kernel yield in the current year was shown to be the primary determinant of N demand with over 90% of tree N partitioned to fruits. The amount of N exported in fruits (kernel + hull + shell) from the orchard varied between 50 and 75 kg N per 1000 kg kernel depending on the season and N status of the tree. In trees receiving optimal (but not excessive) N application, and producing high yield, N export averaged 68 kg N per 1000 kg kernel yield. This value of 68 kg N represents the annual increment in N in perennial tree growth, as well as the total N in fruit and other non-yield plant parts removed from the orchard for each 1000 kg commercial kernel yield. The amount of K exported from the field varies between 67 and 83 kg K per 1000 kg kernel depending on the K status of the trees. In trees receiving optimum K, export was found to be 74 kg K per 1000 kg kernel yield; this value

includes sufficient K for all fruit, perennial organs and annual tissues. Unlike N, the majority of the K in the almond fruit is present in the hull; hence, if hulls are returned to the orchard floor, the N export from the orchard is reduced to 10–15 kg per 1000 kg kernel.

14.5 Nitrogen Guidelines

N application should follow the 4Rs by applying the right rate at the right time in the right place using the right source. The right rate is directly determined by the current year yield of the orchard. The total demand for N in a mature well-managed almond orchard yielding in excess of 1000 kernel kg is 68 kg N per 1000 kg kernel yield (Alonso *et al.*, 2012). At the commencement of the season an estimate of likely yield is made to establish a first target N rate. Subsequently this estimate is updated and the target fertilizer rate is adjusted accordingly.

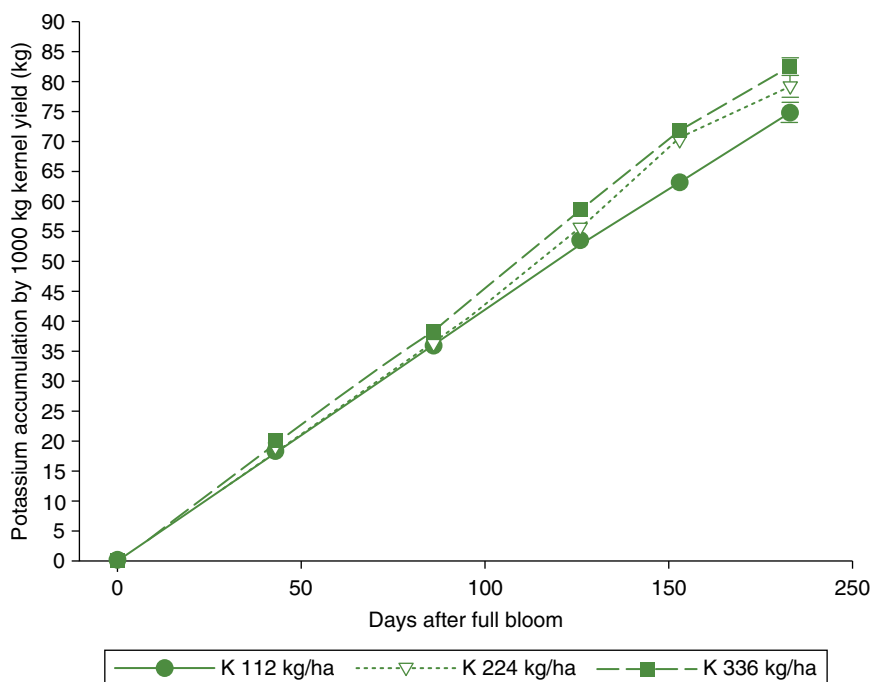


Fig. 14.8. Potassium accumulation in almond fruit by 1000 kg kernel yield throughout the season. Over 70% of the total potassium accumulated in the fruit by 126 days after full bloom.

N applications should be timed to match the period of greatest N uptake. During the period from bud-swell until 15 DAFB, N stored in perennial organs is the sole source of N for tree growth. Root growth and uptake of N from the soil does not occur until 70% leaf-out has been reached, but increases rapidly thereafter. In the absence of significant N uptake prior to 70% leaf-out, N fertilizer applied before this time has the potential to leach below the root zone, particularly if applications are followed by heavy rainfall or irrigation events.

At a minimum N should be provided in four or more fertilization events:

- 30% from 14 days post-full bloom and full leaf expansion;
- 30% from completion of leaf expansion to shell hardening;
- 28% during the period of nut fill (from May to late June); and
- up to 12% immediately post-harvest while trees are still green and active.

While maintaining adequate N status in the tree is important for the developing buds as

well as N storage for the subsequent spring, the decision to apply post-harvest N should be made on the basis of the N status of the tree in July or August. If budgeted N applications were more than sufficient to fully satisfy yield requirements and maintain an adequate July leaf N analysis ($> 2.4\%$) then the post-harvest N application may not be required. Post-harvest and late winter N applications are at greatest risk for leaching loss since they coincide with a period of diminished uptake and possible rainfall, or application of winter salt leaching irrigations. Minimizing the level of unused N in the soil profile in the winter period is critical to protecting groundwater resources from N.

Given the scale and seasonal variability in N demand in almond and the potential for N applications in excess of demand to be leached, efficient N management demands that N fertilizers be applied in as many split applications as is feasible. The number of applications will be influenced by the soil type in the orchard. In sandy soils with very low nutrient-holding capacity, N should be applied in as many small doses as

possible, timed closely with the period of tree demand. In soils with greater nutrient-holding capacity, and good irrigation practice that does not allow water to pass below the root zone, application of fewer splits is possible (though there is no disadvantage in increasing split applications). Theoretically, the most efficient fertilization strategy is to apply fertilizer in every irrigation event in proportion to current plant demand and in a manner that retains applied fertilizer in the active root zone for as long as possible.

14.6 Potassium Guidelines

The scale and pattern of K demand in almond resembles that of N. Like N, fruit growth and kernel yield is the primary determinant of K demand in mature almond trees. In the long-term experiment mentioned earlier, the amount of K exported in fruits (kernel + hull + shell) from the orchard varied between 67 and 83 kg K per 1000 kg kernel depending on the K status of the trees (Alonso *et al.*, 2012). In trees receiving optimum K fertilizer, K export was found to be 74 kg per 1000 kg kernel yield. This value includes sufficient K for all fruit, perennial organs and annual tissues.

Many soils in almond-production areas have appreciable amounts of exchangeable K and hence the high K demand of almond may not necessarily require an immediate replacement in soils rich in native K. Over the longer term, however, the significant K demand of an almond crop will ultimately require replacement of K mined from soils. In soils with limited exchangeable K, annual K applications to replace K removed in the crop will be required. Knowledge of the K-supplying capacity of your soil, based upon a well-established and locally relevant soil test, is essential as a means to make fertilizer decisions and to ensure continued crop productivity.

As K does not leach readily from soils the potential for leaching loss is not significant except in pure sands. K fertilizer management strategy depends largely on the particular soil type and management options. In very light-textured soils (such as sands), with a higher potential of K leaching, K fertilizers can be applied in many split applications using soluble sources

(if your irrigation system allows). In light (sandy loam) to heavy-textured soils with good CEC, K fertilizers can be applied at the beginning of the season with small amounts of K applied in-season. Clay and calcareous soils have the potential to irreversibly fix large amounts of K. To increase the availability of K in fixing soils, K fertilizer can be applied in narrow strips on both sides of the tree row in the wetted zone, thus creating a concentrated zone of K that will undergo fixation more slowly, making it more available to plants.

Leaf tissue analysis for K in almond frequently results in highly variable and inconsistent results, making the use of leaf analysis K for nutrient management difficult. Better results can be obtained by estimating annual K demand and soil K reserves and providing sufficient K to maintain a long-term K balance in the orchard. Because K does not leach easily, and as many soils have large K reserves, orchard response to fertilization or withdrawal of fertilizers is frequently slow.

In a number of recent experiments, excellent results have been achieved by applying 60% of the total required K as SOP as a concentrated band in the winter or early spring, and applying the remaining 40% K through fertigation in-season with a soluble K source such as potassium nitrate or potassium thiosulphate. Potassium chloride is an inexpensive, soluble K source that can be used in areas with low chloride toxicity risk.

14.7 Nitrogen Inputs from Irrigation Water

Fertilizer strategies should be implemented to meet N demand in an efficient and timely fashion. To achieve this, the quantity and timing of fertilizer applications should be adjusted to take account of the contribution from other non-fertilizer N sources in the environment. Sources of N in addition to synthetic fertilizers include residual fertilizer nitrate in the soil, N fixed biologically by leguminous cover crops, decomposition of organic matter and crop residues, and application of nitrate in the irrigation water.

Nitrate in irrigation water (if present) is functionally equivalent to added fertilizer nitrate;

hence, well water can represent a significant free source of N and must be considered when calculating an N fertilizer budget. N in irrigation water is, however, equally susceptible to leaching loss as fertilizer N. At a concentration of 40 parts per million (ppm), NO_3^- contributes 92 kg of N per ha annually if 10,000 m³ of irrigation water is applied. Nitrate-containing irrigation water that is applied during crop dormancy will not be available to the tree until after leaf-out and hence is susceptible to loss if rainfall and irrigation pushes it below the active root zone.

Agriculture and environmental testing laboratories may report results of water analysis as either $\text{NO}_3\text{-N}$ or NO_3^- . The following conversion factors may be used to calculate the amount of N applied annually in the irrigation water for N concentrations or levels of applied irrigation water:

$$\begin{aligned} 1 \text{ ppm } \text{NO}_3\text{-N in water} \\ = 1.03 \text{ kg N/1000 m}^3 \text{ water applied} \end{aligned}$$

$$\begin{aligned} 1 \text{ ppm } \text{NO}_3^- \text{ in water} \\ = 0.23 \text{ kg N/1000 m}^3 \text{ water applied} \end{aligned}$$

For 10,000 m³ applied water with nitrate concentration of 40 ppm the quantity of N added from the irrigation water would be $= 0.23 \times 40 \text{ ppm} \times (10,000 \text{ m}^3/1000 \text{ m}^3) = 92 \text{ kg}$.

14.8 Monitoring Leaf Nutrient Status

Monitoring the nutrient status of leaves can provide information on the current status and potential for a nutrient deficiency to develop. Currently, only two periods of leaf sample collection and analysis have been validated in almond: a summer sample collected in mid-July to early August, or an early spring sample collected towards the completion of spur leaf expansion. To ensure that the collected sample adequately reflects the nutrient status of the management zone the following protocol must be followed for both 'early spring' and 'summer' samples.

Leaves from five to eight well-exposed non-fruited spurs are sampled from around the canopy of each of 15–30 trees, each tree being a minimum of 30 m apart. If a mean nutrient concentration of the sampled zone is required,

then all leaves can be combined as a single sample for submission to a reputable laboratory. 'Summer' samples can be collected at any time from mid-July to mid-August, corresponding with full fruit maturity to full hull-split. 'Early spring' leaves are sampled at approximately 43 ± 6 days after full bloom when the majority of leaves on non-fruited spurs have reached full size.

For 'summer' samples or for samples that are targeted to identify a specific nutrient deficiency, samples should be analysed for the element of interest and results contrasted with established standards. For 'early spring' predictive tissue sampling, leaves must be analysed for a full nutrient panel (N, P, K, S, Ca, Mg, B, Zn, Cu, Fe and Mn) and then analysed using the UCD-ESP program. Most Californian testing labs use this program. For those that do not, results can be manually entered for calculation into the UCD-ESP program using resources at the Almond Board California website (Nitrogen Calculator) and the Fruit and Nut Center website (FNRIC Nitrogen).

14.9 Integrated Nitrogen Budgeting, Early-season Leaf Sampling and In-season Nitrogen Management

Because of its propensity to contaminate water resources, and its critical role in productivity, N management is the most critical aspect of orchard fertilization. To guide growers in the management of N, the Almond Board of California has funded the development of a detailed N management website (Almond Board Nutrient Management, www.almonds.com/nutrients#tc-management) that was developed on the basis of information provided in this chapter. This website also provides online calculators for N budgeting and early-season tissue sampling that are publically available and routinely updated (Nitrogen Calculator <https://www.sustainablealmondgrowing.org>).

The information in these websites and outlined in this chapter is based upon the 4Rs (right rate, right time, right placement and right source) approach to fertilizer management, which has gained widespread acceptance and provides an ideal basis for managing nutrients in crops (<http://www.ipni.net/4R>). In the 4R approach, fertilizers are applied in proportion to

the demand of the crop (right rate) and timed with periods of nutrient uptake (right time) so that plant demand is satisfied in a timely fashion, avoiding the application of fertilizers in excess of plant capacity for uptake. The choice of the correct fertilizer source (right source) and placement within the active root zone (placement) is essential to maximize fertilizer use and avoid losses. In almond the 4Rs have been supplemented with the early-season sampling protocol as an additional management tool. The steps involved in N management are summarized below.

Establish annual orchard-specific N management

1. Estimate the expected yield at bloom, based upon historic yield trends for each orchard, last year's yield, bloom intensity, climatic conditions.
2. Estimate annual inputs of N in irrigation water, manures, composts, etc., bee activity and grower experience.
3. Calculate preliminary fertilization rates based on 68 kg N per 1000 kg kernel yield less N inputs from other sources (manures, irrigation water, etc.).
4. Make first application of 30% of annual N demand in early spring (15–43 DAFB).

Collect and analyse April leaf samples according to the instructions given above.

5. Conduct in-season yield estimation at full leaf expansion (43 ± 6 DAFB).
6. Use April tissue sampling (5) and early-season yield estimation (6) to optimize the annual N fertilization plan by adjusting the mid-April to July and/or fruit maturity/post-harvest fertilization rates accordingly.
7. Apply 28% of annual N demand during the period of nut fill (from May to late June).
8. If yield was equal to or greater than predicted and if trees remain healthy following harvest, apply up to 12% of annual N demand immediately post-harvest while trees are still green and active.

Examples of several orchard scenarios can be found online at <http://fruitsandnuts.ucdavis.edu/files/165544.pdf> and at the Almond Board of California online N calculator (Nitrogen Calculator).

Manage N applications to ensure that applied N is retained in the active root zone, and minimize N leaching below the root zone

1. Almond does not use soil N until 15 days after full bloom. Prior to that time trees use N that has been stored in perennial organs in the previous year.
2. Almond does not use soil N once leaves begin to senesce after harvest.
3. N applications in excess of plant demand will not be used by the plant and will remain in the soil where they may be lost with subsequent irrigation or rainfall.
4. N applications should be provided in proportion to current tree demand in as many fertilization events as possible.
5. Irrigation and fertilization should be coordinated so that applied N is present in the active root zone and is neither pushed below the root zone nor deposited on the soil surface.

14.10 Occurrence, Identification and Correction of Common Nutrient Disorders

14.10.1 Nitrogen

N deficiency can be readily identified in almond. Early in the season N-deficient trees will have small, pale leaves, shoot growth will be reduced and foliage may be sparse. In summer, trees will show a uniform yellowing of leaves, which will be more prevalent in older leaves and in fruiting spur leaves. In mature trees in which new in-season growth is limited, symptoms may occur uniformly through the entire tree. In autumn, leaves of N-deficient trees will become very pale yellow and will senesce earlier than normal. If all trees in an orchard are deficient, symptoms may be difficult to discern as there will be few healthy trees or leaves to contrast with.

Excess N application may have only a moderate effect on tissue N concentrations and in young trees will result in increased vegetative growth and susceptibility to diseases such as hull rot, which can affect the survival of spurs and reduce yield in subsequent years (Saa *et al.*, 2016).

N deficiency can be corrected by applying N fertilizers as described above.

14.10.2 Potassium

K-deficient leaves begin showing symptoms in late spring to early summer. Leaves become pale (like those with N deficiency), and leaf size and shoot growth are reduced. In slight deficiencies, the first leaves to be affected are generally along the middle of the shoots. In more severe cases, all leaves can be affected. The tip and sub-terminal margins of leaves on more vigorous shoots become necrotic, and this often causes the leaf tip to curl upward, a condition referred to as 'Viking's prow'. Nut size is reduced. 'Butte' is a good indicator cultivar because it displays marked symptoms of deficiency while adjacent trees have only mild symptoms. K-deficient spurs have lower bloom in the following years; hence K deficiency affects the next season's yield more than that of the current year (Reidel *et al.*, 2004).

Almond has a high demand for K and, while deficiencies can occur in any region, they have historically been more prevalent in areas with K-fixing soils (heavy clay soils) and in sandy soils. Young, vigorously growing trees are particularly susceptible.

K is the nutrient removed in highest quantity in harvested nuts from an almond orchard, hence annual application is required for better yield. A soil test performed at the beginning of the season can help to assess the quantity of K to be applied. Apply K fertilizer as described above.

14.10.3 Magnesium

Mg is an activator for many enzymes involved in energy transfer and growth processes. It is the central element in the structure of chlorophyll and hence has an essential role in photosynthesis.

Mg deficiency is usually associated with low pH soils or sandy, neutral soils. Symptoms appear in mid- to late summer, after considerable shoot growth. The basal leaves, especially those on vigorous shoots, begin to show a distinct chlorotic region at their apical and lateral

margins, eventually developing an inverted 'V'-shaped green area along the basal part of the midrib, and they senesce early.

Mg deficiency can be corrected by foliar sprays of Mg fertilizer early in the season. There are many soluble Mg fertilizer sources such as magnesium sulfate that can effectively correct Mg deficiency.

14.10.4 Zinc

Zn is an essential constituent of a large number of enzymes and proteins and plays a key function in flowering, early spring growth and in the maintenance of membrane integrity and hormone synthesis. It has a key function in the maintenance of the plant hormone auxin, which plays a major role in cell elongation and growth. Zn is also required for chlorophyll synthesis, and is essential in plant defence against environmental stress.

Zn deficiency occurs in sandy and alkaline soils. It becomes insoluble in soils with pH above 8.5, and high-pH soils may thus become non-responsive to soil Zn applications.

Symptoms of Zn deficiency appear early in the season and will be exacerbated if soils are cool and wet when spring growth commences. The first symptom of Zn deficiency is a delay in opening of the vegetative and flower buds, which can be delayed by as much as several weeks; thus, a Zn-sufficient tree may be in full leaf while a severely Zn-deficient tree is just beginning to bloom. Late bloom induced by a lack of Zn results in poor pollination and greatly reduced yield. When vegetative buds do open the leaves are small, chlorotic and appear in tufts, giving rise to the terms 'rosette' and 'little leaf'. Mildly Zn-deficient leaves are only slightly reduced in size but have chlorotic areas between the lateral veins. These chlorotic areas merge into larger areas and become pronounced along the margin and apex of the leaves when deficiencies are severe. When young, these leaves often have a distinct, undulating leaf margin. In severely deficient cases, terminal dieback sometimes occurs. As the season progresses, subsequent shoot growth tends to mask the symptoms of Zn deficiency that were present earlier, making diagnosis more difficult. Fruit on severely

Zn-deficient trees are markedly smaller than normal; however, with moderate Zn deficiency while overall fruit set may be reduced, individual fruit size may be unaffected. Zn deficiency mostly occurs early in the spring as the low soil temperature and wet conditions reduce soil microbial activity and plant Zn uptake. When the soil conditions improve, Zn uptake increases and deficiency symptoms disappear.

Foliar applications have been the most effective treatments for correcting deficiency in almond orchards. While a single application is usually sufficient for the growing season, foliar sprays of Zn provide only a temporary annual correction and reapplication may be required every year (Brown *et al.*, 1996b). Application of Zn when the leaves are about to reach full size is more effective than applying Zn to mature leaves. In early spring, before leaves have reached full size, sprays of Zn chelate (Zn EDTA) at 1 kg per 380 l or zinc sulfate (ZnSO_4) at 2.3 kg per 380 l are effective. Take care when using zinc sulfate at this stage as leaf burn can occur. In late spring or early summer, when leaves have attained full size, the effectiveness of most Zn sprays is markedly reduced, though some growers report success using zinc oxide or basic zinc sulfate (2.3 kg per 380 l water) or Zinc chelate (1 kg per 380 l water). There has been a long history of applying large amounts of zinc sulfate (36% Zn) at 4.5 kg per 380 l water at the beginning of normal leaf drop in the autumn (end October–end November). This treatment will cause leaf injury and leaf fall but no injury to the trees. A dormant spray of zinc sulfate (36% Zn) at 5–6.8 kg per 380 l water is also a common application. While these treatments are generally effective to promote leaf drop, they are actually very inefficient and the vast majority of the applied Zn does not enter the tree, but rather is deposited on the soil in the fallen leaves.

Do not use zinc sulfate sprays immediately before or following an oil application as tree damage can occur; apply zinc sulfate two or more weeks after oil spray.

Soil pH (pH > 8.0) can have a profound effect on the occurrence and correction of Zn deficiencies, and applying Zn to these soils to correct deficiency without correction of the underlying high pH can be an slow and expensive process. Soil applications of sulfur or acid to reduce soil pH will frequently improve Zn availability and crop response to soil-applied Zn fertilizers. The widespread use of drip- and micro-irrigation

in the almond industry offers an improved ability to locally reduce soil pH and to improve the availability of native and applied soil Zn. Soil Zn fixation (in which fertilizer Zn becomes unavailable) is increased in soils that go through a wet–dry cycle between irrigation events and is minimized under high-frequency, low-volume irrigation regimes that do not experience extreme wet–dry cycles. Direct injection of soluble Zn fertilizers through the drip- or micro-irrigation system has been effective in many situations.

14.10.5 Boron

B is required for new cell division, cell wall growth and fruit set; it may also have a role in maintaining cell membrane integrity. Reproductive structures have a higher demand for B and are particularly sensitive to B deficiencies. B is extremely important in flowering, pollen viability, pollen tube growth and fruit set (Brown *et al.*, 1996a).

Moderate B deficiency does not produce any recognizable leaf symptoms, but is characterized by low fruit set and excessive nut drop. The lack of crop often results in excessive vegetative growth, giving the tree an appearance similar to that of non-productive syndrome ('bull' condition). In 'Peerless', brown gummy areas develop in the endocarp, with gum extruding to the surface of the nut. The embryos in these affected nuts usually abort, and the nuts drop in April or May. The result may be loss of much of the crop. Other cultivars may blossom well but nuts fail to set and spurs develop a non-productive 'willow twig' appearance. 'Mono' and 'Peerless' may produce partial or malformed nuts.

With severe deficiency, the terminal and sub-terminal leaves on watersprouts and other vigorous shoots develop tip scorch, and the leaf tips curl upward and defoliate. The terminal of an injured shoot subsequently dies back. In the following year several buds below the dieback produce shoots, resulting in a brushy appearance. Trees exhibiting severe B deficiency will be non-productive.

With the exception of a severe B deficiency that results in vegetative symptoms, the goal of a B fertilization programme is to ensure there is adequate B in the flower buds to allow for optimal flowering, pollination and fruit set.

To achieve this, fertilizer treatments should be timed to deliver B to flower buds during their development, which occurs in the period from July to late September. Applications of B after full fruit maturity (hull-split) are superior to earlier sprays since almond fruit are a very strong sink for B and applications made prior to fruit maturity (hull-split) will result in increased fruit B concentrations but may not enhance bud B in the developing flower buds.

B can be applied as a soluble soil or foliar application, provided the application is timed to meet the needs of the developing flower bud. Most commercial B fertilizers available in the market have a high level of solubility and are effective. Injection of B into the fertigation system or foliar B applications have also been shown to be effective. Foliar B applications can also be made during the period from bud-swell until 'popcorn' bloom, though this is somewhat less effective than the August–September time frame. B application in September significantly increased yield in the subsequent year compared to B application in February (Nyomora *et al.*, 1999). Pre-bloom B sprays can be beneficial, particularly if trees entered dormancy with marginal B supplies or if the status of the trees is uncertain. Soil applications in the early spring will not be taken up until after leaf out and hence will not benefit the current year's crop.

Foliar applications of a dilute spray of a soluble B material at the rate of 0.5–1 kg B per 380 l water have been shown to be effective at correcting moderate B deficiency. B is rapidly absorbed by sprayed tissues and redistributed to growing organs, and can be applied to the foliage any time you detect B-deficiency symptoms. This treatment may not affect the current season's crop, but it should benefit the current season's growth and may enhance fruit set in the following year. B is highly mobile in soils and also mobile in almond trees. B is stored in the tree organs during dormancy and reused during flowering in the absence of soil B uptake.

When used at the correct rates, B is safe and will not contribute to B toxicity. B can, however, be toxic and care should always be taken to ensure the correct rate is used and any application is distributed uniformly in the field.

Ongoing monitoring of B concentrations in the hull is an essential component of any B fertilization programme and decisions on the

use of B should be made according to the guidelines in [Table 14.1](#).

14.10.6 Copper

Cu is a component of many enzymes in the plant and plays a role in energy metabolism. Cu is immobile in the phloem and its deficiency occurs in the youngest leaves. Symptoms appear in early summer to midsummer. Leaves at the tip of a current shoot become pale and develop scorch, and defoliation follows. Terminal dieback occurs in mid- to late summer. Poor shoot growth and dieback causes the trees to be stunted and bushy in appearance due to excessive branching below the dieback. The bark of the trunk and scaffolds become dark and rough. In 'Nonpareil' trees about 1 month before bud break, a Cu deficiency causes rough bark and severe gumming from trunk and scaffold limbs. The symptoms of Cu deficiency in leaves are similar to Zn deficiency, but can be separated by the timing of occurrence of Zn deficiency occurring in early spring, while Cu deficiency occurs in midsummer.

Soil applications or foliar sprays can correct this problem. In past years Cu deficiency was corrected by large applications of copper sulfate or copper chelate to the soil. These approaches are no longer recommended as they are environmentally and economically unfavourable. Soluble Cu fertilizers are now widely available for direct injection into the fertigation system and can be effective. Foliar copper chelates at 0.23 kg per 380 l water will also give good correction. Be aware, however, that copper chelate sprays sometimes produce leaf injury and defoliation. With either spray, correction should last at least 2 years. The best long-term correction can be expected from a combination of soil and foliar treatments applied yearly.

14.10.7 Iron

Fe is involved in the manufacturing process of chlorophyll and is required for certain enzyme functions. It is found in the Fe-containing (haem) proteins in plants and is important for the electron transfer system in the chloroplasts and mitochondria.

Fe deficiency can occur in highly calcareous soil with a pH of above 7.5, or in heavy and poorly drained soils. Leaves are chlorotic early in the season and a fine netted veinal chlorosis may develop. The chlorosis may disappear as the season progresses. In severe cases the chlorotic leaves may be uniformly yellow, develop scorch and then abscise. Fe chlorosis symptoms are frequently associated with calcareous soils (in such a case the problem is called lime-induced chlorosis) or in heavy, poorly drained soils and especially in cold, wet springs.

Availability and uptake of Fe can be increased by lowering the soil pH through band application of elemental sulfur or by injecting sulfuric acid into the irrigation system, which will create a zone of low pH and increase solubility of Fe and also other nutrients such as Zn. In soils with high pH and high residual lime, foliar applications of a soluble Fe source or injection of chelates directly into the drip-irrigation system can be effective.

14.10.8 Manganese

Manganese is important in photosynthesis and activates many enzymes in plants, and a deficiency can greatly reduce photosynthesis and yield of the crop. Mn-deficient leaves are pale (as they are when N is deficient) with small, chlorotic areas scattered throughout the leaf creating a mottled appearance suggestive of Zn deficiency. Compared to the chlorotic areas on Zn-deficient leaves these are more concentrated between the main lateral veins. Leaf size and nut size are normal unless the tree is very deficient. 'Peerless' is sensitive to Mn deficiency.

In soils with high pH and high residual lime, foliar applications of a soluble Mn source or injection of Mn chelates directly into the drip-irrigation system can be effective. Mn availability can be increased by lowering soil pH by applying sulfur or injecting sulfuric acid into the irrigation system.

14.11 Nutrient Toxicities

B, Cl and Na are present in excessive amounts in many almond-producing areas in the world

and their levels are increasing as growers increase their use of salt- and B-contaminated ground and surface irrigation water.

14.11.1 Boron toxicity

Irrigation water is the most significant source of B for most almond-producing regions and long-term irrigation with water exceeding 1 ppm B can result in the accumulation of B in almond tissue and the development of toxicity symptoms. B is a cumulative toxicant and symptoms will become increasingly worse as the duration of exposure to high-B irrigation water increases. The choice of rootstock can significantly impact the uptake of B from the soil. Peach rootstocks tend to take up more B, and greater tissue B concentrations result than in almond or peach-almond hybrids. In regions with chronically high soil and water B levels the choice of the correct rootstock is essential for long-term viability.

The principal damage caused by B toxicity is to the shoots of the current season's growth. Shoots die back at the tip, losing all terminal leaves for up to 30 cm. Stem tissue becomes brown and necrotic and sometimes it cracks. Gummy deposits may form around the cracks and in the crotch of the tree. Corky areas develop on the midrib on the lower side of the leaves. Unlike leaves of other nut crops, almond leaves may not exhibit marginal burn though young leaves are often small and have wavy margins. B tends to accumulate in fruit tissue, and hull is a useful and sensitive indicator of B toxicity.

14.11.2 Chloride and sodium toxicity

Excesses of both Cl and Na produce leaf-tip burn that looks similar. In severe cases, the necrotic area at the tip increases during the growing season so that by late summer the terminal half of the leaf, or more, is necrotic.

Na toxicity produces a striated pattern in the scorched area. The striations are perpendicular to the midrib, making it appear that necrosis occurred in waves. 'Mission' and 'Fritz' are quite sensitive to Na toxicity while 'Nonpareil' is relatively tolerant.

Cl toxicity produces a necrosis similar to Na toxicity but generally containing fewer striations. Leaf analysis should be conducted to distinguish between Cl and Na toxicity. Frequently, toxicities of both Na and Cl will be present in salt-affected trees.

Rootstocks also differ markedly in their salt tolerance, with peach rootstocks ('Nemaguard', 'Lovell') being more sensitive to salinity than almond or almond/peach/plum hybrids ('Hansen', 'Empyrean', 'Viking'). Information on relative salinity tolerance of different rootstocks is currently being evaluated and growers should consult the most recently available information when planting into a salinity-impacted location.

Toxicities of B, Na and Cl accumulate over time as the trees transpire water and it is

ultimately the accumulation of these elements in the plant and in the root zone that will damage productivity. Saline damage in almonds is therefore largely a consequence of the quality of the irrigation water and the duration of exposure to the poor-quality water. Availability of water of better quality can diminish and ultimately reverse salinity damage. Soil amendments with gypsum or organic matter can also improve plant performance under saline conditions by improving soil aeration and root growth, which will improve plant tolerance. If salts accumulate in the soil then a leaching treatment must be applied to the soil. Care should always be taken to minimize the application of leaching treatments when there is significant residual N in the profile.

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15 Almond Diseases

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15.1 Introduction

This chapter was coordinated by A. Palacio-Bielsa and is the combined effort of expert plant pathologists in Spain, the USA and France. The aim of this chapter is to provide an overview of up-to-date knowledge on major diseases affecting almond, and it is intended to be applicable worldwide.

The information provided is based on the authors' own experience and on bibliographical sources, including some extensive book contributions. It is divided into five sections. The first section describes diseases caused by graft-transmissible pathogens (viruses, viroids and phytoplasmas) and is authored by M. Cambra, C. Martínez, A. Olmos and V. Pallás. The second section includes bacterial diseases and is authored by A. Palacio-Bielsa and M.M. López. The third section describes diseases caused by fungi and fungal-like organisms with J.E. Adaskaveg, H. Förster and M.A. Cambra as contributors. The fourth section by H. Duval and D. Esmenjaud includes nematodes. The final section with J.E. Adaskaveg and H. Förster as authors describes some disorders with unknown aetiologies. Each

section includes a review of the disease distribution and importance, the causal agent(s), symptoms useful for diagnosis, and ecological and epidemiological information, as well as integrated management practices. Although reference is made to fungicides, too many compounds are regulated differently worldwide and may not be registered for the stated use in all countries. Therefore, current sources of information need to be consulted for updated recommendations.

Almond is a very old and traditional crop in many countries, and orchards are often located on marginal lands, especially in Mediterranean countries. Yields are usually very low and orchards suffer from well-known diseases. However, in recent years, a slow but steady trend toward improved cultural practices (drip-irrigation, planting in better soils, new and more productive cultivars, etc.) is happening to obtain higher yields, similar to those in other growing regions. Changes in production practices have also led to the occurrence of new phytosanitary problems or to the aggravation of certain old problems. Consequently, the objective of this chapter is to provide the reader with updated information on old and new diseases and their management.

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15.2 Graft-transmissible Pathogens: Viruses, Viroids and Phytoplasmas

15.2.1 Introduction

Almond graft-transmissible pathogens (viruses, viroids and phytoplasmas) cause common diseases of worldwide distribution and diseases that are restricted to specific regions. Some of them, especially those that result from mixed infections of different pathogens, are economically important, particularly when they affect yield or induce almond decline. In contrast, common single graft-transmissible pathogens that usually produce inconspicuous symptoms may reduce yield by 10–25% depending on the susceptibility of the cultivar, the pathogen strain involved and ecological conditions. Thus, economic losses are not negligible if the prevalence of these pathogens in the crop is high. Propagation of infected sources of plant material and their exchange over long distances are the main cause of the distribution of these pathogens in the almond industry. Still, natural spread via insects, nematodes or other vectors may also occur for some of them. The use in nurseries of symptomless infected trees, insufficiently controlled, for grafting or propagation contributes considerably to the spread of these pathogens. This section includes description of six characterized agents that are responsible for the main graft-transmissible diseases in almond: three from genus *Illarvirus* (prunus necrotic ring spot virus (PNRSV), prune dwarf virus (PDV) and apple mosaic virus (ApMV)), one from genus *Nepovirus* (tomato ringspot virus, ToRSV), one from genus *Trichovirus* (peach mosaic virus, PcMV) and one phytoplasma ('*Candidatus* Phytoplasma prunorum'). Although phytoplasmas are bacteria, historically they have been studied by virologists, and thus the disease is treated in this section. Brief comments are included for diseases caused by peach rosette mosaic virus (PRMV) from genus *Nepovirus*, '*Candidatus* Phytoplasma phoenicium', apple chlorotic leaf spot virus (ACLSV), plum pox virus (PPV) and hop stunt viroid (HSVd), as well as diagnosis/detection strategies and mitigation measures.

15.2.2 Illarviruses

PNRSV, PDV and ApMV are the most common viruses infecting almond trees worldwide (Savino

et al., 2011). PNRSV is the most widespread virus in almond and causes economically important diseases characterized by chlorotic and necrotic leaf spots, ringspots, diffuse yellowing and mottling (almond calico disease), mosaic, leaf line patterns on the spring foliage and bud failure (Fig. 15.1) associated with poor production (yield is reduced by about 15–20%). PDV is probably the most damaging of the illarviruses and causes similar leaf symptoms to PNRSV with small and usually deformed leaves, and short internodes, resulting in a tree that appears stunted and has a dense canopy compared with uninfected trees (Fig. 15.2). Mixed infections of PNRSV and PDV are associated with bud failure and rosetting (Fig. 15.3) (Martelli *et al.*, 2002), resulting in trees with a denuded appearance and reduced fruit production. ApMV is less prevalent than the other illarviruses. Typical symptoms include bright yellow discolourations, line patterns and ringspots on leaves that are masked at high seasonal temperatures, and bud failure.

These viruses, genus *Illarvirus* and family *Bromoviridae*, are characterized by a tripartite genome and isometric particles. They are phylogenetically closely related with at least 65% sequence similarity at the coat protein level (Pallas *et al.*, 2013). The three viruses are transmitted by vegetative propagation. PNRSV and PDV are pollen- and seed-borne pathogens (Amari *et al.*, 2007; Amari *et al.*, 2009). This aspect contributes to their rapid spread among trees (Mink, 1992).

15.2.3 Nepoviruses

ToRSV was first described in North America and is prevalent and detrimental in the USA on genus *Prunus* (almond yellow bud mosaic disease), *Malus* spp. and *Vitis* spp. On trees infected at an early stage of development, the virus can cause severe stunting. Leaves are crinkled, distorted and develop in rosettes on the regrowth. They are reduced in size and have chlorotic spots that result in tattering. Branches lack lateral growth, and leaves are concentrated in the terminal part. Yield is severely affected.

ToRSV is a member of the subgroup C of the genus *Nepovirus*, family *Comoviridae*, which has isometric particles about 28 nm in diameter and a bipartite, single-stranded RNA genome



Fig. 15.1. Almond bud failure caused by prunus necrotic ringspot virus (PNRSV). The foliage is concentrated at the terminals of branches that lack lateral growth. Mixed infections by PNRSV and prune dwarf virus (PDV) or infection by tomato ring spot virus (ToRSV) can cause similar or even more severe symptoms.

(Stace-Smith, 1984). The virus is transmitted by vegetative propagation and is a seed-borne pathogen that can also be transmitted by pollen, and over long distances, by the movement of infected material (Savino *et al.*, 2011). The nematode *Xiphinema americanum sensu lato*, a complex of related species, is the natural vector (EPPO, 2005) (see also the section on Nematodes).

Another nepovirus – PRMV – less prevalent than ToRSV, is present in North America (Martelli and Uyemoto, 2011) and has been reported in Turkey (Azerý and Çýçek, 1997). The virus infection results in delayed bud break, mosaic patterns on leaves, rosettes of leaves and stunted shoots. It is naturally transmitted by *Xiphinema americanum sensu lato*, *Longidorus diadecturus* and *L. elongates* (see also the section on Nematodes). In addition, it is a seed-borne pathogen with a reported percentage of seed transmission ranging from 4 to 90% (Martelli and Uyemoto, 2011).

15.2.4 Trichoviruses

Peach mosaic virus (PcMV) was widely prevalent in the USA and Mexico in the past, but has

not been observed in the field in California in the past 40 years. It is present in South Africa where it causes severe reductions in almond yield and fruit quality. The virus induces a mild foliar mosaic, chlorotic spots and blotches. Shoots are slightly shortened and rosetted.

PcMV belongs to the genus *Trichovirus*, family *Flexiviridae* (James *et al.*, 2006). It is transmitted by vegetative multiplication and by mechanical inoculation; and over long distances by movement of infected plant material. The natural vector of PcMV is the peach eriophyid mite *Eriophyes insidiosus* (Uyemoto, 2002), which is not present in Europe, the Mediterranean Basin and Australia.

15.2.5 Phytoplasmas

Phytoplasmas are restricted to the phloem sieve tubes. Unlike bacteria, phytoplasmas cannot be cultured *in vitro*, and they have small genomes. Due to their vascular habitat, these endogenous organisms have a systemic distribution in the plant, and are transmitted from plant to plant by graft inoculation or by phloem-feeding insects.



Fig. 15.2. Healthy almond tree with normal canopy size (first tree on the left) next to two trees of the same age infected by prune dwarf virus (PDV).



Fig. 15.3. Leaf curl and rosette growth (shortened internodes) induced by mixed infection of an almond tree with prunus necrotic ring spot virus (PNRSV) and prune dwarf virus (PDV).

Based on their morphological and ultrastructural resemblance to animal and human mycoplasmas, they were previously called 'mycoplasma-like organisms' but, in 1994, they were recognized as a genuine type of mycoplasma and placed in the class Mollicutes.

'*Candidatus Phytoplasma prunorum*' (former common name: European stone fruit yellows – ESFY) is a non-culturable prokaryotic pathogen that is present wherever stone fruits are grown in Europe and adjacent non-European Mediterranean countries. It is associated with early bud breaking in late winter and with economically important yellows and decline disorders of almond (Marcone *et al.*, 2010). In summer, almond leaves are light green, rolled along the longitudinal axis and curled downward. Leaves become thick with a rough surface, pronounced midribs and major lateral veins (Marcone *et al.*, 2011). Symptoms of the disease usually appear in a sector of the canopy affecting one or two branches. Trees infected for many years have fewer leaves which become chlorotic and are smaller than in healthy trees. In advanced stages, branch die-back occurs with subsequent tree death.

The phytoplasma is naturally transmitted by the psyllid *Cacopsylla pruni*, which is strictly oligophagous on *Prunus* spp. and as an adult usually overwinters on conifers. The natural spread of the disease is associated with high transmission rates.

'*Candidatus Phytoplasma phoenicium*' is another emergent prokaryotic pathogen that has been detected in Lebanon and Iran. The pathogen is associated with a rapid decline of trees after development of witches' brooms, reduced bloom and reduced fruit development (Verdin *et al.*, 2003). The vector has not been yet identified (Foissac *et al.*, 2011).

15.2.6 Other graft-transmissible pathogens

ACLSV is a member of the genus *Trichovirus*, family *Flexiviridae* and is distributed worldwide. In mixed infection with ilarviruses, especially with the prevalent PDV, it induces ringspots and line patterns in leaves. This prevalent virus only spreads by infected propagation material. PPV, the causal agent of sharka disease of genus *Prunus* (García *et al.*, 2014), does not affect almond; in fact, almond could be used as a source of resistance to PPV (Rubio *et al.*, 2003). HSVd has been

detected on almonds in Mediterranean countries (Cañizares *et al.*, 1999) but the impact on yield remains to be established. False positives were observed in almond extracts due to the presence of host RNAs with sequence similarity to HSVd (Cañizares *et al.*, 1999). As with other viroids, HSVd is mechanically transmitted. There are no known insect vectors (Sano, 2011).

15.2.7 Diagnosis, detection and control

The diagnosis and detection of almond viruses, viroids and phytoplasmas has been based for decades on biological assays (indexing) by chip budding graft inoculation of 'GF 305' or 'Nemaguard' peach seedlings or in the hypersensitive 'Shirofugen' cherry indicator (specifically for genus *Ilarvirus*) (Desvignes, 1999; Marcone *et al.*, 2011). Serological enzyme-linked immunosorbent assay (ELISA)-based procedures are extensively used for viruses of woody species (Boscia and Myrta, 1998; Cambra *et al.*, 2011). Transmission electron microscopy has been used to detect and localize phytoplasmas in infected tissues (Marcone *et al.*, 2011). Molecular hybridization approaches and molecular tests based on amplification of nucleic acids are also currently applied for diagnosis and detection of graft-transmissible pathogens (Cambra *et al.*, 2008). These methods include the sensitive real-time RT-PCR for RNA pathogens and real-time PCR for DNA pathogens (Torres *et al.*, 2005; Osman and Rowhani, 2006) as well as user-friendly methods for sample preparation (De Boer and López, 2012). Detection, especially in quarantine programmes, might in the near future rely on next-generation sequencing.

Preventing the introduction of new pathogens and vectors to unaffected regions is based on quarantine strategies and reliable identification of the initially infected plant material, including pollen (EFSA, 2013). For efficient control of graft-transmissible pathogens, propagation and use of planting material under a certification scheme are key strategies when establishing a new orchard. Nurseries producing basic propagation material need to be separated from commercial orchards to prevent infections by vectors and pollen flow from neighbouring diseased trees. Effective control of vector populations, removal of diseased trees and appropriate orchard management (e.g. elimination of succulent rootstock suckers to minimize vector infection)

are management strategies that complement the use of healthy planting material. Almond nursery stocks must be propagated from tested mother trees (preferably grown in protected facilities) and grafted on rootstocks that were produced under a certification scheme. Today, *in vitro* propagation of tested material and subsequent appropriate cultivation in glasshouse or screenhouse facilities is a routine practice in many nurseries that reduces the risks of infection. Thermo-therapy (usually 24–32 days at 38°C) (Martelli *et al.*, 2002), meristem tip culture and micro-shoot-tip grafting *in vitro* (Juárez *et al.*, 1992) have been used for sanitation. The development of almond cultivars resistant to different graft-transmissible pathogens through conventional breeding and/or biotechnological approaches needs to be more actively encouraged and explored and will be a useful approach in the future to prevent these diseases.

15.3 Bacterial Diseases

15.3.1 Introduction

Plant pathogenic bacteria and phytoplasmas are prokaryotic microorganisms that differ in the respective presence and absence of a cell wall. Both are unicellular and lack a nuclear membrane and other membrane-enclosed organelles. Common plant pathogenic bacteria have a cellular membrane surrounded by a rigid cell wall and they can be cultured in common or selective media. Most of them are exogenous bacteria with epiphytic and endophytic phases that colonize the apoplast (intercellular spaces) of plant tissues. Some exogenous bacteria such as *Xylella fastidiosa*, however, are limited to xylem vessels and induce vascular infections.

Presently, five bacteria-caused diseases and two phytoplasma-caused diseases occur on almonds. In this section the main bacterial pathogens of almond trees, based on their incidence, economic damage and threat to agriculture are described: *Agrobacterium* spp., *Pseudomonas amygdali*, *P. syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *pruni* and *Xylella fastidiosa*. Information on phytoplasmas is presented in section 15.2, under 'Graft-transmissible pathogens'.

The current geographic distribution of these pathogens can be found at the EPPO Global

Database <https://gd.eppo.int> (EPPO, 2017). Occurrence of bacterial diseases is sporadic because they are highly dependent on microclimatic conditions. However, when favourable conditions occur, bacteria can be responsible for high economic losses. Effective chemical control of bacterial diseases is limited mostly to copper, alone or associated with ethylene bis-dithiocarbamate compounds, and antibiotics. European Union (EU) legislation, however, prohibits the use of antibiotics. Therefore, management of bacterial diseases relies primarily upon quarantine measures using certified pathogen-free plant material, avoiding dissemination of pathogens by eliminating contaminated plant material from nurseries and plantations and, when available, through the use of host plant resistance.

The economically important product of almond is the kernel as compared to other *Prunus* species where the entire harvested fruits are marketed. Still, a healthy almond hull and mesocarp are vital to protect the kernel against bacteria. Infections of the hull often penetrate into the mesocarp and affect the kernel quality that is essential in many almond-based products. When the kernel is sliced, the endosperm needs to be without blemishes. Thus, phytosanitary measures for propagation and distribution of healthy trees, as well as in-season management programmes to protect leaves and fruits and maintain tree health, are integral parts of bacterial disease management.

15.3.2 Crown gall disease (*Agrobacterium* spp.; syn. *Rhizobium* spp.)

Crown gall is caused by several species of *Agrobacterium* that were formerly included in *A. tumefaciens sensu lato*. Based on more recent taxonomic revisions, tumourigenic bacteria affecting almond belong mainly to the species complex *Rhizobium tumefaciens* and *R. rhizogenes* (Young *et al.*, 2001). Crown gall is a chronic and resurgent problem in almond nurseries and causes severe losses in many countries because affected plants should be destroyed (Pulawska, 2010). Tumourigenic agrobacteria are considered quality pathogens by EU legislation (Anon., 1993), while in other countries they are more drastically controlled by national regulations.

Bacteria belonging to the genus *Agrobacterium* or *Rhizobium* are aerobic, motile, non-spore-forming, Gram-negative rods with peritrichous flagella. The strains causing crown gall disease harbour a plasmid called Ti, and a fragment of it (i.e. T-DNA) is transferred into the plant chromosome upon infection. Expression of T-DNA genes, which encode for synthesis of the plant hormones auxin and cytokinin, causes uncontrolled plant cell division and growth resulting in tumour (gall) formation.

The main symptom of the disease is the formation of tumours or galls. They are usually formed at the crown or on the main or lateral roots (Fig. 15.4) but, in some cases, can develop on aerial parts of the plants. Galls vary in size, from nearly microscopic to 25 cm or more in diameter. Their surfaces are usually smooth when young, turning to rough and dark when older. The disease is most serious for young nursery plants, which may be unfit for marketing. The direct effect of the disease depends on the location, number and size of the galls. When numerous and large tumours are formed, major roots or crowns may be partly girdled, resulting in tree dwarfing and reduced production (Flint, 2002).



Fig. 15.4. Crown gall of almond rootstock caused by *Rhizobium* spp. Tumours at the crown.

Galls and girdled tissue may also serve as infection sites for wood decay fungi. These fungi exacerbate the decline of the tree by weakening the structural integrity of the wood and predisposing trees to environmental damage (e.g. wind storms) or to damage from crop load that results in fallen trees.

These bacteria are typically considered soil- and rhizosphere-borne microorganisms, but they are also able to migrate inside the plants and to survive in different environments (Moore *et al.*, 2001). They are ubiquitous in agricultural and non-cultivated soils where tumourigenic bacteria can survive for years and form tumours when plants with wounds are available. When tumours become old and necrose, the pathogen can return to the surrounding soil where it can survive again in the absence of host plants. The ability to survive in latently infected plants allows for pathogen dissemination with nursery stock.

Crown gall is difficult to control with chemical treatments, since tumours can be induced by a single bacterial cell. Once the host cell is transformed by the T-DNA, the autonomous growth of the transformed plant cell does not require the presence of the bacteria. Therefore, management strategies must focus on prophylactic methods such as avoiding planting in infested soils, disinfection of substrates, disinfection of tools and strict inspection of nursery stock. Diseased seedlings or trees should be discarded. Infections can be minimized by avoiding practices that causes injuries, specifically at root level.

Soil disinfection by heat, solarization or other methods can be an effective measure. However, it is expensive and sometimes not reliable because soils or substrates are prone to recontamination after the treatment, and then disease incidence can be high due to lack of competition from other soil organisms. Addition of olive mill waste water to soil has been reported to significantly reduce the disease on bitter almond (Yangui *et al.*, 2008).

Biological control, based on use of the antagonist strain K84 currently classified as *Rhizobium rhizogenes* (formerly *A. radiobacter*), has been the most effective method for prevention of crown gall disease worldwide for many years (Moore and Warren, 1979; Penyalver *et al.*, 2000). Control is achieved through several mechanisms, including production of the antibiotic agrocin 84, which selectively affects *Agrobacterium* strains (Kerr and

Htay, 1974). However, the transfer of the plasmid that is responsible for its synthesis to *A. tumefaciens* strains in the soil may result in virulent transconjugants that are not affected by the treatment. This can pose problems at midterm. To safeguard this biocontrol efficacy, strain K1026, a deletion mutant of strain K84, was constructed and proposed as a safe substitute (Jones and Kerr, 1989). At present it is registered in Australia, New Zealand and the USA.

Comparative susceptibility of almond rootstocks to crown gall has not been well studied. Peach rootstocks such as 'Lovell', 'Nemaguard', 'S 37' and 'Rancho' showed great variability and are mostly susceptible (Norton *et al.*, 1963), whereas 'Harrow Blood' and 'Myrobalan' seedlings are considered tolerant (Layne, 1974). Experiments in the greenhouse and in the field using several bacterial strains showed that almond and peach rootstocks, bitter almond, 'Fasciuneddu', 'GF 677' and 'GF 557' were highly susceptible to crown gall, and that 'Cadaman' was more resistant (Rhouma *et al.*, 2005).

15.3.3 Hyperplastic bacterial canker of almond (*Pseudomonas amygdali*)

Hyperplastic bacterial canker of almond was first described in Greece (Psallidas *et al.*, 1968) and it has also been reported in Turkey (Gündoğdu and Kaya, 1976) and Afganistan (EPPO, 2017). The disease was considered the most destructive bacterial disease of almond in Greece. It is caused by *Pseudomonas amygdali*, which differs significantly from other pathogenic *Pseudomonas* spp. and is specific to almond (Psallidas and Panagopoulos, 1975). The bacterium is a Gram-negative, strictly aerobic, non-spore forming rod with flagella.

The characteristic symptom of the disease is the formation of swollen cankers on branches, twigs and trunks. Cankers usually initiate around the leaf scars or any other wound. First symptoms appear in late winter as a swelling of the bark around the leaf scars. A longitudinal crack then develops on the bark, extending into the wood. The affected bark tissues split apart and open cankers 0.5–2 cm in length are formed, surrounded by swollen, rough, dark-brown margins (Fig. 15.5). In section, the thickened cortical tissues exhibit a soft-cheese appearance with scattered



Fig. 15.5. Open canker with rough margins; symptoms caused by *Pseudomonas amygdali* on almond cultivar 'Raptopoulou'. (Photo courtesy of M. Holeva, Benaki Phytopathological Institute, Kiphissia, Athens, Greece.)

water-soaked spots which turn brown upon aging (Fig. 15.6). Cankers are active throughout the year and for many years, increasing in size. Canker length on 2-year-old shoots is about 3–5 cm, while they may reach 15–20 cm on older branches and the trunk. Canker formation near the buds results in failed flower production or reduction in flowering and shoot development. No specific symptoms occur on leaves, but severely affected trees have poor growth, reduced foliage and decline gradually.

P. amygdali cannot live epiphytically on almond trees according to the available data (Psallidas, 1997). There is no evidence of soil survival, and infected almond trees are probably the only source of inoculum. Leaf scars are crucial in the infection process in autumn, coinciding with the most favourable environmental conditions. The bacterium overwinters in the cankers and can be disseminated over short distances by rain and wind.

Management relies on an integrated strategy directed to minimize pathogen dissemination and inoculum availability. Propagation material



Fig. 15.6. Section of a canker showing scattered brown areas caused by *Pseudomonas amygdali* on almond cultivar 'Raptopoulou'. (Photo courtesy of M. Holeva, Benaki Phytopathological Institute, Kiphissia, Athens, Greece.)

should be taken from disease-free areas, and movement of material from affected to disease-free areas should be prohibited. Regular inspections should be done in nurseries and orchards. Affected twigs and shoots should be pruned away, preferably before leaf fall.

Copper compounds, particularly Bordeaux mixture, may be used at 3% concentration in autumn at the 75% leaf-fall stage and at 1% in spring at pink-bud stage (Gündoğdu and Demir, 1990), and immediately after hail.

Cultivars 'Marcona', 'Cristomorto', 'Ferra-duel' and 'Ferragnès', in descending order, are considered as having a good degree of resistance (Psallidas and Stylianides, 1985). Cultivars 'Texas' ('Mission'), 'Nonpareil' and 'Pagrati', as well as some Turkish selections (101-23, 120-1, 104-1, 101-9 and 300-1), have been classified as tolerant (Psallidas and Stylianides, 1985; Gündoğdu and Demir, 1990).

15.3.4 Bacterial canker and bacterial blast of stone fruits (*Pseudomonas syringae* pv. *syringae*)

Bacterial canker (also known as gummosis or sour sap) and bacterial blast are serious diseases of almond that occur in major production areas worldwide. Bacterial canker can affect young plants in the nursery and also adult trees. Sometimes spread of the disease is very rapid, and remarkable losses of fruiting wood, branches, scaffolds or



Fig. 15.7. Active canker caused by *Pseudomonas syringae* pv. *syringae* showing reddish flecking at the margin. (Photo courtesy of Jack Kelly Clark, University of California Statewide IPM Program, © 2000 Regents University of California.)

entire trees may occur. The incidence and severity of the disease is higher in areas with a continental climate characterized by the occurrence of winter and spring frosts, as compared with Mediterranean areas (Kennelly *et al.*, 2007).

These diseases are caused by *Pseudomonas syringae* pv. *syringae* van Hall 1902, which is unique among most *P. syringae* pathovars in its ability to cause disease in over 180 species of plants belonging to several unrelated genera (Bradbury, 1986). The bacterium is a Gram-negative, strictly aerobic, straight or slightly curved rod with one or several polar flagella.

The pathogen can form either limited cankers on trunks, scaffolds or small branches, or a more severe syndrome known as 'sour sap' which is generally observed in young trees. Externally, active cankers are usually not visible unless there is some gumming. Internally, the bark and outer layers of wood appear dark reddish brown (Fig. 15.7). In active cankers, small brown flecks in the bark beyond the canker margin are commonly observed. Cankers are active during dormancy but they are not apparent

until early spring when amber-coloured sap is observed. Branches that are girdled by a canker may die, producing brownish watery ooze on the surface of the bark. Inactive cankers appear rough, elongated and sunken into the bark.

'Sour sap' occurs when *P. syringae* pv. *syringae* invades a tree early enough in dormancy to allow the bacteria time to kill a large branch or the entire above-ground portion of the tree. The

dead wood appears brown, moist and sour smelling. In spring, diseased trees produce a watery sap that runs down the tree. The pathogen can cross the graft union but is restricted to the above portion of the tree. Affected trees may produce suckers from the base of the trunk, but their entire above-ground portion may die (Fig. 15.8).

Bacterial blast occurrence depends on cold or freezing temperatures and rain during the

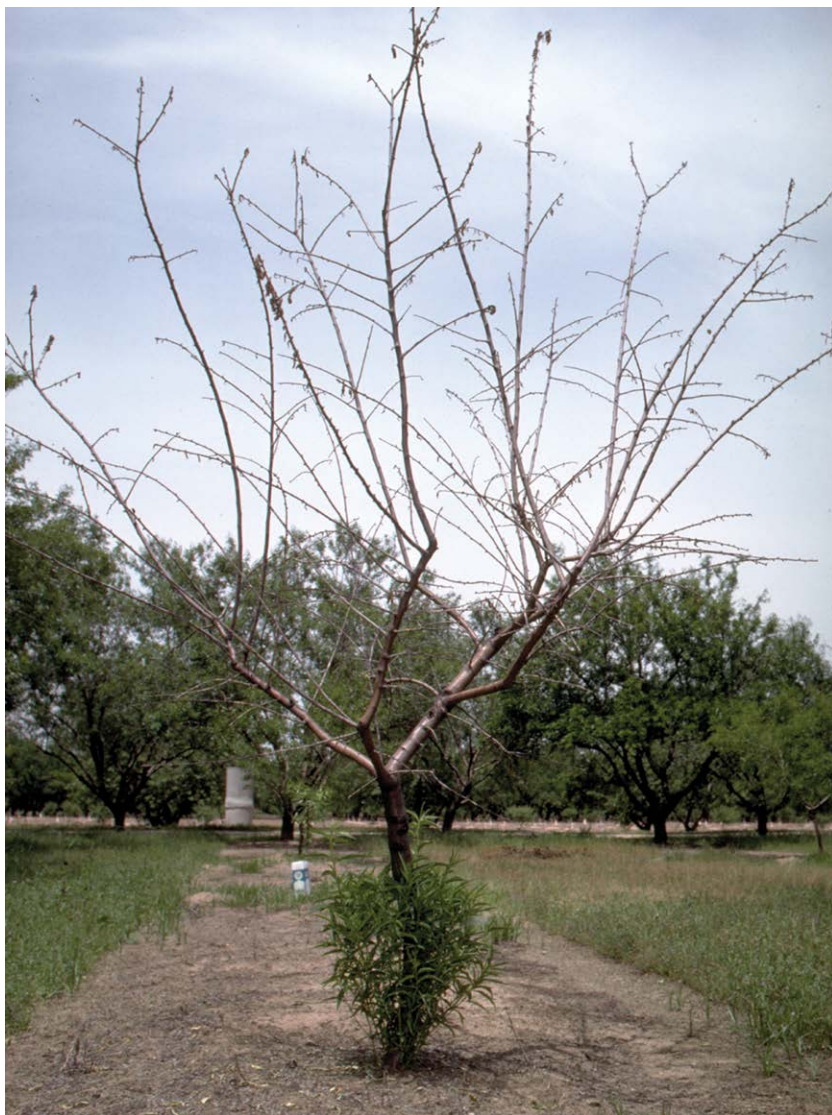


Fig. 15.8. 'Sour sap' showing regrowth from the rootstock of a young tree killed by *Pseudomonas syringae* pv. *syringae*. (Photo courtesy of Jack Kelly Clark, University of California Statewide IPM Program, © 2000 Regents University of California.)

bloom period. *P. syringae* pv. *syringae* attacks just before, during or shortly after bloom and kills buds, flowers, young leaves and shoots (Fig. 15.9). Flowers, apical shoots or opening buds turn black and leaves may develop dark spots that fall out, leaving a hole.

P. syringae pv. *syringae* can survive inside the host as a pathogen and externally as an epiphyte (Crosse, 1966). During summer, the epiphytic population decreases considerably, but increases again in autumn with lower temperatures and rainfall. The pathogen, carried by driving rain and wind, reaches the leaf scars or wounds and moves systemically into the bud and twig where it forms cankers. In the following early spring, as trees emerge from dormancy, callus tissue is formed around the canker and activity of the bacteria is suppressed, although in rare instances the pathogen may become active the following winter.

In general, bacterial canker occurs primarily on stressed trees, and any type of wound can be used by the pathogen for entering into the plant. Winter and spring frost, hailstorms, but also pruning, can dramatically favour its penetration. Most strains of *P. syringae* pv. *syringae* have ice-nucleating activity, forming ice in plant organs colonized by the bacterium at -2°C to -3°C , thus causing rupture of the plant cell wall and enabling tissue colonization. Trees are more susceptible if grown on coarse-textured or sandy soils, nitrogen-deficient soils or on those containing high populations of ring nematodes



Fig. 15.9. Bacterial blast caused by *Pseudomonas syringae* pv. *syringae* showing dead buds. (Photo courtesy of Jack Kelly Clark, University of California Statewide IPM Program, © 2000 Regents University of California.)

such as *Mesocriconema xenoplax* (see also section 15.5 on Nematodes).

Bacterial blast is associated with frost injury. If freezing temperatures occur and the pathogen invades the tree during the bloom period and at bud break, blast occurs in flowers and leaf buds die before opening. If infection occurs during bloom, blast symptoms appear on flowers and young leaves. In both cases, twig and spur die-back may also occur. Cultivars with early bloom are most susceptible to bacterial blast.

Control is mainly based on hygienic measures and copper-based compounds although copper resistance is common in some areas. Cultural practices, such as selecting appropriate soils, controlling nematodes and delaying pruning until late winter or spring when environmental conditions are warmer and drier, are advised. Protecting flowers from frost may provide control of bacterial blast. More information is still needed on disease susceptibility of rootstocks and almond cultivars.

15.3.5 Bacterial spot of almond (*Xanthomonas arboricola* pv. *pruni*)

Bacterial spot of almond is currently widespread in almond-production areas and has been reported in Australia, New Zealand (Young, 1977), India (Jindal *et al.*, 1990), Pakistan (Akhtar *et al.*, 1995), California (USA) (Holtz *et al.*, 2013); and in Europe, in Montenegro (Panič *et al.*, 1998) and Spain (Palacio-Bielsa *et al.*, 2010). It is caused by *Xanthomonas arboricola* pv. *pruni* Vauterin *et al.* 1995 (syn. *Xanthomonas campestris* pv. *pruni* (Smith)). The bacterium is Gram-negative, strictly aerobe, motile and has one polar flagellum. Because of its negative economic impact, *X. arboricola* pv. *pruni* is considered a quarantine organism by the EU phytosanitary legislation (Anon., 2000 and amendments).

Bacterial spot affects leaves, twigs, branches and fruits. Symptoms appear during late spring and early summer, and infections may also occur in autumn. Initial infections begin on leaves as small, angular, water-soaked lesions surrounded by chlorotic tissue. Subsequently, lesions turn necrotic; when they coalesce, large necrotic areas are formed resulting in 'shot-holes' upon detachment of dried tissues (Fig. 15.10). Lesions are visible on both sides of the leaves and are generally clustered in areas that remain wet for



Fig. 15.10. Necrotic leaf lesions on almond caused by *Xanthomonas arboricola* pv. *pruni*.

longer periods, such as the leaf tips and margins. In contrast to peach, yellowing is not observed on almond leaves. Premature leaf drop can occur in severely affected trees. Cankers in shoots and branches are less frequent on almond than on other hosts of this bacterium.

Symptoms on fruits are quite specific (Young, 1977; Anon., 2004; Palacio-Bielsa *et al.*, 2010; Roselló *et al.*, 2012). During spring, infected fruits initially display sunken, corky lesions and oozing gum that streams or clumps (Fig. 15.11). In summer, when the mesocarp dehydrates, the sunken lesions become raised (Fig. 15.12). In some cases, circular dark spots are observed on the endocarp, which can affect the kernel and even the nut (Fig. 15.13). Infected fruits either drop prematurely or remain on the trees after harvest.

The disease is less frequent in arid regions than in areas with temperate and humid climates. *X. arboricola* pv. *pruni* can survive and multiply as epiphytic and endophytic on symptomless tissues of their hosts (Shepard and Zehr, 1994). The bacterium overwinters in buds and leaf scars, as well as in infected fruit that remain on the tree (mummies) where primary inoculum is produced in the spring (Battilani *et al.*, 1999; Roselló *et al.*, 2012). *X. arboricola* pv. *pruni* enters



Fig. 15.11. Fruit infected by *Xanthomonas arboricola* pv. *pruni* showing initial sunken lesions and oozing gum.



Fig. 15.12. Fruit infected by *Xanthomonas arboricola* pv. *pruni* showing raised lesions on dehydrated mesocarp.

through lenticels, stomata, leaf scars or injuries, and migrates internally from infected leaves to twigs (Du Plessis, 1986). A warm spring season with temperatures of 19–28°C, frequent rains, winds and heavy dews favours bacterial multiplication and primary infections of leaves and fruits (Battilani *et al.*, 1999).

The pathogen is easily disseminated, and epidemics may develop rapidly. Chemical control



Fig. 15.13. Dark spots on fruit endocarp infected by *Xanthomonas arboricola* pv. *pruni*, and affected nut.

of the disease based on the use of copper compounds is the most commonly applied strategy in the field. In some growing regions, the use of copper–mancozeb mixtures is highly effective (Adaskaveg, personal communication). In other areas, no effective chemical control is available. Successful control strategies should consider an integrated approach including legislation, suitable agrotechnical measures, disease control in nurseries and breeding for resistance.

EU legislation provides a legal basis for action in areas where stone fruit production may be affected by *X. arboricola* pv. *pruni*, implementing measures to improve the phytosanitary quality of propagation material (Anon., 2000 and amendments).

Sandy soils and imbalance in nutrients (with an excess of nitrogen) render host plants more susceptible to bacterial infections. There is only limited information on susceptibility of almond cultivars. In Australia and California, many growers have been forced to abandon the two most severely affected cultivars, ‘Fritz’ and ‘Ne Plus Ultra’, due to extensive crop loss. ‘Mission’ and ‘Monterey’ were also shown to be susceptible, while ‘Nonpareil’ and ‘Price’ were considered intermediate in their susceptibility (Edstrom, 2007; Holtz *et al.*, 2013). In Spain, the disease can be

very harmful, with severity of infection depending upon cultivar and environmental conditions. Yield losses may be high for the most susceptible cultivars, which include ‘Antoñeta’, ‘Guara’, ‘Marta’, ‘Masbovera’ and ‘Vayro’ (Palacio-Bielsa *et al.*, 2010).

15.3.6 Almond leaf scorch (*Xylella fastidiosa*)

Almond leaf scorch (ALS), caused by *Xylella fastidiosa*, is a very severe disease that causes growth decline, decreased productivity and, in some cases, death of the affected trees. ALS develops slowly through the orchard, but significantly decreases productivity in 10–15 years. It was first described in Los Angeles (USA) in the early 1950s (Moller *et al.*, 1974). Since then, it has been reported in other almond-growing regions in America, and in a few Asiatic countries. The disease has been identified very recently on almond in Europe but only in the south of Italy (Saponari *et al.*, 2013; EPPO, 2017), where olive trees are the main host of the pathogen as well as in Mallorca (Islas Baleares, Spain) (EPPO, 2017). The bacterium is a regulated organism in the EU (Anon., 2000 and amendments).

X. fastidiosa, first cultured and described in 1987 (Wells *et al.*, 1987), is a Gamma proteobacterium of the family *Xanthomonadaceae*. It is xylem-limited, vector-transmitted, Gram-negative and very slow-growing with a temperature optimum of 26–28°C can affect more than 350 hosts (EFSA, 2016). There are three accepted subspecies of *X. fastidiosa*: subspecies *fastidiosa*, *pauca* and *multiplex* (Schaad *et al.*, 2004) based on DNA–DNA hybridization data. However, only subspecies *fastidiosa* and subspecies *multiplex* are so far considered valid names by the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria (ISPP-CTPPB) (Bull *et al.*, 2012). Several additional *X. fastidiosa* subspecies have been proposed based on multi-locus sequence typing (MLST) analysis (Scally *et al.*, 2005; Schuenzel *et al.*, 2015; Yuan *et al.*, 2010), including *sandyi*, *tashke* (*Chitalpa tashkentensis*, Randall *et al.*, 2009) and *morus* (Nunney *et al.*, 2014). In addition, a possible new species of *Xylella* in Taiwan associated with pear leaf scorch may be proposed (Su *et al.*, 2014). Strains of the subspecies *fastidiosa* and *multiplex* have been isolated from almond in California (USA) (Hernandez-Martinez *et al.*, 2006; Chen *et al.*, 2010), and in Italy there have been isolated strains of the subspecies *pauca* (Saponari *et al.*, 2013; Martelli *et al.*, 2016).

Some strains of *X. fastidiosa* cause Pierce's disease (PD) of grape, phony peach disease (PPD), citrus variegated chlorosis (CVC) and olive rapid decline. Moreover, *X. fastidiosa* causes a number of so-called leaf scorch diseases in *Prunus* spp., coffee, ornamental trees and many other plant species, including non-cultivated and ornamental plants that may carry the pathogen without showing symptoms (EFSA, 2016).

The most characteristic symptom of the disease in almond is leaf scorching, which first appears in the summer season. The leaves show chlorotic areas either on the tip or on the sides of the lamina, and the scorched area gradually enlarges over the entire lamina (Fig. 15.14A). Some leaves may drop prematurely, but many remain on the tree until the end of the season. These symptoms are not a specific indication of ALS and at the beginning can be confused with salt toxicity or mineral deficiency. Newly infected trees usually show a single affected terminal branch and, later in the season or in subsequent

years, adjacent branches may become infected. As the disease progresses from year to year, trees show reduced terminal growth, a large number of dead spurs, small branches and dieback. Entire trees may be systemically infected, often within 2–5 years; the yields are greatly reduced on affected branches and finally trees may die.

The bacterium is transmitted persistently by xylem-sap feeders and by propagation practices such as budding and grafting (Mircetich *et al.*, 1976). The insect vectors belong to the families *Cicadellidae*, *Aphrophoridae*, *Cercopidae* and *Cicadidae* within the sub-order *Cicadomorpha*. The sharpshooters *Draeculacephala minerva* and *Homalodisca vitripennis* (formerly *H. coagulata*) can transmit *X. fastidiosa* from grape to almond and within almond plants (Mircetich *et al.*, 1976; Davis *et al.*, 1980; Almeida and Purcell, 2003). Experimentally, other leafhoppers including *Graphocephala atropunctata*, *G. confluens* and *Carneocephala fulgida* and the meadow spittle bug *Philaenus spumarius*, are also able to transmit the bacterium (Purcell, 1980). The polyphagous glassy-winged sharpshooter (*H. vitripennis*) is now considered one of the most efficient vectors in California (Cao *et al.*, 2013), as is *P. spumarius* in Italy (Saponari *et al.*, 2014). Although the majority of species of American vectors reported seem to be absent in Europe, *P. spumarius* and *Cicadella viridis* can be potential local vectors in most European countries (Janse and Obradovic, 2010; Elbeaino *et al.*, 2014; Saponari *et al.*, 2014). Other species of sharpshooters and leafhoppers, however, could also be vectors. The bacterium has been found in a latent stage in many symptomless plants that may serve as reservoirs for vectors.

Once transmitted, *X. fastidiosa* invades only the xylem vessels and, in advanced stages of infection, forms biofilms associated with disease symptoms. Some almond cultivars are better hosts for overwintering populations of the bacterium than others, making them more susceptible to developing systemic ALS (Cao *et al.*, 2011).

Because chemical control of the vector populations is difficult, management of ALS must concentrate on its prevention. This includes not only the use of healthy almond material but also to avoid the introduction of any other infected host in the area. The plants should be produced in *X. fastidiosa*-free areas under strict control, and the establishment of new orchards only in disease-free areas. Once introduced,

eradication of the bacterium, measures for vector contention and contingency plans for the integrated control of local vectors (and of weeds that are reservoirs for *X. fastidiosa*) are necessary. If eradication is not possible, the use of less susceptible cultivars, cultural and hygienic measures, as well as chemical and biological control of vectors, must be integrated. These methods are, however, often only partially successful because the bacterium affects a broad range of symptomless hosts, and insect vectors can be abundant. Freezing temperatures have been shown to be therapeutic for ALS on almond trees (Ledbetter *et al.*, 2009).

Several studies have indicated a range of susceptibility to ALS among almond cultivars, but no resistance is reported. Although there are contradictory data, 'Nonpareil', 'Sonora' and 'Peerless' cultivars have been reported as most susceptible; while 'Butte', 'Carmel', 'Mission', 'Fritz' and 'Thompson' are considered moderately resistant (Groves *et al.*, 2005; Haviland and Viveros, 2005) (Fig. 15.14B).

Agricultural practices and adequate nutrition are required for preventing development of symptoms. Iron deprivation may reduce disease severity by avoiding biofilm formation in the xylem vessels (Toney and Koh, 2006). Severe pruning of newly infected almond limbs has shown promising results depending on the susceptibility of the cultivar (Cao *et al.*, 2013). It has been suggested that using peach as a rootstock with a high-grafted almond scion scaffold system could prevent *X. fastidiosa* spreading from almond scion limbs (Cao *et al.*, 2013).

15.4 Diseases Caused by Fungi and Fungi-like Organisms

15.4.1 Introduction

A large number of fungal pathogens attack flowers, foliage, fruit, branches and trunks of almond trees. Rootstocks of cultivated almond, namely peach, plum and peach-almond hybrids may be invaded by fungal and fungal-like pathogens causing root and crown diseases. The diseases covered in this section are considered important worldwide or only in some countries and most are also found on other species of the genus *Prunus* such as peach and cherry. Some diseases may mainly occur in the nursery, but most diseases occur in established orchards and determine the overall productivity and nut quality of the orchard during its lifespan. Of the diseases discussed, some consistently cause annual losses in production, whereas others occur more sporadically and develop under specific environmental conditions and on certain cultivars. Newer cultivars and high-input farming of almonds, with practices such as high-density plantings, high-nitrogen fertilization and long irrigation periods using more efficient irrigation systems, such as drip or micro-sprinklers, have recently increased the incidence of several diseases in some production areas.

Fungi and fungal-like organisms are eukaryotes (have a true nucleus, mitochondria and other organelles, as well as 80S ribosomes) and



Fig. 15.14. (A) Almond leaf scorch caused by *Xylella fastidiosa*. (Photo courtesy of D. Boscia, CNR – Institute for Sustainable Plant Protection, Bari, Italy). (B) The cultivar 'Nonpareil' (left) is infected and showing symptoms, whereas the adjacent cultivar 'Carmel' (right) is healthy.

belong to two kingdoms – the True Fungi and the Stramenopila (*Chromista*), respectively. These two kingdoms previously were grouped together as ‘Fungi’ because of similarities in growth, mode of nutrient uptake and reproduction by spores. Members of the True Fungi discussed here belong to the phyla *Zygomycota*, *Basidiomycota* and, most importantly, *Ascomycota*. The phyla are characterized by the specific ways that sexual spores (zygospores, basidiospores and ascospores, respectively) are produced. In general, sexual spores of the *Zygomycota* are not produced in any specialized structure. Sexual spores of the *Basidiomycota* may be formed in fruiting structures called basidiomes, and those of the *Ascomycota* are commonly formed in ascoma. Ascoma can be cup-shaped (apothecia), flask-shaped (perithecia) or spherical (cleistothecia, chasmothecia), or are embedded in cavities within fungal tissue (ascostroma). In addition, asexual spores may be formed in large numbers, often in special fruiting structures (e.g. acervuli, pycnidia, sporodochia), and these spores are mostly responsible for the spread of disease and the development of epidemics. *Zygomycota* fungi produce sporangiospores; *Ascomycota* and some *Basidiomycota* may produce conidia in distinctive asexual fruiting structures or freely (not in any structure). Species in all three taxa may produce somatic spores called chlamydospores. Sclerotia are dense survival structures formed by fungal mycelium. Historically, fungi could have two or more names, typically one for the asexual (anamorph) stage and another one for the sexual (teleomorph) stage. Recently, however, the rules for naming fungi have changed and only one name is now accepted for all stages. Pathogens that infect roots are commonly referred to as soil-borne. Those that occur above ground generally produce air- or water-borne propagules that are adapted to wind or rain-splash dispersal.

Within the *Stramenopila*, the phylum *Oomycota* contains important plant pathogens in the genera *Phytophthora* and *Pythium*, as well as other genera. These organisms generally are soil-borne, are adapted to wet environments and thus, are less of a problem when soil water content and orchard irrigation systems are managed properly. Organisms in the *Oomycota* produce asexual, wall-less, flagellate zoospores in sac-like structures called sporangia. Zoospores are capable of swimming towards

roots using chemotaxis. Oospores are sexually produced and are surrounded by a thick wall. These, in addition to asexually produced chlamydospores as described above, are survival structures.

15.4.2 Flower, foliage and fruit diseases

Brown rot blossom blight (Monilinia spp.)

Brown rot is a major disease of all commercially grown *Prunus* species in most regions of the world (Batra, 1991). Blossom blight develops from flower infections. The green hull of almond fruit can also be infected by the brown rot pathogens, causing other diseases such as green fruit rot or hull rot (see below in this section). On stone fruit crops with fleshy fruit, brown rot can result in major losses due to fruit decay. The disease is caused by two species of the *Ascomycota* genus *Monilinia*, *M. fructicola* Winter (Honey) and *M. laxa* (Aderh. & Ruhl.) Honey, which can be differentiated by their cultural (Byrde and Willets, 1977) and molecular characteristics (Fulton and Brown, 1997; Côté *et al.*, 2004; Gell *et al.*, 2007). Both fungi are equally important as blossom blight pathogens of almond, but *M. laxa* is more common in production areas with cooler temperatures in the springtime.

The first symptoms of blossom blight are necrosis of the anthers and browning of the filaments that proceeds to the floral tube, ovary and peduncle. Diseased flowers wither, collapse, turn brown and remain attached to the twigs in a gummy mass as blighted flowers (Fig. 15.15). Sporodochia develop on the dead flowers, especially the shucks (calyx). Nearby leaves wilt, and as disease progresses into the spur and twig, the entire shoot beyond the point of infection often dies. Under the bark, the cankers are light tan with a dark margin, and amber-coloured gum may exude at the margin of dead tissue. The canker may girdle the twig resulting in blight of the distal twig with leaves turning tan to brown and remaining attached. If the branch is not girdled, surrounding healthy tissue will produce callus. On large cankers, sporulation may continue under favourable, wet environments for several years (Fig. 15.16).

Primary inoculum sources in the spring for both pathogen species are blighted flowers, cankers, as well as infected peduncles, twigs



Fig. 15.15. Brown rot blossom blight caused by *Monilinia* spp.



Fig. 15.16. Sporulation on canker caused by *Monilinia* spp.

and fruit that may all produce asexual conidia in cushion-shaped, spore-producing structures called sporodochia. Conidia production of *M. laxa* was found to be highest at 10°C (Tamm and Flückiger, 1993). Conidia are spread by wind and splashing rain to healthy flowers. Sporodochia may produce conidia over several months and spores may remain viable for weeks if not exposed to high temperatures and direct sunlight. *M. fructicola* may also produce sexual fruiting structures (apothecia) and ascospores from fruit mummies and other *Prunus* species on the orchard floor (Biggs and Northover, 1985).

Almond flowers are susceptible to infection at all stages. Open flowers, however, are more susceptible because the brown rot fungi primarily invade through the stamens and pistils. Petals are also very susceptible but they generally abscise before infections advance to other flower parts. The combined effects of temperature and wetness at the time of host susceptibility are responsible for the year-to-year fluctuations in the severity of the disease (Wilson, 1953). The disease is favoured by frequent rains or fog during bloom and develops at a wide temperature range from 4 to 30°C with an optimum of 25°C. Infection is most common as flowers develop at temperatures above 15°C. At 20°C, the brown rot fungi germinate in 3–5 h and infect the host in 7–8 h. Under fluctuating day/night temperatures, 3–6 days may lapse between infection and first disease symptoms of water soaking and necrosis. Higher inoculum levels in the orchard increase the potential for disease and dry periods with sunshine and moderate to low relative humidity limit infection. From the infected flower, especially *M. fructicola* will move into fruit spurs or shoots and may form twig or elliptical branch cankers. Blossom blight rarely reduces the crop load of almond and other *Prunus* species, but successive infections over the years will weaken the trees and cause branch dieback.

Flowers of all almond cultivars are susceptible. Infections of many cultivars often do not abscise quickly enough and, thus, lead to blossom and shoot blights. Some cultivars, however, readily abscise infected flowers by formation of a wound periderm before the infection advances and cankers develop. For example, cultivars 'Fritz', 'Nonpareil' and 'Price' usually sustain less damage. Susceptibility of major almond cultivars is indicated in [Table 15.1](#).

Table 15.1. Susceptibility of almond cultivars to selected flower, fruit and foliar diseases.

Cultivar	Brown rot blossom blight	Jacket rot/green fruit rot	Anthracnose	Shot hole	Scab	Rust ^a	Alternaria leaf spot	Hull rot
'Aldrich'	++	++	++	++	+	+	+	+
'Butte'	+++	+++	++	++	++	++	++++	+
'Carmel'	+++	+++	+++	+++	+++	+++	++++	+
'Drake'	++++	++++	+	++++	++	+++	ND	+
'Fritz'	++	++	++	+	++	+	++	+
'Mission'	++	+	+++	++	++	+	++	++
'Monterey'	++	+++	++++	++	+++	++	++++	+++
'Ne Plus Ultra'	+++	++++	++++	+++	++	+++	ND	+
'Nonpareil'	+	+	++	+	+	++	+++	++++
'Padre'	++	+	+++	+	+	+	++	+
'Peerless'	++	++++	+++	+++	+++	++	++	+
'Price'	+++	++	++++	++	++	+	+++	+
'Sonora'	+++	+++	+++	++	++	++	++++	+
'Winters'	++++	+++	++++	+++	+++	+++	++	+
'Wood Colony'	++++	++++	+++	++	++	++	+++	++

Relative susceptibility of cultivars: ++++ = highly susceptible; +++ = very susceptible; ++ = moderately susceptible; + = low susceptibility; ND = not determined

^aMost cultivars are susceptible to rust during the first years of establishing an orchard when high levels of nitrogen are applied

In integrated brown rot management, growers should consider the planting site, potential microclimates and weather patterns, and cultivars when planting an orchard. Once an orchard is established, the disease history of the previous season determines the risk in the current season. If the disease is not managed, disease levels will carry over and may greatly increase in the subsequent season.

Orchard sanitation practices that include removal of mummified fruit, infected twigs and cankers from trees are important management strategies. Protective fungicide treatments can provide excellent control. The proper use of fungicides with local systemic activity protects flowers, reduces the amount of sporulation formed on the infected tissue and reduces sources of overwintering inoculum. Applications have to be done when susceptible flower parts are exposed and before the occurrence of periods with conducive wetness and temperatures. In most California production areas, one or two applications are done during bloom. If the orchard has no disease history and if low rainfall is predicted, a single, delayed bloom (20–40% of flowers are open) application with a fungicide with locally systemic action may be all that is needed. If disease occurred in the previous season and rainfall is forecast during bloom, a pink bud (5% of flowers are open) and a full bloom (60–80% of flowers are open) application are often required. Fungicides that contain a dicarboximide, demethylation inhibitor (DMI), succinate dehydrogenase inhibitor (SDHI), anilinopyrimidine (AP) or quinone outside inhibitor (QoI) (Fungicide Resistance Action Committee or FRAC Groups 2, 3, 7, 9 or 11, respectively), or combinations of these active ingredients are generally highly effective. Fungicide resistance management practices are essential to prevent the development of insensitive pathogen populations using the newer single-site mode of action materials. Rotations and mixtures of different modes of action greatly reduce the potential of selecting resistant pathogen populations.

Jacket rot and green fruit rot (Botrytis cinerea, Monilinia spp., Sclerotinia sclerotiorum)

Decay of flower parts resulting in jacket rot and subsequent green fruit rot can be a serious problem in wet years or in foggy production areas

with prolonged wet periods during bloom. This disease can affect all stone fruit crops (Ogawa and English, 1960). It is most commonly caused by the Ascomycota fungus *Botrytis cinerea* Pers.: Fr. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel), but the two *Monilinia* species and *Sclerotinia sclerotiorum* (Lib.) de Bary may also be involved. These fungi have a cosmopolitan distribution and thus this disease presumably occurs throughout temperate growing regions of the world wherever wetness occurs during the bloom and early fruit development periods.

In the early stage of the disease, infected flowers whiten, then turn brown. Infection and colonization commonly result in signs of the pathogens such as tan to buff or grey sporulation of *Monilinia* and *Botrytis* spp., respectively. No asexual conidia are produced by *S. sclerotiorum* and primary infections result from ascospores. White mycelium and black sclerotia may be present on diseased fruit. Most importantly, lesions from all three pathogens may occur on the developing fruit where calyces adhere or when fruit are in contact with one another when fruit set is heavy. Lesions are grey to brown, circular and become sunken as they expand. The entire fruit may decay and remain on the tree or may be abscised. Fruit infections result in direct crop loss.

Conidia of *Monilinia* spp. are produced on blighted flowers or twig cankers, whereas those of *B. cinerea* are produced on dead or dying tissues of almond and many other plant species. Ascospores of *S. sclerotiorum* are produced from sexual fruiting structures (i.e. apothecia) that arise from overwintering sclerotia on the orchard floor, especially under vegetation of cover crops. All spore types are wind-disseminated to senescing floral tissues. For the brown rot and grey mould pathogens, germination of conidia occurs with wetness over a wide temperature range with an optimum of 15–20°C. Infection of floral parts by ascospores of *Monilinia* spp. requires 48–72 h of wetness with the temperature seldom limiting. Wetness is not required if relative humidity is above 98%. Once the flower is fertilized and the ovary enlarges, the floral cup senesces, dies and typically splits and falls off. In cool, wet weather, however, the floral cup may persist and be colonized by one or more of the fungal pathogens. As fruit develops and comes in contact with diseased flower parts, the pathogens can grow into the healthy fruit. A brown lesion develops and spreads quickly into the immature fruit (Fig. 15.17).



Fig. 15.17. Green fruit rot of almond caused by *Botrytis cinerea*, Inset: Remnants of decayed flower hypanthial tissue attached to a fruit from where a fruit infection was initiated.

All almond cultivars are susceptible to infection. Cultivars that produce dense fruit clusters such as 'Drake', 'Ne Plus Ultra', 'Peerless' and 'Wood Colony' are highly susceptible (Table 15.1).

Removal of weeds and senescent plant tissues from the orchard floor may be beneficial in reducing inoculum levels for jacket rot and green fruit rot. Removal of the floral cup or jacket by mechanical shaking or by forced air with sprayers or helicopters is not practical. Often, no fungicides are needed during warm, low-rainfall periods following bloom because the floral cup will dry and dehisce quickly. Without senescent flower tissue, green fruit rot fungi typically cannot grow into the fruit. Under high-rainfall conditions, the disease can be managed by the use of fungicides that have activity against these pathogens with applications at full bloom and petal fall prior to wet conditions. A number of fungicides are available including methyl benzimidazole carbamate (MBC), dicarboximide, SDHI, anilinopyrimidine and DMI compounds (FRAC Groups 1, 2, 7, 9 and 3, respectively). Resistance to MBC fungicides, however, is common throughout California's almond-production areas.

Anthrachnose (*Colletotrichum acutatum*)

Anthrachnose occurs in most almond-growing areas worldwide and can be a serious problem in years with high rainfall late in the spring. Flowers, fruit, leaves and woody tissues of almond can be affected. The disease has caused high losses in California and Israel in the 1980s and 1990s (Förster and Adaskaveg, 1999; Freeman *et al.*, 2000), but since then has been mostly successfully controlled with the use of newer fungicides. Therefore, outbreaks are currently sporadic. In Australia, anthracnose was first reported in 1998 (Hall *et al.*, 1998) and has since been observed in all of the major almond-growing regions of the country. Anthracnose is caused by the Ascomycota fungus *Colletotrichum acutatum* Simmonds. Worldwide, two strains of the pathogen have been identified genetically and morphologically. The grey strain grows at cooler temperatures, whereas the more common pink strain grows at slightly higher temperatures. Both strains occur in California (Förster and Adaskaveg, 1999), whereas only the grey strain has been described from Israel (Freeman *et al.*, 2000) and the pink strain from Australia (McKay *et al.*, 2009).

Infected flowers become blighted similar to brown rot infections but often have orange droplets of conidia present on the floral cup (hypanthium). On developing fruit, orangey, depressed lesions develop, and fruit turn into thin wafers. Numerous lesions may develop on larger fruit that are similarly depressed with sometimes blackish margins. Tan centres of the lesions may profusely exude an amber-coloured gum (Fig. 15.18). Infection of nearly mature fruit usually does not cause



Fig. 15.18. Almond anthracnose caused by *Colletotrichum acutatum* with a heavily infected gumming fruit and wilting of leaves.

fruit drop, but infections develop into the kernel causing internal gumming and a brown to purple discolouration. On leaves, irregular lesions that are at first water-soaked and later yellow to tan-brown occur at the tips and along the margins. Leaf symptoms are thought to be caused by toxic substances excreted by the pathogen that are transported by transpiration from infected flower, fruit and woody tissues (Adaskaveg, 1997).

The pathogen overwinters in mummified fruit, fruit spurs, peduncles and twigs. Asexual conidia are formed in acervuli and are disseminated by splashing rain. At warm average daily temperatures (above 17°C), flowers, leaves and fruit at all stages of development are susceptible to infection. Affected tissues drop or remain on the tree. The main damage is to fruit, causing direct crop loss. Late-season fruit infections may also cause reduced kernel quality. From flower and fruit infections, the pathogen can grow into the spur and branch. Thus, the disease can also cause twig, branch and limb dieback and may have long-term effects on orchard productivity. The pathogen *C. acutatum* has a wide host range, but the importance of alternate hosts as sources of inoculum for almond is not clear because many genotypes exist within the species (Sreenivasaprasad and Talhinhas, 2005). Most cultivars are susceptible, but highly susceptible ones include 'Carmel', 'Mission', 'Monterey', 'Ne Plus Ultra', 'Peerless', 'Price', 'Winters' and 'Wood Colony' (Table 15.1).

Anthrachnose can be managed by planting less susceptible cultivars, reducing overwintering inoculum by removal of fruit mummies and dead branches, and using low-angle sprinkler irrigation that reduces canopy wetness. Application of protective fungicides during warm-temperature and high-rainfall periods is also a management strategy. Multi-site fungicides including chlorothalonil (FRAC M5), captan (FRAC M4) and ziram (FRAC M3), as well as DMI (FRAC 3) and strobilurin (QoI; FRAC 11) are effective.

Shot hole (Wilsonomyces carpophilus; syn. Thyrostroma carpophilum)

Shot hole is found at all locations where almonds are grown, and most almond cultivars are susceptible. The disease is associated with long wetness periods. Frequent and extended spring rainfall often results in serious outbreaks of shot hole. Long wetness periods may also be provided by high-angle sprinkler irrigation. The disease mainly

affects leaves and fruit, whereas infections of shoots and floral cups only develop with highly favourable environments and during severe epidemics. New outbreaks do not immediately affect yield of the orchard, but severe epidemics with early tree defoliation weaken fruitwood and small branches, as well as flower bud formation and flowering in the following spring. Shot hole is caused by the Ascomycota fungus *Wilsonomyces carpophilus* (Lév.) Adaskaveg, Ogawa & Butler (reclassified as *Thyrostroma carpophilum* (Lév.) B. Sutton 1997), the same pathogen that infects other stone fruits such as peaches, nectarines and apricots.

Shot hole lesions mostly occur on leaves and fruit. Lesions on leaves are circular and tan in colour and surrounded by a halo. Multiple lesions may coalesce. The colour of the halo and the development of small holes depend on the temperature at which the lesions develop. At cool temperatures (15°C), lesions have purplish margins and remain attached to leaves. At higher temperatures (20°C), halos are chlorotic and lesions commonly abscise, resulting in the characteristic 'shot hole' symptom. During wet weather and at moderate temperatures, the black fruiting structure (sporodochium) erupts in the centre of the lesion. Shot hole lesions resemble those caused by some herbicides or other phytotoxic chemicals, but the presence of sporodochia is the characteristic of a fungal infection.

Symptoms on fruit are tan, slightly raised corky spots, mostly on the upper side of fruit (Fig. 15.19). Spots may coalesce, and heavily infected young fruit drop or are deformed. Infected fruit sometimes gum profusely, but the kernels are usually healthy. Twig lesions are uncommon and are superficial (Fig. 15.20).



Fig. 15.19. Shot hole symptoms on almond fruits caused by *Wilsonomyces carpophilus*.

The pathogen survives host dormancy as conidia in buds and in infected twigs of almond trees (Wilson, 1937; Highberg and Ogawa, 1986). Conidia may remain viable for several months when kept dry. In the spring, they are rain splash-dispersed to newly developing flowers and leaves. If inoculum is present at critical levels in the previous season and at leaf emergence in the spring, climatic conditions determine disease progress. Leaf infections require 8–12 h of wetness at 20–25°C, and symptoms develop in 5–14 days. Early dehiscence of leaf infections mostly eliminates the formation of conidia-producing sporodochia on shot hole lesions. In cool, wet environments, however, lesions remain attached to leaves and sporodochia commonly develop in the centre of each lesion. Secondary conidial inocu-

lum is spread to healthy tissues, and the disease cycle can be repeated multiple times over the season. If the epidemic starts early in the season, these repeated cycles will cause a high severity of leaf and fruit infections that will lead to premature leaf and potentially fruit drop.

Susceptibility of almond cultivars to shot hole ranges from high to low (Table 15.1). Planting of less susceptible almond cultivars and low-angle sprinkler irrigation are cultural practices that can be used. Leaves and fruit need protection during the spring rainy season. The timing and number of fungicide treatments depend upon the amount of rain and disease history in the orchard. Generally, orchards in high-rainfall areas require more frequent applications. The shot hole fungus does not infect shoots or fruit spurs of most almond cultivars during tree dormancy as it does on peach and nectarine. Therefore, a dormant treatment with a copper fungicide is not essential for disease control in almond, but it may improve control in some orchards if included with an appropriate spring programme. Inoculum levels of the pathogen will be reduced the following spring with an application of zinc sulfate at the end of October to remove leaves and initiate tree dormancy prior to the rainy season in late autumn and winter. An inoculum-based model based on the occurrence of sporodochia on leaves in the fall and the risk of disease at flowering in the subsequent spring season has been developed (Fig. 15.21). If inoculum is present on leaves in the autumn season, the risk is high the following spring.



Fig. 15.20. Cankers on almond stems caused by *Wilsonomyces carpophilus*.

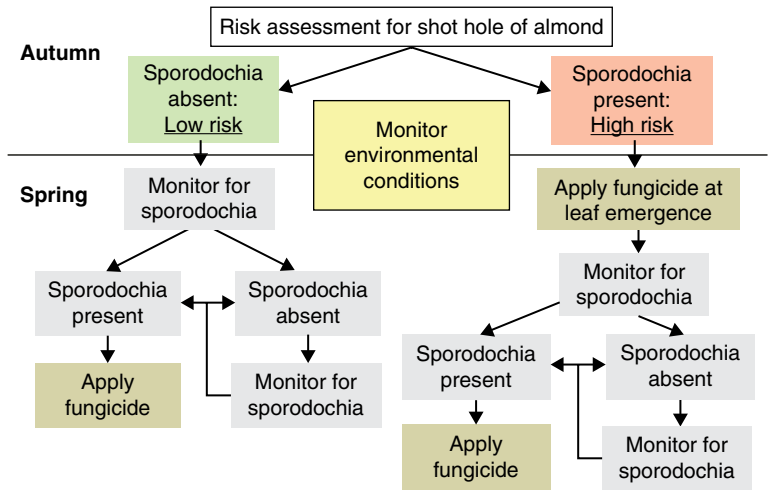


Fig. 15.21. Risk assessment model for managing shot hole of almond based on the detection of inoculum (sporodochia) and environmental conditions (rainfall and temperature) conducive to the disease.

and a fungicide with activity against shot hole such as the multi-site dithiocarbamates captan and chlorothalonil (FRAC M3, M4 and M5, respectively), and the single-site mode of action dicarboximide, DMI, QoI and polyoxin (FRAC 2, 3, 11 and 19, respectively) should be selected and applied at full bloom with leaf emergence. If no inoculum is present on leaves in the autumn season, then the application of fungicides with activity against shot hole can be delayed until the petal-fall period when leaf symptoms develop and sporodochia form within the lesions.

Scab (*Fusicladium carpophilum*; *syn.* *Cladosporium carpophilum*)

Scab is an important fungal disease of almonds and other *Prunus* spp. including peaches and nectarines in warm areas with high rainfall. The disease can also be problematic when orchards are sprinkler-irrigated or when extended periods of irrigation are supplied with micro-sprinklers. Favourable disease conditions occur in locations with high humidity (e.g. along rivers, or low spots in the orchard where dew accumulates) and where air circulation is poor, such as in high-density plantings or where soils have inadequate drainage. Scab epidemics result in premature leaf fall (Ogawa *et al.*, 1955). Defoliation for several years will debilitate trees by reducing tree vigour and lowering yields. Fruit infections do not cause direct damage to the crop. Scab is caused by the Ascomycota fungus *Fusicladium carpophilum* (Thüm.) Oudem. (formerly *Cladosporium carpophilum* Thüm.). The sexual stage *Venturia carpophila* E.E. Fisher has rarely been observed.

Leaf lesions begin as indistinct, light yellow blotches, 2–5 mm in diameter, on the underside of the blade that are best seen using transmitted light. As lesions mature and produce spores, they become dark brown to greyish black to olive green with a slightly greasy appearance (Fig. 15.22). Infections are generally localized under the leaf cuticle and epidermis. If numerous infections are present on the leaf, the entire leaf will abscise. Thus, in contrast to shot hole, no abscission zone is formed around the lesion that will excise the infection. Lesions on fruit are circular, 2–4 mm in diameter, and have a dark grey to black sooty appearance and coalesce into larger dark blotches. Fruit infections may be confused with the superficial growth of other fungi on the hull (Fig. 15.23).



Fig. 15.22. Leaf lesions of almond scab caused by *Fusicladium carpophilum*.



Fig. 15.23. Fruit lesions of almond scab caused by *Fusicladium carpophilum*.

Twig lesions are superficial, brown in colour and elliptical. The lesion edge or sometimes the entire lesion turns dark upon conidia production in the spring.

The pathogen overwinters in shoot infections that were initiated in the previous season. Shoot lesions begin developing primary conidial inoculum in the spring at high humidity and as temperatures increase (approximately 2–5 weeks after petal fall). Conidia are produced over a wide range of relative humidity. They are disseminated by wind and water splash and can infect leaves, green shoots and fruit. They germinate between 15 and 30°C with an optimum temperature of 25–30°C (Lawrence and Zehr, 1982). Maximum conidial germination occurs in free water, but conidia can also germinate at 94–100% relative humidity. Infections are superficial but with high disease severity, leaves will abscise. Symptoms develop slowly 4–6 weeks after infection and are first visible in late spring (Fig. 15.24). Fruit infections cause only cosmetic damage and are non-economical, but they supply more inoculum for leaf and shoot infections during the season. Shoot infections do not permanently affect the host, but they are sloughed off with secondary growth and bark formation of the shoot in the 2nd year. Thus, the fungus does not survive in the twig lesions for another season. All almond cultivars are susceptible to scab, but cultivars 'Aldrich', 'Nonpareil' and 'Padre' are less susceptible (Table 15.1).

Cultural practices, including planting design, have the goal of reducing humidity in the orchard. Trees are best planted in the direction

of prevailing winds to increase airflow down the rows and reduce humidity and wetness periods after rain events or irrigation periods. Pruning of trees also improves air movement and fungicide coverage in tree canopies.

Dormant applications with copper (FRAC Group M1) or chlorothalonil (FRAC Group M5), both mixed with agricultural oil, reduce and delay scab sporulation until late spring and improve the performance of protective in-season applications of fungicides. Spring applications should be initiated with the onset of twig sporulation. Wetttable sulfur (FRAC M2), phthalimide (FRAC M4), chlorothalonil (FRAC M5), MBC (FRAC 1), DMI (FRAC 3), SDHI (FRAC 7), QoI (FRAC 11) and guanidine (FRAC U12) fungicides are effective. Widespread resistance to MBC and QoI fungicides has been reported, whereas resistance to SDHI fungicides is currently more sporadic.

Rust (*Tranzschelia discolor*)

Rust is found worldwide on all cultivated species of genus *Prunus*. On almond, infections only occur on leaves and the disease does not directly



Fig. 15.24. Sporulating twig lesion of almond scab caused by *Fusicladium carpophilum*. Conidia produced in these lesions provide the primary inoculum in the spring.



Fig. 15.25. Almond leaf rust caused by *Tranzschelia discolor*. Lower leaf surface (left) with brown uredinia and uredinospores, and upper leaf surface (right) with angular chlorotic lesions.

damage fruit. Rust outbreaks are sporadic but can be very severe and prematurely defoliate trees. Defoliation over several years will weaken trees and lower yields. Orchards at high risk are those near rivers and streams and those in which humidity is relatively high in late spring and summer. Late-season rains may initiate an outbreak of rust. Newer cultivars and high-input farming of almonds with practices such as high-density plantings, high-nitrogen fertilization and long irrigation periods using drip or micro-sprinklers have caused more consistent outbreaks. Some cultivars like 'Sonora' may keep their leaves into January and thus, provide hold-over inoculum from one season to the next. Almond rust is caused by the Basidiomycota fungus *Tranzschelia discolor* Tranz. & Lit. Specialized forms of this pathogen have been identified that mostly affect almond, peach or prune (Smith, 1947; Bolkan *et al.*, 1985). The form that infects almond is known as *T. discolor* f. sp. *dulcis*, whereas forms affecting peach and prune are *T. discolor* f. sp. *persicae* and f. sp. *domestica*, respectively. Cross-infection among hosts, however, can still occur.

Leaf lesions are visible on both surfaces and are first pale yellow and later bright yellow. They are small and angular because they are delimited by the vascular bundles that are not colonized. The rust pustule erupts through the epidermis on the lower leaf surface below the lesion and the lesion then develops a rusty appearance (Fig. 15.25). The pustule produces bright orange, single-celled urediniospores during the season and two-celled, black teliospores at the end of the season.

Although the life cycle of *T. discolor* is complicated, with five different spore stages occurring on two different hosts (Arthur, 1934), urediniospores are presumably the only stage involved in the disease cycle on almond. Twig infections as they occur on peach have never been described on almond. Apparently, urediniospores can survive for several months on buds and bark, allowing the pathogen to overwinter on almond. Inoculum is spread by wind, rain and overhead irrigation. Germination occurs over a wide range of temperatures (8–38°C, with an optimum of 13–26°C) and requires wetness or a saturated atmosphere. Leaves are susceptible all season and symptoms develop 7–10 days after infection. Under favourable environmental conditions, many generations of urediniospores may be produced in a season. These repeatedly cause new

infections on almond and are responsible for epidemics. Teliospores that are produced at the end of the season do not infect almond, and they probably have no function in the disease cycle of this crop. Susceptibility of selected almond cultivars to rust is shown in Table 15.1.

Similar to shot hole management, removal of leaves with zinc sulfate applications in late October before late autumn and winter rains reduces inoculum and subsequently reduces the risk of disease in the following growing season. Planting designs that increase airflow and reduce humidity should be considered for integrated disease management. Applications of fungicides beginning at 5 weeks after petal fall until late spring can effectively manage the disease. A single application of an ethylene-bis-dithiocarbamate (EBDC) (FRAC Group M3) fungicide such as mancozeb at the 5 weeks after petal fall timing can successfully delay infections until the fall season. Outbreaks of rust, however, are very sporadic at most locations and a calendar-based management programme is not warranted. A more efficient strategy is the routine monitoring of leaves for rust infections from spring to early summer. Then at the very onset of disease detection (approx. 1% incidence), sulfur (FRAC Group M2), DMI (FRAC Group 3), SDHI (FRAC Group 7) or QoI (FRAC Group 11) fungicides should be applied. Applications of fungicides when a high incidence of disease is already present in an orchard are usually less effective and may lead to resistance development in the pathogen population.

Alternaria leaf spot (*Alternaria* spp.)

Alternaria leaf spot has been observed wherever almonds are grown. In California, it was noted as a new disease in the late 1980s and was first associated with severe defoliation in the mid-1990s (Teviotdale *et al.*, 2001). *Alternaria* leaf spot causes economic damage in locations with high humidity in the spring and summer; for example, where dews frequently occur, where air circulation is poor and where trees require frequent and extended irrigations throughout the summer. Losses from *Alternaria* leaf spot are often the result of high-input farming that includes high-density plantings, high-nitrogen fertilization and long irrigation sets. *Alternaria* leaf spot may almost completely defoliate trees

by mid- to late summer. Branches without leaves are more prone to sunburn damage. With early summer defoliation, trees respond by producing new leaves from undifferentiated buds that develop over the summer. Thus, fewer flower buds may be produced and crop production may decrease in the subsequent growing season. Crop yields may decrease dramatically if the disease occurs repeatedly year after year. Severe defoliation may also cause nut drop or interfere with normal hull-split. Fruit infection is less common and does not result in economic damage.

Alternaria leaf spot is caused by several closely related species in the genus *Alternaria* including *A. alternata* (Fr.:Fr.) Keissl., *A. tenuissima* (Nees & T. Nees: Fr.) Wiltshire and *A. arborescens* E.G. Simmons (Teviotdale *et al.*, 2001). These species may occur in the same orchard and also on the same tree. They are common in many environments and infect a wide range of other hosts, including orchard weeds.

The disease develops in late spring and throughout the summer. Leaf infections result in brown lesions 3–5 mm in diameter that may coalesce into larger irregular necrotic areas. Lesions are circular to semi-circular along leaf margins and have a narrow dark border of discoloured host tissue (Fig. 15.26). As the summer progresses, the pathogens sporulate and the lesions turn sooty-black from production of asexual conidia. On cultivar 'Wood Colony', with high disease incidence on leaves, twig lesions have been observed in California that were similar in appearance to scab lesions.

Inoculum of the pathogens is omnipresent in the orchard on senescent and dead organic

matter. Spores are airborne and are readily disseminated. Infection occurs at temperatures above 16°C in the presence of free water on the leaf surface. There is a latent period of approximately 25 days before symptoms develop.

All almond cultivars are susceptible to *Alternaria* leaf spot but vary in their degree of susceptibility. The most susceptible ones include 'Butte', 'Carmel', 'Monterey' and 'Sonora'. Less susceptible cultivars are 'Aldrich', 'Fritz', 'Mission', 'Padre', 'Peerless' and 'Winters' (Table 15.1).

Alternaria leaf spot has to be managed using integrated approaches. Less susceptible cultivars can be planted. A major goal is to improve airflow in the orchard to reduce humidity and leaf wetness periods. For this, tree density should be reduced, rows should be oriented parallel to prevailing winds, soil prepared to improve water penetration, tree canopies pruned and weeds controlled. Irrigation systems should deliver water in short, less frequent periods that allow for drying of the orchard soil between irrigations.

Fungicides with different modes of action are available in some countries for management of *Alternaria* leaf spot. These include DMI (FRAC Group 3), QoI (FRAC Group 11), SDHI (FRAC Group 7) and chitin synthase inhibitor (e.g. polyoxin; FRAC Group 19) compounds, as well as several pre-mixtures. In some production areas, resistance has developed in some populations of the pathogens to QoI and some SDHI sub-groups. Two to four fungicide applications per season are generally required.

Fungicides need to be applied prior to infection periods. Because *Alternaria* leaf spot is greatly influenced by microclimatic conditions such as temperature and wetness, the Disease Severity Value (DSV) Model that was developed for *Alternaria* diseases of tomato (Pitblado, 1992) has been adapted and modified for almonds (Adaskaveg, 2008) and has been used successfully in California.

Red leaf blotch (*Polystigma amygdalinum*)

Red leaf blotch is not a problem in California, but is considered a serious leaf disease of almond in the Middle East and it has been found in many countries (Cannon, 1996). This is because newer almond cultivars are increasingly being grown in the Mediterranean area in the past few decades. Newer cultivars provide improved yields, but



Fig. 15.26. *Alternaria* leaf spot caused by *Alternaria* spp. Trees defoliate prematurely and produce new leaves.

some are very susceptible to red leaf blotch. Conversely, in California, these new cultivars represent only a small percentage of the new plantings. Red leaf blotch is caused by the Ascomycota fungus *Polystigma amygdalinum* P.F. Cannon.

The disease mainly occurs on leaves, whereas fruit, twigs and branches are rarely affected. Initial infections that begin as pale green spots and are visible on both sides of the leaves subsequently turn yellow or orange. Lesions can have different shapes and sizes, but they do not extend to more than 2–3 cm in diameter (Fig. 15.27). In severe stages of disease, lesions coalesce, necrotic areas are formed and leaves drop prematurely. Defoliation for several years may debilitate trees and yield losses may occur.

The fungus overwinters as ascospores in stromata in decayed leaves on the soil surface. Ascospores are released in early spring and function as the primary inoculum. During spring, high humidity conditions favour maturation and discharge of ascospores at petal fall, coinciding with leaf emergence. First leaf symptoms are observed in June and continue to develop during the summer months. Irregular-shaped conidiostromata start forming asexual conidia in early summer, but this secondary inoculum is not considered to have a major role in further infections (Ashkan and Assadi, 1974).

There are great differences among cultivars in their degree of susceptibility to the disease with late-blooming cultivars generally more susceptible. ‘Guara’ is highly susceptible; ‘Tarraço’ ‘Francoli’, ‘Marinada’ and ‘Masbovera’ are less susceptible; ‘Constanti’, ‘Desmayo Largueta’,

‘Ferraduel’, ‘Ferragnès’ ‘Marcona’, ‘Marinada’ and ‘Vayro’ are variable in their susceptibility.

Planting site, microclimates and weather patterns should be considered when establishing an orchard, and less susceptible cultivars should be selected. Cultural management practices such as removing infected leaves on the orchard floor will contribute to reduced fungal inoculum levels in the spring.

Chemical control measures are mainly aimed at primary infections associated with ascospore discharge. Therefore, treatments have to be timed around petal fall. Additional sprays may have to be applied depending upon the duration of ascospore release, presence of susceptible tissue and environmental conditions conducive to infection and disease development. The multi-site mode of action contact compounds captan (FRAC M4) and thiram (FRAC M3) are the only fungicides currently registered for the control of red leaf blotch in Spain.

Almond leaf curl (Taphrina deformans)

This springtime disease, caused by the Ascomycota fungus *Taphrina deformans* (Burk.) Tulasne, is more common on peach and nectarine but may also occur less severely on almond and only rarely on apricot. Leaf curl of almond is found occasionally in New Zealand and Europe, and is rare in the USA. Except in rainy years, almond leaf curl is not a serious disease.

Leaves, fruit and young twigs can be affected. Symptoms appear first on young developing leaves in the spring. Infected leaves progressively pucker and thicken, causing the leaf to curl. Leaves can show a range of colours from light green to yellow, red and purple. As infection progresses, leaf surfaces may be covered by a powdery, white or greyish layer of sexually produced spore sacs (asci). Leaves then turn yellow or brown and abscise prematurely. Fruit infections are extremely rare. They result in irregular yellowish or reddish blistered lesions that can cover up to half of the fruit’s surface. Infected green shoots appear thickened and distorted, usually with deformed leaves at their tips.

The epidemiology of almond leaf curl has not been well studied, but probably resembles that of peach leaf curl. Ascospores that survived over the summer germinate with the first autumn rains and give rise to bud conidia known as blastospores.



Fig. 15.27 Almond leaves infected by *Polystigma amygdalinum* showing yellow or orange-coloured spots.

The fungus then overwinters as blastospores between the bud scales in a saprophytic yeast-like phase. In spring, when the almond buds burst, the fungus starts its parasitic phase. The blastospores germinate and penetrate young leaf tissues and the fungus develops as parasitic mycelium. Infection is favoured by low temperatures and rainy periods. Only juvenile plant tissues are susceptible to infection. If no spore germination occurs at bud break, little damage results for the current growing season. Ascospores and conidia can remain inactive for several years on the tree until conditions are favourable for infection to occur. This explains why leaf curl may be severe one year, even though it was not noticed the previous growing season.

On peach, the disease is mainly managed by preventive fungicide treatments that are applied during tree dormancy. Treatments after symptom development are ineffective. Based on epidemiological data on almond, treatments should be applied at the phenological stages most susceptible to fungal infection, i.e. pre-bloom to fruit set. In case of severe outbreaks, treatments can be done at leaf fall or tree dormancy to reduce inoculum levels. Only the multi-site contact activity fungicides folpet (FRAC M4) and copper compounds (FRAC M1), as well as dodine (FRAC U12), are presently registered for the control of this disease on almond in Spain.

Hull rot (*Monilinia fructicola*, *Rhizopus* sp., *Aspergillus niger*)

Hull rot occurs in most almond-growing areas worldwide and is most severe in well-managed, vigorous, productive orchards with soft-shelled cultivars, especially 'Nonpareil'. Hull rot destroys fruitwood and reduces productivity of an orchard over time, but does not directly affect the kernels. Hull rot also increases the number of 'holdtight' nuts remaining on the tree after harvest. These 'holdtights' are overwintering sites for the navel orange worm (Browne *et al.*, 1977). Hull rot is most commonly caused by the Zygomycota fungus *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. and the Ascomycota fungus *Monilinia fructicola* Winter (Honey) (Fig. 15.28). Other fungi that have been occasionally associated with hull rot are *Rhizopus circinans* Tiegh., *Rhizopus arrhizus* A. Fischer, *Monilinia laxa* (Aderh. & Ruhl.) Honey and *Aspergillus niger* Tiegh. (Mirocha and Wilson, 1961). The two *Monilinia* species also cause brown rot blossom blight.

Rhizopus-infected hulls have a dull light-grey blotch on the outer surface. Dense, dark grey to black mycelium and spores are present between the shell and hull (Fig. 15.29). Brown rot lesions on the hull are tan and covered by masses of buff-coloured spores when humidity is high. The lesion on a hull infected by *A. niger* has a pinkish cast and the spore masses inside the



Fig. 15.28. Almond hull rot caused by *Monilinia* spp. Dieback is present on the associated spur and, at high humidity, the pathogen sporulates on the outside hull surface.



Fig. 15.29. Almond hull rot caused by *Rhizopus stolonifer*. The pathogen sporulates profusely inside the hull.

hull are jet black. Clusters of leaves that are associated with infected fruit begin to wilt and die, but remain attached to the shoot. The shoot or spur may also die, and if multiple strikes occur on a branch, wood 2–5 cm in diameter may be killed. Badly infected trees have a scorched appearance by harvest, and many of the nuts become difficult to remove from the tree even though they may appear healthy and mature. Fumaric acid and possibly other toxins have been implicated in the development of dieback symptoms (Mirocha *et al.*, 1961). These toxins may diffuse from infected fruit into spurs, twigs and branches.

Rhizopus species commonly occur in the soil on organic matter, and spores are readily wind-disseminated. The primary source of *M. fructicola* inoculum is not known because hull rot caused by this fungus also occurs in orchards where *M. laxa* is the primary cause of blossom blight. Additionally, oftentimes no other stone fruit crops are grown nearby that could be inoculum sources for *M. fructicola*. Nitidulid beetles have also been implicated in the spread of the hull rot pathogens, but their overall impact on hull rot has not been determined. After the almond hulls split, *R. stolonifer* infects hulls along the crack, at other injury sites, or at the inner surface of the senescing hull. The brown rot fungi infect the outer hull at earlier stages of fruit development with favourable wet environments or during later fruit development along the hull split where the tissue is senescing. Susceptibility of selected almond cultivars to hull rot is shown in Table 15.1.

Historically, cultural practices were the main focus for managing the disease. Hull rot damage is reduced when the interval from hull-split to harvest is shortened. Cultural practices that reduce dust, such as not mowing between 1 month prior to hull-split and harvest, or that reduce humidity and increase air movement in the orchard, may be beneficial. Irrigation close to harvest intensifies hull rot damage. Therefore, the interval between the last pre-harvest irrigation and harvest should be as long as possible. Strict deficit irrigation practices have been promoted in the past (Teviotdale *et al.*, 1995). However, with today's high-production orchards where tree-wilting may have long-term effects on productivity, this strategy is not acceptable to many growers. Still, reduced watering is necessary for managing the disease. Excess nitrogen fertilization also

intensifies hull rot. Chemigation with nitrogen needs to be suspended by mid-spring.

Fungicides have been developed for managing hull rot in recent years. DMI, SDHI, QoI and polyoxins (FRAC Groups 3, 7, 11 and 19, respectively), as well as selected pre-mixtures have activity in reducing hull rot caused by *Rhizopus* and *Monilinia* species. Timing of fungicide applications should be in early to mid-June for hull rot caused by brown rot fungi on 'Nonpareil', whereas for hull rot caused by *R. stolonifer*, an early hull-split application and possibly a second application after 2 weeks is effective in reducing the disease. Fungicide treatments should not be used alone, but should be used in an integrated programme.

Leaf blight (*Seimatosporium lichenicola*)

Leaf blight caused by the Ascomycota fungus *Seimatosporium lichenicola* (Corda) Shoemaker & E. Müller occurs in areas with high rainfall and usually is not a serious problem. Only the anamorph stage is found on almond. Leaves wilt and die suddenly, but usually do not drop. Petioles of infected leaves remain on the tree until spring. Leaf and flower buds at the base of the petiole may be killed. Small dark asexual fruiting bodies of the pathogen develop at the base of the petiole. Spores are disseminated by rain and infect petioles of new leaves in the spring. *S. lichenicola* survives from one year to the next in the petioles of the blighted leaves that remain on the trees and in the affected part of the shoot (Ogawa *et al.*, 1959). Broad-spectrum fungicides such as captan and ziram applied during the petal fall period are highly effective for disease management.

15.4.3 Trunk and scaffold diseases

Ceratocystis canker (*Ceratocystis fimbriata*)

Ceratocystis canker was widespread in California before the introduction of modern mechanized harvesting equipment. Before this time, the crop was harvested by striking the trunk and scaffold branches with rubber mallets to shake the nuts off the tree. The injuries to the bark and branches were subsequently colonized by *Ceratocystis fimbriata* Ellis & Halst. Thus, the disease is also known as 'mallet wound canker'. *Ceratocystis* canker still occurs in orchards and is associated

with bark injuries that occur from improper shaking or from cultivation equipment. Visibly broken bark or injuries to the bark that are not visible, such as 'slip bark', are susceptible to infection. Under favourable environments and high inoculum levels, pruning wounds may also be infected.

Cankers are characteristic for the disease and start as elliptical, water-soaked injuries that are darker than the surrounding healthy tissue. Symptoms of established infections include dark red-brown bark and blackened heartwood that extends beyond the canker margin. In cross-section, the infections have a 'pie-shaped' appearance due to compartmentalization of the host tissue. The host responds to the infection by producing amber-coloured gum that is secreted in 'ball-like' deposits around the perimeter of the canker (Fig. 15.30). The cankers persist over several years, and expand during the summer and autumn. They may develop around the entire circumference of the branch or trunk. Overall, the time it takes to completely girdle the branch or trunk is dependent on the size of the infected tissue.

The pathogen grows into the cambium and survives in the current and previous year's

xylem tissue and bark of perennial tree cankers. It is rarely found in the darkened heartwood. The fungus is associated with insect and mite vectors. Sap-feeding nitidulid beetles such as *Carpophilus freemani* Dobson and the drosophilid fly, *Chrysomya procnemoides* Wheeler are the most important vectors. Developing mycophagous larvae or adults of these insects feed on the fungus and later excrete its spores in their frass. Larvae that ingested the pathogen may carry it through pupation and emerge with it as adults. Adults may also be contaminated by the pathogen. In general, insects are attracted to bark injuries where they will deposit the spores of the fungal pathogen and subsequently feed on the fungus as it grows in the tree wound. Following bark injury, wounds are susceptible for 8–14 days.

The disease can be managed by avoiding bark injuries from mechanical shakers. If the bark is injured, the damaged tissue should be removed to the edge of the injury to promote callus formation. Additionally, pruning should not be done before or during rainy weather. Cankers are difficult to remove and may require multiple surgeries over several years before all of the infected tissue is removed. Exposing wood may also be colonized by secondary wood rotting fungi or by other pathogens such as *Phytophthora* species that may also contribute to the decline of the tree. Tree surgeries include removing the bark and some of the wood underneath the bark to a depth of approximately 0.5–1 cm and removal of tissue approximately 2.5 cm beyond the margin of the canker. This is best done between December and February when there is little activity of the host, vectors or the pathogen. The value of wound dressing has not been established as beneficial because wound healing may be inhibited.

Band canker (*Botryosphaeria dothidea*)

Band canker has only been reported from California almond-growing areas. The disease is also likely to occur in other regions because the pathogen has a broad host range and a wide geographic distribution. Vigorously growing 4- to 6-year-old cultivar 'Nonpareil' trees and orchards near wooded areas are most commonly affected. Economic damage from this disease, however, has been sporadic and localized. Band canker is caused by the Ascomycota fungus *Botryosphaeria dothidea* (Moug.: Fr.) Ces. & De Not. (anamorph



Fig. 15.30. *Ceratocystis* canker of almond caused by *Ceratocystis fimbriata*. Profuse gumming is seen on the trunk and major scaffold branches.

Fusicoccum sp.). Recent taxonomic revisions have shown that several species closely related to *B. dothidea* may also be involved. Still, all of the species involved probably have a similar biology.

Bands of cankers extend around half or more of the circumference of the trunk or of scaffold branches. Thus, unlike other cankers, their largest extension is perpendicular to the long axis of the trunk or branch. Cankers seem to originate from growth cracks, but not from pruning wounds. Cankers often profusely produce amber-coloured gum (Fig. 15.31). Trees are rarely killed, but when the canker extends into the wood, large branches may be girdled and die. The pathogen forms asexual fruiting structures (pycnidia) in the outer bark that may exude small, white spore tendrils.

Inoculum of the pathogen probably originates from the many other hosts of the pathogen, including other tree crops such as walnuts. Conidia are rain splash-disseminated, whereas the sexual ascospores can also be distributed by wind. Infections likely occur in the spring. Cankers are only active during the summer and usually are not re-activated the following season. Some cankers, however, may be chronic and result in branch and tree death.

Band canker is considered of minor importance and no effective management measures are

known. Pruning and removal of infected plant material from the orchard has been recommended to reduce inoculum sources.

Silver leaf disease and other wood decays (*Chondrostereum purpureum*, *Laetiporus sulphureus*, *Lenzites betulina*, *Oxyporus latemarginatus*, *Perenniporia* spp., *Phellinus* spp., *Schizophyllum commune*, *Stereum* spp., *Trametes* spp.).

Wood decays occur wherever almonds are grown. They are initiated when wood-exposing injuries are colonized by certain Basidiomycota fungi. Decays are mostly observed in older trees after extensive fungal colonization but may develop anytime during the lifespan of the tree. Common decay fungi include: *Chondrostereum purpureum* (Pers.:Fr.) Pouz., *Laetiporus sulphureus* (Bull.:Fr.) Murr., *Lenzites betulina* (Fr.) Fr., *Oxyporus latemarginatus* (Dur. & Mont. Ex. Mont.) Donk, *Perenniporia* spp., *Phellinus* spp., *Schizophyllum commune* Fr., *Stereum* spp., *Trametes* spp. and others. Wood decays are important because they compromise the structural integrity of the tree, which may result in broken scaffold branches and toppled trees. Ultimately, they can determine the productivity and the lifespan of the tree and the entire orchard if left unmanaged.



Fig. 15.31. Band canker of almond caused by *Botryosphaeria dothidea*. The canker develops mostly horizontally around the trunk circumference.

Wood decay on almond is similar to that on all woody plants. Two types of decay have been characterized on living trees depending on the decay organism involved: white rots and brown rots (Adaskaveg and Ogawa, 1990; Gramaje *et al.*, 2012). White rots are the most common. The decay is moist to dry, soft or spongy, and is lighter in colour than healthy wood, to almost bleached white. All of the major structural components of the wood cells including cellulose, hemicellulose and lignin are degraded. The strength of white-rotted wood is lost in advanced stages of decay. Brown-rotted wood is darker brown than healthy wood. It is dry, crumbly and has longitudinal and traverse cracks across the grain. Brown-rotted wood loses its strength rapidly. Generally, wood decays lead to dieback, decreased tree vigour, limb breakage, uprooted trees and tree trunks broken at the soil line. Often these symptoms are associated with severe environmental conditions such as wind storms or heavy rain storms. Heavy crop loads or mechanical harvesting can also lead to structural failures of trees with wood decay.

Additional symptoms may develop with specific pathogen species involved such as *C. purpureum*, the cause of silver leaf disease. As the name indicates, leaves of infected trees become silvery in appearance, which is most noticeable on new growth in the spring (Fig. 15.32). Wood of infected trees shows discolouration and eventually white rot. Wood discolouration in branches is often angular to pie-shaped in cross-section (Fig. 15.33).



Fig. 15.32. Silver leaf disease of almond caused by *Chondrostereum purpureum*. The pathogen is colonizing the woody tissues and produces a toxin that causes the leaves to develop a silvery appearance.

Signs of these organisms include sexual spore-producing basidiomes that range in shape from annual flat to bracket-like fruiting bodies, to perennial conks attached to trunks, or limbs near the entry points of the wood-exposing injuries. The spore-producing tissues of the basidiomes include smooth surfaces, gills, pores and, occasionally, teeth-like projections.

In general, wood decay fungi enter trees through wood-exposing wounds. Airborne, sexual spores known as basidiospores may be deposited on these injuries where they germinate, form mycelia and grow into the wood. Enzymatic degradation of cell-wall components, namely cellulose and hemicellulose, provide sugar residues and the energy for growth of these fungi. As they degrade the wood, they form decay columns. From this food base, they often form reproductive structures or basidiomes where more basidiospores are produced, thus allowing the disease cycle to continue.

There are exceptions to this disease cycle: some *Basidiomycota* fungi are able to decay wood and invade living tissue such as the tree cambium and cause death to living cells. For example, *Armillaria mellea*, the oak root rot pathogen, produces mycelial fans that grow into the cambium and rhizomorphs that grow along infected roots to new healthy roots where new infections are initiated (see 'Armillaria root rot' below). Another exception is silver leaf disease caused by *C. purpureum*. The fungus affects a wide range of other cultivated and non-cultivated hardwood tree species, and the disease cycle is similar to that described above. As the fungus colonizes



Fig. 15.33. Wood discolouration on almond branch caused by *Chondrostereum purpureum*.

wood, however, a toxin is produced by the pathogen that is translocated to the leaves and causes the upper epidermis to separate from the palisade mesophyll layer of the leaf. The separated layers reflect light differently, causing the leaves to have a silvery appearance. In the trunk, the pathogen is localized in the older secondary xylem. Decay of the trunk may extend into the scaffold branches and into the roots. Leathery, bracket-like, short-lived fruiting bodies of the fungus may develop on tree trunks and scaffold branches that produce basidiospores.

A wide range of perennial hosts of most wood decay fungi, inoculum production from basidiomes over a long period, the impossibility of protecting all wound surfaces and the inability to eradicate established infections make wood decays difficult to control. Management practices should focus on prevention by starting with clean nursery stock, minimizing large wood-exposing wounds and using proper pruning practices. Ideally, pruning cuts should be made only to small branches, only during dry conditions and not immediately prior to wet environments. This will allow the wounds to heal before attempted colonization by wood decay fungi. Horizontal pruning cuts that may accumulate water should be avoided. The benefits of tree paints and other wood sealants have not been proven and these treatments may interfere with host wound healing. Fungicides cannot penetrate the highly compartmentalized wood, are not persistent enough to protect the wood over a long period of susceptibility and have not been shown to be efficacious against wood decay fungi. The most effective pruning wound treatments are biocontrol agents such as *Trichoderma* spp. that act by pre-colonizing the wood and site-excluding the pathogen. Commercial products have been registered specifically for this use in the USA.

15.4.4 Root and crown diseases

Phytophthora diseases (*Phytophthora* spp.)

Phytophthora root and crown rots are destructive soil-borne diseases of all stone fruit and many other tree crops in nurseries and orchards worldwide. Additionally, infections of aerial portions of trees may occur, and this is known as 'aerial Phytophthora'. In California it is

estimated that these diseases kill more almond, apple, cherry, peach and walnut trees than any other disease (Strand, 2002). Losses are sporadic, but more than 10% of trees may die in a severely affected orchard. Losses in nurseries can be much higher. Trees can be affected at all ages. They decline and eventually die when uptake and transport of nutrients is disrupted. Phytophthora root and crown rots, as well as aerial Phytophthora can be caused by a number of species in the genus *Phytophthora* including *P. cactorum* (Lebert & Cohn) J. Schröt., *P. cambivora* (Petri) Buisman, *P. cinnamomi* Rands, *P. citricola* Sawada, *P. citrophthora* (R.E. Sm. & E.H. Sm.) Leonian, *P. cryptogea* Pethybr. & Lafferty, *P. drechsleri* Tucker, *P. megasperma* Drechs., *P. parasitica* Dastur and *P. syringae* (Kleb.) Kleb. (Erwin and Ribeiro, 1996; Browne and Doster, 2002; Strand, 2002). Ecologically, these species differ in their geographic distribution, characteristic temperature optima, capability to cause root and/or crown rots or aerial branch infections, seasonal occurrence and virulence.

Primary symptoms include root rot, crown rot and trunk and branch cankers (Fig. 15.34). With root rot, portions of the outer cortical tissue of feeder roots are disintegrated leaving only the white, inner thin stele of the root protruding from the decaying tissue. In early stages of root infection, diseased trees are difficult to distinguish from healthy ones. With disease progression, foliage becomes chlorotic and sparse, and trees develop open canopies and show poor terminal growth. Trees either show dieback and decline progressively over several seasons or die suddenly in late spring or summer following



Fig. 15.34. Root rot and crown rot of almond caused by *Phytophthora* spp.

years with excessive wet weather. These symptoms are often not very characteristic and may be confused with other diseases and disorders. Crown rots develop at the base of the trunk where major roots originate. The bark is killed and discolouration may extend into the outer layers of the xylem tissues. As the decay expands, a canker develops that is often accompanied by the production of copious amounts of amber to brown gum. If the canker encircles the trunk, the tree is girdled and dies. If the lesion ceases expansion or the pathogen dies, callus tissue will grow into the dead areas of the bark in an attempt to heal the wound. Cankers are usually limited to the crown tissues, but sometimes may extend up the trunk and occasionally into scaffold branches. Trunk and scaffold cankers may also develop higher in the canopy independent of root and crown infections. Cankers are irregularly shaped and their margins often exhibit a zonate pattern of light and dark lines. The different canker diseases of almond are often difficult to distinguish and general characteristics of almond cankers are presented in Strand (2002).

In a particular location, several species of *Phytophthora* may be present endemically. Other species may be introduced by infested irrigation water, soil or plant material. *Phytophthora* spp. are mainly soil-borne organisms. They survive as chlamydospores, hyphae or oospores in root debris in the soil. The severity and rate of disease development depends in part on the rootstock and the species of *Phytophthora* involved. Soil moisture, however, is the most important factor. At high soil moisture levels, abundant sporangia are produced that release numerous zoospores. Zoospores are motile and are attracted by root exudates to host plant roots where they encyst and infect mainly the fine feeder roots. After each rain or irrigation, new generations of sporangia are produced that will restart the disease cycle. Zoospores are considered the main infective propagules, but other structures (i.e. germinating chlamydospores, oospores or sporangia) may also cause infections. When inoculum reaches aerial portion of trees by infested irrigation or flood waters, soil particles or by rain splash, infections of the upper trunk or branches may occur. Cankers do not develop under warm conditions of late spring and summer. Cankers, however, can be perennial when caused by other *Phytophthora* spp. such as *P. citricola* and *P. cactorum*. Although

Phytophthora spp. are pathogenic on their own, in many cases *Phytophthora* root rots are increased with root injuries caused by nematodes or other organisms. *Phytophthora* diseases are generally favoured by low to moderate temperatures.

Phytophthora root and crown rots are managed by the use of tolerant rootstocks, soil water management and sometimes by fungicides. The plum rootstock 'Marianna 2624' is currently the most resistant rootstock used for almonds. Ideally, orchards should be established on well-drained soils that are never water-saturated for more than 24 h. In soils with poor drainage, trees should be planted on ridges or raised beds. Flood or furrow irrigation increases the disease, whereas irrigating with mini-sprinklers and allowing the surface soil to dry between irrigations will reduce root rot. In addition, sprinklers should never be directed towards tree trunks and crotches. Control of root and crown rots may also be achieved with the use of systemic fungicides such as mefenoxam or phosphonates that are applied to the soil, injected, or sprayed or painted on gummosis lesions. In problematic areas, fungicides should be used together with nematicides or a soil fumigant to protect replants during the first 2 years of growth. Once *Phytophthora* is present in an orchard, eradication will be impossible.

Armillaria root rot (*Armillaria mellea*)

Armillaria root rot (syn. shoestring root rot, oak root rot) is an important disease of almond and many other woody agricultural and non-agricultural plants worldwide. The disease is not as widespread as many others, but can be very serious where it occurs. Trees can be killed at all ages. Severe outbreaks are often observed on land planted successively with almonds where inoculum was allowed to build up gradually, or where forest trees or oak woodlands were recently removed. In contrast to *Phytophthora* root rot, *Armillaria* root rot is also common on well-drained soils. Affected trees may die over a period of one to a few years.

Until the late 1970s, the Basidiomycota fungus *Armillaria mellea* (Vahl:Fr.) Kummer was considered the only causal organism of *Armillaria* root rot. Based on compatibility assays, the species was subdivided into ten biological species (Anderson and Ullrich, 1979) that have since

been shown to be well-defined morphologically and genetically distinct species. On almond, however, only *A. mellea sensu stricto* (Vahl:Fr.) P. Kumm. has been reported.

Early symptoms of *Armillaria* root rot are similar to those of other root diseases and include poor terminal growth and undersized, curled leaves on all major limbs. Infected trees may collapse suddenly during summer months with a majority of leaves still attached. In older orchards with widely spaced trees, the disease may expand in a circular fashion, but in high-density orchards the disease often progresses along tree rows. White mycelial fans that develop in the cambium between the bark and wood on the crown are highly diagnostic signs of the disease (Fig. 15.35). In advanced stages of colonization, white rot wood decay occurs and the wood is soft, spongy and bleached whitish in colour (Morrison *et al.*, 1991). The decay is a result of the degradation of cellulose, hemicellulose and lignin. Roots may have black, shoestring-like mycelial strands called rhizomorphs attached to the surface. Typically, in late summer to early fall, clusters of brown mushrooms develop at the base of infected trees.

A. mellea persists in the soil for many years as mycelium in infected plant tissues or as rhizomorphs. The pathogen is most successful in invading healthy tissue if it first becomes established saprophytically. Thus, very young trees are often killed on replant sites or older trees after they have grown for 5 years or more. Infections of healthy roots originate from infected root segments in the soil, from root to root contact or from contact with rhizomorphs.



Fig. 15.35. White mycelium of *Armillaria mellea* under the bark of infected almond tree.

Spread by root-to-root contact or rhizomorphs was estimated to be 0.8–3.2 m/year in a peach orchard (Kable, 1974). Basidiospores that are produced on the fruiting bodies are not considered major infection propagules in fruit orchards (Rizzo *et al.*, 1998; Termorshuizen, 2000).

Management strategies for *Armillaria* root rot are not very effective. If sites are seriously infested they are usually abandoned. The most effective strategy is to avoid planting new orchards on infested soil. Options to manage the disease on replant sites are limited and often only marginally effective. Chemical control options with moderate efficacy include pre- and post-plant fumigations to reduce inoculum in the soil (Munnecke *et al.*, 1970; Adaskaveg *et al.*, 1999). However, many fumigants face increased regulation or, in the case of methyl bromide, are phased out. Cultural management practices include removal of the previous trees' roots (> 1 cm in diameter) before replanting, planting trees on raised beds and removal of trees surrounding an infection centre. All almond rootstocks can be affected by *Armillaria* root rot, but 'Marianna 2624' is somewhat less susceptible.

Rosellinia white root rot (*Rosellinia necatrix*)

This is a serious disease of many deciduous fruit trees in most warm-temperate and subtropical regions. It is associated with a high mortality rate in young trees in the initial years of establishment in replant sites where peach and peach-almond hybrids are commonly used as rootstocks. In addition to stone fruits, the pathogen can also affect many other agricultural and non-agricultural plant species, and no host specialization exists. Its causal agent, the soil-borne Ascomycota fungus *Rosellinia necatrix* Prill. (anamorph *Dermatophora necatrix* Harting), has been reported in Europe, where it seems to be more common in areas with a Mediterranean climate. Outside Europe, it is often reported on stone fruits in Japan, Brazil and South Africa. However, the disease is rarely found on stone fruits in California.

Aerial symptoms are not distinctively different from those caused by *A. mellea*. Trees infected by *R. necatrix* show varying degrees of discolouration, wilting of leaves, slowing of growth and general dieback. Declining or dead trees are usually grouped in patches. These symptoms are attributable partly to the physical destruction of

the root system and partly to the synthesis of phytotoxins transported by the sap. According to the nature and age of the tree, disease development can be slow with death occurring after several years of decline, or quickly with sudden wilting following a period of drought or the first onset of fruiting. Below-ground, *R. necatrix* causes a root and collar rot, forming a characteristic white, woolly mycelium around the affected roots that gives the disease its name (Fig. 15.36). Unlike *A. mellea*, the fungus remains localized in the bark (parenchyma, phloem and cambium), but does not colonize the woody tissues. In the bark, but not at the cambium level, *R. necatrix* appears as numerous small finger-like fans, whereas in the soil surrounding the roots the fungus occurs as white or greyish mycelial strands. Under suitable moisture and temperature conditions, white mycelium may appear at ground level near the crown and trees can be removed easily from the soil due to severe root deterioration.

R. necatrix can survive for several years in rotted roots in the ground. The mycelium can complete the whole infection cycle. Upon meeting a young root, the mycelium enters directly by colonizing the outer layers, then the hyphae branch freely into the cortex, destroying it as hyphae grow towards larger roots. The fungus spreads through the soil and from infested roots of adjacent trees. *R. necatrix* requires a high level of oxygen, so it is most of the time restricted to the topsoil. Since mycelium development is favoured in soils with high moisture content, modern systems of almond irrigation have aggravated the disease.



Fig. 15.36. Almond root and collar rot caused by *Rosellinia necatrix* showing a characteristic white, woolly mycelium.

Methods for disease prevention include avoiding planting orchards on sites where susceptible hosts were grown previously, soil drainage, limiting irrigation and organic fertilization and removing roots of the previous crop as completely as possible. Eradication of *R. necatrix* by sanitation is easier than with *A. mellea*, because the mycelium is usually confined to the topsoil and also because the fungus does not survive in the xylem tissues. Disinfection by solarization can be an effective method since the mycelium of *R. necatrix* is highly sensitive to heat. It has been reported that the inoculum could be eliminated even at 60-cm depth after 6 weeks of solarization (López-Herrera *et al.*, 1999). Partial or total chemical soil disinfection has also been reported with different fumigants, but many of them face increased regulation or are phased out. *R. necatrix* is sensitive to various kinds of antagonistics, i.e. bacteria, nematodes, *Trichoderma* spp. (Sztejnberg *et al.*, 1987) and basidiomycetes. The potential almond rootstocks *Prunus cerasifera*, and the plum-almond hybrid 'Replantac' (Rootpac® R) have a level of tolerance to *R. necatrix* (Pinochet, 2010).

Verticillium wilt (Verticillium dahliae)

Verticillium wilt, sometimes referred to as 'black heart', of almond and other stone fruit crops occurs in many parts of the world and can cause serious economic losses, although it is more of sporadic occurrence. The fungal pathogen *Verticillium dahliae* Kleb. attacks many other tree crops, field and vegetable crops (e.g. cotton, tomato, pepper, potato, melon) and other plants. Therefore, almond trees may develop Verticillium wilt when planted in locations where highly susceptible crops were grown previously for a number of years, and inoculum in the soil has accumulated to high levels. The disease is most serious on young trees, especially of cultivars 'Ne Plus Ultra' and 'Carmel' (Stapleton, 1997).

The first symptom of Verticillium wilt is a sudden wilting of leaves on one or more branches during hot weather in late spring or summer. Subsequently, leaves turn yellow or brown and curl up, but usually remain on the tree. The pathogen gradually moves up into the branch. If many roots are infected, several scaffold branches may die back. Longitudinally cut branches of diseased trees show brown to

black streaks in the sapwood. In cross-section, a portion of the outer xylem is stained dark brown to black (Fig. 15.37). Young trees can be killed by *Verticillium* wilt, whereas older trees show poor growth and low productivity.

V. dahliae is a soil-borne pathogen that produces resistant structures (microsclerotia) that can survive in the soil for many years in the absence of hosts. The amount of microsclerotia in the soil determines the risk for *Verticillium* wilt in an orchard. For almond, three microsclerotia per gram of soil is considered high risk. Microsclerotia are stimulated to germinate in the presence of root exudates. The pathogen invades the cortical cells of young roots where it may produce more microsclerotia, and then the current year's xylem vessels. Here it produces conidia that are transported into the tree trunk. Conidia are short-lived and are not considered to have a major role in infection and dissemination of the fungus. Colonization of the xylem results in disruption of water and nutrient transport. Growth of *V. dahliae* is favoured by cool spring weather, but the disease can develop at up to 30°C. At higher temperatures, the pathogen dies in the upper part of infected trees and the tree may recover the following season. The disease cycle, however, can recur each growing season. Older trees may eventually outgrow the disease when roots grow deeper into the soil where inoculum levels are lower. All almond rootstocks are highly susceptible, except 'Marianna 2624'.

The most effective management practice for *Verticillium* wilt is to avoid planting in soils

where other susceptible crops were grown for a number of seasons. Removal of as many roots from a previous perennial crop as possible may help reduce pathogen populations in the soil. Inoculum of the pathogen can be reduced using chemical fumigation, solarization, flooding fallow fields, growing grass crops for several seasons, or any combination of these treatments. For young trees, minimizing tree stress through maintenance of soil fertility and soil moisture will help trees tolerate the disease and encourage their recovery. The disease rarely develops on trees more than 5 years old.

15.5 Nematodes

15.5.1 Introduction

Almond production has become more intensive, and traditional dry-land farming has evolved to high-yield, irrigated orchards growing in diverse soils. Almond seedlings are still extensively used, particularly in dry, rocky, calcareous soil conditions, but improved rootstocks have been developed from other species of the genus *Prunus* specifically for almond (Kester and Grasselly, 1987). The economic impact on crops of nematodes is frequently underestimated because these pathogens are soil-borne, and above-ground symptoms, including reduction in tree vigour, crop yield and, sometimes, plant death, are non-specific. Several nematode genera are associated with severe losses in stone fruit orchards worldwide. This chapter focuses on major nematode pests found in almond orchards. These are the root-knot nematodes (RKN) (*Meloidogyne* spp.), which are sedentary endoparasites, the root-lesion nematodes (*Pratylenchus* spp.), which are migratory endoparasites, and the dagger (*Xiphinema* spp.) and ring (*Mesocriconema xenoplax*) nematodes, which are migratory ectoparasites.

15.5.2 Root-knot nematodes (*Meloidogyne* spp.)

RKN (*Meloidogyne* spp.) are found in temperate, tropical and equatorial areas and are considered the most damaging among nematodes worldwide (Lamberti, 1979). RKN reduce

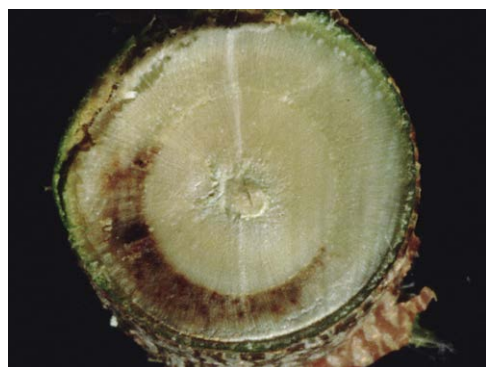


Fig. 15.37. Cross-section through branch of *Verticillium dahliae*-infected almond showing discoloured outer xylem.

fruit production in several economically important species of genus *Prunus*, including almond (McKenry and Kretsch, 1987; Scotto La Massè, 1989).

The three predominant RKN affecting *Prunus* species are *M. arenaria*, *M. incognita* and *M. javanica*, which all feed on many hosts and reproduce asexually via mitotic parthenogenesis. Additionally, several less important species have been reported. These include the northern RKN *M. hapla*, which reproduces by meiotic parthenogenesis or amphimictically, and develops poorly on genus *Prunus* (Esmenjaud *et al.*, 1994). Another species, reported on peach in Florida in the 1960s as overcoming the resistance of 'Nemaguard' and 'Okinawa' rootstocks (Sharpe *et al.*, 1969) and recently described as the peach RKN *M. floridensis* (Handoo *et al.*, 2004), has not yet been found in almond orchards. This new species has been detected in several areas in Florida and neighbouring south-eastern states of the USA (Nyczepir *et al.*, 1998; Stanley *et al.*, 2009). Because RKN species cannot be reliably distinguished by symptoms or morphological and morphometrical characters, biochemical (esterases) (Esbenshade and Triantaphyllou, 1990) and molecular (SCARs) (Adam *et al.*, 2007) tools have been developed. Several cytogenetic studies have indicated high variations in the number of chromosomes due to polyploidy and aneuploidy (Triantaphyllou, 1985), showing that each RKN species is composed of clones.

Infections by RKN on almond cause typical below-ground root galls (Fig. 15.38 A, B, C) and result in above-ground stunted growth of young almond trees. Infections during early root development may lead to extensive tree death in an orchard. In addition to reduction in tree vigour, early tree defoliation may occur that ultimately results in yield losses. Symptoms are enhanced in sandy soils, especially under drought conditions.

RKN are involved in replant disease and are a serious problem in warm, well-drained, sandy soils. Furthermore, RKN have been shown to facilitate penetration of the bacterium *Agrobacterium tumefaciens* into root tissues and subsequent development of crown gall in almond (Orion and Zutra, 1971), peach and plum (Rubio-Cabetas *et al.*, 2001).

Meloidogyne spp. have a sedentary endoparasitic feeding habit. Most are extremely polyphagous and parasitize several thousand plant

species from hundreds of botanical families that are either crop plants (annuals or perennials) or weeds (de Guiran and Netscher, 1970; Sasser, 1979). Because of their obligate mitotic reproduction (Triantaphyllou, 1985), the major species, *M. arenaria*, *M. incognita* and *M. javanica*, have been designated as the parthenogenetic *Meloidogyne* complex. The RKN life-cycle is summarized in Fig. 15.39. Eggs, deposited in egg masses, survive in the soil or in plant residues. The mobile second-stage juveniles (J2s) hatch directly from the eggs and move to root tips where they penetrate and migrate intercellularly within root tissues. J2s become sedentary and initiate feeding cells in future vascular areas and develop into swollen third- and fourth-stage juveniles. Finally, they evolve into pear-shaped adult females that can each lay several hundred eggs in a single egg mass, which is usually deposited on the root surface. Adult females feed with their protruded stylet, which perforates the cell wall but not the cell plasma membrane. Feeding juveniles and adults induce the formation of a few 'giant cells' ('feeding site') around the nematode head.

For some plant species, resistant cultivars are available that do not support development of *Meloidogyne* spp., and resistant rootstocks have been identified for *Prunus* spp. Few studies have been conducted on the histology of resistance and none has dealt with resistant rootstocks derived from almond. In 'Myrobalan' plum (carrying the *Ma* resistance gene), early penetration by *M. arenaria* J2s was shown to be similar to susceptible host accessions (lacking the *Ma* resistance gene) (Voisin *et al.*, 1999). A hypersensitive-like response in the *Ma*-carrying material completely blocks the formation of mobile J2s and prevents their maturing into more advanced developmental stages (Khallouk *et al.*, 2011).

RKN can survive for several years as eggs in egg masses in the soil under Mediterranean and temperate conditions. They may also survive between two successive tree crops on numerous annual crops including weeds on the orchard floor.

Because of the increasing national and international exchange of plant material, RKN populations are expected to be disseminated extensively. This can be prevented by the use of certified nematode-free plant material. Once introduced into an orchard soil, eradication of nematodes is difficult. Therefore, management strategies rely on the



Fig. 15.38. Roots of a peach rootstock showing extensive root-knot symptoms caused by *Meloidogyne* spp. on a peach rootstock (A); isolated galls (B); protruding female and egg mass (C, arrowhead). (Photos courtesy of INRA Sophia Antipolis, France.)

combination of different methods to keep the nematode density below economic damage levels.

Pre- and post-plant use of chemical nematicides can be effective in managing RKN in almond. However, because most nematicides have been progressively removed from the market, alternatives to chemical control methods are increasingly being favoured.

Planting a non-host, such as cereals, or leaving the soil fallow prior to planting an almond orchard is recommended to reduce nematode populations to low levels. However, weeds

must also be controlled because many of them are good hosts that allow nematode survival between successive plantings (Nyczepir and Halbrecht, 1993).

Resistant rootstocks are the most economic and environmentally sound method for managing RKN in *Prunus* spp. and for avoiding pathogen dissemination. Rootstock resistance has been investigated in several countries (Esmenjaud *et al.*, 1994; Esmenjaud *et al.*, 1997; Nyczepir and Esmenjaud, 2007) and has been characterized (Table 15.2). For example, the resistant rootstock

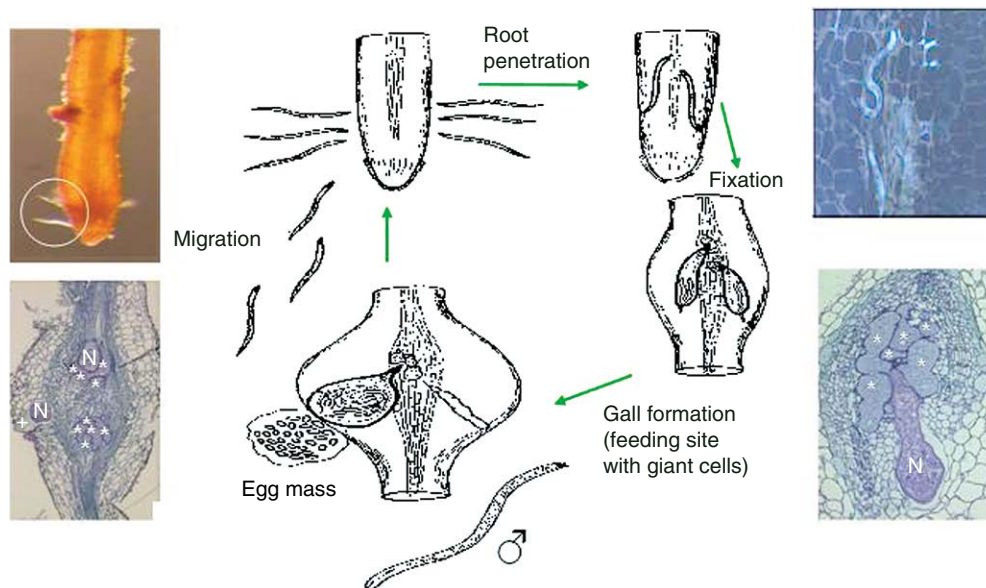


Fig. 15.39. Biological cycle of root-knot nematodes (*Meloidogyne* spp.). N: Nematode; *: giant cells; +: Egg mass.

'Nemaguard' has been widely used in California because of the high RKN frequency in almond orchards (McKenry and Kretsch, 1987).

The RKN impact on stone fruits has justified specific breeding efforts using the best sources of resistance. The corresponding major genes have been identified and mapped in several species of genus *Prunus* (Table 15.3). In almond, bitter cultivars of the 'Alnem' series resist *M. javanica* (Kochba and Spiegel-Roy, 1976) and *M. arenaria*, but are susceptible to *M. incognita* (Scotto La Massèse *et al.*, 1984). Resistance to *M. javanica* is conferred by a single gene (Kochba and Spiegel-Roy, 1975) later designated *RMja* (Van Ghelder *et al.*, 2010). Other genes for RKN resistance are the peach gene *RMia* in 'Nemaguard', which confers resistance to *M. arenaria* and *M. incognita* and the 'Myrobalan' plum gene *Ma* that confers resistance to all tested RKN species (Claverie *et al.*, 2011). The *Ma* gene also controls the peach RKN *M. floricola* (Esmenjaud *et al.*, 2009), while the *RMja* and *RMia* genes are ineffective against this latter species. In the USA, breeding programmes have focused on the development of peach or peach × almond rootstocks using resistant peach germplasm. For example, for 'Nemared' (Ramming and Tanner, 1983) 'Nemaguard' was used as source, whereas 'Hansen 2168' and 'Hansen 536'

(Kester and Asay, 1986), as well as 'Flordaguard' (Sherman *et al.*, 1991) all relied on 'Okinawa' and *P. davidiana*. In Europe, 'Cadaman' (peach × wild peach *P. davidiana* (Edin and Garcin, 1994)) and 'Garnem' (Garfi almond × 'Nemared' peach (Felipe, 2009)) have been selected for resistance to *M. arenaria* and *M. incognita* (Table 15.3). Based on the *Ma* and *RMia* genes, new resistant interspecific rootstocks (notably plum × almond-peach) for stone fruits are being developed in France (Esmenjaud *et al.*, 2009). The *Ma* gene also has a protective effect against the bacterium *A. tumefaciens* in the presence of RKN infections (Rubio-Cabetas *et al.*, 2001). This effect of *Ma* and presumably of other RKN resistance genes against *Meloidogyne*-transmitted crown gall is an additional argument for gene introgression into *Prunus* species rootstocks.

15.5.3 Root-lesion nematodes (*Pratylenchus* spp.)

Two species of root-lesion nematodes (RLN), *Pratylenchus penetrans* and *P. vulnus*, can cause severe damage to *Prunus* spp. The ecological requirements of *P. penetrans* limit its distribution to cold temperate regions where almonds do not

Table 15.2. Host suitability of genus *Prunus* rootstocks to RKN.

Species/accession	Origin	Propagation	Nematodes			
			MA	MI	MJ	MF
Almond						
<i>(Prunus dulcis)</i>						
‘Garrigues’	Spain	Seedling	H	H	H	H
‘Alnem 1’	Israel	Seedling	R	H	R	H
‘Alnem 88’	Israel	Seedling	R	H	R	H
Peach						
<i>(P. persica)</i>						
‘Cadaman’	France/Hungary	Cutting	R	R	R/H	H
‘GF 305’	France	Seedling	R/H	R/H	H	
‘Harrow Blood’	Canada	Seedling		H		
‘Lovell’	USA	Seedling		H	H	H
‘Missour’	Tunisia, Morocco	Seedling			H	
‘Montclar’	France	Seedling	H	H	H	
‘Nemaguard’	USA	Seedling	R	R	R/H	H
‘Nemared’	USA	Seedling	R	R	R/H	H
‘Okinawa’	USA	Seedling	R	R	R	H
‘Rancho Resistant’	USA	Seedling		R	H	
‘Rubira’	France	Seedling	H	H	H	
‘Shalil’	USA	Seedling		R	H	
‘Siberian’	Canada	Seedling				
Almond–peach hybrids						
<i>(P. dulcis × P. persica)</i>						
‘Adafuel’	Spain	Cutting	H	H	H	H
‘Cachirulo’	Spain	Seedling			R	
‘Felinem’	Spain	Cutting	R	R	R/H	H
‘Garnem’	Spain	Cutting	R	R	R/H	H
‘GF 557’	France	Cutting	R	R	H	H
‘GF 677’	France	Cutting	H	H	H	H
‘Hansen 2168’	USA	Cutting	R	R	R	
‘Hansen 536’	USA	Cutting	R	R	R	
‘Titan’ (hybrids)	USA	Seedling	R	R	R	
Plum–peach hybrids						
<i>(P. cerasifera × P. persica)</i>						
‘Citation’	USA	Cutting		R		
‘Isthara’	France	Cutting	R	R		
Plums						
<i>(P. cerasifera)</i>						
‘Myrabi’	France	Cutting	H	H	H	H
‘Myrobalan P.2175’	France	Cutting	R	R	R	R
‘Myrotop® P.2980’	France	Cutting	R	R	R	R
‘Myrobalan 29C’	USA	Cutting		R	R	
‘Myrobalan’ seedling	France	Seedling	H	H	H	
‘Myrobalan 605’	Spain	Cutting	H	H		
‘Myrobalan B’	UK	Cutting		MR		
<i>P. cerasifera × P. munsoniana</i>						
‘Marianna 2624’	USA	Cutting	R	R	R	
‘GF 8-1’	France	Cutting	R	R	R	
<i>P. domestica</i>						
‘Brompton’	UK	Cutting	R	R	R	
‘GF 43’	France	Cutting	H	H	H	

Continued

Table 15.2. Continued.

Species/accession	Origin	Propagation	Nematodes			
			MA	MI	MJ	MF
<i>P. insititia</i>						
‘Montizo’	Spain	Cutting	R	R	R	
‘Montpol’	Spain	Cutting	R	R	R	
‘PSM101’	Spain	Cutting	R	R	R	R

MA: *Meloidogyne arenaria*; MI: *M. incognita*; MJ: *M. javanica*; MF: *M. floridensis*; H: host; R: resistant; MR: moderately resistant

Table 15.3. Sources for resistance to root-knot nematodes in *Prunus* spp., corresponding genes with their spectrum and genus *Prunus* spp. map location.

Species	Accession	Resistance and gene spectrum				Linkage group
		<i>M. arenaria</i>	<i>M. incognita</i>	<i>M. javanica</i>	<i>M. floridensis</i>	
Myrobalan plum	‘P2175’	Ma				LG7
	‘P2980’					
Peach	‘Nemared’	RMia		R/H	H	LG2
	‘Nemaguard’			R	H	LG2
	‘Okinawa’	RMia		H	H	LG2
	‘Shalil (GF 557)’					
	‘Yunnan’	RMia		R	H	LG2
					H	LG7
Almond	‘Juseitou’		H			
	‘Alnem 1’					
	‘Alnem 88’	RMja?		RMja		
	‘Alnem 201’					

R: resistant; H: host; R/H: phenotype depending on the isolate

grow. By contrast, even though the economic impact of *P. vulnus* has not been estimated for almond, this species has been shown to be an economically important pest of peach in the USA (McKenry, 1989), Spain (Marull *et al.*, 1990; Pinochet *et al.*, 2000) and France (Scotto La Massèse, 1975). *P. vulnus* reproduces in rosaceous crops and woody perennials in Mediterranean climates and survives on many annuals. This RLN can be easily identified by its specific morphological and morphometrical features (Corbett, 1974). In field microplots in the USA, *P. vulnus* was associated with reduced growth of peach trees grafted on ‘Guardian’, ‘Lovell’ and ‘Nemaguard’ peach rootstocks (Nyczepir and Pinochet, 2001), and with high nematode numbers and early damage of ‘Nemared’ peach, ‘Garnem’ and ‘Monegro’ peach–almond hybrid rootstocks in Spain (Pinochet *et al.*, 1996). A high diversity in pathogenicity among isolates

infecting *Prunus* spp. rootstocks has been reported (Pinochet *et al.*, 2000; Nyczepir and Pinochet, 2001).

Symptoms of *Pratylenchus* spp. infection include reduction in the number of feeder roots, darkening of root tissues and necrotic lesions. These migratory endoparasites, feeding on cortical parenchyma cells, cause complete destruction of the root tissues (Marull and Pinochet, 1991). Trees show a marked nutrient deficiency and reduced shoot growth, decrease of vigour and a reduction in fruit number and size.

P. vulnus reproduces sexually. Eggs that are laid singly within cortical root tissues can directly produce J2s. Developing juveniles moult three more times between feeding intervals before becoming adults, and all life stages are present in the parenchyma tissues (Fig. 15.40 A, B) (Marull and Pinochet, 1991). This nematode is more damaging in sandy than in finer-textured soils.

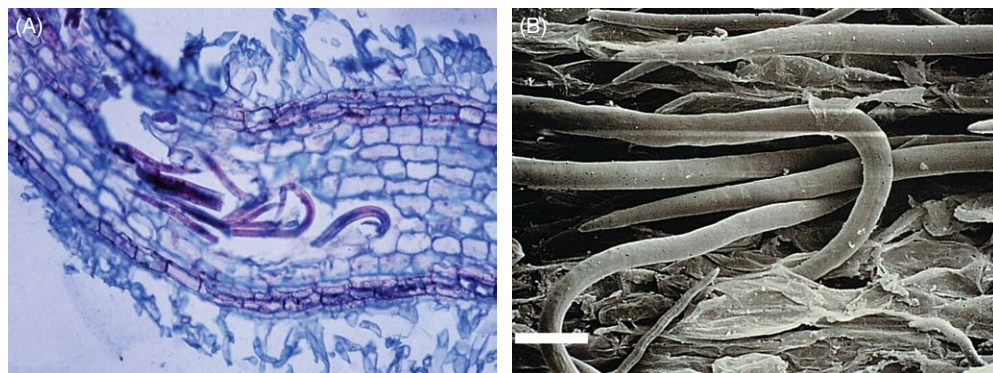


Fig. 15.40. Histological sections of rootlets parasitized by the root-lesion nematode *Pratylenchus vulnus* showing extensive damage in cortical root tissues. Light micrograph (bar 200 μ m) (A); scanning electron micrograph (bar 60 μ m) (B). (Photos courtesy of J. Pinochet, IRTA Cabrils, Spain.)

Pre-plant soil fumigation and post-plant application of nematicides have been widely used previously to provide adequate control of *Pratylenchus* spp., notably in peach (Nyczepir, 1991). Important nematicides that were widely used in the past are no longer registered in some countries and current sources of information need to be consulted for updated recommendations.

Several studies have aimed to identify sources of resistance to *P. vulnus* in rootstock material (McKenry, 1989; Pinochet *et al.*, 2000; Nyczepir and Pinochet, 2001). Potential resistance sources in the wild *Prunus* species *P. fremonti* and *P. tomentosa* (Scotto La Massè, 1975) and in 'Redglow' plum (Pinochet *et al.*, 2000) have unsatisfactory selection features. Moreover, the high variability of the pathogenicity among different isolates renders a reliable evaluation of plant material difficult (Pinochet *et al.*, 2000). Because resistance is only partial, breeding for resistant commercial rootstocks will be a long-term strategy. Using closely related *Prunus* spp. materials carrying or lacking the *Ma* plum gene, no relationship could be evidenced between RKN resistance and host suitability to *P. vulnus* (Stalin *et al.*, 1998) because *Ma* had no effect on RLN multiplication. Such data are crucial for breeding strategies aimed at incorporating multiple resistances to both RKN and RLN in genus *Prunus*.

15.5.4 Ectoparasitic nematodes

On almond, two major ectoparasitic nematodes have been reported (McKenry and Kretsch,

1987): the dagger nematodes *Xiphinema* spp. and the ring nematode *Mesocriconema xenoplax*.

Xiphinema spp.

Dagger nematodes can transmit nepoviruses (see above – section 15.3) that can be very detrimental to stone fruits. Dagger nematodes in *Prunus* spp. comprises the species *X. diversicaudatum*, originating from Europe, and a few species (mainly *X. americanum*, *X. rivesi* and *X. californicum*) of the *X. americanum*-group (*sensu lato*) from North America. This latter group, considered as a single species until 1979, has been split into several distinct species (Lamberti and Bleve-Zacheo, 1979).

X. diversicaudatum is a species of temperate climates in Europe from where it was introduced to woody plants of North America (Dalmasso, 1970; Taylor and Brown, 1997). Nematode species can be identified using PCR primers designed from ribosomal genes (Wang *et al.*, 2002). *X. americanum sensu stricto* is common in North and South America but has also been reported in South Africa, and *X. rivesi* is present in the north-eastern USA, eastern Canada and in Europe, where it has presumably been introduced (Taylor and Brown, 1997). *X. californicum* is frequent along the western seaboard of the Americas. Molecular tools for the identification of species in the *X. americanum* group are lacking.

X. diversicaudatum reproduces by amphimixis and has a long life-cycle (up to 3 years) depending on the environmental conditions.

Other *Xiphinema* species reproduce by parthenogenesis. Interestingly, *X. americanum sensu stricto*, *X. californicum* and *X. rivesi* have only three juvenile stages and not four as usually encountered in European species such as *X. diversicaudatum* (Halbrendt and Brown, 1992). When numbers are high, most *Xiphinema* spp. induce swollen and curled feeder root tips.

Nepovirus diseases

Nematodes acquire nepoviruses by feeding on infected plants, and viruses are subsequently transmitted during feeding on healthy trees. All stone fruits and many other woody plants are hosts of *Xiphinema* spp. Nepoviruses that are vectored by *Xiphinema* spp. are found in natural reservoirs such as common dicot weeds as latent infections. Most nepoviruses associated with genus *Prunus* (see above – section 15.3) have been observed and described in North America, e.g. ToRSV (Taylor and Brown, 1997) and PRMV (Nyczepir and Halbrendt, 1993). The low-prevalent strawberry latent ringspot virus (SLRV), vectored by *X. diversicaudatum* (Crossa-Raynaud and Audergon, 1987), is the only spreading nepovirus of genus *Prunus* in Europe occasionally infecting almond trees.

Control of nepoviruses in almond can be done by eliminating hosts that they have in common with their nematode vector. This is quite difficult, for example, for SLRV and *X. diversicaudatum*, which share many hosts within the Rosaceae family (Bernhard *et al.*, 1985). Direct control of the vector by pre-plant fumigation is no longer possible. Therefore, fields where rosaceous species have been cultivated previously should be avoided in the establishment of new almond orchards.

Mesocriconema xenoplax

The ring nematode *Mesocriconema xenoplax* is present in many orchards of *Prunus* species. In the south-eastern USA this species has been involved in the severe decline of peach termed 'peach tree short life' (PTSL) (Nyczepir and Esmenjaud, 2007). Peach trees parasitized by this nematode are predisposed to bacterial canker infection (*Pseudomonas syringae* pv. *syringae*) and cold injury or both. In California, presence of *M. xenoplax* has been reported particularly in almond orchards

grown in sandy soils, grafted on peach rootstocks (mainly 'Nemaguard') and exhibiting bacterial canker symptoms (McKenry and Kretsch, 1987).

15.6 Disorders with Unknown Aetiologies

A number of additional disorders affecting different parts of almond trees have been reported. Most of these are of more localized impact, but some can be economically important and limit crop production under favourable environments. Disorders such as corky spot, foamy canker, lower limb dieback and almond replant syndrome have unknown aetiologies.

Corky spot symptoms include small yellow leaf spots that turn necrotic and drop out. Premature leaf drop may also occur with new leaves subsequently emerging. This cycle may occur repeatedly in a season and severely weaken a tree. The syndrome develops mainly on 'Nonpareil' and 'Ne Plus Ultra' cultivars, is associated with scions on almond rootstock and is found in California, mostly in some northern growing regions.

Foamy canker has been described mostly from second- and third-leaf trees of cultivar 'Carmel' throughout California. Profuse watery, reddish gum is produced from trunk and scaffold cankers in late spring and summer (Fig. 15.41). A whitish foam develops from the canker that has an alcoholic odour suggesting that fermentation is occurring. The outer layer of wood adjacent to the cambium is soft, watery and whitish in colour. Canker activity tapers off in the fall and cankers do not reactivate the following year. Tree replacement or pruning and retraining are management practices.

Lower limb dieback mainly occurs on cultivars 'Padre' and 'Butte', but other cultivars such as 'Nonpareil', 'Carmel' and 'Aldrich' are also affected. Leaves of affected branches first turn yellow and then tannish brown. As the name implies, lower limbs die back to the point of attachment. Sometimes brown spots develop in the cambium and are visible once the bark is removed. Hull rot is considered to be involved in part with lower limb dieback or even has a major role, especially on cultivars 'Nonpareil', 'Carmel' and 'Aldrich'. Water-soluble acids, such as fumaric acid, which are produced with fungal infections,



Fig. 15.41. Foamy canker (disorder with unknown aetiology).

may have lasting effects between seasons, especially when incidence of infections on single branches is high. Symptoms of lower limb dieback may be confused with shading out, but the disorder may also develop on branches with adequate sunlight. Possible causes include overly wet soil, low light or possibly herbicide injury. Often secondary organisms including *Botryosphaeria* and *Phomopsis* spp. may be recovered from symptomatic branches but isolations have been inconsistent. Occasionally, scale insects are also associated with a high incidence of lower limb dieback.

Almond replant syndrome, in general, is similar to the peach replant problem because the same rootstocks are often used for both crops. The disorder is not consistently associated with known pathogens and is observed when almond orchards are planted on sites where almonds were grown previously, especially when 'Mariana 2624' rootstocks are used. Young trees in non-fumigated soil will often develop symptoms of replant syndrome that include stunting, wilting, chlorosis and defoliation in the summer, after winter planting. Affected trees have smaller trunk diameters and fewer roots, and may never

gain high productivity. Fumigation of planting sites with chloropicrin, by itself or in combination with other non-methyl bromide alternative fumigants, has been successful in managing the disorder (Browne *et al.*, 2006). In the latter study, there was no correlation between the occurrence of nematodes and incidence of replant syndrome. Thus, apparently, this disorder appears to be mediated by biological agents other than nematodes.

15.7 Concluding Remarks and Outlooks

In a globalized world, free-trade agreements can facilitate the entry, establishment and spread of pathogens. This, in turn, may lead to emerging or re-emerging diseases, which are an increasing reality for growers and phytopathologists worldwide.

A guiding principle for disease prevention is that when key inoculum sources have been identified, effective measures must be taken to prevent further spread and subsequent disease outbreaks, and some almond diseases can be managed effectively if detected at an early stage and diagnosed correctly. Therefore, reliable detection, correct diagnosis and identification of diseases are of paramount importance for initiating appropriate prevention measures and mitigation, or integrated management strategies.

Plant quarantine policies and regulations have been implemented in many countries to keep pathogens from spreading and/or to prevent exotic pathogens from being introduced with plant material. To achieve this goal, complex control systems have been designed, which often include guidelines for sampling as well as for rapid, sensitive and specific pathogen detection and diagnosis. However, there is a need for validated diagnostic tools, and more standardization is still required. Advances in these topics will not only have a direct effect in plant health, but could also be applied to environmental studies to obtain new information on the hidden life of pathogens, which could help in designing more appropriate sampling and control methods. In general, it is expected that sequencing the pathogens' genomes will have a strong impact in the near future in improving

knowledge about their life-cycles and their interactions with plants. Comparative analyses will identify species-specific sequences, and functional genomics the genes involved in pathogenesis, fitness, survival and other characteristics.

With the continued cancellation of agricultural chemicals in some countries, especially those in the EU, because of their potential negative environmental and human health impacts based on hazard assessments, the number of available treatments is becoming more restricted for almond growers. Still, in other almond production regions, new, safe pesticides and integrated pest management (IPM) strategies have been identified based on risk assessments that show that proper use of pesticides will not lead to negative consequences. Regulatory efforts

should be internationally harmonized in their assessments. At the same time, research should continue to evaluate new alternative disease management strategies that are effective, economical and pose no negative effect to humans and the environment. Non-chemical strategies including use of biological control agents, rotation/cover crops, soil solarization and especially rootstocks and cultivars tolerant or resistant to the most important diseases (when available) are promising approaches. Preventive approaches will remain essential and using pathogen-free plant material produced under a certification scheme is becoming indispensable, but then integrated management systems need to be improved at local level to succeed in the control of the most important almond diseases.

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16 Almond Pests

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16.1 Introduction

The aim of this chapter is to provide an overview of current knowledge on major pests affecting almond which is intended to be applicable worldwide. The chapter includes two main groups of pests: arthropods and vertebrates. The information presented is based on published sources as well as the authors' experience. It is divided into two sections relating to differences between the two groups of pests. The first section addresses arthropod species, including insects and mites, and is authored by F. Zalom, with the support of E. Núñez, who address European pests. The second section, addressing vertebrate pests, is authored by R. Baldwin.

16.2 Arthropod Pests and Their Management in Almond Orchards

16.2.1 Introduction

Arthropod pests play an important role in almond production as they are capable of seriously impacting the yield and quality of nut production as well as infesting harvested nuts. While specific insects and mites differ in their importance among different production regions

worldwide, arthropods are typically present in all locations to fill ecological niches that are associated with fruiting trees in general. The impact of these insects and mites may vary, however, depending on almond cultivar, production practices and climate. Of course, their economic impact also varies with the intensity of management employed in response to crop value and desired quality.

Not surprisingly, the cultivated almond, *Prunus amygdalus* Batsch, shares a number of insect and mite pests in common with stone fruit, in particular its close relative, the peach, *P. persica* (L.) Batsch. These include arthropods that feed on foliage, woody structures and roots. Many of the same insects also feed upon the fruit with the important difference, of course, that concern is for direct damage to the fleshy part of the fruit (mesocarp) in peach and other *Prunus* species as opposed to the kernel (embryo) within the seed shell (endocarp), as is the case for almond. Insects also infest almonds post-harvest and in storage. As a result, the presence of the same arthropod species on almond and peach in a given production area may well result in differential economic impacts between these crops, and their management may vary accordingly.

In this section, rather than simply describing the insects and mites that are pests of almonds and the damage they cause, which is already well

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presented in a number of extension-type books and articles published throughout the world (see Marsh *et al.*, 2002), we will describe and compare the communities of arthropods comprising various ecological niches within almond orchards with reference to species of significance in various growing locales and then present a season-long strategy for their management.

16.2.2 Early-season Nut Feeders

Lepidoptera

The larvae of a number of moth pests that feed on the leaves of almond trees may also feed on young fruit early in the season. They usually do not require control intervention because of their leaf-feeding habit, but because larvae of the overwintered generation can feed on developing nuts and hollow them out. Many of the damaged nuts are lost in the late spring drop, reducing yield. In the Mediterranean region of Europe, the almond-tree leaf skeletonizer, *Aglaope infausta* L., a moth of the Zygaenidae family, can be an important pest of almonds when present and feeding on foliage and young fruit (Fig. 16.1). Losses of leaves and fruit from larvae feeding early season can be extensive (Meliá Massiá and Almajano Contreras, 1972). In California, larvae of several leafrollers (family Tortricidae), including the oblique-banded leafroller, *Choristoneura rosaceana* Harris and the fruit tree leafroller, *Archips argyrospila* (Walker), also feed on young fruit causing them to drop (Zalom *et al.*, 2015).



Fig. 16.1. Damage to foliage and young fruit by larvae of the almond-tree leaf skeletonizer, *Aglaope infausta*, can cause extensive losses in the Mediterranean region of Europe. (Photo by Henri Robert from <http://aramel.free.fr/INSECTES11-14-1.shtml> and used with permission of Alain Ramel.)

Both species have a wide host range including other *Prunus* species. When early instar larvae are present on any of these species, they can be controlled with *Bacillus thuringiensis* Berliner sprays with less impact on natural enemies than the broad-spectrum insecticide sprays that would also be effective earlier in the season. An orchard's history of damage occurring during previous years is a good indicator of the need to control these insects.

Larvae of the polyphagous common quaker, *Orthosia cerasi* Fabricius (family Noctuidae), are reported as feeding on young almonds and occasionally cause important yield reductions in Spain. A pheromone is available that allows monitoring of adult emergence and timing of insecticide applications when damaging populations are present (Martín Gil *et al.*, 2015).

Hemiptera

Leaf-footed plant bugs (family Coreidae) and stink bugs (family Pentatomidae) are infrequent but economically damaging pests in California orchards when they feed on developing almonds early in the season before shells harden. These include the leaf-footed plant bugs *Leptoglossus clypealis* Heidenmann, *Leptoglossus occidentalis* Heidenmann and *Leptoglossus zonatus* (Dallas) (Zalom *et al.*, 2015), as well as several stink bugs including the green stink bug, *Acrosternum hilare* (Say), and the consperse stink bug, *Euschistus conspersus* Uhler (Fig. 16.2). These bugs tend to



Fig. 16.2. Feeding by Hemiptera of the families Coreidae and Pentatomidae such as this leaf-footed plant bug can result in dark spots on the kernel, wrinkled kernels, and nut drop. (Photo by Jack Kelly Clark, Copyrighted by the Regents, University of California.)

overwinter as adults in protected areas outside of orchards and then migrate to feed and oviposit on various hosts including almond. Feeding by all of these species results in similar injury. Hemipterans feed by inserting their stylets into plant tissue and injecting salivary secretions, then withdrawing the pre-digested nutrients. Feeding on the developing almond embryo causes the nut to abort or gum internally, producing a bump or gumming on the shell. Many of these young damaged nuts will drop, reducing yield. After the shell hardens, bug feeding may still cause dark spots on the kernel or wrinkled, unmarketable kernels within the shells. Leaf-footed plant bugs are considered the more serious pest because they tend to feed earlier in the season and cause more extensive nut drop.

Hymenoptera

The almond seed wasp (*Eurytoma amygdali* Enderslein) is an important pest of almonds in several countries of south-eastern Europe, the Middle East and the former Soviet Union. Oviposition takes place in early spring in unripe green almonds causing heavy premature drop in certain varieties (Tzanakakis *et al.*, 1997). The larvae feed on the developing seed and damage can reach as much as 90% in soft shell cultivars. Mummified infested almonds that contain diapausing larvae are not easily removed during harvest and many will remain on the tree. Removing these mummies before adults emerge is an effective control measure. Chemical control is, however, the most commonly used control method (Athanasios *et al.*, 2008). Recent studies have shown that a single treatment at the beginning of adult emergence can provide a significant level of control (Duval and Millan, 2010). Since the timing of application is critical, adult emergence should be monitored. Although the female sex pheromone of the almond seed wasp has been identified and tested in the field, monitoring is usually done by placing cages or bottles with infested mummies in the field and counting emerging adults.

16.2.3 Pre-harvest nut feeders

Almond kernels become very susceptible to insect invasion after shell dehiscence (hull-split).

This is particularly problematic in cultivars with a soft shell and incomplete shell seal. Unfortunately, most of the preferred cultivars produced in California and Australia including 'Nonpareil' and many others are soft-shelled and are attacked by a variety of insects. Among the lepidopterous insects that attack mature, new crop almonds are the navel orangeworm, *Ameylois transitella* (Walker), the peach twig borer, *Anarsia lineatella* Zeller, the carob moth, *Ectomyelois ceratoniae* (Zeller), and the oriental fruit moth, *Grapholita molesta* (Busck). In Australia, dried fruit beetles of the genus *Carpophilus* have become a serious problem recently as planted hectares have increased and almond orchards mature. Growers have reported annual losses as great as 30%.

Direct insect damage to kernels is far less important in Europe and other almond-growing regions where a major difference is shell hardness of the cultivars that are planted. Major almond cultivars planted in Spain include 'Marcona' and 'Desmayo Langueta', which account for 40% of production and these cultivars do not appear to be particularly susceptible to pre-harvest feeding by lepidopterous insects. Local cultivars grown elsewhere in Europe, North Africa and the Middle East are also typically hard-shelled and so are not heavily damaged by insects before harvest. With few exceptions, all commercial cultivars are self-incompatible, and cross-pollination by compatible cultivars is essential for acceptable production. Modern orchards are often planted with three cultivars so that bloom periods overlap. This complicates management of nut-feeding insects since timing of hull-split and harvest of these cultivars differs by cultivar, and therefore the degree of exposure to possible pests will also vary (Curtis and Barnes, 1977). For example, later harvested soft-shell cultivars fully exposed to the third generation of navel orangeworm in California tend to be more heavily damaged than earlier harvested cultivars, while hard-shell pollinators such as 'Mission' and 'Padre' are highly resistant.

Navel orangeworm

Of the lepidopterous insects associated with soft-shelled almonds, perhaps the most important is the navel orangeworm (Fig. 16.3) because its feeding is also accompanied by webbing and



Fig. 16.3. Navel orangeworm (*Ameylois transitella*) larva feed within almonds leaving frass and webbing. (Photo by Jack Kelly Clark, Copyrighted by the Regents, University of California.)

frass that can serve as a substrate for aflatoxin B_1 , a potent carcinogen produced by *Aspegillus flavus* Link and *Aspergillus parasitica* Speare (Marsh *et al.*, 2002). Several countries around the world have rules and regulations governing aflatoxin B_1 in foods that include the maximum permitted levels present for certain foods including almond, and this becomes an important issue in marketing. For example, the European Union has set maximum permitted levels for aflatoxin B_1 in nuts, dried fruits, cereals and spices ranging from 2–12 $\mu\text{g}/\text{kg}$, while the USA has set a maximum permitted level of 20 $\mu\text{g}/\text{kg}$ for aflatoxin B_1 in combination with the other aflatoxins (B_2 , G_1 and G_2) in all foods. While aflatoxin B_1 presents a health risk, the potential problem is kept under control by effective screening of navel orangeworm-infested kernels during processing and, most importantly, by effective prevention of damage by several cultural controls and optimally timed insecticide interventions.

The navel orangeworm is a pyralid moth that is native to the south-western United States and Mexico, and was first described in Arizona in 1899 (Wade, 1961; Rice *et al.*, 1996). It is also a pest of walnut (*Juglans regia* L.), fig (*Ficus carica* L.), pomegranate (*Punica granatum* L.) and pistachio (*Pistacia vera* L.), and these and other hosts on which it may develop serve as potential external sources of overwintering and invasion of almond orchards, depending on their proximity to them. Navel orangeworms can have up to four generations a year in the warmest almond-growing

areas. The first generation emerges from eggs laid by adults of the overwintering generation on almonds from the previous crop season that remain in the orchard. These old nuts are referred to as 'mummy' nuts, and they represent the only potential in-orchard source of moths that will infest the new crop. Females of the first generation, in turn, lay their eggs on remaining mummy nuts, and they can re-infest previously infested nuts. Indeed, navel orangeworms prefer laying their eggs on damaged nuts including those that have already been fed upon by other insects and birds (Hamby and Zalom, 2013). Moths of the second generation usually emerge at approximately the same time as initiation of 'Nonpareil' hull-split, although the precise date may vary by 7–10 days. This is earlier than hull-split of most pollinator cultivars, which typically occurs a few days to weeks later. It is the larvae of this generation as well as the next (third) generation that invades the new crop, and which is overwhelmingly responsible for economic damage to almonds (Curtis *et al.*, 1984). Larvae of the third and fourth generation, when present, overwinter in nuts that remain on the trees following harvest. Overwintering navel orangeworm larvae do not enter a reproductive diapause, so they can continue to develop when temperatures exceed 12.78°C and moths can be observed emerging when sufficient degree-days greater than this lower developmental threshold are accumulated to complete the life-cycle (about 607 degree-days centigrade on mummy nuts). Navel orangeworms develop more quickly on new crop nuts, with a generation time of only 410 degree-days centigrade (Sanderson *et al.*, 1989).

The weakest point in the navel orangeworm seasonal cycle is during overwintering in mummy nuts. At this time, natural control by bird and rodent depredation, as well as natural infection by *Bacillus thuringiensis*, is common. It is also the time when mummy nut removal can be used to reduce the overwintering population and also remove potential sites of invasion by the first generation in the spring (Zalom *et al.*, 1984). Mummy nut removal by shaking or hand-poling prior to bloom has become a common practice by growers in California, where the navel orangeworm is the most significant insect pest. Another important cultural practice for avoiding damage is early harvest together with

rapid nut pickup. Hull-split in the northern hemisphere begins from late June to late August depending on latitude, cultivar, weather and horticultural conditions such as water stress. In most modern commercial orchards, harvest is done mechanically using shakers, sweepers and pickup machines. Once gathered, the almonds are either stored on farm or at hullers until they can be hulled. Harvesting as soon as possible once 100% of the nuts at the 2–3 m level of the tree canopy are at hull-split, followed by rapid pickup, is important to restrict navel orangeworm damage by limiting the time that nuts are exposed to oviposition and potential invasion by larvae of the third generation. If the harvested nuts cannot be hulled immediately, and must be stored, then fumigation of these nuts in storage might be necessary to eliminate navel orangeworms that might be present, and to prevent further infestation.

Sanitation and early nut harvest with rapid pickup are sufficient to keep damage at acceptable levels when low to moderate orchard populations are present and external sources of migrant moths are minimal. However, when navel orangeworm population levels are high, external sources of infested hosts are nearby, or demand for very low infestation levels (less than 1%) occurs due to high almond prices, chemical sprays are usually necessary (Zalom *et al.*, 2015). The preferred timing for a navel orangeworm spray is when sound fruit begin to split in the top south-west quadrant of the tree. That egg-laying is also occurring at this time should be confirmed using navel orangeworm egg traps. Black navel orangeworm egg traps contain almond press-cake and almond oil, which can simulate a mummy nut when placed in the orchard. Other possible times for insecticide sprays for navel orangeworm are when eggs of the first generation are hatching in the spring, and at hull-split of the pollinator cultivars, or the predicted start of the third-generation flight. These sprays can also be timed using a degree-day model. Male navel orangeworm flights can be monitored with a pheromone lure placed in a wing-style trap. The male pheromone is also available for use in mating disruption, and has proven to be effective in reducing damage when there is no significant external source of mated females that could enter a treated orchard (Higbee and Burks, 2008).

Carob moth

Also a pyralid, the carob moth is the Mediterranean ecological equivalent of navel orangeworm as these species share many similarities with regard to seasonal cycle, life-cycle, behaviour and appearance, as well as their significance in damaging new crop almonds following hull-split (Fig. 16.4). A native of the Mediterranean region, it is a major pest of figs, pomegranates and pistachios, where they are grown within its geographic range, and as with navel orangeworm, these other fruits serve as excellent alternate crop hosts (Gothilf, 1969). Although also introduced into California, where it has become the major pest of dates, carob moth has not become a problem in almonds there. Their diapausing larvae overwinter in mummified nuts and fruit, including stone fruit such as any peach or apricot (*Prunus armenica* L.) mummified fruit remaining on trees. One study in Australia documented as much as 47% of overwintering almond mummies contained live larvae in a heavily infested orchard. Moths of this overwintering generation emerge in spring as temperatures warm, and these go on to produce three generations a year. There can also be a partial fourth generation, most of which become the overwintering larvae that complete the seasonal cycle in almonds.

Females of the overwintering generation oviposit and develop only on almond mummies until the new crop begins to split. Females of this first generation also oviposit on mummies until



Fig. 16.4. The adult carob moth (*Ectomyelois ceratoniae*) looks very similar to an adult navel orangeworm. (Photo by Thomas Eichlin, used with permission of the Statewide IPM Program, University of California.)

hull-split, at which time they begin to oviposit on new crop nuts. The resulting larvae enter the hulls and eventually penetrate the shells or enter through cracks in the shell seal to feed directly on the kernels. As is the case with navel orangeworm, the larvae feed deeply into the kernels and leave frass that is susceptible to fungal infection, rendering the infested nuts unusable. Moths of the second generation produce a third generation that also infests unharvested almonds (Gothilf, 1984). In Australia, females of the third flight are at peak emergence at about the time of 'Nonpareil' harvest, so an early and rapid harvest can help limit damage (Madge *et al.*, 2015). Later harvested pollinators are particularly susceptible to infestation by the third-generation moths.

As might be expected, carob moth management is similar to that of navel orangeworm. For both species, damage can be significant on soft-shell cultivars, while cultivars with hard shells as well as those with good shell seal, limit kernel damage. Sanitation by eliminating mummy nuts in orchards, or cull or stockpiles in nearby orchards, reduces sources of moths that might infest the crop in the next year. Insecticides targeting carob moth at hull-split, as well as protecting the split nuts before harvest, can be useful to limit damage when populations are sufficiently great in orchards or when there is an important source of migrants from outside the orchard.

Male carob moth activity can be monitored using a sex pheromone lure placed in a large delta-style trap. This pheromone has been used effectively in dates and is being studied for implementation on almonds (Madge *et al.*, 2015).

Peach twig borer

The peach twig borer is a gelechiid moth that damages peaches, nectarines and other *Prunus* sp. fruit throughout the USA as well as in Europe and elsewhere in the Mediterranean area (Fig. 16.5). In cooler almond-growing regions it can cause high levels of kernel infestation on soft-shell cultivars if left untreated. It was the major insect pest of California almonds before the introduction of organophosphate insecticides, reportedly causing statewide damage from 10% on 'Ne Plus' nuts to 27% on 'Nonpareil' nuts during the 1930s and 1940s (Rice *et al.*, 1996). Like navel orangeworm, their larvae feed directly



Fig. 16.5. Peach twig borer (*Anarsia lineatella*) larvae emerging from their over-wintering hibernaculae feed on the shoot tips causing characteristic flagging. (Photo by Jack Kelly Clark, Copyrighted by the Regents, University of California.)

on kernels by entering the nut through the end where the hull has loosened from the spur or through the suture following hull-split, and they are most damaging on soft-shell cultivars with poor shell seal. Unlike navel orangeworm, peach twig borer larvae only feed on the surface of the kernels near the pellicle and they do not deposit frass or webbing as do navel orangeworms, so infection by *Aspergillus* sp. fungus is not a concern. However, damage by peach twig borer is believed to make the nuts more attractive to subsequent oviposition and invasion by navel orangeworm.

Peach twig borer overwinters as first or second instar larvae in hibernaculae they build from frass and wood chips in limb crotches of first- to third-year wood (Marsh *et al.*, 2002). A larva may leave the hibernacula briefly on warm winter days. The over-wintering larvae finally emerge during bloom and migrate up small limbs and twigs where they feed on swollen buds

and newly emerged leaves near the terminal of shoots. As terminal growth continues, the larvae enter the tip and mine the interior of the shoot, resulting in a characteristic flagging or wilting of the terminal leaves. Adults of the overwintering generation emerge from the shoots from late March through mid-May, depending on location, and lay eggs, and the resulting larvae again enter and mine new shoots. Many moths of this first generation that emerges in June and July, and of the second generation that emerges in August, lay eggs on maturing nuts. The larvae enter the hulls or move directly into the nuts and feed on the kernels. The fourth-generation moths lay eggs that produce the overwintering larvae that emerge during the following bloom.

Following the introduction of organophosphate insecticides, sprays targeting emergence of the first-generation larvae dramatically reduced damage from peach twig borer, and the use of these products at hull-split for navel orangeworm control further reduced their damage (Rice *et al.*, 1996). Later, most California growers began to use organophosphates in combination with horticultural mineral oil during orchard dormancy as scales and certain other mite and Lepidoptera species could also be controlled, and this practice was also thought a 'safer' practice for natural enemies and humans (since there were fewer orchard operations occurring during that period). The dormant organophosphate and oil spray became standard practice until the 1990s when concern for unintended environmental effects began to surface (Epstein *et al.*, 2001). While concerns for the effects of the organophosphates on raptors during orchard dormancy was later shown to be largely unsubstantiated, the risk of run-off into surface waters from stone fruit and almond orchards following winter rainfall events became known. Although it is now accepted that the organophosphate and oil dormant sprays can be used without substantial run-off in almond production areas that receive little rainfall during winter, where soils are not prone to run-off, or where certain mitigation practices are used, the use of dormant sprays has declined significantly. Using a dormant treatment with an environmentally sound insecticide remains acceptable.

Many orchards are not treated for peach twig borer, and although there is no recommended sampling method or associated treatment threshold,

using past history of damage is a good approach for deciding if a treatment should be considered. Bloom sprays of the biological insecticide *Bacillus thuringiensis* without oil have been shown to control peach twig borer effectively, but as a rule, no other insecticides should be used during bloom because of their risks to pollinators (Barnett *et al.*, 1993). Male moth flights of peach twig borer can be monitored using pheromone lures placed in wing-style traps, and degree-days can be used to predict the best time for spring treatment. Peach twig borer development occurs at temperatures between 10 and 31.1°C, and the average generation time is about 582 degree-days. The optimum treatment timing is 204 degree-days after the start of the spring flight, and this often occurs within a few days or weeks of the recommended timing for navel orangeworm so that susceptible larvae of that insect will be killed as well (Hamby *et al.*, 2015). Pheromone mating disruption is available and is an option, but it is seldom used in almonds because of the cost relative to current conventional insecticide options.

Oriental fruit moth

The oriental fruit moth is a major pest of stone fruit worldwide, particularly peaches and nectarines. It is only occasionally a pest of almond, however, and its damage is sporadic and unpredictable (Zalom *et al.*, 2015). It is thought that the almond orchards at most risk of damage are those located near infested peach or nectarine orchards that are harvested before the almonds, or where peach shoot terminals have hardened due to some tree stress. Five to six generations a year occur in most almond-growing areas, and it is not uncommon to find larvae in shoot strikes that very much resemble those of peach twig borer except that they tend to be deeper into the shoots and occur somewhat after those of peach twig borer in each generation. Oriental fruit moth larvae may also be found feeding within the hulls of almonds, but they rarely enter the shells to feed on the kernel. Its damage is similar to that of peach twig borer; the larvae do not produce webbing and their frass is very fine. Several parasitoids and predators provide control of oriental fruit moth when they are present in almond orchards, but these may be disrupted by broad spectrum insecticide sprays.

Sap beetles

Carpophilus beetles of the family Nitidulidae are worldwide pests of grains and fruits, both pre- and post-harvest, and are well known as stored product pests (Fig. 16.6). Several species including the Australian sap beetle (*Carpophilus davidsoni* Dobson), the confused sap beetle (*Carpophilus mutilatus* Erichson) and the dried fruit beetle (*Carpophilus hemipterus* L.) have been serious pests of ripening stone fruit in southern Australia and have recently become serious pests of almonds there as well, where they feed and breed on them both pre- and post-harvest (Brown, 2015). The adult beetles enter the fruit at hull-split by chewing through the hull beneath the stem, through the suture, or at sites where the almond has been damaged due to mechanical or biological factors. Sap beetles can also serve as vectors of brown rot (*Monilinia* spp.), which develops at the entry sites. *Carpophilus* beetles in Australia overwinter as mature larvae, pupae or adults, and are capable of moving more than 4 km. Their biology together with their broad host range makes control a challenge.

Because *Carpophilus* beetles can move within and between fruit and almond orchards, their movement and distribution within an orchard should be determined. Monitoring is accomplished with a funnel trap that contains a synthetic food volatile-based co-attractant or fermented apple juice replaced weekly, and an insecticide strip. A minimum of one trap deployed per hectare is recommended, with individual



Fig. 16.6. Sap beetles such as *Carpophilus mutilatus* have become serious almond pests in Australia due to their direct feeding on kernels. (Photo by Jack Kelly Clark, Copyrighted by the Regents, University of California.)

traps located at a height of about 1.5 m and at least 15 m from the edge of a block. Early season, the sap beetles may be found in almond mummies on moist ground near drip emitters, so presence of the beetles may help identify blocks where monitoring traps should be installed. History of insect damage and hull rot are also indicators of blocks where traps should be placed.

As with the navel orangeworm, sanitation is very important for sap beetle control. Piles of hulls, damaged kernels and other debris in the vicinity of orchards can become an important breeding site and a refuge for sap beetles during winter and early spring. Ground mummies may also become infested with *Carpophilus* and serve as a source of beetle invasion of the new almond crop.

Insecticide sprays can be applied for control when beetle populations are high or there is continuing migration into the orchard from external sources. A male-produced aggregation pheromone deployed in a large funnel trap, which also contains the same synthetic co-attractant solution and insecticide strip used for monitoring, has been used as an attract-and-kill technique to manage *Carpophilus* beetles in stone fruit, but its efficacy for almonds has not been evaluated to date. The pheromone is attractive to at least nine *Carpophilus* species, making its utility quite robust. The attract-and-kill technology must be deployed before the crop becomes susceptible to avoid competition with volatiles from the ripening fruit in order to be effective, and the pheromone also becomes less attractive once adult beetles become well established.

Interestingly, *C. hemipterus* is the most common sap beetle species in Spain and California, and a major pest of fig, but it is not known to feed on almonds in the field.

Ants

Ants became important in California orchards, especially in the drier production areas when tillage decreased and with the vastly increased use of drip and micro-sprinkler irrigation. Two species are of particular concern, the southern fire ant, *Solenopsis xyloni* McCook, a native species to the western USA, and *Tetramorium caespitum* (L.), which is of European origin (Rice *et al.*, 1996). Both species feed on other resources in the orchard until almonds have been

shaken from the trees. They begin to feed on the kernels once they are on the ground, and the amount of damage is a function of the number of ants present in the orchard and the amount of time that the nuts remain on the orchard floor. Ants begin feeding on the pellicle, exposing the kernel, and workers will continue to feed until the entire nutmeat is consumed. They do not leave webbing or frass. Almond cultivars with a tight shell seal tend to be less affected than those with a soft shell or poor shell seal. Rapid removal of the nuts from the orchard floor is an effective control (Zalom and Bentley, 1985) but attractive ant baits containing an insect growth regulator or other type of insecticide can be used preventatively to reduce ant populations in the orchard.

16.2.4 Post-harvest nut feeders

Almond kernels, either shelled or unshelled, are susceptible to insect feeding in storage, and the pests can be distinguished according to those that infest almonds in the field and those that infest them in storage (Burks and Johnson, 2012). Post-harvest infestations of almonds by insects that were initially in the field often occur by species that attack them on trees, such as the carob moth, the navel orangeworm and the *Carpophilus* sap beetle. If these insects are present in the unshelled nuts during the period between harvest and shelling, they will continue to infest and re-infest the stored nuts resulting in very high levels of damage, unless they are controlled in some manner such as fumigation, drying or controlled atmosphere. Typically, these pests will not readily re-infest almonds once they are shelled and in storage.

Almonds are also attacked by insects that are primarily considered to be storage pests. These include the Indianmeal moth (*Plodia interpunctella* (Hübner)), the almond moth (*Cadra cautella* (Walker)), the tobacco moth (*Ephesia luteola* (Hübner)), and the raisin moth (*Ephesia figulilella* Gregson). These species are found in all almond production areas worldwide. Of these, the Indianmeal moth is the most common to a variety of post-harvest commodities and the most widely distributed (Simmons and Nelson, 1975). The biology and resulting damage by the almond

moth is similar to that of the Indianmeal moth, but the range of commodities it damages is narrower, and it favours more humid conditions than do the other species. The tobacco moth is a more temperate species that does not develop as quickly as the other species under warmer conditions, and it is rarely a pest of stored almonds. The raisin moth may be found in the field in the hulls of mature almonds, but it does not typically feed on kernels. It is occasionally found in stored almonds, but it is not able to establish a breeding population in storage (Soderstrom *et al.*, 1987). Once almonds are shelled and placed into sealed packaging, moths will not enter these stored products, but they can sometimes enter through very small openings that may be present in the packaging in order to infest the nuts.

16.2.5 Foliar feeders

Almond fruit bud differentiation begins in summer and continues through autumn and winter, therefore leaf injury resulting from foliar feeding during the season can impact yield in subsequent seasons. Growth of young trees as indicated by tree girth can also be affected. A complex of leaf-feeding arthropods often similar to those affecting other tree crops in a given region may also attack almonds, and these typically include mites, aphids and leafhoppers.

Mites

A number of mites representing several Acari families are considered pests of almonds. Among these, the web-spinning spider mites of the genus *Tetranychus* are the most damaging worldwide (Fig. 16.7), but the relative importance of each *Tetranychus* species varies in importance locally (Marsh *et al.*, 2002). *Tetranychus urticae* Koch, the two-spotted spider mite, a cosmopolitan species, is perhaps the most important of the web-spinning spider mites, globally, and they occur in all major production areas. As is the case for all pest mites, their damage results from piercing leaf cells and feeding on the chlorophyll within, leaving leaf surfaces with a stippled appearance; however, damage varies by species. Heavy feeding can result in defoliation in late summer or early autumn, especially under conditions of



Fig. 16.7. Spider mites of the family Tetranychidae damage almond leaves by piercing leaf cells and feeding on the chlorophyll within, leaving leaf surfaces with a stippled appearance. (Photo by Jack Kelly Clark, Copyrighted by the Regents, University of California.)

water stress. The *Tetranychus* spider mites also produce webbing, often heavy when present at great densities, hence the common name for this genus. The two-spotted spider mite is generally favoured by hot and dry conditions that may be exacerbated by water stress and dusty situations. Adult females overwinter in diapause as reddish-orange adults on leaf litter or orchard floor vegetation. They become active again near bloom, and move up trees where they begin to lay eggs and become more widely distributed on the new foliage. They reproduce rapidly and their fecundity is high, so populations can become damaging by midsummer. Other closely related species of Tetranychidae are known to exceed the two-spotted spider mite in importance, regionally, where they may be better adapted to local conditions than the two-spotted spider mite. Some examples include the Pacific mite *Tetranychus pacificus* McGregor, which is a problem primarily in California's southern San Joaquin Valley; *Eotetranychus carpini* Oudemans, which is damaging in almond production regions surrounding the Mediterranean Sea into Central Asia; and the strawberry spider mite *Tetranychus turkestanii* Ugarov & Nikolskii, which is an occasional problem in southern and eastern Mediterranean production areas. In general, economic damage to almond trees by all *Tetranychus* species is similar, as is their biology and management. In the absence of disruptive insecticides, natural enemies often control mite infestations. Mite predators in almonds include

several species of thrips, predatory beetles, flower flies and lacewings (Zalom *et al.*, 2015). Predatory mites of the family Phytoseiidae are particularly important, however, and have been widely studied as augmentation biological control agents.

Other mites of the Acari family Tetranychidae are also pests of almonds, their importance again varying by region and time of year. These include the European red mite, *Panonychus ulmi* (Koch), and the brown mite, *Bryobia rubrioculus* (Scheuten), which are distributed worldwide on deciduous fruit and nut hosts (Rice *et al.*, 1996). They tend to be greater problems in cooler regions and during spring to early summer, with populations often declining as temperatures become hot and dry. Unlike the web-spinning spider mites, these other tetranychids do not produce much webbing. Both overwinter in the egg stage and hatch occurs during bloom. The immature mites move up the bark and feed on foliage, preferring the cooler parts of the tree. Although stippling caused by their feeding resembles that of the web-spinning spider mites, defoliation rarely occurs, even under very great population pressure. As is the case with the *Tetranychus* species, cultural practices such as dust control and adequate irrigation help prevent outbreaks, and biological controls and chemical controls are also important to their management. Both European red mite and brown mite, and other species that overwinter as eggs, are effectively controlled by horticultural mineral oil applied during orchard dormancy, which smothers the eggs; and additional miticide treatments are seldom needed during the season if dormant oil is applied (Zalom *et al.*, 2015).

Mites in the Acari family Eriophyidae seldom cause severe damage to almond trees. For example, massive populations of the peach silver mite *Aculus cornutus* (Banks) can occur on occasion in California orchards resulting in a general yellowing of almond leaves through their feeding. *Phyllocoptes graniti* Keifer, the almond erineum mite, causes deformed leaves and forms erineum patches on the undersurfaces of the leaves of almond in Italy and Spain (Vacante, 2016). By contrast, the almond and plum gall mite, *Acalitus phloeocoptes* (Nalepa), is reported to cause severe damage to almond trees in southern and central Europe (Castagnoli and Oldfield, 1996). The main symptoms of their feeding are

permanent bud galls and deformed fruit spurs. In contrast to the relatively limited damage by this species in plum, almond trees do not recover and large populations may cause them to die in a few years if left untreated.

Aphids

A number of different aphids have been reported to feed on almond foliage worldwide. Of these, the green peach aphid, *Myzus persicae* Sulzer, is present in all growing areas. Other aphid species that can become locally important include the mealy aphid *Hyalopterus amygdali* (Blanchard), the black peach aphid *Brachycaudus persicae* (Passerini), the short-tailed almond aphid *Brachycaudus amygdalinus* (Schouteden), the leaf curl plum aphid *Brachycaudus helichrysi* (Kalt), *Myzus varians* (Davidson) and *Myzus amygdalinus* (Nevsky). These species are also found on other *Prunus* species including peach and plum grown in the same locations.

Aphids are of concern when present at high densities in spring following bloom, and shoot infestations at this time are an indication that an insecticide treatment should be considered. Excessive vigour induced by chemical fertilization favours aphid development and can lead to greater population levels. Aphids rarely cause economic damage to mature almond trees or later in the growing season, and are typically kept under good biological control by natural enemies such as green and brown lacewings (Neuroptera) and a number of Hymenoptera parasitoids that are often specific to the particular pest. One study from Spain found seven green lacewing species feeding on aphids in almond, and all were relatively common in the production area. Aphid outbreaks can occur following application of a broad-spectrum insecticide applied for some other insect pest.

Lace bugs, leafhoppers and sharpshooters

Other leaf-feeding Hemiptera including lace bugs (family Tingidae), leafhoppers and sharpshooters (both family Cicadellidae) may be present on almonds. Their feeding results in yellowing and stippling to leaves that is superficially similar to that resulting from mites, as well as leaf distortion, and at high densities lace bugs can produce excessive amounts of honeydew.

Many sharpshooters may also feed on shoots in addition to leaves.

A lace bug *Monosteira unicastata* (Mulsant and Rey), also referred to as the almond tiger, has been reported to cause tree defoliation throughout the Mediterranean region (Sánchez-Ramos *et al.*, 2014). Adults of this species overwinter on the trunk of almond trees, and there are two to three generations a year. Treatment is recommended when more than 10% of the shoots become infested.

Several species of leafhoppers are reported to feed on almond trees. Leafhoppers are sometimes referred to as 'green mosquitoes' in Spain, and the most abundant species reported in one study was *Asymmetrasca decedens* Paoli (Jacas *et al.*, 1997). Green mosquitoes that feed on young shoots at high densities cause small and twisted leaves and stunted shoot growth. These damaged leaves turn yellow, then dry. Damage to young trees and replants can be especially severe by reducing their growth. In California, leafhoppers and sharpshooters rarely cause economic damage to almonds through direct feeding, but sharpshooters are known to transmit the xylem-limited bacterium *Xylella fastidiosa* Dixon, which causes almond leaf scorch when both the insects and pathogen are present. This disease can lead to the death of the tree, although almond leaf scorch incidence is not widespread.

16.2.6 Branch, limb or trunk feeders

Scales and mealybugs

Scales and mealybugs are Hemiptera of the sub-order Sternorrhyncha and superfamily Coccoidea. Adult female scales are almost always immobile and permanently attach to the plant they are feeding upon, secreting a waxy coating for protection. The first instars called crawlers emerge with functional legs from under the waxy covering of the female and search for a favourable spot to establish and feed. Once settled, the females become sessile and moult, then begin to excrete their characteristic covering. The most important pest species on almond trees are the members of the hard-scale family Diaspididae, including the San Jose scale *Diaspidiotus perniciosus* Comstock (Fig. 16.8), the olive scale *Parlatoria oleae* Colvée, and the white peach



Fig. 16.8. The San Jose scale, *Diaspidiotus perniciosus*, is one of several scale insects that damage almond trees when their populations grow on twigs and limbs. (Photo by Jack Kelly Clark, Copyrighted by the Regents, University of California.)

scale or mulberry scale *Pseudaulacaspis pentagona* (Targioni). All have worldwide distributions. Soft scales in the family Coccidae are also reported as almond pests including the European fruit lecanium *Parthenolecanium corni* (Bouche), which is distributed widely and is a pest of other *Prunus* species as well (Rice *et al.*, 1996). All of these scales are most damaging when large populations build on twigs and limbs. The process of feeding on its vascular system and injecting a toxin results in loss of tree vigour and productivity. Untreated infestations can kill fruit spurs and limbs within 1–3 years.

Scale insects are often under good biological control with naturally occurring predators and parasitoids unless broad-spectrum insecticides are applied (Badenes-Perez *et al.*, 2002). They can also be controlled by horticultural mineral oil sprays applied during orchard dormancy or by insect growth regulators targeting the crawler stage (Zalom *et al.*, 2015).

Bark beetles

Bark beetles, including the almond bark beetle *Scolytus amygdali* Geurin-Meneville, the larger shot hole borer *Scolytus mali* (Bechstein) and others, can become problems on old or weakened trees, resulting in loss of limbs or tree death (Fig. 16.9). The adult beetles emerge from trees in spring after spending the winter in galleries they create as larvae, feeding on the inner bark and surface of the wood, then bore into weakened



Fig. 16.9. *Scolytus* bark beetles can weaken or kill older trees by larval feeding creating characteristic galleries. (Photo by Jack Kelly Clark, Copyrighted by the Regents, University of California.)

parts of trees where they lay their eggs along the sides of tunnels. These adult entry sites may be identified by the presence of gumming produced as a defence by the tree. Another generation emerges in summer and a similar life-cycle is repeated with larvae of the second generation overwintering within the tree.

In cases where bark beetle invasion is due to stress from cultural conditions, it may be possible to prune out damaged limbs and improve the irrigation, fertility or soil conditions causing the tree stress to occur. When damaged wood with an active beetle infestation is pruned, it should be removed from the orchard and destroyed since adult beetles can still emerge successfully. When bark beetle infestation is due to tree age, pathogen infection or root feeding by insects or nematodes, it may not be possible to save the affected tree. Removing and destroying dying trees can reduce further spread.

Tree borers

Two Lepidoptera species, the prune limb borer *Bondia comonana* (Kearfott) and the American plum borer *Euzophera semi funerals* (Walker), are localized pests in young almond orchards in California (Zalom *et al.*, 2015). Female moths are attracted to fresh wounds and lay their eggs on rough bark nearby. Their larvae enter the wound and feed on the cambium, and damage may heal. However, when numerous entry wounds and prolonged infestation occurs at scaffold crotches or graft unions of young trees, they may break with wind or heavy crop loads.

The adults may also bore into callus formed under bark resulting from shaker injury, providing an entry site for *Ceratocystis* canker fungus.

The peach twig borer, mentioned previously as a fruit feeder, also mines new almond shoots including watersprouts. Although considered a predictor of potential kernel damage when they occur in large numbers, shoot damage is usually inconsequential to mature trees. However, extensive shoot mining in first through third leaf trees can impair their structure by forcing development of lateral shoots, which becomes problematic as they mature.

16.2.7 Root-feeding insects

Almond trees are deep-rooted and are most productive on well-drained light to medium textured soils, but soil conditions vary considerably even within production regions. Lighter and sandier soils tend to favour certain types of insects that spend a portion of their life-cycles in the soil or feeding on roots.

Mediterranean flathead root borer

The Mediterranean flathead root borer *Capnodis tenebrionis* L., sometimes known as ‘stub-born worm’, is distributed throughout inland almond-producing areas of countries surrounding the Mediterranean Sea (Fig. 16.10). This beetle of the family Buprestidae is particularly important in drip-irrigated orchards, and can cause severe damage to young trees. The adults feed on buds, shoots and leaves, while the larvae feed upon the root system. Adults lay eggs in the soil within 50 cm of the trunk and at an average depth of 3–12 cm. Once larvae emerge, they must find roots and begin to feed within 3 days, and they then survive on the roots for 2 years before pupating. Trees with heavy root infestation are characterized by progressive weakening that makes them particularly vulnerable to attack by bark beetles and other insects (Garrido, 1984). Problems with Mediterranean flathead root borer are exacerbated by drought conditions and proximity to abandoned orchards that have been infested by this pest.

While microbial biological controls such as the entomopathogenic fungi *Beauveria bassiana*



Fig. 16.10. The Mediterranean flathead root borer, *Capnodis tenebrionis*, can cause severe damage to trees through their root feeding in inland almond-producing areas of the Mediterranean zone. (Photo by Yves Banière from <http://aramel.free.fr/INSECTES11-14-1.shtml> and used with permission of Alain Ramel.)

(Balsamo-Crivelli) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin or entomopathogenic nematodes can be used to reduce larval populations, infested orchards will typically need to be replaced in time. When replacing orchards that have been previously infested, European rootstocks should be substituted for hybrid rootstocks and physical barriers should be installed to prevent egg-laying on tree trunks.

Scarabs

The family Scarabaeidae is a large and diverse group of beetles consisting of over 30,000 species worldwide, some of which feed on roots of plants causing economic damage. In California, the ten-lined June beetle, *Polyphylla sobrina* Casey, is a localized pest of almonds and peaches, which is capable of destroying entire orchards (Zalom *et al.*, 2015). Adult males emerge from the soil to mate with females that return to the soil to oviposit. The larvae feed on the roots of almond trees for 2 years before pupating in late spring of the second year prior to adult emergence. An infestation is often recognized when a small group of trees starts to decline and die, then the infestation spreads slowly across the orchard. It is difficult to control larvae in the soil with microbial or conventional insecticides because most are found more than 20 cm beneath the surface. White grubs, the larvae of

several scarab species, are noted as root-feeding soil pests in Spain.

16.2.8 Soils, floor management and their effects on arthropod pests

Soils also influence the amount of water that must be applied to irrigated orchards to maximize production, although in many older Mediterranean regions almonds are produced without irrigation. Irrigation is one of the most important cultural factors responsible for increasing yields, while insufficient water can affect trees in several ways that favour certain arthropod pests. For example, water stress can result in earlier hull-splitting as well as poor shell seal in many cultivars. Earlier hull-split exposes the nut to earlier invasion by navel orangeworm as well as a longer time period of vulnerability, which can result in greater kernel damage. Poor shell seal due to water stress also facilitates feeding by other lepidopterous larvae including peach twig borer and oriental fruit moth, increasing the occurrence of nut damage. It is standard practice in irrigated orchards to withhold water prior to harvest to prepare the orchard floor for almond pickup. During this period, trees often undergo sufficient water stress to promote web-spinning spider mite development, and a miticide often must be applied to prevent leaf drop from occurring before irrigation is resumed following harvest.

Non-tillage and strip weed control is commonly used in conventional irrigated orchards, which consists of a herbicide treated strip down the tree row and an unirrigated vegetated cover maintained during winter and spring that is mowed on occasion. The plants in the row strip are most commonly volunteer native vegetation, but can also be seeded with grasses or, occasionally, a combination of grasses and legumes, or another flowering species that provides horticultural benefits. The vegetative cover reduces dust in the orchards, which can contribute to web-spinning spider mite outbreaks, and also provides a more humid environment that favours the decomposition of mummy nuts harbouring overwintering navel orangeworm larvae that have fallen to the orchard floor. Flowering cover crops can also serve as an augmentative nectar and pollen source for natural enemies.

16.2.9 A season-long arthropod management approach

Integrated pest management focuses on the prevention of pest damage by considering the environment in which the crop is grown and understanding the biology of the pests and their natural enemies which are present in a given production area. With a perennial tree crop such as almonds, a good seasonal starting point is orchard dormancy. This is the time of year when observations from previous years can be assessed to determine which pests were problematic, what factors might have led to them becoming pests and how well tactics applied for their management might have mitigated damage. With this knowledge, a season-long management approach can be developed.

Orchard dormancy is a good time to monitor for number of mummy nuts that could harbour insects that overwinter in them or use them as a resource in the spring, and to remove them if possible. It is also a good time to take spur samples for scales or mite eggs and to apply a horticultural mineral oil spray for their control, if needed. Tree crotches can be examined for peach twig borer hibernaculae if they are present in the area, and an insecticide may be added to the horticultural mineral oil to control them. If an insecticide is used at this time, the product and method used should consider the potential of run-off should sufficient rainfall occur, and potential effects on pollinators if they are present in the orchard. Branches, scaffolds or older trees that are infested by borers can be removed at this time.

Post-bloom and during early fruit development, monitoring can begin for insects and mites that are present in the area. Traps for key pests, if they are available, can be placed in the orchard at this time and monitored to determine their presence and activity. For certain key pests, the traps can be used with degree-day models to time-spring sprays. Monitoring of key pests should continue through hull-split and harvest. The progress of almond fruit development should be monitored as well to determine the initiation of hull-split, a key timing for treatment of several important insects that will attack the new crop at this time. Continue to monitor the progression of hull-split on all cultivars, and shake nuts from the trees as early as possible to

avoid further infestation by insects that damage the kernels. Thorough nut removal, including rapid pickup and removal of nuts from the orchard floor, will help minimize damage. Assess trunk damage from shakers or harvest operations, and provide mitigation measures. Record the amount of nut damage and the insects responsible from samples of harvested nuts, as well as interventions attempted for their control during the season for review during orchard dormancy, preferably by individual block and cultivar. If storing nuts before hulling, consider fumigation or another approach to prevent harvested nuts from becoming further infested by kernel-feeding insects.

The ecological niches available to be filled by insects are common to virtually all almond orchards, although the species that ultimately fill those niches and become pests may vary among production regions. Management of a species that occupies a particular niche in one locale may well be informed by research on another species that fills a similar niche elsewhere. Better knowledge and consideration of the insects and mites present locally, their biology, and options for sustainable management, provide the best opportunity for economical and environmentally sound almond production.

16.3 Vertebrate Pest Management

16.3.1 Introduction

Bird and mammal pests are found in and around virtually all almond orchards, though they may not always present a significant problem. Damage caused by birds generally results in loss of a portion of the current crop and does not affect future production. Tree injury by rodents, rabbits, deer or wild pigs is often more serious, either killing the tree outright or causing permanent damage that lowers yields for years following the initial damage.

Numerous bird species can cause damage to almonds depending on the location of the orchard. Common forms of damage include dis-budding trees, consumption of nuts and drill damage to the trunks of trees from woodpeckers and sapsuckers. Several rodents and rabbits eat roots, nuts or bark and can kill young trees

outright. Rodent burrows and mounds interfere with orchard maintenance and harvesting operations and inflict structural damage by gnawing on drip- or micro-sprinkler irrigation lines. Deer strip young trees of foliage and can stunt or even kill saplings (University of California Statewide IPM Project, 2002).

A programme incorporating the points that follow will not only result in fewer vertebrate pest problems but make orchard management more economical:

- Correctly identify the species causing the problem.
- Alter the habitat, when feasible, to make the area less favourable to the pest species.
- Take early action and use the management approaches appropriate for the orchard and time of year, with due consideration of the environment.
- Establish a monitoring system to detect re-infestation so you can determine when additional corrective measures are necessary.

Vertebrate management equipment and supplies (baits, fumigants, traps etc.) are available at local outlets, such as farm supply and hardware stores, or online. In addition, certain local, regional, or government agencies make certain rodent pesticides available to growers. For further information or sources of special control materials, consult your local authorities on the subject.

16.3.2 Legal aspects of vertebrate pest management

Familiarity with local regulations will be required before engaging in a management programme geared towards vertebrate pests. Some species may be considered game animals (e.g. tree squirrels and deer), and therefore will have hunting restrictions associated with them. Others may be protected by government regulations (e.g. migratory birds). In short, special permits may be required to remove some damaging species, while permits will not be required for other vertebrates.

Trapping and the use of pesticides are often used to manage vertebrate pests. Permits may not be required for trapping many burrowing rodent species (e.g. rats, ground squirrels, pocket

gophers, voles), but they are often required for other species (e.g. rabbits and birds). It is also important to remember that only pesticides that are registered can legally be used for vertebrate pests. You will need to consult with your local authorities to determine what is legal for use in your area.

In some areas, almond orchards are located within the range of threatened or endangered species. When in such areas, special precautions must be taken to eliminate any potential hazard to these species. You will need to contact your local agencies to determine any potential species of concern in your orchards and what impacts this might have on your management programmes.

Keep in mind that reliance on any single management approach is generally not a good strategy as vertebrates can adapt to avoid these tools over time. Additionally, not all individuals in a population will be equally susceptible to a specific management approach. As such, the use of an integrated pest management strategy is strongly recommended for all vertebrate pests to maximize efficacy, reduce management costs and ensure the longevity of these management tools while minimizing potential negative impacts on the native ecosystem.

16.3.3 Vertebrate species of concern

Burrowing rodents

One of the groups of vertebrates most likely to cause damage is burrowing rodents. This could include a wide variety of species including voles, pocket gophers, ground squirrels and many mouse and rat species (Figs 16.11–16.13).

BIOLOGY. Rodents tend to be very fecund with large or multiple litters annually being common. This allows populations to build up rapidly, resulting in substantial damage in some orchards. Some burrowing rodents have general cycles where there is a lag in reproduction. This often coincides with winter months. As such, if removal efforts are desired, they are often most practical when targeted during these time periods given that rodent populations are at their lowest numbers during this timeframe. However, some species hibernate during winter (e.g. ground



Fig. 16.11. Meadow vole. (Photo by J.K. Clark, Copyright the Regents, University of California.)



Fig. 16.12. Pocket gopher. (Photo by J.K. Clark, Copyright the Regents, University of California.)

squirrels). If managing a hibernating species, you must wait until the animals are active for removal efforts to be effective (Baldwin, 2016).

DAMAGE. Burrowing rodents cause various forms of damage depending on the species. Direct losses include consumption of nuts, which reduces yields, and girdling of tree trunks and branches, which can result in mortality of branches or entire trees (Fig. 16.14). Some species (e.g. pocket gophers) will feed directly on root systems, thereby reducing vitality of the tree or leading to direct mortality. Research has shown that direct damage can lead to a substantial reduction in profits (4.2–8.7% mean loss when voles, pocket gophers or ground squirrels are present) depending on the species causing the damage (Baldwin *et al.*, 2014).



Fig. 16.13. California ground squirrel. (Photo by J.K. Clark, Copyright the Regents, University of California.)



Fig. 16.15. Gnawing damage to drip irrigation pipe. (Photo by J.K. Clark, Copyright the Regents, University of California.)



Fig. 16.14. Pocket gophers often consume roots and girdle trees below ground. The dirt has been removed from around the base of this tree to show this root and trunk damage. (Photo by J.K. Clark, Copyright the Regents, University of California.)

Burrowing rodents also cause indirect forms of damage including chewing on irrigation lines and sprinklers, resulting in substantial damage to infrastructure and loss of valuable

water resources (Fig. 16.15). Their burrow systems can also damage trees and result in a loss in irrigation water, which can lead to increased soil erosion from channelling. For larger open burrow systems (e.g. ground squirrels), nuts can be lost down burrow openings during harvest, while larger burrow openings and mounds from pocket gophers can be a hazard to farm equipment and farm labourers. This indirect damage can be extreme, but it has not often been quantified (Baldwin *et al.*, 2014).

A variety of tools are available for managing burrowing rodents depending on the species causing damage. These tools are outlined below.

HABITAT MODIFICATION. This can be a good initial step toward an effective management programme depending on the species encountered. Some species prefer relatively open orchard floor habitats (e.g. ground squirrels) while others prefer dense vegetation (e.g. voles). Adjusting orchard floor habitats accordingly can assist management efforts. Keep in mind that some plants can be preferred food sources for many burrowing rodent species (e.g. clover and other nitrogen-fixing plants), so eliminating them can reduce the desirability for these pests within orchards. Additionally, many rodents prefer pruning piles for safe harbourage. Eliminating these, both from orchard interiors as well as orchard borders, can reduce carrying capacity for these pests while also increasing the overall effectiveness of management programmes.

EXCLUSION. The use of hard plastic or wire tree protectors can effectively reduce or eliminate girdling damage to newly planted trees. Such damage is common when voles, and occasionally mice, are present in orchards in large numbers. Tree protectors must be buried at least 15 cm below the ground to keep voles and mice from digging underneath and inside the structures. If not buried, damage can be more extreme than if not used at all. The use of such devices is expensive so substantial damage must be anticipated before their use would be considered economically beneficial. Exclusion of other burrowing rodents is not generally considered practical or effective.

REPELLENTS. Many chemical repellents are registered for burrowing rodents, but none has yet proved effective. Vibrating stakes are also sold to evict rodents from treated areas but also have not been proved useful. At this time, repellents do not appear to be an effective management tool for burrowing rodents.

FRIGHTENING DEVICES. No auditory or visual frightening devices are effective at deterring burrowing rodents from orchards.

PREDATORS. Many predators feed on burrowing rodents including a suite of carnivore and raptor species. Predator composition varies depending on the orchard location. Although the use of predators to manage rodent populations has long been discussed, few data exist to indicate that they can effectively reduce and subsequently maintain burrowing rodent populations at densities that are acceptable to most growers.

ALTERNATIVE CULTURAL PRACTICES. Flood irrigation can be a useful tool for reducing burrowing rodent populations. During flood events, some rodents will drown while survivors will often be forced out into the open where they will be susceptible to predation.

When possible, soil tillage can also disrupt burrow systems and may result in the direct mortality of some burrowing rodents. The required depth of soil tillage will vary depending on the species causing damage: the deeper the burrow systems, the deeper the tillage will need to be. Such tillage is often not practical or possible

within an existing orchard, but can be a good strategy before replanting a new orchard so as to reduce the number of burrow systems and existing rodent populations before replanting.

SHOOTING. Most burrowing rodents are too small or numerous to be managed effectively through shooting. Ground squirrels, however, can be managed effectively through a shooting programme if the shooter is persistent. As with all removal techniques, the goal should be a minimum removal rate of 70% of the population (and preferably more than that) to benefit long term from the population reduction. As such, continual efforts are needed if shooting is to be employed as a removal technique. Often other approaches, such as burrow fumigation and toxicants, are more practical options.

TRAPPING. Trapping laws and requirements can vary tremendously across regional and federal governments, so be sure to check what is legal and appropriate in your area. When rodent numbers are relatively low, trapping can be an efficacious tool. For some species (e.g. voles and mice), numbers will likely be too great for trapping to be practical, although this can vary regionally depending on the species causing the damage. There are many different kinds of traps available for managing burrowing rodents depending on the species and location, but they all generally fall into two categories: kill traps and live traps. Kill traps are designed to quickly kill the animal in the trap. These traps are advantageous because they euthanize the animal directly rather than relying on the trapper to euthanize the animal after capture. They also are often considered somewhat more effective than live traps. However, these traps are lethal and can be indiscriminate in what they capture. As such, when capture of non-target species is likely, live traps may be a better option, as non-target species can be removed unharmed from such traps. Common examples of live traps include cage or box traps. Common kill traps include body-gripping, snap and pincer-style traps, although there are many examples of kill traps. When using live traps, the trapper is responsible for euthanizing the animal humanely after capture. Local authorities should be consulted to determine what traps and euthanasia approaches are appropriate and effective in a particular area.

FUMIGATION. Burrow fumigation can be a very effective tool for treating burrow systems of some rodent species including ground squirrels, pocket gophers and sometimes, rats. Burrow fumigation tends to be less practical for voles and mice given the large number of burrow systems that have to be treated and the relatively shallow nature of the burrow systems for these species. However, burrow fumigation is occasionally used for them as well.

Burrow fumigation is by far most effective when soil moisture is high. If soil is too dry, the gases can dissipate rapidly through cracks and pores in the soil at a rate too great to maintain toxic levels. As such, fumigants are most practical for use during the wet season or following substantial irrigation events.

Several burrow fumigants are available for use, although some of the commonly used options include aluminium phosphide, gas cartridges and pressurized exhaust. Overall, aluminium phosphide may be the most effective, but it is also highly regulated as to how and where it can be used. Gas cartridges work well for some species (e.g. ground squirrels and sometime rats) but less well for others. Machines that inject pressurized exhaust into burrow systems are increasingly being used as well and appear to be moderately to highly effective depending on the species involved. Local authorities should be consulted to determine what is legal for use in a given area.

TOXIC BAITS. The use of toxic bait (typically referred to as rodenticides) is one of the most widely used methods for managing burrowing rodents, largely due to the high efficacy and minimal treatment cost of this approach when compared to other removal options. Several different active ingredients can be used depending on local restrictions. Some of the more commonly used materials include the first-generation anticoagulants diphacinone, chlorophacinone and warfarin. These are considered chronic feed materials. This means the rodents must consume the bait multiple times over the course of several days to get a lethal dose, thereby requiring multiple applications at set intervals to ensure an effective baiting programme. The acute toxicants zinc phosphide and strychnine are also used to manage burrowing rodents, although the applicability of these will depend on the species for which they are being applied. In general,

strychnine is only available for pocket gopher control and is not available for use in all regions; zinc phosphide is the more widely used acute toxicant. Because they are acute toxicants, they only require a single application to be effective.

An effective bait programme requires consumption of the bait by the target species. Because annual diet can vary for some burrowing rodents, bait application is not always successful throughout the year depending on the target species (e.g. bait application is not generally effective against ground squirrels in winter or spring when they are hibernating or feeding on green vegetation). Also, some toxicants have a detectable odour or taste to them (e.g. zinc phosphide), thereby making bait acceptance more challenging. As such, proper bait selection, timing and application strategies are needed to maximize the efficacy of a baiting programme.

Toxic baits for all rodents except pocket gophers are generally applied above ground through one of three approaches: spot treatments, broadcast applications and bait stations. Spot treatments are used when relatively few burrow systems need to be treated. This approach requires spreading a pre-measured amount of bait around the opening of the burrow system or along runways. The key for this approach is to spread the bait very widely according to label requirements so as to greatly reduce or eliminate the potential consumption of lethal doses of the bait by most non-target species. When larger areas need to be treated, broadcast applications are often used. This approach requires the use of a calibrated seed spreader that distributes the appropriate amount of bait over a defined area. This is a quick and easy method for bait dispersal. Bait stations are also used for first-generation anticoagulants, although they are not typically used for zinc phosphide. With bait stations, the goal is to eliminate non-target access to bait by housing within a station. All can be effective approaches, but bait stations are likely to have the greatest utility for an almond orchard, as spot and broadcast treatments are not generally allowed during the bearing season. However, they could be used on orchard perimeters and after harvest, but before green-up the following spring.

Pocket gopher bait application relies on placement of baits directly into burrow systems, usually through an all-in-one probe and bait dispenser. If large areas need to be treated, a burrow

builder can also be used. The burrow builder is a device pulled behind a tractor that creates an artificial burrow at a set depth. Bait is deposited at set intervals along the artificial burrow. Experience is needed when using the burrow builder to ensure that the machine is run at the proper depth and in the proper soil conditions. Regardless of the bait application approach, strychnine is generally considered the most effective bait option.

Because of substantial differences in regulations across countries, and even regions within countries, it is worth emphasizing that all growers should consult with their local authorities before determining what bait application approach is appropriate for them.

Tree squirrels

Several species of tree squirrels will feed on nuts when orchards are located close to forested or riparian areas (Fig. 16.16). Damage is not generally as great as with burrowing rodents, but can be extreme when they are found in large numbers.

BIOLOGY. Tree squirrels, as with most rodent species, are relatively fecund. They have one to two litters per year with around three to five young per litter. This allows for relatively rapid population build-up when conditions are ideal. Tree squirrels spend most of their time in trees, where they nest in tree cavities or in leaf nests called dreys. However, some species spend considerable

time on the ground foraging as well (Salmon *et al.*, 2006; Baldwin, 2016). Tree squirrels are generally active year round in almond-growing areas.

DAMAGE. The most common form of damage with tree squirrels is direct feeding on nuts. Because they will cache nuts for later use, the number of nuts removed is often much greater than what they consume. Tree squirrels will also strip bark from trees, ultimately leading to mortality of some branches or even entire trees. As with any rodent, they may chew on irrigation tubing (see Fig. 16.5) but this damage is far less common than with other rodent species.

In contrast to burrowing rodents, there are relatively few tools available for managing tree squirrel species. Habitat modification is limited, as preferred habitats include forested and riparian areas that cannot be removed. Exclusion is not practical for tree squirrels given their jumping and climbing abilities. Repellents and frightening devices do not work, and no cultural practices are available. Tree squirrels do have a number of natural predators, but these predators have not been shown to significantly reduce tree squirrel populations in an orchard. Additionally, no rodenticides are registered for tree squirrel control in orchards and, because they do not live in burrow systems, fumigants are not an option. As such, the only practical management options are shooting and trapping.

SHOOTING. Removal of tree squirrels through shooting is difficult and time-consuming, but it is possible. Small-calibre rifles, shotguns and high-powered air rifles are all potential options, but the quieter the gun, the more successful the shooter is likely to be; loud guns will scare off squirrels and will make shooting additional individuals difficult. Special permits acquired from local regulatory agencies may or may not be required; be sure to check before removal efforts are conducted. Alternatively, tree squirrels can generally be removed during the legal hunting season as long as standard hunting restrictions are followed.

TRAPPING. Several different traps can be used to remove tree squirrels including, but not limited to, box traps, tunnel traps and wire-cage traps.



Fig. 16.16. Eastern fox squirrel. (Photo by J.K. Clark, Copyright the Regents, University of California.)

Box and tunnel traps are examples of kill traps. They can be placed up in trees and are baited with a food source, often nuts or fruit of some sort. Wire-cage traps are live traps that are placed on the ground. They can be baited with the same foods. Tree squirrels captured in wire-cage traps must be humanely euthanized after capture, usually through shooting or through the use of a carbon dioxide gas chamber. Because many traps can be set at once, thereby potentially allowing many individuals to be captured per day, trapping is often the preferred tool for tree squirrel removal. As with any management tool, be sure to check with local authorities before conducting trapping efforts as special restrictions may be in place for tree squirrels in a given area.

Hares and rabbits

Young trees are particularly susceptible to debarking and limb clipping by several species of hares and rabbits. For our purposes, we will henceforth refer to all of these species collectively as rabbits.

BIOLOGY. Although not rodents, rabbits have similarly high reproductive outputs, with one to several litters possible per year, and one to eight young born per litter depending on the species. Rabbit populations sometimes fluctuate dramatically and often reach high levels every 5–10 years. Rabbits tend to be most active from early evening to early morning, so they are often not directly observed causing damage. Rabbits are cecotrophic; they consume soft fecal pellets to maximize digestion of cellulose and to increase the protein content of their diet (Salmon *et al.*, 2006; Baldwin, 2016).

DAMAGE. Rabbit damage to almonds is almost always limited to orchards less than 4 years old. Rabbits may debark trunks during the first winter following planting, and clipping of small branches and leaves may be evident as the tree breaks dormancy. As opposed to some rodent damage, rabbits often partially girdle tree trunks rather than completely girdling them. Girdling caused by rabbits is usually higher on the trunk than that caused by voles.

As with rodents, several management tools are available to reduce damage from rabbits. These management options are detailed below.

HABITAT MODIFICATION. Unfortunately, little can be done to deter rabbits from using almond orchards. If orchards are particularly overgrown, removal of vegetation might reduce damage from some rabbit species.

EXCLUSION. As with rodents, the use of hard plastic or wire tree protectors can effectively reduce or eliminate girdling damage to newly planted trees. Rabbits are substantially larger than mice and voles, so the protectors must extend higher up the tree. The exact height will vary depending on the species, but the protectors should extend above the height of the rabbit when standing on its hind legs.

Where rabbits are a constant and continuing threat to young trees, fencing the entire orchard may be the best management approach. An effective barrier can be constructed from 3.8-cm wire mesh that extends approximately 91 cm above ground. This fence must also be buried approximately 15 cm below ground to keep rabbits from digging under the fence. The efficacy of the underground portion of the fence is increased if a 15-cm lip is turned outward at a right angle at the bottom of the fence; the bend should be away from the area where you are attempting to exclude rabbits from. This lip decreases the likelihood that a rabbit will be able to dig under and around the fence.

REPELLENTS. Chemical repellents may provide temporary relief from rabbits. They are typically sprayed or painted onto the trunks or foliage following label instructions. A variety of repellents exist so some experimentation may be needed to determine what works best in a particular situation. It is important to remember that repellents work best when alternative food sources are available. If limited food options are present, repellents are less likely to be effective. Also, repeat applications will be needed as new growth occurs, as well as following substantial rainfall or sprinkler irrigation events. Even solar radiation will break down repellents over time, so be sure to follow label instructions to determine necessary reapplication intervals. Because of the amount of labour and cost required for repellent applications, they often are not the most practical management tool, but they can be an effective option when alternative tools are not available.

FRIGHTENING DEVICES. No auditory or visual frightening devices are effective at deterring rabbits from orchards.

PREDATORS. Many predators feed on rabbits including a suite of carnivore and raptor species. Predator composition varies depending on the orchard location. Although predators certainly do help to keep rabbit populations from reaching artificially high numbers, at present there is little information available to suggest that predators can effectively reduce rabbit numbers to a level that is acceptable to most almond growers.

ALTERNATIVE CULTURAL PRACTICES. There are no cultural practices that seem to have much impact on rabbit populations.

SHOOTING. Rabbit populations can be reduced through extensive shooting efforts, usually through the use of small-calibre rifles or high-powered air rifles. The quieter the firearm, the more effective a shooting programme is likely to be. Shooting efforts are generally focused on low-light hours of the morning and evening when rabbits are active. In some areas, spotlight hunting at night is legal and can be an effective tool. Be sure to check all local regulations before using shooting as a management tool.

TRAPPING. Trapping is not the most practical tool for managing rabbits, although capture of true rabbit species (e.g. cottontails and European rabbit) is possible using box or cage traps. Traps placed underneath natural cover will maximize capture success. A number of baits can be used to lure the rabbit into the traps including various vegetables and fruits. Captured rabbits should be euthanized humanely via shooting or through the use of a carbon dioxide gas chamber. A permit may be required to remove rabbits depending on local regulations. Be sure to check with the local authorities to determine the proper steps for rabbit removal.

FUMIGATION. Rabbits are not burrowing animals *per se*, but true rabbits (e.g. cottontails and European rabbits) do have their young in warrens below ground. Fumigation of these warrens is legal in some areas (e.g. Australia). Which (and if) fumigants are used varies across

countries, but aluminium phosphide is often the preferred option. Be sure to check with local authorities to determine what is appropriate for your location.

TOXIC BAITS. Baiting, usually with first-generation anticoagulants (e.g. chlorophacinone, diphacinone and pindone) or zinc phosphide, can be the most practical and economical way to treat large numbers of rabbits over large areas, although results may be erratic. These baits often come in grain or pelletized formulations, although they can sometimes be used to coat fruits and vegetables as well. Baits are typically only used along orchard edges; they cannot be used within the orchard itself during the bearing season.

Baits are usually placed in open self-dispensing feeders, shallow trays or nursery flats, although specific baiting techniques can vary across countries. The feeders should be positioned in areas frequented by rabbits, such as along trails and resting and feeding sites. If rabbits fail to feed after a few days, the feeders should be moved to another area where the bait is more readily accepted. Keep in mind that with anticoagulants, the bait supply must remain constant so as to ensure repeated access to the bait. Such a baiting programme should continue until feeding ceases, which can be up to 4 weeks after the initiation the programme. Zinc phosphide is an acute toxicant that kills within 24 h of consumption. As such, baiting programmes that use this material often yield results far more rapidly. However, zinc phosphide has a distinctive odour and taste that rabbits may avoid, so results with zinc phosphide are often more erratic.

Because baits can be accessed by other species, it is recommended that bait stations be covered or removed during daylight hours. This will remove non-target concerns associated with diurnal species. Remember that baiting laws vary tremendously across different regions and countries. As such, be sure to contact your local authorities to determine what is appropriate for your area.

Deer

Deer can cause significant damage to an almond orchard located in foothill or coastal districts with brush, riparian or forested areas nearby that provide cover. Regional game-management laws

limit management tools available to growers and make combating deer damage expensive.

BIOLOGY. Deer are generally most active during low-light hours of dawn and dusk, as well as at night (Salmon *et al.*, 2006). However, if they habituate to humans, they may become active during the daytime as well. Generally, deer will hide out in brushy areas during the daytime and move into orchards towards evening and night. Deer are not prolific breeders, having only one or two young per year.

DAMAGE. Deer can completely defoliate young trees, and they can stunt, distort or kill a tree through repetitive browsing. Although relatively rare, buck deer can severely scar the bark of young trees when they rub their antlers on trunks and lower limbs. Deer may browse on older trees, but the damage to them is usually less severe than that caused to young trees.

Because of their status as game species, lethal removal is not usually the preferred method for reducing deer damage. Instead, exclusion, frightening tactics and repellents are often used to deter deer from almond orchards. These and other potential options are detailed below.

HABITAT MODIFICATION. Almond orchards do not provide sufficient hiding cover for deer to use during daylight hours. Instead, deer are generally reliant on adjacent thick cover provided by shrubby, forested or riparian areas during the daytime. Such cover generally cannot be effectively managed to reduce the likelihood of deer using these areas. As such, habitat modification is not usually an effective strategy for deterring deer use of almond orchards.

EXCLUSION. Fencing is the most effective method for reducing or eliminating deer damage in an orchard, but it is expensive. As such, substantial damage must be anticipated to justify the expense of fencing. Many different designs for fencing are available (see Craven and Hygnstrom, 1994 for examples). A 2.5-m-high wire fence is one design frequently used to keep deer out of select areas. A 1.8-m-high mesh fence can be heightened to 2.5 m by adding around three strands of barbed or smooth wire to the top. Deer may occasionally clear a 2.5-m-high fence when

being chased or if the fence is on steep, sloping ground. In these situations, to completely remove the possibility of deer entering an orchard requires an even taller fence, or an alternative design that utilizes a 45°, 0.5-m extension of braces and wire angled toward the area outside of the orchard. Such additions obviously add to the cost of the fence and are often not included. However, it is incumbent on the grower to have a plan for removing deer from an enclosed orchard as such removals can be difficult. One option is to remove the fence in the corner of the orchard that sits at the highest elevation. The deer can then be funnelled towards this opening, although this is easier said than done. Growers must check deer fences periodically to be sure they remain intact; damaged wire, broken gates, soil washout beneath fences, etc. permit access for deer and must be repaired immediately.

Tree protectors can also provide some relief when damage is restricted to small areas. Common materials include hard plastic sleeves or wire mesh. The wire mesh should extend about 4 feet up the tree to prevent rubbing by bucks.

REPELLENTS. A number of repellents are available for use in deterring deer damage, but they all generally elicit either fear, pain, discomfort or some combination of these responses. Repellents that elicit a fear response (e.g. those based on rotten-egg formulations or dried blood) are typically most effective. Repellents that elicit a pain response (e.g. those containing capsaicin) can be effective if applied at a high concentration. Repellents with post-ingestive consequences (e.g. those containing Thiram) are moderately effective. Predator odours from urine or fecal matter exhibit variable efficacy but are generally not the most effective option. Keep in mind that repellents are not likely to eliminate damage, but, at best, will reduce this damage. Efficacy of repellents is also impacted by many factors including the availability of alternative food sources, frequency of rainfall or sprinkler irrigation events, habituation of deer to the field, and speed at which new growth occurs on treated trees. As such, they should not be relied on exclusively to mitigate damage from deer.

FRIGHTENING DEVICES. Noise-making devices such as propane exploders and electronic distress calls

are not typically effective for more than a day or so because deer rapidly habituate to frightening devices. If only a few deer are involved, having someone patrol newly planted areas at night with a spotlight to frighten deer away may prove effective, though expensive.

For small orchards (< 32 ha), dogs can be effectively used to frighten deer away. Dogs must be fenced in to keep them from roaming away from the target area. Options for fencing include a woven wire fence, electric fencing or invisible fencing. Effective breeds will vary but could include retrievers and other medium-sized active dogs. The dogs do not require extensive training, but not all dogs are appropriate for field protection; they must be out in the elements at all times and must show an interest in chasing offending animals away while not acting aggressively toward workers in the orchard.

SHOOTING. In some circumstances, removal of a damaging deer or two might solve a problem, but this is rarely an effective solution. Additionally, a special permit often must be issued by local authorities to remove depredating deer. This can be difficult to obtain for a game species such as deer.

Wild pigs

The wild pig (also called wild boar, Russian boar, feral hog and feral pig) is a cosmopolitan species. It is native to large parts of Europe, Asia and North Africa, but was widely introduced, and is considered an invasive species, in the USA, South America, Australia and New Zealand. It is a common species in Europe and adjacent non-European Mediterranean countries (CAB International, 2015). Throughout much of their range, including Spain and the USA, wild pig populations have increased in the past few decades.

Wild pigs can live in a wide variety of habitats; their populations can increase rapidly in suitable conditions, and they can cause damage, particularly to almond orchards located near forested areas.

BIOLOGY. Wild pigs are opportunistic omnivores whose seasonal dietary shifts occur as food items become either scarce or more abundant. Wild pigs frequently rely on a variety of agricultural crops, including almond nuts.

DAMAGE. Wild pigs cause various forms of damage to almonds. Common losses occur through direct consumption of nuts. However, wild pigs can also break branches that will lower yields for several years after feeding, or they may even kill young almond trees when they rub on the trunk, severely scarring the bark.

Wild pigs may be native or non-native depending on where the orchard is located. Furthermore, they may be managed as a game species, as a pest species, or somewhere in between. As such, it is imperative that the landowner determines what management options are legal in a given area. As a rule, the only proven effective options for wild pig management include exclusion, shooting, trapping and (only in Australia at the time of writing) toxic baits. Chemical repellents and frightening devices have not proved effective.

EXCLUSION. The use of temporary electric fences is one of the most effective methods for keeping wild pigs out of almond orchards. The use of electric fencing requires placement and maintenance efforts as the presence of weeds can short out the system. Strands associated with the electric fences should be set at approximately 25 and 50 cm from the ground; an electric shock surpassing 6000 V is considered effective against wild pigs. Non-electrified fencing can also be somewhat effective at restricting movement of wild pigs into almond orchards. Keep in mind that all fencing activities are costly and may not be appropriate for all situations. West *et al.* (2009) provide additional details on fencing designs that might work for a given situation.

SHOOTING. Shooting may be the most commonly used tool for managing wild pigs world-wide. However, shooting is very labour-intensive and is not appropriate in all situations. Local officials should be contacted before initiating a shooting programme to ensure that all pertinent regulations are followed.

TRAPPING. One of the most commonly used tools to manage wild pigs is trapping. Various trap designs can be used including box traps, large corral traps and snares (see West *et al.*, 2009 and Hamrick *et al.*, 2011 for an overview of trapping techniques), although what is legal for use varies depending on local regulations.

Be sure to consult local officials to determine what is effective and legal for use in a given area.

TOXIC BAITS. The use of toxic bait for wild pig management has received greater attention over the past several years due to the expanding problem that wild pigs represent, although only Australia currently has any toxicants registered (sodium fluoroacetate (1080) and sodium nitrite) for wild pig management in areas where almonds are grown. Substantial restrictions are in place for their use, so be sure to read the label thoroughly and consult local wildlife experts before using these products.

It is important to remember that wild pig numbers must be substantially reduced (~90% or more in a given area) to observe long-term benefits from removal efforts. No single approach will generally result in such a high removal rate. As such, an integrated pest management approach that utilizes various removal tools, combined with exclusionary fencing where appropriate, will likely provide the best results.

Birds

The damage birds cause is often a reflection of the surrounding habitat and whether that habitat increases bird numbers by providing nest sites, cover for loafing or roosting, ample food, etc. Damage may start when the almonds are small and continue through harvest (University of California Statewide IPM Project, 2002). Because solutions to most bird problems are not species-specific and rely extensively on frightening, the birds that are almond pests are not discussed separately but as a group (Fig. 16.17–16.20). Shooting and trapping are lesser-used techniques that are discussed as well.

DAMAGE. Losses often result from feeding on swelling fruiting buds prior to bloom or from birds removing the almonds from the tree or knocking them to the ground. Crop losses from birds can be substantial; in well-managed, bearing orchards, birds may, collectively, be far more destructive than mammals, partly because current bird control methodology often produces poor and inconsistent results. In fact, a research survey study noted an average revenue loss of 9.6% when birds were present and causing damage within nut orchards.



Fig. 16.17. White-crowned sparrow. (Photo by J.K. Clark, Copyright the Regents, University of California.)



Fig. 16.18. Woodhouse's scrub jay. (Photo by W.P. Gorenzel, Copyright the Regents, University of California.)

Some bird species will disbud almond trees prior to bloom. The birds commonly move from cover, such as wildlands, brushy areas and evergreen trees along a watercourse, to feed on the nearest almond trees. Hence damage is nearly always heaviest on margins of the orchard, adjacent to suitable bird habitat or power lines the birds use for resting. Disbudding is generally localized, and orchards surrounded only by other orchards with no adjacent wildlands may suffer little or no measurable damage. However, unless the birds are observed eating buds or unless the trees are inspected very closely during the winter, damage may go undetected. Disbudding first becomes apparent when the trees are in full bloom; fed-upon trees have noticeably fewer blooms in the upper canopy.

Many bird species also damage the nuts once they set on the tree. Damage is most severe



Fig. 16.19. Crows. (Photo by J.K. Clark, Copyright the Regents, University of California.)



Fig. 16.20. Yellow-billed magpie. (Photo by J.K. Clark, Copyright the Regents, University of California.)

from hull-split through harvest. The variety of almond, especially its shell texture and hardness, has a bearing on the birds it attracts and the extent of damage they cause. As might be expected, paper- and soft-shelled almonds usually suffer the most severe damage; however, no variety is entirely immune from damage.

It should be noted that some bird species, such as crows, feed on mummies that may be left on the trees following harvest. Some growers think that since crows remove mummies, it compensates for the sound nuts they eat, since mummies can provide overwinter harbourage for navel orangeworm larvae. In the long run, growers are probably better off if they use other methods to rid the orchard of mummies and waste nuts left on the ground post-harvest. A supply of almonds left on trees serves as winter food and may contribute to increased bird damage the following year by improving the winter survival rate of these bird species and by encouraging nearby nesting in spring. It is important not to

ignore birds' potential for learned behaviour. If they can feed on almonds in an orchard during winter, they will learn to prefer almonds and, in particular, the orchard that they were feeding in.

Monitoring is essential to minimize damage from bird pests. The best monitoring approach uses field personnel to watch for pest species. To detect disbudding, monitoring in the winter months is necessary. As almonds set, systematic monitoring is essential. One strategy involves establishing a schedule of bi-weekly orchard drive-throughs during the period of the day when bird activity is expected. Birds should be counted and recorded during these drive-throughs. Certain bird species often enter the orchard from one point or from one direction, depending on the adjoining habitat; therefore, counts of birds entering or leaving the orchard can also be made from stationary observation points. From these counts, the severity of the problem and its rate of increase can be estimated. Mechanical or digital counters can be helpful for keeping track of numbers. The count of birds actually seen feeding in selected

parts of the orchard can be applied to the entire area normally frequented by the birds. The number of almonds or hulls found on the ground throughout the orchard can also provide a clue to the extent and severity of the bird problem.

HABITAT MODIFICATION. The presence of non-crop trees and shrubby habitats adjacent to almond orchards can serve as loafing sites for some bird species, subsequently allowing for easy invasion into the orchard where they can cause damage. Removal of these loafing sites can help reduce damage, particularly from species that disbud trees over winter.

EXCLUSION. Netting can be used to exclude birds from trees but is not a practical option for almond orchards.

REPELLENTS. There are no repellents currently registered for use in almond orchards.

FRIGHTENING DEVICES. Most bird control in almond orchards presently relies on dispersing birds with frightening sounds made by live ammunition or cracker shells, propane cannons, or electronic distress calls (Salmon *et al.*, 1986; Baldwin *et al.*, 2009). Visual frighteners sometimes augment sound-producing methods. Reflective flagging or tape and large 'scare-eye' balloons attached to poles above treetops, one device per 1.2–1.6 ha, may offer temporary relief, although the reduction in damage provided by these tools tends to be minimal. Some visual devices, such as pop-up scarecrows or air dancers, produce motion that may also temporarily reduce damage. Alternatively, dead crows can be hung as effigies at or above the canopy tops and can serve as effective deterrents of crows as well. The most efficient way to use any visual device is to activate it 7–10 days before harvest. If implemented much sooner than this, the birds will begin to habituate to these devices.

The effectiveness of frightening methods depends on a number of factors:

- Are there nearby alternative feeding sites for birds?
- How great is the birds' affinity for the immediate area in terms of nearby nesting, traditionally high numbers in the area, etc.?
- Have the birds already become accustomed to feeding in the orchard?
- Have the most appropriate frightening methods or combination of methods been selected?
- Are the frightening methods being used to maximum effectiveness?

The grower can do little about factors cited in the first two questions, but those cited in the last three are manageable. Frightening should begin before the birds have established a regular feeding pattern. Once feeding habits are well established, birds are much more difficult to drive from the orchard.

Not surprisingly, shooting to produce noise is one of the most frequently used frightening techniques because it uses commonly available equipment and results are immediate. What is not commonly known, however, is that shooting with live ammunition, cracker shells or whistle bombs consists of two elements of harassment: periodic loud blasts and the presence of humans, and possibly a motor vehicle. For a period of time after shooting has been employed, some birds, like crows, often disperse as soon as they see the shooter or the shooter's vehicle approach. They may return as soon as they see the human and the vehicle leave.

Loud electric truck-type air horns mounted on ATVs or pickups can be effective if blasted periodically, thus cutting down on the amount of cracker shells or live ammunition needed. When bird feeding occurs at specific periods of the day, patrols can be focused on these time periods. However, be aware that birds are quick to alter their feeding patterns to avoid patrols.

The major problem with all frightening techniques is that, when used day in and day out, most birds habituate to them. Thus, their effectiveness diminishes with time. Their effectiveness also diminishes as more growers in the same general area use the same techniques. The grower with the most innovative frightening strategies will often be most successful. However, growers should be aware that some bird damage is likely to occur even with the most innovative strategies.

To prolong the effectiveness of frightening methods, the grower should introduce as many variations as is practical. For example, if a shooter normally rides around the orchard on

an ATV, he or she should occasionally abandon the vehicle and walk through the orchard shooting. Instead of using normal shotgun ammunition, using cracker shells occasionally produces a second blast above the birds and can provide added benefits.

Variations of frightening approaches should also be employed intermittently. Rather than using exploders and electronic distress calls simultaneously, employ them in a rotational scheme. For example, use electronic distress calls for 5–7 days, and then use propane exploders for the next 5–7 days. Compared to simultaneous use, rotation usually results in superior and longer-lasting bird dispersal, reduced operating costs and extended equipment life. To maximize the effectiveness of a rotational programme, the grower should determine the number of days in each sequence by observing the birds' response. When birds begin to habituate to a particular technique, it is time to switch to an alternative approach.

Growers often make the mistake of using too few propane cannons or alarms. The minimum for effectiveness is around one cannon per 2–3 ha. Because birds cause damage during daylight hours only, auditory frightening devices should only be operated during daylight hours. Propane exploders should be on tripods or elevated high off the ground, preferably above the canopy; this will allow the sound to carry further. Keep in mind that the use of sound-making devices may not be possible where residences are nearby or local ordinances prohibit them.

Although this discussion has emphasized the effectiveness of using frightening approaches on a rotational basis, growers should not hesitate to employ, intermittently, appropriate combinations (e.g. a sound-producing device with a pop-up scarecrow, electronic distress calls combined with effigies hung above the canopy). [Table 16.1](#) describes a strategy whose effectiveness derives from combinations and change. This is just an example; many combinations could be developed that would be effective in a given orchard.

PREDATORS. A few raptor species prey regularly on some smaller bird species. However, given the extensive canopy of almond orchards, these raptors are not able to remove anywhere near

Table 16.1. Sample schedule for combining frightening devices.

Week	Activity
1	Roving patrol firing shotgun or cracker shells
2	Electronic distress calls
3	Propane exploders only
4	Roving patrol firing shotgun or cracker shells plus the use of propane exploders

enough birds to have a measurable effect on pest populations. Occasional mammalian predators also capture birds when feeding on the ground, but these losses have little or no impact on pest bird populations.

SHOOTING. Lethally removing depredating birds through shooting can occasionally be successful at reducing damage from resident bird populations that are present in relatively low numbers. Be aware that shooting requires extensive effort. Also, shooting of some bird pests may not be legal, while other bird species may require special permits to remove lethally. Be sure to contact your local authorities before engaging in a shooting programme. However, where permissible, the shooting of a few depredating birds can enhance the effectiveness of ongoing frightening programmes. For this reason alone, shooting may be a worthwhile inclusion in bird management programmes.

TRAPPING. When dealing with certain resident bird species (e.g. house finches), trapping can be relatively effective in reducing damage. Trapping, and any removal technique for birds, will only be effective for resident birds. If the bird population is migratory, those birds that are causing damage one day may be gone the next, thereby rendering removal efforts ineffective.

Trapping, like frightening techniques, can be labour-intensive, and it requires a significant outlay to cover trap costs. A trapping programme must be well planned to produce the desired results at the lowest cost. A cooperative programme involving several neighbouring growers works best. Once a programme is in place, it can be quite efficient. Because trapping depends on drawing birds into the area, this approach is not compatible with frightening methods.

Modified crow traps similar to those for trapping starlings are most commonly used. A combination of canarygrass and rapeseed often provides an excellent bait source for drawing birds into the traps. Once a few birds are caught they serve as decoys to attract others. Therefore, a few decoy birds should be kept in the traps with ample food and water. Excess birds are then euthanized humanely, usually via a carbon dioxide gas chamber. Keep in mind that trapping regulations

vary tremendously across regions and countries. Therefore, growers must consult with local authorities before attempting any trapping programme to determine the legality of this approach, as well as what species requirements are in place to employ a trapping programme.

TOXIC BAITS. Although bait programmes do vary regionally, toxic bait application is not generally used to mitigate bird damage to almond orchards.

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17 Almond Harvesting

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17.1 Introduction

Mechanization is a basic step in almond production to consolidate its sustainability in the global economic framework. Mechanization reduces production costs and solves important social and labour problems, since the availability of skilled workers is continuously decreasing in all producing countries. In addition, mechanical operations have lower costs compared to manual labour costs. These trends are likely to continue in the future as even the availability of people devoted to almond growing is decreasing in some regions. Since mechanization saves time and effort in all orchard management operations, workers can be more devoted towards improving their skills and enhancing their working security. Consequently, mechanization must be applied to all operations whenever possible, but mainly in those operations with higher production costs and manual labour inputs.

Manual harvesting in traditional Mediterranean almond growing reaches 30–35% of total direct production costs (Fig. 17.1). In addition, this operation requires a large number of workers during a short period of time, an increasing problem due to the difficulty in finding part-time seasonal workers. The number in traditional orchards that are not harvested is increasing due

to lack of work force, low production and physical difficulties such as poorly formed trees and difficult terrain. Harvest mechanization offers many advantages for orchard management: improved worker safety; reduced labour requirements and production costs; and obtaining a high-quality product. The different harvesting machines require optimal operation to fulfil all these objectives.

California almond harvesting operations are nearly entirely mechanized. Orchards have the advantage of growing on valley soils with nearly level terrain which facilitates mechanical harvest. California almond orchards are very productive and the industry has been expanding steadily over the past 30 years. Harvest consists of mechanical shaking, sweeping, nut pickup and hauling to a processor. There is a minimal amount of hand raking required during harvest to ensure there are no nuts left behind. Harvest costs for these in-field operations are 18% of total direct production costs (Connell *et al.*, 2012).

17.2 Optimal Harvesting Time

In addition to orchard preparation, almond harvest involves nut removal from the tree as well as

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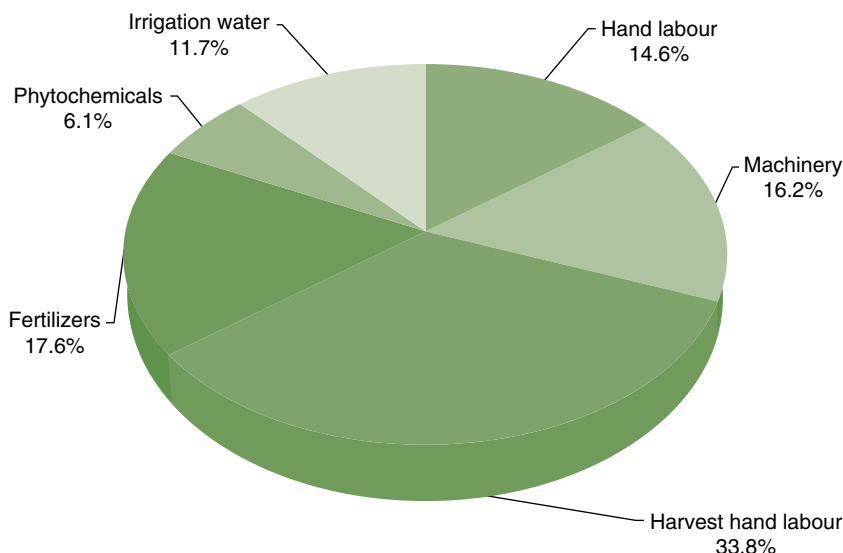


Fig 17.1. Distribution of the production costs in an irrigated almond orchard with hand harvesting.

operations to obtain a stable marketable product. This process includes two basic operations:

- Hulling (removal of the in-shell nut's hull or mesocarp).
- Moisture reduction to acceptable commercial levels.

At present, almond harvest in most orchards is mechanical, as manual harvest is no longer economically feasible. It takes place from mid-July in early-ripening cultivars to mid-October for late-ripening cultivars. Other than very limited commercialization of green almonds in May–June in France and North Africa, most almonds are marketed dry, requiring the complete ripening of the nut. Ripening is not only characterized by the final accumulation of kernel components, such as lipids and carbohydrates, but also by water loss, although not to complete dryness. In the Mediterranean region, this takes place mostly while nuts remain on the tree, while in California final drying of the mesocarp (hull) occurs on the orchard floor once nuts are shaken from the trees.

In the Mediterranean region, the optimal harvesting time is when most fruits show a relatively dry dehiscent hull (Fig. 17.2), with little nut fall before nut maturity and harvest, allowing the maximum efficiency of the harvesting machines. Consequently, the parameters related



Fig. 17.2. Almond nuts showing completely dehiscent and nearly dry mesocarps ready for a shake and catch harvest.

to almond maturity must be considered. Almond fruits receive nutrients produced by the leaves through the peduncle as they increase in size and volume and synthesize all kernel components related to nut quality. However, at 25–28 weeks after full bloom, this metabolism slows down and, depending on cultivar, senescent processes start. During this period the activity of auxin decreases and that of abscisic acid and ethylene increases. At the peduncle, the cementation layers become friable and the cell wall of some cells is degraded; an abscission layer forms, spreading to the bordering tissues until reaching the xylem

vessels, thus leading to nut drop. The hull begins dehydration at the same time, opening along the suture line. The retention force of the peduncle during this period of time changes dramatically, reducing its strength to such low values that at the final stage of senescence a single breeze may be enough to detach the nuts. The rate of decrease of this retention force depends on the cultivar (Tsatsarelis, 1984).

Special attention must be paid to the final steps of nut ripening, from 1% dehisced hulls to the stage of 100% dehisced hulls. Harvesting may be carried out during this dehiscing period. A simple method to evaluate nut readiness for harvest consists of enclosing several branches of representative trees with bags of wide mesh, periodically counting the fallen nuts.

In California, almonds are considered ready for harvest once 100% of the hulls have begun to dehisce. Kernel components have fully accumulated by this time; however, the hulls of different nuts on the same tree are in various stages of dehydration. Although hulls are not fully dehydrated, ‘Nonpareil’, the first Californian cultivar to mature, reaches maximum nut removal with mechanical shaking at this stage (Connell *et al.*, 1989). Under Californian conditions, hulls dehydrate more rapidly on the ground than in the tree thereby advancing harvest and maintaining optimum kernel quality.

Another option for evaluating the ripening rate of each cultivar is the almond hull-split prediction model of the Department of Plant

Sciences, University of California, Davis. The model predicts a date for initiation of almond hull-split at a location, based on user input of the date of full bloom and temperature readings during the first 90 days after full bloom collected by a local California irrigation management information system (CIMIS) weather station, also designated by the user. This model will only work 90 days after bloom. This hull-split prediction model is based on the concept that the rate of fruit development from bloom to 1% hull-split is highly dependent upon early spring temperatures (Tombesi *et al.*, 2006). The rate of fruit development between 1% and 100% hull-split can also vary greatly among years and cultivars (Connell *et al.*, 2010). To estimate the approximate time that a cultivar will be ready for harvest, this hull-split prediction model may be coupled with the information in Table 17.1 to deduce the date of 100% hull-split. Hull-split as defined here is the time when nuts with green hulls develop the first crack along the suture that penetrates to the shell. The rate of nut maturation for each cultivar in the trial is represented as the number of days between 1% and 100 % hull-split.

In the Mediterranean region, combining the parameters of nut quality with the time required to carry out all harvest operations will inform the optimum time for harvest for each cultivar. A shake and catch harvest must be started as soon as these optimum conditions have been reached to finish harvest before nut fall reaches 2–3%, thus avoiding a significant crop

Table 17.1. Nut maturation of almond cultivars in the Chico Regional Almond Variety Trial represented as the number of days between 1% and 100% hull-split. (Connell *et al.*, 2010.)

‘Carmel’ group 15–19 days short maturation	‘Butte’ group 20–22 days medium maturation	‘Nonpareil’ group 23–25 days long maturation	‘Monterey’ group 26–30 days very long maturation
‘Kochi’	‘Durango’	‘Donna’	‘Sano’
‘Rosetta’	‘UC2-19E’	‘UC25-75’	‘Monterey’
‘Carmel’	‘Padre’	‘Avalon’	‘Winters (UC 13-1)’
‘Jenette’	‘UC1-87’	‘Price’	‘Yokut’
	‘Mission’	‘Plateau’	
	‘Moreley’	‘Jiml’	
	‘Kapareil’	‘Savana’	
	‘Wood colony’	‘Johlyn’	
	‘Butte’	‘Ruby’	
	‘UC2-43W’	‘Chips’	
	‘UC1-102W’	‘Nonpareil’	
	‘Aldrich’	‘Kahl’	
	‘Kapareil/PA’	‘Livingston’	
	‘Sonora’		

loss. These conditions define the best harvesting time because organoleptic characteristics of the nuts are also optimal. Therefore, the optimal harvesting time may be defined as the stage when a very high percentage of fruits remain on the limbs, when kernels are of high quality and when the crop is ready to be harvested by the appropriate machine.

In California, since nuts are shaken to the orchard floor while hulls are split but still green, there is less concern about pre-harvest nut fall since the crop dries on the orchard floor. Once hull and kernel dehydration is adequate all nuts are mechanically swept into rows in the orchard middles, are picked up by machine and are then delivered for hulling.

Late-ripening cultivars may be subject to rains during harvest. The probability of rain is greater in some producing areas and the probability increases as the season advances. Rain interferes with all harvest operations, delaying and slowing operations and requiring additional drying of the nuts. These inconveniences result in cost increases while potentially reducing nut quality. Consequently, when selecting a cultivar for planting its ripening date and the rate at which it matures from hull-split initiation to 100% hull-split should be considered.

17.3 Definition of the Time for Beginning Harvest

Weekly observations of nut ripening will allow pre-harvest work to be completed and for harvest to begin in a timely fashion. In the Mediterranean region, this decision can be based on observing the initial hull-split (1% of nuts), the evolution of dehiscence, hull dehydration and the beginning of natural nut fall before nut fall affects the quantity and quality of the crop. In California, once initial hull dehiscence reaches 100% in the lower interior of the tree, a mechanical shake harvest can commence since hulls will complete drying on the orchard floor. These indices are easy to follow and can identify the right moment to begin harvest.

The time for harvesting depends on the cultivar and farm operations in relation to the availability of machinery and labour. An adequate amount of equipment must be available for rapid harvesting and concentrating the operations in the right moment. In California, it is

common to have two to four cultivars present in each orchard since the majority of orchards comprise self-incompatible cultivars. Cultivar selections must be made at planting so that cultivar maturity is different enough so that each cultivar can be harvested sequentially without mixing the nuts of different cultivars.

Adverse climatic conditions can hinder all harvesting operations. Autumn rains not only hamper harvesting, but can also adversely affect nut quality. It is nearly always advisable to advance harvest as much as possible in order to avoid these problems and to ensure kernel quality since delays and a lengthy harvest period cannot be foreseen.

17.4 Traditional Mediterranean Harvesting Systems

The traditional Mediterranean region harvesting systems, including concepts related to the different harvest operations, are described below. Considering this information, the needs for mechanical harvest and for different solutions to overcome the problems of manual harvest are clearly needed. Traditional harvesting systems use two approaches: ground picking and knocking down.

17.4.1 Ground picking

With this system, nuts are allowed to fall naturally to the ground as they ripen and they are then handpicked once or twice. Ground picking is very costly since the efficiency of hand labour employed in this hard task is very low. Consequently, this system is applicable only in rare cases such as very large trees or in areas with difficult access. This is not viable from a commercial point of view.

17.4.2 Hand knocking and use of mesh

This system was most commonly used in the Mediterranean region until the massive introduction of mechanical devices in the 1990s. The worker handles a stick varying from 1–3 m or even 4 m in length, knocking the branches to make the nuts fall down. A good knocker must beat the fruiting branches laterally to avoid

damage to the tree and to the next year's crop. Damage can occur in some cultivars when nuts are difficult to knock or if workers use poor practices. Even in a good operation, 2–5% of the following year's crop may be lost when shoots are broken by knocking the branches (Lamp *et al.*, 2001). This loss can reach 10% in cultivars with strongly held nuts or if workers do not carry out the knocking properly.

Mesh is spread under the tree and extended beyond the canopy to gather all nuts dropping from the tree. The knockers must work around the tree to knock down nuts from the whole canopy, rendering the operation very time consuming. Leaves and shoots may be removed before folding the mesh and emptying the contents in a trailer for transport to the processor for hulling. Mesh extension and folding may be minimally mechanized, depending on the ground condition, but most of the work is manual. The output of this harvesting operation ranges from 24 kg to 63 kg/h per worker, depending on the amount of crop (Table 17.2). The time required for harvesting the same amount of almonds decreases significantly as the crop increases, although this relationship is not linear.

Hand knocking on mesh was adopted in orchards where other types of mechanization were not applicable. At present, one of the major problems of almond growing is the cost of manual harvest which may reach 40% of the direct costs of almond farming. Considering the social development and labour availability in rural areas of the Mediterranean region, mechanization has become an essential requirement for orchard management.

17.5 Harvesting Steps

In the Mediterranean region several almond harvest mechanization attempts have been considered in the past and continue to be considered today. However, the growing conditions of the almond orchards in the different growing regions are very diverse, with trees of all ages; on slopes or flat ground; in small or large plots; on dry land under rainfed conditions; or irrigated. These different conditions have prevented the design of an integral harvester able to pick all nuts in a single run. Consequently, each situation must be examined independently with orchard condition considered in relation to harvesting method chosen. The time needed for each step determines the priorities established for harvesting each orchard.

In California's Central Valley, all almond orchards are irrigated and they are planted on nearly level ground or occasionally on gently sloping terrain. Commercial orchards range in size from 4 ha to over 400 ha. These growing conditions lend themselves to standardized harvesting methods that are similar throughout the Central Valley. In the 1960s the first attempts at mechanical harvest used wire cable shakers mounted on tractors. Later, mechanical knockers mounted on a boom came into use. Today, harvest is conducted exclusively with mechanical trunk shakers, sweepers and pickup machines.

Three main harvesting steps must be considered: ground preparation, picking up nuts that have fallen to the ground and harvesting nuts from the tree.

Table 17.2. Time requirements (min) for almond harvesting by knocking, depending on the amount of crop.

Task	Crop (kg/tree)					
	10	20	30	40	50	60
Mesh transport	5.00	5.00	5.00	5.00	5.00	5.00
Mesh extension	3.55	3.55	3.55	3.55	3.55	3.55
Knocking	11.63	16.76	21.28	26.87	32.12	36.85
Mesh folding	3.80	3.80	3.80	3.80	3.80	3.80
Transport to trailer	1.30	2.60	3.90	5.20	6.50	7.80
Total min/tree	25.28	31.71	37.53	44.42	50.97	57.00
Total min/kg	2.53	1.59	1.25	1.11	1.02	0.95
h/ha	66	82	98	116	133	148

17.5.1 Floor preparation

Planting density must be considered first, as the distance between rows must be wide enough to facilitate the use of trunk multidirectional shakers. The tasks related to floor preparation mainly depend on the type of orchard floor management applied to the orchard. Thus, the type of soil and its depth, and rainfed or irrigated conditions may affect tilling and orchard floor preparation. In many Mediterranean regions, the presence of slopes and of intercrops must also be taken into account, as well as the potential yield in the orchard.

Traditional tilling is by far the most common orchard floor management system applied in non-irrigated orchards in the Mediterranean region, although there is considerable variability. Cross-tilling is mostly applied in winter and spring with a cultivator. In summer two ploughings with a spine harrow are made within 20–30 days, followed by a roller to compact the ground to facilitate harvest. In the past, ploughing with a disc harrow was very common, but it is not used at present as there can be severe root breakage and soil deterioration, facilitating erosion.

In California, orchard floor management is a critical component of harvest operations since some nuts fall naturally and the rest are shaken to the orchard floor to dry. A no-tillage system of orchard floor management is most common, with strip weed control practised in the tree rows, while the orchard middles are mowed to control weeds. Prior to harvest all weeds are eliminated with a contact herbicide application to the orchard middles so that the orchard floor is smooth, firm and weed free to accommodate nut drying and mechanical sweeping and pickup of the crop.

17.5.2 Picking up naturally fallen nuts in the Mediterranean region

Picking up nuts that fall before harvest, even in small percentages, requires large amounts of hand labour, thus increasing the total cost of harvest. Unfortunately, machines designed so far do not satisfactorily solve this issue. Both pneumatic and mechanical machines obtain very low outputs due to design problems as well as to the low density of fallen nuts per unit surface area.

Another solution is to place plastic mesh under the trees before any nut fall. Although technically ideal, this solution comes at a high cost because of the large surface needed to be covered by the mesh; from 60 m² to 80 m² in large trees. The possibility of recovering a large percentage of fallen nuts, which otherwise would be lost, is a key factor in considering the use of permanent mesh. Undoubtedly a more economical solution would be starting harvest before any appreciable quantity of nuts may have fallen. The cost of placing a mesh or shaking a tree is the same whether 100% of the crop is on the tree or if some crop has already fallen.

It may be interesting to extend the harvesting season as a means to improve the amortization of the machinery investment. In this case, the use of abscission retardants might extend the harvesting season. This kind of application has already been studied in other fruit trees, but not so far in almond; it could potentially improve the economics of almond harvesting.

17.5.3 Nut harvest from the tree

Removing nuts from the tree is the main operation in almond harvesting. The goal is to capture all the nuts to maximize the return to the almond producer. Since manual hand labour at harvest is such a high percentage of costs when knocking nuts with sticks, harvesting is the orchard operation receiving the greatest effort towards mechanization in the Mediterranean region. This has given rise to a large number of devices and machines, some fully operational but others still under development.

Manual devices

The first attempts at mechanized harvest were manual harvest aids designed similarly to the original manual operations of collecting and sticking. These devices all failed due to their very low efficiency and to the large amount of damage caused to the plant and fruits. Any machine aiming at knocking the nuts where they are borne is not viable. This approach was completely unfeasible due to the large volume of the tree canopy (50–70 m³) and the small size of almond nuts (a few cm³).

Factors affecting shaking machine efficiency

Unlike simple manual systems, shaking machines use a different concept to remove nuts. Shakers have been developed and used to harvest other fruits for quite some time; therefore, many different sizes of machines using various shaking systems have been tried in almond. The most effective ones are powerful multidirectional shakers (Fig. 17.3) so, as a consequence, most of this discussion will be related to using these machines.

Experience with multidirectional shakers has shown that they remove more than 95% of nuts in orchards with homogeneous trees and optimal conditions for shaking. As many as 50–60 trees can be harvested per hour with minimal limb breakage. Besides nuts, only leaves and shoots previously damaged by pests and diseases fall during shaking. If less than 90% of the nuts are removed it is usually because the shaking force did not fully reach all limbs. Nuts remaining are often grouped in localized places, rendering a complementary knocking with sticks profitable.

Both tree size and structure affect shaking efficiency. In small or medium-sized trees nearly 100% of nuts fall when trees are shaken; as tree

size increases, shaking efficiency decreases. Upright trees have better shaking efficiency than trees with roundish or hanging canopies. Shaking force is transferred more efficiently to vertical or upright branches than to horizontal or bending branches. Greater nut removal is attained from straight branches rather than from curving branches, and this should be considered when developing the best tree structure for shaking trees for harvest.

Pruning can be used to direct growth of primary, secondary and tertiary branches into a more upright structure that will shake more efficiently. A few main branches must be selected for strength to avoid excessive bending. Bending branches can be eliminated and horizontal branches shortened so the trunk will be clearly visible for approaching and clamping on the shaker head. A vertical trunk must be trained with enough height before branching to allow an easy approach of the shaker head with adequate space for opening an inverted umbrella if such a system is used to catch nuts.

Planting density and tree size can affect shaking efficiency and economical operation since more nuts can be harvested from larger trees with each machine operation. Large trunk



Fig. 17.3. Shaking head of a powerful multidirectional shaker.

shakers require wide manoeuvring spaces and the number of trees shaken per hour is not largely affected by planting distance. Therefore, planting distance for modern orchards in the Mediterranean region must be wide enough to manoeuvre shakers and to allow for larger trees, but not so wide that productivity per hectare is reduced. A planting density of 200–300 trees/ha provides these conditions, giving a planting distance of 6–7 m and a canopy volume of 40–50 m³/tree.

In California, maximum productivity is achieved when planting distance is close enough for the trees to capture at least 80% of the sunlight that would otherwise reach the orchard floor. During the 1970s almonds in California were commonly planted at a density of 187 trees/ha at a planting distance of 7.3 m². Since that time planting density has increased, followed by an increase in production as more sunlight was captured by the trees. Today, a common spacing is approximately 5.5 m down the row by 6.5 m between rows resulting in a planting density of 280 trees/ha. A little over 6 m is required between rows to adequately manoeuvre harvest equipment. Some orchards are planted closer down the row with spacing sometimes as close as 4–5 m between trees in the row.

Collection of fallen nuts

Independent of the method of nut removal from the tree, in the Mediterranean region nuts may be collected on plastic mesh previously placed under the trees. The hand labour required to extend the mesh, collect the nuts and move the mesh is approximately 12–13 min per tree. In the knocking system this represents from 10–25% of the total hand labour required, depending on the crop. In the shaking system (Table 17.3) the amount of hand labour required is similar. If shakers have a receptacle to collect the nuts, their efficiency is affected by tree size, as they may only be used with trees of limited canopy. Additionally, approximately 1 min is needed for the deployment and retreat of the receptacle (inverted umbrella), which represents a large amount of time and cost, taking into account the cost of each minute of hand work (Table 17.4).

Since nuts in California orchards are shaken directly to the orchard floor for drying, the condition of the orchard floor prior to harvest is critical. The orchard floor must be smooth and firm with no depressions that could trap nuts and it must be clean with no weeds so that drying is facilitated (Fig. 17.4). Nuts shaken to the

Table 17.3. Time requirements (min) for almond harvesting by trunk shaking and manual mesh extension and collection.

Task	Crop (kg/tree)					
	10	20	30	40	50	60
Mesh movement	12.35	12.35	12.35	12.35	12.35	12.35
Shaking	1.11	1.11	1.11	1.11	1.11	1.11
Transport to trailer	4.47	8.38	13.25	19.03	25.55	32.83
Total min/tree	17.93	21.84	26.71	32.49	39.01	46.29
Total min/kg	1.79	1.09	0.89	0.81	0.78	0.77
Total hand labour/ha (h)	47	57	69	84	101	120
Total shaker/ha (h)	2.89	2.89	2.89	2.89	2.89	2.89

Table 17.4. Time requirements (min) for almond harvesting by trunk shaking and inverted umbrella.

Task	Crop (kg/tree)					
	10	20	30	40	50	60
Shaking	1.11	1.11	1.11	1.11	1.11	1.11
Transport to trailer	0.11	0.23	0.34	0.45	0.56	0.68
Total min/tree	1.22	1.34	1.45	1.56	1.67	1.79
Total min/kg	0.12	0.07	0.05	0.04	0.03	0.03
Total shaker/ha (h)	3.18	3.47	3.77	4.06	4.35	4.64



Fig. 17.4. A young Californian almond orchard at harvest. The surface is level, firm and weed free, with nuts drying on the orchard floor.

orchard floor are typically allowed to dry for 5–10 days prior to the sweeping and pickup operations. Collection of the nuts is done by mechanical sweeping, with a minimal amount of hand raking, followed by a mechanical pickup operation.

Mechanical sweeping and pickup during harvest in California is the equivalent of the nut collection systems described above for the Mediterranean region. In the sweeping operation nuts are blown from around the tree and swept into a row in the orchard middles (Fig. 17.5). A minimal amount of hand raking is done to collect nuts missed by the sweeper. While harvest costs are 18% of total direct production costs, the breakdown of these costs is as follows: shaking 21%, hand raking 3%, mechanical sweeping 16%, and pickup and transporting nuts 60% of the harvest costs (Connell *et al.*, 2012). A pickup machine gathers the nuts from the row in the orchard middles and loads them into a conveyer nut cart that is towed behind the pickup machine (Fig. 17.6). A conveyer chain and suction is built into the pickup machine to separate fine soil, small rocks, leaves and small twigs from the nuts as they are conveyed into the nut cart.

A stick chain to separate larger sticks from the nuts may be built into the conveyer nut cart. Sticks, leaves, twigs and fine soil are left behind in the orchard as the nuts are transported out of the orchard by a shuttle wagon (Fig. 17.7) and either delivered to an on-farm huller or trucked to a commercial huller to remove the hulls. In-shell nuts and kernels produced by the hulling process are then delivered to a nut handler for further processing and marketing.

Transport and hull separation

When nuts are harvested on mesh placed on the ground, a first improvement would be to hull nuts at the farm's headquarters. Nuts and the accompanying impurities could be dumped from the mesh into trailers and transported to the farm headquarters for hulling prior to drying and storage. Hulling machines with high performance and low cost are available. The contents of the mesh can also be dumped in large-volume pallet boxes (300–350 kg), requiring tractors with packing pitchforks to move the boxes to headquarters. Once collected in the middle of the mesh, sucking equipment could be used to



Fig. 17.5. A Weiss McNair (model JD-80) mechanical sweeper blows nuts from the tree row and sweeps nuts into a row in the orchard middle. (Photo courtesy of Weiss McNair, Chico, CA, USA.)



Fig. 17.6. A Flory model 6500 pickup machine (Flory Industries, Salida, CA, USA), towing a conveyor cart from Thomas Manufacturing Co. (Chico, CA, USA), picks up a row of nuts, separates leaves, twigs and soil and leaves debris behind in the field while loading the conveyor cart.



Fig. 17.7. A nut Bankout shuttle wagon from Thomas Manufacturing Co. (Chico, CA, USA) approaches a conveyor cart to offload accumulated nuts for transport from the orchard for hulling.

pile nuts in the trailer, with the possibility of discarding leaves and broken limbs. Mechanical loaders might also be used with the same goal. Tables 17.2, 17.3 and 17.4 show the analysis of time requirements for several operations of the different harvesting systems.

In California, the shuttle wagon delivers nuts either directly to an on-farm huller or to an elevator for loading trucks parked alongside the orchard for subsequent hauling to a huller. Large commercial hulling operations are more common today since a large investment in equipment including colour sorters is required to do an excellent job of hulling. These commercial hullers can improve the quality and value of the in-shell or kernel product that is delivered to processors, improving the economic return to the growers.

17.6 Mechanical Harvesting

Mechanical harvesting is the only alternative for reducing production costs, overcoming the shortage of hand labour and reducing the problems related to unfavourable climatic conditions during harvest (Table 17.5). All aspects of almond harvest

must consider scientific evaluation of new methods to optimize harvesting efficiency. Tree responses to mechanical operations must also be taken into account to achieve maximum crop productivity with any harvesting method adopted.

17.6.1 Nut removal from the tree

Nut removal is accomplished by forces of traction, flexion and rotation acting on the nut peduncle. The traditional picking systems use mainly traction forces, while sticking transmits traction and flexion forces, as well as air flows. The shaking machines mainly use torsion forces together with traction and flexion forces. Among the large number of trials on almond harvest, the shaking systems seem the most convenient and efficient in removing nuts from the tree. In the first trials, shaking was obtained by wire cable or impact shakers. Later inertia shakers were developed, becoming the most widely used at present. Through the rotation of eccentric masses, vibrations are generated with a frequency of 30–40 Hz and an oscillation of several mm. The oscillation depends on the mass and the weight of the eccentric masses of the shaker, as well as on the

Table 17.5. Harvesting costs of different systems in irrigated orchards with different productions (tree density of 156 trees/ha).

System	Crop (kg/tree)						Average (€/kg)
	10	20	30	40	50	60	
Knocking + mesh (manual)	0.29	0.18	0.15	0.13	0.12	0.11	0.16
Trunk shaking + mesh (manual)	0.35	0.20	0.15	0.13	0.12	0.11	0.18
Trunk shaking + inverted umbrella	0.15	0.14	0.06	0.05	0.04	0.04	0.08

mass of the shaker and of the shaken tree. According to experimental trials:

$$S = 2Me r/(Mv + Ma) \quad \text{Eqn 17.1}$$

where S = oscillation (m), Me = eccentric mass (kg), r = mass eccentricity (m), Mv = shaking head mass (kg) and Ma = shaken tree mass (kg).

Nut removal takes place when efficient combinations of frequency and oscillation are attained. Enough acceleration is more easily accomplished when frequencies are like the system resonance or a multiple of it. The components of the torsion forces are more efficient in making nuts fall than the forces of only traction or flexion. Therefore, vibrations originating at the shaking point of the trunk must be transmitted to the fruiting points through the limbs, amplifying the oscillation and generating forces in horizontal and vertical directions.

The shakers most widely spread at present are made of two eccentric masses rotating in opposition, or of a single eccentric mass rotating on an axis. These two types give efficient combinations, based in one case on a wide oscillation and in the other on a high frequency. The shakers of the second type are easier to build and require lighter supports.

17.6.2 Machine types

Inertia trunk shakers

These are the most widely used almond shakers in both the Mediterranean region and in California and there are two general types:

- With two vibrating masses triggered by a single hydraulic engine through a pulley of returning movement. The two masses may have different weights and rotate at

different speeds. The vibrations produced are multidirectional with an effective power of 15–30 kW, a frequency of 15–30 Hz and an oscillation of 20–30 mm. The total weight of the shaking head is 400–600 kg.

- With a vibrating mass triggered by a hydraulic engine. They work at high frequencies, > 20 Hz, with orbital movements. Their weight ranges from 100–300 kg, requiring power of 30–50 kW. These are easier to manage.

Inertial trunk shakers are made by several manufacturers in California. In California these machines are self-propelled with an enclosed air-conditioned cab for the machine operator. Tractor-mounted shaker heads are not used in the California almond industry. Monoboom® shakers are built for trunk or limb shaking on larger trees (Fig. 17.8). These shakers typically move down the orchard middle, shaking one row of trees as they go. Other models are built with side-mounted shaker heads so the machine can be driven directly down the orchard middle, parallel to the tree row, with the shaker head telescoping out to shake trees as it goes (Fig. 17.9). The pads on the clamp have a rubber sling over the pad with lubrication between the pad and sling to reduce the chance of trunk bark injuries occurring during shaking. Since nuts are shaken directly to the orchard floor, rotating brushes sweep the nuts out from in front of the wheels as the machine moves forward or backward through the orchard.

Other types of harvesters

There are four other main types of harvesters in the Mediterranean region. The first is derived from grape harvesters (vintagers); they were first adapted for olive harvesting and are widely used in those industries (Fig. 17.10). At present



Fig. 178. This self-propelled Monoboom® shaker from Orchard-Rite® (Yakima, Washington, USA) will effectively handle either trunk or limb shaking as needed. (Photo courtesy of Orchard-Rite®.)



Fig. 179. The Orchard-Rite® Bullet®, a self-propelled side-mounted tree shaker can move rapidly down the tree row working quickly and effectively on trees that can be trunk shaken. (Photo courtesy of Orchard-Rite®.)



Fig. 17.10. Almond harvester adapted from grape harvesters, under development by the manufacturer Fitasem (Barbastro, Huesca, Spain).

trials are underway to adapt them for almond harvesting, increasing the number of vibrating sticks from four to 10–12. The vibrating space has also been increased, reaching a width of 0.8 m and a height of 2–2.5 m. In some models the height has been increased up to 3.5 m. The shaking system has been improved in some models to reach a frequency of 400–500 cycles/min and to change the curvature of the knocking bars to knock down the nuts with a

controlled vibration exerted in a cyclical and alternate way. The nut-catching system has also been improved by adopting wheels and baskets of different shapes to reduce nut loss. The machines can work on slopes by using levelling and anti-sliding systems to ensure their stability. Nuts are collected at 50 cm above the ground and are cleaned by two suction pumps before being deposited in two containers of 1600 l.

Another system is derived from coffee harvesters. This group of machines has two vertical axes with plastic teeth placed in a radial way slipping along the canopy. The vibration of the axes is transmitted to the fruiting branches, provoking nut fall.

The third system is an over-the-tree harvester with a trunk shaker and catching frame (Fig. 17.11). It was developed by the firm Tenías of Ejea de los Caballeros (Zaragoza, Spain) and was first presented at the International Show of Agricultural Machines of Zaragoza in 2001, where it was recognized as a technical novelty that year. The machine consists of a self-propelled compact structure offering high performance because it does not require any special operation from shaking one tree to the next. All the processes take place continuously, with a single worker able to harvest 5–7 trees/min. The machine consists of two vertical arcades joined by a girder in the upper side and two longitudinal stretcher frames with trays to catch the falling

nuts, discharging at their edges onto conveyers and then to hoppers at the end, without raising the nuts.

One of the trays holds a stand with a longitudinal track on which the trunk shaker moves along. The transversal clamps of the shaker are above the frames and close onto the trunk whenever a palpating sensor detects it. In this way, the trunk is shaken without stopping the forward movement of the harvester. The vibration time is regulated by an adjustable timer. The space between frames is covered by flexible bands with an inverted slope to prevent nuts from falling on the ground while directing them to the tray. With this harvester, since it goes over the trees, the planting distance may be reduced despite the large size of the machine. The distance between rows may be reduced to 5 m and between trees to 2.5–3 m.

The last type of harvester is a very large machine, required when tree size is too large for the 'Tenías' harvester. In this case, both the



Fig. 17.11. Almond harvester under development by the manufacturer Tenías (Ejea de los Caballeros, Zaragoza, Spain).

height and the width of the machine are increased. A machine of this type is MagTec's Colossus®, operating on olives in Australia and Argentina (Fig. 17.12). It has a structure of 4 m × 4 m, with lateral vibrating shaker rods on a rotating drum that operates on the tree canopy as the harvester moves forward.

17.7 Nut Collection and Harvest Efficiency

In the Mediterranean, once nuts are shaken from the tree, they must be collected and sent to a dryer or to a processing plant. Depending on the harvesting system nuts may be collected in a variety of different ways including falling onto mesh, into inverted umbrellas, onto trays or onto catching frames in harvesters.

In California, nuts with hulls in various stages of dehydration are shaken from the trees and allowed to dry on the ground for several days prior to collection. This is important for the California industry because when nuts are delivered to a huller to remove the hulls, the hulls must be dry. Almond hulls in California have a

significant value as part of the feed ration for dairy cattle. Once hulls are removed from the in-shell nuts the dry hulls are immediately piled under cover next to the huller and are then sold as cattle feed during the months prior to the following year's harvest.

17.7.1 Nut collection following trunk shaking

When plastic mesh is used for nut collection the mesh may be of different sizes and width depending on conditions. Most often, a separate mesh is placed on each side of the tree by four workers who pour the nuts directly into containers or trailers and then move the mesh from one tree to the next. The major difficulty is coordination between the workers extending the mesh and the shaker movements. The work is more difficult on slopes and with a wet orchard floor. Depending on the amount of crop, output ranges between 35 kg and 78 kg/h/worker.

A roll-out mesh represents an improvement over the previous system by reducing labour. In this case, the mesh is rolled on rollers placed



Fig. 17.12. A MaqTec Colossus® harvester from Maq Tec (Venado Tuerto, Sante Fe, Argentina) continuously harvesting 'Barnea' olives in Argentina. (Photo courtesy of L. Ferguson.)

laterally to the trailer. The mesh is extended manually and placed under the trees. Once shaken, nuts are poured into the trailer by workers with the help of rollers rolling up the mesh. This system is more efficient than the previous one, with an output of 90–120 kg/h/worker depending on the amount of crop.

Another improvement in nut collection is the inverted umbrella. The umbrella is connected to a container placed just under the shaking head, very close to the soil (Fig. 17.13) so that shaking and nut accumulation take place simultaneously. The container has a storage capacity of 150–300 kg and once full is poured into a trailer. This efficient system combining shaking and nut collection has an output of 250–950 kg/h/worker depending on the amount of crop. Limitations are that trees must not be too large and must be trained with enough trunk space for clamping and umbrella deployment.

A trunk shaker may also be combined with a catching frame to receive the nuts. With this

system two vehicles are required, working in parallel down the almond tree row. Both are flexible machines, adaptable to trees of different size, reaching good operating speed and are easily transportable.

In California, nuts are not collected at the time of shaking. After drying on the ground for 5–10 days they are collected in a separate mechanical sweeping operation followed shortly by a mechanical pickup operation. Nuts are removed from the field by a shuttle wagon for further processing.

17.7.2 Shaker efficiency

Trunk shakers are an important component of almond harvesting. Only a few seconds are needed for harvesting a large percentage of nuts with trunk shakers. Many improvements have been added over the years to increase their efficiency, reliability, manoeuvrability and adaptation to different plantings. Efficiency has been



Fig. 17.13. A model M6 Almond shaker from Topavi (Autol, La Rioja, Spain) with an inverted umbrella for nut reception.

increased by adopting the optimal combination of frequency and oscillation, by the application of high power (50–80 kW) and by reduced mass of the shaking head. Construction has been simplified and more durable materials have been adopted, thus increasing the reliability of the machine.

Shaking heads and clamping have been improved so that trunk damage is reduced through the use of softer and larger protection pillows. Manoeuvrability has increased notably with the use of lighter shaking heads, thus allowing adaptation to medium-sized tractors and thus expanding the use of these machines. The lighter shaking heads are useful as both trunk and limb shakers for harvest in larger trees and on trees of irregular shape.

These machines typically provide 85–95% nut removal depending on the orchard and harvest management. The success of trunk shakers has also forced the evolution of nut collection systems, including improvements in the use of mesh and the adoption of inverted umbrellas.

The powerful self-propelled trunk shakers employed in California typically remove 99% of the nuts from the tree. In addition to excellent nut removal, the shaker heads have been improved with lubrication of the clamping pads, which helps minimize bark damage to the tree trunk.

17.7.3 Harvesters

Harvesters have the great advantage of working continuously, reaching a speed of 1–1.3 km/h, faster than during olive harvesting (Fig. 17.14). If working with small canopies, such as those of 2–3.5 m in height and 0.8–1.2 m in width, the vibrations of these machines derived from grape harvesters are extremely efficient, reaching nut removal higher than 95%, even with cultivars of drooping growth habit. Operational harvesting time is relatively constant, about 1.5–2 h/ha.

The main limitation of this high-density modified grape harvester system is that it will only work on very small trees. Most rootstock/scion combinations found in almond orchards are too vigorous to maintain a small enough tree size to be harvested with this system. Early trials have shown good fruiting ability with 'Belona', 'Guara' and 'Soleta' on the rootstocks 'RootPac® 40' and 'RootPac® 20', which induce less vigour

than the almond seedling rootstocks and the peach × almond hybrids, such as INRA GF 677, 'Garnem' and 'Adafuel'.

Perhaps the most serious problem is the need to maintain stable production in a very limited canopy volume. Since production is directly related to the volume of the fruiting canopy and the amount of sunlight intercepted by the trees, managing pruning to maintain production over time may be quite difficult. Scientific experimentation to confirm successful long-term results with this high-density system is not available at present.

The 'Tenías' harvester of Ejea de los Caballeros described previously is intermediate in size and may be a good compromise between high-density and large trees for continuously operating harvesters in the Mediterranean region.

The very large harvesters (such as Colossus®) have not had success in Europe. In Argentina and Australia results are promising since these harvesters allow harvesting of large trees which cannot be harvested with machines of standard size. They are only useful in very large orchards, however, due to the size, cost and transportation difficulties that are inherent in using these large harvesters.

17.8 Agronomical Aspects

Trunk shakers and harvesters require different orchard systems to work efficiently. Their application is primarily related to the characteristics of the orchard including tree size, orchard spacing, terrain and productivity. Since harvesters are still in the developmental stage, the orchard aspects will be discussed primarily in relation to trunk shakers.

Trunk shakers are able to harvest a certain number of trees/min almost regardless of the amount of crop on the trees. Therefore, the more productive the tree, the higher the productivity of the shaker and the easier it is to cover the costs of the shaking operation. At some minimal level of crop, mechanical harvesting is not cost effective. Cultivar genetics, climatic conditions, terrain and soil fertility, and the availability of irrigation all affect productivity. In each region, the best-adapted cultivars must be planted and optimum orchard management



Fig. 17.14. Almond harvester. Model under development by Fitasem (Barbastro, Huesca, Spain).

must be practised for a cultivar to express its best potential.

Shakers can be applied to trunks or to large scaffold limbs, depending on the size of the trees. Large shakers can achieve good nut removal when trunk shaking. Shaker attachment to large limbs requires more time and attention to branch orientation, as well as more complex nut reception when nut collection is done at the time of shaking. Limb shaking large trees is feasible but costs are greater than when trunk shaking.

Planting distances of 7×6 and 6×6 m provide enough space for trunk shakers to operate efficiently. Wider distances would facilitate machine movement, but machine operation must not compromise production capacity if mechanical harvest is to be cost effective.

Trunk shakers provide efficient nut removal with canopy volumes up to $40\text{--}50\text{ m}^3$, easily obtainable with most cultivars. With larger volumes, nut removal may be reduced. In long-lived orchards, larger volumes can easily be obtained when irrigation and fertilization are not limiting. As an example, a 'Guara' orchard at a planting distance of 7×7 m (200 trees/ha) in a fertile soil, has the potential of developing $11,000\text{ m}^3$ of canopy volume/ha. The tree canopy may be considered a geometric cap (Porrás Piedra, 1994) with a height $h = 3/2 r$ (r = canopy radius), and a volume of:

$$V = 1/3 \pi h^2 (3r - h) \quad \text{Eqn 17.2}$$

Taking into account that the real tree diameter with this planting distance would be 5 m, the

tree volume would be 55.22 m³, and the leaf surface (Porrás Piedra, 1994) would be 60 m² per tree.

Trunk shakers require trees with a single trunk. Trunk height must be 1.2 m from the ground to the first branch, especially if mechanical equipment is used for nut collection. The canopies must be open, with good light exposure and three to four upright-growing main scaffold branches must be selected with rigid secondary and tertiary branches. Fruiting wood must be prevalent on these branches and nuts must predominate in the medium–upper zone of the canopy. Trunk shakers work more efficiently on cultivars with an erect growth habit and branch angles no wider than 45°. Branches must not be too flexible and nuts must ripen relatively uniformly throughout the entire canopy. Cultivars must not drop nuts prematurely, as any nut drop greater than 10% seriously hampers orchard profitability in a shake and catch harvesting system.

Harvesting using trunk shakers may begin when trunk diameters reach 9–12 cm and trees are 5–6 years old. If trunks remain uniform and healthy, shakers may be used effectively for 30–40 years. If trunk wood is damaged, the shaker head may slip at the attachment point and the transmission of vibrations is decreased. Trunk shakers are most efficient when used on level ground but are feasible on up to 30% slopes when fitted to caterpillars with light shaking heads.

For manual sticking the tree does not have special requirements, but it is preferable that nuts be localized in regular planes, vertical or slightly inclined.

Harvesters derived from grape harvesters have given good results so far with the cultivars assayed but they require a continuous hedge, with a predetermined height and width. The fruiting surface must begin at least at 50 cm above ground to allow for nut collection below the canopy. Rigid lateral branches growing towards the middle of the row must be removed.

of oscillation and frequency without creating tree trunk bark damage that can weaken the tree and lead to disease (Fig. 17.15). Actively growing trees are more sensitive to bark damage than are trees later in the season. Once physiological cambial activity has diminished, bark is held more tightly on the trunk. Limb breakage is favoured by wide oscillations at any frequency; hence shaking persistence increases the probability of damage. Weak branches and graft unions are most sensitive to potential breakage. Laterally grafted branches of trees that have been top worked for cultivar replacement are particularly sensitive to breakage at the graft union. Root systems do not appear to suffer from trunk shaking unless the tree is too small.

Knocking by sticks causes wounds in branches and breaks fruiting spurs; as long as damage is less than 2–5% it can be tolerated.



Fig. 17.15. Bark damage caused by shaking. Crushed bark became infected with *Ceratocystis* canker. The margin of the fungal canker produces gumming and is visible when bark is removed.

17.9 Mechanical Damage

Maximum nut removal from fruiting branches must be accomplished by an effective combination

Some cultivars are more sensitive to injury than others and moist conditions can contribute to the development of pests and diseases. If trees are too large for a harvester the machine will cause limb breakage. This may be avoided by proper pruning, but if breakage or wounds are created by the harvester, a copper treatment must be applied right after harvest to avoid fungal infections.

If soils are too wet, heavy machinery may also induce soil compaction. This can be reduced by using equipment fitted with large flotation tyres that reduce the weight per surface area.

17.10 Vibration Transmission

Although fluctuating widely, vibration transmission is highly efficient from the trunk to the main branches but it decreases as it reaches the tertiary branches and leaves. Transmission of vibration is inconsistent and variable depending on the direction of branch growth and on the rigidity, length and flexibility of the fruiting branches.

The natural frequency of oscillation at the trunk averages 26 cycles/s, but this decreases to 16 cycles in the main branches, and even less in the lower limbs and leaves. Consequently, critical oscillations in resonance are not likely throughout the whole tree but will only occur at certain points in the canopy. Depending on pruning, transmission is reduced as the distance to the central axis increases. It is also less as branch direction changes and if fruiting limbs are long and bending.

Therefore, the most efficient vibration transmission occurs when tree architecture is a narrow vase shape with branches that are not too long and are growing erect rather than bending. Good canopy architecture can offset a cultivar tendency to shake poorly. Lack of pruning and an excessively dense canopy can reduce nut removal. The elimination of bending branches by adequate pruning and selection of rigid limbs generally increases harvesting efficiency. Old trees do not transmit vibrations uniformly so nut removal in older orchards is less efficient.

17.11 Conclusions

Controlling harvesting costs is essential in all almond-producing countries to keep almond growing profitable and to meet increasing demands in the future. A high level of mechanization using the best machinery for the conditions is essential, and cultivars and cultural practices must continually improve to keep orchards competitive (Fig. 17.16). Studying the ripening process has defined optimum harvest as the time when nut quality is maximized, nut removal is acceptable and natural nut fall is minimized if shake and catch harvesting is practised.

Shaking is the most efficient harvesting system, requiring a precise relationship between oscillation and frequency to avoid damage to the trunk, branches, leaves and, to a lesser extent, the root system. Shakers have significantly improved through weight reductions and reduced power requirements with multidirectional vibrations increasing the efficiency of the transmission of energy and improving nut removal.

The collection of nuts mechanically reduces the need for hand labour and greatly reduces costs. Agronomical improvements such as growing cultivars most adapted to mechanical harvesting, pruning for efficient vibration transmission and machinery performance, and increasing productivity will all lower costs to make almond growing more economically sustainable. A trunk shaker with an inverted umbrella to collect nuts is an efficient improvement for harvesting in the Mediterranean region. Continuously moving harvesters have the potential for further improvements as new orchards compatible with these machines are developed and if horticultural production problems can be resolved.

Traditional orchards with planting densities of 150–200 tree/ha are compatible with some mechanical harvesting systems although productivity may not be optimized. New orchards planted with densities of 240–270 trees/ha will capture more sunlight on almond leaves and will therefore be more productive. Nearly all cultivars are adaptable to these higher densities, and orchards can be especially productive when irrigation is possible and the climate is suitable for successful almond growing.

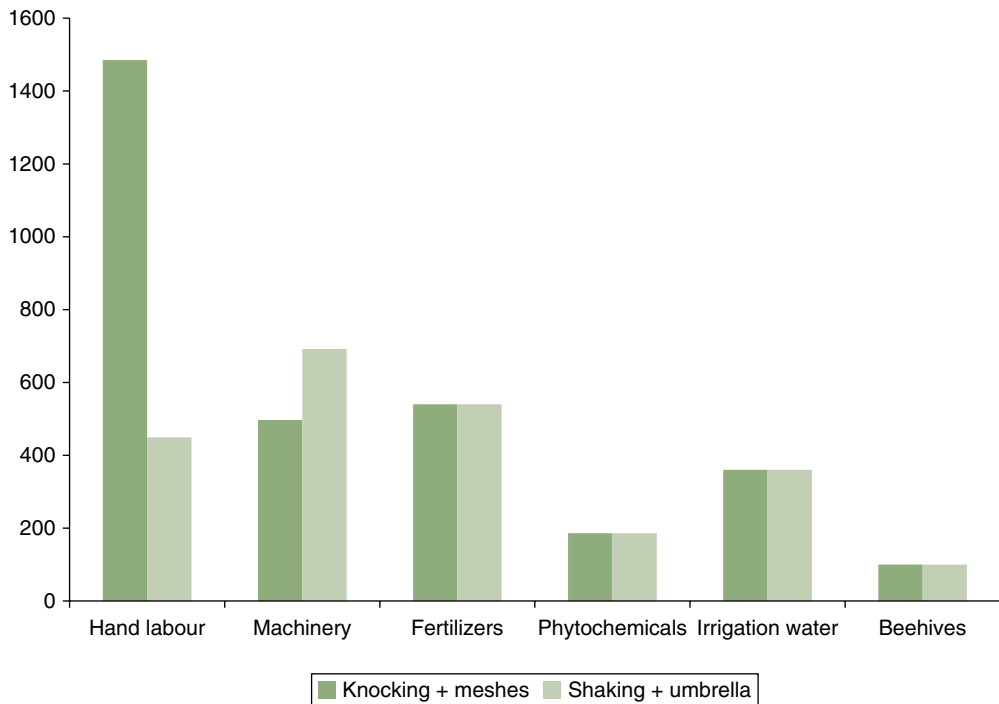


Fig 17.16. Direct production costs in an irrigated almond orchard depending on the harvesting system (€/ha).

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18 Chemical Composition of Almond Nuts

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18.1 Introduction

The edible part of the almond nut is the kernel, considered an important food crop with a high nutritional and medicinal value. The oldest and most extensive medical system that first recorded the health uses for almonds derives from the ancient Greeks and then the Persians, and later in traditional Chinese medicine and Indian Ayurvedic medicine (Albala, 2009). From medieval times to the 18th century, almond nuts were a source of substitute 'milk' (Mori *et al.*, 2011), and almond was also used as thickener before starch was 'discovered' (Albala, 2009). Almond consumption has almost doubled in the past 20 years (Almond Board of California, 2005), a fact that highlights almond evolution from a convenient snack food to an important member of an emerging food group increasingly recognized as essential for good health. The regular consumption of almonds has been associated with a wide range of health benefits including protection from cancer (Davis and Iwahashi, 2001; Davis *et al.*, 2003), obesity (Ren *et al.*, 2001; Fraser *et al.*, 2002; Kendall *et al.*, 2003), diabetes (Lovejoy *et al.*, 2002; Scott *et al.*, 2003) and heart diseases (Spiller *et al.*, 1992; Fulgoni *et al.*, 2002; Hyson *et al.*, 2002; Jenkins *et al.*, 2002; Sabate *et al.*, 2003). Almond may be consumed raw or

cooked, blanched or unblanched, combined and/or mixed with other nuts. It can also be transformed and incorporated into other products or to produce marzipan and nougat (Schirra, 1997). The high nutritive value of almond kernels arises mainly from their high lipid content, which constitutes an important source of calories, but does not contribute to cholesterol formation in humans due to their high level of unsaturated fatty acids, mainly monounsaturated fatty acids (Sabate and Hook, 1996). Although high in energy, humans compensate for energy from almonds due to their high satiety value (Hollis and Mattes, 2007). Furthermore, the absorption of energy from almond kernels is inefficient and they may raise resting energy expenditure with chronic consumption (Cassady *et al.*, 2009). Indeed, acute and longer-term almond ingestion helps regulate body weight (Sabate *et al.*, 2003), and modulates fluctuations in blood glucose (Jenkins *et al.*, 2006), total cholesterol TL:HDL ratio and triglycerides (Foster *et al.*, 2012).

Almond kernels must be of high quality to fulfil industry requirements and to be attractive for consumers (Socias i Company *et al.*, 2008). In addition to the kernel physical traits (Schirra, 1997), the chemical composition of the kernel is essential when considering the different industrial applications and the high diversity of almond

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confectioneries. Taking into account the high concentration of lipids in the almond kernel, the quality of the almond oil appears as the most important feature in the evaluation of almond quality. Several elements of the lipid fraction of the kernel have been suggested as parameters for quality evaluation in almond kernels, including the oil content, the percentage of oleic acid in the lipid fraction, the ratio of oleic/linoleic acids (O/L) and the tocopherol concentration (Kodad and Socias i Company, 2008; Socias i Company *et al.*, 2008).

Reviews of the composition of almond nuts have been provided by Saura-Calixto *et al.* (1988), Schirra (1997) and Yada *et al.* (2011), but these overviews were primarily descriptive without attempts to assess quality, particularly as it relates not only to industrial requirements but also to breeding goals and approaches. Extensive variability in the chemical composition has been demonstrated among cultivars (Souty *et al.*, 1971; Saura-Calixto and Cañellas, 1982; Romojaro *et al.*, 1988a, b; Schirra *et al.*, 1988; García-López *et al.*, 1996; Abdallah *et al.*, 1998; Kodad *et al.*, 2011a). The importance of differences in geographical origin (Abdallah *et al.*, 1998; Kodad *et al.*, 2011a) as well as climatic and growing conditions (Gall and Grasselly, 1977; Grasselly and Crossa-Raynaud, 1980; Barbera *et al.*, 1994; Kodad *et al.*, 2011b) have been demonstrated. Despite this extensive information, little is known concerning the genetic control and inheritance of biochemical components of almond quality.

Although differences in the chemical make-up of almond hulls and shells affect final market quality, biochemical differences in the kernel composition have received most of the attention because, as it is the consumed product, there is a clear need to maximize sensory and phytonutrient value and to minimize food safety concerns (Socias i Company *et al.*, 2008). For all these reasons, this review summarizes the current knowledge of the almond kernel chemical composition and factors affecting its variability.

18.2 Lipid Content and Fatty Acid Profile

Almond kernels are a rich lipid source, essentially composed of mono- and polyunsaturated

fatty acids (Sabate and Hook, 1996). The lipid fraction is also a major determinant of kernel flavour, particularly following roasting (Socias i Company *et al.*, 2008). Recent studies carried out in Iran by Moayedi *et al.* (2011) into the oil content and composition of three wild almond species: *Amygdalus scoparia* (AS), *A. hausknechtii* (AH) and *Prunus dulcis* (Pd), reported that the oil content from the different almond species ranged from 44.4% in AS to 51.4% in Pd, stating that the oil level in domesticated almond was somewhat higher than that in the wild almond species. The oil fraction in almond kernels consists primarily of storage lipids, which are present as intracellular oil droplets (approximately 1–3 mm in diameter) in the cotyledon tissues of the kernels (Pascual-Albero *et al.*, 1998; Ren *et al.*, 2001). During the development of the almond kernels, oil content remains low (< 10% of dry matter) for several months after fruit set, but then increases rapidly until about a month before harvest and then continues to increase gradually (Saura-Calixto *et al.*, 1984b; Kumar *et al.*, 2000; Egea *et al.*, 2009) or remains constant (Cherif *et al.*, 2009).

Lipid content is a very important factor in the confectionery industry, because higher oil contents result in less water absorption by the almond paste (Alessandroni, 1980). Kernels with a high percentage of oil can be used to produce nougat or to extract oil, which is used in the cosmetic and pharmaceutical industries. However, kernels with a low percentage of oil are required to produce almond milk, a dietetic product, because the caloric level of almond milk must be similar to that of cow's milk (Cotta Ramusino *et al.*, 1961). Kernels with low lipid contents are also suitable for production of almond flour and several kinds of food because of their correlated high protein content (Longhi, 1952). The lipid fraction may constitute 40% or more of the kernel dry weight (DW), with a considerable variability among cultivars and provenance (Table 18.1), as shown by many studies carried out in all almond-producing countries, including Argentina (Maestri *et al.*, 2015), France (Souty *et al.*, 1973), Egypt (Nassar *et al.*, 1977), Greece (Nanos *et al.*, 2002), India (Kumar *et al.*, 1994; Kumar *et al.*, 2000), Iran (Mehran and Filsoof, 1974), Italy (Schirra and Agabbio, 1989; Barbera *et al.*, 1994; Ruggeri *et al.*, 1998), Portugal (Cordeiro *et al.*, 2001; Egea *et al.*, 2009), the USA

Table 18.1. Range of variability of major macronutrients of almond kernels (g/100 g almond kernel). (Updated from Yada *et al.*, 2011.)

Nutrient	Range of variability	Origin	References
Oil content	40–67 ^a	Spain	García-López <i>et al.</i> (1996), García-Pascual <i>et al.</i> (2003), Kodad <i>et al.</i> (2006), Kodad <i>et al.</i> (2011a), Kodad and Socias i Company (2008), López-Ortiz <i>et al.</i> (2008), Romojaro <i>et al.</i> (1988b), Sánchez-Bel <i>et al.</i> (2008) and Soler <i>et al.</i> (1989)
	56–61 ^a	Greece	Nanos <i>et al.</i> (2002)
	54.75–64.73 ^a	France	Kodad <i>et al.</i> (2011a)
	56.23–66.8 ^a	Italy	Kodad <i>et al.</i> (2011a)
	42–47 ^b		Barbera <i>et al.</i> (1994), Ruggeri <i>et al.</i> (1998) and Schirra and Agabbio (1989)
	58.33–63.6 ^b	Portugal	Cordeiro <i>et al.</i> (2001) and Egea <i>et al.</i> (2009)
	48–59 ^a		Kodad <i>et al.</i> (2011a)
	35–66 ^b	USA (California)	Abdallah <i>et al.</i> (1998), Ahrens <i>et al.</i> (2005), Hall <i>et al.</i> (1958), López-Ortiz <i>et al.</i> (2008), Sathe (1993), Sathe <i>et al.</i> (2008) and Venkatachalam and Sathe (2006)
	57.3–63.6 ^a		Kodad <i>et al.</i> (2011a)
	57–63.9 ^a	Ukraine	Kodad <i>et al.</i> (2011a)
	63–66 ^a	India	Kodad <i>et al.</i> (2011a)
	48.7–64.5 ^a	Morocco	Kodad <i>et al.</i> (2013)
	63–66 ^a	Argentina	Kodad <i>et al.</i> (2011a)
	48–57 ^b		Maestri <i>et al.</i> (2015)
	20.19–62 ^a	Iran	Mehran and Filsoof (1974), Abaspour <i>et al.</i> (2012) and Kiani <i>et al.</i> (2015)
	56.1–59.8 ^a	Tunisia	Ayadi <i>et al.</i> (2006)
	48–61 ^a	Turkey	Çelik and Balta (2011) and Askin <i>et al.</i> (2007)
	55–59 ^a	Egypt	Nassar <i>et al.</i> (1977)
Protein content	16–31 ^b	Turkey	Askin <i>et al.</i> (2007) and Aslantas <i>et al.</i> (2001)
	16–23 ^a	USA (California)	Ahrens <i>et al.</i> (2005), Hall <i>et al.</i> (1958), Sathe (1993), Venkatachalam and Sathe (2006)
	15.7–21.1 ^a	Spain	Riquelme <i>et al.</i> (1985)
	17.16–29.31 ^b		Kodad <i>et al.</i> (2004)
Carbohydrate Sugars soluble	16–25 ^a	Italy	Ruggeri <i>et al.</i> (1998) and Drogoudi <i>et al.</i> (2013)
	14.1–35.1 ^b	Morocco	Kodad <i>et al.</i> (2013)
	10–29 ^a	Greece	Drogoudi <i>et al.</i> (2013)
	2.1–7.4 ^b	California	Ahrens <i>et al.</i> (2005), Amrein <i>et al.</i> (2005), Venkatachalam and Sathe (2006)
	2.6–4.0 ^b	Greece	Kazantzis <i>et al.</i> (2003) and Nanos <i>et al.</i> (2002)
	3.6–12 ^a	India	Kumar and Uppal (1995)
	2.1–5.5 ^b	Italy	Amrein <i>et al.</i> (2005), Barbera <i>et al.</i> (1994) and Ruggeri <i>et al.</i> (1998)
	2.5–7.1 ^b	Portugal	Cordeiro <i>et al.</i> (2001) and Egea <i>et al.</i> (2009)
	1.8–7.6 ^b	Spain	Amrein <i>et al.</i> (2005), Romojaro <i>et al.</i> (1988a), Sánchez-Bel <i>et al.</i> (2008), Saura-Calixto <i>et al.</i> (1981), Saura-Calixto <i>et al.</i> (1983) and Soler <i>et al.</i> (1989)
Fibre, total dietary	11–14 ^a	Italy	Ruggeri <i>et al.</i> (1998)

^aDry weight basis

^bFresh weight basis

(California) (Abdallah *et al.*, 1998; Sathe *et al.*, 2008), Spain (Romero *et al.*, 1988b; García-Pascual *et al.*, 2003; Kodad and Socias i Company, 2008; López-Ortiz *et al.*, 2008), Tunisia (Ayadi *et al.*, 2006), Turkey (Agar *et al.*, 1998), Serbia (Čolić *et al.*, 2012) and Morocco (Kodad *et al.*, 2013), with a general variation in the lipid fraction between 20% and 68% DW. Kodad *et al.* (2011a) studied 73 cultivars from different origins grown at the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) germplasm collection to evaluate the variability of the lipid fraction under the same environmental conditions, reporting that the variability of the lipid fraction among the Spanish cultivars ranged from 54.75% to 66.40%; from 54.75% to 64.73% for the French cultivars; from 56.23% to 66.80% for the Italian cultivars; from 58.33% to 63.66% for the Portuguese cultivars; from 57.36% to 63.60% for the Californian cultivars; from 57% to 63.9 for the Ukrainian cultivars; and from 63 to 66% for the Indian cultivars (Table 18.1). Nearly all cultivars from France, Italy and the USA showed a mean value of oil content higher than 60%. These results showed the inconsistency of the opinion often manifested that the Californian cultivars have lower oil contents than the European cultivars, as reported by García-López *et al.* (1996) when studying 19 Californian cultivars grown under the same conditions in the experimental fields in Murcia, Spain.

It has been generally reported that oil content in almond kernels depends on the genotype, but showing high variability by year (Abdallah *et al.*, 1998; Sathe *et al.*, 2008; Kodad *et al.*, 2011a) and growing conditions (Kodad *et al.*, 2010). This trait appears to be under polygenic control, with a high heritability estimate ($h^2 = 0.57$) (Font i Forcada *et al.*, 2011). Recently, Font i Forcada *et al.* (2012) identified one quantitative trait locus (QTL) on chromosome 6 believed to control oil content in the almond kernel. However, some almond cultivars, such as 'Mission', have shown high environmental stability in their oil content as compared to other cultivars (Schirra and Agabbio, 1989; Abdallah *et al.*, 1998), but other cultivars, including 'Desmayo Rojo', 'Lauranne', 'Retsou', 'Khoukhi' and 'Le Grand', showed improved behaviour under irrigated conditions rather than under drought conditions (Kodad *et al.*, 2011a). These results

indicate that the environmental stability of the oil content in almond mainly depends on the genotype. More recently, Zhu *et al.* (2015) have reported that moderate deficit irrigation had no detrimental impact on almond kernel lipid content, but that severe and extreme deficiencies influenced lipid content.

Almond oil has been reported to be very rich in monounsaturated fatty acids (MUFAs), especially in oleic and linoleic acids, whereas saturated fatty acids, especially palmitic, palmitoleic and stearic, are very low (Yada *et al.*, 2011) (Table 18.2). These five fatty acids constitute more than 95% of the total lipid fraction, depending on the cultivar, and eight minor fatty acids have also been reported (Saura-Calixto *et al.*, 1988; García-López *et al.*, 1996; Abdallah *et al.*, 1998; Martín-Carratalá *et al.*, 1998). The degradation of the fatty acids to peroxides results in the production of several products affecting the quality of the almond kernel (Sung and Jeng, 1994), including a rancid taste (Harris *et al.*, 1972). Oil oxidation is affected by several factors, such as the percentage of unsaturated fatty acids, light, oxygen, metallic ions, temperature and enzymes (Gou *et al.*, 2000; Zacheo *et al.*, 2000). In commercial almond cultivars, grown under different growing and environmental conditions, the profile of the major fatty acids was similar and varied depending on several factors (García-López *et al.*, 1996; Abdallah *et al.*, 1998; Prats-Moya *et al.*, 1999; Askin *et al.*, 2007; Sathe *et al.*, 2008; Kodad *et al.*, 2011a; Maestri *et al.*, 2015; Zhu *et al.*, 2015). As a general rule, oleic acid ranged between 50 and 80% of the total fatty acid content. Linoleic, palmitic and stearic acids are present at levels of 9–26%, 5–9% and 1.5–5%, respectively (Table 18.2). Study of the 77 almond cultivars of different origin grown in the international almond collection of CITA (Kodad *et al.*, 2011a) showed that the range of variability of oleic acid depended considerably on the provenance, the widest range of variability being observed among the cultivars from the USA (62.86–77.35%), followed by those from France (65.31–76.99%), Greece (64.00–74.97%), Spain (67.42–74.92%) and Portugal (67.66–74.10%). The highest mean value for the content of oleic acid was found for the cultivars from Italy (72.93%), followed by those of France (72.42%), Spain (72.20%), Portugal (70.39%), the USA (70.26%) and Greece (69.39%), although the

Table 18.2. Fatty acid profile and range of variability of almond kernel oil.

Fatty acid	Range of variability (% of total fatty acids)	Origin	References
Linoleic	12–22.5	Argentina	Maestri <i>et al.</i> (2015) and Kodad <i>et al.</i> (2011a)
	15.4–35.1	USA (California)	Abdallah <i>et al.</i> (1998), Sathe <i>et al.</i> (2008) and Kodad <i>et al.</i> (2011a)
	12.6–27	Turkey	Askin <i>et al.</i> (2007), Karatay <i>et al.</i> (2014) and Çelik and Balta (2011)
	19.2–22.4	Morocco	Kodad <i>et al.</i> (2013)
	11–23	Spain	Prats-Moya <i>et al.</i> (1999), García-López <i>et al.</i> (1996), Soler <i>et al.</i> (1989), Kodad <i>et al.</i> (2011a) and Kodad <i>et al.</i> (2014)
Oleic	11.9–24.4	Iran	Kiani <i>et al.</i> (2015) and Mehran and Filsoof (1974)
	68–77.5	Argentina	Maestri <i>et al.</i> (2015) and Kodad <i>et al.</i> (2011a)
	57.4–77.3	USA (California)	Abdallah <i>et al.</i> (1998), Sathe <i>et al.</i> (2008) and Kodad <i>et al.</i> (2011a)
	64–80.6	Turkey	Askin <i>et al.</i> (2007), Karatay <i>et al.</i> (2014) and Çelik and Balta (2011)
	68–70.7 65–77	Morocco Spain	Kodad <i>et al.</i> (2013) Prats-Moya <i>et al.</i> (1999), García-López <i>et al.</i> (1996), Soler <i>et al.</i> (1989), Kodad <i>et al.</i> (2011a) and Kodad <i>et al.</i> (2014)
Palmitic	67.6–80.8	Iran	Kiani <i>et al.</i> (2015) and Mehran and Filsoof (1974)
	6.01–7.26	Argentina	Maestri <i>et al.</i> (2015) and Kodad <i>et al.</i> (2011a)
	5.67–7.8	USA (California)	Abdallah <i>et al.</i> (1998), Sathe <i>et al.</i> (2008) and Kodad <i>et al.</i> (2011a)
	4.4–5.3	Turkey	Askin <i>et al.</i> (2007), Karatay <i>et al.</i> (2014) and Çelik and Balta (2011)
	6.3–7.5 5.4–7.1	Morocco Spain	Kodad <i>et al.</i> (2013) Prats-Moya <i>et al.</i> (1999), García-López <i>et al.</i> (1996), Soler <i>et al.</i> (1989), Kodad <i>et al.</i> (2011a) and Kodad <i>et al.</i> (2014)
Stearic	6–8.1	Iran	Kiani <i>et al.</i> (2015) and Mehran and Filsoof (1974)
	1.06–1.77	Argentina	Maestri <i>et al.</i> (2015) and Kodad <i>et al.</i> (2011a)
	1.1–2.6	USA (California)	Abdallah <i>et al.</i> (1998), Sathe <i>et al.</i> (2008) and Kodad <i>et al.</i> (2011a)
	0.26–1.89	Turkey	Askin <i>et al.</i> (2007), Karatay <i>et al.</i> (2014) and Çelik and Balta (2011a)
	1.9–2 1.54–2.5	Morocco Spain	Kodad <i>et al.</i> (2013) Prats-Moya <i>et al.</i> (1999), García-López <i>et al.</i> (1996), Soler <i>et al.</i> (1989), Kodad <i>et al.</i> (2011a) and Kodad <i>et al.</i> (2014)
	1–1.89	Iran	Kiani <i>et al.</i> (2015) and Mehran and Filsoof (1974)

differences between countries were not significant. Only in the cultivars from India was the content of oleic acid significantly lower than for the other countries. Additionally, the widest ranges of variability for linoleic acid was observed in the cultivars of the USA (14.04–26.63%) and France (14.29–24.71%), and the smallest in those of Portugal

(17.03–23.22%) and Spain (15.37–22.44%). The lowest values for the content of linoleic acid were found among the cultivars from Italy, France and Spain, with values significantly lower than those in the cultivars of some other countries.

The fatty acid profile is considered of the highest interest in the oil oxidation process.

Kester *et al.* (1993) suggested that the oleic acid/linoleic acid ratio (O/L) was a good index of resistance to oil rancidity, with high ratios being preferable. In other nut species, such as hazelnut, the criterion adopted has been the ratio of unsaturated fatty acids/saturated fatty acids (Bonvehí and Coll, 1993; Peshern *et al.*, 1995). Oleic and linoleic acid contents depend primarily on the genotype, are also affected by the climatic conditions of the year (Abdallah *et al.*, 1998; Sathe *et al.*, 2008; Kodad *et al.*, 2011a; Maestri *et al.*, 2015) and environmental condition of the growing region (Abdallah *et al.*, 1998; Sathe *et al.*, 2008; Kodad *et al.*, 2010; Yada *et al.*, 2013). Font i Forcada *et al.* (2011) reported that the heritability of these components is slow to medium, indicating that they are under polygenic control. Recently, Font i Forcada *et al.* (2012) identified QTLs in two different linkage groups (LG2 and LG7) believed to control both the oleic and linoleic acid percentages in the almond kernel. As a general rule, the O/L ratio varied considerably among almond genotypes and cultivars (Kodad *et al.*, 2011a), allowing the possibility for almond breeding to focus on improving kernel oil composition (Abdallah *et al.*, 1998; Kodad and Socias i Company, 2008; Socias i Company *et al.*, 2008).

18.3 Vitamins

Almost all studies carried out on vitamin content in almond are limited to those having an antioxidant effect, mainly tocopherols, since they play an important role in the protection of the oil against oxidation and deterioration. Tocopherols are natural monophenols showing antioxidant activities (Reische *et al.*, 1998), with several homologues depending on the position and number of methyl groups. The main tocopherol homologues detected in almond in decreasing importance are α -, γ -, δ - and β -tocopherol. Their main biochemical function is believed to be the protection of polyunsaturated fatty acids against peroxidation (Kamal-Eldin and Appelqvist, 1996) and they have protective roles in biological systems. Recent data indicate that they also have hypocholesterolaemic, anti-cancer and neuroprotective properties (Sen *et al.*, 2007). Tocopherol concentrations have been determined

in many oils and have been correlated with their antioxidant activity, suggesting the importance of the ratio of % total tocopherols/% polyunsaturated fatty acids (Senesi *et al.*, 1996). In almond, tocopherol concentration plays an important role in protecting lipids against oxidation and thus lengthening their storage time (Senesi *et al.*, 1996; Zacheo *et al.*, 2000; García-Pascual *et al.*, 2003).

α -Tocopherol is the most biologically active form of vitamin E and is used preferentially in the human body over the other forms (Brigelius-Flohé *et al.*, 2002). The range of variability of this homologue has been reported for different almond cultivars and genotypes from different countries (Kodad *et al.*, 2006; Font i Forcada *et al.*, 2011; Kodad *et al.*, 2011b; Yada *et al.*, 2011; Kodad *et al.*, 2013; Yada *et al.*, 2013; Kodad *et al.*, 2014; Maestri *et al.*, 2015) (Table 18.3). At high temperatures γ -tocopherol has been reported to be much more effective as an inhibitor of polymerization and protection against oxidation than α -tocopherol (Lampi and Kamal-Eldin, 1998; Warner *et al.*, 2003). The range of variability of this homologue was: 4.4–33.4 mg/kg oil in local Moroccan almond seedlings (Kodad *et al.*, 2013), 4.64–14.92 mg/kg in some European almond cultivars (Kodad *et al.*, 2011b), 1.97–62.6 mg/kg in almond breeding selections (Kodad, 2006; Font i Forcada *et al.*, 2011), 5.4–58.83 mg/kg in other Spanish almond cultivars (Kodad *et al.*, 2014) and 0.36–0.72 mg/kg in some Californian almond cultivars (López-Ortiz *et al.*, 2008). For δ -tocopherol, the range of variability is reduced as compared to the other homologues and ranged from 0.22 to 2.2 mg/kg oil in Moroccan almond cultivars (Kodad *et al.*, 2013); 0.1–1.8 mg/kg in almond breeding selections (Font i Forcada *et al.*, 2011), 0.1–2.2 mg/kg in Spanish almond cultivars (Kodad *et al.*, 2014) and 0.01–0.04 mg/kg in some Californian almond cultivars (López-Ortiz *et al.*, 2008).

Tocopherol concentration in almond oil depends on the genotype and the climatic conditions of the year (López-Ortiz *et al.*, 2008; Kodad *et al.*, 2011b; Yada *et al.*, 2013; Kodad *et al.*, 2014; Maestri *et al.*, 2015), as well as on the environmental conditions of the growing region (Kodad *et al.*, 2011b; Yada *et al.*, 2013). To date, studies have shown that drought and heat are the most important stressors affecting the expression of the chemical compounds in fruit trees,

Table 18.3. Tocopherol isomer concentration in almond kernel and kernel oil.

Tocopherol isomer	Range of variability		Origin	References
	mg/100 g kernel	mg/100 g oil		
α -tocopherol	8.5–19		Spain	López-Ortiz <i>et al.</i> (2008)
		33.53–55.17		Kodad <i>et al.</i> (2014)
		30.9–65.67	Morocco	Kodad <i>et al.</i> (2013)
	18–32		USA (California)	Yada <i>et al.</i> (2013)
	25–84		Italy	Zacheo <i>et al.</i> (2000)
		35–47.1		Rizzolo <i>et al.</i> (1991)
		37–57.1	Argentina	Maestri <i>et al.</i> (2015)
		20	Australia	Zhu <i>et al.</i> (2015)
β -tocopherol	5–8		Italy	Zacheo <i>et al.</i> (2000)
	0.12		Australia	Zhu <i>et al.</i> (2015)
γ -tocopherol		0.61–5.02	Spain	Kodad <i>et al.</i> (2006) and Kodad <i>et al.</i> (2014)
	0.14–0.84			López-Ortiz <i>et al.</i> (2008)
		7.5	Italy	Senesi <i>et al.</i> (1996)
		0.24–1.35	Morocco	Kodad <i>et al.</i> (2013)
	0.57		Australia	Zhu <i>et al.</i> (2015)
	δ -tocopherol	0.02–0.16		Spain
		0.02–2.2		Kodad <i>et al.</i> (2006) and Kodad <i>et al.</i> (2014)
		0.01–0.03	Morocco	Kodad <i>et al.</i> (2013)

including almond. The effect of drought stress on the oil percentage in almond kernels and on its composition is not clear, since the results of different studies undertaken in this field are ambiguous. Tocopherol concentration has been reported to be affected by drought stress in olive oil (Romero *et al.*, 2003) but not in *Brassica napus* (Richards *et al.*, 2008), by temperature in soybean (Almonor *et al.*, 1998) and by a combination of both in shea butter (*Vitellaria paradoxa* C.F. Gaertn.) (Maranz and Wiesman, 2004). In almond, Kodad *et al.* (2006) found higher tocopherol concentrations in kernels harvested in years with higher temperatures, suggesting that this environmental factor could affect tocopherol synthesis during kernel development. Later, Kodad *et al.* (2010) undertook a study on the effect of two contrasting environments, with drought and heat conditions in Morocco, and irrigated and cold conditions in Spain, reporting that the tocopherol content (mainly of α -tocopherol) increased under the warmer climate conditions of Morocco. More recently, Maestri *et al.* (2015) reported that, in arid north-western Argentina, higher α -tocopherol concentrations (~646 $\mu\text{g/g}$ on average) were found when almond kernel development mostly coincided with

spring and summer months with warmer mean temperatures. Additionally, Zhu *et al.* (2015) reported that there was no obvious relationship between almond tocopherol content and the degree of water deficiency. These authors claimed that the minor tocopherol homologues appeared to be slightly more responsive to deficit irrigation than the main homologue, α -tocopherol, but their small proportion had little impact on the total amount of tocopherol content.

The three main tocopherol homologues detected in almond oil are quantitative traits at the genetic level (Font i Forcada *et al.*, 2011), and more recently five different QTLs believed to control the tocopherol concentration in the almond kernel oil have been identified (Font i Forcada *et al.*, 2012). Font i Forcada *et al.* (2011) reported that the amount of γ -tocopherol showed high heritability estimates, with $h^2 = 60.0\%$, whereas α -tocopherol showed lower heritabilities ($h^2 = 20.5\%$). Thus, selection for high γ -tocopherol concentration in a breeding progeny could easily be attained if adequate parents were chosen. Consequently, these components could be considered as selection criteria in almond breeding programmes, as suggested by Socas i Company *et al.* (2008).

Almond kernels are also a source of vitamins B1 (thiamine), B2 (riboflavin), B6 (pyridoxine) and niacin. Vitamin B6 is involved in reducing levels of homocysteine, an amino acid that, if found in high amounts in the body, can be detrimental to heart health (Whitney and Rolfes, 2002). Hall *et al.* (1958) analysed the vitamin components of 'Nonpareil' almonds from California and evaluated thiamin, riboflavin, niacin, pyridoxine, pantothenic acid, folic acid (folate) and biotin in raw, unblanched almonds. Yada *et al.* (2013) reported in a recent review of the almond chemical composition that the values reported by these authors are generally comparable to United States Department of Agriculture (USDA) SR values. Hall *et al.* (1958) reported that the concentration of vitamin B1 and B2 ranged, respectively, from 0.19 to 0.25 mg/100 g almond and 1.0–1.1 mg/100 g almond. Daud *et al.* (1977) reported that the vitamin B6 content of natural and processed almonds varied between 0.08 and 0.16 mg/100 g almond. Approximately 12% of vitamin B6 was lost following blanching, while approximately 25% was lost following roasting (Daud *et al.*, 1977). Only minor loss is observed when comparing nutrient concentrations of whole raw almonds with blanched (peeled) almonds. The ranges of biotin content of several Italian almond cultivars varied between 0.12 mg/100 g and 0.90 mg/100 g almonds (Rizzolo *et al.*, 1991); however, this range is smaller in Californian almond cultivars and varied from 0.01 mg/100 g to 0.05 mg/100 g almond (Hall *et al.*, 1958; Hoppner *et al.*, 1994).

18.4 Phytosterol Content

Phytosterols belong to the family of triterpenes, present in plants as components of cell membranes and playing an important role in plant function. They control membrane fluidity and permeability. Phytosterols also have an important role in the human body. The present interest in phytosterols is mainly because dietary phytosterols inhibit cholesterol absorption, which leads to decreased plasma low-density lipoprotein (LDL) cholesterol levels and thereby a potentially decreased risk of developing cardiovascular diseases (Gylling *et al.*, 1995; Ntanos *et al.*, 2003). Moreover, several studies reported that phytosterols

might protect against the development of colon cancer (Awad and Fink, 2000) and have a positive effect on the human immune system (Carr *et al.*, 2010). The main natural sources of phytosterols in the human diet are plant oils, including nuts (Piironen *et al.*, 2000).

In almond, studies on the kernel phytosterol content and composition are scarce. The phytosterol content and profile is presented in Table 18.4. Fernández-Cuesta *et al.* (2012) studied 160 almond cultivars and selections from different countries, reporting that the phytosterol content ranged from 1126 to 2777 mg/kg. Normen *et al.* (2007) reported a value of phytosterol content of 2080 mg/kg in a single sample of almond kernels from a local market. Studies carried out on different samples of almond kernels from the US market reported an average value of phytosterol content of 2107 mg/kg (Robbins *et al.*, 2011). Oil phytosterol levels between 2178 and 2750 mg/kg have been reported for raw almond oils (Miraliakbari and Shahidi, 2008), whereas a phytosterol content of 1999 mg/kg has been reported for commercial almond oil (Dulf *et al.*, 2010). Fernández-Cuesta *et al.* (2012) reported that oil phytosterol varied from 2013 to 4497 mg/kg in 160 almond genotypes. Recently, Kodad *et al.* (2015) reported that oil phytosterol concentrations in local Moroccan almond seedlings ranged from 2184 to 3583 mg/kg of oil, with an average of 2723 mg/kg of oil. At the genetic level, Font i Forcada *et al.* (2015) reported that the phytosterol content in almond oil is a quantitative trait, finding seven markers associated with different genes that could control phytosterol concentrations. The phytosterol fraction was mainly made up of β -sitosterol (55.9–84.6%) and Δ^5 -avenasterol (8.5–28.2%) (Fernández-Cuesta *et al.*, 2012). Similarly, Kodad *et al.* (2015) reported that, in local almond seedlings from Morocco, the phytosterol fraction consisted mainly of β -sitosterol (78.90–87.26%) and Δ^5 -avenasterol (6.26–13.04%). These authors reported that almond oil contains other minor phytosterols such as campesterol (1.4–5.8%), stigmasterol (0.3–0.7%), Δ^7 -campesterol (0.9–7.5%), clerosterol (0.5–2.6%) and Δ^7 -avenasterol (0.2–4.6%). Other studies (Normen *et al.*, 2007; Dulf *et al.*, 2010; Robbins *et al.*, 2011) have reported a phytosterol fraction mainly made up

Table 18.4. Phytosterol content in the almond oil and the almond kernel.

Component	Mean value		Origin	References
	mg/100 g oil	mg/100 g kernel		
Campesterol	2.67		Morocco	Kodad <i>et al.</i> (2015)
	2.7		CITA genebank	Fernández-Cuesta <i>et al.</i> (2012)
Clerosterol	0.89		Morocco	Kodad <i>et al.</i> (2015)
	1.3		CITA genebank	Fernández-Cuesta <i>et al.</i> (2012)
Kernel phytosterol	1524.48		Morocco	Kodad <i>et al.</i> (2015)
	1860–1904 ^a		CITA genebank	Fernández-Cuesta <i>et al.</i> (2012)
Oil phytosterol	272.33 ^b		Morocco	Kodad <i>et al.</i> (2015)
	310.8–313.2 ^b		CITA genebank	Fernández-Cuesta <i>et al.</i> (2012)
Stigmasterol	0.21		Morocco	Kodad <i>et al.</i> (2015)
	0.8		CITA genebank	Fernández-Cuesta <i>et al.</i> (2012)
		1.9–6.3	USA (California)	Yada <i>et al.</i> (2013)
β-Sitosterol	82.14		Morocco	Kodad <i>et al.</i> (2015)
	72.4–73.9		CITA genebank	Fernández-Cuesta <i>et al.</i> (2012)
		128–157	USA (California)	Yada <i>et al.</i> (2013)
Δ ^{5,24} -Stigmastadienol	1.11		Morocco	Kodad <i>et al.</i> (2015)
	0.3–0.9		CITA genebank	Fernández-Cuesta <i>et al.</i> (2012)
Δ ⁵ -Avenasterol	10.62		Morocco	Kodad <i>et al.</i> (2015)
	14.8–16.2		CITA genebank	Fernández-Cuesta <i>et al.</i> (2012)
Δ ⁷ -Avenasterol	1.08		Morocco	Kodad <i>et al.</i> (2015)
	1–1.8		CITA genebank	Fernández-Cuesta <i>et al.</i> (2012)
Δ ⁷ -Campesterol	0.60		Morocco	Kodad <i>et al.</i> (2015)
	2.5–3.2		CITA genebank	Fernández-Cuesta <i>et al.</i> (2012)
		4.7–6	USA (California)	Yada <i>et al.</i> (2013)
Δ ⁷ -Stigmasterol	0.69		Morocco	Kodad <i>et al.</i> (2015)
	1–1.5		CITA genebank	Fernández-Cuesta <i>et al.</i> (2012)

^aPercent kernel^bmg kg⁻¹ oil

of β-sitosterol (72.1–83.9%) and Δ⁵-avenasterol (9.9–12.9%) and to a lesser extent campesterol (2.5–3.4%) and stigmasterol (1.6–3.4%). However, other studies have reported different phytosterol profiles characterized by absence or lower levels of Δ⁵-avenasterol (Maguire *et al.*, 2004; Miraliakbari and Shahidi, 2008; Cherif *et al.*, 2009).

Studies on the effect of the genotype and the environment on phytosterol content and profile in almond are even scarcer. Fernández-Cuesta *et al.* (2012) reported that phytosterol content, oil phytosterol content and the concentration of individual phytosterols, except Δ⁷-campesterol and clerosterol, depend on the genotype. These authors also claimed that the year effect was significant for all individual phytosterol components, except for clerosterol, but not for kernel and oil phytosterol contents. Recently, Yada *et al.* (2013) reported that there

were no significant location effects on β-sitosterol, when studying seven cultivars in three different growing conditions in California. However, a significant year effect was observed on β-sitosterol under Californian (Yada *et al.*, 2013) and Spanish conditions (Fernández-Cuesta *et al.*, 2012). Recently, Kodad *et al.* (2015) reported that phytosterol concentration varied significantly between different local almond populations grown under different environmental conditions, suggesting that the environment can affect phytosterol concentrations in almond. All these results indicate that β-sitosterol and Δ⁵-avenasterol, the two main phytosterol components, mostly depend on the genotype, but are also largely influenced by the climatic conditions. Thus, these compounds could be considered under polygenic control. Concerning the minor phytosterol components, the genotype effect was only significant for stigmasterol and

Δ^7 -campesterol. The location effect was significant for Δ^7 -stigmastenol and Δ^7 -campesterol, but no location effect was observed for any of the minor phytosterol compounds detected. The absence of location effect observed for campesterol and stigmasterol coincided with those reported by Yada *et al.* (2013).

18.5 Minerals

The almond kernel is considered a good source of mineral elements (Table 18.5), playing an important role for human health. Almost all of these mineral compounds found in plant tissues are obtained by the plant from the soil and the water applied in production. Mineral content is sometimes expressed in general terms as ash content, the inorganic residue remaining after incineration of the plant tissue (Yada *et al.*, 2011). In almond kernels the value of the mineral content is very similar, with estimates of 3.05–3.45 g/100 g obtained for some Spanish cultivars (Romojaro *et al.*, 1977; Saura-Calixto *et al.*, 1981; Kodad, 2006), 3.4 g/100 g in Lebanese-grown almonds (Cowan *et al.*, 1963), 2.3–3.7 g/100 g in some cultivars grown in Italy (Barbera *et al.*, 1994; Ruggeri *et al.*, 1998) and 3.8 g/100 g in Turkish almond genotypes (Aslantas *et al.*, 2001). Almond kernels are an important source of calcium, magnesium and

manganese (Saura-Calixto and Cañellas, 1982; Prats-Moya *et al.*, 1997; Schirra, 1997; Drogoudi *et al.*, 2013; Yada *et al.*, 2013; Mahmoud and Yassin, 2016). Copper, iron, potassium, phosphorus and zinc are also accumulated in significant amounts in almond kernels during fruit growth and ripening (Schirra *et al.*, 1994). Recent studies have used the atomic absorption spectrometry (AAS) method to determine calcium, iron, magnesium, zinc, copper and manganese, and flame atomic emission spectrometry (FAES) to determine sodium and potassium from Indian almond samples (Agunbiade and Olanlokun, 2006; Mahmoud and Yasin, 2016). The values of these mineral compounds reported were: 0.17% for potassium, 0.25% for magnesium, 245.65 µg/ml for sodium, 845.45 µg/ml for calcium, 92.12 µg/ml for zinc, 70.62 µg/ml for iron and 9.21 µg/ml for copper. In Pakistan almond kernels, the concentration of copper was 0.522–2.633 µg/ml, cobalt 0.01–1.001 µg/ml, chromium 0.02–0.909 µg/ml, lead 1.001–10.002 µg/ml and cadmium 0.722–8.332 µg/ml (Manzoor *et al.*, 2013). All these results show that almond kernel is rich in alkaline metals. The amount of potassium was reported to be high (four times more) as compared to the amount of sodium, a very important component because it is necessary for the function of all living cells and the regulation of blood pressure in the body (Mahmoud and Yasin, 2016).

Table 18.5. Mineral contents in almond kernels.

Nutrient	Range of variability mg/100 g	References
Calcium	160–670	Prats-Moya <i>et al.</i> (1997), Saura-Calixto <i>et al.</i> (1981), Drogoudi <i>et al.</i> (2013) and Yada <i>et al.</i> (2013)
Copper	0.9–1.3	Hall <i>et al.</i> (1958) and Yada <i>et al.</i> (2013)
Iron	2.6–4.9	Hall <i>et al.</i> (1958), Saura-Calixto <i>et al.</i> (1981) and Yada <i>et al.</i> (2013)
Magnesium	166–610	Prats-Moya <i>et al.</i> (1997), Saura-Calixto <i>et al.</i> (1981) and Drogoudi <i>et al.</i> (2013)
Manganese	1.2–3.04	Prats-Moya <i>et al.</i> (1997), Saura-Calixto <i>et al.</i> (1981), Saura-Calixto and Cañellas (1982) and Yada <i>et al.</i> (2013)
Phosphorus	360–1235	Prats-Moya <i>et al.</i> (1997), Saura-Calixto <i>et al.</i> (1981) and Drogoudi <i>et al.</i> (2013)
Potassium	430–940	Prats-Moya <i>et al.</i> (1997), Saura-Calixto <i>et al.</i> (1981), Saura-Calixto and Cañellas (1982) and Drogoudi <i>et al.</i> (2013)
Sodium	1.0–20	Prats-Moya <i>et al.</i> (1997), Saura-Calixto <i>et al.</i> (1981) and Saura-Calixto and Cañellas (1982)
Zinc	2.0–4.0	Saura-Calixto <i>et al.</i> (1981), Saura-Calixto and Cañellas (1982) and Yada <i>et al.</i> (2013)

With respect to heavy metals, the amount of chromium is much higher than the amount of other heavy metals present in almond. Chromium is suitable biochemical nutrition material to promote weight loss, muscle development and to treat the symptoms of type 2 diabetes, and is the best-selling mineral supplement after calcium.

18.6 Protein and Amino Acids

Protein is the second major chemical component of the almond kernels after the lipid fraction. The major protein fraction identified in almond has been globulins and albumins (88–91% of total protein) (Saura-Calixto *et al.*, 1982). The protein content in almond kernels is usually determined indirectly through the determination of the amount of total nitrogen (N) by analytical methods, being commonly multiplied by the specific nitrogen-to-protein conversion factor of 5.18, as recommended by Jones (1941) and reviewed by Yada *et al.* (2011). The earliest work by Osborne and Campbell (1896) established that the dominant protein in almonds is a globulin named amandin, containing 19.3% of nitrogen, thus corresponding to a conversion factor of 5.18. Yada *et al.* (2011) reported that the general factor of 6.25 used in protein calculations is based on a 16% nitrogen content of many common proteins; however, the use of the factor $N \times 6.25$ for almonds would overestimate the protein content.

Protein content is inversely correlated with the lipid fraction and the ratio between these two components ($R1: \% \text{ lipids} / \% \text{ protein}$) is important in the preparation of some processed products because the absorption of water by almond paste is dependent on the balance between these two components (Alessandrini, 1980). For marzipan production, a low index would be desired, whereas for nougat production, a high index would be preferred. This index shows a great variability, with values ranging from 1.60 to 6.33, with a variability coefficient of 5% for genotypes (Kodad *et al.*, 2004), higher than the variability of 4.17% for years (Kodad, 2006). Almond protein contains a good balance of essential amino acids with the exception of methionine, being easily hydrolysed by common digestive protease, producing high-quality protein hydrolysates in relation

to the essential amino acid balance (Sathe, 1993). The most common amino acids in almond proteins are glutamic acid, aspartic acid and arginine (reviewed by Socias i Company *et al.*, 2008). Small amounts of non-protein nitrogen have also been found (Schirra, 1997). The essential amino acids account for about 30% of the protein. Some differences among cultivars grown in the same region have been reported (Ruggeri *et al.*, 1998).

Protein content of the commercial almond cultivars ranged from 13% to 29% on a dry weight basis (Table 18.1) depending on authors (Schirra, 1997; Saura-Calixto *et al.*, 1988; Kodad *et al.*, 2004) and the conversion factors (ranging from 5.18 to 6.25) for estimating protein content from total sample nitrogen (Socias i Company *et al.*, 2008). The average total nitrogen value of the different almond samples ranged between 3% (Saura-Calixto *et al.*, 1988), 3.35% (Kodad, 2006), 3.6% (Riquelme Ballesteros *et al.*, 1982), 3.69% (Casares and López, 1952), 4.23% (Aslantas *et al.*, 2001), 4.28% (Romojaro *et al.*, 1977), 4.4% (Souty *et al.*, 1971) and 4.62% (Barbera *et al.*, 1994). Protein contents of 16–23 g/100 g have been reported in studies of commercial California-grown almond cultivars (Hall *et al.*, 1958; Sathe, 1993; Ahrens *et al.*, 2005; Venkatachalam and Sathe, 2006). For European cultivars, Riquelme *et al.* (1985) reported a range of variability of 15.7–21.1 g/100 g in nine major almond cultivars grown in southern Spain. In northern Spain, Kodad *et al.* (2004) reported a range of variability from 17.16–29.31% in eight almond cultivars. In Italy, Ruggeri *et al.* (1998) reported a range of variability from 18.5 to 20.0 g/100 g in four commercial cultivars. More recently, Drogoudi *et al.* (2013) reported that the value of this component varied between 16 and 25 g/100 g. Özcan *et al.* (2010) reported a range of variability of crude protein from 12.71 to 16.34 g/100 g in four European almond cultivars grown in Turkey. Other studies carried out in local almond seedlings and selections have been reported in the literature. Higher mean crude protein content (32% DW) was reported by Kumar and Uppal (1995) for various Indian almond selections. Thirteen Turkish almond selections analysed by Aslantas *et al.* (2001) had protein contents ranging from 19 to 24 g/100 g (DW). Askin *et al.* (2007) reported

a wider range of protein content variability (16–31%) in 26 native genotype selections from Turkey. Kodad *et al.* (2013) analysed 41 native almond genotypes grown in different geographical regions in Morocco and reported a range of variability of crude protein from 14.1 to 35.1%. The value of the protein content in Greek cultivars presents a large variability ranging from 10 to 29 g/100 g (DW) (Drogoudi *et al.*, 2013). All these results indicate the high range of variability of protein content depending on the origin of the genotype and the environmental conditions of the growing region, as reported by Kodad (2006) and Saura-Calixto *et al.* (1988). Font i Forcada *et al.* (2011) reported that the heritability estimate of protein content in almond is very low ($h^2 = 12.1\%$), confirming the strong effect of environmental conditions on its expression. More recently, Font i Forcada *et al.* (2012) found three loci for protein content mapped on LG6 and LG7.

18.7 Phenols

Polyphenol compounds are produced through the phenylpropanoid pathway and encompass a wide range of chemical classes, including phenolic acids such as benzoic and hydroxycinnamic acids, flavonoids such as flavonols and anthocyanins, stilbenes and lignans (Manach *et al.*, 2004). Polyphenols, especially ortho-diphenolic compounds, have been shown to be strong, active, free-radical scavengers (Gordon *et al.*, 2001; Frankel, 2005). Almond kernels are a good source of phenolic substances (Bolling *et al.*, 2010). Almond phenolics are mainly polyphenolics of the flavonoid type (Wijeratne *et al.*, 2006; Bolling *et al.*, 2010). In the almond teguments, the flavonoids and other polyphenols comprise 0.2–0.8% of the DW of the tegument (Garrido *et al.*, 2008). The total phenol and polyphenol content in almond kernels varied from 60.2 to 175.1 mg GAE/100 g, and from 14.6 to 27.2 mg/100 g almonds, respectively (Kodad, 2006; Milbury *et al.*, 2006; Bolling *et al.*, 2010). Catechin was the major phenolic acid, ranging from 11.1 to 227.2 µg/g, followed by caffeic acid (2.9–32.1 µg/g), epicatechin (2.0–23.5 µg/g) and gallic acid (2.4–6.1 µg/g) (Yildirim *et al.*, 2010).

In the almond kernels the predominant flavonoids were isorhamnetin-3-o-rutinoside and isorhamnetin-3-o-glucoside (in combination), catechin, kaempferol-3-o-rutinoside, epicatechin, quercetin-3-o-galactoside and isorhamnetin-3-o-galactoside at 16.81, 1.93, 1.17, 0.85, 0.83 and 0.50 mg/100 g of kernel fresh weight (FW), respectively (Milbury *et al.*, 2006). Later, Bolling *et al.* (2010) reported that isorhamnetin-3-O-rutinoside was the most abundant flavonoid in almond kernels, present at 28–49% of total polyphenols among different cultivars. The flavonoids and total polyphenols are reported to be more dependent on the cultivar than on the environmental conditions (Bolling *et al.*, 2010). A study of ten Portuguese almond cultivars found four- and 18-fold differences in flavonoids and total phenols between cultivars, respectively (Barreira *et al.*, 2008). Analysis of 18 Iranian almond genotypes showed a 4.6-fold variation in the phenolic content of hulls and a similar variability in the antioxidant activity of extracts (Sfahlan *et al.*, 2009). In Californian cultivars, the total phenols and polyphenols varied by 2.7-fold and 1.9-fold, respectively, in a single year across eight almond cultivars (Milbury *et al.*, 2006; Bolling *et al.*, 2010).

18.8 Carbohydrates and Fibre

The only carbohydrate forms present in almond kernels that can be digested, absorbed and metabolized by humans to provide a source of energy are sugars, starch and some sugar alcohols. The non-starch polysaccharides are indigestible and therefore unavailable as an energy source, but they promote physiological effects that are beneficial for human health (Yada *et al.*, 2011). Soluble sugars, while present in relatively low amounts (Table 18.1), are sufficient to make kernels sweet tasting (Schirra, 1997). The soluble sugars content varied between 1.8 g/100 g for 'Desmayo Langueta' from Spain (Amrein *et al.*, 2005) and 13 g/100 g for local almond breeding selections from Turkey (Balta *et al.*, 2009). Most soluble sugars are non-reducing, with sucrose representing more than 90% of the total (Socias i Company *et al.*, 2008). Other sugars include raffinose, glucose, fructose, sorbitol and inositol (Schirra, 1997; Saura-Calixto

et al., 1984a). Different studies have reported that carbohydrates varied during kernel development, resulting in a drastic decrease of all sugars in the 2 months prior to final maturity (Kazantzis *et al.*, 2003; Egea *et al.*, 2009). At harvest, Egea *et al.* (2009) found that sucrose was the major sugar component (1.6–2.6 g/100 g, DW); glucose and fructose contents were insignificant; and mannose and arabinose were no longer detected. The contents of these components are reported to be under the influence of irrigation strategies. Sánchez-Bel *et al.* (2008) reported that sucrose and glucose contents in kernels of 'Guara' grown under drip-irrigated orchards were higher than those from non-irrigated orchards. The effect of the year and location were reported to be non-significant on the expression of the sucrose content (Yada *et al.*, 2013), considered the major sugar component.

Along with the polysaccharides, there are 10 g of dietary fibre/100 g of almond kernel (Socias i Company *et al.*, 2008). About 80% of the fibre is insoluble and 20% is soluble fibre. This mixture of fibres positively affects colonic health and cholesterol levels (Saura-Calixto *et al.*, 1988). Romojaro *et al.* (1988a) reported that the sum of crude fibre and pentosans is considered as dietary fibre. Almond fibre is mainly composed of the polysaccharides cellulose, hemicelluloses and lignin, frequently accompanied by pectic substances, impurities and side groups linked to the acid chain (Vidal Valverde *et al.*, 1982). Saura-Calixto *et al.* (1984c) compared neutral detergent fibre (NDF) and acid detergent fibre (ADF) determinations with crude fibre analysis. The value of dietary fibre contents in almond kernels varied between 10.8 g/100 g and 13.5 g/100 g (Ruggeri *et al.*, 1998).

18.9 Hull and Shell Biochemical Constituents

The almond hull represents the fruit mesocarp and is made up of the portion of the almond nut surrounding the wooden shell; this is used in different ways. It can be a byproduct feedstuff, defined as having value as an animal feed and obtained during the harvesting or processing of a commodity in which human food or fibre is derived. Almond hulls are becoming increasingly

important as animal feed (Aguilar *et al.*, 1984), emphasizing the need to optimize their digestibility and nutrient value. The chemical composition of almond hull was determined to estimate the nutritive value of the hull as animal feed and results show that it contained 2.1–8% crude protein (CP) (Fadel, 1999; Getachew *et al.*, 2002), 1.69–2.9% ether extract (EE) (Reed and Brown, 1988; Getachew *et al.*, 2004), 28–38.49% NDF (Reed and Brown, 1988; Getachew *et al.*, 2004) and 48.7–57.8% non-fibrous carbohydrate (NFC) (Reed and Brown, 1988). However, the carbohydrate content in hulls is typically higher than 25% and is composed of glucose (14%), fructose (9%), sucrose (5%), sorbitol (5%) and inositol (3%) (Sequeira and Lew, 1970). Saura-Calixto and Cañellas (1982) and Saura-Calixto *et al.* (1984a) analysed the water-soluble carbohydrate composition of almond hulls, shells, integument and kernels of mixtures of cultivars grown on the island of Mallorca, Spain. For the hull component, they found that the principal sugars and sugar alcohols were fructose, glucose, sucrose, inositol and sorbitol, with minor amounts of xylose, arabinose and raffinose. The almond hulls are used as a natural source of sweetener concentrate and dietary fibre (Takeoka and Dao, 2003). They contain triterpenoids (Takeoka *et al.*, 2000), lactones (Sang *et al.*, 2002) and phenolics (Sang *et al.*, 2002). The almond hull sampling and analysis programme has confirmed that they contain significant amounts of fermentable sugars, ranging from 25% to 33% of the DW of the hull portion of the samples (Offeman *et al.*, 2014).

Recent studies reported that the almond hull can be used as a diet for feeding sheep during off-season periods because of the high content of NFC, as well as greater digestibility in sheep (Yalchi, 2011). In a study by Getachew *et al.* (2004) about the relationship between the chemical compositions of several ruminant feeds, the NFC content of almond hulls (48.70%) was higher than that of alfalfa (26.80%) and almost the same as that of sugar beet pulp (43.80%). A recent study reported that almond hulls showed greater DM disappearance than alfalfa and an even a greater soluble fraction than sugar beet pulp. The higher NFC and lower NDF content of almond hulls are subjected to a higher soluble fraction, indicating that almond hulls are

an agricultural byproduct that can be used as feed material for ruminants (Jafari *et al.*, 2015).

Between the outer hull and the nutritious kernel lies the shell or endocarp, primarily composed of cellulose, hemicellulose and lignin. Shell strength is a function of the proportion of these chemicals, shell morphology, fibre content and the outer shell adherence (Ledbetter, 2008). The shell varies in cracking strength between cultivars, and terms such as 'paper shell' and 'extreme hard shell' are used as descriptors for the shell types of various cultivars (Gülcan, 1985). The kernel percentage (kernel weight/nut weight) is used as an indicator of relative shell strength and this value provides almond handlers with a general guide to the almond cultivar's shell cracking requirements (Ledbetter, 2008).

Almond shell carbohydrates are mainly cellulose and hemicellulose, which constitute a potential source of industrial chemicals, particularly furfural (Schirra, 1997). The high xylan content of almond shells makes them a suitable substrate for the production of xylose (Pou-Llinás *et al.*, 1990), furfural (Quesada *et al.*, 2002) or for fractionation into cellulose, pentosans and lignin (Martínez *et al.*, 1995). More commonly, almond shells are considered a waste product and are composted, used as mulch, ploughed back into the orchard or used as a fuel. Several potential antimicrobial compounds have been reported to be associated with almond shells (Sachdiva, 1968; Bhatia, 1977), which could affect orchard soil ecology if allowed to form in significant quantities in new almond cultivars.

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19 World Almond Market

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19.1 Introduction

In order to make good almond marketing decisions, it is important to have a comprehensive understanding of the world almond market and how it works. In this chapter, we will look at the history and growth of the almond market, its structure and behaviour, then some of the specific decisions a grower or processor makes within this context, and finally ways to minimize risk in an almond market known for volatile prices.

19.2 History and Growth

One of the most remarkable features of the world almond market is the growth over the past 35 years. California production grew from an average of 155,000 metric tonnes (t) in the period 1979–1982 to 909,000 t in 2011 – a six-fold increase, and an annual growth rate of 6%. The annual growth rate from the 1997 crop to the 2011 crop is 7%. Shipments showed similar increases over the same periods, keeping carryover inventories low. Another remarkable feature of the world almond market is the concentration of production – California produces two-thirds of the world supply and exports 70% of its crop.

What has permitted this growth? Almonds are versatile in the variety of uses – as an

ingredient in confectionery, ice cream, bakery, nut mixes, snacks and cereal – in addition to whole almonds in bulk and in consumer packs, as shown in the estimated usage by the USA market below:

Snack (roasted/salted)	23%
Confectionery	20%
Food service	20%
Cereal	14%
Dairy (ice cream)	10%
Bakery	8%
Consumer bulk	5%

Other factors favouring growth in almond usage around the world are:

- Almond exports have expanded in a period of reduction in trade barriers (North American Free Trade Agreement (NAFTA) and World Trade Organization (WTO)), making it easier to expand foreign markets.
- As incomes grow in the developing economies, diets increase in protein, thus increasing demand for almonds, which are a good source of protein as well as being non-perishable, and easy to store and transport.
- In California, with the support of ABC-funded production research and the Ag Extension (University of California Cooperative Extension and Agricultural Experiment Station) system, growers have been able to increase

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- yields, thus keeping prices attractive for expanding sales.
- The ABC has worked to expand markets through its activities in the areas of nutrition research, market research, advertising and promotion.

Now let’s look at some of these market features in more detail.

19.3 Market Structure

The world almond market is characterized by the pure competition model. There are many sellers, many buyers and good information throughout the marketplace. This makes for a very competitive and very efficient market. Starting with production, in California there are about 5500 growers cultivating almost 360,000 ha of producing almonds. Blue Diamond Growers Cooperative (BDG) has an estimated 3500 growers, each growing about an average of 20 ha. About 2000 independent growers grow approximately 140 ha each.

There are over 100 handlers listed with the ABC. An estimated 60 handlers are of commercial size, offering services to other growers and handling 2250 t of shelled almonds or more. Nine handlers (including BDG), processing over 22,700 t each, handle 48% of the crop (Table 19.1). This illustrates the dispersed nature of the market at the processor level.

We do not have similar figures for the buyer/user level but it is also dispersed among many firms. The big three in the USA are Hershey, M&M Mars and Kraft Foods (the latter includes Planters); however, their market share is not enough to dominate. In the export markets, buyers are equally fragmented.

Table 19.1. Distribution of the almond crop in California according to handler size. (Almond Board of California Almanac, 2016.)

Handler size (tm)	Number of handlers	Crop handled (%)
< 500	22	1
500–1000	53	22
11,000–22,000	18	35
23,000–36,000	3	10
> 36,000	4	33

Most markets go through a maturity cycle of almond use, beginning with top quality (Nonpareil Supreme or Extra #1 Grade) for gift and luxury use. This was true for Mexico, India and China. As the market grows and competition increases, lower-priced varieties and sizes are offered, thus increasing total demand for all varieties and grades. Ultimately, almonds are used as an ingredient in locally produced products, and a full range of almond products are consumed from top-quality whole almonds to manufactured (sliced, diced, powder, paste) to byproducts (whole and broken).

The majority of almonds are used by food companies as ingredients in their own branded products, especially in the mature markets (USA, western Europe, Japan). For this reason, the market does not give much advantage to almond processors with a consumer brand. Processor-branded almond packs are a small part of the market. This enables independent handlers to compete with larger companies because of the commodity demand for ingredients. These food companies look for price, quality and service, and a brand name is less important. There are several examples of well-known consumer brands, such as Dole, that have decided to leave the almond-growing and/or processing business.

19.3.1 Price discovery

One of the first questions of a person entering the almond business, is ‘where can I get price information?’ Unfortunately, there is no reliable printed source of real-time price information for growers or handlers because there is no regulated trading exchange as there is for agricultural products with a larger market volume, like wheat, corn and cotton. Companies have asked Chicago commodity trading exchanges about the possibility and their reply is that the market is not large enough and does not have enough liquidity – not enough volume trading on a daily basis. So, price discovery is accomplished by calling around and getting individual reports of sales by growers or by handlers. Some handlers write a newsletter or post one on their website giving current market information. In California the almond

industry is characterized by friendly relationships among competitors, whether growers or handlers, so there is a healthy exchange of information and one can quickly learn whose information is accurate and which sources are reliable.

19.3.2 Nature of demand

Almonds are a commodity but have characteristics of value-added products because each grower or handler earns a reputation for his or her level of quality and service. The differentiating factors for a grower are the varieties grown, the level of insect damage, the kernel size (bigger is usually more valuable) and the level of mechanical damage from the hulling and shelling process (split, broken, chipped). Most handlers pay premiums for low percentages of insect and mechanical injury. In some crop years, some handlers pay a premium for larger sizes.

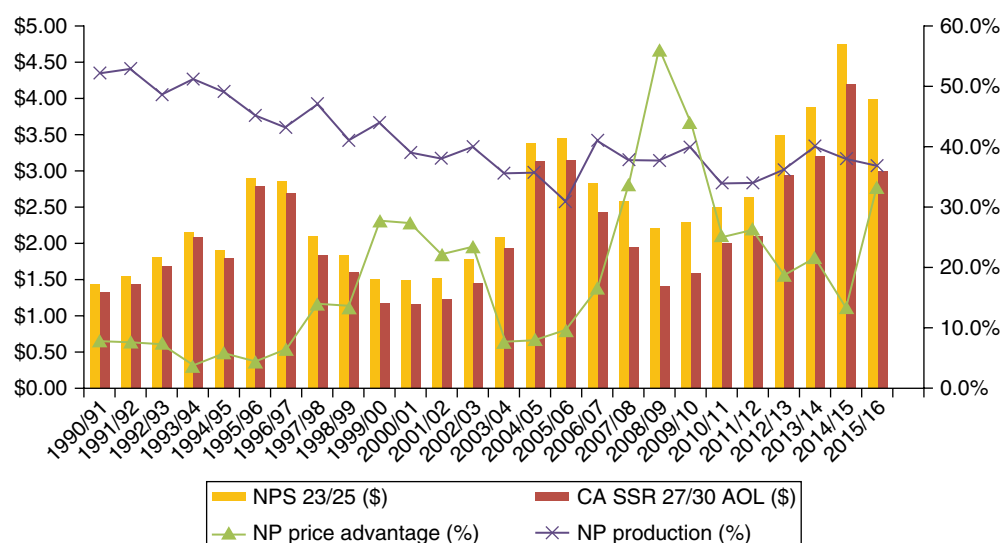
Almonds are sold by variety, grade and size. 'Nonpareil' is in a class by itself and always has a premium price over the other cultivars at the grower and processor level. This premium has varied over the years from as low as

US\$.44/kg to as high as US\$1.30/kg (see Fig. 19.1, showing recent increase in the 'Nonpareil' price premium as its percentage of the total crop has declined).

The next major variety group contains the pollinators that are long in shape: 'Carmel', 'Monterey', 'Price', 'Sonora', 'Wood Colony' and 'Aldrich'. They have a premium of about US\$.11 to US\$.22/kg over the short, round-shaped cultivars: 'Butte', 'Padre', 'Fritz' and 'Mission'. Each grade is about US\$.11/kg apart, as are the sizes, although in a year with an abundance of small kernels, the premium for larger sizes can be US\$.44/kg (e.g. from 23–25 to 20–22 kernels per 28.35 gm).

19.3.3 European market trends

Western Europe is the largest export market for California almonds, consuming about 224,000 t from 2011–2015 crop years (Almond Board of California Position Reports). This compares with about 274,000 t average for the US market for 2011–2015 crop years. In a recent paper presented at the ABC Annual Conference in December 2015, Joanna Clifton of Innova Market



NPS = 'Nonpareil' Supreme; CASSR = California select Shellerun; AOL = and/or larger; NP = 'Nonpareil'

Fig. 19.1. Averaged annual price of 'Nonpareil' versus other cultivars. (From ABC, 2015.)

Insights summarized new almond product introductions in the period 2010–2015.¹ European launches of new products containing almonds represented 45% of global launches in 2015, much greater than their approximate 25% share of global consumption. By comparison, North American and Asian markets launched 20% each of the global total. Within the European market, Germany, the UK, France and The Netherlands dominated the new introductions. Categories of new products listed below are similar to market share of these categories:

Confectionery	30%
Bakery	19%
Snacks	16%
Bars	11%
Cereals	8%
Desserts/ice cream	6%
Other (spreads, etc.)	11%

The fastest-growing category in the 2010–2014 period is snack introductions (up 21%), and within that group of products, snack products containing almonds with health claims grew 26%, illustrating growing consumer interest in healthier eating. Emerging sub-categories within almond product introductions showing rapid growth in the period 2013–14 are: spreads (almond butter), almond milks and fruit-based snacks.

19.3.4 The Spanish market

Most Spanish production is marketed through grower associations which are grouped in two main unions at the national level: Cooperativas Agroalimentarias (Agro-food Cooperatives) and AEOFROUTE (Asociación Española de OPAs de Frutos Secos y Algarrobas, Spanish Association of Growers Associations of Nuts and Carobs). The external trade (Table 19.2) requires special examination because, in recent years, imports have exceeded exports, a strange situation for the second largest almond producer. This discrepancy is because these figures reflect the exports and imports of almonds, both shelled and in-shell, but not of the many manufactured almond products. Spain has become a secondary almond market, as it receives a large amount of almonds to be later exported, both raw and processed.

Despite the larger amount of imports, their value is lower than that of the exports, due to the higher price of the exported almonds (mainly Spanish cultivars) than of the imported almonds (mainly Californian cultivars). This difference reflects the economic value of Spanish production, mainly due to the taste and composition of the Spanish cultivars as a result of the higher fat content, quality, stability and presence of antioxidants.

Exports are mostly directed to the European market, primarily to Germany, which is also a market for re-exporting to third countries. The largest demand is for blanched kernels of large size, raw or sugared, as well as for half-processed products such as flour and small pieces (slivers, slices and cubes). The market is increasingly demanding high-quality products, where the two traditional Spanish cultivars Marcona and Desmayo Langueta stand out. Recently, newly bred cultivars such as Belona and Soleta, of similar or higher quality, also stand out in the market.

19.3.5 Price elasticity of demand

Buyers' response to price changes varies with the type of market. In developed markets (the USA, western Europe and Japan) the majority of the almonds are used as an ingredient in food products. Accordingly, the demand is relatively price inelastic – buyers will pay higher prices when necessary to purchase the volume needed for their production lines, and will not buy much additional stock when prices are lower. In contrast, in developing markets (China, India, Middle East) where snack and gift almonds are the primary use and the ingredient market is limited, the demand is relatively price elastic. As prices increase, buyers purchase less and substitute competing snack nuts or even other seeds; and, as prices go down, buyers purchase more and expand the flexible snack market using price incentives. Accordingly, when the crop is large and prices are lower, we see expansion in the new markets dominated by snack use; and when the crop is short and prices are high, we see ingredient markets continue to buy similar quantities while snack markets reduce consumption.

Table 19.2. Spanish external trade of almonds for 2010–2012. (From Secretaría de Estado de Comercio.)

	2010				2011				2012			
	Exports		Imports		Exports		Imports		Exports		Imports	
	t	1000 €	t	1000 €	t	1000 €	t	1000 €	t	1000 €	t	1000 €
Kernel	60,189	230,071	65,478	187,438	59,874	244,482	74,329	233,939	58,763	280,922	74,034	269,697
In-shell	539	1470	3059	6302	203	796	1748	4023	425	1394	4233	7346
Total		231,541		193,740		245,278		237,962		282,316		277,043

19.3.6 Nature of supply

Growers plant almonds when they look profitable. The best measure of profitability is crop value per bearing hectare rather than price per kg/pound. Since California produces most of the world supply, a short crop is almost always accompanied by a higher world price, thus maintaining the crop value per hectare. California almond growers can have a steady profitable income with a large crop at low to moderate prices, as well as with a small crop at high prices. Periods of increased new plantings accompany periods of steady high crop value per hectare (Table 19.3).

The 5-year period 1993–1997 saw steady value per bearing hectare over US\$5000 and was accompanied by increased plantings during 1994–1999. Almond hectares were static from

1985 to 1996 (165,000–175,000 estimated hectares in production) then grew to 220,000 by 2002 (30% expansion) then flattened again. The next strong income period were the six crops in 2003–2008, with value per hectare exceeding US\$7000/ha and accompanied by heavy plantings during 2004–2007. Of course, production costs grew substantially over the period as well. Producing hectares grew from 244,000 in 2006 to 308,000 for the 2010 crop (26% expansion). Income per hectare increased substantially in 2011–2014 crop years but we have not seen the accompanying increase in new plantings because the 4 years of drought (2012–2015) have restricted expansion. For statistical purposes, almonds are considered bearing in their fourth year. For example, trees planted in 2004 are ‘producing’ in the 2007 crop, even though a small third

Table 19.3. California almond hectares and farm value (Almond Board of California and US Department of Agriculture (USDA) National Agricultural Statistics Service, NASS). (Adapted to SI from California acreage and farm value table, Almond Board of California Almanac 2015.)

Crop year	Hectares			Value in US\$	
	Bearing hectares	First-year plantings	Average trees/hectare	Farm price (US\$)	Value per bearing hectare
1988–89	168,000	6200		2.31	3573
1989–90	164,000	6400		2.24	2890
1990–91	164,000	9200		2.05	3594
1991–92	162,000	5400		2.62	3441
1992–93	160,000	5300		2.86	4258
1993–94	165,000	7600		4.27	5566
1994–95	173,000	12,700		2.95	5506
1995–96	167,000	14,400		5.46	5205
1996–97	171,000	14,000		4.58	5877
1997–98	177,000	15,300		3.43	6486
1998–99	184,000	19,100		3.10	3778
1999–00	194,000	13,500		1.89	3503
2000–01	204,000	9400		2.13	3228
2001–02	212,000	4700		2.00	3448
2002–03	218,000	3900	253	2.44	5441
2003–04	220,000	5700	258	3.45	7185
2004–05	228,000	10,600	258	4.86	9485
2005–06	236,000	13,600	260	6.18	10,574
2006–07	244,000	12,800	262	4.53	9146
2007–08	256,000	5800	262	3.85	9270
2008–09	284,000	8700	267	3.19	8151
2009–10	300,000	7300	270	3.63	7553
2010–11	308,000	5300	270	3.94	9314
2011–12	320,000	6000	277	4.38	12,375
2012–13	328,000	4900	280	5.68	14,509
2013–14	340,000	4400	280	7.06	18,552
2014–15	348,000	7900	285	7.02	16,727

leaf harvest is common. The growers' ability to increase yields over time, coupled with increased number of trees per hectare, has contributed to the higher crop value per hectare in addition to the market price contribution.

Another factor that augments increases in bearing hectares during and following periods of high crop value per hectare is that growers delay removal of older orchards when prices are high. Almonds are considered economically productive for about 25 years. Accordingly, 4% new plantings annually would be required to just maintain the producing base.

Other important factors affecting new plantings are the availability of bank loans for orchard development and the relative profitability of alternative crops. In California there have not been many other profitable crop options over the past 20 years of growth of almond hectares. Since the 1970s' tax incentives for orchard development were changed to require capitalization of development costs, the expansion has been mainly by owner operators rather than by investment funds.

Foreign development of almond plantings has been much slower than in California. Except for Chile and Australia, where California's cultivars are planted, most other regions do not have the right combination of climate (long growing season yet with sufficient winter chill) and cultivars developed for profitable production. The Mediterranean area and central Asian traditional cultivars are not as productive as the California cultivars; hence their planting is not as profitable as in California compared with alternative crops; however, new cultivars are being introduced that will improve profitability. In Chile and Australia, as well as in Mediterranean and central Asian regions, almonds are limited by availability of irrigation water needed to achieve top production. Recent expansion of Australian production will, however, put it in second position ahead of Spain soon (USDA, 2015). Turkey and China are experimenting with expanding almond production but have not increased significantly. Nevertheless, at some point we could see more competition outside California than we have today.

Sources of information regarding supply are available through the ABC. On about 1 May each year, the US Department of Agriculture (USDA) National Agricultural Statistics Service (NASS), in cooperation with the California

Department of Food and Agriculture, makes a hectares report by county and variety. They adjust the producing hectares estimate for the previous year and make an estimate for the current year. These calculations are based upon annual grower surveys and other data to assist with the estimates.

On about 10 May each year NASS releases a California almond crop estimate based upon a grower survey asking growers in the sample to estimate their yield per hectare as a percentage of the previous year. They then apply this calculated yield to the new hectares estimate. NASS also conducts a field survey of two trees per orchard in their sample orchards around the state to make an objective measurement of the almond crop. This is released at the end of June each year and also gives kernel weights and other quality measurements. NASS has been quite accurate in its crop estimates (within 10% from 1996 to 2016) and the market has become dependent upon this information. Crop and hectares surveys and estimates are financed by the ABC.

Data on foreign production are harder to find and less accurate, with the exception of Australia whose Almond Board produces similar statistics to those of the ABC (www.AustralianAlmonds.com.au). For other countries, the best source is the USDA Foreign Agricultural Service (FAS). That leaves out some almond-producing countries such as Iran where the USA does not have a full diplomatic presence. United Nations Food and Agriculture Organization (FAO) figures are only as accurate as official reports from the specific country, and therefore not very reliable. Using FAS data, the ABC and the International Nut Council (INC) estimate the California crop at about 80% of the world supply (USDA, 2015). If we add in countries not included in these reports, California produces about two-thirds of the world supply of almonds. By either measure, California dominates the market and the size of its crop is the primary determinant of the supply side of the market price equation.

19.3.7 Role of the Almond Board of California

The ABC was formed in 1950 when Italy produced the largest crop. There were about six handlers

for California and the California Almond Growers Exchange (now BDG) marketed the majority of the crop. The ABC administers the federal marketing order for almonds, and is supervised by the USDA. The ABC must be continued by a grower referendum every 5 years and it is empowered to collect assessments (0.066/kg in 2016) from handlers. Its functions include funding production, nutrition, technical and market research; advertising and promotion of almonds; collection and dissemination of production and shipping data; and estimating crop and hectares planted. The ABC is considered a model commodity board and is given much credit for the expansion of the world almond market.

Inasmuch as California is the dominant world almond producer, the crop estimates, crop receipts and shipment reports issued by the ABC are critical pieces of information for a commodity market seeking to judge supply and demand then adjust prices accordingly. The early May and late June crop estimates, as well as the monthly position reports showing crop receipts and handler shipments to the domestic and international trade, are often turning points in market prices. The two crop estimates can move the market if they are significantly different from what the market is expecting. Actual crop can be estimated within 3–4% in early December when the November receipts are reported. By this time about 90% of estimates is received. Shipments are reported monthly, showing domestic shipments and exports by country. These data provide the most reliable market information and are summarized in the annual almanac. The market price can move as much as US\$0.22/kg to US\$0.45/kg within a week following a surprise in shipments or crop size, as reported by the ABC Position Report. (Reports are available on the ABC website www.almondboard.com.)

19.3.8 The market cycle

The single most influential factor in determining world almond market price is the weather during bloom – late February and early March – in California's Central Valley. Since this is the major source of supply and almost all almond cultivars are self-sterile (or self-incompatible), and growers rely on bees to carry pollen between cultivars,

good bee-flight weather is required to set a good crop. Poor weather (rain, fog, frost) during the critical 2-week bloom can reduce the crop by 25% from its potential. Accordingly, bloom is the most critical part of the market cycle and sets the market price for the crop year. If pollination takes place in uniformly good weather, the prices can come down quickly. If the weather is decidedly poor during most of the bloom, the price will move up quickly. Following mixed conditions, the market reaction can be delayed until April when nuts enlarge enough to indicate crop size.

As mentioned earlier, other critical market turning points are the crop estimates and the crop receipts reports at the end of harvest. On the demand side, monthly shipment reports released by ABC on about the tenth day of each month can cause changes in prices if they are surprisingly high or disappointingly low.

The demand for almonds (as well as other tree nuts) is concentrated in the September–December period, when about 40% of the crop is shipped. Accordingly, sales activity tends to be highest during the August–October period.

19.3.9 Supply, demand and market price

World almond demand has been steadily increasing and tends to be less volatile than the annual size of the California almond crop. Accordingly the swings in market price are more dependent upon supply variation than on demand changes (Fig. 19.2). These charts show the volatility of the almond market.

19.3.10 Growers' marketing decisions and risk management

Almond growers have long been in a strong position in the marketplace. With the steady growth of demand, periods when supply exceeds demand tend to be short-lived and most years are a 'seller's market' in which the grower has many market outlets from which to choose. The California almond industry has always had a strong cooperative (BDG), which has always been the largest single handler. BDG almost

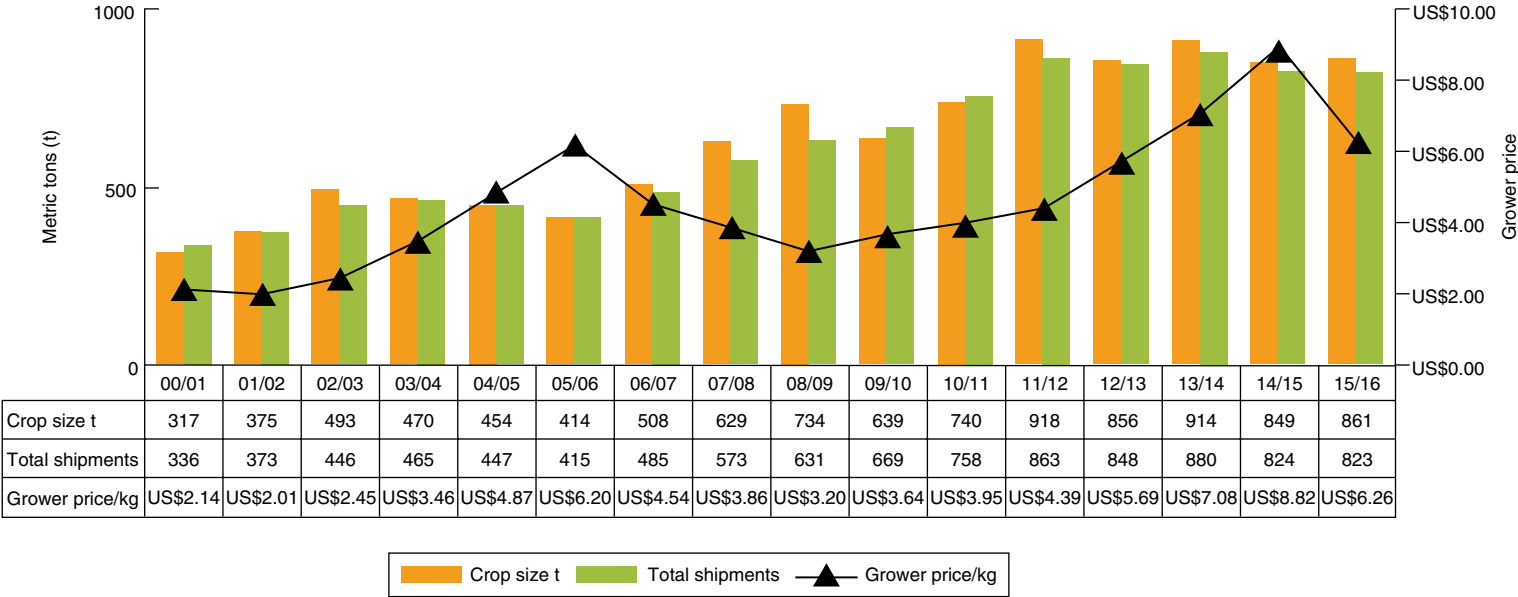


Fig. 19.2. Annual almond crop size in California 2000–2015 (ABC Almanac), annual shipments (ABC Almanac) and annual grower prices (USDA National Ag Statistics Service, California Almond Price Report).

always welcomes new growers who wish to join. In addition, there are over 60 independent handlers who are usually looking for more volume. If a grower wishes to become more involved in processing and marketing, there are opportunities for custom processing by a few of the commercial handlers. Since the cost of entry into processing is low compared to other agricultural commodities, growers can form partnerships or enter into processing on their own. Most of the independent processors are growers who have vertically integrated into shelling and/or processing.

Once a grower has decided upon a handler (or more than one handler), he or she may also have a choice of sales plans. Most handlers offer a 'season pool' in which sales are made over an 8–12-month period, progress payments are sent to the grower, then a final payment is made based upon the season average. This is the safest plan as it is never the lowest (nor, of course, the highest) price but a good average year in and year out. The major alternative to the season pool is the 'call pool', offered by many handlers, in which a grower delivers the crop to the handler(s), watches the market then advises his or her handler when to sell. Most of these growers will sell two to four different times during the year, thus getting their own market average to minimize risk of hitting a down market with a majority of one's crop unsold. Two other sales alternatives in lesser use are: (i) handler offers a grower a 'floor price' or minimum, then pays all or part of the increase (if any) in the market average; and (ii) handler offers to match or exceed the price paid by a single or group of two to four other handlers. These offers by a handler are impossible to cover back to back in the market with sales, thus putting the handler in a risky position.

If the grower elects to sell via the call pool, there are key market points to watch:

- Sales of a given crop prior to bloom are risky because of potential significant market increase in the event of poor weather during bloom. Nevertheless, it can be a good hedge against a full crop with lower prices if prices before bloom are profitable. A safe percentage to hedge in this way is 10–20% at the most to minimize risk.
- Sales from post-bloom to the June crop estimate carry somewhat less risk but do not give the seller (and buyer) the benefit of knowing the crop estimates.
- Sales during the July–January period carry the least risk because most almonds are sold during this period and are likely to be made close to the annual average price on a collective basis.
- Growers who wait to sell after the bloom for the next crop year are taking the risk of a big next crop and lower prices. Conversely, they can profit from higher prices due to poor pollination or from strong shipments and shortages at the end of the crop year just prior to the next harvest.

19.4 Summary

Growing almonds comes with financial risk because of the variability of market prices from one crop year to the next, as well as within an individual crop year. This market price risk is counterbalanced by the many factors that have favoured the growth of the world market and the ability of California growers to not only remain competitive but to dominate the world market. Factors responsible for this world market growth and California's advantages discussed in this chapter are:

- steady growth in world demand, which shows no sign of ceasing;
- good nutritional content of almond and acceptance in diets of most cultures;
- adaptability of almond to many uses in many products and ease of storage, use and transport;
- as an ingredient, almond adds value to finished products;
- gradual lowering of trade barriers;
- ability to increase yields with the help of research, irrigation and new cultivar development;
- effective federal marketing order providing information, research, promotion, etc.;
- better crop value per hectare than most alternative crops; and
- minimum competition from other almond-growing regions.

Note

¹ A European Odyssey: From Shipments to Supermarkets. Slide presentation at 2015 Almond Board of California annual conference by Joanna Clifton, Innova Market Insights, Arnhem, The Netherlands.

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20 Processing and Industrialization

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20.1 Introduction

Almonds have been used from time immemorial and are used in a large variety of derived products. They are used not only for human consumption, but also in the pharmaceutical and cosmetic industries, and applications from long ago are known. In olden times, in Mediterranean countries, in all weddings, the newly weds offered their guests five sweet almonds as a symbol of health, fertility, good luck, wealth and happiness. In contrast, bitter almonds were associated with misfortune and death. Consequently, many authors have included bitter almonds in their literary writings, such as Gabriel García Márquez in his *Love in the Time of Cholera*: 'It was unavoidable: the smell of bitter almonds reminded the fate of the upset loves...'

Almonds are considered to be a basic ingredient of a Mediterranean diet. Their versatility renders them the favourite choice for cooking, pastries and drinks. Almonds are ranked over any other nut because of their diversity of uses, being the mostly demanded nut by technologists, food developers, cooks and even scientists. They have a delicate and healthy taste and add a special richness to any product due their oily quality. The popularity of almonds reflects consumers' preference for their excellent taste in many different products, including:

- snacks, cereals and sweets;
- salads and salad dressings;
- soups;
- gravies to thicken stews;
- crunchy and delicious coatings and gratins for meat and fish;
- desserts, cakes, creams, ice creams, sugared almonds; and
- turrón (a confection made from a sugar or honey paste into which nuts are mixed), nougat, pastries and confectioneries.

Unsurprisingly, world almond demand has increased steadily – and beyond all expectations – in recent years (see Chapter 3). A study by *Innova Market Insights* showed that in 2011 almonds reached a world record of 4200 different new products.

The organoleptic and qualitative characteristics of almonds have always been the focus of comparative studies and examination by technologists, mainly in industries addressing:

- the high quality of the finished product;
- the long shelf life of the finished product; and
- the possible shortage of the supplied product.

The solution to these problems is evidently related to the typology, characteristics and origin of the almonds. Clearly, therefore, the choice

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of a cultivar must not be autarkical or, even worse, nationalist. This must be an objective and impartial choice with the final industrial aim of favouring the consumers' taste and preference, reaching the highest quality in the finished product. Based on our experience, it is possible to assert that the Spanish turrón industry shows a wide preference for 'Marcona'. This preference is mainly due to the organoleptic and analytical characteristics of its composition, and was considered to be possibly irreplaceable in some industries, particularly in some specific sectors such as turrón and marzipan production, until a similar cultivar such as 'Belona' had been introduced. These industries have an elaboration process several centuries old, with design and improvement of some tools, machinery and manufacturing systems perfectly adapted to the quality and industrial behaviour of this cultivar being developed over centuries. The experience of the professionals carrying out their work, mainly in the turrón industry, is also important. As a consequence, when addressing the requirement of an adequate shelf life, the industry tries to avoid the use of some Spanish and foreign cultivars, since their composition or organoleptic quality is not adequate for the process. The high content of antioxidants such as tocopherols in 'Marcona' may have contributed to its high industrial quality. Therefore, this quality typology has been searched for in some breeding programmes for new cultivars, such as 'Belona' in Spain and 'Sweetheart' in California.

To improve quality, correct characterization and traceability of the raw material is essential. Processing industries must solve the delicate problem of controlling the qualitative traits of all the materials and processing steps, requiring the standardization and certification of the different products by the appropriate organizations.

In the Californian market, the United States Department of Agriculture (USDA) has established quality regulations to carry out the required controls in relation to demand. From the associative point of view, the distribution circuit of almonds in California is particularly interesting. The 'California Almond Growers Exchange' fully centralizes and controls all the almond marketing in the USA, establishing precise regulations for its relations with the associated growers (see Chapter 19).

In addition to the kernels, almond hulls are used in animal feeds and the shells as an energy source when burnt, as well as abrasive once powdered. Shells are also used as a component of conglomerate boards.

The main attribute rendering almonds so special is that they combine taste, texture and versatility with nutrition. This combination of positive attributes is further improved by the ease of consumption. Whereas manual shell removal is tedious and only feasible with paper-shell cultivars, there is a wide variety of industrial confectioneries ready to be consumed on the market. Furthermore, there is increased knowledge of the benefits of almond consumption, and consumers increasingly realise that almonds are healthy from the dietary point of view.

20.2 Industrial Preparation

20.2.1 Reception

After harvest, almond nuts are delivered to the different reception centres, such as cooperatives, processing plants or simple distribution points, from where the nuts are sent to the cracking plants (Fig. 20.1). In all cases, before permanent storage, the department of quality and reception must evaluate the characteristics of the delivery to establish its compliance with the reception standards agreed on previously. This evaluation is carried out by different samplings relating to moisture, shelling percentage, cultivar, foreign materials, rotten kernels, etc. The physico-chemical parameters of the almond nuts are analysed; all consignments not fulfilling the strict quality parameters adopted for each standard being rejected. In this way the quality and homogeneity of the product is ensured.

If the consignment is too moist, the nuts must be dried before storage. This is done by natural drying in thin layers under the sun or in silos, where temperate air at 35–38°C flows over them for several hours until the excessive moisture is extracted. Drying was generally done by the growers in their own facilities, but recently reception centres have been introducing drying silos for in-shell almonds, allowing the growers to transport their product directly from the farm to the cracking plant. In contrast, in California



Fig. 20.1. Almond reception plant. (With kind permission from SIRVENT ALMENDRAS S.A. – <http://www.sirventalmendras.com>.)

drying is mainly done on the ground after tree shaking at harvest (see Chapter 17).

After drying, almonds must be stored in dry and well-aerated places. Generally, the cracking plants are close to the drying facilities, but this is not always so.

20.2.2 Cracking

Before cracking, almond nuts are moistened to increase kernel elasticity and avoid possible kernel breakage during cracking. They are then passed into the cracking machines (Figs 20.2 and 20.3) and forced to go through punched circular bars. One part of these bars is fixed and the other is mobile; when the nut goes through the hole between them, it is pressed and cracked. Each group of bars has a different diameter, thus requiring previous calibration to force the nuts to pass through the appropriate hole. Once cracked, the mixed kernels and shell pieces go through separating devices (Fig. 20.4), which separate the kernels from the shell pieces at a high percentage, working by a system of screening and aspiration. After this first separation a

final one is done to obtain kernels free of shell pieces. This operation was done manually over a conveyor belt in the past, but now defect detection is mostly done with electronic devices.

The next step is a second kernel selection to discard any foreign material such as stones, sticks, pieces of glass and hulls (Fig. 20.5). The darker kernels (probably rotten) are also removed, as well as kernels broken or damaged during the cracking process (Fig. 20.6). This operation is done using electronic devices with laser detectors. After this separation, depending on the specific requirements of the consignment, a last manual inspection over a conveyor belt may be made to refine the final product.

Kernel drying is a requirement because almond nuts are moistened before cracking. Depending on the industrial design, it may be done before or after kernel separation from the shell pieces. In most cases kernels are then calibrated because of the industry demand for homogeneous consignments. The calibration machine consists of different sieve lines with circular holes of different sizes allowing the kernels to be separated by size. This operation is of commercial interest since larger sizes normally obtain higher prices.



Fig. 20.2. Almond processing plant. Cracking. (With kind permission from SIRVENT ALMENDRAS S.A. – <http://www.sirventalmendras.com>.)



Fig. 20.3. Almond processing plant. Sampling. (With kind permission from SIRVENT ALMENDRAS S.A. – <http://www.sirventalmendras.com>.)



Fig. 20.4. Selection plant. (With kind permission from SIRVENT ALMENDRAS S.A. – <http://www.sirventalmendras.com>.)



Fig. 20.5. Different foreign materials. (With kind permission from SIRVENT ALMENDRAS S.A. – <http://www.sirventalmendras.com>.)

The system for soft-shell cultivars is different – not involving a previous moistening due to the very fragile condition of the shell. Cracking is mainly done by subjecting the nuts to the concomitant action of two rubbing strengths working in opposite directions. Kernel separation and calibration is carried on as already described.

20.2.3 Blanching

The objective of blanching is the separation of the tegument or seed coat from the rest of the kernel. The seed coat is brown or yellowish and is removed to obtain a fully white kernel (Fig. 20.7). This process is carried out by natural means



Fig. 20.6. Mechanical damage. (With kind permission from SIRVENT ALMENDRAS S.A. – <http://www.sirventalmendras.com>.)



Fig. 20.7. Blanched 'Marcona' kernels. (With kind permission from TDC Association, <http://www.tdc.es>.)

and is fully controlled to guarantee the moisture of the kernel, its integrity and its organoleptic properties such as taste, colour and odour. The physical conditions of the operation such as temperature and time of moistening must, therefore, be controlled very carefully since kernel moisture must not exceed 6.5%.

Once the kernels are selected by size, they are immersed in boiling water. This operation has a double function: it softens the seed coat and makes its removal easier, and also removes any hydrocyanic acid caused by the presence of bitter almonds, since this soluble acid is eliminated by hydrolysis. There are several devices in use, based on subjecting the moistened kernels to the concomitant action of two rubbing strengths working in opposite direction at different speeds. The softened seed coats are broken and later removed by an air stream. A very important problem, not easy to solve, is to perfectly blanch the nut. The removal of unblanched kernels

can be achieved by electronic devices, although not all kernels imperfectly blanched are finally removed, thus requiring a final manual inspection. After blanching, if the kernels are not immediately used, they must be dried to avoid becoming mouldy. The percentage of moisture must not go beyond about 6% in a good consignment, since higher moisture results in kernel elasticity and difficulty in breaking.

20.3 Quality Guidelines for Almond Reception

Almonds must follow at least two selection steps: (i) ensuring the absence of imperfect kernels and of any foreign material; and (ii) selecting each individual kernel by modern, high-precision electronic devices, rejecting all kernels lacking maximum quality. The level of selection depends on the commercial requirements of each consignment and its industrial use, such as flour, dices, slices, dragées and turrón.

20.3.1 In-shell almonds

Almonds from different crop years will not be accepted. In-shell almonds must be dry, healthy, without impurities and with the hull removed. Mixing different cultivars, mainly of different shell hardness, is unacceptable. Moisture tolerance depends on the time of reception. The maximum moisture accepted is 6.50% from 15 August to 28 February in the northern hemisphere, and 6.25% after this date. Any excess over that level

will be discounted, and if the difference over the threshold is higher than 3.01% the entry may be rejected. The official Spanish regulation establishes the tolerance levels for mixtures of cultivars and presence of double kernels (Table 20.1). Since ‘Marcona’ and ‘Desmayo Langueta’ are more highly prized than other cultivars, the presence of a percentage of any other cultivar is penalized, and may include triage costs. The presence of hulls and of almond pieces is also penalized.

20.3.2 Shelled almonds

All entries of shelled almonds must fulfil food legislation requirements in regard to aflatoxins, traceability, genetically modified organisms, pesticide residues, heavy metals, microbiological contamination and any other regulation affecting food quality and safety. The maximum moisture tolerance is 6.75% for all cultivars, the ideal percentage between 5.75% and 6.50%, and relates to the same time period as in-shell almonds: 6.75% from 15 August to 28 February and 6.50% after that date and for the old crop. Moisture excess may be also penalized in relation to moisture weight and drying costs. The presence of foreign materials such as in-shell almonds, shell pieces, stones, sticks, plastic pieces, threads, glass pieces and any

Table 20.1. Maximum tolerance for double kernels and mixture of different cultivars. (With kind permission from RCPGIJTA, <http://www.ijjona.com>.)

Cultivar	Tolerance
Bitter (only for Comunas)	2%
Comunas	12%
Planeta	34% (4% double, 30% Comunas)
Marcona	8%
Soft-shell	5% (Rof)
Fitas (soft-shell)	0% (hard shell)
Desmayo Langueta	6%
Jordana	15%
Rumbeta	3%
Ramillete	6%
Atocha	6% (3% doubles, 3% Comunas)
Colorada	18% (15% doubles, 3% Comunas)
Guara	20%
Mallorcás	20%

other component not coming from the almond kernel and obviously not suitable for human consumption is also negatively valued. The presence of foreign materials will also be penalized, since the tolerance threshold for all cultivars is 0%, including weight losses and triage costs.

When considering kernel suitability, the unsuitable kernels are those that are rotten, mouldy, rancid, gummy, moth-eaten and spotted. The threshold of tolerance is 0.40% for all cultivars, in addition to discounts for possible triage costs and penalization.

Almond pieces are defined as kernels lacking more than one-third of the whole. The tolerance varies according to the cultivars, from 2.5% in ‘Marcona’ and ‘Desmayo Langueta’ to 6% in bitter samples. Mechanical damage is considered as the lack of more than one-third of the kernel and with a breakage larger than 5 mm, with a maximum tolerance of 5%. The lack of seed coat is not considered damage. The presence of pieces is also penalized. In relation to dust and small pieces, the weight of everything passing through holes of 5 mm in diameter is discounted for all cultivars. Only a 5% of calibration error is allowed. Efforts to increase quality have sharply reduced the acceptability of the presence of bitter almonds; consequently, all consignments with any bitter almonds will not be considered to be the norm.

The acid level is measured as the acid percentage in the oleic acid fraction, with a maximum tolerance of 0.7%. The presence of peroxides is measured as milliequivalents (meq) of O²/kg for the extracted oil, with a maximum of 4 meq. The methods required by food legislation will be applied to the analysis.

Only neutral, new and white bags 62 × 105 cm and tare of 150 g may be used. For larger consignments, bags 1.80 m high, 1 m deep × 1 m wide and tare of 2.5 kg are used. Previously used bags are not permitted. Poor transport conditions could be a reason for rejection as ruled by the CODEX ALIMENTARIUS (FAO, Food and Agriculture Organization).

20.3.3 Sampling

In certifying almond consignments, adequate sampling must be carried out. Correct sampling is a difficult operation requiring the adoption of

careful standards because a sufficiently representative sample must be obtained. Most commercial operations are decided on the sample taken from the bulk of the consignment, remaining the reference for any discrepancy arising during the commercial transaction. Any mistake during sampling will result in possible misunderstandings, delays and controversial price adjustments. The commercial almond sector has recognized some sampling methods as effective for the determination of almond consignments and it is highly recommended that they are adopted in all transactions. It is, however, difficult to establish unique rules for sampling, valid in all cases; therefore, in some especial circumstances some modifications to the mostly accepted rules may be accepted.

The guidelines have the objective of regulating the general conditions for sampling to make possible the estimation of almond quality, since almonds are considered as any other primary industrial staple. The consignment must be examined in its whole volume, but in portions of a maximum of 3000 kg, both for in-shell and shelled almonds. The samples must be wholly representative of the portion from where they have been obtained. Consequently, from an entry of 3000 kg as a maximum, all the partial samplings must be carefully mixed to obtain a global sampling of about 20 kg from which, through successive reductions, a final sampling of about 1 kg will receive laboratory analysis. All sampling devices should be clean, dry and free of any foreign material or odour. Additionally, all sampling operations must be done in such conditions that the samples, the sampling devices and the containers will be protected from any possible accidental contamination such as rain, dust, etc.

For sampling consignments in normal and big bags, sharp cylindrical and conical drills specially designed for this sampling are used, as well as manual shovels. For sampling of bulk consignments, mechanical or manual shovels are used, as well as cylindrical and conical drills, devices for mechanical sampling and other devices designed for taking successive samples during nut pouring. For mixing and division of the samples, shovels, crosses (for division in quarters) and other devices for dividing are used, such as pouring-reducing devices.

Most between-country almond consignments are handled by maritime transport with containers of about 20,000 kg. Sampling can be

made when unloading the containers or once the consignment arrives at its destination. The whole consignment is grouped in lots of about 3000 kg each for the partial samples. In the case of almonds loaded in trucks, sampling can be made when transferring the almonds from the container to the trucks or when the trucks reach their destination. In any case, the maximum size of any lot must be 3000 kg.

Sampling must be carried out by expert people appointed by the seller and the buyer. Since the composition of any lot is practically homogeneous only in a few cases, enough partial samples must be taken to obtain a global sample representative of all the lots. Sampling of any partial lot with specific features must be done separately, such as any damage by seawater or by any other reason during transportation or storage, in bad conditions or previously dispersed by any reason and later joined. Consequently, any damaged lot must be evaluated separately and never mixed with the better-quality lots.

In each case the partial samplings must be carried out according to the lot. In the case of bags, partial samples must be taken from at least 10% of the bags, independently of their size. If the bags are open, the sample must be taken with the shovel or the cylindrical or conical drills. If the bags are closed, the sample can be taken with a sharp drill specially designed for sampling bags.

With bulk consignments, if the almonds are in movement the partial samples must be taken through all sections of the almond flow, at fixed intervals depending on the flow speed. If sampling is done when the consignment is in the ship hold or when unloading, the partial samples must be taken from the greatest number of places as possible and also at fixed intervals depending on the unloading speed. In truck consignments, sampling must be done at three different levels with a cylindrical or conical drill, at the centre of the bulk and at approximately 50 cm from the walls. If sampling is done in the truck hopper before weighing, the partial samples must be taken with cylindrical drills, shovels or mechanical sampling devices. The method of sampling in silos and stores will necessarily depend on the local conditions.

In the case of samples for laboratory analysis, the global sample must be mixed and reduced by division until the volume required by the laboratory is reached. The number of samples for analysis and arbitration must be specified. For

in-shell and unblanched almonds, it is recommended to sieve the global sample before reduction and later add the sieved fine residue in its exact proportion to the laboratory sample. All these preventive measures are to ensure that all samples contain the same percentage of fine elements of lower quality. The samples for laboratory must be packed in very compact bags, paper bags, cardboard packs, polyethylene bags, metal boxes or glass bottles and vials. Samples devoted to moisture determination or to any other analysis possibly influenced by moisture modifications must be packed in watertight containers with hermetical closing in relation to moisture. The containers must be fully filled and the closing must be sealed to avoid any modification of the initial moisture content. The labels for proper sample identification must be of sufficient quality and size to allow its correct and permanent identification. The samples must be sent to the laboratory as soon as possible and, unless in exceptional cases, not later than 48 h after sample preparation, excluding the days of commercial closing.

The sample for analysis is obtained from the laboratory sample. First, any gross impurity must be removed if there is any present. Later the sample is reduced with the devices already mentioned, taking care that the final sample is representative of the whole initial sample. The reducing-dividing devices must guarantee the uniform repartition of all the components of the initial sample for the sample for analysis.

After removal of any gross impurity (if present) and weighing, the laboratory sample must be mixed to ensure the final sample is as homogeneous as possible, and after successive divisions the amount of sample required for each specific determination is prepared. For impurity separation the method to be followed has been defined by the International Organization for Standardization, Geneva, in the recommendation ISO R-168, 'Oil seeds-determination of the content of impurities'.

20.4 Almond Products

20.4.1 Bitter almonds

In the past bitter almonds were mainly used in pastries, where they were added to give their particular scent to the different products. Bitter

and semi-bitter almonds are especially interesting in this industry because they possess a cyanogenic heteroside, amygdaloside, whose hydrolysis produces a strong aromatic odour due to its decomposition in benzaldehyde and hydrocyanic acid.

At present the retail sale of bitter almonds only has some importance for home-made Christmas shortbreads, where some traditional recipes include bitter almonds for obtaining this particular flavour. In industrial confectionery, however, bitter almonds have not been used for a long time, being substituted by 'bitter almond oil' as this has a more intense flavour. In this way, this flavour is added in a more concentrated and pure way. Since 'natural bitter almond oil' is normally obtained from apricot kernels (always cheaper), for economic reasons, bitter almonds are no longer used to obtain almond oil.

Trade in bitter almonds, not mixed with sweet almonds, only has some importance for the industries preparing raw almond paste, such as raw marzipan with a bitter almond taste. According to German standards, this paste may contain a percentage of bitter almonds not higher than 12.5% of the total amount of almonds. Without any other formality, this product is simply commercially known as 'raw marzipan'.

20.4.2 Appetizers

Almonds are not considered as superfluous appetizers but an indispensable food for healthy and equilibrated nutrition due to their high content of proteins (of very high biological value), vegetal fibre, unsaturated fatty acids, minerals and vitamins (mainly E, A, B1 and B2) (see Chapter 18). Furthermore, food chains and retailers are also aware of the present consumer trend towards consuming more snacks between meals. Snacks are an important reason for the increased demand for almonds in the global market and are driving the search for new products, taking advantage of the great possibilities of almond as a versatile vehicle of many different tastes from sweet to salty, or even to hot and spicy.

As a consequence, almonds may be roasted or fried, adding also a large variability of flavours to be served as appetizers. Roasting for only a few minutes increases almond flavour and adapts the kernel texture to many different

uses and transformation. Many different flavourings may be added, such as salt, garlic, onion, smoke, curry and paprika, etc.

20.4.3 Industrialization

Many different almond products have different uses in the confectionery industry, as well as in the chocolate industry, although rather fewer in the latter. Almond flour is used in turrón and pastries (such as the tarte de Santiago from north-east Spain), marzipan and different kinds of Christmas shortbreads. Slices, sticks and diced almonds (Fig. 20.8) are used as decorative elements in many confectionery and bakery products, such as tea pastries, bonbons, ice creams, cakes and biscuits. The industries producing sugared almonds ('peladillas' or 'dragées') and bonbons only use whole almonds, as

is the case for the artisan industries producing crunchy pastries. For economic reasons almond halves and unblanched almonds are also used in producing sugared almonds, both white and covered with brown caramel ('garrapiñadas', 'Alcalá almonds' or 'rushi'). Almond slices, or their halves, are used in the decoration of some types of biscuits, pastries and macaroons. Diced almonds and almond flour are used in some biscuits and pastries (fairy cakes, etc.) as well as in some types of turrón, such as worker and stone turrón ('turrón de obrador' and 'turrón a la piedra') and different types of marzipan ('cascas de Valencia', 'pan de Cádiz', 'pasteles de Gloria', 'pasteles de yema', etc.). Diced almond is mainly used in the decoration of pastries and ice creams, with an increasing consumption. Almonds are also used, mixed with dry figs, in producing 'fig bread', a confectionery documented in Alicante at least since the year 1492.

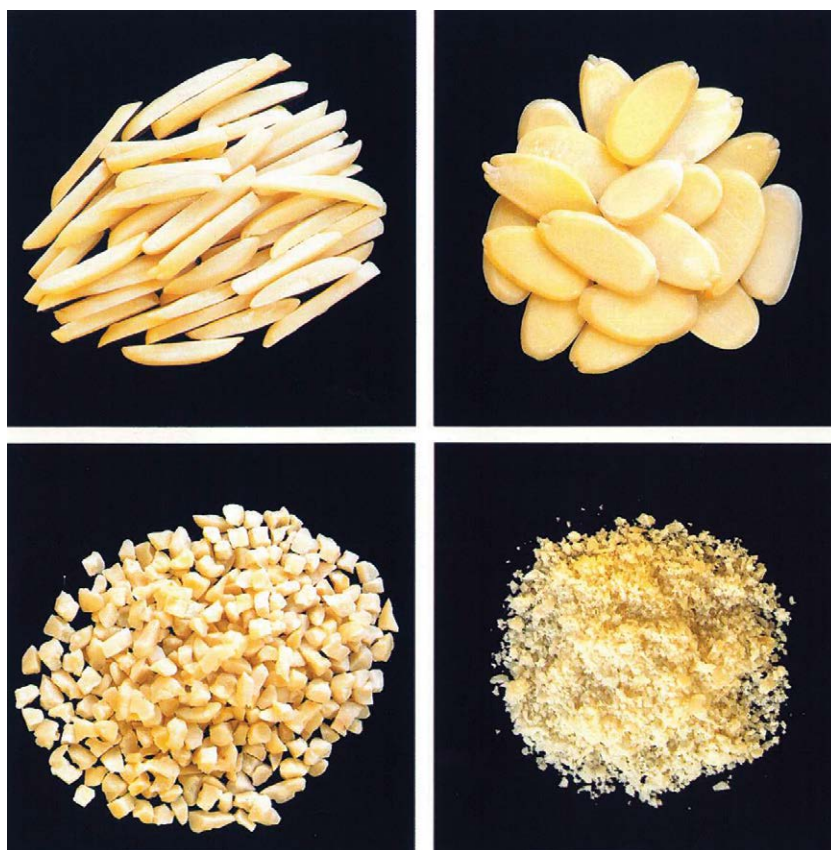


Fig. 20.8. Different almond additives. (With kind permission from TDC Association, <http://www.tdc.es>.)

Almond flour is another solution for the bakery and pastry industries seeking products free from gluten. Almond flour is simply ground raw and blanched almond kernels, but the appearance is different from wheat flour since the granulometry is larger. However, there are different degrees of milling for almond flour – the smallest sizes being used in such products as macaroons, marzipan and ice cream.

The Spanish industry differentiates four main almond types:

1. 'Marcona', the typical Mediterranean almond, referred to as a standard in comparison with the other cultivars due to its undeniable properties of taste, texture and quality. This cultivar is valued by most almond industries, including those of turrón, chocolate, cakes and pastries and Christmas shortbreads, because of its nutritional quality and high fat content. In addition, roasted as well as blanched fried 'Marcona' kernels constitute a much-appreciated snack.
2. 'Desmayo Largueta' (or simply 'Largueta' or 'Desmayo'), characterized by its long shape and peculiar and pleasant taste, occupying a very relevant place among all nuts because it is highly valued in the elaboration of high-quality bonbons, sugared almonds, chocolates and confectioneries. Its excellent taste, as well as its characteristic easy seed coat removal once roasted, make this cultivar an appetizer of very high quality.
3. 'Common almonds' ('comunas'), the denomination for a great number of cultivars not individually recognized but characterized by sweet taste and good-quality traits, making them the raw material for many almond industries (turrón, pastries, marzipan, ice cream and appetizers).
4. 'California almonds', including all types from California, such as 'Nonpareil', 'Carmel', 'Monterey', 'Sonora' and 'Butte'. They are appreciated because of their uniformity, a valued trait for many almond industries (pastries, chocolate, ice cream, etc.).

20.4.4 Turrón

The French term 'nougat' is applied to nuts or fruit pieces in a sugar paste. Therefore, it is not strictly a synonym of 'turrón'. Usually the paste is obtained by cooking sugar and honey, egg white or albumin, to which roasted, blanched almonds are later added.

The definition of soft turrón or 'turrón de Jijona' is due to a geographical name, a city close to Alicante in south-east Spain. This has a long tradition of more than five centuries of creating different kinds of turrón that are well known and greatly appreciated. According to accounts of historical events mentioning turrón since then, the first turrón known was of the 'tough' type, made with whole unblanched almonds (later with whole blanched almonds) and called 'turrón de Alicante' since the end of the 16th century. Later, and with the improvement in working tools and the contribution of fire to the process, it was discovered that the simultaneous milling and cooking of the basic turrón paste produced a more refined and creamy product, 'soft' turrón, finally named 'turrón de Jijona'. Thus, the appellation 'Jijona' started to be used in the 17th century to designate the soft turrón produced in Jijona, distinguished from the turrón and nougat of other regions because of its white-brown colour and fine texture.

In one of the first stages of preparation of the raw materials, almond roasting (Fig. 20.9),

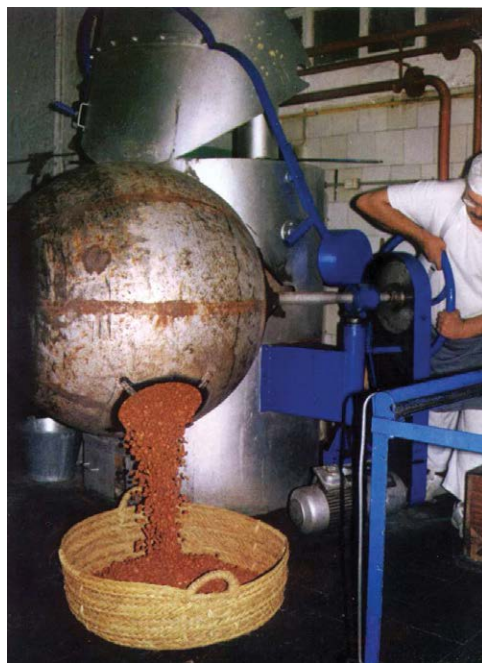


Fig. 20.9. Almond roasting for turrón production. (With kind permission from RCPGIJTA, <http://www.jijona.com>.)

several elements must be taken into account since they may cause a certain variability in the organoleptic characteristics of the product, such as the almond cultivar, the kernel moisture content, and the temperature and length of roasting. Some properties may be particularly affected, such as the colour and the flavour. In the next step, the mixture of honey, water, egg white and sugar with almonds begins to define the peculiarity of the turrón, either of Jijona or of Alicante (Fig. 20.10). The parameters of temperature, time of reaction and quality of the raw material will mainly affect the colour, taste, flavour and texture of the final turrón. The following step, milling, will essentially affect the texture of the final product. Finally, there is the 'boixet' stage, on which will depend not only the output, but also – to a large degree – the organoleptic quality of the turrón, mainly texture and taste.

The only difference between the 'turrón de Alicante' and the 'tortas' is the format. The first is offered in rectangular bars, whereas the 'tortas' are offered in circular format and maintain the integrity of the whole almonds, since in the production of the rectangular bars the mass of turrón is cut at the appropriate size.

In Spain the commercial classes of turrón are established by the Technical and Sanitary Regulation established by the Ministry of Agriculture

for producing turrón and marzipan, defining four qualities or commercial categories. The only differences are the minimum percentages of almond and honey present in the final product to ensure a product fully answering to market demands (defined in Table 20.2). A new category 'improved supreme' has been defined, with the characteristics defined in Table 20.3.

Some particular cultivars are preferred for each type of turrón. For turrón requiring almond roasting (Jijona and stone turrón), cultivars such as 'Marcona' are preferred due to their quality. Their shape, texture and analytical properties confer to their kernels an exceptional behaviour during roasting and following manipulation along the different phases of turrón elaboration. For turrón with raw kernels and marzipan, cultivars such as 'Ramillete' are preferred because their kernels are sweeter and whiter than others.

The process of creating 'turrón de Alicante' and 'tortas' has changed very little for centuries. First the blanched almonds are roasted in large rotating cylinders heated by a hot fire. Separately, a mixture of sugar and honey – according to the specificities of the product quality – is cooked at a high heat in a rotating blender. By evaporation of the added water, this mixture reaches the desired point of caramelization.



Fig. 20.10. Mechanical cooking of sugar, honey and egg white. (With kind permission from RCPGIJA, <http://www.jijona.com>.)

Once this point is reached, the roasted whole kernels are added and the mixture is turned over for homogenization (Fig. 20.11). This warm mass is placed in aluminium boxes introduced in a cooling tunnel where the mass is solidified. After this cooling the final product is already ‘turrón de Alicante’ and is cold cut in bars for later packaging, or moulded before cooling into circular formats for ‘tortas’.

Table 20.2. Commercial categories of turrón and marzipan according to their almond content (%). (With kind permission from RCPGIJTA, <http://www.jijona.com>.)

Product	Supreme	Extra	Standard	Popular
Soft turrón	64	50	44	30
Tough turrón	60	46	40	34
Marzipan	45	38	25	9
Yolk marzipan	40	33	20	5
Other marzipans	45	38	25	5

The following operations aim at breaking up the structure of the tough turrón to obtain the specific characteristics of the soft turrón (‘turrón de Jijona’). Consequently, instead of moulding or cutting the mass of rough turrón as just described, the mass is extended in a sheet of approximately 1 cm thickness over cooling tables

Table 20.3. Commercial categories of turrón and marzipan according to their contents of almond and honey or sugar (%). (With kind permission from RCPGIJTA, <http://www.jijona.com>.)

Product	Improved supreme	Supreme	Extra
Turrón of Jijona	69–17	64–10	50–10
Turrón of Alicante and Guirlache	65–17	60–10	46–10
Stone turrón ^a	52	45	38
Yolk turrón ^a	52	40	33
Snow turrón ^a	52	45	38
Walnut turrón ^a	52	40	33

^aOnly sugar is used in these products, not honey



Fig. 20.11. Mass of tough turrón. (With kind permission from RCPGIJTA, <http://www.jijona.com>.)

or surfaces. Once cool, these sheets are milled in granite or stone mills (Fig. 20.12) and later in refining and blender machines, thus obtaining a mass with a very similar aspect to the 'turrón de

Jijona', but with a much larger granulometry (Fig. 20.13). This mass is further processed in refining machines until the desired texture is obtained (nearly fluid).



Fig. 20.12. Turrón mill. (With kind permission from RCPGIJTA, <http://www.jijona.com>.)



Fig. 20.13. Mixture of syrup and almond paste. (With kind permission from RCPGIJTA, <http://www.jijona.com>.)

The next step takes place in the homogenizers, which maintain the product in as near a homogeneous state as possible and, in addition, control the flow to the following step. During the milling phase, the oil present in the almond kernels is released, giving rise to the formation of a complex emulsion of water and oil, in which drops of liquid syrup, proteins and cellular fragments of almond kernels appear to be dispersed in an oily continuous phase. This resultant mass is introduced into a special tool developed in Jijona and called a 'boixet' (Fig. 20.14).

The mass of milled turrón is introduced into cauldrons of 50–60 kg capacity, the 'boixets', to undergo a second moderate cooking at low, adjustable heat. After 2.5 h of cooking, thanks to the movement of a large pestle (the 'boix'), the almond oil is slowly released and mixed with the sugars until reaching an adequate compactness and texture. This point is known as that of 'phase

inversion or curding' of the mass. It is attained when the mass reaches a critical temperature as well as structural changes generated during shaking which modify the relation constant effort/speed gradient. It is known that this type of process is hastened by increasing shaking. However, although the shaking type is not fundamental for obtaining a final product of adequate quality, the combination of such elements as composition, particle size, process temperature and shaking speed has a decisive influence on the duration of the process. Therefore, the technical control of the whole process is very difficult since many highly correlated variables are involved in it, and a small variation in one of them may convey divergent behaviours in the final product. This fact stresses the importance of an expert surveillance of the whole process, provided by the experience of some renowned workers who have reached the level of turrón masters ('maestros turroneros').

It is important to stress that at the inversion point the syrup drops have a low viscosity, and it is important not to surpass a given temperature, because a vitreous sugar matrix does not facilitate the emulsion. This vitreous matrix would result in a brittle structure, which will not help in maintaining the internal structure and in facilitating the oil exudation. Consequently, a gummy continuous phase is required, helping in the homogeneous distribution of all the elements in the mass. The joint observation of temperature and moisture present in the mass allows a higher or lower viscosity of the continuous phase and, therefore, a larger or lower stability of the emulsion structure. If the mass is heated too much, then an excess of moisture is evaporated and the continuous phase does not reach the right gummy structure, the sugar matrix is vitreous and the emulsion of the disperse phase/organic phase/oil is not facilitated.

If the kind of turrón is granulated, then a second amount of roasted and ground almonds (almond grain) is added to the resultant mass, ensuring that the new mixture is fully homogeneous to reach the final point of 'turrón completion'.

The product leaving the 'boixet' is placed in moulds with perforated bases. These moulds are placed on shelves where they cool down for 1–2 days. During this period the mass reaches the appropriate consistency and the excess almond



Fig. 20.14. 'Boixets'. (With kind permission from RCPGIJA, <http://www.jijona.com>.)

oil pours off through the holes. The larger the almond percentage present in the elaborated product, the longer the time for resting and oil exudation in the exudation cars, thus this parameter is related to the almond cultivar selected and to the final elaboration of the turrón. After this period of oil exudation, the mass of Jijona turrón has reached the adequate consistency to proceed to the final cutting of bars of different sizes for commercial distribution (Fig. 20.15).

The soft turrón is really an oil–water emulsion, similar to the composition of any cosmetic product, since the main component – almond kernels – is also an emulsion of the same kind, where the dispersed phase is the content of fat or oil. In this emulsion the oil drops are dispersed inside the continuous phase, such a net formed mostly by sugars, fibre, proteins and water.

At the beginning of turrón manufacture, the process of milling and refining aims at breaking the structure of both the almond kernels and the caramel (sugar and honey) of the previous step to obtain a turrón cream of a specified granulometry. A phase change is then taking place due to the composition of the raw materials and to their milling, giving rise to a water–oil emulsion where the dispersed phase is made up of the drops of syrup, proteins and almond cellular fragments (fibre and mineral ashes), over a continuous phase, that of almond oil. The interaction between the polar parts of the different amino acids making the proteins, the sugars and the fatty acids, with the non-polar parts of the different almond components, gives rise to inter-molecular links between the different components which stabilize the structure of the achieved emulsion, thus decreasing its superficial tension.



Fig. 20.15. Cut soft turrón. (With kind permission from RCPGIJTA, <http://www.jijona.com>.)

In addition to these two basic types of turrón, many other different kinds are produced depending on different additions: dry fruit, yolk, chocolate, etc. (Fig. 20.16).

20.4.5 Marzipan

Marzipan is the fine and compact mass resulting from the mixture of blanched and ground raw almonds with sugar of any kind, and is used for a diversity of sweets. Since sugar was not widely known in Europe until the 16th century, mostly as a substitute for honey, real marzipan (as known at present) could not be made before that date. However, there are early examples of similar products, such as an almond and honey product made by Jews in the 5th century BC for the Passover celebration. Some sources place the origin of marzipan in Venice, where 'marcipane' (bread of St Mark) was a typical sweet for the celebration of St Mark, the patron saint of Venice. Other sources place the origin of marzipan in the German city of Lübeck, or in the Spanish city of Toledo where today an important marzipan industry is in operation.

The first marzipan recipe dates back to 1525. The first instruction for marzipan fabrication was put in force in Toledo in 1613, ruling that marzipan had to be made with almonds from Valencia and white sugar, and nothing else. The basic guidelines for its manufacture have not changed, but some different forms have been introduced for commercial presentation.

The ground almonds and the sugar (preferably also ground) are mixed with a small quantity of water, and are worked for a long time until a homogeneous mass is obtained. Cinnamon and lemon peel may be added. Once the mass has settled for several hours, different figures are made, placed on an oven plate, painted with whisked egg white and baked for about 2 min at 200°C. More recently, marzipan has been used to make eel-like figures, with a filling of fruit, egg cream or pumpkin ('angel hair'¹), of circular form and decorated with crystallized fruit, whisked egg white and icing sugar.

20.4.6 Cakes and pastries

Almonds – mainly as almond flour – are used in producing many biscuits, cakes and pastries,



Fig. 20.16. Different types of turrón and almond confectioneries. (With kind permission from RCPGIJTA, <http://www.ijjona.com>.)

including a large variety of fairy cakes, mainly at Christmas time. The objective of this chapter is not to give a list of recipes for these cakes, but a special biscuit in some Mediterranean regions is almond cake made of ground almonds, sugar, egg yolk and whisked egg white, cinnamon and lemon peel, baked at 170°C for about 35 min.

20.4.7 Ice cream

In addition to the decoration of different kinds of ice creams with diced and sliced almond, almond-based ice creams are also made. A typical dessert in some Mediterranean regions is more an almond sorbet than an ice cream. It is a milk-based product, obtained after boiling milk with sugar, cinnamon and lemon peel. After cooling, it is mixed with ground almonds and placed in a deep freezer.

20.4.8 Almond milk

Almond milk is one of the richer vegetal sources of calcium. Thus, almond milk is considered a substitute for cow's milk whenever this is not tolerated by the consumer, as in the case of lactose intolerance or of difficult protein digestion. Due to its high fibre content it is also used as a laxative and anti-inflammatory in the digestive and urinary systems. It has a higher content of vitamin E than many nuts, thus playing an important antioxidant role. Almond milk is also a refreshing drink and there has been increased consumption recently. From almond milk other products may be derived, such as concentrated milk, syrups and sweet milk ('horchata').

Almond milk is lighter and more easily digested than cow's milk (Table 20.4). It can be consumed in a similar way to cow's milk: with cereals, in cooked foods, in soups or even in sauces. The intake of two glasses of almond milk contributes approximately 35% of the daily recommended calcium intake, in addition to other minerals such as potassium and phosphorus. It is very useful in the prevention of osteoporosis and control of cholesterol because of the presence of a high percentage of unsaturated essential fatty acids. It is also recommended for people with coeliac disease since it does not

Table 20.4. Characteristics of almond milk in comparison to other milks. (With kind permission from RCPGIJA, <http://www.jijona.com>.)

Table of values	Almond milk	Cow's milk	Milk without lactose
Calories (Kcal) ^a	90	100	110
Calcium ^b	45%	30%	30%
Vitamin D ^b	25%	25%	25%
Vitamin E ^b	50%	0	0
Saturated fats ^a	0.3 g	1.5 g	1.5 g
Cholesterol ^a	0 mg	10 mg	15 mg
Sugar ^a	7 g	13 g	12 mg

^aFor 1 l milk

^bPercentage of daily requirements

contain gluten, lactose or casein. It is also low in sodium and is also a source of high-quality proteins, balanced in relation to the composition of essential amino acids.

The beneficial role of almond milk stands out in relation to:

- cardiovascular diseases (excess cholesterol);
- people intolerant to cow's milk or lactose;
- people with coeliac disease having problems with milk products; and
- people generally looking for substitutes for milky products.

In hospitals it is mainly useful in post-operational processes, where patients require nutritious, easily digested food. It is also ideal to avoid hypo-nutrition in sick patients since it is rich in fibre and re-mineralizing. As it is low in sodium and high in potassium, almond milk is appropriate in cases of potassium loss such as diarrhoea, vomiting and diuretic treatments involving potassium elimination; and in cases where sodium may be a problem, such as arterial hypertension and illness producing an increase in extracellular fluid, such as cardiomyopathy, kidney diseases and hepatic cirrhosis. Almond milk consumption also results in a great improvement in cases of food allergies. In digestive problems, it is of great help in gastrointestinal dyspepsia, gastritis and regulation of intestinal peristalsis.

Due to its calcium/phosphorus ratio it is an ideal food for several groups of people, such in the stages of growth and puberty, where both minerals play an essential role in the formation and remodelling of bones. In pregnant and nursing mothers almond milk may partially cover

their higher mineral requirements; and, in people of advanced age, a calcium-rich diet represents an important preventive measure against the development of osteoporosis. Almond milk also has an important percentage of soluble and insoluble fibre, thus protecting the intestinal walls, mainly at the colon, and regulating the absorption speed of sugars and cholesterol. Almond milk has a very pleasant taste and is an excellent cardio-protector due to its high vitamin E content. It is an excellent option for those children with allergic problems and intolerance to lactose.

The preparation of almond milk starts with kernel washing. After that, kernels are disinfected and later ground in a mill (generally of the crosshead type), incorporating at the same time a uniform water volume, in an approximate ratio of 3 l water/kg almonds. This facilitates the process, avoiding compression of the mass and its retention in the mill. Later the combination of milled almond and water is left to macerate for a short time, depending on the duration of the previous soaking. If soaking is longer than 8 h, maceration may be avoided, proceeding directly to the next operation: shaking. The shaker consists of a cylindrical container with central stirrers at low agitation in order to suitably mix the mass coming from the mill. At the same time a water spray sweeps away all the almond and fat

components. The crushed mass obtained is introduced into a continuous or individual press and is forced through a sieve in order to separate the liquid from the solid residue (Fig. 20.17). In the continuous press the pulp is sieved by a mesh of stainless steel to produce the liquid extract. The individual press consists of a cylindrical device with tilting shovels inside sweeping the internal perforated surface leaving at the same time the solid residue on the cylinder wall whereas the water is poured outside like a shower. After pressing, the first extract is obtained and it is sieved again.

For sweet milk ('horchata') production, the milk obtained as just described is mixed with 100–150 g of sugar/l, adding cinnamon and lemon peel to taste. After shaking, this solution is sieved through a very fine mesh to eliminate any gross solid impurity. After that, the soft milk is cooled to 0°C or even a little less. The production of sweet milk must be done daily since it only maintains all its properties for 36 h due to possible microbial contamination.

The present regulations for sweet milk production require that all the machinery and tools must be of stainless steel to attain the highest hygienic standards and easy cleaning. Therefore, all the old wood and tin tools have disappeared from use.



Fig. 20.17. Sieve press for 'horchata' preparation. (With kind permission from TDC Association, <http://www.tdc.es>.)

20.4.9 Gastronomy

Almond is an ingredient widely used in Mediterranean cuisine, being found in many different dishes and sauces such as almond soup, chicken fricassee, white garlic soup and Catalan romesco sauce. Almonds may be a garnish for fish like trout, for meat like chicken or pigeon, for vegetables like cardoon and for many different preparations like couscous, stuffing or flavoured butters.

Ground almond is ideal for coating meat and fish, making a covering free from gluten. Although most recipes with ground almond are for sweet products, it is also an interesting component of salty recipes, such as for making denser gravies, creams or pâtés. Besides contributing an exquisite taste, density and texture, almonds also enrich the dish with nutrients such as proteins, minerals (calcium, potassium, iron, manganese), vitamins (E, B1, B2), fibre and mono- and polyunsaturated fatty acids.

20.4.10 Cosmetics

Nut oils, mainly almond oils, are classic ingredients in dermo-aesthetics and continue to be used as emollients, nutrients and protectors of hair and every kind of skin. The presence of natural components in cosmetics is also an advertising benefit attracting great acceptance among consumers. As a consequence, the providers of raw materials for the cosmetics industry are continuously developing new products, in many cases taking foods as the starting point for their developments.

Almond oil is produced through pressing and extraction of ripe almond kernels. The best extraction method for vegetal oils, to maintain all their properties and components undamaged, is through cold pressing at no more than 27°C. This is so even if the oil yield with this method is lower than with other methods such as extraction with organic solvents (e.g. hexane) and later concentration of the obtained extracts.

The more important variables in this extraction process are the amount of kernels and the solvent volume. The most-used solvent is n-hexane due to its low cost, moderate toxicity and easy availability. Among its interesting

physical properties low density and superficial tension stand out, as compared to water, which makes the processes of extraction and solution easier. It has also a low vaporization heat value, thus decreasing the amount of energy required to take the solvent to the required temperature.

For oil extraction at room temperature, the almonds are finely ground in a mechanical mill to increase the contact surface between solid and solvent. The extraction solvent is added and the resultant mass is shaken by a magnetic stirrer for 15 min. The mass is sieved and the resultant solid is rinsed with hexane. The liquid phase is transferred to a balloon glass and the solvent is distilled by a rota-evaporator. Almond oil density is of 0.92 g/cm³ at 20°C.

For the extraction of the oil by reflux the hexane is introduced with the ground almonds in a kettle. A ball refrigerant is connected to the reaction kettle and the system is placed in a shaking bain-marie until reaching the reflux temperature of the solvent (69°C). These conditions are maintained for 15 min. Shaking and heating are then suspended, the mixture is left to cool down and later is vacuum filtered and washed with hexane. The extract obtained is transferred to a balloon and distilled by means of a rota-evaporator. If a higher oil yield is desired, the extraction process must be repeated under the same conditions. Almond oil thus obtained may be kept for 12 months at room temperature.

The cosmetic industry also uses almond oil obtained as a byproduct of turrón production, resulting from the exudation of the Jijona turrón. Specifically, sweet almond oil is the exudation from the Jijona turrón mass obtained in the 'boixet' and subjected to oil loss by gravitational exudation to give consistency to the turrón mass and allow easier cutting for bar moulding.

Almond oil is one of the most neutral oils without known contraindications, and has a long-standing reputation. It is rich in unsaturated fatty acids, with a high percentage of essential fatty acids such as linoleic and linolenic acids. It also contains water, proteins, a high percentage of fibre, vitamins B1, B5, B6, D and E, thiamine, niacin and minerals such as zinc, iron, potassium, magnesium, sulphur, copper and calcium. This composition makes almond oil a natural medicine with a regenerating and moistening effect. Consequently, it has many applications in the production of lotions and creams

for beauty treatments because of its moisturizing, softening, anti-inflammatory and anti-aging properties, preventing premature aging.

For centuries almond oil has been highly regarded because of its good nutritive and medicinal properties, mainly for skin treatments. As a food complement, it is excellent because of its high protein content, being a good substitute for animal proteins in vegetarian diets. As an anti-inflammatory it may be added to other vegetal oils to clear tissue mucus. As a laxative, it is of help in diseases of the digestive system.

Almond oil is suitable as a base oil and may be mixed with other vegetal oils before direct application to the skin, improving skin appearance and general condition, leaving it moistened, well-nourished and smooth; it also contributes to elasticity, mainly in cases of dryness, dehydration or peeling. It is recommended in all skin types, but especially in sensitive, fragile and dry skins, because of its nourishing and curative properties; thus it is very advisable for old people and for those with skin problems. It lubricates and protects eroded or scaly skins and is very suitable for treating problems of irritation and allergy.

Almond oil is much used in massage because of its lightness and viscosity level, which facilitate hand sliding. It has emollient and anti-inflammatory properties, and can soften the skin without obstructing the pores. It is specifically used in aromatherapy lotions. Pure almond oil, due to its cicatrizing effects, is used in removing the 'cradle cap' (dermatitis, *crusta lactea*) that forms on babies' heads, since it is inert and non-toxic. It may be applied on dry skin before bathing; on moist skin; after a shower; or during a moistening bath. It is a natural make-up remover.

In addition to reducing inflammation and relieving skin irritations, almond oil moistens and softens every type of skin. It is soothing, very useful in decreasing the effects of irritation and treating dry skin eczemas, psoriasis and other dermal sicknesses, due to its strong antioxidant effect. It is also recommended for treating wounds and burns due to its nutritious and softening effect. It may be applied on stretch marks and other problematic places, as well as for relieving rheumatic pains due to its anti-inflammatory action. It prevents wrinkles and is very useful on sensitive skins, such

as in babies and children. Almond oil has tonic effects and contributes brightness and softness to damaged, burnt, scaly or brittle hair, and is also an eyelash strengthener.

Natural creams are made from natural ingredients and are mainly conceived for caring for and protecting the skin. The formulation of the cosmetic product consists of an emulsion of oil in water following the guidelines of the Regulatory Commission. Preparation of the cosmetic product is defined by four phases:

- water;
- oil;
- gel formation; and
- incorporation of the active principle (almond oil).

Almond oil is a natural and healthy alternative to other oils such as animal oils or those derived from crude oil.

Different kinds of creams may be used to soften and protect dry lips, hydrate the hands and rejuvenate the skin. The latter is achieved with the peptidic extracts from almond, formulated to firm and restructure mature skins, to protect skins and for the production of cosmetics to combat the effects of pollution. They may also be formulated into restorative hair treatments, hair conditioning treatments, or soothing and nutritious products for the scalp.

Bitter almond has recently been incorporated into the cosmetic industry thanks to the properties of one of its components, mandelic acid (α -phenil-hydroxyacetic acid), obtained after diluting an extract of bitter almond in hydrochloric acid. The name 'mandelic' acid comes from the German name for almond, 'Mandel'. This acid has many applications in the industry and is of special interest in the treatment of skin defects such as acne and hyperpigmentation, as well as in peeling. It also has blanching and anti-inflammatory properties.

20.4.11 Pharmacy

Almonds have been used in natural medicine for generations. Almond milk, for example, has been used for coughs. Pharmaceutical preparations are generally complex formulations which aim to give each active substance the

best medical presentation to attain the desired therapeutic effect.

Both the ingestion of whole kernels and of almond oil are used in the prevention of cardiovascular diseases and inflammation of the digestive and urinary systems. Almond oil also has anti-fever, anti-cough and breast-softening effects, and can be used as an antioxidant and

laxative. Its topical application for skin diseases has already been mentioned. The ingestion of almond oil as a cholesterol reducer can probably be traced back to the 15th century, this effect being based on the high percentages of omega-9, omega-6 and omega-3 fats in its composition. Almond oils may also be used as vehicles for oily injections.

Note

¹ 'Angel hair' refers to a kind of jam made from pumpkin. In Spain the species *Cucurbita maxima* is mostly used.

Index

- Achaemenian Empire, map 49
Adesoto 101 217
AEOFRUSE 452
agricultural practices 335
Agrobacterium 212–214, 217
Agrobacterium tumefaciens 94, 219
agronomical aspects, harvesting 424–425
allele sequencing 195–196
almond additives 469
Almond Board of Australia (ABA) 91, 92
almond leaf scorch (ALS) 333–335
almond milk 477–478
almond oil 479–481
Alnem series 62
Alternaria leaf spot 345–346
amandola 53
Amplified Fragment Length Polymorphism (AFLP) markers 150
Amyelois transitella 121
amygdalai 50
Amygdali amari 56
Amygdalus communis L. 1, 48
anilinopyrimidine (AP) 339
anthracnose 340–341
Aphelocoma californica 47
Apicius 54
apothermum 54
appetizers 468–469
apple chlorotic leaf spot virus (ACLSV) 325
apple mosaic virus (ApMV) 322
apricot (*Armeniaca*) 1
Ardècheoise (cultivars) 59, 61, 81
Argentina 94–103
 acreage 96
 cultivars in use 100–101
 development of industry 97–100
 history 94–95
 imports and exports 103
 production 95, 97
 provinces and planted area 97
 statistics 101, 103
Armillaria mellea 352, 354–355
Armillaria root rot 354–355
arthropod pests and their management
 branch, limb or trunk feeders 385–387
 early-season nut feeders 376–377
 foliar feeders 383–385
 post-harvest nut feeders 383
 pre-harvest nut feeders 377–383
 root-feeding insects 387–388
 season-long management approach 388–389
 soils, floor management and their effects on
 arthropod pests 388
Asia, modern cultivation 63–64
Asiatic stage, dissemination 50–53
Asociación Española de OPAs de Frutos Secos y
 Algarrobas, Spanish Association of Growers
 Associations of Nuts and Carobs, *see*
 AEOFRUSE
Aspergillus flavus 130
Aspergillus niger 348
association mapping (AM) 151, 153, 156–157
Atocha (cultivars) 79, 213–214
atomic absorption spectrometry (AAS) method 437
Australian Almond Growers Association 92
Australian almond story 87–94
 almond-growing regions 89
 breeding programme 93
 cultivation 91–92
 future prospects 94
 history 87
 industry support and research 92–94

- Australian almond story (*continued*)
 orchard in Sunraysia region 89
 pests and diseases in 94
 production 88–89, 91
 autogamous cultivars, breeding 199–200
 autumn cutting 232
 Avalon (cultivars) 113, 117
 Ayurvedic medicine 52
- bacterial diseases 326–335
 ALS 333–335
 bacterial canker and bacterial blast of
 stone fruits 329–331
 bacterial spot 331–333
 crown gall disease 326–328
 hyperplastic bacterial canker 328–329
 badam 44, 51
 band canker 350–351
 Bartre (cultivars) 12, 14, 120, 128
 bearing habit 124–126
 Belona (cultivars) 113, 182, 200, 268,
 423, 452, 461
 biological control 327
 bitter almonds 48, 54, 56, 57, 468
 blanching 464–465,
see also industrial preparation
 Blanquerna (cultivars) 113, 195, 200
 Blue Diamond Growers Cooperative
 (BDG) 450
 ‘boixet’ 474
 bomboniere 56
 boron toxicity 318
Botryosphaeria dothidea 350–351
Botrytis cinerea 94, 267
 branch, limb or trunk feeders
 bark beetles 386
 scales and mealybugs 385–386
 tree borers 386–387
 Brandis Jordan (cultivars) 87
 breeding 131–137
 autogamous cultivars 199–200
 floral biology and selection cycles 133–134
 for flowering time 178–183
 genetic markers 149–150
 methodology 134–137, 178
 and molecular markers 151–159
 objectives 133
 prospects 138
 breeding programmes 58–59
 Australian 93
 flowering time 172
 French 200
 in Italy 182
 objectives and achievements 112–114, 117
 self-compatibility 201
 Spain 78–79, 182
- Britz (cultivars) 61
 brown rot blossom blight 337, 339
Bryobia rubrioculus 94
 bud and flower 12–14
 development 24
 evolution of 24
 phenological stages 26
 pistils in single flower 16
 shapes and colours 15
 twin flowers 16
 bud dormancy 256
 bud drop 265
 budding methods 238
 bulk segregant analysis (BSA) 176
- Cacopsylla pruni* 325
 cakes and pastries 476–477
 California
 annual almond crop size in 457
 cultivars 84
 distribution in 450
 growing regions 83
 hectares and farm value 454
 modern cultivation 63
 production 82
 rootstocks 84
 California Almond Growers Exchange 461
 Cambra (cultivars) 113, 195, 200
 canopy morphology 11–12
Capnodis tenebrionis 80, 213
 carbohydrates and fibre
 dietary fibre 440
 polysaccharides 439–440
 Carmel (cultivars) 113, 346, 356, 364, 451, 470
 carob moth 379–380
 management 380
 Mediterranean region 379
Ceratocystis 250
Ceratocystis canker 349–350
Ceratocystis fimbriata 349–350
 characteristics, almonds 460
 characteristics, rootstocks 213–223
 clonal rootstocks 215, 217–219, 222–223
 seedlings 213–215
 Chellaston (cultivars) 92, 213, 269
 chemical composition
 carbohydrates and fibre 439–440
 consumption 428
 hull and shell biochemical constituents 440–441
 lipid content and fatty acid and profile 429–433
 minerals 437–438
 phenols 439
 phytosterol content 435–437
 protein and amino acids 438–439
 variability 429
 vitamins 433–435

- cherry (*Cerasus*) 1
- Chile 103–108
- almond-producing areas 104, 105
 - cultivars in use 104, 106
 - development of industry 107–108
 - evolution of area planted 104
 - history 103–104
 - orchard in Rancagua 106
 - statistics 108
- chilling requirements 171, 172, 174, 177, 179–180, 257–259
- chip budding 238
- chlorine and sodium toxicity 318–319
- Chondrostereum purpureum* 352
- citrus variegated chlorosis (CVC) 334
- Cladosporium carpophilum* 343–344
- climate change, flowering 183–184
- clonal propagation 111
- clonal rootstocks 210, 215, 217–219, 222–223
- to biotic and abiotic stresses 220–221
 - clones of peach x almond hybrids 218–221
 - hybrids between species of *Prunus* 223
 - plum 215–217
 - Prunus* hybrids 222–223
 - Prunus* interspecific hybrids 217–218
 - water availability and edaphic factors 215
- cold acclimation 256
- cold hardiness 255–261
- Colletotrichum acutatum* 94, 340–341
- Comunas (type of almond) 77, 470
- Conformitas Agrarias Communitatis* 235
- consumption 428
- Controller[®] 6 rootstocks 223
- Cooperativas Agroalimentarias (Agro-food Cooperatives) 452
- corky spot symptoms 364
- cosmetics 479–480
- almond oil 479–480
 - bitter almonds 480
 - cosmetic product 480
- cracking 462–464, *see also* industrial preparation
- creta argentina* 54
- Cristomorto (cultivars) 35, 61, 126–127, 191
- critical value (CV) curves 296
- crop coefficient (K_c) 279–280
- cross-compatible cultivars 27, 60
- cross-pollination 63, 70, 188
- crown gall disease 326–328
- cultivars 12
- in Argentina 100–101
 - Australian 87, 88, 90
 - blooming date 102
 - branching habit 13
 - in California 84
 - callus and gumming 123
 - characteristics of almond 58–59
 - in Chile 104, 106
 - classification 116
 - cross-compatible 27, 60
 - early-flowering 170, 182
 - extra-late-flowering 170, 175
 - French 79
 - full-bloom coincidence 27
 - full flowering time 170
 - growing habits of 13
 - homozygous sweet kernel 200
 - Italian 79
 - late-flowering 63, 97, 100, 113, 170, 182
 - origin and characteristics 115
 - 'paper shell' 100
 - Puglian 59
 - qualities 114
 - seedling-producing 214
 - self-incompatible 27
 - Sicilian 59
 - in Spain 78
 - susceptibility 338, 342
 - in Syria 75
- cultivated almonds, flowering time of 168–169
- cultivation
- Australian almond story 91–92
 - Greek nut 48–50
 - historical and cultural references 45–48
 - modern 61–64
 - new world dissemination 60–61
 - old world dissemination 50–60
 - prehistoric evidence 43–44
 - wild badam 44–45
- cutting propagation 228
- cyanogens 176
- demethylation inhibitor (DMI) 339
- denitrification 302
- Densipac hybrid 222
- Dermatophora necatrix* 355–356
- Desmayo Largueta (cultivars) 12, 17, 28, 35, 59, 77, 79, 112, 123, 125, 138, 153, 190, 268, 469
- Diaspidiotus perniciosus* 94
- dicarboximide 339
- diseases 321–366
- bacterial diseases 326–335
 - disorders with unknown aetiology 364–365
 - by fungi and fungi-like organisms 335–357
 - graft-transmissible pathogens 322–326
 - nematodes 357–364
- DNA marker 149
- DNA methylation 198
- DNA polymorphisms 160
- domestication 118
- dormancy 256
- double kernel 123

- double tunnel system 232
 Duro Italiano (cultivar) 190, 192
- early-flowering cultivars 136, 170, 182
 early-season nut feeders
 hemiptera 376–377
 hymenoptera 377
 leaf skeletonizer, *Aglaope infausta* 376
 lepidoptera 376
 early selection methods 179–182
 Eastern Europe, modern cultivation 63–64
Ectomyelois ceratoniae 94
 ectoparasitic nematodes 363–364
 nepovirus diseases 364
 Xiphinema spp. 363–364
 effective pollination period (EPP) 265–266
 Emilito INTA (cultivars) 101
 environmental requirements 254
 cold hardiness and dormancy 255–261
 light 254–255
 rains 267–268
 soil properties limiting production 268–270
 spring frosts and protection 261–265
 temperature 255–267
Eolophus roseicapilla 94
 ethylene-bis-dithiocarbamate (EBDC) 345
 Europe, rootstock development in 223–224
 evapotranspiration (ET) 279–281
 Exinograd (cultivar) 191
 extra-late-flowering cultivars 170, 175
- Felisia (cultivars) 95, 113, 200
 Ferraduel (cultivars) 97, 101, 113
 Ferragnès (cultivar) 12, 97, 101, 113, 262, 267
 Ferralise (cultivars) 113, 193, 258
 Ferrastar (cultivars) 113
 fertilization 29–30, 129
 fertilizer 304–310
 Filippo Ceo (cultivar) 191, 192
 flower, foliage and fruit diseases 336–349
 Alternaria leaf spot 345–346
 anthracnose 340–341
 brown rot blossom blight (*Monilinia* spp.) 336–339
 hull rot 348–349
 jacket rot and green fruit rot 339–340
 leaf blight 349
 leaf curl 347–348
 red leaf blotch 346–347
 rust 344–345
 scab 343–344
 shot hole 341–342
 flower buds or fruits 263–267
 flower density 128
 flower initiation 18–19, 21–25
 luxury stage 21
 requirement for 22
- flowering 25–27
 anthesis 27
 dates 25, 128, 257
 frosts 26
 percentages of open flowers 25
 time 26, 128, 257
 flowering almond, *see Prunus triloba*
 flowering time 128–129, 168
 breeding for 178–183
 cultivars 170
 of cultivated almonds 168–169
 environmental conditions 169
 genetic control 170–178
 impact of climate change 183–184
 limitations in delay 182–183
 seedlings 174
 variability 169–170
 of wild almonds 168
- Flowers 12–14
 colours of 19
 sizes of 19
 foamy canker 364, 365
 fog system 231
 foliage density 126
 foliar feeders 383–385
 aphids 385
 lacebugs, leafhoppers and sharpshooters 385
 mites 383–384
- 4th-generation sequencing (FGS) 161
 fragmentability 123
 Francolí (cultivars) 129
 fruit 14–16
 development 30, 32–35
 endocarp 15–16
 Mediterranean shelling plants 16
 mesocarp 14
 pericarp 14
 shell hardness 15
- Fruit and Nut Center website
 (FNRIC Nitrogen) 313
 fruit development period 266
 fruit lesions 343
 fruiting bud protected by ice 98
 fungal diseases 94
 fungi and fungi-like organisms, diseases 335–357
 fungicides 339
Fusicladium carpophilum 343–344
Fusicoccum amygdali 130
- gametogenesis 25
 gametophytic self-incompatibility (GSI) 117
 Garrigues (cultivars) 79, 129, 158, 214
 gastronomy 479
 Genco (cultivar) 117, 191, 195
 gene mutation 129
 general linear models (GLM) 156

- genetic control of flowering time 170–178
 - correlation 175
 - flowering process 170–172
 - genomics, transcriptomics and metabolomics 175–176, 178
 - heritability and environmental influence 172–173
 - regression 173
- genetic markers 149–150
- genetics 117–130
 - ploidy and genome size 117
 - qualitative characters 117–118
 - quantitative characters 118–130
 - variation and characterization 138, 149
- genetics of self-compatibility 192–199
 - allele identification by PCR 194–195
 - allele sequencing 195–196
 - double expression of *S_j* allele 196–197
 - historical 192–193
 - inbreeding effects 193
 - mapping 194
 - origin 197–198
 - partial 198–199
 - pollen component 196
 - RNase activity 194
- genome-wide selection (GWS) 131
- genomics 175–176, 178
- germinated pollen grains 30
- germplasm 112
- GF-677 hybrids (rootstocks) 209, 218–219
- Graeco-Persian culture 50
- graft compatibility 211–212
- graft-transmissible pathogens 322–326
 - diagnosis, detection and control 325–326
 - ilarviruses 322
 - nepoviruses 322–323
 - phytoplasmas 323, 325
 - trichoviruses 323
- grafted plants, production 236–237
 - grafting 237
 - mother plants in fields 237
 - scion plant material 236–237
- Greek (cultivars) 62
- Greek medicine 52
- Greek nut 43, 53
 - cultivation 48–50
- green fruit rot 339–340
- The Groupe de Recherche et d'Etudes Meditteranneen pour l'Amandier (GREMPA) 63
- growing degree hours (GDH) 171, 257
- growth cycle, annual 23
- Guara (cultivars) 77, 95, 97, 101, 106, 129, 262, 268
- gummosis 329

- hard-shell cultivars 114, 121
- hardwood leafless cuttings 232–235
- harisa* 54

- harvest date 266
- harvesting 99
 - agronomical aspects 424–425
 - floor preparation 411
 - manual harvesting 406
 - mechanical damage 425–426
 - mechanical harvesting 416–421
 - mechanization 406
 - nut collection and harvest efficiency 421–423
 - nut harvest from tree 411–416
 - optimal harvesting time 406–409
 - picking up naturally fallen nuts 411
 - production costs in orchard, hand harvesting 407
 - time for beginning harvest 409
 - traditional Mediterranean harvesting systems 409–410
 - vibration transmission 426
- hazelnuts 95
- heat requirements 171, 172, 177, 257–259
- heirloom almond cultivars 57
- heritability and environmental influence 172–173
- high resolution melting (HRM) analysis 159–161
- Hippocrates 52
- historical and cultural references 45–48
 - bitter almonds 48
 - early-flowering habit 45, 46
 - human gatherers 47
 - medieval Christian artistic convention 47
 - prayer cap or *topi* 45, 47
 - symbolic importance 46
- homozygous sweet kernel cultivars 200
- honey bees (*Apis mellifera*) 28
- hop stunt viroid (HSVd) 325
- horticultural traits 117–118
- hull and shell biochemical constituents 440–441
 - diet for feeding sheep 440
 - fruit mesocarp 440
 - shell strength 441
- hull rot 348–349
- hull-split 379
- humidity 234
- hybridization 2, 48–49, 61, 191, 197, 199
- hyperplastic bacterial canker 328–329

- ice cream 477
- improvement programmes 62, 132
- in vitro* plant production 235–237
- in vitro* propagation techniques 213, 228
- inbreeding effects on self-compatibility 193
- Indian Ayurvedic medical system 52
- Indian meal moth 94
- indole butyric acid (IBA) 230
- industrial preparation
 - blanching 465
 - cracking 462–464
 - reception 461–462

- industrialization 469–470
 - almond products 468–481
 - industrial preparation 461–465
 - quality guidelines for almond reception 465–468
- industry, development
 - Argentina 97–100
 - Australian almond story 92–94
 - Chile 107–108
- Innova Market Insights* 460
- Institut de Recerca i Tecnologia Agroalimentàries (IRTA) 79
- Intergovernmental Panel on Climate Change (IPCC) 183
- International Nut Council (INC) 455
- interspecific hybridization 133–134
- introgression line (IL) 152
- invertebrate pests 94
- Iran, production 74
- iron deprivation 335
- irrigation efficiency (Ea) 279–282
- irrigation system 99, 279–282
- isozyme markers 150

- jacket rot 339–340
- Javier INTA (cultivars) 101
- Jose Dias (cultivar) 190
- 'June drop' 35
- juvenile period 179–182
- juvenility 18

- kernel-meat production 74
- kernels 7–8, 16–17, 43–44
 - aborted and semi-developed 33
 - bitterness 118
 - colours 22
 - domestication 47
 - double 23, 123
 - mutation 47
 - nutrient composition 48
 - pellicle and texture 122–123
 - production 75, 76, 91
 - shape, size and weight 22, 121–122
 - shell and kernel colour 122
 - shell hardness and percentage of 120–121
 - by Spanish growing regions 77
 - twin 123–124
- khasta badam* 44
- koufeta* 55
- Kyrenia II* replica of almond 51

- large-scale sequencing methods 161
- late-flowering cultivars 63, 97, 100, 113, 168–184
- late-flowering progenies 173
- late-flowering progenitors 178
- leaf blight 349
- leaf curl 347–348
- leaf lesions 343
- leafing, time of 180–182
- lime-induced chlorosis 212
- lipid content and fatty acid profile 429–433
 - kernels with low lipid contents 429
 - lipid content 429
 - macronutrients, variability of 430
 - MUFAs 431
 - oil oxidation 431
 - QTL 431
 - and range of variability 432–433
- lithographic reproduction 55
- local cultivars 56
- Lovell rootstock 210
- low-vigour plums (rootstocks) 215, 217
- lower limb dieback 364

- MADS genes 176
- manual harvesting 406
- Marcona (cultivars) 59, 62, 77, 112, 131, 138, 262, 266, 461, 469
- Mardía (cultivar) 79, 97, 101, 200, 267
- Marinada (cultivars) 128–129, 133
- marker-assisted breeding (MAB) 150–151
- marker-assisted introgression (MAI) 150–152
- marker-assisted selection (MAS) 149–151
- market structure, *see also* world almond market, history and growth
 - Almond Board of California, role of 455–456
 - annual almond crop size in California 457
 - California almond hectares and farm value 454
 - 'call pool', key points 458
 - data on foreign production 455
 - distribution in California 450
 - European market trends 451–452
 - factors affecting new plantings 455
 - growers' marketing decisions and risk management 456–458
 - market cycle 456
 - nature of demand 451
 - new products, categories 452
 - 'Nonpareil' vs. other varieties 451
 - price discovery 450–451
 - price elasticity of demand 452
 - processor-branded almond packs 450
 - Spanish external trade 453
 - Spanish market 452
 - supply, demand and market price 456
- Marta (cultivars) 127, 129
- Martinelli L (cultivars) 100
- Marzipan 476
- Mazzetto (cultivar) 191, 192

- mechanical damage
 - knocking by sticks 425
 - limb breakage 425
 - soil compaction 426
- mechanical harvesting 416–421
 - adapted from grape harvesters 419
 - almond harvester 420
 - inertia trunk shakers 417
 - MagTec's 'Colossus' 421
 - nut removal from the tree 416–417
 - Orchard-Rite® Bullet® 418
 - over-the-tree harvester 420
 - self-propelled Monoboom® shaker 418
 - types of harvesters 417–421
- mechanization 406
- medicinal uses 52–54
- Mediterranean region 75, 77–81
 - France 81
 - Greece 81
 - Italy 81
 - local cultivars 75
 - modern cultivation 61–63
 - modern growing regions 75
 - Morocco 80
 - Portugal 81
 - shelling plants 16
 - Spanish production 75, 77–80
 - traditional culture system 75
 - Tunisia 80
- Mediterranean stage, dissemination 53–56, 59–60
- Meloidogyne arenaria* 94, 214
- Meloidogyne incognita* 94, 214
- Meloidogyne javanica* 94, 214
- Menut (cultivars) 12
- Mesocriconema xenoplax* 215, 331, 364
- metabolomics 175–176, 178
- methodology, breeding 134–137
 - crossing and growing seedlings 134–135
 - early selection 135–137
 - marker-assisted selection 137
 - parental choice and cross design 134
 - seedling evaluation and selection 135
- micro-irrigation systems 288
- microarray systems 162
- mgdala* 53
- minerals 437–438
 - AAS method 437
 - chromium 438
 - contents in almond kernels 437
 - potassium 437
- minimal tillage 252
- mist propagation 231
- mixed linear models (MLM) 156
- modern cultivation 61–64
 - California 63
 - Eastern Europe and Asia 63–64
 - Mediterranean 61–63
- molecular breeding 149
 - impact of new technologies 161–162
 - linkage map 154, 155
 - SNP markers and HRM 159–161
- molecular hybridization 325
- molecular markers 137, 151–159
 - AM 153, 156–157
 - MAI 151–152
 - MAS 151
 - QTL mapping 152–153
 - SNP markers and HRM 159–161
 - traits 157–159
- Monilinia laxa* 130, 267
- monounsaturated fatty acids (MUFAs) 431
- Monterey (cultivars) 83, 222, 288, 333, 341, 346, 451, 470
- Montizo (rootstocks) 217
- morphology 10–17
 - bud and flower 12–14
 - canopy 11–12
 - fruit 14–16
 - kernel 16–17
 - root 10–11
 - traditional propagation 10
- mycoplasma-like organisms 325
- naphthalene acetic acid (NAA) 230
- National Institute of Agricultural Technology (INTA) 100
- navel orangeworm (NOW) 289
 - over-wintering in mummy nuts 378
 - sanitation and early nut harvest 379
- near-isogenic line (NIL) 152
- necrotic leaf lesions 332
- Nemaguard (rootstocks) 84, 94, 97, 106, 210, 214–215, 223–224, 295, 319, 328, 358, 360, 364
- Nemared rootstocks 97
- nematodes 357–364
 - ectoparasitic 363–364
 - RKN 357–360
 - RLN 360, 362–363
- nepovirus diseases 322–323, 364
- new world dissemination 60–61, 81–84
- next-generation sequencing (NGS) 160
- Nicotiana glauca* 194
- nitrogen 300, 303–306, 314–315
 - accumulation in almond fruit 310
 - cycle 301
 - deficiency 314–315
 - guidelines 310–312
 - inputs from irrigation water 312–313
 - losses from almond orchard 302–303
 - sources of fertilizer 304–305
 - transformation into nitrate in soils 301–302
 - uptake by plants 303–304

- non-equilibrium pH gradient electrofocusing (NEPHGE) 194
- non-irrigated orchards 253, 254
- Nonpareil (cultivars) 12, 34, 83, 91, 93, 95, 97, 100, 104, 107, 113, 114, 120, 123, 125, 131, 138, 288
- nut collection and harvest efficiency
- harvesters 423
 - inverted umbrella 422
 - shaker efficiency 422–423
 - 'Tenías' harvester 423
 - trunk shaking 421–422
- nutrient composition 48
- nutrient management 292
- nutrient toxicities 318–319
- Occhiorosso (cultivar) 190, 191
- old world dissemination 50–60
- Asiatic stage 50–53
 - Mediterranean stage 53–56, 59–60
- optimal harvesting time 406–409
- hull-split 408
 - hulling 407
 - late-ripening cultivars 409
 - moisture reduction 407
 - nut maturation 408
 - ripening rate evaluation 408
- orchard(s) 188
- characteristics 212
 - planting 240–243
 - practices 80
 - sanitation 339
 - soil management 252–253
 - systems 218
 - training and pruning systems 243–252
- Osmia cornuta* 28
- 'paper shell' cultivars 100
- partial self-compatibility 198–199
- peach 1
- and almond stones 45
 - rootstocks 79, 212
 - seedlings 214–215
 - twig borer 380–381
- peach-almond hybrid rootstock 91
- peach mosaic virus (PcMV) 323
- peach tree short life (PTSL) 212, 364
- Penta (cultivars) 114, 175, 178, 183, 217
- Pestaneta (cultivars) 190
- pests management
- arthropod pests and their management 375–389
 - vertebrate pest management 389–403
- pharmacy 480–481
- 'phase inversion or curding' 474,
see also turrón
- phenols 439
- phenotypic evaluation 178
- phony peach disease (PPD) 334
- photosynthetic active radiation (PAR) 255
- Phylla 46
- Phytophthora* 214, 217
- phytophthora diseases 353–354
- phytosterol content 435–437
- effect of the genotype and environment 436
 - role 435
 - studies on 435–436
- Pierce's disease (PD) 334
- Pink 'Marcona' flowers 19
- pistil bending 18
- plant water stress 286
- planting, orchard
- density 241
 - design 241–242
 - and GPS devices 242
 - post-planting care 242–243
 - previous steps 240
 - in sloping plots 242
- Plodia interpunctella* 94
- plum as almond rootstock 79, 215–217
- genetic and regional origin 216
 - low-vigour plums 215, 217
 - vigorous plums 217
- plum (*Prunus sensu stricto*) 1
- pneuma* 52
- pollen component of self-incompatibility 196
- pollen grains 25
- pollen-style (in)compatibility 188–201
- pollination 27–29
- cross-compatible cultivars 27
 - honey bees 28
 - requirement for 29
 - self-incompatible cultivars 27
 - temperatures for 29
 - tests 190
- pollizos (rootstocks) 215, 217
- polymerase chain reaction (PCR)
- allele identification 194–195
 - PCR-based markers 149, 195
- Polystigma amygdalinum* 346–347
- 'Pomona' peach rootstock 106
- 'poor man's almond' 44
- Poria (cultivars) 62
- post-harvest nut feeders 383
- potassium
- accumulation in almond fruit 311
 - cycle 303
 - deficiency 315
 - guidelines 312
 - sources of fertilizer 305–306
 - uptake by plants 303–304
- Pratylenchus vulnus* 214, 219, 222

- pre-harvest nut feeders
 - ants 382–383
 - carob moth 379–380
 - navel orangeworm 377–379
 - oriental fruit moth 381
 - peach twig borer 380–381
 - sap beetles 382
- production and growing regions
 - in 2001–2010 72–73
 - in 1947–1996 71
 - in Argentina 95, 97
 - Asia and eastern Europe 74–75
 - in Australia 88–91
 - California 82, 83
 - climatic conditions 70
 - costs in orchard, hand harvesting 407
 - Mediterranean region 75, 77–81
 - New World 81–84
 - producing countries 74
 - trends 84–85
- products
 - additives 469
 - almond additives 469
 - almond milk 477–478
 - appetizers 468–469
 - bitter almonds 468
 - cakes and pastries 476–477
 - cosmetics 479–480
 - gastronomy 479
 - ice cream 477
 - industrialization 469–470
 - Marzipan 476
 - pharmacy 480–481
 - turrón 470–475
- propagation techniques 228–239
 - scion 237–239
 - seedling 228–230
 - vegetative 230–237
- protein and amino acids 438–439
- Prune dwarf virus (PDV) 322–324
- pruning intensity 247
- pruning systems 243
 - equipment 250–252
 - first dormant pruning 245–247
 - heading cuts 243, 244
 - for mature trees 249–250
 - mechanical pruning 251–252
 - season pruning 245
 - second dormant pruning 248
 - summer pruning 243
 - thinning cuts 243, 244
 - third dormant pruning 248–249
 - types of pruning cuts 243
- Prunus arabica* 10
- Prunus argentea* 8
- Prunus bucharica* 7–8
- Prunus communis* 169
- Prunus dulcis* 56, 117
- Prunus fenzliana* 4, 6, 7
- Prunus kotschyi* 8
- Prunus kuramica* 7, 9
- Prunus necrotic ring spot virus (PNRSV) 322–324
- Prunus scoparia* 10
- Prunus triloba* 10
- Prunus webbii* 8, 9
- pseudo-self-fertility 198
- Pseudomonas amygdali* 328–329
- qualitative characters and inheritance 117–118
- quality guidelines for almond reception
 - in-shell almonds 465–466
 - sampling 467
 - shelled almonds 466
- quantitative characters 118–130
 - characters to nut 119
 - characters to tree 124
 - cold hardiness 129–130
 - disease and pest resistance 130
 - double kernel 123
 - flower density 128
 - flower type 127–128
 - flowering characteristics 127
 - flowering time 128–129
 - foliage 126
 - fruit set 129
 - heritability 119
 - kernel pellicle and texture 122–123
 - kernel (shape, size and weight) 121–122
 - nut hull 119–120
 - shell and kernel colour 122
 - shell hardness and percentage of kernel 120–121
 - time of maturity 126–127
 - tree growth and bearing habit 124–126
 - twin kernel 123–124
 - vigour 124
- quantitative trait loci (QTL) mapping 152–153, 175, 177, 431
- quinone outside inhibitor (QoI) 339
- radiative frosts 264
- rains
 - deficiency and excess 268
 - untimely rains 267–268
- random amplified polymorphic DNAs (RAPDs) 150, 175–176
- ‘raw marzipan’ 468
- recipes 53, 55
- red leaf blotch 346–347
- regrafting 238–239
- replant syndrome 365
- Replantpac hybrid (rootstocks) 222

- reproductive physiology 17–35
 - blooming 25–27
 - fertilization 29–30
 - flower initiation 18, 21–25
 - fruit development 30, 32–35
 - pollination 27–29
 - vegetative growth 35–37
- Restriction Fragment Length Polymorphism (RFLP) markers 149–150
- Rhizopus stolonifer* 348
- ripening, time of 127
- risk assessment model 342
- RMja* gene 224
- RNAse activity 194
- Rof (cultivars) 128
- root 10–11
- root and crown diseases 353–357
- root-feeding insects
 - Mediterranean flathead root borer 387
 - scarabs 387–388
- root-knot nematode (RKN) 209, 357–362
- root-lesion nematodes (RLN) 360, 362–363
- Rootpac® 20 orchard systems 218
- Rootpac® 40 orchard systems 218
- rootstock development 209–225
 - breeding programmes 223–224
 - characteristics, *see* characteristics, rootstocks
 - in Europe 223–224
 - genotypes 210, 211
 - selection criteria 210–213
 - in the USA 224
- rootstock production by *in vitro* culture 235–236
 - collection of shoots 235
 - disinfection of explants 235
 - elongation phase 236
 - establishment 235–236
 - growth phase 236
 - multiplication phase 236
 - origin of starting material 235
 - plant material in laboratory 235
 - rooting and acclimatization phase 236
- Rosellinia necatrix* 355–356
- Rosellinia* white root rot 355–356
- rust 344–345
- sandy soils 268
- scab 343–344
- scion plant material 236–237
- scion propagation 237–239
 - budding 238
 - grafting 238
 - regrafting 238–239
- Sclerotinia sclerotiorum* 267
- season-long management approach 388–389
 - ecological niches 389
 - orchard dormancy 388
 - post-bloom and during early fruit development 388
- seed dormancy 256
- seed germination 180, 181, 193
- seedling(s) 87, 95, 124, 213–215
 - almond 213–214
 - crossing and growing 134–135
 - evaluation and selection 135
 - flowering time 174
 - peach 214–215
 - rootstocks 210–211
- seedling-producing cultivars 214
- seedling propagation 228–230
 - germination 229
 - stratification 229–230
- Seimatosporium lichenicola* 349
- selection criteria for rootstocks 210–213
 - good sanitary status 213
 - graft compatibility 211–212
 - nursery characteristics 210–211
 - orchard characteristics 212
 - resistance to biotic and abiotic factors 212–213
- self-compatibility 191–192
 - breeding programmes 201
 - genetics, *see* genetics of self-compatibility
- self-incompatibility 117–118, 188–190
 - breeding 117
 - cultivars 117
 - transmission 118
- self-incompatible cultivars 27
- semi-hardwood cuttings 230–232
 - double tunnel system 232
 - mist propagation 231
- semi-soft cultivars 121
- Sequence Characterized Amplified Region (SCARs) 150
- shell, almond
 - apex sting on 22
 - pores, types of 21
 - types of 20
 - ventral suture, types of 21
- shell hardness 120
- shoot-bearing habits 126
- shoots 14
- shot hole 341–343
- Sicilian (cultivars) 59
- silver leaf disease 351–353
- simple sequence repeat markers (SSRs) 150, 176
- Single Nucleotide Polymorphism (SNP) markers 150
- soaking almonds 52
- soft-shell cultivars 114, 121
- soil(s)
 - floor management and their effects on arthropod pests 388
 - pH 294–295
 - properties and almond production 268–270
 - salinity 295

- saturation 280
- sensors 280
- Soleta (cultivars) 113, 200, 262
- sour sap 329–330
- South Africa 108–109
- Southern hemisphere
 - in Argentina 94–103
 - Australian almond story 87–94
 - in Chile 103–108
 - in South Africa 108–109
- Spanish production 75, 77–80
 - almond surface 77
 - cultivars 77, 78
 - EU project 75
 - geographical distribution 78
 - kernel production 75, 76
 - orchard practices 75
- species, classification 3–4
- sporulating twig lesion 344
- sporulation on canker 337
- sprinkler frost protection 98
- stigma-anther positions 17
- Stigmata carpophila* 130
- stone fruits, bacterial canker and bacterial blast 329–331
- stone tools 44
- strategic deficit irrigation (SDI) 286
- strawberry latent ringspot virus (SLRV) 364
- succinate dehydrogenase inhibitor (SDHI) 339
- sugar-coated almonds 56
- sugared almonds 17
- summer pruning 243
- sustained deficit irrigation (SDI) 92
- sweet milk ('horchata') 478
- Sweetheart (cultivars) 121, 131

- T-budding 238
- tao'ze badam* 44
- Tardona (cultivars) 114
- Tarraco (cultivars) 127
- taxonomy 1–2
- temperature inversion 265
- Tetranychus urticae* 94
- Texas (cultivars) 12, 34, 126, 152, 214, 329
- third-generation sequencing (TGS) 161
- Thompson (cultivars) 63, 74, 100–101, 123
- Thyrostroma carpophilum* 341–343
- tillage 252
- time for beginning harvest 409
- Tomato ring spot virus (ToRSV) 322, 323
- traditional Mediterranean harvesting systems 409–410
 - ground picking 409
 - hand knocking and use of mesh 409–410
- training young trees 243–244
- transcription factors (TFs) 176
- transcriptomics 175–176, 178

- Tranzschelia discolor* 344–345
- tree growth and bearing habit 124–126
- tree nutrition 291–292
 - boron 316–317
 - copper 317
 - iron 317–318
 - magnesium 315
 - management strategies 300–304
 - manganese 318
 - monitoring leaf nutrient status 313
 - nitrogen 300, 303–306, 314–315
 - nutrient management 292–293
 - nutrient toxicities 318–319
 - plant tissue analysis 295–299
 - potassium 300, 304–306, 315
 - 4Rs of nutrient management 304–312
 - soil analysis 293–295
 - visual diagnosis of nutrient status 300
 - zinc 315–316
- tree stress 286
- trichoviruses 323
- trunk and scaffold diseases 349–353
 - band canker 350–351
 - Ceratocystis canker 349–350
 - silver leaf disease 351–353
- Tuono (cultivars) 12, 64, 127, 129, 159, 191, 192, 195
- turrón
 - boixet 474
 - commercial categories 472
 - cut soft turrón 475
 - definition 470
 - roasting for turrón production 470
 - turrón mill 473
- turrón de Alicante 470
- twin almond nuts 17
- twin flowers 16
- twin kernel 123–124

- Unani medicine 52
- unproductive period 18–19
- The USA, rootstock development in 224

- Valencias (type of almond) 77
- variability 429
 - of flowering time 169–170
- vegetative covers 252
- vegetative growth 35–37
 - branching pattern 36
 - bud bursting 35–36
 - internal regulation 37
- vegetative propagation 230–237
 - hardwood leafless cuttings 232–235
 - semi-hardwood cuttings 230–232
 - in vitro* plant production 235–237

- vertebrate pest management
 - legal aspects 389–390
 - threatened or endangered species 390
 - trapping and use of pesticides 389
 - vertebrate species of concern 390–403
- vertebrate species of concern 390–394
 - birds 399–403
 - burrowing rodents 390–394
 - deer 396–398
 - hares and rabbits 395–396
 - tree squirrels 394–395
 - wild pigs 398–399
- Verticillium dahliae* 94, 356–357
- Verticillium wilt 356–357
- Vialfas (cultivars) 113, 117, 200
- vibration transmission 426
- vigorous plums 217
- vitamins 433–435
 - effect of drought stress 434
 - source, kernels 435
 - tocopherol homologues 434
 - tocopherol isomer concentration 43, 434
 - vitamin B6 435
- walnuts 95
- water requirements 279–284
 - development of fruit buds, impacts on 286
 - factors and considerations of water management 287–289
 - growth and yield, impacts on 284
 - in-season kernel development, impacts on 284–286
 - irrigation in water-limited environments 286–287
 - vegetative growth, impacts on 284
- White Nonpareil (cultivar) 87
- wild almonds 2, 4, 6–8, 10
 - distribution 5, 44
 - flower variability 5
 - flowering time of 168
 - fragile nature 64
 - hybridizations 2
 - kernels 44, 118
 - leaf variability 5
 - nut variability 5
 - nuts 4
 - trade 43
 - traits 2
- wild badam 44–45
- willow-type branching 12
- Wilsonomyces carpophilus* 94, 341–343
- wood decays 351–353
- wood discolouration 352
- world almond market, history and growth 449–450
 - estimated usage by the USA market 449
 - factors favouring growth in almond usage 449–450
- Xanthomonas arboricola* pv. *pruni* 94, 331–333
- Xiphinema* spp. 363–364
- Xylella fastidiosa* 326, 333–335