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A Global Perspective

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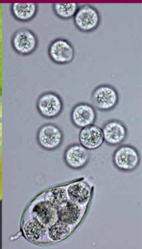

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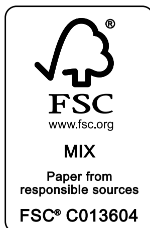

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Phytophthora

A Global Perspective

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Preface

As a researcher interested in the epidemiology of *Phytophthora*, I have to hide my joy at finding a new farm or greenhouse in the midst of a serious epidemic. Here the situation is entirely different and it has been a pleasure and privilege to work with these authors to overview *Phytophthora* globally. The book begins with an account of the early history of *Phytophthora* research and the tumultuous events setting the genus in motion. In keeping with its controversial inception, the chapter on taxonomy and phylogeny makes a compelling case that our current notion of *Phytophthora* as a genus is illusory. This chapter sets the stage for the importance of molecular tools on these enigmatic pathogens, and the following chapters discuss species identification, population-level investigation, interspecific hybrids and the impact of diverse *Phytophthora* species on crops, forests, nurseries, greenhouses and natural areas worldwide.

An important theme is that plants are moving around the globe at an unprecedented rate, and *Phytophthora* often hitches a ride. It is interesting to see how different countries and industries are dealing (or not dealing) with this reality. As the tools for identifying *Phytophthora* species become increasingly refined, many new species are being discovered and there is no doubt the current 120+ formally described species are just a fraction of those occupying various niches worldwide.

For me, the involvement of Olaf Ribeiro is significant because his and Dr Donald C. Erwin's 1996 book *Phytophthora Diseases Worldwide* became available just as I was embarking on my own journey into the world of *Phytophthora*. Their book was (and is) incredibly helpful in making this genus accessible, and, as the literature on *Phytophthora* continues to grow rapidly, I'm hopeful that this book will provide a useful glimpse of the genus worldwide.

Colour photos to accompany the chapters in this book can be found on the editor's webpage (www.Plantdestroyer.com).

Kurt Lamour
2012

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1 A Historical Perspective of *Phytophthora*

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1.1 Introduction

The origin of the genus *Phytophthora* is closely associated with what is now known as the Irish Potato Famine. The famine resulted from severe potato blights in the years 1845–1846 and led to Ireland losing as many as a quarter of its eight million inhabitants to starvation and emigration. A poignant account of the famine is presented by Cormac Ó Gráda (1999). Though it is popularly believed that the potato blight first occurred in Ireland, the first recorded cases of potato epidemics are from the east coast of North America in 1843–1845 (Bourke, 1991). At the time many thought the problem was the result of extensive inbreeding of the potato resulting in diminished vigour. A report by J.E. Teschemacker suggesting the blight may be of fungal origin was quickly dismissed (cited in Bourke, 1991). The historical aspects of the ensuing controversy as to the cause of the blight are fascinating and involve several well-known scientists, each with a different theory as to the origin of the problem. Suffice to note that it cost the reputations of several well-known scientists who espoused a fungal origin for this malady. A detailed account of the controversy, the scientists involved and theories proposed are provided by Bourke (1991) and Nelson (1995).

The devastation of the potato crop in Ireland was originally investigated in 1845 by David Moore, curator of the Royal Dublin Society's Botanic Gardens at Glasnevin (Moore, 1846). He suggested a fungus as the potential culprit and for several years corresponded with the Reverend Berkeley, a noted amateur mycologist, seeking confirmation that a fungus was the cause of the malady. Even though Berkeley agreed with Moore, he was loathe to publish his findings since fungi as plant parasites were heretofore unknown. This was an era when people thought that microorganisms only appeared after a plant started to decline and were never the cause of the decline. Indeed, a commission comprising well-known scientists rejected a fungus as the cause of the problem and attributed the blight to 'atmospheric influences'. Meanwhile potato blight spread across Europe resulting in the death of millions who depended on the potato for sustenance. It should be noted that wheat, meat and dairy produce were available, but the Irish peasants had no money to purchase these commodities and the repeal of the Corn Laws by the British Parliament and the importation of American maize (corn) provided no real help.

The Reverend Miles Berkeley (1846) was the first to formally recognize fungal infection as the cause of the famine. His painstaking observations of the disease process (then known as 'potato murrain') are impressive, especially considering his lack of a good compound microscope. However, his publication describing the cause of the blight as a fungus in the genus *Botrytis* was met with scepticism by several scientists who clung to a non-fungal theory for the origin of the malaise. Berkeley's

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findings may have been dismissed because he was never formally trained as a mycologist. Consumed by describing mushroom species, he eventually amassed an incredible collection of some 10,000 species (Buczaki, 1991).

Berkeley's theory eventually gained credence, and during the period 1845–1876 the potato blight fungus was alternately described as *Botrytis infestans* (Mont. 1845), *Botrytis devastatris* (Lib. 1845), *Botrytis solani* (Hart. Verhandl. 1846), *Botrytis fallax* (Desm. 1846), *Peronospora trifurcata* (Unger. 1847), *Peronospora fintelmannii* (Casp. Rabenh. 1852), *Peronospora infestans* (Caspo. Rabenh. 1854) and *Peronospora devastatrix* (Casp. Ber. Verhandl. Konigl. Preuss. 1855) (Tucker, 1931).

1.2 The Birth of and the Discipline of Plant Pathology

It was not until two decades later that Anton de Bary (1876 cited in Tucker, 1933), a noted German mycologist, formally named the potato blight fungus *Phytophthora infestans*. The new name replaced *Botrytis infestans* as originally proposed by Montagne (1845) and *Peronospora* by Unger (1847) and is derived from the Greek φυτόν (phytón) 'plant' and φθορά (phthorá) 'destruction', which aptly conjugates as 'the plant-destroyer'. In addition, this name change proved a historic event as it initiated the formal science of plant pathology. Once the genus name received wide acceptance scientists soon began describing new species. The first species were *Phytophthora cactorum* in 1870 (Lebert and Cohn, 1870), followed by *Phytophthora nicotianae* (Breda de Haarn, 1896 cited in Tucker, 1933), *Phytophthora phaseoli* (Thaxter, 1889) and *Phytophthora colocasiae* (Raciborski, 1900 cited in Waterhouse, 1970).

Meanwhile, the genus name *Phytophthora* prompted an interesting discourse between mycologists attempting to correctly place the genus. *Phytophthora* was included in the family Peronosporaceae by de Bary. Fischer (1892 cited in Tucker, 1931) separated *Phytophthora* from *Pythium* based on the way swimming zoospores are released from the sporangia. In *Pythium* the zoospores form in a vesicle that bulges out from the opening of the sporangia, whereas in *Phytophthora* the zoospores differentiate in the sporangium. Pethybridge (1913 cited in Tucker, 1933) favoured the retention of species with paragynous antheridia in the Peronosporaceae under the new genus *Nozemia* and those with amphigynous antheridia in a new family that he proposed to name Phytophthoraceae. However, when Lafferty and Pethybridge (1922) observed some amphigynous antheridia in cultures of *P. cactorum* (known to predominately have paragynous antheridia) the genus *Nozemia* was discarded. Soon thereafter, Fitzpatrick (1923) came to the conclusion that *Phytophthora* and *Pythium* should be combined into a single genus in the family Pythiaceae, a division of the Peronosporales. This classification appears to have been accepted by many researchers studying this family at the time. Meanwhile, Leonian (1925), having observed the close similarity of *Pythiacystis citrophthora* to *Phytophthora*, combined them and named the new species *Phytophthora citrophthora*.

Between 1900 and 1925, 12 new *Phytophthora* species were described including: (i) *Phytophthora syringae* (Klebahn, 1909 cited in Tucker, 1931); (ii) *Phytophthora gonapodyides* (Petersen, 1910); (iii) *Phytophthora arecae* (Coleman, 1910); (iv) *Phytophthora parasitica* (Dastur, 1913); (v) *Phytophthora erythroseptica* (Pethybridge, 1913 cited in Tucker, 1933); (vi) *Phytophthora lepironiae* (Sawada, 1919 cited in Ho and Jong, 1992); (vii) *Phytophthora palmivora* (Butler, 1919); (viii) *Phytophthora cryptogea* (Pethybridge and Lafferty, 1919 cited in Tucker, 1933); (ix) *Phytophthora capsici* (Leonian, 1922); (x) *Phytophthora cinnamomi* (Rands, 1922); (xi) *Phytophthora citrophthora* (Smith and Smith, 1925); and (xii) *Phytophthora hibernalis* (Carne, 1925).

1.3 Identification to Species

By 1925 the genus *Phytophthora* was firmly established and the most important features were described by Tucker in a monograph on *Phytophthora* published in 1931. His

study of 150 isolates represented most of the species described up to that point and resulted in several criteria being established as valid features for identifying *Phytophthora s*pecies. These included: (i) temperature relations; (ii) type of growth on culture media; (iii) the antheridial type; and (iv) the character of the sporangia (papillate or non-papillate). In some cases pathogenicity to certain plant species was also used to further differentiate species. Tucker also established the validity of some of the *Phytophthora* species that were previously described under different genera and species such as *Pythiacystis citrophthora* (Smith and Smith, 1906), *Kawakamia colocasiae* (Rac.) Saw. 1911, and *Blepharospora terrestris* (Sherb.) Peyr., 1920.

While the debate on the proper classification of *Phytophthora* continued unabated, Leonian (1925) at West Virginia University examined the physiological aspects of *Phytophthora* species with the goal of trying to identify discrete characteristics useful for identification. His work documents the variability inherent in the genus and between isolates of the same species and resulted in a publication on the identification of *Phytophthora* species in 1934. To avoid what appeared to be a confusing use of terms when describing *Phytophthora* species, Blackwell (1949) published a timely paper presenting precise definitions for describing various features. Meanwhile, Lilly and Barnett continued the physiological studies initiated by Leonian and their collaboration culminated in a book on the physiology of fungi in 1951. This book was a valuable resource to those seeking information on the physiology of *Phytophthora* species as well as fungal physiology in general. It also provided a chapter detailing several exercises for students interested in performing experiments on various aspects of fungal physiology.

This was also a time when several important discoveries were made. Black (1952) published a significant paper on the relationship between pathogenicity of *P. infestans* and dominant genes present in the potato plant. He postulated 16 potential races (0; 1.2; 3.4; 1.2.3; 1.2.3.4; etc.) based on his knowledge of potato lines which carried four single dominant genes for resistance. Thus Race 1.2.3.4 overcomes resistance to any combination of the four dominant genes in the host plant. In a few years all 16 races were recovered in nature. Since then the number of new races has greatly increased as new genes for resistance are added into the potato plant, an eventuality predicted by Toxopeus (1956). This situation was further complicated with the discovery of genes for horizontal resistance. Meanwhile, Galindo and Gallegly (1960) published their discovery of the sexual stage of *P. infestans* in Mexico, a significant finding that enabled the mating of several other species that hitherto were not known to have sexual organs (Galindo and Zentmyer, 1964). The culmination of a large amount of research on homothallism, heterothallism and interspecific hybridization in the genus *Phytophthora* was published by Savage *et al.* (1968); research that provided the impetus for much of the work that followed in subsequent years.

1.4 Variability of *Phytophthora*

The number of *Phytophthora* species described steadily increased, and by 1960 there were 50 new species. Waterhouse (1963) reviewed the species described up to 1960 and helped sort out synonyms or invalidly described species and brought order to the genus by developing a key based on the morphological features of the sporangia and antheridia. This publication spurred research on all aspects of the genus *Phytophthora*. During this period the variation inherent in this genus was described by Erwin *et al.* (1963), Caten (1971) and Erwin (1983), further increasing the difficulty in accurately describing species. At this time a controversy arose as to the basic inheritance in *Phytophthora*. Published research offered evidence of sexual recombination, heterokaryosis, vegetative recombination and cytoplasmic inheritance. The vegetative mycelial stage was interpreted to be haploid, diploid and

even polyploid (Gallegly, 1968; Laviola, 1968; Romero and Erwin, 1969; Shaw and Khaki, 1971; Elliott and MacIntyre, 1973; Brasier and Sansome, 1975; Sansome 1976; Long and Keen, 1977). It is now accepted that *Phytophthora* is unique in being diploid in the vegetative stage unlike other fungi.

Ribeiro (1978) collated much of the available information on taxonomy, genetics, ultrastructure, morphological characteristics, isolation techniques, isolation media, culture media, methods to induce asexual and sexual production, as well as techniques for studying various other aspects of *Phytophthora*, into a reference book. This manual proved to be a valuable resource to *Phytophthora*, researchers worldwide. During this period, Newhook *et al.* (1978) published a revised taxonomic key based on detailed morphological features and included six new species. The key was presented in a tabular form for ease of identification. Subsequently, Stamps *et al.* (1990) revised Newhook's tabular key and added 19 new species.

During the decade 1970–1980 the physiology of *Phytophthora* spp. was elucidated by various researchers, and chemically defined media were developed that allowed a precise study of the nutritional requirements for growth and reproduction (Ribeiro *et al.*, 1975, 1976; Ribeiro, 1983). Concurrently, the requirements for sexual reproduction were being elucidated by Brasier (1969, 1972). This was also the decade when studies were initiated at the University of California, Riverside, to measure intraspecific variation in several hundred isolates of *P. cinnamomi*, *Phytophthora megasperma* and *P. palmivora* recovered from different hosts worldwide. This work generated several notable papers that illustrate how difficult it is to precisely define discrete morphological and physiological characteristics of each species. During this period the department of plant pathology at the University of California, Riverside, rose to the forefront of research on all aspects of *Phytophthora*, covering its biology, epidemiology, aetiology, physiology, genetics and pathogenicity. In 1981 a seminal international conference was held at the University of California, Riverside, to recognize the increasing worldwide importance of *Phytophthora* spp. and to honour the numerous contributions made by Professor George Zentmyer towards our understanding of this genus. Over 300 scientists from 24 countries attended and the proceedings were edited by Erwin *et al.* (1983). It was apparent that while much information was available on this genus, there was much work that needed to be done, and this conference was the impetus for much of the work in the following decade.

1.5 Not a Fungus

Until the mid-1980s *Phytophthora* was placed with other oomycetous microorganisms in the kingdom Myceteae. Although there is not space to address this here, multiple lines of evidence showed that the oomycetes were different from the other fungi and *Phytophthora* was placed in the newly described kingdom *Chromista* (Cavalier-Smith, 1986; Barr, 1992; Dick, 1995). This radical revision considered *Phytophthora* species to be 'fungus-like' rather than true fungi. A detailed discussion placing *Phytophthora* into the kingdom *Chromista* is given by Erwin and Ribeiro (1996). The current consensus is that *Phytophthora* belongs to the Kingdom Stramenopila, Phylum Oomycota.

Further complications in the taxonomy of *Phytophthora* also arose when several researchers observed hybridization between species. This added another layer of complexity when attempting to describe new *Phytophthora* species, and natural hybrids are discussed elsewhere in this book (Boccas, 1981; Érsek *et al.*, 1995; Brasier *et al.*, 1999; Érsek and Nagy, 2008).

1.6 The Molecular Era

The 1990s saw the adaption of molecular techniques to better understand the taxonomy of *Phytophthora* species. Isozyme analyses of various *Phytophthora* spp. demonstrated the relationships between

various morphologically similar and dissimilar species (Förster and Coffey, 1991; Oudemans *et al.*, 1994; Förster *et al.*, 1995). This was followed by molecular studies of mitochondrial and nuclear DNA, work that refined the molecular taxonomy for a number of species (Förster *et al.*, 1995). This work was further expanded by Cooke *et al.* (2000) and Förster *et al.* (2000). These papers also demonstrated the inherent complexity within this genus. It now remained to be seen whether the relationships between the various molecular groupings that were identified could be correlated with the classical morphological groups originally erected by Waterhouse (1963) and revised by Newhook *et al.* (1978) and Stamps *et al.* (1990). The first attempt in trying to correlate DNA fingerprints with morphological traits for the identification of *Phytophthora* species was made by Gallegly and Hong (2008). Their publication describes morphological as well as the DNA fingerprints for each member of the genus. However, work still needs to be done to sort out the various forms, strains and subspecies that have been described for each species.

More recently, Lamour and Kamoun (2009) published a comprehensive book on tools and techniques for genetic and genomic research into the oomycetes. This book provides current information on molecular methods and also provides resources to better understand these diverse organisms. Molecular analysis has resulted in a better understanding of the genetic structure of *P. capsici* (Quesada-Ocampo *et al.*, 2011; Lamour *et al.*, 2011) and *P. infestans* (Nowicki *et al.*, 2012). Both these species are discussed in greater detail elsewhere in this book. The importance of the molecular aspects of *Phytophthora* is now evidenced by the establishment of publicly available reference genomes for *Phytophthora ramorum*, *Phytophthora sojae*, *P. capsici* and *P. infestans*, and web resources to assist with identification including http://phytophthora-id.org and the online *Phytophthora* database at Penn State University, which is a forensic database supporting the identification and monitoring of all aspects of *Phytophthora* species (http://www.phytophthoradb.org/). Meanwhile, Cline *et al.* (2008) published a synopsis of *Phytophthora* with accurate scientific names, host ranges and geographic distributions, which highlights the significance of Phytophthora diseases worldwide. The significance of *Phytophthora* is also evidenced by the number of books and monographs published on *Phytophthora* in various countries (Lucas *et al.*, 1991; Ho *et al.*, 1995; Irwin *et al.*, 1995; Erwin and Ribeiro, 1996; Jee *et al.*, 2000; Mehrotra and Aggarwal, 2001; Drenth and Guest, 2004).

An update of all new species published in 2010 describes the phylogeny based on internal transcribed spacer (ITS) sequences, the morphology of each species and a list of natural hybrid *Phytophthora* species (Érsek and Ribeiro, 2010). Meanwhile, Kroon (2010) performed a detailed study of a broad range of *Phytophthora* species utilizing nuclear DNA and mitochondrial DNA (mtDNA), and proposed a revised classification based on molecular phylogeny as well as morphological traits and niche preference. Molecular approaches will eventually bring order to the taxonomy of this genus and also help refine the taxonomy of species hybrids.

1.7 Summary

It has been over 150 years since de Bary first described *P. infestans*, and in the intervening years a prodigious amount of research has been accomplished on the taxonomy, biology, morphology, ecology, physiology, pathology and ultrastructure. Despite many years of intense research, complete control of *Phytophthora* is often elusive. For example, the cost of *Phytophthora* to the potato crop alone amounted to US$6.7 billion annually (USA Blight, 2012). The reasons for the difficulty in controlling *Phytophthora* include several unique traits such as: (i) homothallism; (ii) heterothallism; (iii) rapid creation of various reproduction structures such as oospores, sporangia and chlamydospores (Fig. 1.1); (iv) the rapid evolution of new races and/or strains; and (v) the ability to produce hybrid species.

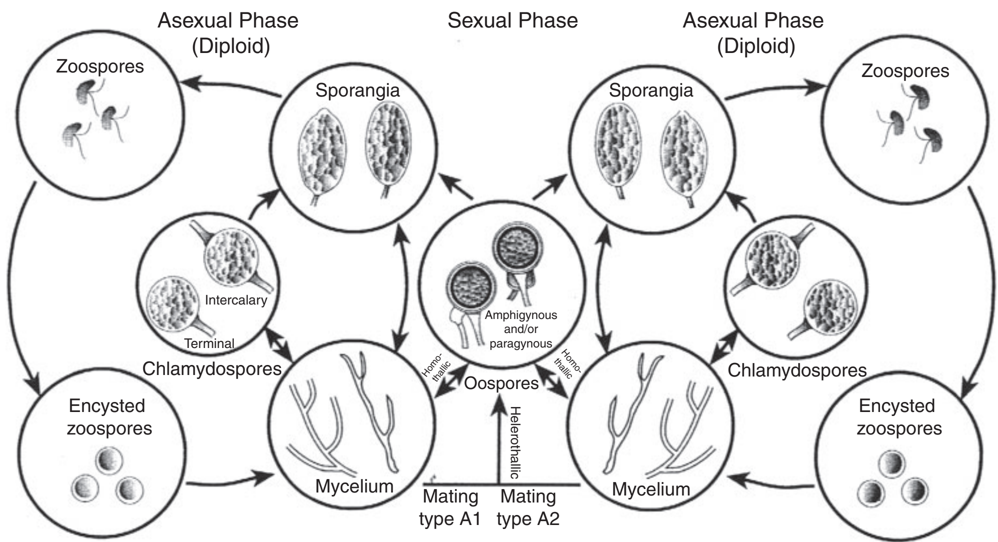

Fig. 1.1. Representative life cycle for *Phytophthora*.

Phytophthora species also are unique in being diploid in the vegetative stage and in being 'fungus-like' rather than true fungi. Cell walls do not have chitin, a component common to most fungi, and since *Phytophthora* does not require sterols for growth, it is not affected by sterol-inhibiting fungicides that are effective against other fungal genera. It is also resistant to polyene antibiotics such as pimaricin, vancomycin and ampicillin.

Since 2000 *Phytophthora* has once again increased in importance with the discovery of *P. ramorum* (Rizzo *et al.*, 2002), a new species that has devastated the oak trees in California and is now found on several horticultural species. The host range for *P. ramorum* has recently been found to include larch and Sitka spruce in Britain, a situation that may greatly alter the forested landscape in some parts of Britain and elsewhere. Another new pathogen, *Phytophthora alni*, is responsible for a lethal root and collar rot of alder species in Europe, presenting a serious threat to all alder species in the western hemisphere (Brasier and Kirk, 2004). These are just two of several new *Phytophthora* species that will have grave consequences for forestry in the near future and both are discussed further elsewhere in this book. By 1996 58 valid *Phytophthora* species had been described (Erwin and Ribeiro, 1996). This number is now well over 100, and there is no doubt that *Phytophthora* will be a continued global threat for many decades.

References

Barr, D.J.S. (1992) Evolution and kingdoms of organisms from the perspective of a mycologist. *Mycologia* 81, 1–11.

Berkeley, M.J. (1846) Observations, botanical and physiological, on the potato murrain. *Journal of the Horticultural Society* 1, 9–34.

Black, W. (1952) A genetical basis for the classification of strains of *Phytophthora infestans*. *Proceedings of the Royal Society of Edinburgh* B65, 36–51.

Blackwell, E. (1949) Terminology in *Phytophthora*. *Commonwealth Mycological Institute Mycological Paper* No. 30, 24 pp.

Boccas, B. (1981) Interspecific crosses between closely related heterothallic *Phytophthora* species. *Phytopathology* 71, 60–65.

Bourke, A. (1991) Potato blight in Europe 1845, the scientific controversy. In: Lucas, J.A., Shattock, R.C., Shaw, D.S. and Cooke, L.R. (eds) *Phytophthora*. Cambridge University Press, Cambridge, UK, pp. 12–24.

Brasier, C.M. (1969) Formation of oospores *in vivo* by *Phytophthora palmivora*. *Transactions of the British Mycological Society* 52, 273–279.

Brasier, C.M. (1972) Observations on the sexual mechanism in *Phytophthora palmivora* and related species. *Transactions of the British Mycological Society* 58, 237–251.

Brasier, C.M. and Kirk, S.A. (2004) *Phytophthora alni. Mycological Research* 108, 1172–1184.

Brasier, C.M. and Sansome, E. (1975) Diploidy and gametangial meiosis in *Phytophthora cinnamomi*, *P. infestans* and *P. dreschleri*. *Transactions of the British Mycological Society* 65, 49–65.

Brasier, C.M., Cooke, D.E.L. and Duncan, J.M. (1999) Origin of a new *Phytophthora* pathogen through interspecific hybridization. *Proceedings of the National Academy of Sciences USA* 96, 5878–5883.

Breda de Haan, J. van (1896) De bibiziekte in de Deli tabak veroorzaakt door *Phytophthora nicotianae*. *Mededeelingen uit's Lands plantentuin* 15. 107 pp. (in Dutch). (Cited in Tucker, 1933.)

Buczaki, S.T. (1991) The Reverend Miles Berkeley. In: Lucas, J.A., Shattock, R.C., Shaw, D.S and Cooke, L.R. (eds) *Phytophthora*. Cambridge University Press, Cambridge, UK, pp. 1–11.

Butler, E.J. (1919) Report of the Imperial Mycologist 1918–1919. *Scientific Reports of the Research Institute, Pusa 1918–1919,* 68–85.

Carne, W.M. (1925) A brown rot of citrus fruit in Australia (*Phytophthora hibernalis* n. sp.). *Journal of the Royal Society of Western Australia* 12, 13–41.

Caten, C.E. (1971) Single zoospore variation in *Phytophthora infestans* and attenuation of strains in culture. *Transactions of the British Mycological Society* 56, 1–7.

Cavalier-Smith, T. (1986) The kingdom *Chromista*, origin and systematics. In: Round, I. and Chapman, D.J. (eds) *Progress in Phycological Research*, Vol. 4. Biopress, Bristol, UK, pp. 309–347.

Cline, E.T., Farr, D.F. and Rossman, A.Y. (2008) A synopsis of *Phytophthora* with accurate scientific names, host range, and geographic distribution. *Plant Health Progress*. Available at: http://www.plantmanagementnetwork.org/sub/php/review/2008/phytophthora/ (accessed 1 January 2012).

Coleman, L. (1910) Diseases of the areca palm. I. Koleroga rot disease. *Annals of Mycology* 8, 591–626.

Cooke, D.E.L., Drenth, A., Duncan, J.M., Wagels, G. and Brasier, C.M. (2000) A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* 30, 17–32.

Dastur, J.F. (1913) *Phytophthora parasitica* n. sp., a new disease of the castor oil plant. *Memoirs of the Department of Agriculture in India, Botanical Series* 5, 177–231.

de Bary, A. (1876) Researches into the nature of the potato fungus, *Phytophthora infestans*. *Journal of the Royal Agricultural Society of England*. Series 2. 12, 239–269. (Cited in Tucker, 1933.)

Dick, M.W. (1995) Sexual reproduction of the *Peronosporomycetes* (chromistan fungi). *Canadian Journal of Botany* 73 (Suppl. 1), 5712–5724.

Drenth, A. and Guest, I. (eds) (2004) *Diversity and Management of* Phytophthora *in Southeast Asia*. *Australian Centre for International Agricultural Research (ACIAR) Monograph* No. 114, 238 pp.

Elliott, C.G. and MacIntyre, D. (1973) Genetical evidence on the life-history of *Phytophthora*. *Transactions of the British Mycological Society* 60, 311–316.

Érsek, T. and Nagy, Z. (2008) Species hybrids in the genus *Phytophthora* with emphasis on the alder pathogen *Phytophthora alni*, a review*. Eurpean Journal of Plant Pathology* 122, 31–39.

Érsek, T. and Ribeiro, O.K. (2010) An annotated list of new *Phytophthora* species described post-1996. *Acta Phytopathologica et Entomologica Hungarica* 45, 251–266.

Érsek, T., English, J.T. and Schgoelz, J.E. (1995) Creation of species hybrids of *Phytophthora* with modified host ranges by zoospore fusion. *Phytopathology* 85, 1343–1347.

Erwin, D.C. (1983) Variability within and among species of *Phytophthora.* In: Erwin, D., Bartnicki-Garcia, S. and Tsao, P.H. (eds) Phytophthora*, Its Biology, Taxonomy, Ecology, and Pathology.* APS Press, St Paul, Minnesota, pp. 149–165.

Erwin, D.C. and Ribeiro, O.K. (1996) *Phytophthora Diseases Worldwide*. APS Press, St Paul, Minnesota, 562 pp.

Erwin, D.C., Zentmyer, G.A., Galindo, J. and Niederhauser, J.S. (1963) Variation in the genus *Phytophthora*. *Annual Review of Phytopathology* 1, 375–396.

Erwin, D.C., Bartnicki-Garcia, S. and Tsao, P.H. (eds) (1983) Phytophthora*, Its Biology, Taxonomy, Ecology, and Pathology*. APS Press, St Paul, Minnesota, 392 pp.
Fischer, A. (1892) Die Pilze Deutschlands, Oesterreich und der Schweiz. IV. *Phycomycetes, Peronosporaceae*. *Rabenhorst's Kryptogamentflora* 1, 392–490. (Cited in Tucker, 1931.)
Fitzpatrick, H.M. (1923) A survey of the evidence indicating that *Phytophthora* should be merged with *Pythium*. *Phytopathology* 13, 34 (abstract).
Förster, H. and Coffey, M.D. (1991) Approaches to the taxonomy of *Phytophthora* using polymorphism in mitochondrial and nuclear DNA. In: Lucas, J.A., Shattock, R.C., Shaw, D.S. and Cooke, L.R. (eds) *Phytophthora*. Cambridge University Press, Cambridge, UK, pp. 164–183.
Förster, H., Learn, G. and Coffey, M.D. (1995) Toward a better understanding of the evolutionary history of species in the genus *Phytophthora* using isozymes, DNA RFLPs and ribosomal DNA spacer sequences. In: Dowley, L.J., Bannon, E., Cooke, L.R., Keane, T. and O'Sullivan, E. (eds) *Phytophthora infestans*. Boole Press, Dublin, Ireland, pp. 42–54.
Förster, H., Cummings, M.P. and Coffey, M.D. (2000) Phylogenetic relationships of *Phytophthora* species based on ribosomal ITS I DNA sequence analysis with emphasis on Waterhouse groups V and VI. *Mycological Research* 104, 1055–1061.
Galindo, J. and Gallegly, M.E. (1960) The nature of sexuality in *Phytophthora infestans*. *Phytopathology* 50, 123–128.
Galindo, J. and Zentmyer, G.A. (1964) Mating types in *Phytophthora cinnamomi*. *Phytopathology* 54, 238–239.
Gallegly, M. (1968) Genetics of pathogenicity of *Phytophthora infestans*. *Annual Review of Phytopathology* 6, 336–396.
Gallegly, M. and Hong, C. (2008) Phytophthora*, Identifying Species by Morphology and DNA Fingerprints.* APS Press, Minnesota, 158 pp.
Ho, H.H. and Jong, S.C. (1992) A re-evaluation of *Phytophthora* species described by K. Sawada in Taiwan. *Mycotaxon* 43, 297–316.
Ho, H.H., Ann, F.J. and Chang, H.S. (1995) The genus *Phytophthora* in Taiwan*. Institute of Botany, Academia Sinica, Monograph*, Series 15, 86 pp.
Irwin, J.A.G., Cahill, D.M. and Drenth, A. (1995) *Phytophthora* in Australia. *Australian Journal of Agricultural Research* 46, 1311–1337.
Jee, H.J., Cho, W.D. and Kim, C.H. (2000) *Phytophthora Diseases in Korea.* National Institute of Agricultural Science and Technology, Suwon 442-0707, Korea, 227 pp. (in Korean).
Klebahn, H. (1909) Die neue Zweig-und Knospenkrankheit (A new twig and bud disease). *Krankheiten des Flieders*, Berlin, pp. 18–75. (in German). (Cited in Tucker, 1931.)
Kroon, L.P.N.M. (2010) The genus *Phytophthora*: phylogeny, speciation and host specificity. PhD dissertation, Wageningen University, the Netherlands, 184 pp.
Lafferty, H.A. and Pethybridge, G.H. (1922) On a *Phytophthora* parasitic on apples which has both amphigynous and paragynous antheridia; and on allied species which show the same phenomenon. *Scientific Proceedings Royal Dublin Society* 17, 29–43.
Lamour, K. and Kamoun, S. (eds) (2009) *Oomycete Genetics and Genomics, Diversity, Interactions, and Research Tools*. John Wiley and Sons, Hoboken, New Jersey, 574 pp.
Lamour, K.H., Stam, R., Jupe, J. and Huitema, E. (2011) The oomycete broad-host-range pathogen *Phytophthora capsici. Molecular Plant Pathology* 13, 329–337.
Laviola, C. (1968) Studies of the genetics of *Phytophthora infestans.* PhD thesis, West Virginia University, Morgantown, West Virginia, 72 pp.
Lebert, H. and Cohn, F. (1870) On the rot of cactus stems. *Beitraege zur Biologie der Pflanzen* 1, 51–57. (in German).
Leonian, L.H. (1922) Stem and fruit blight of peppers caused by *Phytophthora capsici* sp. nov. *Phytopathology* 12, 401–408.
Leonian, L.H. (1925) Physiological studies on the genus *Phytophthora*. *American Journal of Botany* 12, 444–498.
Leonian, L.H. (1934) Identification of *Phytophthora*. *West Virginia Agricultural Experimental Station Bulletin* 262, 36 pp.
Lilly, V.G. and Barnett, H.L. (1951) *Physiology of the Fungi*. McGraw-Hill, New York, 464 pp.
Long, M. and Keen, N. (1977) Evidence for heterocaryosis in *Phytophthora megasperma* var. *sojae. Phytopathology* 67, 670–674.

Lucas, J.A., Shattock, R.C., Shaw, D.S. and Cooke, L.R. (eds) (1991) *Phytophthora*. Cambridge University Press, Cambridge, UK, 447 pp.

Mehrotra, R.S. and Aggarwal, A. (2001) *Phytophthora Diseases in India*. Bishen Singh and Mahendra Pal Singh, Debra Dun 248 001, India, 322 pp.

Montagne, J.F.C. (1845) Note sur la maladie qui ravage les pommes de terre et caracteres du *Botrytis infestans. Memoirs of the Institute of France* 609, 98–101, 312–313. (In French.)

Moore, D. (1846) Remarks on the potato disease. *Gardeners' Chronicle* 26, 557–558.

Nelson, E.C. (1995) The cause of the calamity, the discovery of the potato blight in Ireland, 1845–1847, and the role of the National Botanic Gardens, Glasnevin, Dublin. In: Dowley, L.K.J., Bannon, E., Cooke, L.R., Keane, T. and O'Sullivan, E. (eds) *Phytophthora infestans*. Boole Press, Dublin, pp. 1–121.

Newhook, F.J., Waterhouse, G.M. and Stamps, D.J. (1978) Tabular key to the species of *Phytophthora* de Bary. *Mycological Paper* 143. Commonwealth Mycological Institute, Kew, Surrey, UK, 20 pp.

Nowicki, M., Foolad, M.R., Nowakowska, M. and Kozik, E.U. (2012) Potato and tomato late blight caused by *Phytophthora infestans*. An overview of pathology and resistance breeding. *Plant Disease* 96, 4–17.

Ó Gráda, C. (1999) *Black '47 and Beyond, The Great Irish Famine in History, Economy, and Memory*. Princeton University Press, Princeton, New Jersey, 302 pp.

Oudemans, P., Förster, H., and Coffey, M.D. (1994) Evidence for distinct isozyme subgroups within *Phytophthora citricola* and close relationships with *P. capsici* and *P. citrophthora. Mycological Research* 98, 189–199.

Petersen, H.E. (1910) An account of Danish freshwater Phycomycetes with biological and systematical remarks. *Annales Mycologici* 8, 494–560.

Pethybridge, G.H. (1913) On the rotting of potato tubers by a new species of *Phytophthora* having a method of sexual reproduction hitherto undescribed. *Scientific Proceedings of the Royal Dublin Society* 13, 529–565. (Cited in Tucker, 1933.)

Pethybridge, G.H. and Lafferty, H.A. (1919) A disease of tomato and other plants caused by a new species of *Phytophthora*. *Scientific Proceedings of the Royal Dublin Society* 15, 487–503. (Cited in Tucker, 1933.)

Quesada-Ocampo, L.M., Granke, L.L., Mercier, M.R., Olsen, J. and Hausbeck, M.K. (2011) Investigating the genetic structure of *Phytophthora capsici* populations. *Phytopathology* 101, 1061–1073.

Raciborski, M. (1900) Parasitische Algen und Pilze, Java. *Batavia* p. 9. (Cited in Waterhouse, 1970.)

Rands, R.D. (1922) Streepkanker vn Kaneel, veroorzaakt door *Phytophthora cinnamomi* n. sp. (Stripe canker of cinnamon caused by *Phytophthora cinnamomi* n. sp.). *Mededelingen van het Instituut voor Plantenziekten* 54, 41 pp. (In Dutch.)

Ribeiro, O.K. (1978) A sourcebook of the genus *Phytophthora*. J. Cramer, Vaduz, Liechtenstein, 417 pp.

Ribeiro, O.K. (1983) Physiology of asexual sporulation and spore germination in *Phytophthora*. In: Erwin, D.C., Bartnicki-Garcia, S. and Tsao, P.H. (eds) Phytophthora*, Its Biology, Taxonomy, Ecology and Pathology.* APS Press, Minnesota, pp. 55–70.

Ribeiro, O.K., Erwin, D.C. and Zentmyer, G.A. (1975) An improved synthetic medium for oospore production and germination of several *Phytophthora* species. *Mycologia* 67, 1012–1019.

Ribeiro, O.K., Zentmyer, G.A. and Erwin, D.C. (1976) The influence of qualitative and quantitative radiation on reproduction and spore germination of four *Phytophthora* species. *Mycologia* 68, 1162–1175.

Rizzo, D.M., Garbelotto, M., Davidson, J.M., Slaughter, G.W. and Koike, S.T. (2002) *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus in* California. *Plant Disease* 86, 205–214.

Romero, S. and Erwin, D.C. (1969) Variation in pathogenicity among single-oospore cultures of *Phytophthora infestans*. *Phytopathology* 59, 1310–1317.

Sansome, E. (1976) Gametangial meiosis in *Phytophthora capsici*. *Canadian Journal of Botany* 54, 1535–1545.

Savage, E.J, Clayton, C.W., Hunter, J.H., Brenneman, J.A., Laviola, C. and Gallegly, M.E. (1968) Homothallism, heterothallism, and interspecific hybridization in the genus *Phytophthora*. *Phytopathology* 58, 1004–1021.

Sawada, K. (1919) Preliminary report of Phytophthora diseases of *Cyperaceae*. *Formosan Agricultural Magazine* 146, 8–14. (In Japanese.) (Cited in Ho and Jong, 1992.)

Shaw, D.S. and Khaki, I.A. (1971) Genetical evidence for diploidy in *Phytophthora*. *Genetical Research* 17, 165–167.

Smith, R.E. and Smith, E.H. (1906) A new fungus of economic importance. *Botanical Gazette* 42, 215–221.
Smith, R.E. and Smith, E.H. (1925) Further studies of *Pythiaceous* infection of deciduous fruit trees in California. *Phytopathology* 15, 389–404.
Stamps, D.J., Waterhouse, G.M., Newhook, F.J. and Hall, G.S. (1990) Revised tabular key to the species of *Phytophthora*. *Mycological Paper* 162. Commonwealth Mycological Institute, Kew, Surrey, UK, 28 pp.
Thaxter, R. (1889) A new American *Phytophthora*. *Botanical Gazette* 14, 273–274.
Toxopeus, H.J. (1956) Reflections on the origin of new physiologic races in *Phytophthora infestans* and the breeding for resistance in potatoes. *Euphytica* 5, 221–237.
Tucker, C.M. (1931) Taxonomy of the genus *Phytophthora* de Bary. *University of Missouri Agricultural Experimental Station Research Bulletin* No. 153, 208 pp.
Tucker, C.M. (1933) Distribution of the genus *Phytophthora*. *University of Missouri Agricultural Experimental Station Research Bulletin* 184, 80 pp.
Unger, F. (1847) Beitag zur Kenntnis der in der Kartoffelkrankheit vorkommenden Pilze und der Ursache ihres Enstehens. *Botanica Zeitschrift* 5, 314.
USA Blight (2012) USA Blight: a national project on late blight of tomato and potato in the United States. United States Department of Agriculture (USDA). Available at: www.usablight.org/ (accessed 1 January 2012).
Waterhouse, G.M. (1963) Key to the species *Phytophthora* de Bary. *Mycological Paper* 92. Commonwealth Mycological Institute, Kew, Surrey, UK, 22 pp.
Waterhouse, G.M. (1970) The genus *Phytophthora* de Bary. *Mycological Paper* 122, 1–59. Commonwealth Mycological Institute, Kew, Surrey, UK, 104 pp.

2 Taxonomy and Phylogeny of *Phytophthora* and Related Oomycetes

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2.1 Introduction

Phytophthora is the second largest genus in the Peronosporaceae and currently comprises more than 100 species. The discovery of new species has increased dramatically over the past decade, primarily due to the application of molecular tools. There have been numerous attempts to place *Phytophthora* within the taxonomic framework of the oomycetes and to subdivide it into smaller entities or informal groups, but it has only been through large-scale phylogenetic analyses that the major evolutionary trends are becoming visible. In particular, it is now clear that the downy mildews and *Phytophthora* are closely related. Some downy mildew and *Phytophthora* species share key characteristics and there is increasing evidence that *Phytophthora* is multiply paraphyletic with respect to the downy mildews. Since the goal of phylogeny is to identify monophyletic taxa, these findings may lead to a subdivision of *Phytophthora* into as many as seven smaller genera, with the most well-known species of *Phytophthora*, *Phytophthora infestans*, retaining the current genus name. In this chapter the most important developments in the taxonomy and phylogeny of *Phytophthora* and closely related oomycetes are outlined.

2.2 *Phytophthora* and its Taxonomic Placement Among the Peronosporalean Oomycetes

The genus *Phytophthora* was described in 1876 (de Bary, 1876) as the fourth oomycete genus in what is now the Peronosporaceae. The tremendous attention given to this group is due to the large number of destructive diseases it causes on a huge variety of crops. The type species, *P. infestans*, is the most notorious as it played an important role in the Irish Potato Famine during the middle of the 19th century (see Ribeiro, Chapter 1, this volume). *Phytophthora* species, in contrast to most downy mildews that feed exclusively on living cells, feed on living host cells at the beginning of the infection process (biotrophic phase) and then kill the host cells to absorb nutrients from the dead tissue (necrotrophic phase). This so-called hemibiotrophy contrasts with *Pythium* species, which are usually necrotrophic during all stages of colonization.

The extended biotrophic phase and the whitish fluff of asexual propagules developing on the lower surface of host plants led mycologists to challenge the classification in *Botrytis* (Montagne, 1845), initially including *Phytophthora* in the genus *Peronospora* (for reference see de

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Bary, 1876 and discussion in Chapter 1). *Peronospora*, described in 1837, was the first genus of downy mildews (Corda, 1837), followed by *Bremia* (Regel, 1843), *Basidiophora* (Roze and Cornu, 1869) and *Phytophthora* (de Bary, 1876) with *P. infestans* as the type species. Since then *Phytophthora* has generally been considered separate from the biotrophic downy mildew pathogens. *Phytophthora* and the more necrotrophic genus, *Pythium*, were originally placed in the family Peronosporaceae alongside the obligate biotrophic downy mildews (de Bary, 1863). This placement was retained until the Pythiaceae were described based on the morphological distinctiveness (e.g. lack of clearly differentiated sporangiophores) (Schröter, 1893). As members of the genus *Phytophthora* share several morphological traits with some species of *Pythium* (e.g. poorly developed sporangiophores), and most species of the genus are almost as readily cultivable as *Pythium* species, *Phytophthora* was placed into the Pythiaceae (e.g. by Waterhouse (1973) and Dick *et al.* (1984)), a classification scheme still used in current textbooks.

The second group of obligate plant parasitic oomycetes, the white blister rusts, was established in 1893 when Schröter (1893) introduced the family Albuginaceae. Dick *et al.* (1984) grouped the Albuginaceae and Peronosporaceae together in the Peronosporales, distinct from the Pythiales, which contained *Pythium*, *Phytophthora* and several additional genera. However, even in the absence of phylogenetic evidence, several scientists suggested that the placement of *Phytophthora* within the Pythiaceae and distinct from the obligate biotrophic Peronosporaceae was not a natural classification (Gäumann, 1952). It was revealed at the turn of the century that *Phytophthora* is much more closely related to the downy mildews than to *Pythium* (Cooke *et al.*, 2000; Hudspeth *et al.*, 2000, 2003; Riethmüller *et al.*, 2002), and that *Albugo* is highly distinct from the downy mildews (Riethmüller *et al.*, 2002) warranting an order of its own, the *Albuginales* (Thines and Spring, 2005). Nonetheless, taxonomic schemes based on biotrophy versus necrotrophy are still widely used. Subsequent phylogenetic studies confirm the close relationship of *Phytophthora* and the downy mildew genera (Göker *et al.*, 2003, 2007; Hulvey *et al.*, 2010; Runge *et al.*, 2011).

While the taxonomy and phylogenetics of downy mildews have been mostly brought to congruence during the past decade (Voglmayr, 2008; Thines *et al.*, 2009b), the genus *Phytophthora* has so far been untouched with respect to major taxonomic rearrangements, which are possibly needed in light of their relationships with the downy mildews.

2.3 Phylogenetic Relationships Among the Peronosporaceae

According to the taxonomic scheme presented by Hulvey *et al.* (2010), which is refined in Fig. 2.1, the Peronosporaceae currently include the downy mildews and the genera *Halophytophthora*, *Phytophthora* and *Phytopythium*. Several genera that share some characteristics with *Phytophthora* have been segregated from the latter genus before the advent of molecular phylogenetic methods, for example the genera *Halophytophthora* (Ho and Jong, 1990), *Kawakamia* (Miyabe and Kawakami, 1903) and *Peronophythora* (Chen, 1961). Thus far, no species of the uncultivable genus *Kawakamia* (type species parasitic to *Cyperaceae*), which never gained broad recognition, have been included in phylogenetic investigations. The genus *Peronophythora* is monotypic, containing only its type species, *Peronophythora litchii*. This species is set apart from the monophyletic downy mildews and was placed among *Phytophthora* species by Göker *et al.* (2007), who consequently considered *Peronophythora* to be synonymous with *Phytophthora*. The genus *Halophytophthora* currently contains about 15 species, which are united mainly by their ecological preference (saline and brackish habitats) rather than clear-cut morphological (or genetic) differences. As a

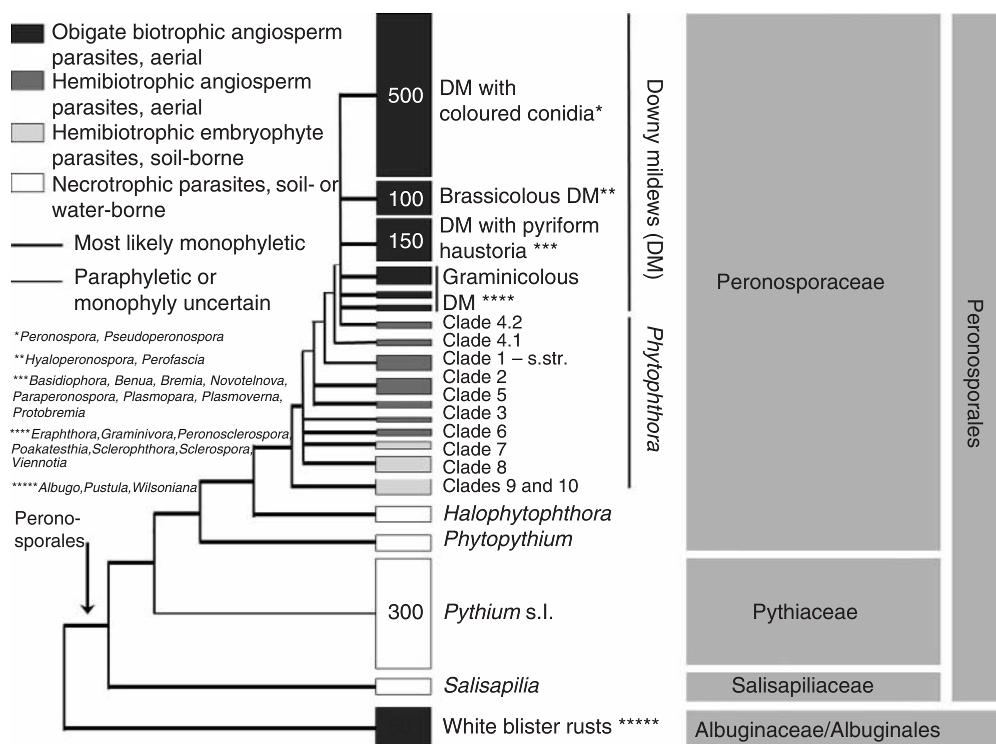

Fig. 2.1. Phylogenetic framework of the relationships within the Peronosporales compared with the white blister rusts. DM, Downy mildew.

consequence, this genus is probably highly polyphyletic. Initial investigations of the relationships of *Halophytophthora* to other members of the Peronosporaceae indicate the type species is distinct from *Phytophthora* and forms, together with some additional species, a well-defined clade within the Peronosporaceae (Göker *et al.*, 2007; Hulvey *et al.*, 2010; Nigrelli *et al.*, 2013). Other species of this genus do not belong to the Peronosporaceae and have thus been transferred to the newly described family, Salisapiliaceae (Hulvey *et al.*, 2010).

2.4 *Phytophthora* – Phylogenetic Relationships and Subgeneric Classification

Species of *Pythium* similar to *Phytophthora* and characterized by an extended biotrophic phase during plant colonization, for example *Pythium vexans* were often seen as the link between the two genera and seemed to justify the inclusion of both within the Pythiaceae. However, recent molecular investigations (Bala *et al.*, 2010; Hulvey *et al.*, 2010) indicate these species are distinct from both genera and are the sister group of a clade containing *Halophytophthora*, *Phytophthora* and the downy mildews (Hulvey *et al.*, 2010). Of any of the groups mentioned above *Phytophthora* is most closely related to the downy mildews, and their close relationship is also corroborated by the synapomorphic presence of canonical RxLR-type effectors in both groups (Tyler *et al.*, 2006; Haas *et al.*, 2009; Baxter *et al.*, 2010; Thines and Kamoun, 2010).

Expansion of the lineage giving rise to *Phytophthora* and downy mildews was so rapid that even in recent phylogenetic investigations disentangling the two groups

– which contain about two-thirds of the known oomycete diversity – is difficult and often relies on indirect evidence. Cooke *et al.* (2000) first showed that *Phytophthora* is probably paraphyletic with respect to the downy mildews. This means that some *Phytophthora* species are branching basal to a clade that contains *Phytophthora* species and downy mildews. Göker *et al.* (2007) provided further evidence for paraphyly in their multigene analyses of representative downy mildew and *Phytophthora* species. Recently Runge *et al.* (2011) added two downy mildew genera (*Pseudoperonospora* and *Hyaloperonospora*) to the most comprehensive dataset for *Phytophthora* species, the dataset of Blair *et al.* (2008), and reanalysed the data. Their multigene approach suggests that *Phytophthora* is at least six times paraphyletic with respect to the downy mildews. In contrast, a phylogenomic investigation comparing the genomes from three species of *Phytophthora* to a genome of *Hyaloperonospora arabidopsidis* led the authors to conclude that *Phytophthora* appears to be monophyletic (Seidl *et al.*, 2011). However, larger taxon sampling may help overcome the problem of highly divergent evolutionary rates in the downy mildews and *Phytophthora*, and multigene analyses with a few variable genes might provide a more robust view of the evolutionary relationships within the Peronosporaceae.

The close relationship of *Phytophthora* and the downy mildews is also reflected by some taxa in either group that have intermediate characteristics (Thines *et al.*, 2009a). For example, the *Cyperaceae*-infecting species, for example *Phytophthora cyperi*, which were placed into a separate genus, *Kawakamia* (Miyabe, 1903), have not been cultured on artificial media. These species are morphologically similar to the genus *Sclerophthora*, a downy mildew genus with many plesiomorphic traits. There are reports of successful cultivation of *Sclerophthora macrospora* (Tokura, 1975), but so far these experiments could not be successfully repeated. Due to morphological similarities with *Phytophthora*, especially with respect to the asexual stage (e.g. hardly differentiated, hyphal sporangiophores), *S. macrospora* was also included in the monograph of *Phytophthora* by Erwin and Ribeiro (1996). So far, the phylogenetic position of *Sclerophthora* among downy mildews and *Phytophthora* could not be clarified, owing to the accelerated rate of mutations in this lineage. However, the sister genus of *Sclerophthora*, *Eraphthora*, shows more clearly differentiated sporangiophores and thus its classification as a downy mildew has never been questioned (Telle and Thines, 2012). Other graminicolous downy mildews, in particular *Poakatesthia penniseti* and *Viennotia oplismeni*, also exhibit features typical for *Phytophthora*, including intracellular mycelium and indeterminate sporangiophore growth (Thines *et al.*, 2009a). The exact relationships between *Phytophthora* and the different groups of downy mildews has yet to be investigated in detail. Multigene analyses indicate the graminicolous downy mildews occupy basal positions with respect to the three crown groups of downy mildews (downy mildews with pyriform haustoria, ~150 species; downy mildews with coloured conidia, ~500 species; and brassicolous downy mildews, ~100 species) (Göker *et al.*, 2007). This led Thines *et al.* (2009a) to speculate that downy mildews might have arisen from *Phytophthora*-like ancestors on graminaceous hosts. However, there is no direct evidence for this hypothesis as neither *Kawakamia* nor *Sclerophthora* could be included in multigene analyses. Also, *Peronophythora litchii* could not be compared to a broader set of *Phytophthora* species, as the datasets of Göker *et al.* (2007) and Blair *et al.* (2008) have insufficient overlap for detailed analyses.

The need for discrete characters to clearly differentiate *Phytophthora* species arose in the middle of the 20th century as the number of species increased. Host range, which is often a useful trait for the downy mildews, was clearly not adequate as many *Phytophthora* species have much broader and in some cases overlapping host ranges. This led Waterhouse (1963) to introduce a classification and identification

scheme in her monograph of *Phytophthora* that subdivided *Phytophthora* into six groups (I–VI), mainly based on sporangial characteristics (papillate, semipapillate or conidial) and whether or not the sporangia are deciduous (caducous or non-caducous). This scheme proved useful and was adopted by many researchers. In time it became clear that morphological characters have many drawbacks (e.g. high intraspecific variation, lack of production in culture, etc.), and it was no surprise when the first phylogenetic investigation using a representative sample of *Phytophthora* species revealed the Waterhouse system was not a natural classification scheme (Cooke *et al.*, 2000). Subsequent multigene analyses support this finding (Kroon *et al.*, 2004; Blair *et al.*, 2008) and have led to a revised classification scheme describing ten phylogenetic clades (1 to 10) for the genus. The relationships of the phylogenetic clades and the integrity of some of them (e.g. clades 9 and 10) has yet to be unequivocally resolved, and clear-cut morphological differences between the different phylogenetic clades have not been identified. In general, the more basal clades (8–10) are predominantly soil-borne and have wider host spectra than the crown clades (1, 2, 4), which contain most of the air-borne species of *Phytophthora*. The intermediate clades have intermediate characteristics, while clades 3 and 5 are mostly air-borne and clades 6 and 7 are predominantly soil-borne. None of the phylogenetic clades shows a one-to-one congruence with any of the groups defined by Waterhouse (1963), but the more basal clades 6 to 10 correspond well to groups V and VI, while clades 1 to 5 correspond to groups I to IV. The only obvious trend is a general tendency of a more *Pythium*-like habit among the more basal groups (shorter biotrophic phase, non-caducous sporangia) and the presence of several characters reminiscent of downy mildew among the crown groups (e.g. differentiated sporophores, haustoria and caducous sporangia).

Runge *et al.* (2011) used several phylogenetic methods and confidence tests to infer that the downy mildews, represented by the two divergent genera, *Hyaloperonospora* and *Pseudoperonospora*, are the closest relatives of members of clade 4 of *Phytophthora*. This position was also suggested by the previous analyses of Cooke *et al.* (2000) using a smaller data set and a single gene. Future analyses using more loci and a more comprehensive sample of the downy mildew genera should help to draw a clearer picture regarding the extent of paraphyly of *Phytophthora*.

However, if one agrees that taxonomic groups should be ultimately monophyletic, the description of several new genera will be necessary to create monophyletic groups within the Peronosporaceae. Currently it seems unlikely that clear-cut morphological or physiological characteristics can be found to discriminate between the monophyletic *Phytophthora* groups, and they may have to be defined solely on molecular synapomorphies (i.e. diagnostic bases in loci easily accessible for phylogenetic reconstructions). The alternative solution, to include all downy mildews and *Phytophthora* species into a single large genus, *Peronospora*, does not seem to be preferable, given the much larger amount of name changes and the currently well-defined, morphologically distinct, monophyletic genera of the downy mildews.

2.5 Diversity and Species Concepts in *Phytophthora*

Currently, the genus *Phytophthora* contains more than 100 species, and this number continues to grow (Érsek and Ribeiro, 2010). Especially in the more basal clades, many new species have been described based on morphological and phylogenetic evidence (Brasier *et al.*, 2005; Belbahri *et al.*, 2006; Dick *et al.*, 2006). For other clades species delimitation can be more difficult. This is especially true when there is little molecular or morphological differentiation, and in these cases physiological properties such as host range and growth properties on a variety of media may again become more important. A good example for this is the *P. infestans* species cluster, which includes

P. infestans, *Phytophthora andina*, *Phytophthora ipomoeae*, *Phytophthora mirabilis* and *Phytophthora phaseoli*. Closely related species pairs, like *P. infestans* and *P. andina*, require reconsideration based on population studies from a broad sampling of strains from a variety of hosts. Ultimately, taxa at the species rank should be distinguishable on the basis of genetic information and any kind of other synapomorphies.

So far the diversity of *Phytophthora* species is hard to estimate, and some recent studies have even observed multiple new species (Jung *et al.*, 2011; Nigrelli and Thines, 2013). While some of these species apparently have wide distribution ranges, others may be limited to specific areas (Erwin and Ribeiro, 1996).

Extrapolating from recent findings, it is likely that the majority of *Phytophthora* species have yet to be discovered. Some of the recently emerging pathogens, like *Phytophthora ramorum* in the USA, *Phytophthora alni* in Europe and *Phytophthora cinnamomi* in Australia, have probably been spread by humans, with severe effects on forest ecosystems. A clear understanding of the evolutionary relationships and diversity within this group is important to all aspects of identification, mitigation and control.

References

Bala, K., Robideau, G.P., Lévesque, A., Cock, A.W.A.M. de, Abad, Z.G., Lodhi, A.M., Shahzad, S., Ghaffar, A. and Coffey, M.D. (2010) *Phytopythium* Abad, de Cock, Bala, Robideau & Levesque, gen. nov. and *Phytopythium sindhum* Lodhi, Shahzad & Levesque, sp. nov. *Persoonia* 24, 136–137.

Baxter, L. *et al.* (2010) Signatures of adaptation to obligate biotrophy in the *Hyaloperonospora arabidopsidis* genome. *Science* 330, 1149–1151.

Belbahri, L., Moralejo, E., Calmin, G., Oszako, T., García, J.A., Descals, E. and Lefort, F. (2006) *Phytophthora polonica*, a new species isolated from declining *Alnus glutinosa* stands in Poland. *FEMS Microbiology Letters* 261, 165–174.

Blair, J.E., Coffey, M.D., Park, S.Y., Geiser, D.M. and Kang, S. (2008) A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. *Fungal Genetics and Biology* 45, 266–277.

Brasier, C.M., Beales, P.A., Kirk, S.A., Denman, S. and Rose, J. (2005) *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. *Mycological Research* 109, 853–859.

Chen, C.C. (1961) A species of *Peronophythora* gen. nov. parasitic on litchi fruit in Taiwan. *Special Publication of College of Agriculture, National Taiwan University* 10, 1–37.

Cooke, D.E.L., Drenth, A., Duncan, J.M., Wagels, G. and Brasier, C.M. (2000) A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* 30, 17–32.

Corda, A.C.J. (1837) *Icones Fungorum Hucusque Cognitorum* vol. 1. J.G. Calve, Prague, 1, p. 20.

de Bary, A. (1863) Recherches sur le développement de quelques champignons parasites. *Annales des Sciences Naturelles (Botanique)* 20, 5–148.

de Bary, A. (1876) Researches into the nature of the potato fungus *Phytophthora infestans*. *Journal of the Royal Agricultural Society of England*, Series 2, 12, 239–269.

Dick, M.A., Dobbie, K., Cooke, D.E.L. and Brasier, C.M. (2006) *Phytophthora captiosa* sp. nov. and *P. fallax* sp. nov. causing crown dieback of *Eucalyptus* in New Zealand. *Mycological Research* 110, 393–404.

Dick, M.W., Wong, P.T.W. and Clark, G. (1984) The identity of the oomycete causing 'Kikuyu yellows', with a reclassification of the downy mildews. *Botanical Journal of the Linnaean Society* 89, 171–197.

Érsek, T. and Ribeiro, O.K. (2010) An annotated list of new *Phytophthora* species described post-1996. *Acta Phytopathologica et Entomologica Hungarica* 45, 251–266.

Erwin, D.C. and Ribeiro, O.K. (1996) *Phytophthora Diseases Worldwide*. American Phytopathological Society Press, St Paul, Minnesota.

Gäumann, E.A. (1952) *The Fungi: a Description of their Morphological Features and Evolutionary Development.* Hafner, New York.

Göker, M., Weiß, M., Oberwinkler, F., Voglmayr, H. and Riethmüller, A. (2003) Taxonomic aspects of *Peronosporaceae* inferred from Bayesian molecular phylogenetics. *Canadian Journal of Botany* 81, 672–683.

Göker, M., Voglmayr, H., Riethmüller, A. and Oberwinkler, F. (2007) How do obligate parasites evolve? A multi-gene phylogenetic analysis of downy mildews. *Fungal Genetics and Biology* 44, 105–122.
Haas, B.J. *et al*. (2009) Genome sequence and comparative analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature* 461, 393–398.
Ho, H.H. and Jong, S.C. (1990) *Halophytophthora* gen. nov., a new member of the family *Pythiaceae*. *Mycotaxon* 36, 377–382.
Hudspeth, D.S.S., Nadler, S.A. and Hudspeth, M.E.S. (2000) A *cox*2 molecular phylogeny of the *Peronosporomycetes*. *Mycologia* 92, 674–684.
Hudspeth, D.S.S., Stenger, D.C. and Hudspeth, M.E.S. (2003) A *cox*2 phylogenetic hypothesis for the downy mildews and white rusts. *Fungal Diversity* 13, 47–57.
Hulvey, J., Telle, S., Nigrelli, L., Lamour, K. and Thines, M. (2010) *Salisapiliaceae* – a new family of oomycetes from marsh grass litter of southeastern North America. *Persoonia* 25, 109–116.
Jung, T., Stukely, M.J.C., Hardy, G.E.S.J., White, D., Paap, T., Dunstan, W.A. and Burgess, T.I. (2011) Multiple new *Phytophthora* species from ITS clade 6 associated with natural ecosystems in Australia: evolutionary and ecological implications. *Persoonia* 26, 13–39.
Kroon, L.P.N.M., Bakker, F.T., van den Bosch, F.T., Bonants, P.J.M. and Flier, W.G. (2004) Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. *Fungal Genetics and Biology* 41, 766–782.
Miyabe, K. and Kawakami, T. (1903) *Kawakamia* Miyabe. A new genus belonging to the *Peronosporaceae*. *Botanical Magazine, Tokyo* 17, 306.
Montagne, J.F.C. (1845) Observations sur la maladie des pommes de terre. *Journal Universel des Sciences et des Sociétés Savantes en France et à a l'Étranger. 10 Section, Sciences Mathématiques, Physiques et Naturelles* 13 (609), 312–314.
Nigrelli, L. and Thines, M. (2013) Tropical oomycetes in the German Blight – climate warming or overlooked diversity? *Fungal Ecology*, in press.
Regel, E. (1843) Beiträge zur Kenntnis einiger Blattpilze. *Botanische Zeitung* 1, 665–667.
Riethmüller, A., Göker, M., Weiß, M., Oberwinkler, F. and Voglmayr, H. (2002) Phylogenetic relationships of the downy mildews (*Peronosporales*) and related groups based on nuclear large subunit ribosomal DNA sequences. *Mycologia* 94, 834–849.
Roze, E. and Cornu, M. (1869) Sur deux nouveaux types génériques pour des familles Saprolegniées et des Péronosporées. *Annales des Sciences Naturelles (Botanique)* 5, 72–91.
Runge, F., Telle, S., Ploch, S., Savory, E., Day, B., Sharma, R. and Thines, M. (2011) The inclusion of downy mildews in a multi-locus-dataset and its reanalysis reveals high degree of paraphyly in *Phytophthora*. *IMA Fungus* 2, 163–171.
Schröter, J. (1893) Peronosporinae. In: Engler A. (ed.) *Die Natürlichen Pflanzenfamilien*, Vol. 1. Wilhelm Engelmann, Leipzig, Germany, pp. 108–119.
Seidl, M.F., Van den Ackerveken, G., Govers, F. and Snel, B. (2011) A domain-centric analysis of oomycete plant pathogen genomes reveals unique protein organization. *Plant Physiology* 155, 628–644.
Telle, S. and Thines, M. (2012) Reclassification of an enigmatic downy mildew species on lovegrass (*Eragrostis*) to the new genus *Eraphthora* with a key to the genera of the Peronosporaceae. *Mycological Progress* 11, 121–129.
Thines, M. and Kamoun, S. (2010) Oomycete–plant coevolution: recent advances and future prospects. *Current Opinion in Plant Biology* 13, 427–433.
Thines, M. and Spring, O. (2005) A revision of *Albugo* (*Chromista*, *Peronosporomycetes*). *Mycotaxon* 92, 443–458.
Thines, M., Voglmayr, H. and Göker, M. (2009a) Taxonomy and phylogeny of the downy mildews (Peronosporaceae). In: Lamour, K. and Kamoun, S. (eds) *Oomycete Genetics and Genomics: Biology, Interactions with Plants and Animals, and Toolbox*. Wiley, Hoboken, New Jersey, pp. 47–75.
Thines, M., Telle, S., Ploch, S. and Runge, F. (2009b) Identity of the downy mildew pathogens of basil, coleus, and sage with implications for quarantine measures. *Mycological Research* 113, 532–540.
Tokura, R. (1975) Axenic or artificial culture of the downy mildew fungi of gramineous plants. *Tropical Agricultural Research Service* 8, 57–60.
Tyler, B.M. *et al*. (2006) *Phytophthora* genome sequences uncover evolutionary origins and mechanisms of pathogenesis. *Science* 313, 1261–1266.
Voglmayr, H. (2008) Progress and challenges in systematics of downy mildews and white blister rusts: new insights from genes and morphology. *European Journal of Plant Pathology* 122, 3–18.

Waterhouse, G.M. (1963) Key to the species *Phytophthora* de Bary. *Mycological Paper* 92. Commonwealth Mycological Institute, Kew, Surrey, UK, 22 pp.

Waterhouse, G.M. (1973) Peronosporales. In: Ainsworth, G.C., Sparrow, F.K. and Sussman, A.S. (eds) *The Fungi: an Advanced Treatise.* Vol. 4B. *A Taxonomic Review with Keys: Basidiomycetes and Lower Fungi.* Academic Press, New York, pp. 165–183.

3 Molecular Identification of *Phytophthora*

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3.1 Introduction

With approximately 124 described species of *Phytophthora*, reliance on morphological characteristics for species identification is challenging, even for experienced researchers. This is especially true for species where morphological features overlap or there is intraspecific variation in morphology. The availability of multiple searchable sequence databases, and lower costs of sequencing, has significantly expanded the potential of molecular techniques for isolate identification. In addition, the sequence databases are a useful resource for the development of diagnostic markers. While DNA sequence-based approaches are the most accurate for species identification, gel-based techniques can also be useful as long as the limitations for accurately differentiating closely related species are taken into account. Due to space constraints, a comprehensive literature review is not presented, but additional information may be found in reviews by Cooke *et al.* (2007) and O'Brien (2009), and a recent review that relates more to this chapter may be found in Martin *et al.* (2012).

3.2 Sequence-based Identification of Species

The most accurate methods for the molecular identification of *Phytophthora* to the species level are DNA sequence based. To ensure correct results the sequence database(s) used to search a sequence must be curated to ensure the reference isolates are correctly identified. There are several sequence database web sites based on curated reference isolate sequences and some have supporting information on morphological features and biology. The *Phytophthora* Database (www.phytophthoradb.org) houses data for nuclear (60S ribosomal protein L10, β-tubulin, enolase, HS protein 90, large subunit rRNA, TigA gene fusion, translation elongation factor 1α) and mitochondrial (cox1, cox2, nad1, nad9, rps10 and secY) loci from reference cultures that were the basis for the most recent multigene phylogeny of the genus (Blair *et al.*, 2008). *Phytophthora*-ID (www.phytophthora-id.org) has data for the internal transcribed spacer (ITS) region and cox1 and cox2 spacer region (the latter are from the same isolates supporting the *Phytophthora* Database; Grünwald *et al.*, 2011), while Q-Bank (www.q-bank.eu) has data for the ITS region, β-tubulin, elongation factor 1 alpha and cox1. Recently, the ITS region and a portion of the cox1 gene were sequenced for a number of species (Robideau *et al.*, 2011) and deposited in the Barcode of Life Database (www.boldsystems.org). All of these sites provide useful resources for identification of isolates to a species level using BLAST analysis, and the sequences can be downloaded for further use.

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There are several important concepts to keep in mind when doing sequence-based identification by BLAST analysis. Some loci are better suited than others for delineating species, especially those that are closely related. For example, the ITS region will not differentiate some of the clade 1C species or separate *Phytophthora fragariae* from *Phytophthora rubi*. *Phytophthora* is diploid and it is possible to encounter heterozygous bases in the same position in some nuclear loci, especially if the species is heterothallic. While on a large scale this could be reflective of a hybrid isolate with distinct alleles from different species (a situation covered elsewhere in this book), on a small scale (a few bases) it is more likely to be an indication of heterozygosity. In addition, the taxonomic description of species has not kept pace with phylogenetic analyses, and some described species are actually species complexes consisting of distinct evolutionary lineages. While many new species have been described in the past 10 years (the number of described species has essentially doubled in this time) there are a number of complexes where accurate species identification is problematic, including *Phytophthora cryptogea*/*Phytophthora drechsleri*, *Phytophthora capsici*, *Phytophthora citricola*, *Phytophthora citrophthora* and *Phytophthora megasperma*. With species complexes BLAST analysis may provide equal results for distinctly different evolutionary lineages. In addition, it is important to keep the length and identity of a sequence in mind when using BLAST as use of a partial sequence may lead to a high score and an erroneous conclusion. When using the ITS region this problem can be enhanced if highly conserved flanking coding regions are included in the BLAST query. To help address some of these issues it can be helpful to compare the results obtained with several loci before drawing conclusions of species identity. For some species complexes, identifying isolates to a specific phylogenetic lineage will only be possible once the taxonomic descriptions are revised.

3.3 Gel-based Molecular Techniques for Species Identification

Although less commonly used, gel-based techniques have a place for identification with some species or when evaluating a large numbers of isolates – especially if other approaches for species identification confirm the accuracy of results. PCR-RFLP (restriction fragment length polymorphism) of the ITS region has been used to identify some species (Cooke *et al.*, 2000), although it may not be possible to differentiate those that are closely related. Genus-specific ITS primers have been reported and should be useful when looking at environmental samples (Drenth *et al.*, 2006, Scibetta *et al.*, 2012). The mitochondrially encoded *cox2* and *cox1* gene cluster is another locus that has proved useful for species identification, but due to the lack of primer specificity this approach cannot be used with environmental samples (Martin and Tooley, 2004). A set of nested genus-specific primers that amplify primarily the spacer region was useful in PCR-RFLP analysis (*Apo*I and *Dra*I can differentiate many but not all species; F. Martin, unpublished) (Martin *et al.*, 2004). Single-stranded conformation polymorphism (SSCP) analysis using the ITS (Kong *et al.*, 2003, 2005; Gallegly and Hong, 2008; Ghimire *et al.*, 2009) or the *cox1* and *2* spacer region (Reeser *et al.*, 2011) can also be useful for species identification. This approach has been used with the ITS region to investigate the population structure of *Pythium* species in Ohio soil (Broders *et al.*, 2007) and should be useful in similar types of studies with *Phytophthora*. Using an automated sequencer to collect the data on SSCP band migration can improve the accuracy of the results (T. Kubisiak cited in Martin *et al.*, 2009). Using a similar approach, Rytkönen *et al.* (2012) used denaturing gradient gel electrophoresis (DGGE) of a PCR-generated ITS1 amplicon to differentiate most of the 16 *Phytophthora* spp. they tested. Although the technique was unable to differentiate some closely related species, it

did identify some species directly from infected tissue.

A variety of markers have been used to measure intraspecific variation, including: (i) RFLP; (ii) random amplified polymorphic DNAs (RAPD); (iii) amplified fragment length polymorphisms (AFLP); (iv) simple sequence repeats (SSRs); (v) intersimple sequence repeats (ISSRs); and (vi) single nucleotide polymorphisms (SNPs) (for examples see Cooke *et al.*, 2007; O'Brien *et al.*, 2009; Martin *et al.*, 2012). For greater detail on these techniques and their application in population analysis see Lamour, Chapter 4, this volume.

3.4 Mitochondrial Haplotypes

Mitochondrial haplotypes have been used to characterize populations of *P. infestans* (reviewed in Gavino and Fry, 2002; Wattier *et al.*, 2003) but have not been widely used with other species. Perhaps one reason for this is that mitochondria are uniparentally inherited from the maternal parent (Förster and Coffey, 1990), and haplotype analysis may be more useful for clonally reproducing species compared with a sexually recombining population. Schena *et al.* (2008) and Giresse *et al.* (2010) identified several mitochondrial regions that may be useful for identifying haplotypes. Martin (2008) sequenced the mitochondrial genomes of two *Phytophthora ramorum* isolates and identified 28 polymorphic loci that differentiated four mitochondrial haplotypes in a collection of isolates representing the three nuclear genotypes. High resolution DNA melting analysis (HR-DMA) of two loci was found to accurately identify these haplotypes with less effort and expense than DNA sequencing (F. Martin, unpublished). Comparative analysis of mitochondrial genomic data from a number of *Phytophthora* spp. identified specific regions that were more prone to interspecific variation, and these regions were examined to evaluate their level of intraspecific variation (F. Martin, unpublished). Some of these loci were used by Martin and Coffey (2012) to identify 45 mitochondrial haplotypes in 62 isolates of *Phytophthora cinnamomi*. Likewise, Mammella *et al.* (2011) used a similar approach to identify 20 mitochondrial haplotypes from 50 isolates of *Phytophthora nicotianae* recovered from Italy. When an additional 46 isolates from a broader geographic area were examined, a total of 50 haplotypes were observed (M. Mammella *et al.*, unpublished). In a study using four mitochondrial genes to evaluate the phylogeny of the genus (*cox2*, *nad9*, *rps10* and *secY*; F. Martin, J. Blair and M. Coffey, unpublished) 69% of the 64 species where multiple isolates were sequenced exhibited SNPs, suggesting that mitochondrial haplotypes could be used with a number of species (especially since most intraspecific variation is found in spacer regions rather than in the coding regions that were examined in this study). Efforts to evaluate the correlation between mitochondrial haplotypes and nuclear genotype are underway for multiple species in several labs and should provide additional insight on the utility of using these markers for population analysis.

3.5 Molecular Detection

Diagnosticians, regulatory personnel and those responsible for making disease management decisions may not always have the resources to culture individual isolates, and the ability to identify pathogens directly from infected tissue is crucial, especially since some species can be difficult to recover at certain times of the year. The impact of invasive species such as *P. ramorum*, *Phytophthora alni* and *Phytophthora kernoviae* in the past 10 years has spurred research efforts devoted to molecular diagnostics, and a number of species-specific diagnostic markers have been reported (reviewed in Martin *et al.*, 2012).

3.5.1 Diagnostic loci

Historically the ITS region has been used to develop species-specific markers for

Phytophthora (Cooke *et al*., 2007; O'Brien *et al*., 2009; Martin *et al*., 2012). This is due in part to: (i) the large number of sequences deposited in public databases; (ii) its high copy number relative to many other nuclear loci (thereby improving detection sensitivity); and (iii) a level of interspecific sequence variation that has enabled species-specific primers to be developed for multiple species. Additional loci that have been used to separate species include: (i) β-tubulin and elicitin (Bilodeau *et al*., 2007); (ii) the ras-related protein *Ypt*1 gene (Schena *et al*., 2006); and (iii) the spacer region between the mitochondrial encoded *cox1* and *cox2* genes (Martin *et al*., 2004; Tooley *et al*., 2006). Further details on diagnostic markers developed from these loci (including sequence alignments used in their development) may be found on the *Phytophthora* Database (www.phytophthoradb.org). Diagnostic markers have also been developed for individual species based on random clones and RAPDs; however, this approach is not useful to develop markers for multiple species.

Work is currently underway to develop a TaqMan real time PCR diagnostic marker system for *Phytophthora* based on gene order differences between *Phytophthora*, plants and *Pythium* (F. Martin, unpublished). The idea is that regions with differences may provide targets that can be amplified using a wider range of conditions, without losing specificity. The mitochondrial genomes of 19 *Phytophthora* and 15 *Pythium* spp. were sequenced and highly conserved gene order differences were identified (F. Martin, unpublished). The multiplex assay includes a *Phytophthora* genus-specific amplicon with annealing sites for genus and species-specific TaqMan probes, as well as a control amplicon to validate DNA amplification (G. Bilodeau, F. Martin, M.D. Coffey and C. Blomquist, unpublished). An extensive sequence database of the amplified region for most species in the genus has been developed and used to design species-specific TaqMan probes for 14 species with *in silico* analysis suggesting that probes specific for an additional 65–70 species may be possible (the sequence database is also useful for identification of amplified environmental templates by BLAST analysis). Evaluations with various *Pythium* and plant species has confirmed the genus-specific detection, while evaluations with DNA for multiple isolates representing over 100 species has validated the species specificity of the TaqMan probes thus far developed. This marker system is currently in the final stages of validation with environmental samples.

3.5.2 Detection technology

Although conventional PCR can be useful for diagnostics, real time PCR is increasingly the technology of choice. The cost of real time PCR thermal cyclers is becoming more affordable, and the technology offers fast sample processing, greater sensitivity, the ability to multiplex and identify multiple targets in the same amplification, high throughput analysis and quantification capabilities. Real-time assays using a labelled probe (e.g. TaqMan, Molecular Beacon, Scorpion) are desirable compared with SYBR Green due to enhanced specificity and the option to multiplex different diagnostic markers. The development of techniques that do not rely on expensive equipment and are less technologically complex to use would broaden the applicability of molecular diagnostic tools and enhance their potential for field-based diagnostics. One such technology that is under evaluation for detection of *P. ramorum* is isothermal loop mediated amplification (LAMP). Tomlinson *et al*. (2010) reported a technique where the nitrocellulose membrane from a serological test was the source of target DNA (thereby eliminating the need for DNA extraction) and LAMP was used to detect the pathogen. Another isothermal amplification technique that may be useful for detection of *Phytophthora* is the nicking enzyme amplification reaction (NEAR; Spenlinhaur *et al*., 2011). Other isothermal amplification technologies such as recombinant protein amplification (RPA; Piepenburg *et al*., 2006)

or helicase dependent amplification (HDA; Tong *et al.*, 2011) may be useful as well, and kits for these are commercially available.

Array-based detection methods can also be useful to identify isolates and have the advantage of options to identify multiple species in a single assay or, when evaluating cultured isolates, determine if isolates are hybrids. Several arrays that included *Phytophthora* spp. have been reported (reviewed in Martin *et al.*, 2012), and recently a macroarray that uses three loci (*cox1*, *cox1* and *cox2* spacer region, and the ITS region) was developed that is capable of identifying 84 out of 97 species evaluated (W. Chen, Z.R. Djama, M.D. Coffey, F.N. Martin, G.J. Bilodeau, L. Radmer, G. Denton and C.A. Lévesque, unpublished). In a modification of traditional array design, Szemes *et al.* (2005) developed a technology referred to as padlock probes. Species-specific sequences at the termini of the probe anneal to the target sequence and the probe is circularized. Internally located universal primers are then used to amplify the circularized probe and a unique sequence referred to as a 'zip code' is used for species-specific detection either by annealing to a probe (for example, TaqMan real time PCR; van Doorn *et al.*, 2007) or for hybridization to an array (Bonants *et al.*, 2011).

3.5.3 Considerations for marker development and use

Most molecular marker systems detect single species. While this specificity is important to determine if a particular species is present, it may not provide all the information that it would be useful to have. The ideal detection system will simultaneously: (i) detect all species in a genus; (ii) target multiple loci to ensure accuracy of the results; and (iii) contain a control marker to ensure the sample DNA is amplifiable. Just as importantly, it should be highly reproducible and not dependent on narrowly defined amplification parameters for performance. Several genus-specific primers have been reported (Martin *et al.*, 2004; Drenth *et al.*, 2006; Kox *et al.*, 2007; Schena *et al.*, 2008; Bilodeau *et al.*, 2009), but some of these have non-specific amplification of *Pythium* spp. and should be tested to confirm specificity. A recent report by Scibetta *et al.* (2012) described the use of two genus-specific primers based in the ITS region that were effective in evaluating species diversity in soil and riparian ecosystems. They sampled water using a backpack sprayer with an inline filter to capture pathogen propagules. DNA was extracted and genus-specific amplicons cloned and sequenced to characterize species diversity. The genus-specific amplicon used for the mitochondrial-based diagnostic TaqMan marker system noted above (G. Bilodeau, F. Martin, M.D. Coffey and C. Blomquist, unpublished) would also be useful for this purpose and complement the nuclear-based ITS results. In addition, it would be beneficial to have primers useful for a wider spectrum of oomycete genera to analyse *Phytophthora* community structure in the broader context of oomycetes in an ecosystem. Amplification primers for a range of oomycetes have been reported by Arcate *et al.* (2006), Bent *et al.* (2009) and Giresse *et al.* (2010).

In addition to specificity, it is also important that diagnostic markers be sensitive enough to detect pathogens when present in low amounts. This is particularly important for detection of quarantine species but can also be useful when large numbers of samples need to be processed and bulking prior to DNA extraction would reduce the workload. Sensitivity is greatly enhanced by the use of high copy number targets such as rDNA, elicitin or mitochondrial loci. When using a TaqMan assay another possibility for enhanced sensitivity is the selection of master mix, as Bilodeau *et al.* (2012) observed that the use of Real Master Mix without Rox (5 Prime, Gaithersburg, Maryland) lowered the C_t (the cycle threshold) by approximately four rounds compared with amplification of the same template with TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, California). When changing the amplification master mix it is important to

reconfirm specificity as we have encountered alteration in specificity when the brand of master mix was changed.

With the ability to measure fluorescence during amplification, real time PCR provides an opportunity for quantifying the amount of the pathogen present in samples; however, it is important to take into consideration possible differences in copy number at both an intra- and an interspecific level. This could be particularly important when using ribosomal DNA (rDNA) as the target as copy number variation has been reported for this locus in Eumycota fungi, and there is indirect evidence this occurs for *Pythium* spp. (reviewed in Martin *et al.*, 2012) and *Phytopythium vexans* (Spies *et al.*, 2011). When working on a TaqMan assay for quantification of *Verticillium dahliae*, Bilodeau *et al.* (2012) observed that the copy number of the rDNA varied among isolates from approximately 24 to 73 copies per haploid genome, and while this potentially could shift the C_t of an amplification by as much as 1.8, it did not have any effect on the accuracy of the quantification of this pathogen in California soils. Variation in a target locus at an intraspecific level may also limit the utility for analysing community structure. Additional research is needed to evaluate if mitochondrial DNA copy number is constant among propagules or lesions of differing age before this genome could be used as a target for quantification purposes.

Another important consideration for quantification assays is the use of a control to measure amplification efficiency. Different soils or tissue samples may have differing amounts of PCR inhibitors, which in turn could influence the amount of template ultimately amplified. The internal control can be added to the master mix and ideally would have the same amplification primers as the pathogen template (but a different TaqMan probe). While this type of internal control has not been reported for use with *Phytophthora* quantification, it has with other pathogens and should be adaptable (Haudenshield and Hartman, 2011; Bilodeau *et al.*, 2012).

3.6 Future Directions

In recent years the availability of curated sequences for nuclear and mitochondrial loci has significantly increased our ability to identify isolates to a species level and to identify new species. However, there are still species complexes where additional research is needed to help align the results from phylogenetic analysis with more updated taxonomic descriptions. Thus far 14 loci have been used for phylogenetic studies, and as more genomes are sequenced additional phylogenetically informative loci will be identified and should facilitate clarification of these species complexes.

The increase in available sequence data and renewed interest in the development of methods for pathogen detection brought on by concerns about exotic species has led to significant improvements in real time PCR and array-based diagnostic capabilities. However, to fully realize the potential of these assays, further work is needed to reduce the complexity and cost and, ideally, to allow onsite analysis. While isothermal amplification methods are promising, additional work is required to fully evaluate/validate the potential of the technology. One limitation with many diagnostic assays is that they can't differentiate between DNA from viable and non-viable cells. Since DNA can survive for differing periods of time depending on the environment, confirmation of viable cells may be important. Vettraino *et al.* (2010) and Chimento *et al.* (2012) approached this for *Phytophthora cambivora* and *P. ramorum*, respectively, by targeting the mRNA of the *cox2* or *cox1* gene for reverse transcription followed by PCR amplification. Additional efforts to develop assays enabling this distinction would be beneficial. With the continued improvements in DNA sequencing technology, data management software and reductions in cost, it is likely that large-scale sequencing for elucidation of species communities and perhaps detection of high-risk quarantine species may become more commonplace.

References

Arcate, J.M., Karp, M.A. and Nelson, E.B. (2006) Diversity of Peronosporomycete (oomycete) communities associated with the rhizosphere of different plant species. *Microbial Ecology* 51, 36–50.

Bent, E., Loffredo, A., Yang, J.I., McKenry, M.V., Becker, J.O. and Borneman, J. (2009) Investigations into peach seedling stunting caused by a replant soil. *FEMS Microbiology Ecology* 68, 192–200.

Bilodeau, G., Pelletier, G., Pelletier, F., Lévesque, C.A. and Hamelin, R.C. (2009) Multiplex real-time polymerase chain reaction (PCR) for detection of *Phytophthora ramorum*, the causal agent of sudden oak death. *Canadian Journal of Plant Pathology* 31, 195–210.

Bilodeau, G.J., Levesque, C.A., de Cock, A.W.A.M., Duchaine, C., Briere, S., Uribe, P., Martin, F.N. and Hamelin, R.C. (2007) Molecular detection of *Phytophthora ramorum* by real-time polymerase chain reaction using TaqMan, SYBR Green, and molecular beacons. *Phytopathology* 97, 632–642.

Bilodeau, G.J., Koike, S.T., Uribe, P. and Martin, F.N. (2012) Development of an assay for rapid detection and quantification of *Verticillium dahliae* in soil. *Phytopathology* 102, 331–343.

Blair, J.E., Coffey, M.D., Park, S.Y., Geiser, D.M. and Kang, S.C. (2008) A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. *Fungal Genetics and Biology* 45, 266–277.

Bonants, P.J.M., Gaszczyk, K., Mendes, O., Verstappen, E. and Schoen, C.D. (2011) Multiplex detection of *Phytophthora*: padlock probe based universal detection multiplex array (PUMA). *Phytopathology* 101, S18 (abstract).

Broders, K.D., Lipps, P.E., Paul, P.A. and Dorrance, A.E. (2007) Characterization of *Pythium* spp. associated with corn and soybean seed and seedling disease in Ohio. *Plant Disease* 91, 727–735.

Chimento, A., Cacciola, S.O. and Garbelotto, M. (2012) Detection of mRNA by reverse-transcription PCR as an indicator of viability in *Phytophthora ramorum*. *Forest Pathology* 42, 14–21.

Cooke, D.E.L., Duncan, J.M., Williams, N.A., Hagenaar-De Weerdt, M. and Bonants, P. (2000) Identification of *Phytophthora* species on the basis of restriction enzyme fragment analysis of the internal transcribed spacer regions of the ribosomal RNA. *EPPO Bulletin* 30, 519–523.

Cooke, D.E.L., Schena, L. and Cacciola, S.O. (2007) Tools to detect, identify and monitor *Phytophthora* species in natural ecosystems. *Journal of Plant Pathology* 89, 13–28.

Drenth, A., Wagels, G., Smith, B., Sendall, B., O'Dwyer, C., Irvine, G. and Irwin, J.A.G. (2006) Development of a DNA-based method for detection and identification of *Phytophthora* species. *Australasian Plant Pathology* 35, 147–159.

Förster, H. and Coffey, M.D. (1990) Mating behavior of *Phytophthora parasitica*: evidence for sexual recombination in oospores using DNA restriction fragment length polymorphisms as genetic markers. *Experimental Mycology* 14, 351–359.

Gallegly, M. and Hong, C. (2008) Phytophthora*. Identifying Species by Morphology and DNA Fingerprints.* APS Press, St Paul, Minnesota.

Gavino, P.D. and Fry, W.E. (2002) Diversity in and evidence for selection on the mitochondrial genome of *Phytophthora infestans*. *Mycologia* 94, 781–793.

Ghimire, S.R., Richardson, P.A., Moorman, G.W., Lea-Cox, J.D., Ross, D.S. and Hong, C.X. (2009) An *in-situ* baiting bioassay for detecting *Phytophthora* species in irrigation runoff containment basins. *Plant Pathology* 58, 577–583.

Giresse, X., Ahmed, S., Richard-Cervera, S. and Delmotte, F. (2010) Development of new oomycete taxon-specific mitochondrial cytochrome *b* region primers for use in phylogenetic and phylogeographic studies. *Journal of Phytopathology* 158, 321–327.

Grünwald, N.J., Martin, F.N., Larsen, M.M., Sullivan, C.M., Press, C.M., Coffey, M.D., Hansen, E.M. and Parke, J.L. (2011) *Phytophthora*-ID.org: a sequence-based *Phytophthora* identification tool. *Plant Disease* 95, 337–342.

Haudenshield, J.S. and Hartman, G.L. (2011) Exogenous control increase negative call veracity in multiplexed, quantitative PCR assays for *Phakopsora pachyrhizi*. *Plant Disease* 95, 343–352.

Kong, P., Hong, C., Richardson, P.A. and Gallegly, M.E. (2003) Single-strand-conformation polymorphism of ribosomal DNA for rapid species differentiation in genus *Phytophthora*. *Fungal Genetics and Biology* 39, 238–249.

Kong, P., Richardson, P.A. and Hong, C.X. (2005) Direct colony PCR-SSCP for detection of multiple pythiaceous oomycetes in environmental samples. *Journal of Microbiological Methods* 61, 25–32.

Kox, L.F.F., van Brouwershaven, I.R., van de Vossenberg, B.T.L.H., van den Beld, H.E., Bonants, P.J.M. and de Gruyter, J. (2007) Diagnostic values and utility of immunological, morphological, and molecular methods for *in planta* detection of *Phytophthora ramorum*. *Phytopathology* 97, 1119–1129.

Mammella, M.A., Cacciola, S.O., Martin, F. and Schena, L. (2011) Genetic characterization of *Phytophthora nicotianae* by the analysis of polymorphic regions of the mitochondrial DNA. *Fungal Biology* 115, 432–442.

Martin, F.N. (2008) Mitochondrial haplotype determination in the oomycete plant pathogen *Phytophthora ramorum*. *Current Genetics* 54, 23–34.

Martin, F.N. and Coffey, M.D. (2012) Mitochondrial haplotype analysis for differentiation of isolates of *Phytophthora cinnamomi*. *Phytopathology* 102, 229–239.

Martin, F.N. and Tooley, P.W. (2004) Identification of *Phytophthora* isolates to species level using restriction fragment length polymorphism analysis of a polymerase chain reaction-amplified region of mitochondrial DNA. *Phytopathology* 94, 983–991.

Martin, F.N., Tooley, P.W. and Blomquist, C. (2004) Molecular detection of *Phytophthora ramorum*, the causal agent of sudden oak death in California, and two additional species commonly recovered from diseased plant material. *Phytopathology* 94, 621–631.

Martin, F.N., Coffey, M.D., Zeller, K., Hamelin, R.C., Tooley, P., Garbelotto, M., Hughes, K.J.D., Kubisiak, T., Bilodeau, G.J., Levy, L., Blomquist, C. and Berger, P.H. (2009) Evaluation of molecular markers for *Phytophthora ramorum* detection and identification: testing for specificity using a standardized library of isolates. *Phytopathology* 99, 390–403.

Martin, F.N., Abad, G., Balci, Y. and Ivors, K. (2012) Identification and detection of *Phytophthora*: reviewing our progress, identifying our needs. *Plant Disease* 96, 1080–1103.

O'Brien, P.A., Williams, N. and Stj Hardy, G.E. (2009) Detecting *Phytophthora*. *Critical Reviews in Microbiology* 35, 169–181.

Piepenburg, O., Williams, C.H., Stemple, D.L. and Armes, N.A. (2006) DNA detection using recombinant proteins. *PLoS Biology* 4, e204.

Reeser, P.W., Sutton, W., Hansen, E.M., Remigi, P. and Adams, G.C. (2011) *Phytophthora* species in forest streams in Oregon and Alaska. *Mycologia* 103, 22–35.

Robideau, G.P. *et al*. (2011) DNA barcoding of oomycetes with cytochrome *c* oxidase subunit I and internal transcribed spacer. *Molecular Ecology Research* 11, 1002–1011.

Rytkönen, A., Lilja, A. and Hantula, J. (2012) PCR-DGGE method for *in planta* detection and identification of *Phytophthora* species. *Forest Pathology* 42, 22–27.

Schena, L., Hughes, K.J.D. and Cooke, D.E.L. (2006) Detection and quantification of *Phytophthora ramorum*, *P. kernoviae*, *P. citricola* and *P. quercina* in symptomatic leaves by multiplex real-time PCR. *Molecular Plant Pathology* 7, 365–379.

Schena, L., Duncan, J.M. and Cooke, D.E.L. (2008) Development and application of a PCR-based 'molecular tool box' for the identification of *Phytophthora* species damaging forests and natural ecosystems. *Plant Pathology* 57, 64–75.

Scibetta, S., Schena, L., Chimento, A., Cacciola, S.O. and Cooke, D.E.L. (2012) A molecular method to assess *Phytophthora* diversity in environmental samples. *Journal of Microbiological Methods* 88 (3), 356–368.

Spenlinhaur, T.R., Judice, S., Lampton, P., Hardingham, J., Estock, M., Kovacs, S., Hoyos, G., McFadd, T.K. and Parker, B.O. (2011) The use of isothermal DNA amplification (NEAR) in plant disease diagnostics. *Phytopathology* 101, S215.

Spies, C.F.J., Mazzola, M. and McLeod, A. (2011) Characterisation and detection of *Pythium* and *Phytophthora* species associated with grapevines in South Africa. *European Journal of Plant Pathology* 131, 103–119.

Szemes, M., Bonants, P., de Weerdt, M., Baner, J., Landegren, U. and Schoen, C.D. (2005) Diagnostic application of padlock probes – multiplex detection of plant pathogens using universal microarrays. *Nucleic Acids Research* [Online] 33, e70 doi:10.1093/nar/gni069.

Tomlinson, J.A., Dickinson, M.J. and Boonham, N. (2010) Rapid detection of *Phytophthora ramorum* and *P. kernoviae* by two-minute DNA extraction followed by isothermal amplification and amplicon detection by generic lateral flow device. *Phytopathology* 100, 143–149.

Tong, Y., Lemieux, B. and Kong, H. (2011) Multiple strategies to improve sensitivity, speed and robustness of isothermal nucleic acid amplification for rapid pathogen detection. *BMC Biotechnology* 11, 50.

Tooley, P.W., Martin, F.N., Carras, M.M. and Frederick, R.D. (2006) Real-time fluorescent polymerase chain reaction detection of *Phytophthora ramorum* and *Phytophthora pseudosyringae* using mitochondrial gene regions. *Phytopathology* 96, 336–345.

van Doorn, R., Szemes, M., Bonants, P., Kowalchuk, G.A., Salles, J.F., Ortenberg, E. and Schoen, C. (2007) Quantitative multiplex detection of plant pathogens using a novel ligation probe-based system coupled with universal, high-throughput real-time PCR on OpenArrays. *BMC Genomics* 8, 276. Available at: http://www.biomedcentral.com/1471-2164/8/276 (accessed 1 January 2012).

Vettraino, A.M., Tomassini, A. and Vannini, A. (2010) Use of mRNA as an indicator of the viability of *Phytophthora cambivora*. *Acta Horticulturae* 866, 431–434.

Wattier, R.A.M., Gathercole, L.L., Assinder, S.J., Gliddon, C.J., Deahl, K.L., Shaw, D.S. and Mills, D.I. (2003) Sequence variation of intergenic mitochondrial DNA spacers (mtDNA-IGS) of *Phytophthora infestans* (oomycetes) and related species. *Molecular Ecology Notes* 3, 136–138.

4 Characterizing *Phytophthora* Populations

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4.1 Introduction

Phytophthora is aptly named the plant destroyer. When a virulent species encounters a susceptible host, under the proper conditions, the scale of destruction is often impressive. Chapter 2 overviews modern approaches to identifying *Phytophthora* species based on autosomal and/or mitochondrial loci. For species identification multi-copy loci are advantageous, and as long as the loci are simultaneously conserved within and variable between the evolutionarily distinct species everything works well. Things are quite different when it comes to analysing variation within a species – especially at the population level. Traits or markers suitable for characterizing populations are generally not appropriate for species identification because they are either too variable or not variable enough.

A population is a group of individuals sharing a unique pool of genetic variation. For *Phytophthora*, populations often contain a large proportion of mitotically derived isolates known as clonal lineages. Depending on the species and situation these may be distributed locally on nearby lesions, a few plants or a field, or widely across a state, region or entire country. Examples of widely distributed clonal lineages are *Phytophthora infestans* and *Phytophthora ramorum* in the USA and Europe, and *Phytophthora capsici* in Peru and Argentina (Lamour and Kamoun, 2009) (see Cooke and Andersson, Chapter 7, and Granke *et al.*, Chapter 11, this volume). In other scenarios *Phytophthora* populations include genetically unique individuals produced via sexual recombination as well as clonal lineages. This is the situation for *P. infestans* at some locations in Mexico and Europe, and *P. capsici* at many locations in the USA (Chapters 7 and 11, this volume). In the cases of *P. infestans* and *P. capsici*, populations vary considerably depending on the presence or absence of sexual recombination. If the right tools are available and sampling is conducted properly, a population study can illuminate how epidemic populations survive and spread and may reveal new ways to limit disease. There have been numerous investigations of intraspecific variation for species of *Phytophthora* including surveys and population analyses. Both provide useful insights and will be discussed further.

And finally, a growing body of evidence indicates significant changes can occur at the genetic level during mitotic growth and that the population of nuclei within a single *Phytophthora* individual may be highly diverse (Chamnanpunt *et al.*, 2001; Catal *et al.*, 2010; Lamour *et al.*, 2012). Although the mechanisms driving the diversity during asexual growth are not known, the implications for population studies and adaptive evolution may be profound.

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4.2 Traits for Characterizing Populations

To study a population is to study a collection of individuals. Individuality in *Phytophthora* is often established using a two-step process. An isolate is recovered from a single plant (or a discrete lesion) and a single zoospore or hyphal-tip culture isolated. This ensures subsequent analyses are based on isolates stemming from one or a few nuclei. In general, analyses are based on the assumption that individuals are diploid, although triploids are found with some species. There is no accessible haploid life stage.

Ideal traits for characterizing populations meet a number of criteria: (i) the traits are polymorphic; (ii) the variable states are derived from a common ancestor; (iii) the mode of inheritance is known; (iv) the traits are unlinked and selectively neutral; and (v) the variable states can be measured unambiguously. For molecular markers the variable states are called alleles. A significant challenge with many *Phytophthora* species is making crosses in the laboratory (e.g. *P. ramorum*), and it may be difficult or impossible to test for simple Mendelian inheritance and linkage between traits. Roughly half the *Phytophthora* species are primarily self-fertile (homothallic), a process that quickly fixes (or removes) novel alleles. Most other species require outcrossing to reproduce sexually (heterothallic). There are a few species that appear unable to produce sexual oospores (sterile) and these include *Phytophthora citrophthora* and *Phytophthora pinifolia* (see Ahumada *et al.*, Chapter 17, this volume). The mating strategy, inbreeding or outbreeding, or some combination of the two, has a significant impact on the level of polymorphism within individuals and on the genetic structure of populations (Goodwin, 1997).

4.2.1 Phenotypes

Prior to the advent of molecular tools various phenotypes and abilities were used to characterize diversity. These include: (i) spore shape and ornamentation; (ii) growth optima; (iii) host preference; (iv) fungicide resistance; and (v) mating type. Many studies (and much practical experience) indicate these characters can vary widely within a single isolate and rarely meet the criteria for good markers. Even mating type, often used to characterize outbreeding populations, can switch in *P. capsici* isolates undergoing loss of heterozygosity (LOH) across the mating type region (Lamour *et al.*, 2012). Plasticity for mating type has also been reported in field isolates of *Phytophthora colocasiae* (Lin and Ko, 2008) and it may be unstable in other outcrossing *Phytophthora* species.

An example of a stable phenotypic character is the metalaxyl/mefenoxam resistance reported in Michigan populations of *P. capsici*. A series of crosses between resistant and sensitive isolates identified a single incompletely dominant resistance gene of major effect when isolates were screened with a high dose of mefenoxam (Lamour and Hausbeck, 2000). Analysis of genetically diverse mefenoxam-resistant populations found no obvious fitness cost for resistance and it appears resistant populations are unlikely to revert to sensitivity (Lamour and Hausbeck, 2001). Despite limitations, when used in conjunction with appropriate molecular markers, phenotypes are very helpful to paint a full picture of a population's diversity.

4.2.2 Proteins and DNA fragments

Some of the earliest molecular-level studies of intraspecific variation utilized isozyme markers. These are based on the migration of homologous (and active) proteins in a gel matrix. Although the resolution of isozyme profiles can be laborious compared with some of the newer genetic approaches, the markers are useful for measuring variation and are employed with some species today (see for example Érsek and Man in 't Veld, Chapter 5, this volume). The most common DNA-level markers are based on length variation for homologous DNA fragments.

These include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats or microsatellite (SSR) markers. In most cases, the markers are based on autosomal DNA, although mitochondrial DNA has been used (see Martin, Chapter 3, this volume). Same-sized fragments are considered identical and the location of the DNA fragments in the genome is often not known. Because the markers are based on an estimation of fragment length, it can be difficult to compare results produced on different analytic platforms.

A limitation for any marker based on DNA fragment size is convergence. Identically sized fragments may not represent the same ancestral evolutionary event. For example, an SSR marker may lose or gain different parts of the repeat sequence, resulting in identically sized alleles. A similar problem occurs when similarly sized fragments (but not identical) are scored as identical. Accurately sizing fragments can be quite challenging when many fragments are resolved in a single reaction (e.g. AFLP). Whether it is true convergence or simply a scoring error, the result is a false impression of similarity. Nonetheless, many fragment-length-based markers are used successfully to characterize populations of *Phytophthora*.

4.2.3 Single nucleotide variation and the whole genome

High quality reference genomes are now available for four *Phytophthora* species (*P. ramorum*, *P. infestans*, *P. capsici* and *Phytophthora sojae*) and more are in the works (see Ludowici *et al.*, Chapter 13, this volume). Because *Phytophthora* is diploid almost any sequencing project will reveal heterozygous sites within a single isolate. A heterozygous site within an isolate is referred to as a single nucleotide variant (SNV), and an SNV only becomes a single nucleotide polymorphism (SNP) marker after the allele frequencies are measured in a natural population. Within a species almost all SNPs are bi-allelic, and there are many techniques to unambiguously measure SNP genotypes. Rare alleles tell little about a population, and SNPs with a minor allele frequency of at least 5% are generally employed – although, the closer the alleles are to 50%, the better (Brookes, 1999).The large number and wide distribution of SNPs in many eukaryotic genomes, coupled with a variety of analytic approaches to unambiguously score genotypes, has made SNP markers increasingly popular for many organisms. With the continued trend of low-cost sequencing and high through-put SNP-typing platforms, the use of SNPs is likely to increase for *Phytophthora* (Van Tassell *et al.*, 2008).

Recently, SNP markers have been used to characterize variation at the population level for *P. capsici* (Hurtado-Gonzales *et al.*, 2008; Gobena *et al.*, 2011, 2012). In addition, a large collection of SNV sites (>20,000) has been identified through extensive genome-level focused re-sequencing, verified for simple Mendelian inheritance in a laboratory cross and placed into 18 linkage groups (Lamour *et al.*, 2012). The laboratory cross was made between isolates of *P. capsici* recovered from cucumber in Michigan and pumpkin in Tennessee. Surprisingly, there was a polymorphic site, on average, every 25 bp, with >17,000 loci heterozygous in one parent or other. Similar focused re-sequencing of seven field isolates of *P. capsici* and one isolate of *Phytophthora tropicalis* revealed a heterozygous site approximately every 200 bp with many fixed differences between *P. tropicalis* and *P. capsici*. The number of polymorphic markers in *P. capsici* is impressive, to the point of being overwhelming. How do you choose a set of markers to analyse a population? At this point, sampling the *P. capsici* genome is analogous to sampling a field where disease blankets the field. The genome sequence and linkage map provide the geography and sampling should include as many widely spaced loci as possible from across the genome. Once widely spaced loci are identified, the next step is measuring the allele frequencies within individual

populations. Just like any class of marker, the allele frequencies may vary significantly between populations.

4.3 Species and Surveys

In the past most *Phytophthora* species were described in conjunction with a specific plant disease (Erwin and Ribeiro, 1996). *P. infestans* is a good example where the economic and social consequences demanded that a proper name be put on the culprit (see Ribeiro, Chapter 1, this volume). More recently, especially with increased surveillance for invasive species, new *Phytophthora* species are being discovered and named that may not be causing significant problems (see Thines, Chapter 2, this volume). A good example is *Phytophthora foliorum* (Donahoo *et al*., 2006). *P. foliorum* was recovered from nurseries in California and Tennessee while surveying for the sudden oak death pathogen *P. ramorum*. The then unknown species was not causing large-scale damage to nursery crops and became significant because it tested positive for *P. ramorum* using an early *P. ramorum* nested PCR diagnostic assay. *P. foliorum* is homothallic and produces abundant oospores in culture, whereas *P. ramorum* is heterothallic and does not. Sequence analysis of the internal transcribed spacer (ITS) region revealed high similarity (although, not exact) in the nested target region, which allowed amplification and the false positive reaction. With ongoing surveys of natural waterways and soil, previously undescribed species are coming to light, and *P. foliorum* serves as a cautionary example of how unknown closely related taxa may masquerade as an invasive species (see Martin, Chapter 3, this volume).

4.4 Population Analyses

4.4.1 Clonal lineages

A survey is often the best way to begin a population analysis. If isolates at geographically separate locations across multiple years have identical multi-locus genotypes for known polymorphic markers, it is likely that clonal reproduction drives the population structure. This does not mean all members of the clonal lineage are identical. Evidence from field and laboratory studies indicate asexually produced propagules can vary extensively for phenotypes and molecular-level variation (Caten, 1971; Dobrowolski *et al*., 2002; Ivors *et al*., 2004; Goss *et al*., 2009; Lamour *et al*., 2012). For *P. capsici* whole genome re-sequencing of asexually derived isolates indicates loss of heterozygosity can radically alter the genotype and phenotype of isolates grown on nutrient media in the laboratory (Lamour *et al*., 2012). Loss of heterozygosity (LOH) describes a phenomenon where short (*c*.300 bp) or long (>1 Mbp) stretches of contiguous autosomal DNA have switched to one of the two possible haplotypes and are now homozygous at loci heterozygous in the 'mother' isolate. Among a collection of sexual progeny and field isolates, >11,000 SNV sites showed LOH after serial subculturing and long-term storage (Lamour *et al*., 2012). Figure 4.1 shows variation in pathogenicity to pepper for isolates of *P. capsici* following storage, subculturing and extensive LOH. For the homothallic species *P. sojae* a high frequency of gene conversion was documented in hybrid isolates produced via outcrossing (Chamnanpunt *et al*., 2001). Gene conversion refers to changes occurring in relatively short blocks of contiguous DNA (<1 kbp).

For species with a clonal structure it may be difficult to track the movement or survival of populations using the relatively small number of markers appropriate for analysing outcrossing populations. Low-cost sequencing (and re-sequencing) has the potential to revolutionize the analysis of clonal populations. A genome approach can reveal novel mitotically derived genomic variation (eg. LOH or novel mutations) making it possible to track clonal lineages within clonal lineages.

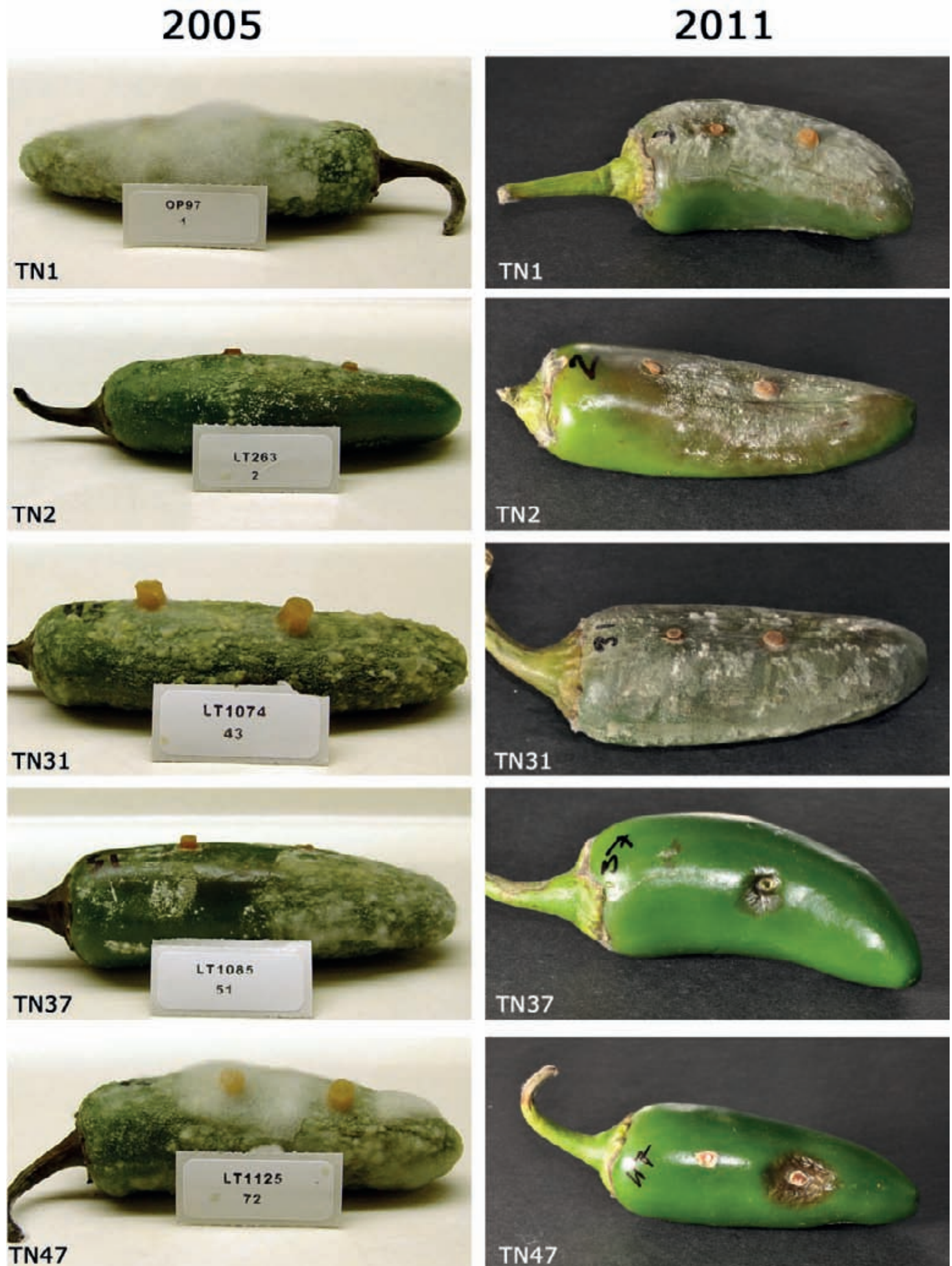

Fig. 4.1. Association of loss of heterozyosity (LOH) with loss of pathogenicity in *Phytophthora capsici*. In 2005 and 2011 *P. capsici* isolates were inoculated onto wounded (site distal to stem) and unwounded (site proximal to stem) sites on healthy jalapeno fruits. TN1 and TN2 are field isolates and TN31, TN37 and TN47 are sexual progeny produced in 2004. In 2011 the genomes for TN37 and TN47 had approximately 10% of the heterozygous nucleotide sites switched to homozygosity.

4.4.2 Sexual recombination

If a survey reveals isolates with variable genotypes, a finer scale population analyses is needed to determine the population dynamics. For example, genetic analysis of *P. capsici* and *P. tropicalis* isolates recovered from locations worldwide over many years revealed a high level of genotypic diversity (Quesada-Ocampo *et al.*, 2011). Finer scale sampling and analyses indicate *P. capsici* in Michigan and New York persist by producing sexual oospores, and epidemics are initiated by a pool of genetically diverse individuals. Clonal lineages rise to prominence locally, only to die off during the winter or crop rotation periods. The genetically diverse oospore inoculum remains dormant in the soil for years making re-entry with a susceptible host risky (Lamour and Hausbeck, 2002, 2003; Dunn *et al.*, 2010; Gobena *et al.*, 2011).

The populations of *P. capsici* in the USA are quite different from those in Peru and Argentina, where genotype analysis (AFLP and SNPs) of *P. capsici* collected from widely separated sites indicates single clonal lineages dominate across multiple years. In Peru this seems to make sense because host plants are present year round and there is no obvious selection pressure (e.g. winter or fallow period) for the thick-walled oospore (Hurtado-Gonzales *et al.*, 2008; Hulvey *et al.*, 2011). At some point in the past a single A2 mating type clonal lineage was widely dispersed and limits the production of diverse peppers (*Capsicum annuum*, *Capsicum baccatum* and *Capsicum pubescens*) and multiple varieties of tomato. The situation in Argentina is less clear: there, a fallow period exists between pepper crops, and an A1 clonal type has risen to dominance across a wide geographic area and has persisted for at least 3 years (Gobena *et al.*, 2012). For both countries the mechanism for dispersal is unknown. As stated above, clonality makes tracking the history or movement of isolates difficult, and a deeper analysis using markers across the genome (or whole genome sequences) may be helpful.

4.5 Population Dynamics

4.5.1 Sampling

Once it is known (or suspected) that diversity exists within a field (or forest or greenhouse) it is important to gather adequate samples to fully characterize the population. *Phytophthora* spreads using sporangia and zoospores, and disease often follows the patterns of water dispersal (see Fig. 10.1 in Sanogo and Bosland, Chapter 10, this volume). For fine-scale investigations the area to be studied can be laid out on a grid using permanent landmarks as an anchor. Collections should include as many diseased samples as possible from as many grid quadrants as possible, keeping in mind the common modes of asexual dispersal. It is difficult to specify an exact number of samples to clearly outline a population's structure as this may depend on when the samples are collected (beginning versus height of an epidemic). Bear in mind that one can always throw samples away but never go back in time to collect more. If possible, samples should be collected from the same site at multiple times during the season and at the same site in subsequent years. Because *Phytophthora* isolates may change in culture, it is best to extract genomic DNA as soon as possible. As molecular technologies advance, the best approach may be to assess isolate diversity within the context of the infected plant. For example, a 12-plex SSR PCR assay is currently used to genotype blight lesions in Europe (Li, 2012). In the near future it may be feasible to employ genome-level sequencing of the infected plant(s) and the analysis will include *Phytophthora*, the host, and any other intermingled organisms.

4.5.2 Allele frequency

Phytophthora is generally diploid throughout its life cycle, and the analysis of the population dynamics (e.g. sexual recombination, population sub-structuring, migration, etc.) relies on measures of allele frequency.

There are exceptions. For example, some *P. infestans* isolates are triploid (Hamed and Gisi, 2012). Allele frequency calculation will be invalidated by the presence of clones – meaning that clone correction is required. It's useful to have a reference panel of diverse isolates from a wide geographic origin to develop novel markers with a higher probability of being polymorphic. This can also be accomplished at the population level, and for a study of *P. capsici* populations on Long Island, New York, an A1 and an A2 isolate were selected from different fields and candidate SNPs identified by sequencing single copy genes and choosing heterozygous loci (Gobena *et al.*, 2011). This ensured that the markers had some level of polymorphism but did not ensure high levels of variation. Analysis of 14 SNP markers revealed alternate allele frequencies ranging from 4% to 28%. An additional step would be to test each candidate SNP on a panel of ten isolates, determine a putative allele frequency and only go forward with markers showing a predetermined level of polymorphism (e.g. >20%). Here again, the clonal component of the population can skew estimates. For example, in the case of *P. capsici* on Long Island there were 54 clonal lineages ranging in size from two to 26 members and 128 unique multi-locus genotypes from an initial set of 373 isolates (Gobena *et al.*, 2011).

4.5.3 Sex, survival, the Hardy–Weinberg equilibrium (HWE) and fixation indices

Once a population has been clone corrected the allele frequencies can be used to test for sexual recombination and to determine the level of sub-structuring among populations. The classic test for sexual recombination is based on the HWE. The assumptions for HWE are based on an idealized population (any isolate can mate with any other, markers are neutral and unlinked, etc.) but, even when the populations are known to deviate from these assumptions, it has proven to be a useful test (Hartl, 2000; Lamour and Hausbeck, 2002). For a bi-allelic SNP marker the calculations are simple. For example, the HWE formula for a marker with two alleles (**A** and **a**) is $p^2 + 2pq + q^2 = 1$ where p = frequency of **A** and q = frequency of **a**, and the expected proportion of each of the three possible genotypes is calculated as **AA** = p^2, **Aa** = $2pq$ and **aa** = q^2. If the allele frequency q is determined to be 0.40, HWE predicts 48% of the individuals should have a heterozygous (**Aa**) genotype ($2 \times 0.60 \times 0.40 = 0.48$). To test if the observed proportions of each genotype deviate from expectations based on HWE, a χ^2-test (or other statistical tests) can be used (Hartl, 2000). For loci with more than two alleles the formula is expanded (Hartl, 2000). For *P. capsici* most markers tested for populations in Michigan were in HWE (Lamour and Hausbeck, 2002).

If a population is in HWE for a given year, populations from the same location in subsequent years can be compared to determine the impact of migration or population bottlenecks. If there is migration from a genetically distinct population, or if the population has passed through a bottleneck due to poor survival (or many other factors), the allele frequencies will shift. On the other hand, if sufficient sexual propagules survive from season to season the allele frequencies will remain stable. This was tested for a population of *P. capsici* in Michigan over a 4-year period where the two middle years were rotation crops. Here the populations from the first and fourth years were separately determined to be in HWE and the allele frequencies remained stable over 4 years (Lamour and Hausbeck, 2003). The crop in the first year was processing cucumbers and in the fourth year was processing tomatoes, suggesting sufficient oospores survived the 2 years of crop rotation to maintain allele frequencies and there was not a significant shift based on host selection pressure.

For sexual populations (hetero- and homothallic species) the degree of population sub-structuring is estimated using allele frequencies. Population structure analyses require sufficiently detailed sampling to adequately estimate allele frequencies within each population. Population-specific

allele frequencies are compared using tests for inbreeding known as fixation indices (Hartl, 2000). Inbreeding takes place when individuals preferentially mate within a population and allele frequencies gradually drift (shift) to population-specific frequencies, including fixation. Shifts in allele frequency are due to many stochastic factors, including founder effects and population bottlenecks. A low frequency of migration can homogenize allele frequencies (Hartl, 2000). Fine-scale population analyses of geographically separated populations of *P. capsici* in Michigan indicate populations contain discrete pools of genetic variation (Lamour and Hausbeck, 2001).

4.6 Conclusions and Future Directions

The prospects for *Phytophthora* population-level analyses have never been better. Rapid development of lower-cost sequencing and marker technologies coupled with multiple high quality reference genomes opens the door for sensitive population analyses in some species. Important virulence genes can be tracked in conjunction with neutral markers to measure dynamics within individual populations or widely dispersed clonal lineages (Raffaele *et al.*, 2010). It is now possible to envisage studies based on whole genome re-sequencing across entire populations – ushering in the age of population genomics where the impact of various control measures (fungicides or resistant host plants) can be measured directly. Traditionally, these population-level tools have come at a high price economically. Hopefully, the trend of decreasing costs for genetic discovery will continue and there will be similar genetic resources developed for the less advantaged species. One thing that is clear after 150+ years of research is that *Phytophthora* will often find a way around our best control efforts (Goodwin *et al.*, 1995; Fry, 2008). In the future the tools for characterizing populations may advance to the point where control measures are tailored to discrete pools of genetic variation.

References

Brookes, A.J. (1999) The essence of SNPs. *Gene* 234, 177–186.

Catal, M., King, L., Tumbalam, P., Wiriyajitsomboon, P., Kirk, W.W. and Adams, G.C. (2010) Heterokaryotic nuclear conditions and a heterogeneous nuclear population are observed by flow cytometry in *Phytophthora infestans*. *Cytometry Part A* 77A, 769–775.

Caten, C.E. (1971) Single zoospore variation in *Phytophthora infestans* and attenuation of strains in culture. *Transactions of the British Mycological Society* 56, 1–7.

Chamnanpunt, J., Shan, W. and Tyler, B.M. (2001) High frequency mitotic gene conversion in genetic hybrids of the oomycete *Phytophthora sojae*. *Proceedings of the National Academy of Sciences USA* 98, 14530–14535.

Dobrowolski, M.P., Tommerup, I.C., Blakeman, H.D. and O'Brien, P.A. (2002) Non-Mendelian inheritance revealed in a genetic analysis of sexual progeny of *Phytophthora cinnamomi* with microsatellite markers. *Fungal Genetics and Biology* 35, 197–212.

Donahoo, R., Blomquist, C.L., Thomas, S.L., Moulton, J.K., Cooke, D.E.L. and Lamour, K.H. (2006) *Phytophthora foliorum* sp. nov., a new species causing leaf blight of azalea. *Mycological Research* 110, 1309–1322.

Dunn, A.R., Milgroom, M.G., Meitz, J.C., McLeod, A., Fry, W.E., McGrath, M.T., Dillard, H.R. and Smart, C.D. (2010) Population structure and resistance to mefenoxam of *Phytophthora capsici* in New York state. *Plant Disease* 94, 1461–1468.

Erwin, D.C. and Ribeiro, O.K. (1996) *Phytophthora Diseases Worldwide*. APS Press, St Paul, Minnesota, 562 pp.

Fry, W. (2008) *Phytophthora infestans*: the plant (and *R* gene) destroyer. *Molecular Plant Pathology* 9, 385–402.

Gobena, D., McGrath, M.T. and Lamour, K. (2011) Survival and spread of *Phytophthora capsici* on Long Island, New York. *Mycological Progress* 11, 761–768.

Gobena, D., Roig, J., Galmarini, C., Hulvey, J. and Lamour, K. (2012) Genetic diversity of *Phytophthora capsici* isolates from pepper and pumpkin in Argentina. *Mycologia* 104, 102–107.
Goodwin, S.B. (1997) The population genetics of *Phytophthora*. *Phytopathology* 87, 462–473.
Goodwin, S.B., Sujkowski, L.S. and Fry, W.E. (1995) Rapid evolution of pathogenicity within clonal lineages of the potato late blight disease fungus. *Phytopathology* 85, 669–676.
Goss, E.M., Carbone, I. and Grunwald, N.J. (2009) Ancient isolation and independent evolution of the three clonal lineages of the exotic sudden oak death pathogen *Phytophthora ramorum*. *Molecular Ecology* 18, 1161–1174.
Hamed, B.H. and Gisi, U. (2012) Generation of pathogenic F_1 progeny from crosses of *Phytophthora infestans* isolates differing in ploidy. *Plant Pathology*. Available at: doi:10.1111/j.1365-3059.2012.02655.x (accessed 19 September 2012).
Hartl, D. (2000) *A Primer of Population Genetics*. Sinauer Associates, Sunderland, Massachussets.
Hulvey, J., Hurtado-Gonzales, O., Aragon-Caballero, L., Gobena, D., Storey, D., Finley, L. and Lamour, K. (2011) Genetic diversity of the pepper pathogen *Phytophthora capsici* on farms in the Amazonian high jungle of Peru. *American Journal of Plant Sciences* 2, 461–466.
Hurtado-Gonzales, O., Aragon-Caballero, L., Apaza-Tapia, W., Donahoo, R. and Lamour, K. (2008) Survival and spread of *Phytophthora capsici* in coastal Peru. *Phytopathology* 98, 688–694.
Ivors, K.L., Hayden, K.J., Bonants, P.J., Rizzo, D.M. and Garbelotto, M. (2004) AFLP and phylogenetic analyses of North American and European populations of *Phytophthora ramorum*. *Mycological Research* 108, 378–392.
Lamour, K.H. and Hausbeck, M.K. (2000) Mefenoxam insensitivity and the sexual stage of *Phytophthora capsici* in Michigan cucurbit fields. *Phytopathology* 90, 396–400.
Lamour, K.H. and Hausbeck, M.K. (2001) The dynamics of mefenoxam insensitivity in a recombining population of *Phytophthora capsici* characterized with amplified fragment length polymorphism markers. *Phytopathology* 91, 553–557.
Lamour, K.H. and Hausbeck, M.K. (2002) The spatiotemporal genetic structure of *Phytophthora capsici* in Michigan and implications for disease management. *Phytopathology* 92, 681–684.
Lamour, K.H. and Hausbeck, M.K. (2003) Effect of crop rotation on the survival of *Phytophthora capsici* in Michigan. *Plant Disease* 87, 841–845.
Lamour, K.H. and Kamoun, S. (2009) *Oomycete Genetics and Genomics: Diversity, Interactions, and Research Tools*. Wiley-Blackwell, Hoboken, New Jersey.
Lamour, K. *et al*. (2012) Genome sequencing and mapping reveal loss of heterozygosity as a mechanism for rapid adaptation in the vegetable pathogen *Phytophthora capsici*. *Molecular Plant–Microbe Interactions* 25 (10), 1350–1360.
Li, Y. (2012) Multiplex SSR analysis of *Phytophthora infestans* in different countries and the importance for potato breeding. PhD thesis, Wageningen University, Wageningen, the Netherlands.
Lin, M.-J. and Ko, W.-H. (2008) Occurrence of isolates of *Phytophthora colocasiae* in Taiwan with homothallic behavior and its significance. *Mycologia* 100, 727–734.
Quesada-Ocampo, L.M., Granke, L.L., Mercier, M.R., Olsen, J. and Hausbeck, M.K. (2011) Investigating the genetic structure of *Phytophthora capsici* populations. *Phytopathology* 101, 1061–1073.
Raffaele, S., Win, J., Cano, L.M. and Kamoun, S. (2010) Analyses of genome architecture and gene expression reveal novel candidate virulence factors in the secretome of *Phytophthora infestans*. *BMC Genomics* 11, 637.
Van Tassell, C.P., Smith, T.P.L., Matukumalli, L.K., Taylor, J.F., Schnabel, R.D., Lawley, C.T., Haudenschild, C.D., Moore, S.S., Warren, W.C. and Sonstegard, T.S. (2008) SNP discovery and allele frequency estimation by deep sequencing of reduced representation libraries. *Nature Methods* 5, 247–252.

5 *Phytophthora* Species Hybrids: a Novel Threat to Crops and Natural Ecosystems

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5.1 Introduction

Species hybrids were first described in mythology as fantastical creatures that combined body parts of animals (e.g. Pegasus), or a man and animal (e.g. Minotaur, mermaids), and often claimed supernatural powers. Although less commonly known, nature, with or without human contribution, has also given rise to species hybrids, most notably in the animal and plant kingdom. Well-known examples include mule (horse × donkey), triticale (wheat × rye) and quite a few ornamental plants. Whereas plant hybrids have been reported for decades, interspecific hybridization in *Phytophthora* and other eukaryotic microbes has been more difficult to assess due to limited morphological characters. Although Flor (1932) pointed out the potential for hybridization among closely related plant pathogenic fungi based on the appearance of *Tilletia* isolates with atypical morphological phenotypes, and Sansome *et al.* (1991) suggested that the oomycete *Phytophthora meadii* might be a species hybrid, conclusive proof of interspecific hybrids has only arrived in the molecular era. Since the mid-1990s a limited number of species hybrids have been detected in the fungal phyla Ascomycota and Basidiomycota, as well as *Phytophthora* (cf. Schardl and Craven, 2003) and, most recently, *Pythium* (Nechwatal and Mendgen, 2009).

There are now more than 100 species in the genus *Phytophthora* (Érsek and Ribeiro, 2010). Most are harmful plant pathogens that may present serious, and unpredictable, ecological and economic problems if they hybridize. Due to their rarity, atypical morphology or, conversely, morphology resembling an existing species, interspecific *Phytophthora* hybrids are difficult to detect. One clue, at least with homothallic species, is the presence of numerous abortive oospores in culture. Molecular methods such as sequence analysis of nuclear single-copy genes and isozyme analysis have been crucial to identify hybrids. Isozyme analysis has an advantage over other techniques if dimeric enzymes are used, because it is possible to distinguish physical mixtures from crossings by the so-called intermediate, heterodimeric band. Notably, isozymes are proteins, and when two different parental alleles are present (as is the case in hybrids) their gene products combine in three different ways: (i) one homodimeric band originating from one parental allele; (ii) a second homodimeric band (with different mobility) originating from the other parental allele; and (iii) a third intermediate band composed of the gene products of both parental alleles.

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5.2 Artificially Created Hybrids

5.2.1 Sexual crosses

The first *Phytophthora* species hybrids were produced in the laboratory by Goodwin and Fry (1994) through sexual crosses of the sympatric, heterothallic species, *Phytophthora mirabilis* and *Phytophthora infestans*. DNA fingerprinting and isozyme analyses confirmed the majority of the progeny were species hybrids. Notably, the uniparentally inherited mitochondrial DNA was predominantly from the *P. infestans* parent. Interestingly, none of the F_1 progeny infected *Mirabilis jalapa*, the host for *P. mirabilis*, and only a few infected potato or tomato, the hosts for *P. infestans*. More recently, sexual crosses between these *Phytophthora* species produced hybrids able to infect tomato plants and seven out of the eight hybrid F_2 progeny able to infect tomato also infected potato (Kroon, 2010). Tomato may serve as a bridging host for *P. infestans* × *P. mirabilis* progeny. The different results of Kroon (2010) and Goodwin and Fry (1994) may be due to differences in the viability of the isolates. It was concluded that *P. infestans* and *P. mirabilis* are sexually fully compatible and that the two species diverged by a change in host.

Hybrids generated by outcrossing two homothallic species, *Phytophthora sojae* and *Phytophthora vignae*, were confirmed by random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) analyses (May *et al.*, 2003). These hybrids were pathogenic to soybean, the host for *P. sojae*, and cowpea, the host for *P. vignae*. However, the aggressiveness of these hybrids was weaker and substantially more variable when compared with the parental isolates on their respective hosts.

More recent findings based on AFLP analysis revealed oospore progeny from crosses between isolates of two heterothallic species, *Phytophthora capsici* from pumpkin and *Phytophthora tropicalis* from cacao, were apomicts and identical to one or the other parent (Donahoo and Lamour, 2008). In contrast, progeny from crosses between *P. capsici* from pumpkin and *P. tropicalis* from rhododendron were parental as well as hybrid. Hybrids had either one or the other parent mitochondrial DNA (mtDNA) type. Here too the overall pathogenicity and virulence of the hybrid progeny was greatly reduced.

5.2.2 Somatic hybridizations

Somatic fusion was also proposed as a mechanism for hybridization for species that are heterothallic and lack compatible mating types (Brasier, 1992). *In vitro* evidence of somatic hybrids was obtained by the induced fusion of zoospores derived from non-compatible mating-type isolates of the heterothallic species *P. capsici* and *Phytophthora nicotianae* (Érsek *et al.*, 1995). The fusion hybrids resembled *P. capsici* more closely than *P. nicotianae*, and their hybrid nature was confirmed using parent-specific DNA sequences. DNA of *P. capsici* was detected by hybridization of restriction fragment length polymorphisms (RFLPs) with a *P. capsici*-specific DNA probe, whereas *P. nicotianae*-specific DNA was amplified using *P. nicotianae*-specific primers or random primers (Érsek *et al.*, 1995; English *et al.*, 1999). Of the four hybrids tested each was pathogenic to tomato, a plant susceptible to both parental species, and two had an expanded host range that included both radish and lemon, hosts that are susceptible to *P. capsici* or *P. nicotianae*, respectively.

The tractability of zoospore fusion was also demonstrated by the creation of triparental hybrids derived from drug-resistant A2 mating type isolates of the heterothallic species *P. capsici*, *P. nicotianae* and *Phytophthora citrophthora* (Érsek *et al.*, 1997). Viable fusion hybrids expressed differential drug resistance derived from the parental isolates and their hybrid nature was confirmed using species-specific and arbitrary primers. However, the fusion hybrids failed to express pathogenicity to any of the parents' hosts.

5.3 Naturally Evolved Hybrids

Once artificial hybrids were produced in the laboratory, it was an open question whether interspecific hybridization occurs in nature (Goodwin and Fry, 1994; Érsek *et al.*, 1995). This question has been answered definitively with the discovery of natural hybrids between *P. nicotianae* and *Phytophthora cactorum* (Man in 't Veld *et al.*, 1998), *Phytophthora* hybrids pathogenic to alders (Brasier *et al.*, 1999), *Phytophthora hedraiandra* × *P. cactorum* hybrids (Man in 't Veld *et al.*, 2007), and, most recently, *Phytophthora andina* (see Forbes *et al.*, Chapter 6, this volume; Goss *et al.*, 2011).

5.3.1 *Phytophthora alni*, the hybrid pathogen of alders

The most intensively studied natural *Phytophthora* hybrid attacks alder species including *Alnus glutinosa*, *Alnus incana* and *Alnus cordata* (Brasier *et al.*, 1999). The hybrid pathogen was first detected on dying alder trees in southern Britain in the 1990s and within a few years caused 10–15% mortality in the region. Disease symptoms include root and collar rot, abnormally small, yellow and sparse leaves, and tarry spots at the trunk base indicative of dead phloem. The disease has since been found throughout Europe and 'running amok in Europe's woodlands', as the *New Scientist* (Pain, 1999) referred to the immense losses caused by the 'fiendish fungus'. Assessments of internal transcribed spacer (ITS) sequences and genomic polymorphisms initially suggested that the homothallic alder *Phytophthora* might be a hybrid of two alder-non-pathogenic species, the heterothallic *Phytophthora cambivora* and a homothallic *Phytophthora fragariae*-like species (Brasier *et al.*, 1999). Ultimately, the hybrid pathogen was formally described as the new species *P. alni* Brasier & S.A. Kirk (Brasier *et al.*, 2004). Because *P. alni* comprises a range of phenotypically diverse allopolyploid genotypes, the species was split into three subspecies: (i) *P. alni* subsp. *alni* (*Paa*); (ii) *P. alni* subsp. *uniformis* (*Pau*); and (iii) *P. alni* subsp. *multiformis* (*Pam*). These were originally referred to as the 'standard type', the 'Swedish variant' and 'Dutch, German or UK variants' of alder *Phytophthora*, respectively.

Strains of *Paa* occur more commonly across much of Europe and are generally more aggressive than those of *Pau* or *Pam*. *Pau* and *Pam* are also present in several European countries, and *Pau* has been recovered in Alaska, the first record outside Europe (Adams *et al.*, 2008). There are conspicuous morphological differences for gametangia in the three subspecies (Fig. 5.1A and B) and other phenotypic and genotypic features (Brasier *et al.*, 1999).

Subspecies of *P. alni* can be differentiated using several molecular traits. For instance, *Paa* has polymorphic sites in the ITS region, representative of both parental species. In contrast with *Paa*, ITS sequences in both *Pam* and *Pau* are homogeneous and resemble the ITS sequences of either *P. fragariae* or *P. cambivora*, respectively (Brasier *et al.*, 1999, 2004). In addition, subspecies can be differentiated on the basis of AFLP profiles (Brasier *et al.*, 1999), RAPD profiles, isozyme patterns (Nagy *et al.*, 2003; Brasier *et al.*, 2004; Bakonyi *et al.*, 2007) and diagnostic *P. alni*-specific PCR (Ioos *et al.*, 2005; Bakonyi *et al.*, 2006). Genetic studies reveal a great deal of genomic complexity, suggesting that *P. alni* may be a hybrid species in a state of continuing evolution. However, the evolutionary mechanism leading to the formation of subspecies is uncertain. Brasier *et al.* (1999) favoured the view that *Paa* arose via somatic fusion followed by further segregation rather than via a sexual cross between *P. cambivora* and a *P. fragariae*-like species. It's also thought that *Pau* and *Pam* then evolved through subsequent recombination events and chromosome losses in *Paa* leading to reversions towards the *P. cambivora*-like or *P. fragariae*-like parental genotypes.

According to an alternative evolutionary model, *Paa* might have arisen via hybridization of *Pam* and *Pau* with *Pau* evolving from *P. cambivora* and *Pam* representing an ancient reticulation or by autopolyploidization (Ioos *et al.*, 2006).

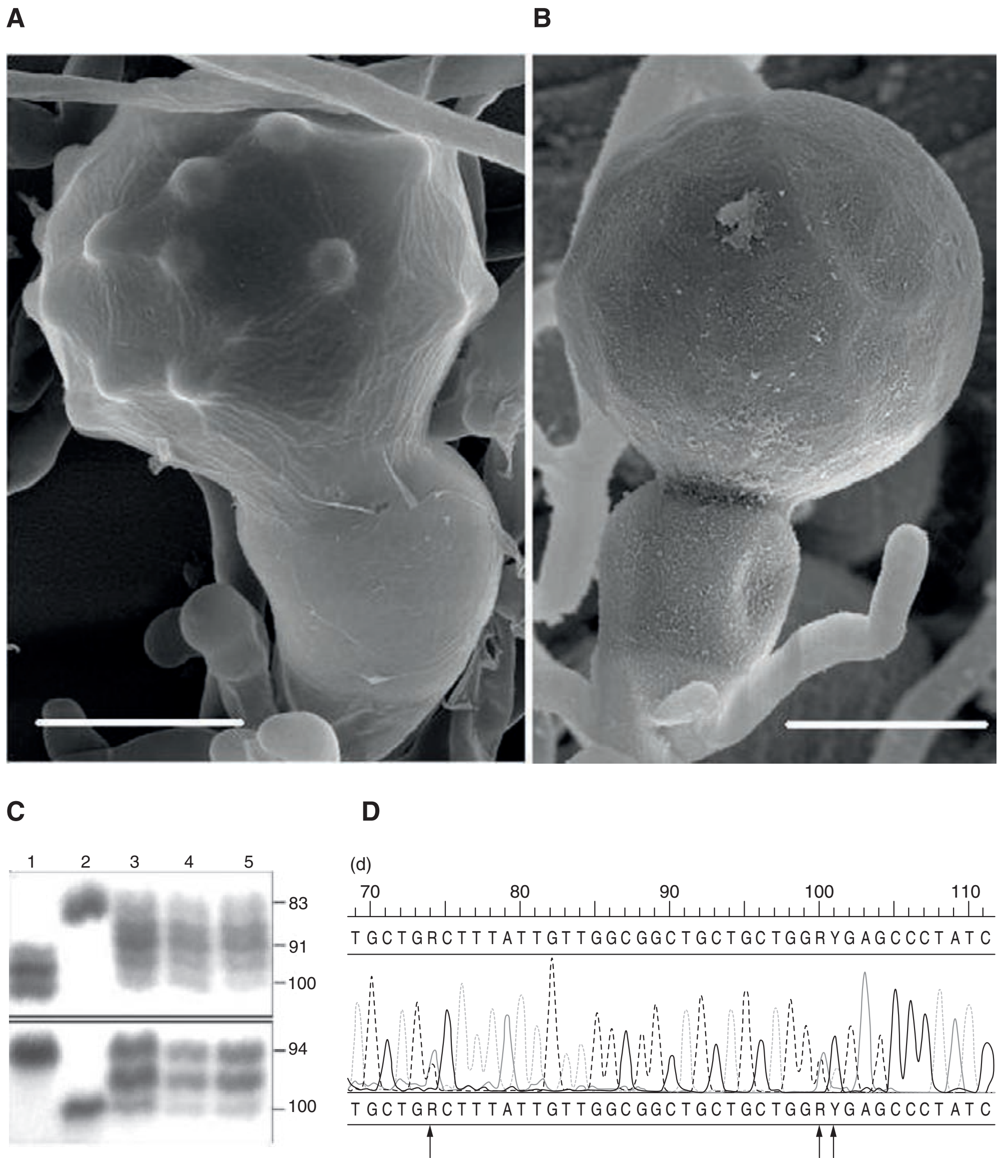

Fig. 5.1. Scanning electron micrographs of *Phytophthora alni* gametangia showing **(A)** the ornamented oogonial surface of *P. alni* subsp. *alni* and **(B)** the smooth oogonial surface of *P. alni* subsp. *uniformis*. The two-celled antheridia is visible for both subspecies. Bars, 20 µm. **(C)** Dimeric malate dehydrogenase (Mdh) enzyme patterns at the *Mdh-1* and *Mdh-2* loci for subspecies of *P. alni*. Lane numbers are on top and relative band mobility is on the right. The banding patterns for subsp. *multiformis* (*Mdh-1*$^{91/100}$ and *Mdh-2*$^{94/94}$, Lane 1) and subsp. *uniformis* (*Mdh-1*$^{83/83}$ and *Mdh-2*$^{100/100}$, Lane 2) are combined in subsp. *alni* isolates (*Mdh-1*$^{83/91/100}$ and *Mdh-2*$^{94/100}$, Lanes 3–5) including two atypical isolates in Lanes 4 and 5 with a *Pau* mitotype. *Mdh-1* bands were visualized by prolonged electrophoresis to let *Mdh-2* bands run off the gel according to the manufacturer's instructions (Helena Laboratories) (reproduced by permission of Akadémiai Kiadó, Budapest, Hungary). **(D)** Electropherogram of part of the ITS of *Phytophthora hedraiandra* × *Phytophthora cactorum* hybrid strains showing additive bases at four positions of which three are shown here (see arrows) where the sequences of the parents differ (reproduced by permission of Springer).

This hypothesis is based on broad-scale analyses of *P. alni* isolates showing that *Paa* has three different alleles for each of four nuclear genes, two of which were also present in *Pam* and a third one that matched a single allele in *Pau*.

Phylogenetic analyses of the mitochondrial genes *cox1* and *nad1* from the hybrid isolates and *P. cambivora* or *P. fragariae* did not show strong similarity (Ioos *et al.*, 2006). mtDNA sequences of *P. alni* isolates from the three subspecies clustered into only two groups, one that included *Paa* and *Pam*, and the other *Pau*. Furthermore, the *Paa* isolates displayed mtDNA RFLP patterns identical to isolates of either *Pam* or *Pau*. The results of Ioos *et al.* (2006) are supported by analysis of the isozyme patterns of malate dehydrogenase (Fig 5.1C). This enzyme expressed in *Paa* included combined expression profiles of *Pam* and *Pau*, whereas the mtDNA restriction profiles of *Paa* matched that of either *Pam* or a *Pau* (Bakonyi *et al.*, 2007). However, not discounting the possibility of a hybridization event between *Pam* and *Pau*, Bakonyi *et al.* (2007) suggest that *Paa* isolates with a *Pau* mitotype might have arisen in bark tissue through the introgression of mitochondria from *Pau* into the nuclear background of *Paa*, as is exemplified with *Ophiostoma* spp. (Bates *et al.*, 1993).

The hybridization event that led to the emergence of *P. alni* is believed to be recent, and it may have occurred in a European nursery, perhaps on raspberry or another host common to the putative parents (Brasier *et al.*, 2004). It is further thought that *P. alni* arrived in Britain, the country of first record, as a result of commercial trade of infested nursery stock. The occurrence of *Pau* in remote regions of Alaska, however, is not likely to be due to infested nursery stock. Rather, the introduction may have occurred from contaminated footwear of tourists, or this subspecies may be native (Adams *et al.*, 2008). Although *P. alni* has not yet been found in continental USA, the pathogen is regarded as a serious threat to riparian alders, largely along the west coast. In addition, greenhouse trials suggest that walnut, chestnut and cherry trees may also be susceptible to the disease.

5.3.2 *P. nicotianae* × *P. cactorum*

Hybridization between these closely related species was first reported in hydroponic greenhouse systems in the Netherlands (Man in ’t Veld *et al.*, 1998). Isolates recovered from *Spathiphyllum* and *Primula* plants differed morphologically from known *Phytophthora* species pathogenic to these ornamental plants. Isozyme and RAPD analyses revealed the unusual isolates represented species hybrids, and mtDNA restriction patterns were identical to *P. nicotianae* confirming *P. nicotianae* as one parent. The heterothallic *P. nicotianae* is an introduced species in the Netherlands that infects both *Spathiphyllum* and *Primula*. In contrast, the homothallic *P. cactorum* is a resident species that does not cause disease on these plants. Similar hybrid isolates were obtained from a *Cyclamen* sp., which is not a known host of either of the parental species (Bonants *et al.*, 2000). Further analyses of the ITS region and AFLP analyses provided evidence of a bi-parental origin of the recovered isolates (Man in ’t Veld *et al.*, 1998; Bonants *et al.*, 2000; Hurtado-Gonzales *et al.*, 2009; Nirenberg *et al.*, 2009). In all cases genes of both parents were present in the hybrid strains. They were formally described in 2009 as *Phytophthora* × *pelgrandis* (Nirenberg *et al.*, 2009), although the morphological description was incomplete (Man in ’t Veld *et al.*, 2012).

In 1998 atypical strains of *P. nicotianae* were reported on loquat (*Eriobotrya japonica*) trees in Taiwan (Chern *et al.*, 1998), and these were later re-identified as *P. nicotianae* × *P. cactorum* hybrids (Man in ’t Veld, 2001). This same hybrid was also found on loquat in Peru. A comparison of the hybrid isolates from Peru and Taiwan using AFLP indicates that independent hybridization events took place (Hurtado-Gonzales *et al.*, 2009). In both cases heavy flooding preceded disease, strongly

suggesting that this was a prerequisite for the physical encounter of the parents of the hybrids. Incidentally, these parents could originate from other infected plants in the neighbourhood. In Taiwan thousands of trees continue to die, and in Peru several hundreds of trees are affected (Fig. 5.2A and B).

A

B

C

Fig. 5.2. (A) Loquat tree (*Eriobotrya japonica*) in Coayllo, Peru, with basal stem rot, caused by *Phytophthora nicotianae* × *Phytophthora cactorum* hybrids, showing dieback in the canopy. **(B)** Death of a loquat tree in Peru caused by *P. nicotianae* × *P. cactorum* (courtesy of Liliana M. Aragon-Caballero, Peru). **(C)** Symptoms caused by a *Phytophthora hedraiandra* × *P. cactorum* hybrid on *Penstemon* sp. (courtesy of Monika Heupel, Germany).

Naturally occurring species hybrids appear to be quite stable. Sequence analysis of the ITS region and the phenol acid carboxylase gene (*Pheca*) of two strains from Taiwan (originally isolated in 1995) demonstrated that both strains retained both parental sequences 14 years after isolation (Hurtado-Gonzales *et al*., 2009). In addition, sequence analysis of β-tubulin of a strain from Taiwan and another (PD94/1116, GenBank JQ681269) from the Netherlands revealed both were hybrid 17 years after their original isolation (Man in 't Veld, unpublished results).

P. nicotianae × *P. cactorum* hybrids are well established and successful. To date, this hybrid has been isolated from: (i) stem base rot (*Spathiphyllum*, *Lavandula*); (ii) root rot (*Lavandula*, *Primula*); (iii) tuber rot (*Cyclamen*); and (iv) leaf spots (*Lewisia*) in the Netherlands. They have also been found elsewhere on *Anigozanthos* (Belgium), *Pelargonium grandiflorum* (Germany), *Buxus sempervirens*, *Chamaecyparis lawsoniana* and *Lavandula angustifolia* (Szigethy *et al*., 2012) and *Rhododendron* (Leonberger, 2010; USA), in addition to *E. japonica* (Taiwan, Peru). Pathogenicity tests showed that *E. japonica* (Chern *et al*., 1998) and *Spathiphyllum* (Man in 't Veld *et al*., 1998) developed disease symptoms. In both cases the pathogen was successfully re-isolated and its identity reconfirmed.

Mitochondrial genes are inherited from only one of the hybrid parents, and analysis of the *cox1* sequences of nine *P. hedraiandra* × *P. cactorum* strains demonstrated that eight strains contained *cox1* of *P. hedraiandra* and one strain contained that of *P. cactorum*, confirming the parents and indicating that two independent hybridization events had taken place. Hybrids of *P. hedraiandra* and *P. cactorum* are reported by the Dutch Plant Protection Service to cause stem base rot (*Idesia*), leaf spots (*Allium cepa*, *Allium porrum*) and wilting shoots (*Rhododendron*), usually resulting in the death of the host. Since its initial discovery, this hybrid has been reported on *Rhododendron* (Kris van Poucke, Belgium, 2010, personal communication; Grazyna Szkuta, Poland, 2012, personal communication), *Kalmia latifolia* (Alenka Munda, Slovenia, 2008, personal communication), *Penstemon* (Fig. 5.2C, Monika Heupel, Germany, 2001, personal communication) and *Dicentra* (Leonberger, 2010; Indiana, USA), indicating it is well established, successful and proliferating on new hosts. Since the first isolation of *P. hedraiandra* × *P. cactorum* hybrids from *Rhododendron* in the Netherlands in 1992, the resident pathogen, *P. cactorum*, has not been isolated from *Rhododendron*, strongly suggesting the hybrids are displacing the resident *P. cactorum* on this host.

5.3.3 *P. hedraiandra* × *P. cactorum*

In 2007 a second hybrid of *P. cactorum* was identified resulting from hybridization with another homothallic species, *P. hedraiandra* (Man in 't Veld *et al*., 2007). It was recently formally described as *Phytophthora* × *serendipita* (Man in 't Veld *et al*., 2012). This hybrid was identified by heterozygous malic enzyme (*Mdhp*) patterns and heterozygous ITS sequences showing additive bases at four positions where the ITS sequence of the parents differed (Fig. 5.1D). Normally, the ribosomal DNA gene repeat that contains the ITS region is homogenized due to concerted evolution.

5.3.4 *P. andina*

The latest identification of a hybrid species is *P. andina* (Goss *et al*., 2011), which was originally described as a heterothallic species from the Andean region in South America (Oliva *et al*., 2010). The hybrid nature of *P. andina* was revealed by four nuclear loci that each contain two distinctly different sequences, one clustering with *P. infestans* and one belonging to a species yet to be identified. *P. andina* and *P. infestans* are both able to infect pear melon (*Solanum muricatum*), and this common host may be the bridge that allowed hybridization (Adler *et al*., 2004). Perhaps it is noteworthy to mention that

the nearby Amazon Basin may harbour a plethora of new *Phytophthora* species, yet to be discovered.

5.4 Concluding Remarks and Future Trends

5.4.1 Sexual or parasexual hybridization?

The genus *Phytophthora* consists of numerous species and only a few have been proven to be involved in interspecific genetic exchange. This is principally due to the fact that species hybridization in nature appears to be episodic in comparison with the more commonly recognized processes of mutation and sexual or parasexual reproduction within individual species.

Before the recognition of natural hybridization, laboratory hybridization was demonstrated using both sexual crosses and somatic (zoospore) fusions. Although the sexual approach is often thought to be the primary way hybrids are created in nature, the alloploidy of *P. alni* isolates suggests that somatic (maybe zoospore) fusion may have occurred between its parental species. Thus far, polymorphism in mitochondrial genes has not been demonstrated and somatic hybridization is not likely.

Furthermore, a preliminary analysis of a hybrid swarm in Australia demonstrated the presence of single base polymorphism in both ITS and *cox1*, suggesting the presence of two different *cox1* genes in some strains (Burgess *et al.*, 2010). This indicates that somatic fusion, presumably by hyphal anastomosis, may occur under natural conditions. Hydroponic systems or heavy flooding and infection of common hosts create the conditions where the physical encounter of the two parents is possible.

5.4.2 Continuing evolution

In *Phytophthora*, hybridization may be advantageous because deleterious mutations are buffered by multiple alleles in the allopolyploid genome and fitness may decrease at a slower rate allowing them to persist in the environment. Due to the merger of the two parental genomes, the genetic diversity within a hybrid population exceeds the genetic diversity of each of the parental species. Hence, hybridization may generate rare genotypes termed 'Red Queens' that are particularly successful (Wuethrich, 1998).

Evolving strains have been discovered for all three known natural hybrids (*P. alni* subsp. *alni*, *P. nicotianae* × *P. cactorum* and *P. hedraiandra* × *P. cactorum*). For instance, in a strain (P669) of *P. alni* subsp. *alni*, the Gpi^{100} alleles were lacking and another two (P668 and P1137) missed the Gpi^{93} allele, as compared with the regular $Gpi^{85/93/100}$ genotype (Man in 't Veld, unpublished results). As opposed to the regular $Mdhp^{92/100}$ genotype of *P. nicotianae* × *P. cactorum*, in one strain (PD 97/8241) the $Mdhp^{100}$ allele was not present. In *P. hedraiandra* × *P. cactorum* strains BBA5/94 and CBS114342 the characteristic well-defined double bases in the ITS were missing, and the latter strain also lacked the $Mdhp^{B}$ allele from *P. hedraiandra*. These changes could have occurred by intercrossing, backcrossing to either one of the parents (introgression), mitotic chromosomal rearrangements and/or by chromosome loss.

Thus far it appears that species hybridization occurs most often between allopatric species from different locations. The *Phytophthora* species hybrids described to date are offspring of an exotic and a native species or two exotic species that occupy the same habitat and niche. No reports have described similar hybridization among indigenous, sympatric *Phytophthora* species, even though such hybrids can be generated in the laboratory. Increasing global horticultural trade increases the chance of introduced species hybridizing with phylogenetically closely related local species. This may be because the allopatric species have diverged without building up premating barriers towards local species. In addition, newly introduced hosts may serve as 'bridge species' for pathogen species that diverged by host specialization.

5.4.3 Divergence of hybrids

Although it was originally proposed by Brasier *et al.* (1999) that *P. alni* subsp. *alni* was the result of hybridization between *P. cambivora* and a taxon close to *P. fragariae*, and that *P. alni* subsp. *multiformis* and subsp. *uniformis* were evolving hybrids, it was later suggested that *P. alni* subsp. *multiformis* and subsp. *uniformis* might be the parents (Ioos *et al.*, 2006, 2007). These two last mentioned subspecies cannot have diverged by host specialization since they have the same host. The only option left is that they must have diverged by geographical isolation. Indeed, subsp. *uniformis* has been found in Eastern Europe and Sweden (and even in Alaska) whereas subsp. *multiformis* is present in Central and Western Europe. Are the hybrids perhaps the result of a collision between the eastern (subsp. *uniformis*) and the western (subsp. *multiformis*) population? If so, it is not exactly clear which event triggered the formation of the *P. alni* subsp. *alni* hybrids in the 1990s.

Regarding *P. nicotianae* × *P. cactorum* and *P. hedraiandra* × *P. cactorum* hybrids, they occupy different niches. *P. nicotianae* × *P. cactorum* hybrids have been found in greenhouses in Europe and in countries with a tropical climate like Peru and Taiwan. Therefore, the most likely scenario is that *P. nicotianae* was moved from the tropics (Indonesia or South America) to the northern hemisphere in Europe in colonial times. It could then survive in greenhouses and interact with *P. cactorum*, which is resident in Europe. However, in Peru and Taiwan *P. cactorum* is probably the introduced species. In contrast, *P. hedraiandra* × *P. cactorum* hybrids are able to survive in the temperate northern hemisphere. *P. hedraiandra* was not reported before its description in 2004 (de Cock and Lévesque, 2004), and this species may have been introduced recently from an unknown centre of origin and hybridized with the resident *P. cactorum*.

Interestingly, most *Phytophthora* species hybrids have acquired the mitochondrial genome of the exotic, introduced parental species. It is intriguing that until now, only *cox1* from *P. nicotianae* has been found in *P. nicotianae* × *P. cactorum* hybrids (Man in 't Veld *et al.*, 1998; Hurtado-Gonzales *et al.*, 2009; Nirenberg *et al.*, 2009; Bakonyi and Szigethy, 2011, personal communication), suggesting the *P. nicotianae* mitochondrial genome may encode factors contributing to virulence (Olson and Stenlid, 2001). Both mitochondrial genomes of *P. ramorum* and *P. sojae* contain unique open reading frames (ORFs), and the mitochondrial genome of *P. nicotianae* may have unique genes contributing to virulence (Martin *et al.*, 2007).

On the whole, hybrids, by their very nature, are unpredictable. They may exhibit increased aggressiveness compared with either parent and they may also be able to explore new niches and new hosts. Interspecific hybridization events are likely to increase with expanding world trade and non-traditional agricultural conditions. Whether produced recently, or existing for long periods and unrecognized, species hybrids can become a dominant component of *Phytophthora* populations in a region and may lead to epidemic outbreaks. Thus, they represent a unique danger for agriculture, horticulture and natural ecosystems.

References

Adams, G.C., Catal, M., Trummer, L., Hansen, E.M., Reeser, P. and Worrall, J.J. (2008) *Phytophthora alni* subsp. *uniformis* found in Alaska beneath thinleaf alders. *Plant Health Progress*. Available at: doi:10.1094/PHP-2008-1212-02-BR (accessed 12 December 2008).

Adler, N.E., Erselius, L.J., Chacon, M.G., Flier, W.G., Ordoñez, M.E., Kroon, L.P.N.M. and Forbes, G.A. (2004) Genetic diversity of *Phytophthora infestans sensu lato* in Ecuador provides new insight into the origin of this important plant pathogen. *Phytopathology* 94, 154–162.

Bakonyi, J., Nagy, Z.Á. and Érsek, T. (2006) PCR-based DNA markers for identifying hybrids within *Phytophthora alni*. *Journal of Phytopathology* 154, 168–177.

Bakonyi, J., Nagy, Z.Á. and Érsek, T. (2007) A novel hybrid with nuclear background of *Phytophthora alni* subsp. *alni* exhibits a mitochondrial DNA profile characteristic of *P. alni* subsp. *uniformis*. *Acta Phytopathologica et Entomologica Hungarica* 42, 1–7.

Bates, M.R., Buck, K.W. and Brasier, C.M. (1993) Molecular relationship of the mitochondrial DNA of *Ophiostoma ulmi* and the NAN and EAN races of *O. novo-ulmi* determined by restriction fragment length polymorphisms. *Mycological Research* 97, 1093–1100.

Bonants, P.J.M., Hagenaar-de Weerdt, M., Man in 't Veld, W. and Baayen, R.P. (2000) Molecular characterization of natural hybrids of *Phytophthora nicotianae* and *P. cactorum*. *Phytopathology* 90, 867–874.

Brasier, C.M. (1992) Evolutionary biology of *Phytophthora*. Part I: Genetic system, sexuality and the generation of variation. *Annual Review of Phytopathology* 30, 153–171.

Brasier, C.M., Cooke, D.E.L. and Duncan, J.M. (1999) Origin of a new *Phytophthora* pathogen through interspecific hybridization. *Proceedings of the National Academy of Sciences USA* 96, 5878–5883.

Brasier, C.M., Kirk, S.A., Delcan, J., Cooke, D.E.L., Jung, T. and Man in 't Veld, W.A. (2004) *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. *Mycological Research* 108, 1172–1184.

Burgess, T., Stukely, M., Jung, T., White, D., Hüberli, D. and Hardy, G. (2010) Molecular characterisation of a *Phytophthora* hybrid swarm in native ecosystems and waterways in Western Australia. In: Proceedings of the 5th International Union of Forest Research Organizations (IUFRO) meeting '*Phytophthora* in Forests and Natural ecosystems', Rotorua, New Zealand, pp. 1–44.

Chern, L.L., Ann, P.J. and Young, H.R. (1998) Root and foot rot of loquat in Taiwan caused by *Phytophthora*. *Plant Disease* 82, 651–656.

de Cock, A.W.A.M. and Lévesque, C.A. (2004) New species of *Pythium* and *Phytophthora*. *Studies in Mycology* 50, 481–487.

Donahoo, R.S. and Lamour, K.H. (2008) Interspecific hybridization and apomixes between *Phytophthora capsici* and *Phytophthora tropicalis*. *Mycologia* 100, 911–920.

English, J.T., Láday, M., Bakonyi, J., Schoelz, J.E. and Érsek, T. (1999) Phenotypic and molecular characterization of species hybrids derived from induced fusion of zoospores of *Phytophthora capsici* and *Phytophthora nicotianae*. *Mycological Research* 103, 1003–1008.

Érsek, T. and Ribeiro, O.K. (2010) An annotated list of new *Phytophthora* species described post-1996. *Acta Phytopathologica et Entomologica Hungarica* 45, 251–266.

Érsek, T., English, J.T. and Schoelz, J.E. (1995) Creation of species hybrids of *Phytophthora* with modified host ranges using zoospore fusion. *Phytopathology* 85, 1343–1347.

Érsek, T., English, J.T. and Schoelz, J.E. (1997) Triparental species hybrids from fused zoospores of *Phytophthora*. *Czech Mycology* 50, 13–20.

Flor, H.H. (1932) Heterothallism and hybridization in *Tilletia tritici* and *T. laevis*. *Journal of Agricultural Research* 44, 49–58.

Goodwin, S.B. and Fry, W.E. (1994) Genetic analysis of interspecific hybrids between *Phytophthora infestans* and *Phytophthora mirabilis*. *Experimental Mycology* 18, 20–32.

Goss, E.M., Cardenas, M.E., Myers, K., Forbes, G.A., Fry, W.E, Restrepo, S. and Grünwald, N.J. (2011) The plant pathogen *Phytophthora andina* emerged via hybridization of an unknown *Phytophthora* species and the Irish potato famine pathogen, *P. infestans*. *PLoS One* 6 (9), e24543.

Hurtado-Gonzales, O.P., Aragon-Caballero, L.M., Flores-Torres, J.G., Man in 't Veld, W.A. and Lamour, K.H. (2009) Molecular comparison of natural hybrids of *Phytophthora nicotianae* and *P. cactorum* infecting loquat trees in Peru and Taiwan. *Mycologia* 101, 496–502.

Ioos, R., Husson, C., Andrieux, A. and Frey, P. (2005) SCAR-based PCR primers to detect the hybrid pathogen *Phytophthora alni* and its subspecies causing alder disease in Europe. *European Journal of Plant Pathology* 112, 323–335.

Ioos, R., Andrieux, A., Marçais, B. and Frey, P. (2006) Genetic characterization of the natural hybrid species *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses. *Fungal Genetics and Biology* 43, 511–529.

Ioos, R., Panabières, F., Industri, B., Andrieux, A. and Frey, P. (2007) Distribution and expression of elicitin genes in the interspecific hybrid oomycete *Phytophthora alni*. *Applied and Environmental Microbiology* 73, 5587–5597.

Kroon, L.P.N.M. (2010) The genus *Phytophthora*; phylogeny, speciation and host specificity. PhD thesis, Wageningen University, Wageningen, the Netherlands.
Leonberger, A.J. (2010) Distribution and host specificity of *Phytophthora* species found in Indiana nurseries, greenhouses, and landscape plantings. PhD thesis, Purdue University, Indiana.
Man in 't Veld, W.A. (2001) First report of natural hybrids of *Phytophthora nicotianae* and *P. cactorum* on loquat in Taiwan. *Plant Disease* 85, 98.
Man in 't Veld, W.A., Venbaas-Rijks, W.J., Ilieva, E., de Cock, A.W.A.M., Bonants, P.J.M. and Pieters, R. (1998) Natural hybrids of *Phytophthora nicotianae* and *Phytophthora cactorum* demonstrated by isozyme analysis and random amplified polymorphic DNA. *Phytopathology* 88, 922–929.
Man in 't Veld, W.A., de Cock, A.W.A.M. and Summerbell, R.C. (2007) Natural hybrids of resident and introduced *Phytophthora* species proliferating on multiple new hosts. *European Journal of Plant Pathology* 117, 25–33.
Man in 't Veld, W.A., Rosendahl, C.H.M and Hong, C. (2012) *Phytophthora* × *serendipita* sp. nov. and *P.* × *pelgrandis*, two destructive pathogens generated by natural hybridization. *Mycologia* 104, 1390–1396.
Martin, F.N., Bensasson, D., Tyler, B.M. and Boore, J.L. (2007) Mitochondrial genome sequences and comparative genomics of *Phytophthora ramorum* and *P. sojae*. *Current Genetics* 51, 285–296.
May, K.J., Drent, A. and Irwin, J.A.G. (2003) Interspecific hybrids between the homothallic *Phytophthora sojae* and *Phytophthora vignae*. *Australasian Plant Pathology* 32, 353–359.
Nagy, Z.Á., Bakonyi, J. and Érsek, T. (2003) Standard and Swedish variant types of the hybrid alder *Phytophthora* attacking alder in Hungary. *Pest Management Science* 59, 484–492.
Nechwatal, J. and Mendgen, K. (2009) Evidence for the occurrence of natural hybridization in reed-associated *Pythium* species. *Plant Pathology* 58, 261–270.
Nirenberg, H.I., Gerlach, W.F. and Gräfenheim, T. (2009) *Phytophthora* × *pelgrandis*, a new natural hybrid pathogenic to *Pelargonium grandiflorum* Hort. *Mycologia* 101, 220–231.
Oliva, R.F., Kroon, L.P.N.M., Chacon, G., Flier, W.G., Ristaino, J.B. and Forbes, G.A. (2010) *Phytophthora andina* sp. nov., a newly identified heterothallic pathogen of solanaceous hosts in the Andean highlands. *Plant Pathology* 59, 613–625.
Olson, Å. and Stenlid, J. (2001) Mitochondrial control of fungal hybrid virulence. *Nature* 411, 438.
Pain, S. (1999) Fiendish fungus. *New Scientist* 15 May, p. 7.
Sansome, E., Brasier, C.M. and Hamm, P.B. (1991) *Phytophthora meadii* may be a species hybrid. *Mycological Research* 95, 273–277.
Schardl, C.L. and Craven, K.D. (2003) Interspecific hybridization in plant-associated fungi and oomycetes: a review. *Molecular Ecology* 12, 2861–2873.
Szigethy, A., Nagy, Z.Á., Vettraino, A.M., Józsa, A., Cacciola, S.O., Faedda, R. and Bakonyi, J. (2012) First report of *Phytophthora* × *pelgrandis* causing root rot and lower stem necrosis of common box, lavender and Port-Orford-cedar in Hungary. *Plant Disease.* Available at: http://dx.doi.org/10.1094/PDIS-07-12-0662-PDN (accessed 15 August 2012).
Wuethrich, B. (1998) Why sex? Putting theory to the test. *Science* 281, 1980–1982.

6 *Phytophthora infestans* and *Phytophthora andina* on Solanaceous Hosts in South America

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6.1 Introduction

This review summarizes research on the closely related pathogens *Phytophthora infestans* and *Phytophthora andina* and their hosts in South America. Hopefully, the chapter brings together information not previously assembled, although it is not exhaustive. Not all areas of South America have contributed directly. For example, there is no direct contribution from Brazil, an important potato- and tomato-producing country. However, the Brazilian pathogen populations are more or less defined and the data are summarized from the literature. An important issue is the uncertain taxonomy of both the hosts and the pathogens, as evidenced by a recent collegial debate on the naming of *P. andina* in the journal *Plant Pathology* (Cárdenas *et al.*, 2011; Forbes *et al.*, 2011). Overall, the resources to investigate solanaceous plants and *Phytophthora* are limited, and it is likely that significant diversity is lurking undiscovered in the Andean region.

6.2 Symptoms, Host Range and Host Preference

P. infestans and *P. andina* (and potentially unclassified but closely related taxa – see below) cause blight-like symptoms on a number of hosts (Table 6.1). Several of the cultivated hosts have different names in South America, and Table 6.2 lists some of most commonly used names in different countries. On most hosts the foliar symptoms are similar to those on potato and tomato and include dark expanding lesions surrounded by water-soaked areas, and, if conditions are favourable, a halo of sporulation (Fig. 6.1). On the more perennial hosts with woody tissues the pathogens can

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Table 6.1. Hosts of *Phytophthora infestans* (Pi), *Phytophthora andina* (Pa) and unclassified isolates (U) reported for seven countries in South America.

Host taxa	Colombia	Venezuela	Bolivia	Ecuador	Peru	Argentina	Chile
Cultivated taxa							
Tuber bearing[a]	Pi	Pi	Pi	Pi	Pi	Pi	Pi
Solanum betaceum	Pi/Pa/U			Pa	Pa		
Solanum quitoense	Pi	Pi		Pa/Pi			
Physalis peruviana	Pi						
Solanum muricatum	Pi			Pi/Pa[b]	Pi		
Solanum lycopersicum	Pi	Pi	Pi	Pi	Pi	Pi	Pi
Wild taxa							
Tuber bearing	Pi			Pi	Pi		
Solanum caripense	Pi			Pi	Pi		
Solanum juglandifolium, *Solanum ochranthum*	Pi			Pi/U			
Solanum section Anarrhichomenum				Pa			
Solanum marginatum	Pi						
Solanum hispidum				Pa			
Datura stramonium	Pi						
Brugmansia spp.[c]				Pa			

[a]*Solanum tuberosum* and according to Ochoa (2003) this also includes: *Solanum chaucha* Juz. et Buk. ($2n = 36$); *S. tuberosum* ssp. *andigena* (Juz. et. Buk.) Hawkes ($2n = 48$); *Solanum phureja* Juz. et. Buk. ($2n = 24$); *Solanum goniocalyx* Juz. et Buk. ($2n = 24$); *Solanum stenotomum* Juz. et. Buk ($2n = 24$); *Solanum hygrothermicum* Ochoa ($2n = 48$); *Solanum ajanhuiri* Juz. et Buk. ($2n = 24$); *Solanum juzepczukii* Buk. ($2n = 36$); and *Solanum curtilobum* Juz. et Buk. ($2n = 60$).
[b]*P. andina* was found attacking *S. muricatum* in one field but across two consecutive seasons (Adler *et al.*, 2002); no other reports are known.
[c]Only on flower petals.

Table 6.2. Common names of cultivated solanaceous hosts of *Phytophthora infestans* and/or *Phytophthora andina* in the Andean region.

Host species	Common name (country)[a]
Tuber bearing	
Solanum tuberosum	Papa (all countries)
S. tuberosum ssp. *andigena*	Papa nativa (PE, EC)
Solanum phureja	Papa (PE), chaucha (EC), papa criolla (CO)
Solanum stenotomum	Papa amarilla (PE), papa nativa (PE)
Others[b]	Papa nativa (PE)
Non-tuber bearing	
Solanum muricatum	Pepino dulce (all countries)
Solanum quitoense	Lulo (CO, VE), naranjillo (EC)
Solanum betaceum	Tomate de arbol (EC, CO, PE), sachatomate (PE)
Physalis peruviana	Uvilla (CO, EC), uchuva (CO), aguaymanto or capulí (PE)

[a]Countries: CO, Colombia; EC, Ecuador; PE, Peru; VE, Venezuela.
[b]According to Ochoa (2003) this includes: *Solanum chaucha* Juz. et Buk. ($2n = 36$); *Solanum goniocalyx* Juz. et Buk. ($2n = 24$); *Solanum hygrothermicum* Ochoa ($2n = 48$); *Solanum ajanhuiri* Juz. et Buk. ($2n = 24$); *Solanum juzepczukii* Buk. ($2n = 36$); and *Solanum curtilobum* Juz. et Buk. ($2n = 60$).

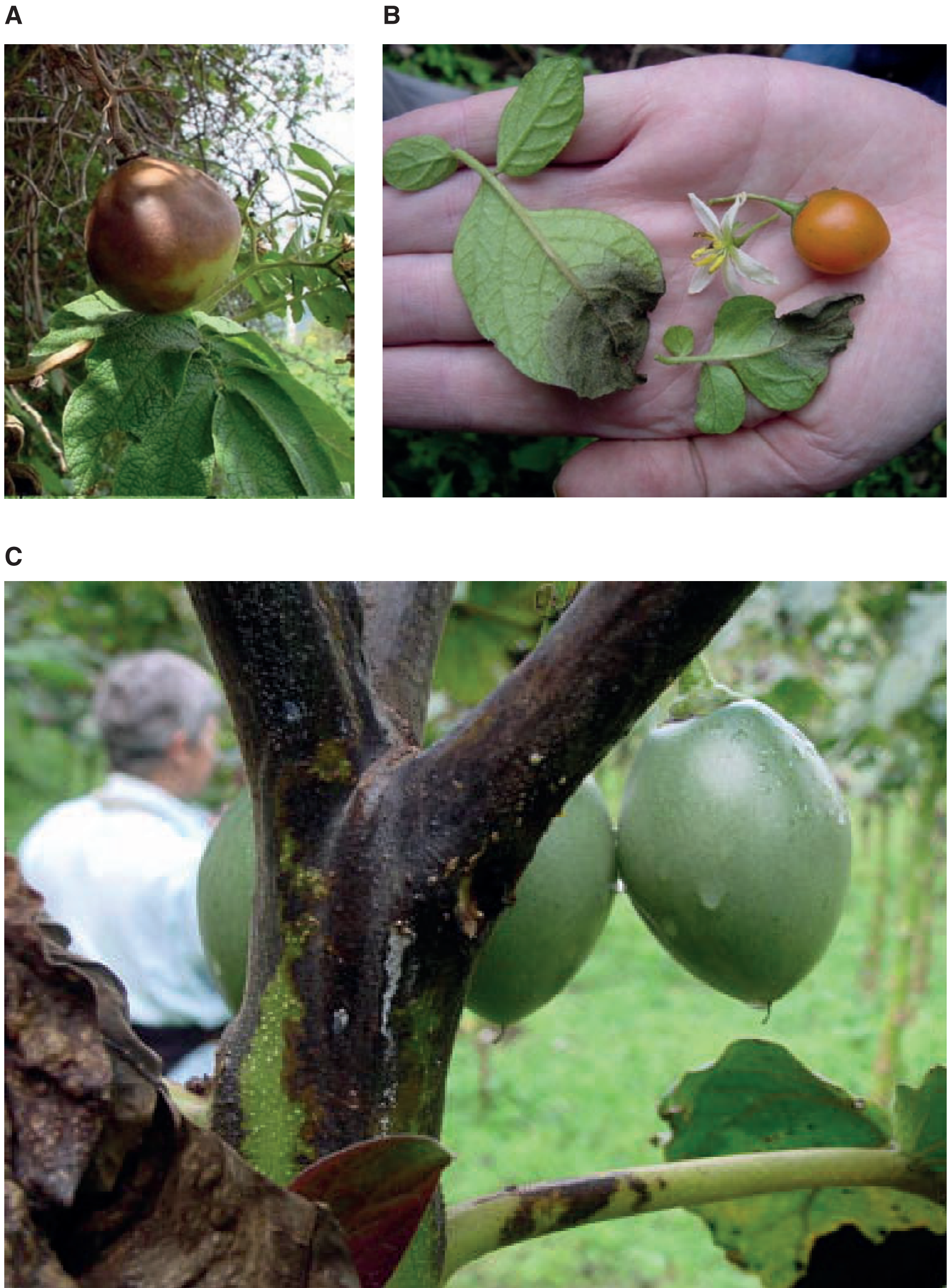

Fig. 6.1. (A) Typical symptoms on fruit of *Solanum ochranthum*; **(B)** leaves of a host in the Anarrhichomenum group; and **(C)** on the stem of *Solanum betaceum* in South America.

cause dark stem lesions. Although not yet confirmed, these lesions may remain latent for long periods, providing a survival strategy for drought periods.

There are many references to *P. infestans* attacking potato and/or tomato and some other hosts in South America. Here the focus is on recent studies using modern tools for pathogen identification. Although *P. infestans* and *P. andina* are found on many solanaceous hosts in South America (Table 6.1) the actual number of host species is imprecise for several reasons. First, the actual number of species within some solanaceous taxa is disputed and continues to evolve with new advances in taxonomy (Bohs and Olmstead, 1997). This is most evident in the widely studied tuber-bearing species. It appears that species numbers were inflated in earlier work (Spooner, 2009), and studies at the International Potato Center (CIP) in Peru and Ecuador reveal limited variation in wild or cultivated tuber-bearing species. For this reason, all tuber-bearing solanaceous hosts are treated as either domesticated tuber-bearing or wild tuber-bearing (Table 6.1). Secondly, there is uncertainty as a result of a lack of resources to search the rich diversity of solanaceous species in the Andean region for blight-causing pathogens. *P. andina* appears to be a hybrid of *P. infestans* and another unidentified species that may still be present in the wild in South America and may attack solanaceous or other hosts (Goss *et al.*, 2011).

Six cultivated taxa are attacked by *P. infestans* and *P. andina*. These include potato, tomato and *Solanum muricatum*, which is commonly attacked by *P. infestans*, with one curious exception in Ecuador (Table 6.1). The other host species are less clear. *Physalis peruviana* is attacked by an A1/A2 population of *P. infestans* in Colombia (Fig. 6.2), but blight has not yet been detected on this host in other countries, even though it is widely grown in Ecuador and Peru. *Solanum betaceum* has only been associated with *P. andina* in Ecuador and Peru (Oliva *et al.*, 2010). In south-western Colombia, isolates morphologically and molecularly intermediate between *P. infestans* and *P. andina* have been collected from *S. betaceum* plants in the departments of Nariño and Putumayo. These isolates have a strong host preference with low virulence and aggressiveness on potato cultivars. However, this host expansion towards *S. betaceum* has not only occurred in these hybrids. Since 2005 isolates clearly classified as *P. infestans* have been collected from *S. betaceum* in the departments of Cundinamarca and Antioquia. Blight-like infections have been identified on *S. betaceum* in Venezuela, but the pathogen has not been identified. Thus, with *S. betaceum* it appears there is a gradient of pathogen diversity in the Andes. In Peru and Ecuador it appears to be a more homogeneous population of genotypes that are classified as *P. andina*. In Colombia, however, there is a mix of *P. andina*, *P. infestans* and apparently intermediate genotypes. *Solanum quitoense* is attacked by *P. infestans* and *P. andina* throughout the central and northern Andes of Ecuador (Oliva *et al.*, 2007) and *P. infestans* in Colombia (Gilchrist Ramelli *et al.*, 2009), whereas only *P. infestans* has been identified in Venezuela. In summary, several cultivated plant species are hosts for both *P. infestans* and *P. andina*, including *S. betaceum*, *S. muricatum* (rare and only found in Ecuador) and *S. quitoense*, thus providing opportunities for inoculum to survive and spread and potentially for gene flow between species.

Pathogen diversity is also complex on non-cultivated hosts and at least eight are attacked by one or more of these pathogen species; however, some of the taxa contain several species (e.g. section Anarrhichomenum and tuber-bearing species). *P. infestans* has been known to attack wild tuber-bearing and other solanaceous species for decades. Molecular investigation of isolates recovered from vine-like plants of several poorly defined species in the *Solanum* section Anarrhichomenum were subsequently identified as *P. infestans sensu lato* because of their obvious divergence from typical *P. infestans* (Adler *et al.*, 2004). These isolates were subsequently classified

Fig. 6.2. (A, B) Typical symptoms of blight on leaves of *Solanum betaceum* and of a species in the Anarrhicomenum group; and **(C)** on fruit of *Solanum muricatum* in South America.

as *P. andina* (Oliva *et al.*, 2010), but, similar to the situation with isolates recovered from cultivated species, not all isolates from wild hosts could easily be classified as *P. infestans* or *P. andina*. For example, in Ecuador some isolates from *Solanum ochantum* could not be classified as either *P. infestans* or *P. andina* based on neutral markers (mostly simple sequence repeats, SSRs) (Chacón *et al.*, 2006; Oliva *et al.*, 2007).

Host preference is strong for a number of the host–pathogen systems in South America. In Ecuador there is a clear host preference for potato and tomato isolates (Oyarzún *et al.*, 1998). In Colombia, isolates of *P. infestans* from potato and *P. peruviana* were preferentially pathogenic on their host

of origin (Vargas *et al*., 2009). Isolates that appear to be genetically intermediate between *P. andina* and *P. infestans* from *S. betaceum* in Colombia also show low pathogenicity on potato and are highly host specific in Ecuador (Adler *et al*., 2004). *S. muricatum* also has a specialized population of the US-1 clonal lineage of *P. infestans* (Adler *et al*., 2004).

6.3 Socio-economic Importance

While there are many studies demonstrating the host range, phenotypic and even genetic diversity of the pathogen populations in South America, there are few studies quantifying losses due to late blight on the various hosts. None the less, a number of surveys or anecdotal observations indicate that late blight is very important in the Andean region. In a geographic information system (GIS)-based study looking at potential late blight severity, parts of South America (e.g. the Andean region, southern Brazil) had among the highest risks compared with locations globally (Hijmans *et al*., 2000). A survey of 131 potato farmers in mountainous areas in Cajamarca, Peru, indicated that late blight significantly limits potato productivity and was their main problem (Ortiz *et al*., 1999). Bailon and Otazu (1992) estimated losses of 6 t/ha from late blight under Peruvian highland conditions.

In times of disease-favourable weather extreme losses are common. In a study carried out several years ago in Ecuador, about 10% of previously identified farmers' fields could not be sampled because they had been devastated by late blight and produced no tubers (Oyarzún *et al*., 2005). We are aware of only anecdotal information of the importance of late blight on *S. betaceum.* In areas particularly favourable for disease development blight appears to be a limiting factor. For example, *S. betaceum* was once common in San Jose de Minas, north of Quito, Ecuador, but has now virtually disappeared because of blight. At this point it is unclear if increased disease intensity is due to changes in the pathogen's population structure, the prevailing climate, cropping intensity or a combination of these factors.

In addition to the risk of crop loss, economic loss resulting from fungicide use is important. Kromann *et al*. (2009) estimate farmers in the Andes spend between 5 and 20% of total production costs on fungicides to control late blight, depending on their financial resources and disease pressure. Depending on weather conditions, farmers in northern Ecuador may spray foliage up to 18 times with translaminar fungicides (Oyarzún *et al*., 2005) and in Colombia up to ten times (Vargas *et al*., 2009). An earlier survey in Peru found farmers on average sprayed six times with fungicides (Nelson *et al*., 2001). However, more recent publications indicate that farmers in Peru often spray more than ten times (Bustamante *et al*., 2008; Pérez *et al*., 2009).

There are serious health risks related to the fungicides used for late blight control in South America. Ethylenebisdithiocarbamates (EBDCs) are among the most commonly used compounds in late blight control in many developing countries. The EBDCs break down into ethylenethiourea (ETU), which is a Type IIB carcinogen and an anti-thyroid compound (Panganiban *et al*., 2004). The EBDCs are also skin irritants and high levels of dermatitis are attributed to these products in Ecuador. Both mancozeb, the most commonly used EBDC, and chlorothalonil, a non-EBDC, are considered highly dangerous for low-input farmers (Wesseling *et al*., 2005). These pesticides are particularly dangerous in developing countries because of repeated exposure of farm workers and family. Studies at CIP indicate most farmers do not have protective clothing for mixing and applying pesticides and that exposed clothing is not washed frequently (Orozco *et al*., 2009). This creates a situation where family members come into contact with the hazardous compounds (Yanggen *et al*., 2004).

In addition to above-ground infections, tuber blight is also an important aspect of the disease. The importance of tuber blight appears to be location specific. A study in

Ecuador indicated that tuber blight was relatively infrequent (Oyarzún *et al.*, 2005); however, a subsequent study demonstrated that latent infection may be more common than previously thought (Kromann *et al.*, 2008).

6.4 Genetic and Phenotypic Diversity of *P. infestans* and *P. andina*

The diversity of these species remains unclear due in part to at least three factors: (i) the natural complexity of the system, which appears to be evolving rapidly; (ii) the increasing number of samples being analysed as new resources are dedicated to field exploration; and (iii) the greater use of higher resolution genotyping and increasingly sophisticated population analyses. The following synopsis may soon be modified if new studies are undertaken.

The population structure of *P. infestans* in South America, similar to other parts of the world, is characterized by major migration events that have occurred in the last few decades. Earlier studies indicate *P. infestans* populations in much of South America were part of the 'old' US-1 lineage (Spielman *et al.*, 1991). Population studies in the 1990s revealed that the US-1 lineage was no longer common on potato in many parts of the continent and that the EC-1 lineage was common in most of the Northern Andes (Forbes *et al.*, 1997), while other lineages were found in Brazil and Argentina, including populations with the A2 mating type (Forbes *et al.*, 1998). The EC-1 lineage appears to have spread north into Colombia (Forbes *et al.*, 1998) and is still common on potato (Vargas *et al.*, 2009). The EC-1 also stretched south through much of Peru. To a lesser extent, other lineages (e.g. PE-3 and PE-7) have also been found in Peru and are primarily associated with native cultivated landraces (Pérez *et al.*, 2001) or wild tuber-bearing species (Garry *et al.*, 2005). A different lineage seems to currently be dominant in Venezuela (Briceño *et al.*, 2009), owing perhaps to frequent seed importation. Chile was long considered an 'island' for the continued presence of the old US-1 population on potato (Forbes *et al.*, 1998), owing to strict quarantine regulations. However, an analysis of isolates collected between 2006 and 2011 revealed that a new pathogen population is now present in Chile (Acuña *et al.*, 2011). The new Chilean population is the A1 mating type but has a Ia mitochondrial haplotype and is not EC-1, which has a IIa haplotype.

While potato populations are characterized by recent migrations, potentially from Europe or North America (Forbes *et al.*, 1997), populations of *P. infestans* on other hosts show no evidence of such changes. For example, tomato and *S. muricatum* are primarily attacked by US-1 (Adler *et al.*, 2004; Gilchrist Ramelli *et al.*, 2009), and there is no indication of displacement by newer populations. The EC-1 clonal lineage apparently does not infect *P. peruviana* as it has not been found in countries where the crop is commonly grown. Furthermore, the *P. infestans* population found on *P. peruviana* in Colombia is of the A2 mating type and therefore not EC-1 (Vargas *et al.*, 2007).

Clonal populations of *P. infestans* typically show little genetic diversity with neutral markers such as SSRs (Blandón-Diaz *et al.*, 2012); however, this is not the case in Ecuador. A recent PhD thesis describes 31 sub-clonal genotypes in a sample of 61 EC-1 isolates from potato (R. Delgado, 2012, personal communication). Similarly high allelic diversity was found within the US-1 population from tomato, *S. muricatum* and *Solanum caripense* (Oliva *et al.*, 2007). This may indicate that host diversity is driving pathogen diversity and may also indicate that the US-1 lineage has been in South America for a long time, possibly even prior to the major migrations of *P. infestans* in the 19th century. Chacón *et al.* (2006) found a group of isolates on the wild host *Solanum ochranthum* (one of three groups from that host) that closely resembled US-1 from tomato but had unexpected SSR alleles.

As mentioned above, the species status of *P. andina* is not clear (Cárdenas *et al.*,

2011; Forbes *et al.*, 2011). *P. andina* is morphologically similar to *P. infestans* and a recent study indicates it is probably a hybrid of *P. infestans* and another unidentified species (Goss *et al.*, 2011). *P. andina* comprises at least three clonal lineages defined by multi-locus genotyping and designated EC-2(1a), EC-2(1c) and EC-3(1a) (Oliva *et al.*, 2010), where '1a' and '1c' refer to mitochondrial haplotypes using the nomenclature of Griffith and Shaw (1998). *P. andina* is thought to originate in the Andes as it has not been found elsewhere (Oliva *et al.*, 2010).

6.5 History and Origin of *P. infestans* and *P. andina*

For several decades the Toluca Valley in Central Mexico was considered the centre of origin for *P. infestans* and the closely related species *Phytophthora mirabilis*, *Phytophthora ipomoea* and possibly *Phytophthora phaseoli* (Grünwald and Flier, 2005). This is in part because until the 1980s the A1 and A2 mating types were only found in the Toluca Valley. Other authors have published evidence of co-evolution between *P. infestans* and potato in the Andes of South America (Abad and Abad, 1997), and a theory recently supported by nuclear and mitochondrial gene genealogies proposed that both *P. andina* and *P. infestans* evolved from a common ancestor in South America (Gómez-Alpizar *et al.*, 2007).

In addition to the studies above, recent reviews describe historical population changes in *P. infestans* at a global level (Fry *et al.*, 2009). One important issue related to *P. infestans* globally is the presence of sexually active populations, particularly in Scandinavia (Lehtinen and Hannukkala, 2004; Widmark *et al.*, 2011). This is potentially significant for South America where in some locations both the A1 and the A2 coexist. For example, in Argentina both A1 and A2 mating types were collected from potato fields (Van Damme and Ridao, 1994). Thus far in South America, only clonal populations have been identified and there is no evidence of oospore involvement in disease epidemics.

As noted, several migrations have apparently occurred within South America, presumably involving pathogen strains introduced from North America or Europe, and most likely with seed. This includes the EC-1 lineage in the central Northern Andes (Forbes *et al.*, 1997), populations in Venezuela (Briceño *et al.*, 2009), populations in the southern cone outside of Chile (Forbes *et al.*, 1997; Suassuna *et al.*, 2004) and a new population in Chile (Acuña *et al.*, 2011). The origin, distribution and evolution of *P. infestans* are still under discussion. Recent evidence indicates that the Irish famine was caused by isolates belonging to mitochondrial haplotype Ia (Ristaino *et al.*, 2001; May and Ristaino, 2004) and not Ib, as previously thought (Goodwin *et al.*, 1994). Given the lack of clarity that still exists on the origin of *P. infestans*, and considering that the centre of origin of potato, the principal host of this plant pathogen, is South America, more research in the Northern Andean region would appear merited.

6.6 Threats and Other Issues

Given the complexity of the pathogen populations, the number of common hosts for both *P. infestans* and *P. andina*, and the level of continuous disease intensity in many parts of South America, hybridization or other forms of gene flow may pose a threat. Host range expansion by *Phytophthora* hybrids has been documented for naturally occurring hybrids and for hybrids created in the laboratory (see Érsek and Man in 't Veld, Chapter 5, this volume). Regardless of the mechanism, there seems to be evidence of host range expansion in these pathogen taxa within South America. A number of *P. andina*'s hosts have been introduced to new regions, particularly *S. betaceum* and to a lesser extent *S. muricatum*. *S. betaceum* is now found throughout the tropics, is commonly eaten in sub-Saharan Africa and is an export crop for New Zealand. Thus far, the Andean

pathogen populations have not been found in these new locations. Similarly, *P. peruviana* has also been globalized and is currently grown in a number of countries, particularly in South Africa. To our knowledge, the specialized form of *P. infestans* attacking this crop exists only in Colombia.

References

Abad, Z.G. and Abad, J.A. (1997) Another look at the origin of late blight of potatoes, tomatoes, and pear melon in the Andes of South America. *Plant Disease* 81, 682–688.

Acuña, I., Gutiérrez, M., Sagredo, B., Fahrenkrog, A., Seco, G., Rivera, V., Manchilla, S. and Sandoval, C. (2011) Monitoreo y determinación de los cambios poblacionales de *Phytophthora infestans* en el cultivo de papa en Chile. *Fitopatología Colombiana* 35, Supplement.

Adler, N.E., Chacón, G., Flier, W.G. and Forbes, G.A. (2002) The Andean fruit crop, pear melon (*Solanum muricatum*), is a common host for A1 and A2 strains of *Phytophthora infestans* in Ecuador. *Plant Pathology* 51, 802.

Adler, N.E., Erselius, L.J., Chacón, M.G., Flier, W.G., Ordoñez, M.E., Kroon, L.P.N.M. and Forbes, G.A. (2004) Genetic diversity of *Phytophthora infestans sensu lato* in Ecuador provides new insight into the origin of this important plant pathogen. *Phytopathology* 94, 154–162.

Bailon, Y. and Otazu, V. (1992) Aspectos economicos del control de la rancha (*P. infestans*) de la papa en los Andes Centrales Del Peru. [Economic aspects of control of potato late blight in the Central Andes of Peru]. *Fitopatologia (Peru)* 27 (1), 33–37.

Blandón-Diaz, J.U., Widmark, A.K., Hannukkala, A., Andersson, B., Högberg, N. and Yuen, J.E. (2012) Phenotypic variation within a clonal lineage of *Phytophthora infestans* infecting both tomato and potato in Nicaragua. *Phytopathology* 102, 323–330.

Bohs, L. and Olmstead, R.G. (1997) Phylogenetic relationships in *Solanum* (Solanaceae) based on ndhF sequences. *Systematic Botany* 22, 5–17.

Briceño, A., Cedeño, L., San Román, M., Moreno, M., Quintero, K., Pino, H. and Fermin, G. (2009) Population structure of *Phytophthora infestans* in the Venezuelan Andes (2004–2007). *Acta Horticulturae* 834, 129–140.

Bustamante, N., Pérez, W. and Aragón, L. (2008) Control químico del tizón tardío (‘*Phytophthora infestans*’) de la papa ‘canchán’ en Huasahuasi, Junín. *Fitopatología* 43, 32–40.

Cárdenas, M., Tabima, J., Fry, W.E., Grünwald, N.J., Bernal, A. and Restrepo, S. (2011) Defining species boundaries in the genus *Phytophthora*: the case of a response to ‘*Phytophthora andina* sp. nov., a newly identified heterothallic pathogen of solanaceous hosts in the Andean highlands’. *Plant Pathology* 61, 215–220.

Chacón, M.G., Adler, N.E., Jarrin, F., Flier, W.G., Gessler, C. and Forbes, G.A. (2006) Genetic structure of the population of *Phytophthora infestans* attacking *Solanum ochranthum* in the highlands of Ecuador. *European Journal of Plant Pathology* 115, 235–245.

Forbes, G.A., Escobar, X.C., Ayala, C.C., Revelo, J., Ordoñez, M.E., Fry, B.A., Doucett, K. and Fry, W.E. (1997) Population genetic structure of *Phytophthora infestans* in Ecuador. *Phytopathology* 87, 375–380.

Forbes, G.A., Goodwin, S.B., Drenth, A., Oyarzún, P., Ordoñez, M.E. and Fry, W.E. (1998) A global marker database for *Phytophthora infestans*. *Plant Disease* 82, 811–818.

Forbes, G.A., Ristaino, J.B., Oliva, R.F. and Flier, W. (2011) A rebuttal to the letter to the editor concerning ‘Defining species boundaries in the genus *Phytophthora*: the case of *Phytophthora andina*’. *Plant Pathology* 61, 221–223.

Fry, W.E., Grünwald, N.J., Cooke, D.E.L., McLeod, A., Forbes, G.A. and Cao, K. (2009) Population genetics and population diversity of *Phytophthora infestans*. In: Lamour, K.H. and Kamoun, S. (eds) *Oomycete Genetics and Genomics: Diversity, Interactions, and Research Tools*. Wiley-Blackwell, Hoboken, New Jersey, pp. 139–162.

Garry, G., Forbes, G.A., Salas, A., Cruz, M.S., Pérez, W. and Nelson, R.J. (2005) Genetic diversity and host differentiation among isolates of *Phytophthora infestans* from cultivated potato and wild solanaceous hosts in Peru. *Plant Pathology* 54, 740–748.

Gilchrist Ramelli, E., Jaramillo Villegas, S., Afanador Kafuri, L. and Arango Isaza, R.E. (2009) Characterization of *Phytophthora infestans* populations in Antioquia, Colombia. *Revista Facultad Nacional de Agronomía, Medellín* 62, 5031–5037.

Gómez-Alpizar, L., Carbone, I. and Ristaino, J.B. (2007) An Andean origin of *Phytophthora infestans* inferred from mitochondrial and nuclear gene genealogies. *Proceedings of the National Academy of Sciences USA* 104, 3306–3311.

Goodwin, S.B., Cohen, B.A. and Fry, W.E. (1994) Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proceedings of the National Academy of Sciences USA* 91, 11591–11595.

Goss, E.M., Cardenas, M.E., Myers, K., Forbes, G.A., Fry, W.E., Restrepo, S. and Grünwald, N.J. (2011) The plant pathogen *Phytophthora andina* emerged via hybridization of an unknown *Phytophthora* species and the Irish potato famine pathogen, *P. infestans*. *PLoS One* 6, e24543.

Griffith, G.W. and Shaw, D.S. (1998) Polymorphisms in *Phytophthora infestans*: four mitochondrial haplotypes are detected after PCR amplification of DNA from pure cultures or from host lesions. *Applied and Environmental Microbiology* 64 (10), 4007–4014.

Grünwald, N.J. and Flier, W.G. (2005) The biology of *Phytophthora infestans* at its center of origin. *Annual Review of Phytopathology* 43, 171–190.

Hijmans, R.J., Forbes, G.A. and Walker, T.S. (2000) Estimating the global severity of potato late blight with GIS-linked disease forecast models. *Plant Pathology* 49, 697–705.

Kromann, P., Taipe, A., Andrade-Piedra, J.L., Munk, L. and Forbes, G.A. (2008) Preemergence infection of potato sprouts by *Phytophthora infestans* in the highland tropics of Ecuador. *Plant Disease* 92, 569–574.

Kromann, P., Taipe, A., Perez, W.G. and Forbes, G.A. (2009) Rainfall thresholds as support for timing fungicide applications in the control of potato late blight in Ecuador and Peru. *Plant Disease* 93 (2), 142–148.

Lehtinen, A. and Hannukkala, A. (2004) Oospores of *Phytophthora infestans* in soil provide an important new source of primary inoculum in Finland. *Agricultural and Food Science* 13, 399–410.

May, K.J. and Ristaino, J.B. (2004) Identity of the mtDNA haplotype(s) of *Phytophthora infestans* in historical specimens from the Irish potato famine. *Mycological Research* 108, 471–479.

Nelson, R.J., Orrego, R., Ortiz, O., Tenorio, J., Mundt, C.C., Fredrix, M. and Vien, N.V. (2001) Working with resource-poor farmers to manage plant diseases. *Plant Disease* 85, 684–695.

Ochoa, C.M. (2003) *Las Papas Del Perú, Base De Datos 1947–1997*. Centro Internacional de la Papa (CIP), Universidad Nacional Agraria La Molina (UNALM), COSUDE, Lima, Peru.

Oliva, R.F., Chacón, M.G., Cooke, D.E.L., Lees, A.K. and Forbes, G.A. (2007) Is *Phytophthora infestans* a good taxonomist? Host recognition in the *Phytophthora*/*Solanum* interaction. *Acta Horticulturae (ISHS)* 745, 465–471.

Oliva, R.F., Kroon, L.P.N.M., Chacón, G., Flier, W.G., Ristaino, J.B. and Forbes, G.A. (2010) *Phytophthora andina* sp. nov., a newly identified heterothallic pathogen of solanaceous hosts in the Andean highlands. *Plant Pathology* 59, 613–625.

Orozco, F.A., Cole, D.C., Forbes, G.A., Kroschel, J., Wanigaratne, S. and Arica, D. (2009) Monitoring adherence to the International FAO Code of Conduct on the distribution and use of pesticides: highly hazardous pesticides in central Andean agriculture and farmers' rights to health. *International Journal of Occupational and Environmental Health* 15, 255–268.

Ortiz, O., Winters, P. and Fano, H. (1999) La percepcion de los agricultores sobre el problema de tizon tardio o rancha (*Phytophthora infestans*) y su manejo: estudio de casos en Cajamara, Peru. *Revista Latinoamericana de la Papa* 11, 97–120.

Oyarzún, P.J., Pozo, A., Ordoñez, M.E., Doucett, K. and Forbes, G.A. (1998) Host specificity of *Phytophthora infestans* on tomato and potato in Ecuador. *Phytopathology* 88, 265–271.

Oyarzún, P.J., Garzón, C.D., Leon, D., Andrade, I. and Forbes, G.A. (2005) Incidence of potato tuber blight in Ecuador. *American Journal of Potato Research* 82, 117–122.

Panganiban, L., Cortes-Maramba, N., Dioquino, C., Suplido, M.L., Ho, H., Francisco-Rivera, A. and Manglicmot-Yabes, A. (2004) Correlation between blood ethylenethiourea and thyroid gland disorders among banana plantation workers in the Philippines. *Environmental Health Perspectives* 112, 42–45.

Pérez, W., Lara, J. and Forbes, G.A. (2009) Resistance to metalaxyl-M and cymoxanil in a dominant clonal lineage of *Phytophthora infestans* in Huánuco, Peru, an area of continuous potato production. *European Journal of Plant Pathology* 125, 87–95.

Pérez, W.G., Gamboa, J.S., Falcon, Y.V., Coca, M., Raymundo, R.M. and Nelson, R.J. (2001) Genetic structure of Peruvian populations of *Phytophthora infestans*. *Phytopathology* 91, 956–965.

Ristaino, J.B., Groves, C.T. and Parra, G.R. (2001) PCR amplification of the Irish potato famine pathogen from historic specimens. *Nature* 411, 695–697.

Spielman, L.J., Drenth, A., Davidse, L.C., Sujkowski, L.J., Gu, W., Tooley, P.W. and Fry, W.E. (1991) A second world-wide migration and population displacement of *Phytophthora infestans*? *Plant Pathology* 40, 422–430.

Spooner, D.M. (2009) DNA barcoding will frequently fail in complicated groups: an example in wild potatoes. *American Journal of Botany* 96, 1177–1189.

Suassuna, N.D., Maffia, L.A. and Mizubuti, E.S.G. (2004) Aggressiveness and host specificity of Brazilian isolates of *Phytophthora infestans*. *Plant Pathology* 53, 405–413.

Van Damme, M. and Ridao, A. (1994) Determination of races and mating type of *Phytophthora infestans* isolated in Argentina. *Fitopatología* 29, 78–82.

Vargas, A.M., Correa, A., Lozano, D.C., Gonzalez, A., Bernal, A.J., Restrepo, S. and Jimenez, P. (2007) First report of late blight caused by *Phytophthora infestans* on cape gooseberry (*Physalis peruviana*) in Colombia. *Plant Disease* 91, 464.

Vargas, A.M., Ocampo, L.M.Q., Cespedes, M.C., Carreno, N., Gonzalez, A., Rojas, A., Zuluaga, A.P., Myers, K., Fry, W.E., Jimenez, P., Bernal, A.J. and Restrepo, S. (2009) Characterization of *Phytophthora infestans* populations in Colombia: first report of the A2 mating type. *Phytopathology* 99, 82–88.

Wesseling, C., Corriols, M. and Bravo, V. (2005) Acute pesticide poisoning and pesticide registration in Central America. *Toxicology and Applied Pharmacology* 207, S697–S705.

Widmark, A.K., Andersson, B., Sandström, M. and Yuen, J.E. (2011) Tracking *Phytophthora infestans* with SSR markers within and between seasons – a field study in Sweden. *Plant Pathology* 60, 938–945.

Yanggen, D., Cole, D.C., Crissman, C. and Sherwood, S. (2004) Pesticide use in commercial potato production: reflections on research and intervention efforts towards greater ecosystems health in Northern Ecuador. *EcoHealth* 1, SU72–SU83.

7 *Phytophthora infestans* and Potato Late Blight in Europe

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7.1 Introduction

The cold, wet summer of 1845 and the arrival of *Phytophthora infestans* sparked a dramatic episode in European history with terrible consequences for millions of people who went hungry or starved due to widespread failure of the potato crop. In the UK and the Republic of Ireland the political and social consequences of the late blight epidemic are still felt almost 170 years later. The disease remains a serious threat to potatoes and tomatoes in Europe, and most growers manage the disease with routine applications of a broad range of anti-oomycete fungicides. In the past decade the plant production industry has been well informed concerning the pathogen's epidemiology, evolutionary potential and population structure, and, in most years, late blight management is adequate. Intensive management is costly and there is pressure to reduce the financial and environmental burden of spraying to manage late blight. Efforts include efficient and appropriate fungicidal spray regimes and the development of resistant cultivars acceptable to European consumers. Our understanding of host resistance and pathogen virulence has increased dramatically in recent years, driven largely by genome sequencing. This knowledge has opened new avenues to select and deploy durable forms of late blight resistance, offering the promise of improved blight management in the future.

7.2 Perspective of Late Blight Disease in Europe

The arrival and subsequent spread of *P. infestans* in Europe has been the subject of intense scrutiny and debate (see Ribeiro, Chapter 1, this volume). Bourke presents evidence that blight may have been present in drier seasons prior to the cold, wet summer of 1845 that prompted the widespread epidemic (Bourke, 1964). Potatoes were introduced into Europe as a novelty in the 16th century and at first they were not widely accepted as a food crop. Ironically, it was their establishment as an 'anti-famine' crop capable of generating a high yield when cereal crops failed that led to the potato's widespread cultivation in the late 18th century. The absence of records of the late blight disease up until the 1840s provides compelling evidence for the introduction of *P. infestans* in 1845 or thereabouts. Growers in Europe have battled against late blight disease ever since. Two factors allowed potato production to continue in the face of late blight: (i) the breeding of more resistant cultivars; and (ii) application of copper-based fungicides. The current commercial impact of *P. infestans* (control costs and crop losses) is conservatively estimated at €1000 million/year across the 6 million ha of commercially grown potato in Europe (Haverkort *et al.*, 2008). Disease loss tends to be greater in Eastern Europe where fungicide inputs are lower. The pathogen is also a scourge of

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outdoor-grown tomato crops, particularly in southern regions of Europe, and is a problem on other solanaceous vegetables such as aubergine or ornamentals such as petunia. Reports of *P. infestans* on solanaceous weeds such as nightshade are common and these hosts may play a role in the epidemiology of late blight (see below).

7.3 History of Population Change of *P. infestans* in Europe

Because of the historical importance of late blight to Europe, forensic analysis has been used to piece together the history of the disease and to try and identify the isolates responsible for the original blight. Mitochondrial restriction fragment length polymorphism (RFLP) analysis of diseased herbaria material collected between 1845 and 1886 from several European countries indicates there is a point mutation between the A1 mating type US-1 lineage of *P. infestans* that was thought to have incited the blight and the isolates found in the herbaria material (May and Ristaino, 2004). Further investigation using autosomal markers or next generation sequencing will allow this hypothesis to be rigorously tested. Prior to the 1980s European *P. infestans* isolates were of the A1 mating type and the US-1 lineage, as defined by isozymes, autosomal DNA-based RFLPs using the RG57 probe and mitochondrial DNA (mtDNA) haplotyping primers (Cooke and Lees, 2004). It is thought that two waves of *P. infestans* migration occurred between the 1840s and the late 20th century. The second major migration occurred when the potato crop failed in the drought year of 1976 and new lineages of the A1 and A2 mating type were brought into Europe on potatoes from the reported centre of diversity of the pathogen in Mexico (Niederhauser, 1991; Goodwin and Drenth, 1997). Novel A1 and A2 isolates were subsequently found in many parts of Europe, and the absence of isolates of the US-1 lineage indicated a population displacement had occurred. The presence of both mating types presented a new threat to European crops due to the potential for sexual recombination and the presence of long-lived soil-borne oospores (Andersson *et al.*, 2009; Brurberg *et al.*, 2011). From 1980 to 1994 the frequency of A2 types was low in most parts but later approached 50:50 in some regions, for example, the Netherlands (Zwankhuizen *et al.*, 2000), Poland (Sujkowski *et al.*, 1994) and Nordic countries (Hermansen *et al.*, 2000). There was also increased genetic diversity probably due to isolates produced via sexual recombination (e.g. Drenth *et al.*, 1994; Brurberg *et al.*, 1999). In other countries A2 isolates were absent (Cooke *et al.*, 2006) or remained at a low frequency, and populations were dominated by a few clones (e.g. Lebreton *et al.*, 1998; Day *et al.*, 2004). Resistance to fungicides in the phenylamide group was widespread in isolates of the A1 mating type but, interestingly, until 2004 A2 isolates from across Europe were predominantly sensitive to these important fungicides (Hermansen *et al.*, 2000; Cooke *et al.*, 2003; University of Arhus, 2012). This suggests resistance among A1 lineages had not been genetically recombined into isolates of the A2 mating type in Europe and phenylamide fungicides may have suppressed the A2 clonal lineages (University of Arhus, 2012).

What were the implications of this 1980s phase of population change to the European potato industry? The transition from a clonal population to one with both mating types was accompanied by an increase in genetic and virulence diversity (Drenth *et al.*, 1994; Sujkowski *et al.*, 1994). Analysis of long-term data sets in the Netherlands suggests the appearance of the ‘new’ populations around 1980 may have been a factor in the end of a so-called ‘10 year truce’ (1969–1978) during which blight disease intensity was markedly lower than earlier or later periods (Zwankhuizen and Zadoks, 2002). Other studies, however, have not indicated clear differences in aggressiveness between ‘old’ and ‘new’ populations (Day and Shattock, 1997). The complete displacement of the US-1 lineage,

however, implies a fitness advantage in the newly introduced populations. Perhaps such fitness is expressed in a very specific set of environmental conditions that has not been reproduced in laboratory-based aggressiveness tests. There is a consensus among researchers that even in carefully controlled studies, discriminating consistent differences in *P. infestans* aggressiveness is challenging. It is likely that there is a fitness cost associated with the accumulation of deleterious mutations in a clonal population (i.e. Muller's ratchet; Goodwin, 1997) but this has yet to be tested objectively.

7.4 Molecular Tools to Track Population Change

As in other regions, population diversity has been monitored using a mix of phenotypic and genotypic markers. Mating type, fungicide sensitivity and virulence against the 11 known resistance genes were the main traits studied. For genotyping, isozymes, RFLPs (using the moderately repetitive RG57 probe), mitochondrial haplotyping and amplified fragment length polymorphisms (AFLPs) were the principal markers. Each genotypic marker has, however, limitations due, for example, to limited diversity or a lack of reproducibility (Cooke and Lees, 2004; see also Lamour, Chapter 4, this volume). A need for objective, locus-specific, co-dominant markers was fulfilled with the advent of microsatellite or simple sequence repeat (SSR) markers in the early 2000s. Using such markers one can PCR-amplify loci from DNA extracted from *P. infestans* cultures, pathogen DNA extracted direct from a blight lesion or from lesions pressed on to an FTA^{TM} card (Whatman, UK; Li, 2012). Accurate sizing of the alleles amplified at each locus via electrophoresis on a gel, or increasingly through fluorescent labelling and capillary electrophoresis, generates a multilocus genotype (MLG) for each isolate. The loci used to date have proven to have an appropriate resolution for discriminating *P. infestans* isolates within Europe (Brurberg *et al.*, 2011) and beyond (Li, 2012). The recent development of a multiplex assay in which 12 SSR loci are amplified in a single PCR is increasing the efficiency of this approach (Li, 2012). The dissemination of this 12-plex protocol along with reference isolates and their SSR profiles on the Euroblight web site (www.euroblight.net) will aid the adoption of a standard fingerprinting system that facilitates harmonization of international datasets (see later comments on the Eucablight database). The amplification of more than two alleles per locus in many European isolates (Cooke *et al.*, 2012a, b) suggests trisomy or polyploidy is frequent in some *P. infestans* populations. Software to examine genetic diversity in SSR datasets of mixed ploidy levels has recently become available (Clark and Jasieniuk, 2011) and should allow more detailed analysis of Europe-wide populations.

7.5 Epidemiology and Management

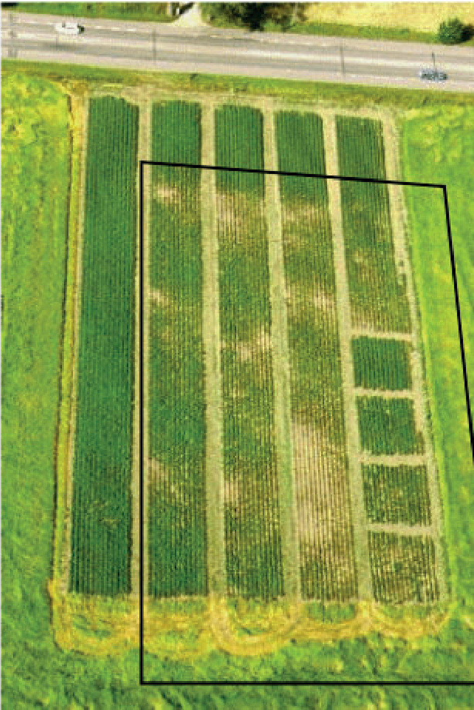
A recent review of the management and epidemiology of late blight in Europe overviews approaches used in several important potato growing regions (Cooke *et al.*, 2011). Current best practice for management is provided under the Potato IPM menu on the Euroblight web site (www.euroblight.net). In this section key findings are summarized.

A critical step in all disease management is to understand and limit all sources of primary inoculum. Within Europe (and particularly the north-west regions) intensive commercial and home-garden potato cultivation provides ample opportunities for carry-over of inoculum from one season to the next, as asexual mycelium in seed, volunteer tubers or discarded tuber piles. Advice centres on: (i) the production and sale of healthy seed potato; (ii) control of volunteer potato plants via long rotations; and (iii) proper treatment of discarded tuber piles. The role of solanaceous weeds such as hairy nightshade as alternative hosts of

P. infestans (Grönberg *et al.*, 2012) should also be considered. In some regions of Europe the soil-borne sexual oospores of *P. infestans* have been demonstrated to cause early and severe blight infection in subsequent crops (Fig. 7.1; Andersson *et al.*, 2009). In particular, this has been evident in Nordic regions, probably because of a combination of short rotations (Hannukkala *et al.*, 2007) and cold winters killing asexual inoculum in infected volunteer and discarded potato tubers. Oospores are also considered a problem in starch-growing regions in the north-east of the Netherlands where 2–3-year rotations are common and, because long-term storage of tubers is not required, blight management lapses towards the end of the season (Cooke *et al.*, 2011). It is not readily apparent why oospores are such a minor component of the primary inoculum in other western regions (Cooke *et al.*, 2007) but longer rotations and reduced viability of the oospore progeny due to aneuploidy in the dominant clones are possible explanations.

Given optimal environmental conditions, blight infection is inevitable in European crops and its management remains a challenge. Outbreak dates are monitored in many European countries with systems in place to alert growers of active late blight or conducive weather in their region (see pathogen surveillance section of Euroblight: www.euroblight.net). Under intensive large-scale production, cultivar choice is shaped more by market requirements than disease resistance and most cultivars have moderate to low levels of resistance to late blight. Frequent fungicide application remains the only practical and effective approach to disease management. Fungicide use in Europe is guided by European legislation

A



B

Fig. 7.1. Demonstration of the role of soil-borne *P. infestans* oospores in subsequent foliar late blight outbreaks. **(A)** Aerial photograph of a potato trial at Uppsala 1996; the black rectangle shows the location of a late blight trial in 1994. **(B)** Close-up of areas heavily affected by late blight (photograph credit Magnus Sandström).

(Directive 2009/128/EC) that establishes a community framework for the sustainable use of pesticides. A key element of this is that each member state develops a 'National Action Plan' to reduce the risks of pesticide use on human health and the environment, and encourages the use of alternative methods of control. Current research in many European Union (EU) states aims to reduce fungicide inputs via better prediction of blight activity (Hansen *et al.*, 2010) to ensure application timings and dose rates are optimal (Nielsen *et al.*, 2010). Supplanting part of the fungicide input with host resistance is another valid approach (Kessel *et al.*, 2010; Nærstad *et al.*, 2010) but requires reliable and up-to-date knowledge of the resistance ratings of cultivars. Awareness of contemporary changes in the pathogen population (see below) and its impact on the stability of blight resistance of some cultivars is critical to the success of such a strategy (White and Shaw, 2010).

The Euroblight potato late blight network is a valuable forum for researchers and industry representatives to meet every 18 months and report the latest European research in areas such as: (i) *P. infestans* populations; (ii) decision support systems; (iii) resistance breeding; and (iv) chemical efficacy. Independent trials on fungicide active ingredients are published as a regularly updated fungicide table that collates the key properties of all products. The Euroblight web site maintained by Jens Hansen at the University of Arhus is a useful resource for timely data and suggestions for late and early blight management (www.euroblight.net).

7.6 Current Research and Future Perspectives

7.6.1 Monitoring contemporary population change

Recent surveys of *P. infestans* using SSR markers revealed a major population change in much of north-west Europe. An A2 clonal lineage termed 13_A2 was first identified in samples from the north-east region of the Netherlands in 2004 and by 2006 and 2007 comprised over 40% of the *P. infestans* population in Great Britain (Cooke *et al.*, 2007, 2012b). The lineage comprises a large proportion of the population in the Netherlands (Li, 2012) and France (Duvauchelle *et al.*, 2009; Montarry *et al.*, 2010), and was reported in Switzerland, Belgium and Germany in 2006 and 2007 (Gisi *et al.*, 2011). Its westward progress continued into Ireland in 2007, becoming established in crops across the island over 2008 and 2009 (Kildea *et al.*, 2010). The 13_A2 lineage has also been reported beyond the borders of Europe (Li, 2012). No differences in overall fitness between the A1 and A2 populations have been identified (Gisi *et al.*, 2011). However, the population spread suggests that individuals of the 13_A2 lineage are more fit and aggressive than individuals of the populations they have displaced. The emergence and rapid spread of this strain serves as an illustration of the potential threat posed by emergence and international movement of not only invasive species but intraspecific lineages of *Phytophthora*. Should the 13_A2 lineage prove well adapted to conditions in potato-growing regions of Asia (e.g. Papua New Guinea) or sub-Saharan Africa, late blight loss will probably increase and the sustainability of potato production will be threatened. Interestingly, a similar displacement has not occurred across all parts of Europe. Nordic regions maintain high levels of diversity and no single clone dominates (Grönberg *et al.*, 2010). Isolates from commercial sites across east and north-east Europe (D.E.L. Cooke, unpublished) and parts of the Netherlands are similarly diverse with less dominance of single clones (Li, 2012). This genotypic variation suggests oospores are important sources of inoculum in these areas. It is currently unclear if the population structures are based on adaptive differences within the pathogen populations, the local physical environments, different systems of potato cropping or some combination of factors. Comprehensive datasets of *P. infestans* isolates and standardized SSR profiles have been collected from many regions of Europe

(see below), which will provide a detailed picture of the population structure on a wider scale.

7.6.2 Coordination and use of databases

European late blight research has been strongly influenced by the EU-funded research projects (EU.ICP.NET and Eucablight) that led to the formation of the Euroblight network. The coordination of activities and cooperative approach has resulted in the establishment of databases of *P. infestans* pathogen diversity and variation in potato late blight resistance. The database and interface (www.eucablight.org) were planned by the project partners and implemented in 2003 (Hansen *et al*., 2007). Updates to the datasets have continued and calculations and data display is 'on the fly' so that summaries of all current data may be viewed. For these resources to reach their potential, improvements to the data upload facilities and the web interface for displaying the results are required. The team at the University of Arhus has designed and built a new .NET technology-based 'crop problem' database with mapping tools to track changes in cereal pathogens (e.g. www.wheatrust.org). The Eucablight database will be migrated to this new format and improved means of uploading data are allowing its expansion. The benefits of sharing population change data on local, national and international scales are clear. From such data we can, for example: (i) infer sources of primary inocula; (ii) identify and track breakdown of cultivar resistance or insensitivity to specific fungicide active ingredients; (iii) share experiences in managing different clonal lineages of the pathogen; and (iv) gain a deeper understanding of the rates and pathways of pathogen spread and the evolutionary mechanisms employed by *P. infestans*.

7.6.3 Effector diversity and new approaches to late blight resistance breeding

Durable late blight resistance is a major goal for potato breeders but remains a great challenge. The rapid breakdown of resistance from *Solanum demissum* deployed in Europe in the 1960s demonstrated the vulnerability of single genes in the face of an adaptable pathogen (Bradshaw *et al*., 1995). Fifty years on, the specific details of the potato late blight major resistance genes and the corresponding *P. infestans* effectors responsible for their breakdown are understood and lessons are being learned. The publication of the genomes of several isolates of *P. infestans* and their potato and tomato hosts is heralding a new era in our understanding of how the pathogen and host interact. These resources are being exploited to identify sources of resistance with the aim of improved durability (Vleeshouwers *et al*., 2011). There are hundreds of pathogen effector genes and part of *P. infestans*' adaptability is thought to rely on redundancy in these genes and their location in gene-sparse regions of the genome (Vleeshouwers *et al*., 2011). To successfully exploit new sources of resistance there is a need to understand the existing effector diversity and mechanisms and rates of mutation within the effector genes. Databases of pathogen isolate diversity such as the Eucablight database (www.euroblight.net), if linked with a database of DNA samples, can be used to track effector evolution in different populations. The theoretical advantages of a strategic and coordinated deployment of novel sources of host resistance (Haverkort *et al*., 2008; Vleeshouwers *et al*., 2011) have been demonstrated (Skelsey *et al*., 2010), which will help improve their durability.

References

Andersson, B., Widmark, A.K., Yuen, J.E., Evenhuis, A., Turkensteen, L.J., Lehtinen, A., Nielsen, B., Ravnskov, S., Kessel, G.J.T., Hansen, J.G., Hermansen, A., Brurberg, M.B. and Nordskog, B. (2009) The role of oospores in the epidemiology of potato late blight. In: Forbes, G.A., Govers, F. and Fry, W.E. (eds) International Society for Horticultural Science (ISHS) III International Late Blight Conference, Beijing, China, 3–6 April 2008. *Acta Horticulturae* 834, 61–68.

Bourke, P.M.A. (1964) Emergence of potato blight, 1843–46. *Nature* 203, 805–808.

Bradshaw, J.E., Wastie, R.L., Stewart, H.E. and Mackay, G.R. (1995) Breeding for resistance to late blight in Scotland. In: Dowley, L.J., Bannon, E., Cooke, L.R., Keane, T. and O'Sullivan, E. (eds) *Phytophthora infestans* 150, Euorpean Association for Potato Research (EAPR) Pathology Section Conference. Boole Press and Teagasc, Ireland, pp. 246–254.

Brurberg, M.B., Hannukkala, A. and Hermansen, A. (1999) Genetic variability of *Phytophthora infestans* in Norway and Finland as revealed by mating type and fingerprint probe RG57. *Mycological Research* 103, 1609–1615.

Brurberg, M.B., Elameen, A., Le, V.H., Nærstad, R., Hermansen, A., Lehtinen, A., Hannukkala, A., Nielsen, B., Hansen, J., Andersson, B. and Yuen, J. (2011) Genetic analysis of *Phytophthora infestans* populations in the Nordic European countries reveals high genetic variability. *Fungal Biology* 115, 335–342.

Clark, L.V. and Jasieniuk, M. (2011) Polysat: an R package for polyploid microsatellite analysis. *Molecular Ecology Resources* 11, 562–566.

Cooke, D.E.L. and Lees, A.K. (2004) Markers, old and new, for examining *Phytophthora infestans* diversity. *Plant Pathology* 53, 692–704.

Cooke, D.E.L., Young, V., Birch, P.R.J., Toth, R., Gourlay, R., Day, J.P., Carnegie, S.F. and Duncan, J.M. (2003) Phenotypic and genotypic diversity of *Phytophthora infestans* populations in Scotland (1995–97). *Plant Pathology* 52, 181–192.

Cooke, D.E.L., Lees, A.K., Shaw, D.S., Taylor, M.C., Prentice, M.W.C., Bradshaw, N.J. and Bain, R.A. (2007) Survey of GB blight populations. In: Proceedings of the 10th workshop of a European network for the development of an integrated control strategy for late blight. *Praktijkonderzoek Plant en Omgeving (PPO)* [*Applied Plant Research*] *Special Report* no. 12, 145–152.

Cooke, D.E.L., Lees, A.K., Lassen, P. and Hansen, J.G. (2012a) Making sense of *Phytophthora infestans* diversity at national and international scales. In: Proceedings of the 13th EuroBlight workshop October 2011, Saint Petersburg, Russia. *Praktijkonderzoek Plant en Omgeving (PPO)* [*Applied Plant Research*] *Special Report* no. 15, 37–44.

Cooke, D.E.L., Cano, L.M., Raffaele, S., Bain, R.A., Cooke, L.R., Etherington, G.J., Deahl, K.L., Farrer, R.A., Gilroy, E.M., Goss, E.M., Grünwald, N.J., Hein, I., MacLean, D., McNicol, J.W., Randall, E., Oliva, R.F., Pel, M.A., Shaw, D.S., Squires, J.N., Taylor, M.C., Vleeshouwers, V.G.A.A., Birch, P.R.J., Lees, A.K. and Kamoun, S. (2012b) Genome analyses of an aggressive and invasive lineage of the Irish potato famine pathogen. *PLoS Pathogens* 8 (10), e1002940.

Cooke, L.R., Carlisle, D.J., Donaghy, C., Quinn, M., Perez, F.M. and Deahl, K.L. (2006) The Northern Ireland *Phytophthora infestans* population 1998–2002 characterized by genotypic and phenotypic markers. *Plant Pathology* 55, 320–330.

Cooke, L.R, Schepers, H.T.A.M, Hermansen, A., Bain, R.A., Bradshaw, N.J., Ritchie, F., Shaw, D.S., Evenhuis, A., Kessel, G.J.T., Wander, J.G.N., Andersson, B., Hansen, J.G., Hannukkala, A., Nærstad, R. and Nielsen, B.J. (2011) Epidemiology and integrated control of potato late blight in Europe. *Potato Research* 54,183–222.

Day, J.P. and Shattock, R.C. (1997) Aggressiveness and other factors relating to displacement of populations of *Phytophthora infestans* in England and Wales. *European Journal of Plant Pathology* 103, 379–391.

Day, J.P., Wattier, R.A.M., Shaw, D.S. and Shattock, R.C. (2004) Phenotypic and genotypic diversity in *Phytophthora infestans* on potato in Great Britain, 1995–98. *Plant Pathology* 53, 303–315.

Drenth, A., Tas, I.C.Q. and Govers, F. (1994) DNA fingerprinting uncovers a new sexually reproducing population of *Phytophthora infestans* in the Netherlands. *European Journal of Plant Pathology* 100, 97–107.

Duvauchelle, S., Dubois, L. and Détourné, D. (2009) Evolution of the population of *Phytophthora infestans* in France measured by epidemiologic and phenotypic markers. In: Forbes, G.A., Govers, F. and Fry, W.E. (eds) International Society for Horticultural Science (ISHS) III International Late Blight Conference, Beijing, China, 3–6 April 2008. *Acta Horticulturae* 834, 149–154.

Gisi, U., Walder, F., Resheat-Eini, Z., Edel, D. and Sierotzki, H. (2011) Changes of genotype, sensitivity and aggressiveness in *Phytophthora infestans* isolates collected in European countries in 1997, 2006 and 2007. *Journal of Phytopathology* 159, 223–232.

Goodwin, S.B. (1997) The population genetics of *Phytophthora*. *Phytopathology* 87, 462–473.

Goodwin, S.B. and Drenth, A. (1997) Origin of the A2 mating type of *Phytophthora infestans* outside Mexico. *Phytopathology* 87, 992–999.

Grönberg, L., Andersson, B., Högberg, N., Widmark, A.K. and Yuen, J.E. (2010) Genotypic variation of *Phytophthora infestans* within and between fields in the Nordic countries. In: Proceedings of the 12th Euroblight workshop. *Praktijkonderzoek Plant en Omgeving (PPO)* [*Applied Plant Research*] *Special Report* no. 14, 151–152.

Grönberg, L., Andersson, B. and Yuen, J.E. (2012) Can weed hosts increase aggressiveness of *Phytophthora infestans* on potato? *Phytopathology* 102, 429–433.

Hannukkala, A.O., Kaukoranta, T., Lehtinen, A. and Rahkonen, A. (2007) Late-blight epidemics on potato in Finland, 1933–2002: increased and earlier occurrence of epidemics associated with climate change and lack of rotation. *Plant Pathology* 56, 167–176.

Hansen J.G., Colon, L.T., Cooke, D.E.L., Lassen, P., Nielsen, B., Cooke, L.R., Andrivon, D. and Lees, A.K. (2007) Eucablight – collating and analysing pathogenicity and resistance data on a European scale. In: Proceedings of conference: 'Computer Aids for Plant Protection', Wageningen, the Netherlands, 17–19 October 2006. *Organisation Européenne et Méditerranéenne pour la Protection des Plantes/European and Mediterranean Plant Protection Organization (OEPP/EPPO) Bulletin* 37, 383–390.

Hansen, J.G., Kessel, G.J.T., Nærstad, R., Schepers, H.T.A.M., Nielsen, B.J. and Lassen, P. (2010) EuroBlight tool for the comparison of late blight sub-models – status and perspectives. In: Proceedings of the 12th Euroblight workshop. *Praktijkonderzoek Plant en Omgeving (PPO)* [*Applied Plant Research*] *Special Report* no. 14, 67–74.

Haverkort, A.J., Boonekamp, P.M., Hutten, R., Jacobsen, E., Lotz, L.A.P., Kessel, G.J.T., Visser, R.G.F and van der Vossen, E.A.G. (2008) Societal costs of late blight in potato and prospects of durable resistance through cisgenic modification. *Potato Research* 51, 47–57.

Hermansen, A., Hannukkala, A., Nærstad, R.H. and Brurberg M.B. (2000) Variation in populations of *Phytophthora infestans* in Finland and Norway: mating type, metalaxyl resistance and virulence phenotype. *Plant Pathology* 49, 11–22.

Kessel, G.J.T., Spruijt, J., Evenhuis, B., van Bekkum, P. and Schepers, H.T.A.M. (2010) Experimental control strategies reducing the fungicide input at a practical scale. In: Proceedings of the 12th Euroblight workshop. *Praktijkonderzoek Plant en Omgeving (PPO)* [*Applied Plant Research*] *Special Report* no. 14, 199–204.

Kildea S., Cooke, L.R., Quinn, L., Little, G., Armstrong, C., Hutton, F., Dowley, L.J. and Griffin, D. (2010) Changes within the Irish potato late blight population. In: Proceedings of the 12th Euroblight workshop. *Praktijkonderzoek Plant en Omgeving (PPO)* [*Applied Plant Research*] *Special Report* no. 14, 147–150.

Lebreton, L., Laurent, C. and Andrivon, D. (1998) Evolution of *Phytophthora infestans* populations in the two most important potato production areas of France during 1992–96. *Plant Pathology* 47, 427–439.

Li, Y. (2012) Multiplex SSR analysis of *Phytophthora infestans* in different countries and the importance to plant breeding. PhD thesis, Wageningen University, the Netherlands.

May, K.J. and Ristaino, J.B. (2004) Identity of the mtDNA haplotype(s) of *Phytophthora infestans* in historical specimens from the Irish Potato Famine. *Mycological Research* 108, 471–479.

Montarry, J.D., Andrivon, D., Glais, I., Corbiere, R., Mialdea, G. and Delmotte, F. (2010) Microsatellite markers reveal two admixed genetic groups and an ongoing displacement within the French population of the invasive plant pathogen *Phytophthora infestans*. *Molecular Ecology* 19, 1965–1977.

Nærstad, R., Le, V.H. and Hermansen, A. (2010) Reduced fungicide input in late blight control (REDUCE 2007–2011) – preliminary results from 2007 to 2009. In: Proceedings of the 12th Euroblight workshop. *Praktijkonderzoek Plant en Omgeving (PPO)* [*Applied Plant Research*] *Special Report* no. 14, 193–198.

Niederhauser, J.S. (1991) *Phytophthora infestans*: the Mexican connection. In: Lucas, J.A., Shattock, R.C., Shaw, D.S. and Cooke, L.R. (eds) *Phytophthora*. Cambridge University Press, Cambridge, UK, pp. 25–45.

Nielsen, B.J., Bødker, L. and Hansen, J.G. (2010) Control of potato late blight using a dose model to adjust fungicide input according to infection risk. In: Proceedings of the 12th Euroblight workshop. *Praktijkonderzoek Plant en Omgeving (PPO)* [*Applied Plant Research*] *Special Report* no. 14, 187–192.

Skelsey, P., Rossing, W.A.H., Kessel, G.J.T. and van der Werf, W. (2010) Invasion of *Phytophthora infestans* at the landscape level: how do spatial scale and weather modulate the consequences of spatial heterogeneity in host resistance? *Phytopathology*, 100, 1146–1161.

Sujkowski, L.S., Goodwin, S.B., Dyer, A.T. and Fry, W.E. (1994) Increased genotypic diversity via migration and possible occurrence of sexual reproduction of *Phytophthora infestans* in Poland. *Phytopathology* 84, 201–207.

University of Arhus (2012) Eucablight project website. Available at: www.eucablight.org (accessed 20 September 2012).

Vleeshouwers, V.G.A.A., Raffaele, S., Vossen, J.H., Champouret, N., Oliva, R., Segretin, M.E., Rietman, H., Cano, L.M., Lokossou, A., Kessel, G., Pel, M.A. and Kamoun, S. (2011) Understanding and exploiting late blight resistance in the age of effectors. *Annual Review of Phytopathology* 49, 507–531.

White, S. and Shaw, D.S. (2010) Breeding for host resistance: the key to sustainable potato production. In: Proceedings of the 12th Euroblight workshop. *Praktijkonderzoek Plant en Omgeving (PPO)* [*Applied Plant Research*] *Special Report* no. 14, 125–132.

Zwankhuizen, M.J. and Zadoks, J.C. (2002) *Phytophthora infestans*'s 10-year truce with Holland: a long-term analysis of potato late-blight epidemics in the Netherlands. *Plant Pathology* 51, 413–423.

Zwankhuizen, M.J., Govers, F. and Zadoks, J.C. (2000) Inoculum sources and genotypic diversity of *Phytophthora infestans* in Southern Flevoland, the Netherlands. *European Journal of Plant Pathology* 106, 667–680.

8 *Phytophthora infestans* in the USA

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8.1 Introduction

Late blight of tomato and potato caused by *Phytophthora infestans* is a recurring and costly problem for growers in the USA. In 2010 over 400,000 ha of potatoes valued at almost US$3.5 billion and 161,000 ha of tomatoes valued at US$1.4 billion were planted in the USA (NASS, 2010), and the majority of commercially grown varieties are extremely susceptible to late blight. A study found economic impacts of late blight to US potato growers (cost of spraying plus losses from disease) averaged more than US$500/ha (Guenthner et al., 2001), making late blight one of the most economically important diseases of this crop.

In the USA late blight is usually caused by a limited number of unique genotypes, referred to as clonal lineages (Fry *et al.*, 1992). Clonal lineages are propagated and transported on infected potato tubers and tomato seedlings and also by the production of asexual sporangia and zoospores on the foliage and stems of infected plants. While certain lineages are historically more common in certain regions of the country, a single genotype can be widely distributed. Both mating types of *P. infestans* have been present in the USA for decades, but sexually propagated oospores have not been identified in fields and at this time do not appear to be an important part of the pathogen's life cycle. Epidemics of late blight are sporadic, and regions may experience heavy disease during years of high rainfall and no late blight for several subsequent years. Clearly, weather is an important driver of late blight. Figure 8.1 shows slides from a presentation created in 1943 by Dr Russ Larson, a University of Wisconsin plant pathologist, to illustrate blight to a broad audience. Figure 8.2 provides an overview of the disease cycle on potato as it occurs in the USA. Although the tenacious oospores may not be a source of primary inoculum, they may play an important role in the creation of new strains.

8.2 History of Late Blight in the USA

The USA played a significant role in the history of late blight as the first reported epidemics began in 1843 in the port cities of Philadelphia and New York and within the same year spread to the surrounding five states. Within 2 years late blight had spread west to Illinois and Wisconsin, south to Virginia, and as far north as the Ontario and Nova Scotia provinces of Canada. It is likely this strain of *P. infestans* then crossed the Atlantic via seed potatoes bound for Belgium, eventually leading to its spread to other European nations, including Ireland. Due to its presence in the oldest collections obtained worldwide, these initial outbreaks are attributed to a currently unnamed lineage with Ia mitochondrial DNA (mtDNA)

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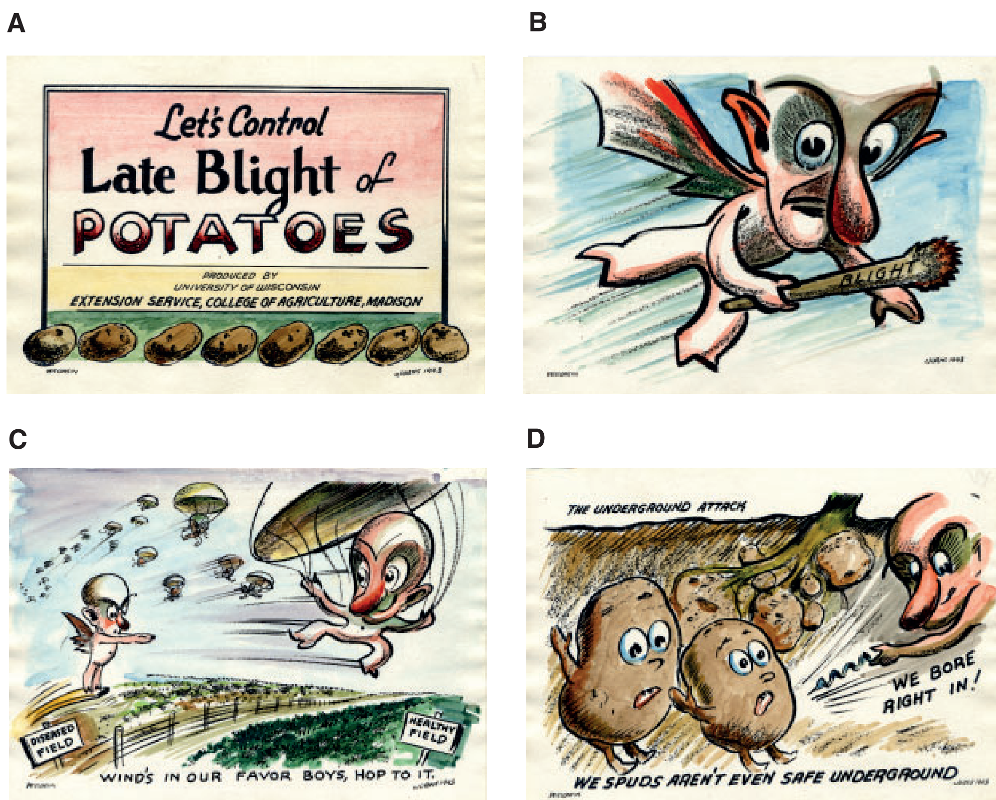

Fig. 8.1. Slides created in 1943 by Dr Russ Larson, a University of Wisconsin plant pathologist, to illustrate blight to a broad audience. **(A)** Illustrates the historical importance of blight in the Midwest, **(B, C)** the importance of the aerial phase of sporangia dispersal and **(D)** the movement of inoculum to tubers.

haplotype (Ristaino *et al.*, 2001). US-1, with a Ib mtDNA haplotype, and other closely related lineages, such as US-3 and CDA-1, were introduced to the USA probably in the early 20th century and then persisted asexually in the USA and Canada until a second major migration event in the late 1970s (Table 8.1) (Goodwin *et al.*, 1994). New lineages of *P. infestans* are currently defined using an analysis of multiple loci including: (i) allozyme genotype (Goodwin *et al.*, 1995); (ii) fungicide sensitivity (Deahl *et al.*, 1993); (iii) mtDNA haplotype (Griffith and Shaw, 1998); and (iv) DNA fingerprinting with the RG57 restriction fragment length polymorphism (Goodwin *et al.*, 1992). Isolates with a unique combination of loci are named with the number following the last published lineage designation.

A second migration of *P. infestans* to the USA occurred in 1978 or 1979 with the introduction of the US-6 lineage. This was the most common genotype recovered from 1987 to 1991 on both potatoes and tomatoes, and evidence suggests it originated from north-western Mexico, where it was widely distributed (Goodwin *et al.*, 1994). The US-4 lineage is also associated with the 1979 epidemic since it was found on tomatoes in California in 1980, but there is no evidence that it persisted beyond this time or spread to other areas.

In the late 1980s and early 1990s new populations of *P. infestans* on tomato and

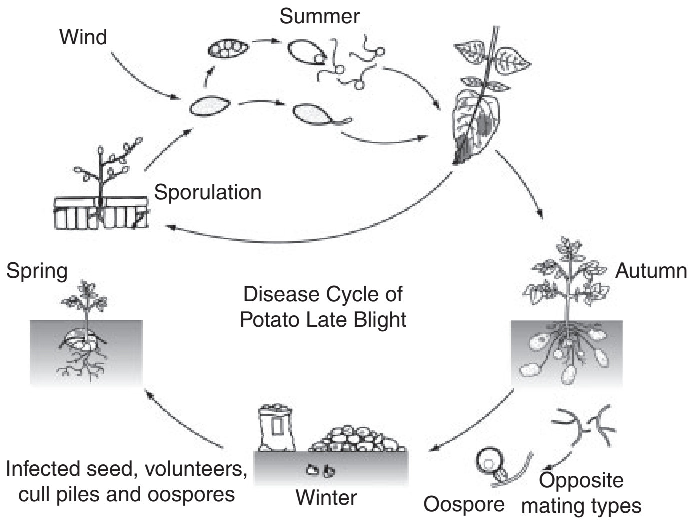

Fig. 8.2. Disease cycle of *Phytophthora infestans* on potato.

Table 8.1. Predominant clonal lineages of *Phytophthora infestans* in the USA 1840–present.

Lineage	Mating type	Host[a]	Date	Mef/Met[b]
US-1	A1	Potato	1840–present	Sensitive
US-6	A1	Potato/tomato*	1979–1994	Sensitive
US-7	A2	Potato/tomato*	1992–1995	Resistant
US-8	A2	Potato*/tomato	1992–present	Resistant
US-11	A1	Potato/tomato	1994–present	Resistant
US-17	A1	Tomato	1996	Resistant
US-18	A2	Tomato	1995–1998	Sensitive
US-22	A2	Potato/tomato*	2009–present	Sensitive
US-23	A1	Potato/tomato*	2010–present	Sensitive
US-24	A1	Potato*/tomato	2010–present	Resistant

[a]Favoured host is marked with an *.
[b]Mefenoxam and metalaxyl sensitivity.

potato emerged and introduced the A2 mating type to the USA and Canada (Deahl *et al.*, 1991). Genotyping later identified US-7 and US-8 from populations isolated in 1992 (Goodwin *et al.*, 1995). Both US-7 and US-8 were A2 mating types with resistance to the fungicide metalaxyl, which was used extensively at the time to control disease. Because of its fungicide resistance and increased aggressiveness, US-8 quickly became the dominant lineage of potatoes in the USA and remained the most common strain from the early 1990s through the mid-2000s.

Despite the introduction of the A2 mating type in the USA and its coexistence with populations of A1 lineages, evidence of sexual recombination was rare. The strongest indication of sexual recombination between lineages of *P. infestans* in the USA was presented by Gavino and colleagues (2000). They provided data supporting the hypothesis that the US-11 lineage from the Columbia Basin in Washington was the

product of sexual recombination between US6 and US7 followed by selection of a progeny that was well suited to the local environment.

8.3 Current Status of Late Blight in the USA

During a more recent migration in 2009 and 2010 (Fig. 8.3) late blight was identified on tomatoes and potatoes in a number of US states and Canadian provinces. The 2009 epidemic began in the early spring in the Southern USA and was initiated in the North-eastern USA in June, followed by Midwestern reports in late July–early August. By the end of 2009 late blight was reported in 29 states, caused primarily by the relatively new *P. infestans* clonal lineage, US-22, a lineage not previously known to occur across such a broad geographical distribution. The rapid spread of late blight in 2009 is attributed to the sale and distribution of infected tomato transplants throughout the North-eastern USA. Shortly after the first reports of the outbreak a greenhouse-grown tomato seedling supplier recalled all remaining plants at a cost of more than US$1 million. The rapid spread of late blight through the tomato distribution system in 2009 and its eventual impact on the tomato and potato industry throughout the USA clearly demonstrated a need for improved oversight and regulation to limit the future risk of such an economically devastating outbreak (McGrath, 2011). The 2010 epidemic did not follow a spatiotemporal pattern similar to 2009 and only 13 US states reported disease. In the Midwestern USA an unusually wet production season contributed to disease problems. In 2010 US reports of late blight remained isolated without further spread to greater geographic areas. While the 2009 epidemic introduced *P. infestans* US-22, which is sensitive to mefenoxam and metalaxyl fungicides and is of the A2 mating type, the 2010 epidemic included the additional genotypes US-23 and US-24. Lineages US-23 and US-24 are the A1 mating type and exhibit moderate sensitivity and resistance to mefenoxam, respectively.

Until recently the management of potato and tomato late blight in the USA was not

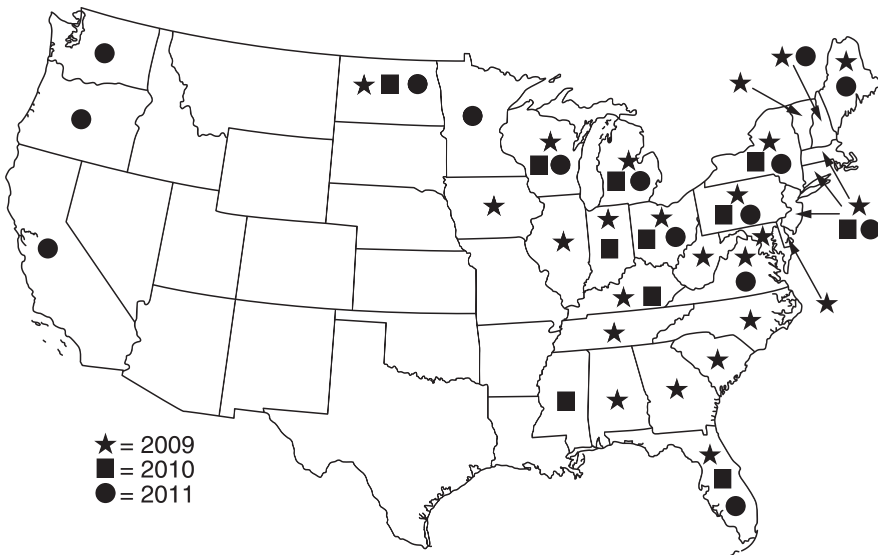

Fig. 8.3. Map of the USA providing an overview of reported late blight during the period 2009–2011.

closely coordinated. This stems from multiple factors including: (i) a predominating clonal lineage prior to the 1990s that was solely virulent on potato; and (ii) the typical commodity division of land grant extension plant pathologists where one specialist group studies tomato (and other vegetable crops) and another group is focused solely on potato. While the commodity division among pathologists is still generally in place, there is increased collaboration among research and extension pathologists. The appearance of multiple new clonal lineages with pathogenicity on both tomato and potato has also spurred increased collaboration, information sharing and has aided our overall understanding of host and pathogen interactions. Similar to recent outbreaks on potato, late blight populations on tomatoes are dynamic, with distinct clonal lineages sometimes changing yearly. Typically, only one clonal lineage is present within an epidemic population (Fry and Goodwin, 1997), although the origin of the diversity in tomato late blight populations is not well characterized. Migration from Mexico is the most logical source, but it might also have come from sporadic sexual reproduction (Fry and Goodwin, 1997).

Five major clonal lineages of *P. infestans* are currently present in the USA (US-8, US-11, US-22, US-23 and US-24). The 2009 national epidemic was composed of primarily US-22. In 2010 multiple clonal lineages were identified across the nation with US-24 dominating, but US-11, US-22 and US-23 were also present. In 2011 these five clonal lineages were identified in at least 14 states across the country (Fig. 8.3). To date, there is no evidence that US populations are undergoing sexual recombination despite the presence of both mating types in some regions. It is most probable that late blight is being spread on living host tissue, such as transplants or infected seed tubers. In 2011 US-23 was the most common lineage identified, followed by US-22, US-24, US-8 and US-11. These lineages are a mixture of A1 and A2 mating types and in several states the two mating types were found in the same region (neighbouring counties) but not in the same field.

8.4 Grower Attitudes Towards Late Blight

In the USA potato and tomato growers are well educated concerning late blight disease management. A recent survey showed that approximately two-thirds have bachelor's degrees and almost a third have worked towards advanced degrees in agriculture or a related field (K. Warren, Michigan, 2012, personal communication). The growers are interested in the basic and applied aspects of late blight control research and participate in powerful lobbying groups that influence state and federal funding. Most growers are fearful of the economic impact of late blight and they understand the importance of community interactions and implementing recommendations focused on limiting spread of disease, varietal resistance, proper cultural management and, when necessary, use of fungicides or plant destruction.

The ability of extension specialists to make proper recommendations for integrated management of late blight in tomato and potato is reliant upon several factors including: (i) an understanding of *P. infestans* genotypes and their host range and virulence; (ii) the efficacy of registered fungicides; and (iii) good lines of communication with growers and crop advisors. In order to prevent epidemics it is crucial to monitor key conditions season-long and to choose the correct fungicide and the correct application interval. The monitoring needs to include: (i) aspects of Blitecast (a computerized forecast of potato late blight; Krause *et al.*, 1975); (ii) accurate reports of new infections; and (iii) up-to-date reports on the spread of the disease. An unanticipated consequence of making late blight detection information public was discovered during the outbreaks of late blight in the late 1980s and early 1990s. During this time, extension specialists and consultants promptly verified, characterized and communicated new finds of late blight, and in several locations the updates were

disseminated to growers using telephone hotlines. While effective, and still utilized in some regions, problems arose when farmers with late blight-infected crops, particularly seed potato, realized the risk of lost profits and in rare cases avoided reporting to sell their crop. During the 1990s this breakdown in accurate tracking may have played a role in the interstate spread of late blight through infected seed, although there is no direct evidence to support this claim. Recent technology allows rapid identification of established and new clonal lineages, which then allows accurate and timely fungicide applications in infected and uninfected fields, and over the past 20 years growers have increasingly realized the benefits of communicating late blight incidence and providing samples for confirmation and characterization.

In the USA extension specialists monitor late blight outbreaks and make recommendations to growers in most potato- and vegetable-producing states. Local environments are quite variable and it is difficult to make effective recommendations on a national level, and many growers support local extension personnel and researchers and consistently choose their recommendations over those from the chemical industry. In addition, coordinated efforts are underway to track outbreaks, characterize strains and identify areas with the right environment for *P. infestans* growth, efforts valuable to researchers and growers making control decisions at the local level.

8.5 Management

Managing late blight is a challenge. Effective control requires informed participants at all levels including: (i) producers; (ii) university extension personnel; (iii) crop consultants; and, most recently, (iv) managers of home garden centres. Regularly released newsletters, updated websites and hotlines with updating late blight status are vital to manage this fast-spreading and aggressive plant disease. For susceptible crops in conventional, organic and other integrated systems, cultural practices to limit survival are essential. Although tomato seeds are not known to harbour late blight (Vartanian and Endo, 1985), infected tomato transplants are carriers and careful inspection is crucial. Potato tubers are living, respiring plant parts that harbour *P. infestans* in a variety of different settings including: (i) storage potatoes for consumption and seed; (ii) potatoes remaining in the field as volunteers; and (iii) cull piles of discarded potatoes (Fig. 8.4). Potatoes with late blight or with great risk of late blight should not be stored but utilized as soon as harvested. If storage is necessary, careful management of environmental conditions is essential. Infected seed potatoes should not be planted, and volunteers and cull piles should be eliminated prior to and throughout the production season. Compost piles with late blight-infected plant material must be managed appropriately and the temperature in the centre of the pile must reach adequate killing heat temperatures (>104°F or 40°C) or viable *P. infestans* can serve as an inoculum source, particularly if the compost is spread over production fields. Anecdotally, tomatoes grown under high tunnels have displayed a delay in late blight onset, while plants in adjacent uncovered fields have become infected. This has been reported in at least three states and is an area that will benefit from further research.

Fungicides are an important component of late blight control in tomato and potato. Conventional tomato and potato producers have several registered fungicides that are quite effective when applied early and throughout the season. In many regions growers use the Blitecast disease forecast tool to initiate late blight fungicide applications. Fungicides are classified as either contact, translaminar or systemic. Contact fungicides provide a barrier to infection on the plant surface, translaminar fungicides have localized movement inside of plant tissues (most currently registered conventional fungicides are either contact or translaminar), and systemic fungicides are xylem mobile with the fungicides moving into newer growth for extended protection. The benefit of using translaminar or systemic fungicides is their length of

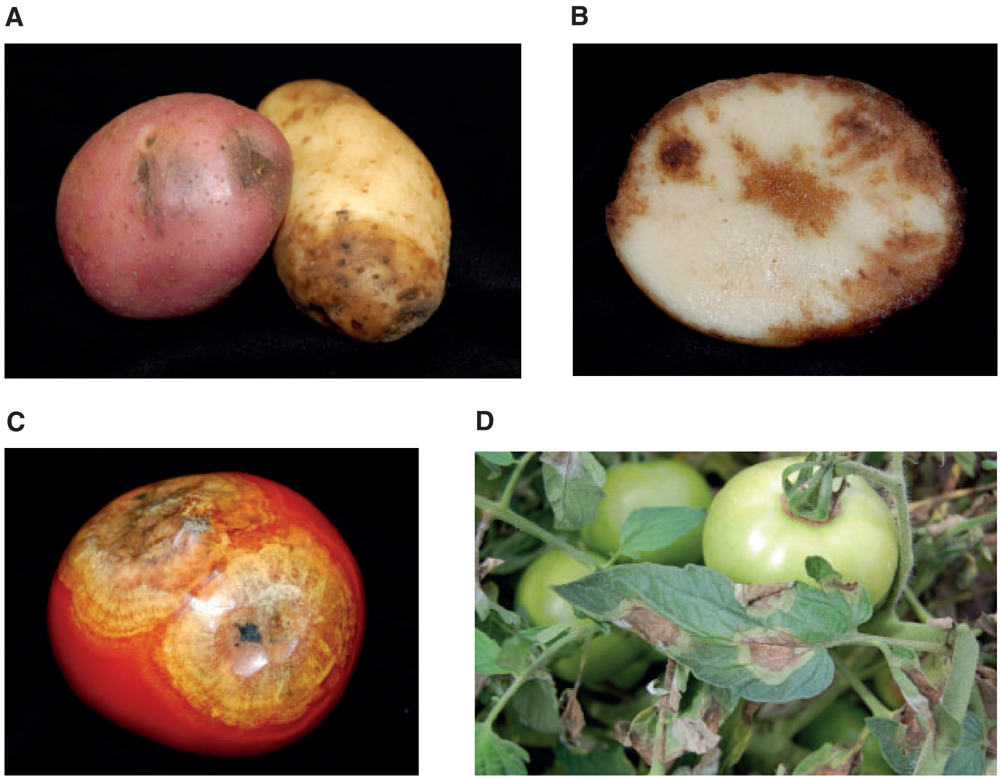

Fig. 8.4. Typical late blight symptoms on potato tubers **(A, B)**, and tomato fruit **(C)** and foliage **(D)**.

activity and ability to limit active infection sites. Generally, it is recommended that producers apply a mixture of a contact fungicide (such as chlorothalonil) and a translaminar (such as dimethomorph) or systemic (propamocarb) to limit active, and prevent new, infections, and to provide protection against a wide range of pathogens. To prevent fungicide resistance it is important to alternate active ingredients. Current active ingredients registered for late blight control in the USA include: (i) chlorothalonil; (ii) mancozeb; (iii) zoxamide; (iv) cyazofamid; (v) cymoxanil; (vi) femoxadone; (vii) dimethomorph; (viii) mandipropamid; (ix) fluopicolide; and (x) propamocarb.

For organic producers, there are several fungicides that are approved for organic use to control late blight in potato and tomato. Research using replicated studies indicates copper is the best of the approved fungicides (Dorn *et al.*, 2007). Although there are many copper-containing fungicides available for organic use, it is important to check with certifying agencies as approved formulations vary from year to year. Applications must occur prior to infection and then should be applied repeatedly. Precipitation and irrigation will degrade protection, and copper products are much less useful after disease onset.

Late blight is a community crop disease and we now know that blight management in the home garden is as important as the commercial farm, be it organic or conventional. Home gardening is on the rise for reasons of health and economy and even though the acreage of late blight-susceptible crops in the individual home garden may

be small, the sum total of home gardening in a state or region is significant and can contribute significant inoculum to epidemics in commercial potato and tomato (Fig. 8.4). Uncontrolled inoculum sources pose a high risk to crop quality and overall costs. Many home gardeners do not want to use fungicides and in these cases it is best to utilize varietal resistance if it is available. If gardeners choose to plant susceptible varieties and disease is detected in their region they have the option to protect plants with fungicides available at home gardening centres. These materials often contain copper or chlorothalonil. It is essential that gardeners read and follow label restrictions, as they must be applied appropriately and repeatedly for effective control.

In all tomato and potato production systems control of solanaceous weeds is important to limit late blight. In addition to potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*), *P. infestans* can infect members of the weedy nightshade family including woody nightshade (*Solanum dulcamara*) and black nightshade (*Solanum nigrum*), as well as the ornamental flower petunia (*Petunia* × hybrid). While strains of *P. infestans* may be specific for a certain host, the close proximity of alternative hosts to potato and tomato fields allows them to act as reservoirs for inoculum or may aid in the diversification of populations by permitting interaction between multiple strains. There is currently no evidence that nightshades act as overwintering hosts for *P. infestans* in colder climates, so primary infections of these hosts probably originate from nearby potato or tomato fields.

As a final note on management, if all control measures fail infected plants should be destroyed. Destruction methods vary (i.e. burning, herbicides, plant removal and burial), but it is crucial to limit further spore production and spread of the late blight pathogen. Some states require the destruction of the first detected late blight case(s), particularly if they occur early in the season.

8.6 Current Research

In the USA late blight research has many applied and basic research components. Field trials to test fungicides and germplasm screening are conducted by university and federal laboratories in several regions. The trials often incorporate postharvest studies for chemical and environmental control of late blight during storage. Many researchers are also working with historical and predictive climate change data to forecast environmental conditions that favour late blight in near- and long-term models. Molecular-based research includes the identification and characterization of host resistance genes as well as differences in pathogen genotypes that condition their ability to suppress host resistance or grow under diverse environmental conditions. Although much of the current research is focused on developing effective methods to control blight, significant research also aims to develop advanced resistant germplasm to alleviate our dependence on preventative pesticide applications.

The United States Department of Agriculture is currently funding a 5-year project to improve late blight management. An important part of this effort is the development of tools to allow specialists to determine the characteristics of newly identified strains and make management recommendations within a short period of time (2–3 days). A core set of simple sequence repeat markers is being developed to fingerprint strains and identify individual genotypes within complex populations. In addition, markers are being developed to track important traits such as mefanoxam resistance. This collaborative effort relies on rapid submission of *P. infestans* specimens from infected plants, and the resulting knowledge will be communicated via a new online management website (www.USAblight.org) to alert growers of new infections and the characteristics of the strains found at those locations.

Although there are currently no biotech (genetically modified) potatoes or tomatoes grown for consumption in the USA,

development of advanced germplasm using transgenic approaches is underway in the public and private sector. One promising source of resistance is derived from the wild potato species *Solanum bulbocastanum*, where three major genes have been identified for late blight resistance (Song *et al.*, 2003; van der Vossen *et al.*, 2003, 2005; Lokossou *et al.*, 2009). These genes, called *RB* (also named *Rpi-blb1*), *Rpi-blb2* and *Rpi-blb3*, confer broad spectrum (*RB* and *Rpi-blb2*) and genotype-specific (*Rpi-blb3*) resistance to *P. infestans*. Although it is anticipated that these genes will provide effective late blight control, lineages of *P. infestans* that can overcome *RB* have been identified. The molecular determinants that allow this to occur have been recently identified, which will allow for DNA-based techniques to rapidly predict whether a specific strain is able to overcome *RB* (Chen *et al.*, 2012). This will allow growers to more easily predict appropriate control procedures in fields that contain potatoes with the *RB* gene.

Traditional breeding for late blight resistance in potato has focused on incorporating wild species germplasm into cultivated varieties. In contrast to other regions of the world, the vast majority of potato breeding in the USA is done within the public sector at universities and federal agencies. Three commercial potato varieties, 'Jacqueline Lee' (Douches *et al.*, 2001), 'Defender' (Novy *et al.*, 2006) and 'Missaukee' (Douches *et al.*, 2010), contain foliar resistance to the aggressive, mefenoxam-resistant genotypes of *P. infestans* first identified in North America in the 1990s. Late blight resistance expressed in these varieties effectively reduces the rate and frequency of fungicide applications, reducing production costs and impacts on the environment (Kirk *et al.*, 2005; Stevenson *et al.*, 2007).

Tomato breeding has focused on the incorporation of the *Ph* genes from *Solanum pimpinellifolium* into lines with desired horticultural qualities including fruit colour and shape. Recently released fresh market cultivars include 'Mountain Magic', 'Plum Regal' and 'Defiant' from the North Carolina State University Tomato Breeding Program. Recent evaluations show these cultivars holding up well against several of the new US clonal lineages.

8.7 Future Perspectives

Due to constant genetic shifts of *P. infestans* populations and decreases in fungicide effectiveness due to selection for resistance in new pathogen strains, late blight can cause a complete loss of the crop. The most effective and environmentally friendly way to prevent widespread devastation by late blight is to incorporate host plant resistance. Several resistance genes are available from wild potato species that can be introduced into cultivars via stable transformation. The sources of these genes are especially important if the public or industry requires that they originate from potato. Overall, the US public is generally accepting of biotech food products, and surveys by the International Food Information Council indicate >75% of respondents support the technology if specific characteristics, such as a reduction in required pesticide applications, can be incorporated into food sources.

Potato and tomato are crops that can benefit greatly from biotechnological approaches, especially with respect to late blight. In potato, cultivars are tetraploid and genetic variability in existing cultivars is low, making traditional breeding difficult and slow. Both potato and tomato are easy to transform, and promising resistance genes are available from wild species, and, due to their reproductive cycles, gene flow is not an issue. Public institutions play an important role in identifying genes for resistance, and companies such as Simplot and BASF are currently pursuing the development and release of late blight resistant cultivars in the USA.

If history is any indication, the deployment of host resistance alone will not solve the late blight problem. Success relies on an integrated approach combining advanced breeding, effective monitoring and timely pesticide applications. If properly implemented, the impact of late blight on the US potato and tomato industries can be greatly reduced.

References

Chen, Y., Liu, Z. and Halterman, D. (2012) Molecular determinants of resistance activation and suppression by *Phytophthora infestans* effector IPI-O. *PLoS Pathogens* 8(3), e1002595.

Deahl, K., Goth, R., Young, R., Sinden, S. and Gallegly, M. (1991) Occurrence of the A2 mating type of *Phytophthora infestans* in potato fields in the United States and Canada. *American Journal of Potato Research* 68, 717–725.

Deahl, K., Inglis, D. and DeMuth, S. (1993) Testing for resistance to metalaxyl in *Phytophthora infestans* isolates from northwestern Washington. *American Journal of Potato Research* 70, 779–795.

Dorn, B., Musa, T., Krebs, H., Fried, P. and Forrer, H. (2007) Control of late blight in organic potato production: evaluation of copper-free preparations under field, growth chamber and laboratory conditions. *European Journal of Plant Pathology* 119, 217–240.

Douches, D., Jastrzebski, K., Coombs, J., Kirk, W., Felcher, K., Hammerschmidt, R. and Chase, R. (2001) Jacqueline Lee: a late-blight-resistant tablestock variety. *American Journal of Potato Research* 78, 413–419.

Douches, D., Coombs, J., Felcher, K., Kirk, W., Long, C. and Bird, G. (2010) Missaukee: a round white potato variety combining chip-processing with resistance to late blight, *Verticillium* wilt and golden cyst nematode. *American Journal of Potato Research* 87, 10–18.

Fry, W.E. and Goodwin, S.B. (1997) Re-emergence of potato and tomato late blight in the United States. *Plant Disease* 81, 1349–1357.

Fry, W.E., Goodwin, S.B., Matuszak, J.M., Spielman, L.J., Milgroom, M.G. and Drenth, A. (1992) Population genetics and intercontinental migrations of *Phytophthora infestans*. *Annual Review of Phytopathology* 30, 107–129.

Gavino, P.D., Smart, C.D., Sandrock, R.W., Miller, J.S., Hamm, P.B., Lee, T.Y., Davis, R.M. and Fry, W.E. (2000) Implications of sexual reproduction for *Phytophthora infestans* in the United States: generation of an aggressive lineage. *Plant Disease* 84, 731–735.

Goodwin, S.B., Drenth, A. and Fry, W.E. (1992) Cloning and genetic analyses of two highly polymorphic, moderately repetitive nuclear DNAs from *Phytophthora infestans*. *Current Genetics* 22, 107–115.

Goodwin, S.B., Cohen, B.A., Deahl, K.L. and Fry, W.E. (1994) Migration from Northern Mexico as the probable cause of recent genetic changes in populations of *Phytophthora infestans* in the United States and Canada. *Phytopathology* 84, 553–558.

Goodwin, S.B., Sujkowski, L.S., Dyer, A.T. and Fry, B.A. (1995) Direct detection of gene flow and probable sexual reproduction of *Phytophthora infestans* in northern North America. *Phytopathology* 85, 473–479.

Griffith, G.W. and Shaw, D.S. (1998) Polymorphisms in *Phytophthora infestans*: four mitochondrial haplotypes are detected after PCR amplification of DNA from pure cultures or from host lesions. *Applied and Environmental Microbiology* 64, 4007–4014.

Guenthner, J., Michael, K. and Nolte, P. (2001) The economic impact of potato late blight on US growers. *Potato Research* 44, 121–125.

Kirk, W.W., Abu-El Samen, F.M., Muhinyuza, J.B., Hammerschmidt, R., Douches, D.S., Thill, C.A., Groza, H. and Thompson, A.L. (2005) Evaluation of potato late blight management utilizing host plant resistance and reduced rates and frequencies of fungicide applications. *Crop Protection* 24, 961–970.

Krause, R.A., Massie, L.B. and Hyre, R.A. (1975) Blitecast: a computerized forecast of potato late blight. *Plant Disease Reporter* 59, 95–98.

Lokossou, A.A., Park, T.-H., van Arkel, G., Arens, M., Ruyter-Spira, C., Morales, J., Whisson, S.C., Birch, P.R.J., Visser, R.G.F., Jacobsen, E. and van der Vossen, E.A.G. (2009) Exploiting knowledge of *R*/*Avr* genes to rapidly clone a new LZ-NBS-LRR family of late blight resistance genes from potato linkage group IV. *Molecular Plant–Microbe Interactions* 22, 630–641.

McGrath, M.T. (2011) Late blight: recent occurrences, management challenges, and future outlook. Available at: http://www.longislandhort.cornell.edu/vegpath/late_blight_pasa2011.pdf (accessed 20 February 2012).

National Agricultural Statistics Service (NASS) (2012) Available at: http://www.nass.usda.gov/Statistics_by_Subject/index.php?sector=CROPS (accessed 19 January 2012).

Novy, R., Love, S., Corsini, D., Pavek, J., Whitworth, J., Mosley, A., James, S., Hane, D., Shock, C., Rykbost, K., Brown, C., Thornton, R., Knowles, N., Pavek, M., Olsen, N. and Inglis, D. (2006) Defender: a high-yielding, processing potato cultivar with foliar and tuber resistance to late blight. *American Journal of Potato Research* 83, 9–19.

Ristaino, J.B., Groves, C.T. and Parra, G.R. (2001) PCR amplification of the Irish potato famine pathogen from historic specimens. *Nature* 411, 695–697.

Song, J., Bradeen, J.M., Naess, S.K., Raasch, J.A., Wielgus, S.M., Haberlach, G.T., Liu, J., Kuang, H., Austin-Phillips, S., Buell, C.R., Helgeson, J.P. and Jiang, J. (2003) Gene RB cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. *Proceedings of the National Academy of Sciences USA* 100, 9128–9133.

Stevenson, W.R., James, R.V., Inglis, D.A., Johnson, D.A., Schotzko, R.T. and Thornton, R.E. (2007) Fungicide spray programs for Defender, a new potato cultivar with resistance to late blight and early blight. *Plant Disease* 91, 1327–1336.

van der Vossen, E., Sikkema, A., Hekkert, B.T.L., Gros, J., Stevens, P., Muskens, M., Wouters, D., Pereira, A., Stiekema, W. and Allefs, S. (2003) An ancient *R* gene from the wild potato species *Solanum bulbocastanum* confers broad-spectrum resistance to *Phytophthora infestans* in cultivated potato and tomato. *Plant Journal* 36, 867–882.

van der Vossen, E.A.G., Gros, J., Sikkema, A., Muskens, M., Wouters, D., Wolters, P., Pereira, A. and Allefs, S. (2005) The *Rpi-blb2* gene from *Solanum bulbocastanum* is an *Mi-1* gene homolog conferring broad-spectrum late blight resistance in potato. *Plant Journal* 44, 208–222.

Vartanian, V.G. and Endo, R.M. (1985) Survival of *Phytophthora infestans* in seeds extracted from infected tomato fruits. *Phytopathology* 75, 375–378.

9 *Phytophthora sojae* on Soybean

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9.1 Introduction

Phytophthora sojae is a soil-borne pathogen specific to soybean and infection occurs on the seed, seedlings, roots and stems. The morphology of the sporangia and the oospores is similar to *Phytophthora megasperma* (Fig. 9.1), and consequently *P. sojae* has multiple synonyms including *Phytophthora megasperma* var. *sojae*, *P. megasperma* f. sp. *glycinea* and *P. sojae* f. sp. *glycines* (authors and dates are reviewed in Erwin and Ribeiro, 1996). *P. sojae* is homothallic (self-fertile) and isolates readily produce oospores in culture and plants. Despite intense inbreeding, there are high levels of pathotype variability in field populations. Although the primary host is soybean, lupin (*Lupinus*) has been confirmed as a host in greenhouse inoculations and once from the field (reviewed in Erwin and Ribeiro, 1996). Yield losses are primarily due to plant loss. In fields with early season seed rot and damping-off, economic losses are greater due to the cost of replanting and lower yields due to the later planting dates. *P. sojae* also reduces yield by reducing the number of nodes, seeds and the seed weight (Tooley and Grau, 1984).

The deployment of resistant soybeans is the main strategy to control *P. sojae*. The forms of resistance in soybean can be complex. The first identified was resistance mediated by single genes known as *Rps* genes. The *Rps* genes are expressed in roots, seedling hypocotyls and cotyledons, and act as an immune type response. There is one exception, *Rps2*, which is only expressed in the roots. In addition to the *Rps* genes there are sources of quantitative resistance. These are referred to as: (i) partial resistance; (ii) rate-reducing resistance; (iii) field resistance; and (iv) tolerance. Quantitative resistance manifests as reduced infection efficiency, lesion length and oospore production and is generally not race- or pathotype-specific. And finally, there are sources of root resistance inherited quantitatively but with different race specificities (Mideros *et al.*, 2007).

Soybean germplasm from China, Japan and the Republic of Korea has been screened extensively for single gene resistance using a limited number of isolates of *P. sojae*. These data are available at the US National Plant Germplasm System web site (www.ars-grin.gov/npgs/index.html). In addition, approximately 1000 accessions have been evaluated for the expression of partial resistance. Interestingly, the closely related non-hosts *Medicago truncatula* and *Lotus japonica* are being studied as potential sources of resistance that may contribute to the expression of a non-host resistance response.

9.2 Distribution of *P. sojae*

P. sojae was first reported in Indiana and soon emerged as the most devastating disease of soybean across many US production regions (Schmitthenner, 1985). At the time commercial varieties were highly susceptible and in some areas the losses exceeded 50%. Currently, in several Midwest states, soybeans are produced on

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Fig. 9.1. Morphological features of *Phytophthora sojae* and symptoms from early season disease. **(A)** A single sporangium releasing zoospores, note the extended length of the pedicel. **(B)** Oospores forming inside root tissue. **(C)** Infection on cotyledon and hypocotyl of a seedling.

more acres than maize and the use of resistant varieties has greatly reduced overall losses. This large-scale deployment of resistant soybean varieties represents one of the most successful management schemes for a soil-borne pathogen. Despite reducing the overall impact of *P. sojae*, losses still occur. Wrather and Koenning (2009) estimate that *P. sojae* significantly reduced yields at sites where the pathogen population adapted to the *Rps* genes and there were insufficient levels of partial resistance in the cultivars being planted.

Historically, races of *P. sojae* were defined using a differential series of soybean lines carrying known *Rps* genes (Grau *et al.*, 2004). For example, Race 1 was virulent on *Rps7*, while Race 2 was virulent on *Rps1b*. The last isolates to be classified using this race scheme were Race 54 and 55, which were baited from soils in Illinois by Leitz *et al.* (2000). In this survey they characterized seven different races from 33 isolates. Due to the increasing complexity of field populations, the race nomenclature was dropped in favour of reporting the most common virulence pathotypes within a region (Table 9.1; Dorrance *et al.*, 2003a). In the past decade researchers in several states have conducted surveys that recovered a large number of isolates with virulence patterns that show more complexity than those from 20 to 40 years earlier. This increase in pathotype complexity is thought to be a direct result of the widespread deployment of *Rps* genes throughout the region. However, there are numerous instances where races virulent to a specific *Rps* gene existed prior to the release of a cultivar carrying the gene (Grau *et al.*, 2004). In Iowa and Ohio pathotype diversity within individual fields is extensive (Dorrance *et al.*, 2003a; Robertson *et al.*, 2009). In Ohio more than 50 pathotypes were detected within a single field. In addition, multiple pathotypes were recovered from individual soil samples. This indicates that a single soybean plant may be attacked by diverse isolates carrying different virulence genes. This adds complexity to breeding for resistance and the *Rps* genes effective in one area of a field may not be effective in another.

Table 9.1. Percentage of *Phytophthora sojae* isolates collected from sites in Ohio during 2010 that are able to overcome different *Rps* genes.

	Rps gene differentials[a]												
Field	*1a*	*1b*	*1c*	*1d*	*1k*	*2*	*3a*	*3b*	*3c*	*4*	*5*	*6*	*8*
A	50.0	91.7	25.0	0.0	91.7	50.0	100.0	16.7	0.0	0.0	33.3	0.0	0.0
B	100.0	75.0	58.3	16.7	75.0	50.0	91.7	25.0	50.0	8.3	50.0	33.3	16.7
C	85.7	85.7	57.1	14.3	100.0	57.1	85.7	14.3	57.1	0.0	57.1	14.3	0.0
D	89.5	84.2	47.4	16.0	73.7	26.3	79.0	42.1	36.8	15.8	31.6	21.1	10.5
E	78.6	71.4	50.0	14.3	71.4	28.6	57.1	21.4	35.7	14.3	7.1	28.6	7.1

[a]Ten to 12 7-day-old seedlings were inoculated in the hypocotyl region with mycelial slurry and rated as susceptible if a girdling lesion developed at the inoculation site. A zero (0%) indicates that all of the seedlings were resistant to all isolates; any value >0% indicates the percentage of the isolates that had a susceptible response.

Extensive losses due to Phytophthora root and stem rot are reported worldwide including Argentina and Brazil in South America, Australia, China, Japan, South Korea and the Middle East. In China *P. sojae* was first identified in 1991. Affected areas can extend to over 150,000 ha annually. More than half of the isolates collected from Heilongjiang province in north-east China were Race 1 (virulence to *Rps7* only), and isolates also had virulence to *Rps1a*, *Rps1c*, *Rps1d*, *Rps1k*, *Rps3a* and *Rps6* (Zhang *et al.*, 2010). Not surprisingly, as soybean production increases, reports of losses and the overall complexity of populations are also increasing.

9.3 Identification and Epidemiology

P. sojae is readily recovered from symptomatic plant tissue using selective media. It grows slowly in culture compared with other root and seedling pathogens (e.g. *Pythium*) taking 3–5 days for visible growth. Oospores are produced abundantly in culture, occasionally chlamydospores, but sporangia rarely form in culture. Sporangia can be induced on some media through washings of 3–5-day-old cultures. *P. sojae* does not grow on full-strength potato dextrose agar (PDA); however, the closely related *Phytophthora sansomeana* does, and this is an easy way to separate the two species. When this disease was first discovered in the USA the key symptom was stem rot (Fig. 9.2). Stem rot occurs late in the season, and on highly susceptible cultivars a brown lesion will form from the bottom of the stem and move up the plant. Stem rot does not occur on plants with high levels of partial resistance or an *Rps* gene that is effective towards the *P. sojae* population. In the field, infections occur on the roots and lesions do not develop on the leaves, although leaves may be used successfully for some assays.

Symptoms on seedlings are similar to those caused by *Pythium* spp. and *P. sansomeana* (Fig. 9.1C). Water-soaked brown lesions and collapsing tissue are common when soils become saturated for at least 24 h or more. Flooding injury occurs when soils are saturated for more than 48 h and CO_2 accumulates in the soil. The key feature separating root rot from flood injury is the condition of the root stele. During flooding the outside cortex of the root will be damaged and can be easily pulled off the plant leaving the hard white root stele (Fig. 9.2C). Root rots caused by pathogens cause the stele to be soft and brown.

In general, high levels of oospores in the soil are thought to serve as the primary source of inoculum throughout the growing season (Tooley and Grau, 1984). Oospores germinate when soils are saturated, producing sporangia. In culture these sporangia have long, non-dehiscent pedicels (Fig. 9.1A). Zoospores, produced within the sporangia, are released into the soil water, where they are attracted to soybean roots via chemotaxis. Several chemotactic compounds have been identified including daidzein, genistein and isoflavones (Morris *et al.*, 1998). With

inoculum readily available and diverse virulence pathotypes present in many fields, environmental conditions and the resistance in the soybean variety are the main factors determining the impact of *P. sojae*.

A

B

C

Fig. 9.2. Symptoms of Phytophthora stem rot. **(A)** Stand reduction due to Phytophthora stem rot on a cultivar with no *Rps* genes and low levels of partial resistance. **(B)** Close-up of the distinctive brown lesion on the stem. **(C)** Symptoms of flooding injury, not caused by *P. sojae*.

9.4 Management

As mentioned above, *P. sojae* has been well managed through the deployment of cultivars with *Rps* genes. The most common genes used are *Rps1a*, *Rps1c*, *Rps1k*, *Rps3a* and *Rps6*. If an *Rps* gene is effective towards a *P. sojae* field population it will provide good protection (Dorrance *et al.*, 2003b). Deployment of resistance gene (R-gene)-mediated resistance places a high degree of selection pressure on the pathogen populations, and often new pathogen populations emerge that can overcome this pressure. This has occurred with each of the *Rps* genes released in soybean and there is currently an emphasis on partial resistance in some regions. Soybean cultivars with high levels of partial resistance performed consistently across environments with or without the presence of an *Rps* gene (Dorrance *et al.*, 2003b).

9.4.1 Tillage and tiling

P. sojae is difficult to recover directly from soil, although baiting with seedlings or leaf discs has been useful to recover isolates and assess the effectiveness of cultural practices. *P. sojae* was baited with leaf discs from >40% of the fields tested in Ohio, Minnesota, Iowa, Missouri and Illinois (Workneh *et al.*, 1998). 'No-till' fields had higher levels of *P. sojae* compared with soil samples collected from fields with conventional tillage. In addition, Dorrance *et al.* (2003a) compared the recovery of isolates with virulence to *Rps1k* and found an association with 'no-till' production systems. In both studies the high incidence and association with shifting virulence pathotypes was due to increased inoculum in the root zone associated with the 'no-till' system. Tiling is a process that involves inserting clay tile or plastic pipe at varying depths across a field to facilitate faster removal of excess water. In some areas this is not possible due to the potential impact on waterways. However, where possible, it can effectively reduce the time a soil is saturated and reduce the incidence of Phytophthora stem rot on susceptible cultivars.

9.4.2 Seed treatment

Following the emergence of *P. sojae* populations with virulence to *Rps1a* and *Rps1c*, producers applied metalaxyl (Ridomil) in furrow providing protection from seed rot, damping-off and early season stem rot. These applications ceased in the mid- to late 1980s when varieties with *Rps1k* were released (effective against all populations at the time) and when growers shifted to much narrower row spacing (from 76 cm (30 inch) to 38 cm (15 inch) or 18 cm (7 inch)), which made the in-furrow applications too costly. Seed treatments with metalaxyl or mefenoxam provide protection to varieties with different types and levels of resistance in areas where rain or irrigation occurred shortly after planting (Dorrance *et al.*, 2009). The rate influences the level of protection and high concentrations of metalaxyl (>15.5 g ai/100 kg) and mefenoxam (>7.5 g ai/100 kg) are required to effectively control *P. sojae*. Several studies indicate metalaxyl applied in furrow can protect susceptible cultivars for extended periods, but with cultivars that have effective *Rps* genes there is no benefit (Dorrance *et al.*, 2003b, 2009). Complicating factors include the emergence of *Pythium* populations insensitive to metalaxyl/mefenoxam and one report of *P. sojae* isolates that began to grow 10 days after placement on metalaxyl/mefenoxam-amended agar plates (Nelson *et al.*, 2008). Insensitive mutants can readily be selected for in the laboratory assays.

9.4.3 Novel approaches

Laboratory studies suggest preventative applications of calcium in the form of $CaCl_2$ and $Ca(NO_3)_2$, and potassium as KNO_3, may reduce the incidence of seedling disease, possibly due to higher concentrations of calcium or potassium in the plant tissue (Sugimoto *et al.*, 2005, 2009). Calcium and potassium also inhibited zoospore release when concentrations were greater than 10 mM on lima bean agar. However, when potassium or other ions were added as chloride salt it increased the incidence of Phytophthora root and stem rot (Canaday and Schmitthenner, 2010). When potassium was added as other potassium or sulfate salts directly to field soil there was no effect on disease incidence. Although the studies have some contrasting results, they raise the issue of caution for fertility applications at planting. Interestingly, the area in Ohio with the highest incidence of Phytophthora root and stem rot is on soils with the highest levels of available calcium.

9.5 Current Research

Extensive genome resources are now available for *P. sojae* and for soybean. These resources have opened the door for many new avenues of research and are leading to a better understanding of the complex interactions within this dynamic pathosystem.

9.5.1 *P. sojae*

A reference genome for *P. sojae* was developed in a community effort led by Dr Brett Tyler at the Virginia Bioinformatics Institute and is discussed in a seminal issue of the journal *Molecular Plant–Microbe Interactions* (Govers and Gijzen, 2006). As *P. sojae* colonizes plant tissue it secretes a number of proteins important for infection. Prior to the sequencing effort a few genes had been cloned (e.g. Crinklers and *Avr1b-1*) and their functions described, and knowledge of the *Avr1b-1* gene provided essential clues that led to the discovery of a signature motif (RXLR dEER domain) for secreted virulence genes and the discovery of 396 additional avirulence homologues (*Avh*) (Jiang *et al.*, 2008). Subsequently, the *Avr1a*, *Avr3a*, *Avr5*, *Avr4* and *Avr6* genes have all been cloned (Qutob *et al.*, 2009; Dou *et al.*, 2010; Dong *et al.*, 2011). Analyses indicate some of these *Avr* and *Avh* genes are clustered while others are distributed to a few regions within the genome and some may be alleles at the same locus.

Efforts are underway to clone and describe these *Avr*/*Avh* genes and the

genetic factors that affect their expression, and to identify other proteins secreted early in the infection process. These may serve as targets for novel control strategies. As part of these efforts, some *Avr* genes have been characterized in a few isolates of *P. sojae*. In some instances there were multiple copies of the gene in a locus, while in some isolates the *Avr* sequence was present but not transcribed giving a susceptible instead of the resistant response. In a recent report characterizing *Avr4/6*, Dou *et al.* (2010) showed that one *Avr* protein has specificity for two *Rps* genes. Evaluation of additional *P. sojae* isolates revealed some that do not have the expected *Avr* sequence even though they trigger a specific R-gene response. This suggests that there are additional *Avr*/*Avh* genes within *P. sojae* populations recognized by these R-genes.

9.5.2 Soybean

There are ongoing efforts to map and clone *Rps* genes and to map quantitative trait loci (QTL) for partial resistance. These efforts are providing markers to rapidly incorporate novel resistance into new cultivars through marker-assisted selection. Locations for most of the *Rps* genes have been mapped and candidate genes have been identified for *Rps1k* and *Rps4*. There are a large number of reported *Rps* genes on chromosome 13 including *Rps3* and *Rps8*, two novel genes, and a region with numerous R-genes for several soybean pathogens and the soybean aphid (Gordon *et al*., 2006). Characterization of these complex loci is underway (M. Graham, 2011, personal communication).

Partial resistance conferred by QTL is highly heritable and effective with most *P. sojae* isolates. More than 20 QTL have been mapped in soybean, with most of the studies focused on the cultivar ‘Conrad’. Interestingly, several QTL that contribute to resistance have also been identified from the susceptible parent. This suggests that resistance expression can be masked or inhibited, and it is important that the mechanisms responsible are identified. To date, several studies have begun to explore this aspect and initially it appears to be quite complex, involving several different resistance pathways (Wang *et al*., 2010).

Resistant cultivars express higher levels of resistance-related proteins following inoculation with *P. sojae*, and preformed suberin was higher in whole roots. Suberin may delay *P. sojae* penetration through the epidermis (Thomas *et al*., 2007). Transcription profiling of soybean genotypes with differential levels of partial resistance revealed ~25,000 genes that had statistically significant responses to infection. Interestingly, there was little difference in transcript abundance between 3 and 5 days after inoculation (Zhou *et al*., 2009) indicating that once the response is initiated it is a sustained response. In a similar study approximately 20,000 genes had significant changes in transcript abundance in the cultivars ‘Conrad’ and ‘Sloan’ compared with mock-inoculated controls (Wang *et al*., 2010). This is in contrast to R-gene-mediated responses where transcriptional changes can occur less than 3 h after inoculation. It appears that key mechanisms limiting *P. sojae* in resistant lines occur at the transition from biotrophy to necrotrophy. To begin to identify candidate genes associated with the partial resistance response, Wang (2011) used expression analysis to find genes underlying two QTLs from resistant and susceptible genotypes. There were single nucleotide polymorphisms in a large proportion of the genes and expression analysis confirmed that many of them had significant difference in expression between resistance and susceptible genotypes. Candidate genes included those involved in: (i) signal transduction; (ii) hormone-mediated pathways; (iii) plant-cell structural modification; (iv) ubiquitination; and (v) basal resistance. Even within one QTL region, the genes associated with resistance appear to involve several layers of defence.

9.6 Future Perspectives

Due to the diversity of the virulence pathotypes, it is a challenge to determine which *Rps* gene to introgress into new

cultivars. It will be important to continue to identify and clone *Rps* genes that are in the soybean genome. Many of these are in difficult regions in which the segregation ratios from soybean populations are distorted (Demirbas *et al.*, 2001; Gordon *et al.*, 2006). This makes the identification of molecular markers that are specific for the *Rps* gene (and not just linked to the *Rps* gene) important and will allow the stacking of *Rps* genes.

As mentioned above, great progress has been made to characterize the *Avr* genes that interact with several of the *Rps* genes. The next key question to address is whether these *Avr* genes are the same within diverse populations. Recent experiments suggest that not all of the *Rps* genes have been identified and that there are additional *Avr* genes that can trigger the resistance response for some of the known *Rps* genes. Many previous studies used a limited number of isolates and small plant populations and in the future the use of larger more diverse collections of *P. sojae* as well as larger segregating populations for mapping should be highly informative.

It's increasingly clear that incorporating high levels of partial resistance into soybean cultivars is important for long-term management of *P. sojae*. The long-term challenge will be detecting and measuring the level of partial resistance within genotypes. When an *Rps* gene is effective it masks the partial resistance component of the resistance response, and this same effect could also be true for novel defence strategies. Therefore, identifying QTL, the correct alleles, promoter sequences and triggers for a defence response will be critical to maintain high levels of partial resistance in the event that *P. sojae* continues to adapt and change.

Development of cultivars with novel resistance and those with partial resistance are a large investment and partial resistance is not effective in limiting colonization early in the plant development. Thus, efforts to bring oomycete seed treatments to the marketplace should continue. Soybean seed is one of the most costly investments for growers, and this added protection will enhance and ensure healthy stands.

References

Canaday, C.H. and Schmitthenner, A.F. (2010) Effects of chloride and ammonium salts on the incidence of Phytophthora root and stem rot of soybean. *Plant Disease* 94, 758–765.

Demirbas, A., Rector, B.G., Lohnes, D.G., Fioritto, R.J., Graef, G.L., Cregan, P.B., Shoemaker, R.C. and Specht, J.E. (2001) Simple sequence repeat markers linked to the soybean *Rps* genes for *Phytophthora* resistance. *Crop Science* 41, 1220–1227.

Dong, S., Yu, D., Cui, L., Qutob, D., Tedman-Jones, J., Kale, S.D., Tyler, B.M., Wang, Y. and Gijzen, M. (2011) Sequence variants of the *Phytophthora sojae* RXLR effector *Avr3a/5* are differentially recognized by *Rps3a* and *Rps5* in soybean. *PLoS One* 6, e20172.

Dorrance, A.E., McClure, S.A. and deSilva, A. (2003a) Pathogenic diversity of *Phytophthora sojae* in Ohio soybean fields. *Plant Disease* 87, 139–146.

Dorrance, A.E., McClure, S.A. and St Martin, S.K. (2003b) Effect of partial resistance on Phytophthora stem rot incidence and yield of soybean in Ohio. *Plant Disease* 87, 308–312.

Dorrance, A.E., Robertson, A.E., Cianzo, S., Giesler, L.J., Grau, C.R., Draper, M.A., Tenuta, A.U. and Anderson, T.R. (2009) Integrated management strategies for *Phytophthora sojae* combining host resistance and seed treatment. *Plant Disease* 93, 875–882.

Dou, D., Kale, S.D., Liu, T., Tang, Q., Wang, X., Arredondo, F.D., Basnayake, S., Whisson, S., Drenth, A., Maclean, D. and Tyler, B.M. (2010) Different domains of *Phytophthora sojae* effector *Avr4/6* are recognized by soybean resistance genes *Rps*4 and *Rps*6. *Molecular Plant–Microbe Interactions* 23, 425–435.

Erwin, D.C. and Ribeiro, O.K. (1996) *Phytophthora Diseases Worldwide*. APS Press, St Paul, Minnesota, 562 pp.

Gordon, S.G., St Martin, S.K. and Dorrance, A.E. (2006) *Rps*8 maps to a resistance gene rich region on soybean molecular linkage group F. *Crop Science* 46, 168–173.

Govers, F. and Gijzen, M. (2006) *Phytophthora* genomics: the plant destroyers' genome decoded. *Molecular Plant–Microbe Interactions* 12, 1295–1296.
Grau, C.R., Dorrance, A.E., Bond, J. and Russin, J. (2004) Fungal diseases. In: Boerma, H.R. and Specht, J.E. (eds) *Soybeans: Improvement, Production and Uses.* Agronomy Monograph No. 16, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, Wisconsin.
Jiang, R.H.Y., Tripathy, S., Govers, F. and Tyler, B.M. (2008) RXLR effector reservoir in two *Phytophthora* species is dominated by a single rapidly evolving superfamily with more than 700 members. *Proceedings of the National Academy of Sciences USA* 105, 4874–4879.
Leitz, R.A., Hartman, G.L., Pedersen, W.L. and Nickell, C.D. (2000) Races of *Phytophthora sojae* on soybean in Illinois. *Plant Disease* 84, 487.
Mideros, S., Nita, M. and Dorrance, A.E. (2007) Characterization of components of partial resistance, *Rps2*, and root resistance to *Phytophthora sojae* in soybean. *Phytopathology* 97, 655–662.
Morris, P.F., Bone, E. and Tyler, B.M. (1998) Chemotropic and contact responses of *Phytophthora sojae* hyphae to soybean isoflavonoids and artificial substrates. *Plant Physiology* 117, 1171–1178.
Nelson, B.D., Mallik, I., McEwen, D. and Christianson, T. (2008) Pathotypes, distribution, and metalaxyl sensitivity of *Phytophthora sojae* from North Dakota. *Plant Disease* 92, 1062–1066.
Qutob, D., Tedman-Jones, J., Dong, S., Kuflu, K., Pham, H., Wang, Y, Dou, D., Kale, S.D., Arredondo, F.D., Tyler, B.M. and Gijzen, M. (2009) Copy number variation and transcriptional polymorphisms of *Phytophthora sojae* RXLR effector genes *Avr1a* and *Avr3a*. *PLoS One* 4 (4), e5066.
Robertson, A.E., Cianzio, S.R., Cerra, S.M. and Pope, R.O. (2009) Within-field pathogenic diversity of *Phytophthora sojae* in commercial soybean fields in Iowa. *Plant Health Progress.* Available at: doi:10.1094/PHP-2009-0908-01-RS (accessed 1 January 2012).
Schmitthenner, A.F. (1985) Problems and progress in the control of Phytophthora root rot of soybean. *Plant Disease* 69, 362–368.
Sugimoto, T., Aino, M., Sugimoto, M. and Watanabe, K. (2005) Reduction of Phytophthora stem rot disease on soybean by the applications of $CaCl_2$ and $Ca(NO_3)_2$. *Journal of Phytopathology* 153, 536–543.
Sugimoto, T., Watanabe, K., Furiki, M., Walker, D.R., Yoshida, S., Aino, M., Kanto, T. and Irie, K. (2009) The effect of potassium nitrate on the reduction of Phytophthora root and stem rot disease of soybeans, the growth rate and zoospore release of *Phytophthora sojae. Journal of Phytopathology* 157, 379–389.
Thomas, R., Fang, X., Ranathunge, K., Anderson, T.R., Peterson, C.A. and Bernards, M.A. (2007) Soybean root suberin: anatomical distribution, chemical composition, and relationship to partial resistance to *Phytophthora sojae. Plant Physiology* 144, 299–311.
Tooley, P.W. and Grau, C.R. (1984) Field characterization of rate-reducing resistance to *Phytophthora megasperma* f. sp. *glycinea* in soybean. *Phytopathology* 74, 1201–1208.
Wang, H. (2011) Identification and dissection of soybean QTL conferring resistance to *Phytophthora sojae*. PhD dissertation, The Ohio State University, Ohio, 260 pp.
Wang, H., Waller, L., Tripathy, S., St Martin, S.K., Zhou, L., Krampis, K., Tucker, D.M., Mao, Y., Hoeschele, I., Saghai Maroof, M.A., Tyler, B.M. and Dorrance, A.E. (2010) Analysis of genes underlying soybean quantitative trait loci conferring partial resistance to *Phytophthora sojae*. *The Plant Genome* 3, 23–40.
Workneh, F., Yang, X.B. and Tylka, G.L. (1998) Effect of tillage practices on vertical distribution of *Phytophthora sojae*. *Plant Disease* 82, 1258–1263.
Wrather, J.A. and Koenning, S.R. (2009) Effects of diseases on soybean yields in the United States 1996 to 2007. *Plant Health Progress.* Available at: doi:10.1094./PHP-2009-0401-01-RS (accessed 1 January 2012).
Zhang, S.Z., Xu, P.F., Wu, J.J., Xue, A.G., Zhang, J.X., Li, W.B., Chen, C., Chen, W.Y. and Lv, H.Y. (2010) Races of *Phytophthora sojae* and their virulences on soybean cultivars in Heilongjiang, China. *Plant Disease* 94, 87–91.
Zhou, L.S., Mideros, S., Bao, L., Hanlon, R., Arredondo, F.D., Tripathy, S., Krampis, K., Jerauld, A., Evans, C., St Martin, S.K., Saghai Maroof, M.A., Hoeschele, I., Dorrance, A.E. and Tyler, B.M. (2009) Infection and genotype remodel the entire soybean transcriptome. *BMC Genomics* 10, 49.

10 Biology and Management of *Phytophthora capsici* in the Southwestern USA

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10.1 Introduction

Phytophthora blight by *Phytophthora capsici* limits the profitable production of many crops in New Mexico, especially chili peppers (*Capsicum annuum* L.) (Sanogo, 2003). Although it is not known when *P. capsici* was first introduced, farmers in the state complained of 'chili wilt' many years before the causal agent was identified. In the 1908 *Bulletin – Agricultural Experiment Station of the New Mexico College of Agriculture and Mechanic Arts* entitled 'Chile culture', Professor Fabían García reports:

> Once in a while a grower complains of some of the plants dying out. This occurs as a rule with plants growing in the lower spots in the field and where the soil is very heavy. It seems that the probable cause is too much water being allowed in the low places, causing the souring of the soil.

The aetiology of 'chili wilt' was addressed in two studies published in 1919 and 1922 by L.H. Leonian, an experiment station biologist at the New Mexico College of Agriculture and Mechanic Arts. In 1919 Leonian attributed the chili wilt to *Fusarium annuum*, while in 1922 he described *P. capsici* as the cause of chili wilt in pepper. In contrast to *F. annuum*, *P. capsici* has subsequently been shown as a causal agent of chili wilt through several studies (Leyendecker, 1947; Nakayama, 1960; Sanogo and Carpenter, 2006). By 1933 chili wilt had become so severe that García published a research bulletin, 'Reduction of chile wilt by cultural methods'. The disease was most severe when the chili pepper crop was grown by the 'old methods' that include level soil and 'native ridge culture'. The native ridge culture consisted of planting chili peppers on ridges about 1.20 m apart and 20–30 cm high. García concluded that roots developing too close to the soil surface were subjected to higher soil temperatures and that this encouraged blight. García suggested growing chili pepper using a 'furrow-ridge' method, which consists of preparing a shallow furrow in which the seeds or transplants are placed. After the plants are established soil is added around the chili plant. This is repeated until the ridges are about 30 cm high. At this point the roots are about 25 cm below the soil level and are less affected by the high soil temperatures on the surface. This method is still used today in the Southwestern USA where furrow irrigation is practised.

Phytophthora blight encompasses both below-ground and above-ground symptoms (Leonian, 1922). Under the semi-arid and arid conditions of the Southwestern USA, below-ground symptoms are more common. However, symptoms on leaves, stems and fruit (Fig. 10.1A and B) are found in production systems using sprinkler irrigation or following summer monsoonal rain. This is due to increased dispersal of

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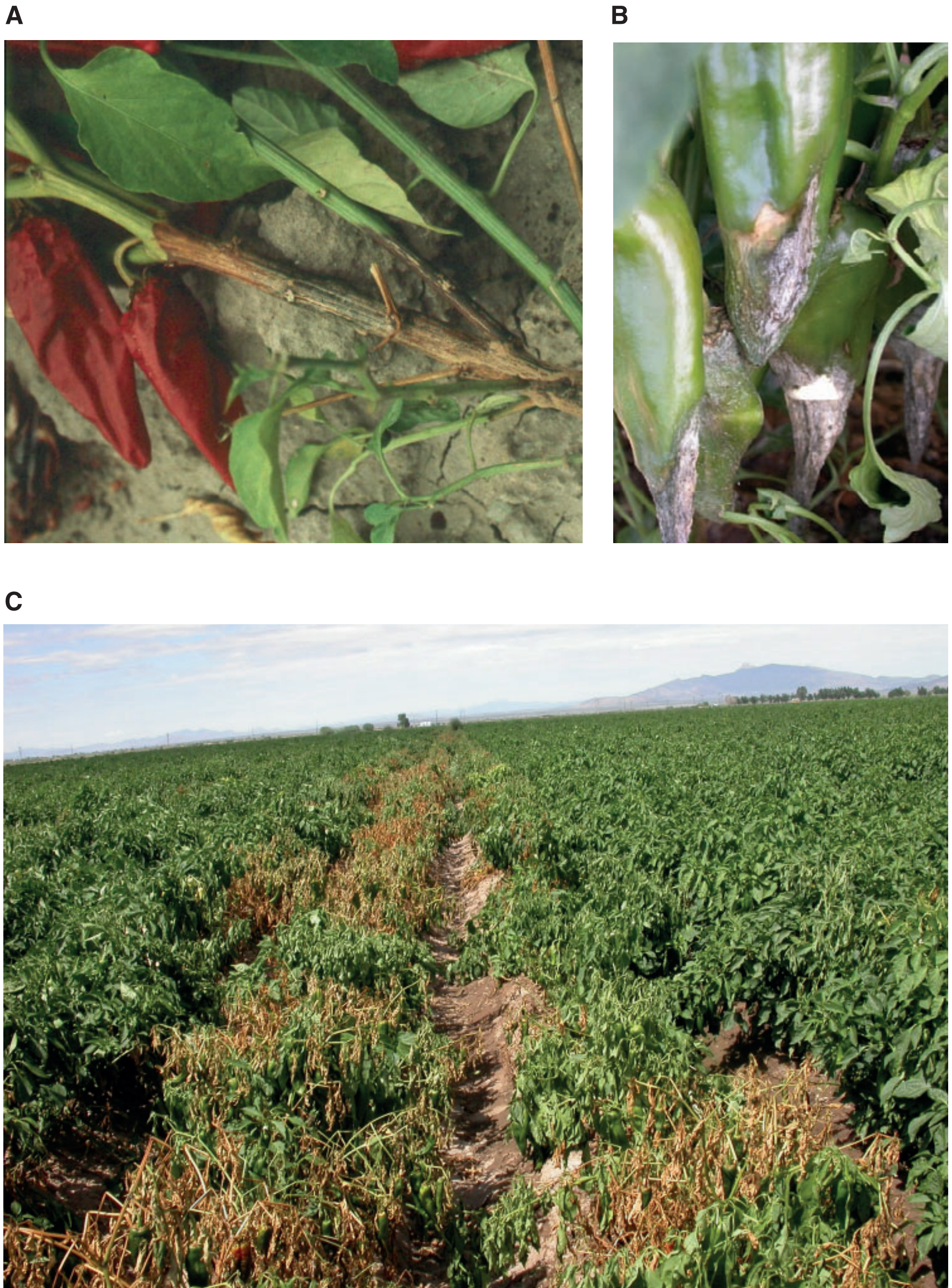

Fig. 10.1. Phytophthora blight of pepper. **(A)** Lesion on pepper stem caused by *Phytophthora capsici.* **(B)** Lesion on pepper fruit with conspicuous mycelium and sporangia production on lesion surface revealed as a whitish mat. **(C)** Early phase of plant infection in a pepper field infested with *P. capsici*; note the row pattern of affected plants.

soil inoculum and increased soil moisture during the overhead irrigation and rain events. In commercial fields, affected plants are generally found in row-bound patterns (Fig. 10.1C).

Initially thought to be strictly confined to chili peppers, it is now clear that *P. capsici* has a broad host range across varied agroecosystems (Erwin and Ribeiro, 1996). Leyendecker (1947) reported an epiphytotic of chili pepper blight in southern New Mexico with 50–60% of green fruit infected. In one field up to 85% of the plants were infected. He reports that the year the epiphytotic was observed, infection took place during a 10-day period of unusually high humidity, with rainfall on 6 of the 10 days. Xie *et al.* (1999) showed that Phytophthora blight decreased yield of green chili pepper by 55% and the combined yield of green and red chili pepper by 36%. Similarly, Biles *et al.* (1992) reported the yield of green chili pepper was reduced by 48–55% by Phytophthora blight. A survey of fields in New Mexico found *P. capsici* to be present in all chili pepper fields, with the proportion of infected plants reaching 90% in some fields (Sanogo and Carpenter, 2006). Similar surveys have not been conducted in other areas of the Southwestern USA. In addition to plant mortality, chili pepper pods on plants infected with *P. capsici* turn red prematurely, and this affects the quality of the pods for pigment colour extraction.

10.2 Ecology of *P. capsici*

10.2.1 Edaphic factors and phenology of *P. capsici*

Several studies have examined the relationship of various edaphic variables such as moisture, salinity and soil chemical composition to the phenology of *P. capsici* and plant infection. High soil moisture is associated with increased Phytophthora blight. For example, a high incidence of tomato (*Solanum lycopersicum*) fruit rot caused by *P. capsici* was observed in a season with excessive precipitation compared with drier growing seasons (Kreutzer *et al.*, 1940). Increased incidence of diseases caused by *Phytophthora* species under high soil moisture conditions can be explained by an increased production and dispersal of sporangia and zoospores (Erwin and Ribeiro, 1996) and/or predispositional effects of soil water saturation on plants (Kuan and Erwin, 1980). Although many studies indicate high soil moisture is conducive to increased spore dispersal, there has been little work on the predispositional effect of high soil moisture on plant infection by *P. capsici*. Sanogo (2006) subjected a susceptible chili pepper cultivar and a resistant line to unsaturated and saturated conditions for 3 and 6 days, and then inoculated with *P. capsici*. The resistant line displayed no disease symptoms under either soil water condition regardless of the growth stage. On the susceptible plants disease severity increased when plants were inoculated at an early growth stage compared with a later growth stage, and at each of the two growth stages disease severity was comparable under nonsaturated and saturated conditions. These results suggest that soil water saturation does not exert a significant predispositional effect on plant infection by *P. capsici*.

Salinity is an important variable in many arid and semi-arid regions including the Southwestern USA. Although salinity has been examined in many pathosystems, it has been little studied in pathosystems involving *P. capsici*. Salinity exerts significant effects on reproduction of *P. capsici* as demonstrated by a decrease in sporangium formation and zoospore production with increasing salinity levels (Sanogo, 2004). In addition, there is an increase in disease severity with increasing salinity levels, amid a decrease in reproduction of *P. capsici*, which indicates that salinity may impose predispositional effects on plants, and these effects are observed regardless of salt tolerance. Furthermore, soil salinity does not appear to change the response of *P. capsici*-resistant plants to infection by *P. capsici*. The relationship of soil chemical composition to reproduction and infection of chili pepper by *P. capsici* was examined by Sanogo (2007b) using agricultural and

non-agricultural soils (from rangeland and forests). The most important finding was that non-agricultural soils are more receptive to asexual reproduction of *P. capsici* than agricultural soils.

10.2.2 Zoospore attachment, wounding and infection by *P. capsici*

Zoospore attachment is important for plant infection by *P. capsici* and may vary according to the host plant (Waugh *et al.*, 1993). It is affected by several factors including wounding and the amount of free water. Higher zoospore attachment was reported on pepper roots than on tomato or cucumber (*Cucumis sativus*) roots (Waugh *et al.*, 1993). Wounding of chili pepper roots is a common occurrence during cultivation, transplanting and weeding operations. Zoospore attachment is greater when roots were inoculated immediately after wounding than when roots were inoculated 48 h after wounding (Adorada *et al.*, 2000). Also, wounding of above-ground plant parts such as fruit has been shown to enhance infection by *P. capsici* (Biles *et al.*, 1993). Another important factor in infection by zoospores is the amount of free water. Disease incidence is greater when fruit are inoculated with large water droplets (100 µl) rather than with small water droplets (10 and 50 µl) of identical zoospore concentrations (Biles *et al.*, 1995). The large amounts of water in flood irrigation and monsoonal torrential rains are clearly important factors driving increased infection.

10.2.3 Biological interactions of *P. capsici* with other pathogenic microorganisms

In a field survey conducted in chili pepper fields in New Mexico, Sanogo and Carpenter (2006) found that *P. capsici* was recovered from roots of wilted plants along with many microorganisms including *Verticillium dahliae* Kleb. and *Rhizoctonia solani* Kühn. In a follow-up study the coexistence of *P. capsici* and *V. dahliae* indicated that wilt development in chili peppers is hastened by the presence of both *P. capsici* and *V. dahliae* attacking the same plant (Sanogo, 2007a).

10.3 Host Range, Genetic Diversity and Population Biology

10.3.1 Host range and population diversity

In the Southwestern USA *P. capsici* attacks cucurbits such as pumpkin (*Cucurbita pepo*) (Sanogo and Clary, 2006; Isakeit, 2007), tomato, winter squash (*Cucurbita maxima* and *Cucurbita* sp.) (Isakeit, 2007), cucumber and watermelon (*Citrullus vulgaris*). Isolates are not specific to one host crop, and Sanogo and Clary (2006) and Isakeit (2007) showed that *P. capsici* isolates from pumpkin caused characteristic symptoms of Phytophthora blight when inoculated on to chili pepper. Similarly, isolates of *P. capsici* from squash induced Phytophthora blight on watermelon and vice-versa (Kreutzer *et al.*, 1940). The diversity of *P. capsici* has been studied by several workers in New Mexico and in the Southwestern USA (Polach and Webster, 1971; Biles *et al.*, 1991; Fernandez-Pavía *et al.*, 2004; Glosier *et al.*, 2008; Nalim *et al.*, 2010). A high genetic variability among populations of *P. capsici* is evident using morphological, physiological and molecular characterization.

One way diversity of *P. capsici* has been characterized is through the use of physiological race typing. This is assessed by screening isolates on a specific set of host cultivars or recombinant inbred lines of pepper (Oelke *et al.*, 2003; Glosier *et al.*, 2008). Oelke *et al.* (2003) were the first to describe physiological races of *P. capsici* using host cultivars. They reported nine races causing the root rot syndrome and four races causing the foliar blight syndrome. Glosier *et al.* (2008) identified 14 physiological races for the Phytophthora root rot syndrome using a set of different *C. annuum* cultivars. However, a host differential based on cultivars is inherently poor because cultivars can change genotype through repeated seed increases, and some

cultivars are abandoned by seed companies making them difficult to obtain. A solution to this problem is a host differential based on recombinant inbred lines, which provides the advantages of combining the maximum genetic variability within the population with homozygous lines able to be replicated permanently without risk of segregation. To help with breeding for resistance against *P. capsici*, a set of recombinant inbred lines were developed at New Mexico State University using a highly resistant landrace from Mexico, Criollo de Morales 334 and 'Early Jalapeño' as a susceptible parent (Sy *et al.*, 2008). The New Mexico recombinant inbred lines (NMRILs) established a stable and reproducible host differential that detects resistance and susceptibility to the specific and independent disease syndromes (i.e. root rot, stem blight and foliar blight). Furthermore, inheritance studies using the NMRILs reveal that race-specific resistance to each disease syndrome is controlled by many loci and/or alleles. The NMRILs characterized 17 isolates of *P. capsici* into 13 distinct physiological races (Sy *et al.*, 2008).

The use of host differentials for determining races of *P. capsici* is an invaluable tool. Nalim *et al.* (2010) conducted studies to develop race-specific molecular fingerprints to provide a rapid avenue for classifying new isolates of *P. capsici* to a given race. Although there was greater nucleotide polymorphism within the intergenic spacer (IGS) region than in the internal transcribed spacer (ITS) region of *P. capsici*, the physiological races examined could not be identified based on a unique DNA-fingerprint (Nalim *et al.*, 2010). Further work in this area may streamline race characterization in *Phytophthora.*

10.3.2 Genetic resistance and gene expression

Despite numerous studies conducted on the inheritance of resistance to *P. capsici* in *C. annuum*, few resistance genes have been identified in plants. Using complementary DNA (cDNA) microarray technology, Richins *et al.* (2010) identified 168 genes differentially expressed following inoculation with *P. capsici* on *C. annuum* roots from one susceptible and two resistant lines. Of the pool of genes identified, 22 were only found in the resistant lines. Time course analysis showed that for many genes transcript levels were similar in susceptible and resistant lines 0 and 4 h after inoculation; however, transcript levels were nearly constant in the susceptible line while there was a marked increase in expression levels in the resistant lines 4–24 h post-inoculation. Additional work to elucidate the interaction of *C. annuum* with *P. capsici* should include recombinant inbred lines and several races of *P. capsici.*

10.4 Management of Phytophthora Blight

10.4.1 Screening of resistant cultivars and accessions

Breeding programmes in New Mexico and Texas have evaluated *Capsicum* and cucurbit germplasm collections for sources of resistance to *P. capsici*. In the process, reliable inoculation techniques have been developed for root rot (Bosland and Lindsey, 1991), foliar blight (Alcantara and Bosland, 1994) and stem blight (Sy and Bosland, 2005). More recently a method to simultaneously screen plants for resistance to several physiological races of *P. capsici* foliar blight has been developed (Monroy-Barbosa and Bosland, 2010). This multiple race approach significantly reduces the number of plants needed to screen for foliar blight resistance and also greatly speeds up the process as the plants can be scored 3 days following inoculation (Monroy-Barbosa and Bosland, 2010). The screen is: (i) precise as only the inoculated leaves show symptoms; and (ii) attractive because resistant plants survived to set fruit.

Although resistant plants can be an environmentally sound and cost-effective method to control Phytophthora blight, breeding for *Phytophthora*-resistant chili pepper is difficult and complex. It is widely known that *P. capsici* attacks every part of the chili pepper plant including roots, stem,

foliage and fruits (Leonian, 1922), and that *P. capsici* causes separate disease syndromes for each. *Capsicum* has evolved independent resistance mechanisms to protect against these various disease syndromes (Walker and Bosland, 1999; Sy and Bosland, 2005), and genes for resistance to Phytophthora root rot will not protect against Phytophthora foliar blight or stem blight.

The complexities of the disease syndromes are further exacerbated by the high levels of diversity both within and between *P. capsici* populations. Deployment of *P. capsici* resistance genes could be addressed by pyramiding combinations of race-specific resistant genes against *P. capsici* isolates for specific geographic regions instead of searching for a universal resistant cultivar. Plant breeding for Phytophthora blight resistance requires the independent evaluation of the different disease syndromes and the different physiological races within the pathogen. Thus, specific locations may require a *C. annuum* cultivar resistant to the specific physiological races found in that area. As there may be several physiological races in one location, the resistance genes will need to be combined to obtain a resistant cultivar. Within *P. capsici* both A1 and A2 mating types exist, and have been found in the same field in New Mexico (S. Sanogo, unpublished). This increases the probability of sexual recombination leading to the emergence of new physiological races. Ultimately, this greater understanding of the genetics of the *P. capsici*–*C. annuum* interaction will support breeding for effective resistance and the development of durable strategies for exploiting resistance in the field.

10.4.2 Crop attributes and *P. capsici*

In New Mexico it is widely believed that hot chili peppers are less susceptible to Phytophthora blight than low-heat chili peppers. A comparison of high- and low-heat peppers to inoculation with *P. capsici* revealed little or no relationship between heat level and susceptibility in either roots or fruit (Tahboub *et al.*, 2008). The determinants of heat in pepper are capsaicinoids, found only in the fruit. There have been few studies examining the direct effect of capsaicinoids on *P. capsici* and plant infection by this pathogen. However, capsaicin-containing capsicum oleoresins (approximately 1,000,000 Scoville heat units with 6.6% capsaicin) were evaluated *in vitro* against mycelial growth of *P. capsici* by Beard (2006), who found that mycelial growth was reduced by 49–83% on medium amended with 15–30% aqueous solutions of the oleoresins relative to non-amended medium. Based on this information, capsicum oleoresins were evaluated *in vivo* for efficacy against Phytophthora blight of chili pepper (Sanogo, 2008). At the six- to eight-leaf stage, transplants of a chili pepper cultivar susceptible to *P. capsici* were drenched with 5 and 15% aqueous solution of capsicum oleoresin. Three days after soil drenching, transplants were inoculated with *P. capsici* and observed for a period of 5 weeks. The area under the disease progress curve was lower for plants grown in soil drenched with capsicum oleoresin at both concentrations than for control plants. Capsicum oleoresin has the potential to reduce Phytophthora blight in production fields, but further testing under field conditions is needed.

10.4.3 Biorationals and induced resistance

Several bacteria-based biofungicides and botanical extracts (e.g. garlic extracts) have been assessed for their efficacy against *P. capsici* as seed, soil and plant treatments (Sanogo, 2008; Sanogo and Liess, 2010). Treating seeds alone is not sufficient to provide season-long protection against *P. capsici*, and a combination of seed, soil and plant treatment is necessary. The most promising botanical extracts for soil treatment were capsicum oleoresins.

Other potential amendments include pecan (*Carya illinoiensis*) by-products (Sanogo *et al.*, 2011). Aqueous extracts, prepared from ground tissue of pecan leaf, husk, shell and woody branches, were

evaluated for activity against *P. capsici*. No sporangia were observed on mycelium plugs in any of the extracts after 48 h incubation, whereas abundant sporangia production was recorded on control plugs incubated in distilled water. These results suggest pecan tissue may be a useful soil amendment to suppress or reduce infection by *P. capsici*.

Other researchers in the region have explored the use of plant activators in the control of *Phytophthora* through induced resistance. In Arizona Matheron and Porchas (2002) showed that acibenzolar-S-methyl significantly reduced Phytophthora blight on pepper.

10.4.4 Irrigation, fungicides and cover crops

High soil moisture is associated with disease, and efficient soil water drainage is essential to reduce Phytophthora blight (García, 1933). Drip irrigation, alternate-row furrow irrigation and lower frequency furrow irrigation all reduce Phytophthora blight compared with every-row irrigation (Biles *et al.*, 1992; Café-Filho *et al.*, 1995; Xie *et al.*, 1999).

Water management may be combined with fungicides to alleviate Phytophthora blight. Biles *et al.* (1992) conducted a 2-year study comparing several fungicides and irrigation practices. The following six treatments were evaluated: (i) every-row irrigation; (ii) alternate-row irrigation; (iii) metam-sodium (preplant) with alternate-row irrigation; (iv) Telone C-17 (preplant) with alternate-row irrigation; (v) metalaxyl; and (vi) copper sulfate. In general, disease incidence was significantly reduced with fungicide treatments used in combination with alternate-row irrigation compared with every-row irrigation with or without fungicides. Reduction in disease incidence and increase in yield were similar in every-row irrigation used alone or used in conjunction with metam-sodium. Extensive evaluations of fungicides active on *P. capsici* have been conducted in Arizona (Matheron and Porchas, 2000) and New Mexico (Uchanski *et al.*, 2009). Although the performance of many of these fungicides has been variable from season to season, they are an essential component to manage *P. capsici*.

The efficacy of bioactive crops such as broccoli (*Brassica oleracea*) and mustard (*Brassica juncea*) to control Phytophthora blight was examined in conjunction with the fumigant Telone C-35 (Ludwig, 2005). The best treatments were winter crop covers of either broccoli or mustard followed by application of Telone C-35. The use of glucosinolates-containing cover crops as green manure has several shortcomings. A good stand establishment is critical to obtain the large biomass necessary for soil incorporation. Furthermore, there may be significant variation in the phenological stage at which the cover crop needs to be cut and incorporated into soil, and this in turn may affect the properties of the green manure.

10.5 Future Directions

The pioneer research conducted by Leonian (1922) and other researchers has greatly contributed to our understanding of the aetiology and biology of Phytophthora blight in the Southwestern USA and other geographical regions within and outside the USA. Over the past nine decades several advances have been made on many facets of the biology of *P. capsici*, including the determination of physiological races within this oomycete species. These advances have a bearing on the management of *P. capsici*. For example, knowing the physiological races in a specific production locale can assist in developing a durable resistance. Management of moisture within production fields remains an essential tenet of controlling *P. capsici*. However, water management must be used in conjunction with other tools to effectively reduce the impact of *P. capsici* on crops. Future endeavours should focus on combined control strategies including host resistance, cultural practices, and chemical and biological tools.

References

Adorada, D.L., Biles, C.L., Liddell, C.M., Fernández-Pavía, S., Waugh, K.O. and Waugh, M.E. (2000) Disease development and enhanced susceptibility of wounded pepper roots to *Phytophthora capsici*. *Plant Pathology* 49, 719–726.

Alcantara, T.P. and Bosland, P.W. (1994) An inexpensive disease screening technique for foliar blight of chile pepper seedlings. *HortScience* 29, 1182–1183.

Beard, M. (2006) The effects of *Capsicum* oleoresins on the soil-borne pathogens *Phytophthora capsici* and *Verticillium dahliae.* MSc thesis, New Mexico State University, New Mexico.

Biles, C.L., Liddell, C.M. and Faubion, G.F. (1991) *Phytophthora capsici* strain characterization in southern New Mexico. *Phytopathology* 81, 1192.

Biles, C.L., Lindsey, D.L. and Liddell, C.M. (1992) Control of Phytophthora root rot of chile peppers by irrigation practices and fungicides. *Crop Protection* 11, 225–228.

Biles, C.L., Wall, M.M., Waugh, M. and Palmer, H. (1993) Relationship of Phytophthora fruit rot to fruit maturation and cuticle thickness of New Mexican-type peppers. *Phytopathology* 83, 607–611.

Biles, C.L., Bruton, B.D., Wall, M.M. and Rivas, M. (1995) *Phytophthora capsici* zoospore infection of pepper fruit in various physical environments. *Proceedings of the Oklahoma Academy of Science* 75, 1–5.

Bosland, P.W. and Lindsey, D.L. (1991) A seedling screen for Phytophthora root rot of pepper, *Capsicum annuum. Plant Disease* 75, 1048–1050.

Café-Filho, A.C., Duniway, J.M. and Davis, R.M. (1995) Effects of the frequency of furrow irrigation on root and fruit rots of squash caused by *Phytophthora capsici*. *Plant Disease* 79, 44–48.

Erwin, D.C. and Ribeiro O.K. (1996) *Phytophthora Diseases Worldwide*. APS Press, St Paul, Minnesota, 562 pp.

Fernandez-Pavía, S.P., Biles, C.L., Waugh, M.E., Onsurez, W., Rodriguez-Alvarado, G. and Liddell, C.M. (2004) Characterization of southern New Mexico *Phytophthora capsici* Leonian isolates from pepper (*Capsicum annuum* L.). *Revista Mexicana de Fitopatología* 22, 82–89.

García, F. (1908) Chile culture. *Bulletin – Agricultural Experiment Station of the New Mexico College of Agriculture and Mechanic Arts* No. 67.

García, F. (1933) Reduction of chile wilt by cultural methods. *Bulletin – Agricultural Experiment Station of the New Mexico College of Agriculture and Mechanic Arts* No. 216.

Glosier, B.R., Ogundiwin, E.A., Sidhu, G.S., Sischo, D.R. and Prince, J.P. (2008) A differential series of pepper (*Capsicum annuum*) lines delineates fourteen physiological races of *Phytophthora capsici*. *Euphytica* 162, 23–30.

Isakeit, T. (2007) Phytophthora blight caused by *Phytophthora capsici* on pumpkin and winter squash in Texas. *Plant Disease* 91, 633.

Kreutzer, W.A., Bodine, E.W. and Durrell, L.W. (1940) Cucurbit diseases and rot of tomato fruit caused by *Phytophthora capsici. Phytopathology* 30, 972–976.

Kuan, T.L. and Erwin, E.C. (1980) Predisposition effect of water saturation of soil on Phytophthora root rot of alfalfa. *Phytopathology* 70, 981–986.

Leonian, L.H. (1919) Fusarium wilt of chile pepper. *Bulletin – Agricultural Experiment Station of the New Mexico College of Agriculture and Mechanic Arts* No. 121.

Leonian, L.H. (1922) Stem and fruit blight of peppers caused by *Phytophthora capsici* sp. nov. *Phytopathology* 12, 401–408.

Leyendecker, P.J. (1947) An epiphytotic of pepper blight caused by *Phytophthora capsici* in southern New Mexico. *Plant Disease Reporter* 31, 421–422.

Ludwig, G. (2005) Evaluation of the effect of *Brassica* residue on the incidence of soil-borne diseases in chile peppers. MSc thesis, New Mexico State University, New Mexico.

Matheron, M.E. and Porchas, M. (2000) Comparison of five fungicides on development of root, crown, and fruit rot of chile pepper and recovery of *Phytophthora capsici* from soil. *Plant Disease* 84, 1038–1043.

Matheron, M.E. and Porchas, M. (2002) Suppression of Phytophthora root and crown rot on pepper plants treated with acibenzolar-S-methyl. *Plant Disease* 86, 292–297.

Monroy-Barbosa, A. and Bosland, P.W. (2010) A rapid technique for multiple-race disease screening of Phytophthora foliar blight on single *Capsicum annuum* L. plants. *HortScience* 45, 1563–1566.

Nakayama, R.M. (1960) Verticillium wilt and Phytophthora blight of chile pepper. PhD dissertation, Iowa State University, Ames, Iowa.

Nalim, A., Sanogo, S. and Bosland, P. (2010) Genotypic characterization of *Phytophthora capsici* races from chile pepper in New Mexico. *Inoculum* 61 (4), 65. Available at: http://msafungi.org/inoculum (accessed 23 September 2012).

Oelke, L.M., Bosland, P.W. and Steiner, R. (2003) Differentiation of race specific resistance to Phytophthora root rot and foliar blight in *Capsicum annuum*. *Journal of the American Society of Horticultural Science* 128, 213–218.

Polach, F.J. and Webster, R.K. (1971) Identification of strains of inheritance and pathogenicity in *Phytophthora capsici*. *Phytopathology* 62, 20–26.

Richins, R.D., Micheletto, S. and O'Connell, M.A. (2010) Gene expression profiles unique to chile (*Capsicum annuum* L.) resistant to Phytophthora root rot. *Plant Science* 178, 192–201.

Sanogo, S. (2003) Chile pepper and the threat of wilt diseases. *Plant Health Progress*. Available at: http://www.apsnet.org/publications/apsnetfeatures/Pages/ChilePepper.aspx (accessed 23 September 2012).

Sanogo, S. (2004) Response of chile pepper to *Phytophthora capsici* in relation to soil salinity. *Plant Disease* 88, 205–209.

Sanogo, S. (2006) Predispositional effect of soil water saturation on infection of chile pepper by *Phytophthora capsici*. *HortScience* 41, 172–175.

Sanogo, S. (2007a) Interactive effects of two soilborne pathogens, *Phytophthora capsici* and *Verticillium dahliae*, on chile pepper. *Phytopathology* 97, 37–43.

Sanogo, S. (2007b) Asexual reproduction of *Phytophthora capsici* as affected by extracts from agricultural and non-agricultural soils. *Phytopathology* 97, 873–878.

Sanogo, S. (2008) Seed and soil treatment with biofungicides and plant extracts for control of Phytophthora blight on chile pepper. In: Proceedings of the International Pepper Conference, 7–10 September 2008, Atlantic City, New Jersey, pp. 27–28.

Sanogo, S. and Carpenter, J. (2006) Incidence of Phytophthora blight and Verticillium wilt within chile pepper fields in New Mexico. *Plant Disease* 90, 291–296.

Sanogo, S. and Clary, M. (2006) Occurrence of Phytophthora blight on pumpkin in New Mexico. *Plant Disease* 90, 1110.

Sanogo, S. and Liess, L. (2010) Biofungicides as transplant and soil treatment in the control of Phytophthora blight on chile pepper. *Phytopathology* 101, S250.

Sanogo, S., Liess, L. and Richman, R. (2011) Mycelial growth and sporangial production of *Phytophthora capsici* as affected by extracts from pecan tissues. *Phytopathology* 101, S159.

Sy, O., Bosland, P.W. and Steiner, R. (2005) Inheritance of Phytophthora stem blight resistance as compared to Phytophthora root rot and foliar blight in *Capsicum annuum* L. *Journal of the American Society of Horticultural Science* 130, 75–78.

Sy, O., Steiner, R. and Bosland, P.W. (2008) Recombinant inbred line differential identifies race-specific resistance to Phytophthora root rot in *Capsicum annuum*. *Phytopathology* 98, 867–870.

Tahboub, M.B., Sanogo, S., Bosland, P.W. and Murray, L. (2008) Heat level in chile pepper in relation to root and fruit infection by *Phytophthora capsici*. *HortScience* 43, 1846–1851.

Uchanski, M.E. (2009) Evaluation of fungicides for management of Phytophthora blight in chile, 2008. *Plant Disease Management Reports* 3, V159.

Walker, S. and Bosland, P.W. (1999) Inheritance of Phytophthora root rot and foliar blight resistance in pepper. *Journal of the American Society of Horticultural Science* 124, 14–18.

Waugh, M.E, Onsurez, K., Biles, C.L. and Liddell, C.M. (1993) Chemotaxis and attachment of *Phytophthora capsici* zoospores to healthy and wounded roots of pepper, tomato, and cucumber. *Phytopathology* 83, 1333.

Xie, J., Cardenas, E.S., Sammis, T.W., Wall, M.M., Lindsey, D.L. and Murray, L.W. (1999) Effects of irrigation method on chile pepper yield and Phytophthora root rot incidence. *Agricultural Water Management* 42, 127–142.

11 *Phytophthora capsici* in the Eastern USA

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11.1 Introduction

Phytophthora capsici was first documented in the eastern USA in Florida in 1931 and was subsequently reported in New York in 1935, New Jersey around 1970, South Carolina in 1994, Michigan in 1997 and Illinois in 1999. Despite the early reports in Florida and New York, no major outbreaks were observed until the 1980s and 1990s. Today *P. capsici* is a significant limiting factor to vegetable production in the eastern USA, causing up to 100% losses in individual fields (Granke *et al.*, 2012). Due to long-term survival of the pathogen in the soil (Lamour and Hausbeck, 2003), once a field becomes infested with *P. capsici* disease is likely to be an annual problem.

11.2 Host Range

Under laboratory and greenhouse conditions *P. capsici* can infect a wide range of hosts including cultivated crops, ornamentals and native plants belonging to diverse plant families (Erwin and Ribeiro, 1996). Diseases caused by *P. capsici* include damping off, foliar blight, and fruit, root and stem rot (Hausbeck and Lamour, 2004). In the eastern USA the most important hosts of *P. capsici* are cucurbits, peppers, tomatoes and succulent beans (Fig. 11.1) (Hausbeck and Lamour, 2004). Recently Fraser fir, weeds (French-Monar *et al.*, 2006) and some solanaceous and fabaceous ornamentals have been reported as likely hosts, but these have not been reported as an economic problem.

11.3 Epidemiology in the Eastern USA

In the eastern USA *P. capsici* can survive for years as oospores in the soil (Lamour and Hausbeck, 2003). Early in the growing season oospores initiate infections, and the pathogen produces asexual sporangia and zoospores that are responsible for rapid polycyclic disease development throughout the growing season (Hausbeck and Lamour, 2004). Infected host tissue may be covered with many sporangia, which may be detached and transported via wind or heavy rain (Granke *et al.*, 2009) or in irrigation water (Wang *et al.*, 2009); however, sporangia are not likely to be spread from field to field or for long distances within a field via the wind (Granke *et al.*, 2009). When in contact with free water, sporangia will differentiate into 20–40 biflagellate motile zoospores (Hausbeck and Lamour, 2004). Both sporangia and zoospores germinate to produce hyphae that penetrate host surfaces directly or enter through wounds or natural plant openings such as the stomata or lenticels. Disease progress is favoured by warm (~25°C optimum) and moist conditions (high relative humidity or rainfall), and rainfall is the most important

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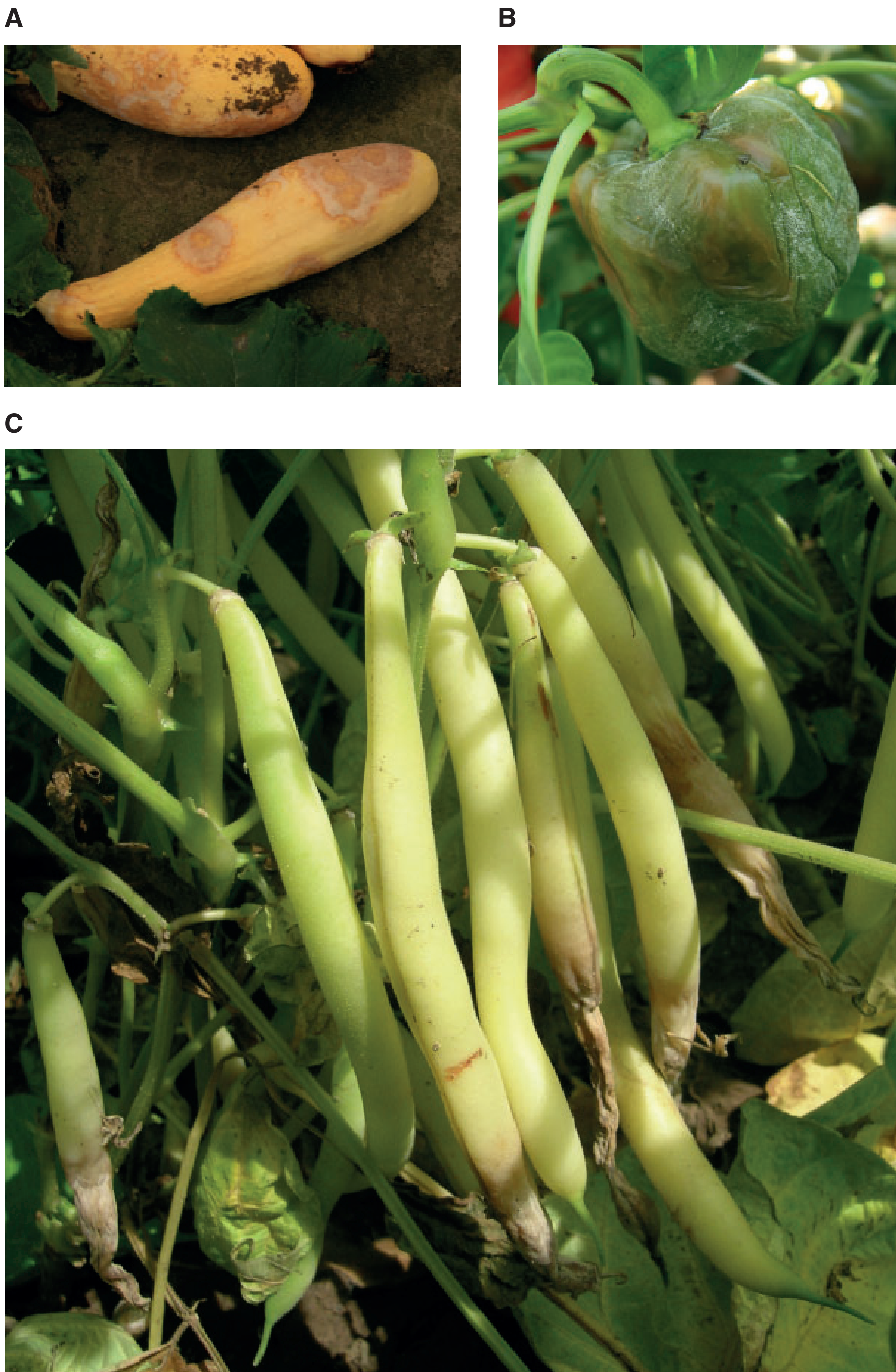

Fig. 11.1. *Phytophthora capsici* causes economic losses of **(A)** squash, **(B)** pepper and **(C)** snap bean in the eastern USA. Symptoms of disease include fruit rot, root and crown rot, and/or foliar blighting.

environmental factor driving sporangial dispersal and disease development (Ristaino and Johnston, 1999).

11.4 Isolating from Plant Tissue, Water and Soil

P. capsici is relatively easy to isolate from most diseased plants. The use of a semi-selective medium such as BARP (benomyl, ampicillin, rifampicin, pentachloronitrobenzene)-amended V8 juice agar helps separate *P. capsici* from true fungi and bacteria. The pathogen may be isolated from water directly via filtering or by baiting using susceptible host fruits. The host fruit is submerged in the water, removed after a period of time, and the pathogen isolated from developing lesions on the fruit (Gevens *et al.*, 2007). The recovery of *P. capsici* from irrigation water may be increased by transferring infected tissues from the fruit bait to pepper seedling stems and then plating pieces of the infected seedling on to agar plates (Wang *et al.*, 2009). The pathogen may be isolated directly from soil via dilution plating or by baiting with host plants or plant material such as leaves and fruits. Recovery of oospores from soil may be increased by incubating saturated soil samples prior to dilution plating, but zoospores and sporangia are better recovered without incubation prior to plating (Larkin *et al.*, 1995). Although *P. capsici* can often be identified using morphological characteristics, especially the lemon-shaped papillate sporangia, molecular methods are more accurate. *P. capsici* has substantial variation in morphological characteristics that overlap with closely related species such as *Phytophthora tropicalis* (Granke *et al.*, 2011).

11.5 Variation in Morphology and Virulence within *P. capsici*

Significant morphological and physiological variation within *P. capsici* has been noted for isolates from the eastern USA and other regions worldwide (Granke *et al.*, 2011). *P. capsici* is heterothallic, and oospores form when an isolate with the A1 mating type and an isolate with the A2 mating type are in close proximity. While some variation in oospore diameter and shape has been noted (Erwin and Ribeiro, 1996), oospore size does not seem to be associated with host or geographic origin (Granke *et al.*, 2011). Extensive variation is observed in *P. capsici* colony morphology, sporangial shapes and sizes, pedicle lengths, optimum growth temperature and sporangial production (Erwin and Ribeiro, 1996). Light and other cultural conditions may affect sporangial morphology and production (Erwin and Ribeiro, 1996). Although chlamydospores have been reported from *P. capsici* isolates recovered from vegetable and non-vegetable hosts, most isolates do not produce chlamydospores. Since some isolates of *P. capsici* do produce chlamydospores, this characteristic cannot be used reliably to separate *P. capsici* from closely related *Phytophthora* spp. such as *P. tropicalis* (Granke *et al.*, 2011). Incubation conditions are important for chlamydospore production (Erwin and Ribeiro, 1996).

Isolates of *P. capsici*, even those recovered from the same field, show significant differences in virulence and pathogenicity to hosts (Ristaino, 1990). Understanding virulence variation within *P. capsici* is key for the selection of isolates for robust host resistance screenings (Foster and Hausbeck, 2010). It is unclear if isolates from cucurbit hosts may be more virulent to cucurbit plants than isolates from solanaceous hosts (Lee *et al.*, 2001), but isolate host family of origin does not appear to correlate with isolate virulence on cucurbit and solanaceous host fruits. Several factors may affect virulence screening of isolates including: (i) the host type and organ screened; (ii) culture storage conditions; and (iii) the environmental conditions during screening. Isolates that have been repeatedly subcultured or in long-term storage for years may show reduced virulence (Ristaino, 1990). Inoculating isolates on to host tissue and then re-isolating the pathogen from the host prior to virulence screening appears to reduce differences in virulence due to

subculturing. Plant breeders should use a variety of *P. capsici* isolates to develop resistant varieties as isolates in some regions may be more virulent than isolates in other regions (Foster and Hausbeck, 2010).

11.6 Genetic Diversity and Population Dynamics

The genetics of *P. capsici* have been investigated since the early 1990s. The morphological characteristics defining *P. capsici* are inclusive of several closely related species, and molecular studies are crucial to delineate *P. capsici sensu stricto* and identify new species. Several phylogenetic studies have investigated the *P. capsici* species complex, and new species closely related to *P. capsici*, such as *P. tropicalis*, have been found. None the less, the high genetic diversity found in *P. capsici* resulting from sexual reproduction in the field makes phylogenetic studies more challenging than in other *Phytophthora* species. *P. capsici* is the only heterothallic *Phytophthora* species that has been shown to regularly complete the sexual stage in the USA. Recombination present in genes commonly used for phylogenetic studies has somewhat obscured the evolutionary relationship of *P. capsici* with sister species. In addition, most phylogenetic studies have not included enough isolates to truly represent the extensive genetic variation of *P. capsici* found in nature.

Populations of *P. capsici* have been studied at a local scale within the USA and within countries where this pathogen is economically important. A recent study using hundreds of isolates from worldwide locations and diverse hosts has confirmed the high genetic diversity of *P. capsici* in the eastern USA and detected the presence of population structure by geography and by host (Quesada-Ocampo *et al.*, 2011). This was in agreement with previous reports of differences in pathogenicity of isolates from cucurbitaceous and solanaceous hosts (Ristaino, 1990), and is consistent with the finding that *P. capsici* is not wind-dispersed between fields, which results in geographically isolated subpopulations (Granke *et al.*, 2009). Genetic stratification by geography and host highlights the importance of including isolates from different subpopulations for development of diagnostic tools, fungicides and host resistance. Management should have a local focus that takes into account regional pathogen population structure and distribution.

The mixed reproductive strategy of *P. capsici* leads to rapid evolution, which may result in populations that overcome fungicides and resistant host varieties. In the eastern USA new pathogen genotypes are frequently created through sexual recombination providing many opportunities for the development of novel strains with increased fungicide resistance and increased virulence. It is important to determine the genotype and phenotype (virulence, resistance to fungicides) of strains present in a certain region and prevent the entrance of plant tissue infected with exotic strains. These new strains could be responsible for initiating devastating epidemics by increasing genetic variability, which could include hard-to-control strains.

The possibility that *P. capsici* may form hybrids in nature with other *Phytophthora* species has not been widely addressed. Hybrids between *Phytophthora* species have been produced in the laboratory and found to have an expanded host range in comparison to the parental strains (Èrsek *et al.*, 1995). Population structure analysis of *P. capsici*, *P. tropicalis* and isolates closely related to both species revealed some shared polymorphisms between *P. capsici* and *P. tropicalis* (Quesada-Ocampo *et al.*, 2011). The observed shared admixture could be due to ancestral polymorphism or to recent recombination events given that it is possible to obtain interspecific progeny in the laboratory when *P. capsici* and *P. tropicalis* are crossed (Donahoo and Lamour, 2008); however, no hybrids have been characterized from nature. The finding of a set of isolates that formed a subpopulation clearly differentiated from, but closely related to, *P. capsici* and *P. tropicalis* (Quesada-Ocampo *et al.*, 2011)

highlights the importance hybridization could have in the evolution of this *Phytophthora* clade.

11.7 Disease Management

Exclusion is key, as *P. capsici* can persist in soil for many years making disease management difficult and expensive (Lamour and Hausbeck, 2003; Hausbeck and Lamour, 2004). Introduction can occur via equipment, diseased cull fruit and contaminated irrigation water. Equipment should be cleaned after use in an infested field. Diseased vegetables and rinse water from vegetable packing sheds should be disposed of off-site and a clean source of irrigation water should be used (Hausbeck and Lamour, 2004). *P. capsici* has been detected in surface water sources used for irrigation including rivers (Gevens *et al.*, 2007), ponds (Wang *et al.*, 2009) and ditches (Gevens *et al.*, 2007) in diverse areas of the eastern USA. If possible, susceptible crops should be watered directly from a well as *P. capsici* has been recovered from some well-fed ponds (Gevens *et al.*, 2007; Wang *et al.*, 2009). Drilling wells is expensive and growers may be reluctant to incur this cost.

Once a site is infested with *P. capsici* an integrated approach that includes cultural techniques, host resistance and chemical control is needed (Granke *et al.*, 2012) (Fig. 11.2). Due to high rainfall in the eastern USA managing the pathogen can be especially challenging. During periods of high rainfall, crops may be lost despite the use of an aggressive and well-designed strategy (Hausbeck and Lamour, 2004). Cultural techniques are focused on water management (i.e. limiting soil saturation). These include planting into well-drained fields and using raised beds with plastic mulch and drip irrigation to limit standing water in the field. Subsoiling between plant beds and along field headlands can decrease the chances of heavy rain causing flooding. To limit splash dispersal, cucurbits can be planted into mowed cover crops and smaller-fruited cucurbits trellised. The use of mechanical harvesters and a relatively narrow profit margin for cucurbits grown for the processing market limits the use of some cultural techniques (i.e. raised plant beds, plastic mulch and trickle irrigation). Other important strategies include: (i) leaving poorly drained areas of the field fallow; (ii) tilling under symptomatic plants; and (iii) including a buffer area to reduce disease spread. The effects of incorporating soil amendments and composts in *P. capsici*-infested fields are unclear: in some studies amendments reduced disease incidence whereas in other studies the disease incidence was greater following incorporation of amendments. Additional studies are needed to clarify whether soil amendments can consistently suppress the pathogen and contribute to disease management (Ristaino and Johnston, 1999).

The use of resistant host varieties (Fig. 11.2A) is desirable as an effective, inexpensive and environmentally friendly tool to limit *P. capsici*. Susceptibility of the host is influenced by: (i) the cultivar; (ii) the plant age; (iii) the plant part affected; and (iv) environmental conditions (Gevens *et al.*, 2006; Foster and Hausbeck, 2010). Resistance to root and crown rot is genetically distinct from resistance to fruit rot or foliar blighting in pepper. Tolerance has been identified in cucumber (Gevens *et al.*, 2006) and pumpkin (Lee *et al.*, 2001), but no sources of high or complete resistance to *P. capsici* have been identified. Some squash (Padley *et al.*, 2009), tomato (Quesada-Ocampo and Hausbeck, 2010) and pepper (Foster and Hausbeck, 2010) varieties show complete resistance to *P. capsici*; none the less, they are not widely used by growers due to their lack of commercially appealing characteristics. In addition, several commercial bell pepper cultivars possess tolerance to *P. capsici*, but growers are reluctant to use these because of marketability concerns (i.e. poor fruit shape and a potential correlation with fruit discoloration or silvering). Pepper varieties such as ‘Paladin’ have tolerance to crown rot caused by *P. capsici* and good horticultural traits, and are grown in *P. capsici*-infested fields. However, some of the pepper varieties marketed as tolerant to

A

B

C

Fig. 11.2. (A) The use of a tolerant cultivar (right side of bed) can assist in managing crown rot of pepper and other diseases caused by *P. capsici*. Yellow squash plants with **(B)** no fungicide applications or **(C)** fungicide applied as a drench.

P. capsici may be susceptible to local populations of *P. capsici* (Foster and Hausbeck, 2010). Identifying new sources of resistance to *P. capsici* to use in breeding programmes for commercially important hosts is needed. As mentioned above, a range of isolates representing the genetic and virulence diversity of *P. capsici* should be used for host resistance screening.

Fungicides are an essential component of an integrated management strategy for *P. capsici* (Hausbeck and Lamour, 2004). Historically, many growers successfully used mefenoxam to control *P. capsici*.

However, populations of *P. capsici* in many vegetable-growing regions are now partially or fully resistant and application of mefenoxam in areas with resistant populations provides little or no disease control. Newer products containing dimethomorph, zoxamide (marketed in a mixture with mancozeb), fluopicolide, ametoctradin, mandipropamid and cymoxanil in combination with famoxadone may be used in alternation with mefenoxam or in areas with mefenoxam-resistant pathogen populations. Copper hydroxide provides limited control when used alone but may be tank-mixed with other fungicides. Alternating fungicides with different modes of action is wise to delay the development of fungicide resistance. Cyazofamid has shown some efficacy for *P. capsici*, but resistant isolates have already been described in North Carolina and Georgia (Kousik and Keinath, 2008).

Foliar fungicide sprays are used to protect the fruit, crown, stem and leaves. Vegetable fruits are especially susceptible to *P. capsici* and require thorough coverage to prevent disease. Fungicides need to be applied before and after a rainfall event to protect plants from inoculum spread by soil splash and drainage water. Fungicides should be applied to pickling-type cucumber fruit when they are 2.5, 7.5 and 12.5 cm in length to ensure that fruits are protected as they mature. Fungicides applied via drip application or as drenches are more effective than foliar sprays in controlling root and crown rot (Fig. 11.2B and C). Currently, fluopicolide is the only fungicide registered for direct soil application to vegetables. Seed treatments can help limit pre- and post-emergence damping off.

Fumigation effectively reduces *P. capsici* inoculum in the soil, thereby limiting disease incidence. Products containing dimethyl disulfide, metam sodium, metam potassium, 1,3-dichloropropene/chloropicrin, idomethane/chloropicrin and chloropicrin have proved to be effective for disease control without crop injury. Fumigants may be applied via injection into the soil or through the drip irrigation system. Fumigants should be applied to well-aerated soil at 50–80% field moisture capacity and moderate temperature. Autumn fumigation is recommended in the north-eastern USA because the soil temperatures are usually too cool in the spring to allow fumigation prior to the first plantings (Granke *et al.*, 2012).

11.8 Future Prospects

The occurrence of *P. capsici* throughout many vegetable-growing regions in the USA has prompted recent research on various aspects of the basic biology of *P. capsici*. However, additional research is needed to further clarify: (i) the taxonomy; (ii) variations in virulence and pathogenicity among physiological races; and (iii) pathogenicity determinants. A better understanding of these components will ultimately facilitate the development of durable host resistance and new fungicides that provide effective control. In the short term, development of new fungicides and delivery mechanisms that place the materials precisely where needed (i.e. soil application for plant health and enhanced foliar coverage for fruit protection) will assist growers in remaining viable in spite of infested fields.

A high-quality reference genome sequence for *P. capsici* and more than 20,000 validated single nucleotide variants (SNVs) have recently been described (Lamour *et al.*, 2012). These resources will be useful for development of molecular tools for gene discovery and the design of molecular markers, which may be used to conduct sensitive investigations of *P. capsici* population and evolutionary biology. In addition, the genome resources provide a foundation to investigate the impact of active sexual reproduction in the field at the genome level and to detect introgression events that may provide evidence of hybridization in nature. And finally, the reference genome will allow association studies, an increasingly popular technique to link phenotype to genotype that could help unravel the genetic basis of virulence, pathogenicity and fungicide resistance in *P. capsici*.

References

Donahoo, R.S. and Lamour, K.H. (2008) Interspecific hybridization and apomixis between *Phytophthora capsici* and *Phytophthora tropicalis*. *Mycologia* 100, 911–920.

Èrsek, T., English, J.T. and Schoelz, J.E. (1995) Creation of species hybrids of *Phytophthora* with modified host ranges by zoospore fusion. *Phytopathology* 85, 1343–1347.

Erwin, D.C. and Ribeiro, O.K. (1996) *Phytophthora Diseases Worldwide*. APS Press, St Paul, Minnesota, 562 pp.

Foster, J.M. and Hausbeck, M.K. (2010) Resistance of pepper to Phytophthora crown, root, and fruit rot is affected by isolate virulence. *Plant Disease* 94, 24–30.

French-Monar, R.D., Jones, J.B. and Roberts, P.D. (2006) Characterization of *Phytophthora capsici* associated with roots of weeds on Florida vegetable farms. *Plant Disease* 90, 345–350.

Gevens, A.J., Ando, K., Lamour, K.H., Grumet, R. and Hausbeck, M.K. (2006) A detached cucumber fruit method to screen for resistance to *Phytophthora capsici* and effect of fruit age on susceptibility to infection. *Plant Disease* 90, 1276–1282.

Gevens, A.J., Donahoo, R.S., Lamour, K.H. and Hausbeck, M.K. (2007) Characterization of *Phytophthora capsici* from Michigan surface irrigation water. *Phytopathology* 97, 421–428.

Granke, L.L., Windstam, S.T., Hoch, H.C., Smart, C.D. and Hausbeck, M.K. (2009) Dispersal and movement mechanisms of *Phytophthora capsici* sporangia. *Phytopathology* 99, 1258–1264.

Granke, L.L., Quesada-Ocampo, L.M. and Hausbeck, M.K. (2011) Variation in phenotypic characteristics of *Phytophthora capsici* isolates from a worldwide collection. *Plant Disease* 95, 1080–1088.

Granke, L.L., Quesada-Ocampo, L.M., Lamour, K.H. and Hausbeck, M.K. (2012) Advances in research on *Phytophthora capsici* on vegetable crops in the United States. *Plant Disease*. Available at: http://apsjournals.apsnet.org/loi/pdis (accessed 31 August 2012).

Hausbeck, M.K. and Lamour, K.H. (2004) *Phytophthora capsici* on vegetable crops: research progress and management challenges. *Plant Disease* 88, 1292–1303.

Kousik, C.S. and Keinath, A.P. (2008) First report of insensitivity to cyazofamid among isolates of *Phytophthora capsici* from the southeastern United States. *Plant Disease* 92, 979.

Lamour, K.H. and Hausbeck, M.K. (2003) Effect of crop rotation on the survival of *Phytophthora capsici* in Michigan. *Plant Disease* 87, 841–845.

Lamour, K.H., Mudge, J., Gobena, D., Hurtado-Gonzalez, O., Shmutz, J., Kuo, A., Miller, N.A., Rice, B.J., Raffaele, S., Cano, L.M., Bharti, A.K., Donahoo, R.S., Finley, S., Huitema, E., Hulvey, J., Platt, D., Salamov, A., Savidor, A., Sharma, R., Stam, R., Storey, D., Thines, M., Win, J., Haas, B.J., Dinwiddie, D.L., Jenkins, J., Knight, J.R., Affourtit, J.P., Han, C.S., Chertkov, O., Lindquist, E.A., Detter, C., Grigoriev, I.V., Kamoun, S. and Kingsmore, S.K. (2012) Genome sequencing and mapping reveal loss of heterozygosity as a mechanism for rapid adaptation in the vegetable pathogen *Phytophthora capsici*. *Molecular Plant–Microbe Interactions* 25 (10), 1350–1360.

Larkin, R.P., Ristaino, J.B. and Campbell, C.L. (1995) Detection and quantification of *Phytophthora capsici* in soil. *Phytopathology* 85, 1057–1063.

Lee, B.K., Kim, B.S., Chang, S.W. and Hwang, B.K. (2001) Aggressiveness to pumpkin cultivars of isolates of *Phytopththora capsici* from pumpkin and pepper. *Plant Disease* 85, 497–500.

Padley, J.L.D., Kabelka, E.A. and Roberts, P. (2009) Inheritance of resistance to crown rot caused by *Phytopthora capsici* in *Cucurbita*. *HortScience* 44, 211–213.

Quesada-Ocampo, L.M. and Hausbeck, M.K. (2010) Resistance in tomato and wild relatives to crown and root rot caused by *Phytophthora capsici*. *Phytopathology* 100, 619–627.

Quesada-Ocampo, L.M., Granke, L.L., Mercier, M.R., Olsen, J. and Hausbeck, M.K. (2011) Investigating the genetic structure of *Phytophthora capsici* populations. *Phytopathology* 101, 1061–1073.

Ristaino, J.B. (1990) Intraspecific variation among isolates of *Phytophthora capsici* from pepper and cucurbit fields in North Carolina. *Phytopathology* 80, 1253–1259.

Ristaino, J.B. and Johnston, S.A. (1999) Ecologically based approaches to management of Phytophthora blight on bell pepper. *Plant Disease* 83, 1080–1089.

Wang, Z., Langston, D.B., Csinos, A.S., Gitaitis, R.D., Walcott, R.R. and Ji, P. (2009) Development of an improved isolation approach and simple sequence repeat marker to characterize *Phytophthora capsici* populations in irrigation ponds in southern Georgia. *Applied and Environmental Microbiology* 75, 5467–5473.

12 Taro Leaf Blight Caused by *Phytophthora colocasiae*

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12.1 Introduction

Taro (*Colocasia esculenta* (L.) Schott) is one of the most important staple food crops in the Pacific Islands and is widely cultivated throughout South America, Asia, Africa and the Caribbean (Plucknett *et al.*, 1970; Kreike *et al.*, 2004). It was the fifth most produced tropical root crop in the world in 2009, with global production of 1.6 billion kg (FAO, 2009). Taro can be grown under flooded (i.e. wetland; Fig. 12.1A) or non-flooded conditions (i.e. upland) over a 6–13-month crop cycle (Plucknett *et al.*, 1970). The corm, or underground stem, is consumed for its starch, and taro leaves serve as a vegetable, providing good sources of dietary fibre and vitamin C (Ferguson *et al.*, 1992).

Worldwide, one of the most important diseases threatening the sustainability of taro production is taro leaf blight (TLB) (Fig. 12.1B) caused by the oomycete pathogen *Phytophthora colocasiae* (Ooka, 1994). In Hawai'i between 25 and 50% of taro corms are lost due to oomycete and fungal diseases (Trujillo, 1967; Miyasaka *et al.*, 2001).

12.2 Loss of Productivity due to TLB

In Hawai'i taro yields have been declining over the past 34 years, partially due to pests and diseases (Fig. 12.1C). According to the Hawai'i Agricultural Statistics Service (2006), total state-wide taro production in 2005 was only 1.8 million kg, the lowest since records began in 1946. Factors contributing to low yield included: (i) rainy weather; (ii) TLB; (iii) taro pocket rot (another disease caused by a *Phytophthora* species); and (iv) apple snail infestation (Hawai'i Agricultural Statistics Service, 2006).

P. colocasiae invaded Hawai'i during the 1920s and very likely contributed to the extinction of dozens of traditional Hawaiian cultivars (CTAHR, 2009). Due to the ease of global transport and trade between countries, TLB has spread to new geographic areas across the Pacific, the Caribbean and Africa (Omane *et al.*, 2012). In 1993 TLB was introduced to American and Western Samoa and devastated the highly susceptible, traditional Samoan taro cultivars. The epidemic halted all taro production in Samoa from 1994 to 1998, and it was only with the introduction of TLB-resistant taro cultivars that production has resumed (Trujillo and Menezes, 1995). A similarly devastating epidemic occurred when TLB reached the Dominican Republic in 2004, resulting in dramatic losses to the susceptible, commercial taro cultivar (R.P. Duverge, Dominican Republic, 2009, personal communication).

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A

B

C

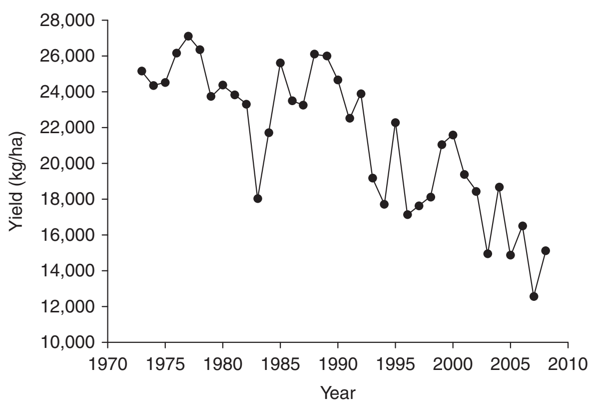


Fig. 12.1. Impact of taro leaf blight (TLB) on taro production. **(A)** Wetland taro field in the Waipio Valley, Hawai'i, in which plants exhibit symptoms of TLB. **(B)** Heavily infected taro leaf. **(C)** Decreased corm yields of taro on a per hectare basis on the island of Kaua'i (data from Hawai'i Agricultural Statistics Service, 1974–2008).

12.3 *P. colocasiae*

P. colocasiae was first described as causing leaf infections on taro in Java in 1900 and is considered to be host specific, primarily infecting foliage (Raciborski, 1900 cited in Erwin and Ribeiro, 1996). Although the disease can be easy to identify on leaves due to the distinctive lesions, it also attacks the stems and corms and these lesions may be less distinctive (Fig. 12.2A). Lesions on leaves often have a distinctive bull's-eye pattern resulting from the varied growth and sporulation that occurs as the moisture and temperature fluctuate throughout the day and night. Figure 12.2B shows a heavily sporulating lesion containing many thousands to millions of deciduous sporangia. This particular taro plant was growing under conditions of optimum humidity and temperature. Under field conditions, particularly when it is dry, the necrotic area may fall out and leaves may have variable sized holes in them. Taro is often grown in flooded conditions and there are few crops that provide an equally optimal arena for the dissemination and development of *Phytophthora* disease (Fig. 12.1A).

Morphologically, *P. colocasiae* forms semipapillate, cauducous, ellipsoid to ovoid sporangia that are 45–70 × 25–37 µm (Gallegly and Hong, 2008). Shapes of sporangium are variable, but generally they have a tapered base with a conspicuous basal plug at the point where the pedicel attaches to the spore. The pedicel length is about 10–12 µm (Gallegly and Hong, 2008). Epidemics of TLB often occur when air temperatures are in the range of 20–24°C (Trujillo, 1965), and under wet conditions the sporangia release zoospores that spread rapidly in water and cause multiple infection sites (Fig. 12.2C).

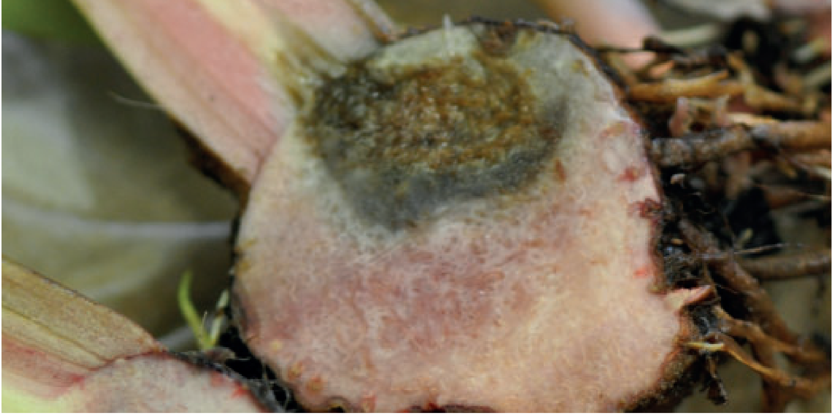
12.4 Diversity of *P. colocasiae*

P. colocasiae is heterothallic (obligatory outcrossing), and sexual oospores are formed in paired cultures of the A1 and A2 mating types. At this point, there have been no reports of progeny produced in the laboratory and the importance of the sexual stage in the life history is not known. Unlike the related vegetable pathogen *Phytophthora capsici*, which is also heterothallic and often relies on the sexual stage to survive cold or fallow periods, *P. colocasiae* may not be under intense selection pressure for dormant spores. This is because *P. colocasiae* has abundant host material year round. Although disease severity peaks during the wet months, lesions can be found even during the dry months on plants in moist niches, such as the lower leaf canopies.

Overall, studies to determine the diversity of *P. colocasiae* are limited. Some of the earliest surveys record the presence of the A1 and A2 mating types. In the 1970s an analysis of 114 isolates collected from Hawai'i, Maui and Kaua'i revealed all were A1 and it was concluded that *P. colocasiae* was probably an exotic invasive pathogen (Ko, 1979). A similar study in Taiwan revealed that all 799 isolates collected were of the A2 mating type (Ann *et al.*, 1986). In 1994 Zhang *et al.* identified the presence of three different mating types, A1, A2 and A0 (unable to produce oospores when paired with either an A1 or A2) on the agriculturally important Hainan Island in China, and the authors suggest this region may be within the centre of origin for *P. colocasiae* (Zhang *et al.*, 1994). Similarly, A2 and A0 mating types were reported from 54 isolates collected from Asia and the Pacific region (Tyson and Fullerton, 2007). A more recent investigation of the diversity of *P. colocasiae* in Taiwan reports seven isolates that appear to be homothallic, capable of producing oospores in a single culture. During mitotic growth these isolates produced asexual progeny that were A1 or A2 mating type, as well as some that were homothallic. All were able to cause disease (Lin and Ko, 2008).

In 2003 Lebot *et al.* utilized random amplified polymorphic DNA (RAPD) markers and isozymes to characterize a large collection of *P. colocasiae* from Indonesia, Papua New Guinea, the Philippines, Thailand and Vietnam (Lebot *et al.*, 2003). The isozyme profiles varied

A



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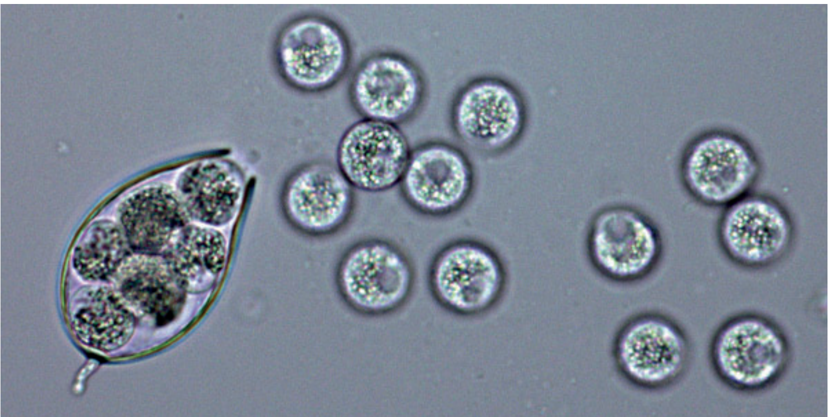

Fig. 12.2. (A) Infected corm collected on Oahu, Hawai'i. **(B)** TLB lesion with very heavy sporangia production collected at the Kaua'i Agricultural Research Center, Kapaa, Hawai'i. **(C)** Photomicrograph of a sporangium of *Phytophthora colocasiae* releasing swimming zoospores.

considerably between countries, and isolates with identical profiles from within the same country were found to be diverse based on RAPD fingerprints (Lebot *et al.*, 2003). A recent report on 14 isolates from India indicates all had unique isozyme and RAPD profiles (Mishra *et al.*, 2010).

12.5 Current and Future Genetic Work on *P. colocasiae*

P. colocasiae is relatively closely related to the vegetable pathogen *P. capsici*, and this shared evolutionary history is proving useful to develop novel genetic resources and markers. A current project is using a restriction-enzyme-based focused sequencing approach to survey the *P. colocasiae* genome for single nucleotide variant (SNV) sites. Alignment of these sequences to the *P. capsici* genome provides a mechanism to compare isolates and to identify polymorphic sites (Lamour *et al.*, 2012). Although these studies are still in progress, it is already clear that *P. colocasiae* has abundant genetic variation at the nucleotide level, and further testing of these markers in natural populations will determine if the allele frequencies are suitable for further investigations and should allow a detailed investigation of population diversity.

As we are learning with *P. capsici*, a high level of nucleotide-level variation presents both challenges and opportunities. It is challenging because breeding efforts must take this genetic diversity into account when developing resistant taro cultivars as pathogen populations may contain sufficient variation to rapidly overcome resistance. From the viewpoint of research, genetic variation becomes an asset as it allows opportunities to conduct sensitive genetic analyses. For example, if *in vitro* crosses are possible, then the genome can be mapped at a fine scale and the factors driving pathogenicity and virulence on different taro varieties could be identified (Lamour *et al.*, 2012). Even without the ability to make crosses, the abundant genetic variation provides a powerful resource to track clonal lineages and test whether sexual recombination plays a role in the success of naturally occurring populations.

12.6 Integrated Pest Management (IPM) Practices to Control *P. colocasiae*

An IPM system can be used to control *P. colocasiae* (as well as other diseases) and includes: (i) field sanitation; (ii) selection of healthy, disease-free vegetative propagules; (iii) use of compost or green manure; (iv) crop rotation; (v) registered pesticides; (vi) removal of diseased leaves; and (vii) TLB-resistant cultivars (Uchida *et al.*, 2002; Nelson *et al.*, 2011). Field sanitation involves removal of almost all of the host tissues (including roots) from the field and is crucial to reduce disease in the next crop. Spores and mycelia within infected tissue survive many months as they are protected by the host tissue from sunshine, high or low pH, salinity and, most importantly, from bacteria that feed on organic matter in the soil.

In Hawai'i vegetative propagules ('huli') are often prepared from the lower 30 cm of the petiole plus 0.5 cm of the upper corm, although in some farms and other areas of the world cormels or sucker plants are planted. Selection of clean and healthy starter plants to establish the new fields is extremely important. When growing taro from 'huli', it is recommended that they should be planted on the day after harvest or within 2 days, since each day depletes their reserves of stored food. A few growers place the 'huli' in water to encourage root growth; however, extra care must be used or damaged roots will allow oomycete pathogens such as *P. colocasiae* to enter the plant.

Compost may be incorporated prior to planting as a means of stimulating the growth and diversity of microorganisms that out-compete *P. colocasiae* and other pathogens. Alternatively, to provide a period without the presence of susceptible

host plants, cover crops such as sunn-hemp (*Crotalaria juncea* L.) or rye (*Secale cereale* L.) can be grown and ploughed under, prior to seed development. Similarly, crop rotation with non-susceptible plants such as maize (*Zea mays* L.), squash (*Cucurbita pepo* L.) or sweet potatoes (*Ipomoea batatas* (L.) Lam.) may reduce the pathogen level.

Although morphologically similar to fungi, *Phytophthora* is related to brown algae and diatoms and is unaffected by most fungicides. Based on the Hawai'i Pesticide Information Retrieval System (HPIRS, 2012) and the National Pesticide Information Retrieval System (M. Kawate, Hawai'i, 2012, personal communication), there are 12 fungicides registered for control of oomycete pathogens on taro (or dasheen) in Hawai'i or the USA. These include mefenoxam (e.g. Twist® or Ridomil Gold®, Syngenta, Greensboro, North Carolina; and MetaStar®, LG International, Englewood Cliffs, New Jersey) and mono- and di-potassium salts of phosphorous acid (Fosphite®, JH Biotech, Ventura, California). Mefenoxam is restricted to one soil application pre-plant or at planting and phosphorous acid can be applied as a root dip (pre-plant), with irrigation, or on foliage at 2–3-week intervals. Pesticides restricted to one application (e.g. mefenoxam) have limited use, since taro is grown over a period of 6–13 months. Both mefenoxam and phosphorous acid have been reported to be effective in controlling *P. colocasiae* (Nelson *et al.*, 2011). However, although these fungicides may be effective in controlling TLB, many growers don't use them due to cost, time and environmental concerns (Nelson *et al.*, 2011; R. Yamakawa, Hawai'i, 2012, personal communication).

Removal of diseased leaves can significantly delay serious disease development but is only effective at the beginning of a disease cycle and is not practical if environmental conditions are conducive to the development of TLB (Nelson *et al.*, 2011). One of the most easily adopted IPM practices to control TLB is growing TLB-resistant cultivars.

12.7 Breeding of Taro for Increased TLB Resistance

In Hawai'i there have been two earlier breeding programmes to develop TLB-resistant taro cultivars. Trujillo *et al.* (2002) crossed the modern commercial Hawaiian cultivar 'Maui Lehua' with the TLB-resistant Palauan cultivar 'Ngeruuch'. Three cultivars from this F_1 cross ('Pa'lehua', 'Pa'akala' and 'Pauakea') were patented and released. However, due to controversies over royalties and ownership of taro, the University of Hawai'i relinquished its patent rights and released the new taro cultivars into the public domain (CTAHR, 2009). These cultivars are problematic because they appear to be more susceptible to *Pythium* soft rots under certain soil conditions (Trujillo *et al.*, 2002), and several producers of poi (i.e. a traditional Hawaiian food made from mashed and fermented taro corms) will no longer purchase Palauan cultivars or hybrids with this parentage because they ferment too rapidly. In contrast, there is one poi producer who uses cultivar 'Pa'akala' due to its improved yield, large corm size and excellent corm quality, and this cultivar maintains its corm quality in the field longer than other varieties (R. Yamakawa, Hawai'i, 2012, personal communication).

In a second taro breeding programme in Hawai'i, Cho *et al.* (2007) introduced TLB resistance from taro collected from Micronesia, Palau, Indonesia, Papua New Guinea, Thailand and Nepal. Three promising cultivars ('BC99-6', 'BC99-7' and 'BC99-9') were produced by a cross between commercial cultivar 'Maui Lehua' and a cultivar (#21) with parental cultivars 'Bangkok' and 'Niue Waula'. Cultivar 'BC99-6' (now called cultivar 'Lehua Hoolehua') is grown in 25% of the taro-growing areas on the Island of Kaua'i, replacing commercial cultivar 'Maui Lehua' (C. Tottori, Hawai'i, 2011, personal communication). These TLB-resistant cultivars exhibit a hypersensitive response to *P. colocasiae*, with a chlorotic area forming around the infection site followed by necrosis that appears to limit the spread of the disease.

12.8 Current and Future Directions to Improve TLB Resistance in Taro

The taro cultivar 'Bun Long', that has been transformed with an oxalate oxidase (OxO) gene from wheat, exhibited dramatically increased resistance to TLB under tissue-culture and growth chamber conditions (He *et al.*, 2011). However, due to controversies in Hawai'i including bans in two counties on genetic engineering research on taro (He *et al.*, 2010) research has shifted to naturally occurring OxO genes within the taro germplasm to test if they may play a role in disease resistance. Preliminary results indicate the presence and expression of an homologous OxO gene within several conventionally bred TLB-resistant taro cultivars (He *et al.*, 2011).

Currently, in the third taro breeding programme in Hawai'i, crosses are being made with taro cultivars exhibiting TLB resistance based on field evaluation. A modified, excised leaf assay to determine TLB resistance (Brooks, 2008; He *et al.*, 2010) is being conducted to evaluate progeny of conventional crosses. Lesion diameter is measured and relative lesion diameter is calculated in comparison to the TLB-susceptible cultivar 'Bun Long' (Brown *et al.*, 2011). Results of excised leaf assays correlate well with field-based ratings of TLB resistance in Hawaiian, Samoan and Palauan cultivars. In addition, a taro population has been developed that segregates for TLB resistance, and single nucleotide polymorphism (SNP) markers are being developed to hopefully identify markers linked to TLB resistance (Brown *et al.*, 2011). Known simple sequence repeat (SSR) markers do not cosegregate with observed TLB resistance in this segregating population. Once identified, genetic markers will be used to select for targeted disease resistance genes (foreground selection) as well as desired traits of the recurrent parent (background selection).

12.9 Summary

Despite its status as the fifth most produced root crop in the world, taro and its pathogen *P. colocasiae* have been largely neglected in genetic studies. New molecular resources (e.g. reference genome sequences, SNPs) will help: (i) characterize the diversity of *P. colocasiae*; (ii) understand factors that drive pathogenicity; and (iii) improve the efficiency of breeding resistant taro through marker-assisted selection.

References

Ann, P.J., Kao, C.W. and Ko, W.H. (1986) Mating-type distribution of *Phytophthora colocasiae* in Taiwan. *Mycopathologia* 93, 193–194.

Brooks, F.E. (2008) Detached-leaf bioassay for evaluating taro resistance to *Phytophthora colocasiae*. *Plant Disease* 92, 126–131.

Brown, A., Veillet, A., Miyasaka, S.C., Bernabe, C. and Shintaku, M. (2011) Screening taro (*Colocasia esculenta*) for resistance to taro leaf blight (TLB) using a detached-leaf disc bioassay and developing DNA markers for use in selection. Abstract p. S77, American Society of Horticultural Science (ASHS), September 2011, Waikoloa, Hawai'i. Available at: http://ashs.org/downloads/2011ASHS_Conference_abstracts.pdf (accessed 27 September 2012).

Cho, J.J., Yamakawa, R.A. and Hollyer, J. (2007) *Hawaiian Kalo, Past and Future*. University of Hawai'i, College of Tropical Agriculture and Human Resources, Honolulu, Hawai'i, *Sustainable Agriculture*, SA-1. Available at: http://www.ctahr.hawaii.edu/oc/freepubs/pdf/SA-1.pdf (accessed 27 September 2012).

College of Tropical Agriculture and Human Resources (CTAHR) (2009) *CTAHR and Taro*. University of Hawai'i, College of Tropical Agriculture and Human Resources, Honolulu, Hawai'i. Available at: http://www.ctahr.hawaii.edu/oc/freepubs/pdf/CTAHR_and_taro.pdf (accessed 27 September 2012).

Erwin, D.C. and Ribeiro, O.K. (1996) *Phytophthora Diseases Worldwide*. APS Press, St Paul, Minnesota, 562 pp.

Ferguson, L.R., Roberton, A.M., Mckenzie, R.J., Watson, M.E. and Harris, P.J. (1992) Adsorption of a hydrophobic mutagen to dietary fiber from taro (*Colocasia esculenta*), an important food plant of the South Pacific. *Nutrition and Cancer* 17, 85–95.

Food and Agriculture Organization of the United Nations (FAO) (2009) Available at: http://faostat.fao.org/site/339/default.aspx (accessed 13 October 2011 for 2009 statistics).

Gallegly, M.E. and Hong, C.X. (2008) Phytophthora: *Identifying Species by Morphology and DNA Fingerprints*. APS Press, St Paul, Minnesota.

Hawai'i Agricultural Statistics Service (2006) *Statistics of Hawai'i Agriculture 2005*. Hawai'i Agricultural Statistics Service, Honolulu, Hawai'i.

Hawai'i Pesticide Information Retrieval System (HPIRS) (2012) Available at: http://state.ceris.purdue.edu/doc/hi/statehi.html (accessed 13 February 2012).

He, X., Miyasaka, S.C., Zou, Y., Fitch, M.M.M. and Zhu, Y.J. (2010) Regeneration and transformation of taro (*Colocasia esculenta*) with a rice chitinase gene enhances resistance to *Sclerotium rolfsii*. *HortScience* 45, 1014–1020.

He, X., Fitch, M., Zhu, Y.J. and Miyasaka, S.C. (2011) The important roles of oxalate oxidase in taro disease resistance. Abstract pp. S78–79, American Society of Horticultural Science (ASHS), September 2011, Waikoloa, Hawai'i. Available at: http://ashs.org/downloads/2011ASHS_Conference_abstracts.pdf (accessed 27 September 2012).

Ko, W.H. (1979) Mating-type distribution of *Phytophthora colocasiae* on the island of Hawai'i. *Mycologia* 71, 434–437.

Kreike, C.M., Van Eck, H.J. and Lebot, V. (2004) Genetic diversity of taro, *Colocasia esculenta* (L.) Schott, in Southeast Asia and the Pacific. *Theoretical and Applied Genetics* 109, 761–768.

Lamour, K., Mudge, J., Gobena, D., Hurtado-Gonzalez, O., Shmutz, J., Kuo, A., Miller, N.A., Rice, B.J., Raffaele, S., Cano, L.M., Bharti, A.K., Donahoo, R.S., Finley, S., Huitema, E., Hulvey, J., Platt, D., Salamov, A., Savidor, A., Sharma, R., Stam, R., Storey, D., Thines, M., Win, J., Haas, B.J., Dinwiddie, D.L., Jenkins, J., Knight, J.R., Affourtit, J.P., Han, C.S., Chertkov, O., Lindquist, E.A., Detter, C., Grigoriev, I.V., Kamoun, S. and Kingsmore, S.K. (2012) Genome sequencing and mapping reveal loss of heterozygosity as a mechanism for rapid adaptation in the vegetable pathogen *Phytophthora capsici*. *Molecular Plant–Microbe Interactions* 25 (10), 1350–1360.

Lebot, V., Herail, C., Gunua, T., Pardales, J., Prana, M., Thongjiem, M. and Viet, N. (2003) Isozyme and RAPD variation among *Phytophthora colocasiae* isolates from South-east Asia and the Pacific. *Plant Pathology* 52, 303–313.

Lin, M.-J. and Ko, W.-H. (2008) Occurrence of isolates of *Phytophthora colocasiae* in Taiwan with homothallic behavior and its significance. *Mycologia* 100, 727–734.

Mishra, A.K., Sharma, K. and Misra, R.S. (2010) Isozyme and PCR-based genotyping of epidemic *Phytophthora colocasiae* associated with taro leaf blight. *Archives of Phytopathology and Plant Protection* 43, 1367–1380.

Miyasaka, S.C., Hollyer, J.R. and Kodani, L.S. (2001) Mulch and compost effects on yield and corm rots of taro. *Field Crops Research* 71, 101–112.

Nelson, S., Brooks, F. and Teves, G. (2011) *Taro Leaf Blight in Hawai'i*. University of Hawai'i, College of Tropical Agriculture and Human Resources, PD-71, 14 pp. Available at: http://www.ctahr.hawaii.edu/oc/freepubs/pdf/PD-71.pdf (accessed 27 September 2012).

Omane, E., Oduro, K.A., Cornelius, E.W., Opoku, I.Y., Akrofi, A.Y., Sharma, K., Kumar, P.L. and Bandyopadhyay, R. (2012) First report of leaf blight of taro (*Colocasia esculenta*) caused by *Phytophthora colocasiae* in Ghana. *Plant Disease* 96, 292.

Ooka, J.J. (1994) Taro diseases, a guide for field identification. University of Hawai'i, Hawai'i Institute of Tropical Agriculture and Human Resources, Research Extension Series 148.

Plucknett, D.L., de la Pena, R.S. and Obrero, F. (1970) Taro (*Colocasia esculenta*). *Field Crops Abstracts* 23, 413–426.

Raciborski, M. (1900) *Parasitische Algen und Pilze, Java's* [*Java's Parasitic Algae and Fungi*]. I. *Batavia*. p. 9. (Cited in Erwin and Ribeiro, 1996.)

Trujillo, E.E. (1965) The effect of humidity and temperature on *Phytophthora* blight of taro. *Phytopathology* 55, 183–188.

Trujillo, E.E. (1967) Diseases of the genus *Colocasia* in the Pacific area and their control. *Proceedings of International Symposium Tropical Root Crops* 2, 13–19.

Trujillo, E.E. and Menezes, T. (1995) Field resistance of Micronesian taros to *Phytophthora* blight. *Phytopathology* 85, 1564.

Trujillo, E.E., Menezes, T. and Cavaletto, C. (2002) *Promising New Taro Cultivars with Resistance to Taro Leaf Blight: 'Pa 'lehua', 'Pa 'akala', and 'Pauakea'*. University of Hawai'i, College of Tropical Agriculture and Human Resources, *New Plants for Hawai'i*, NPH-7, 4 pp. Available at: http://www.ctahr.hawaii.edu/oc/freepubs/pdf/NPH-7.pdf (accessed 27 September 2012).

Tyson, J.L. and Fullerton, R.A. (2007) Mating types of *Phytophthora colocasiae* from the Pacific region, India and South-east Asia. *Australasian Plant Disease Notes* 2, 111–112.

Uchida, J.Y. Silva, J.A. and Kadooka, C.Y. (2002) *Improvements in Taro Culture and Reduction in Disease Levels*. University of Hawai'i, College of Tropical Agriculture and Human Resources, *Plant Disease*, PD-22, 4pp. Available at: http://www.ctahr.hawaii.edu/oc/freepubs/pdf/PD-22.pdf (accessed 27 September 2012).

Zhang, K.M., Zheng, F.C., Li, Y.D. and Ko, W.H. (1994) Isolates of *Phytophthora colocasiae* from Hainan Island in China: evidence suggesting an Asian origin of this species. *Mycologia* 86, 108–112.

13 *Phytophthora nicotianae*

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13.1 Introduction

Phytophthora nicotianae, like many other *Phytophthora* species, is a devastating plant pathogen. With its wide host range, polycyclic nature and long-term survival in the soil, *P. nicotianae* is a factor that must be managed in many agricultural areas around the world. In this chapter we provide an overview of the nature of *P. nicotianae*, its hosts and its infection strategy. We outline current approaches to controlling diseases caused by *P. nicotianae* and review recent research that has advanced our understanding of its cellular and molecular biology and pathogenicity.

13.2 Taxonomy and Phylogeny

P. nicotianae was first described in 1896 by van Brenda de Haan from an isolate collected from tobacco plants in Indonesia, but, unfortunately, the original culture was contaminated with a *Pythium* species (Erwin and Ribeiro, 1996). The pathogen was re-isolated in India from castor bean by Dastur in 1913 and named *Phytophthora parasitica* (see Erwin and Ribeiro, 1996); however, according to the International Code of Botanical Nomenclature, the original description has priority (Waterhouse, 1963). In 1993 Hall re-described the species under the name *P. nicotianae* (Hall, 1993) and this is the name that will be used in this chapter.

Modern molecular phylogenetic analyses of *Phytophthora* indicate that the genus is monophyletic and that the >100 species fall into eight (Cooke *et al.*, 2000; Kroon *et al.*, 2004) or ten clades (Blair *et al.*, 2008). Species composition within the clades does not coincide with Waterhouse's (1963) taxonomic groupings based on morphological and cultural criteria. All three molecular studies place *P. nicotianae* in clade 1, along with *Phytophthora infestans* and *Phytophthora cactorum*.

13.3 Hosts and Economic Losses

P. nicotianae has a broad host range infecting over 255 genera in 90 different families (Cooke *et al.*, 2000). Susceptible plants include important agricultural and horticultural species such as citrus, cotton, apple, cashew, pistachio, tobacco and tomato. *P. nicotianae* is also virulent on *Arabidopsis* (Wang *et al.*, 2011), and this plant–pathogen interaction promises to become an important model system for future research.

P. nicotianae usually infects roots or the crown region of host plants, but it is also capable of infecting fruits, leaves and flowers when inoculum reaches these organs (Fig. 13.1A and B). On tobacco *P. nicotianae* causes black shank in the crown region, and on citrus it most commonly causes foot rot or gummosis (Erwin and Ribeiro, 1996). Although the presence of

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A

B

Fig. 13.1. *Phytophthora nicotianae* infection of leaves and roots. **(A)** Lesions on tobacco leaves following inoculation with *P. nicotianae* zoospores. **(B)** Necrotic lesion on lupin roots 3 days after a 10-min immersion in a suspension containing 500 *P. nicotianae* zoospores/ml.

multiple pathogens often makes the estimation of economic losses difficult, annual economic losses run into the millions of dollars in citrus, tobacco and tomato (Cahill, 1993; Drenth and Sendall, 2004).

13.4 Controlling *P. nicotianae* Diseases

Modern disease management strategies recommend an integrated approach that includes the following: (i) hygiene practices; (ii) use of resistant plants; (iii) ongoing surveillance for timely detection of disease; (iv) quarantine measures to limit disease spread; and, if possible, (v) eradication of the pathogen from infested areas. In the case of *P. nicotianae*, implementation of these strategies is challenging. *P. nicotianae*'s wide host range and the restricted availability of resistant cultivars and rootstocks make disease prevention difficult. Its soil-borne nature, potential latency period and production of motile zoospores impede timely detection and containment. The similarity of its morphology, cultural parameters and disease symptoms to other *Phytophthora* species means that pathogen identification requires expert knowledge and/or access to diagnostic assays. The limited availability of control measures and development of resistance to inhibitory chemicals hinder treatment and eradication. The following discussion focuses on recent advances in strategies pertaining specifically to *P. nicotianae* diseases.

13.4.1 Immunological and DNA-based pathogen diagnosis

Both antibody-based and DNA-based diagnostic assays have been developed for the identification of *P. nicotianae* in conjunction with its isolation from infected soil or plant material using baiting and selective media. One immunodiagnostic assay utilizes species-specific monoclonal antibodies that recognize an antigen on the surface of *P. nicotianae* cysts (Gautam *et al.*, 1999). The assay exploits zoospore chemotaxis. The zoospores are attracted and adhere to a dipstick, which is then processed to give a colorimetric indication of *P. nicotianae* presence and abundance. The assay is quick and sensitive and does not require specialized equipment or expertise. Polyclonal antibodies raised against *P. nicotianae* have also been used in ELISAs to monitor the effects of biological control agents (Sukhada *et al.*, 2011).

Much of the current focus in plant disease diagnosis is on assays based on PCR. The critical factor in the success of these assays is the specificity of the PCR primers. Primers designed for specific and sensitive detection of *P. nicotianae* target the elicitin gene, *ParA1* (Lacourt and Duncan, 1997), and the internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) (Grote *et al.*, 2002). For *P. nicotianae*, as for other soil-borne pathogens, one potential problem for PCR-based assays is interference by soil-derived contaminants. In this regard, use of a nested-PCR approach may improve assay efficacy (Grote *et al.*, 2002).

13.4.2 Chemical control

Implementation of hygienic practices, including improved drainage and irrigation regimes and effective decontamination procedures, help stop the spread of *P. nicotianae* diseases. Crop rotation and the use of resistant or tolerant varieties also help reduce disease incidence (Graham and Menge, 2000; Csinos, 2005); however, in most cases there is a need for additional control strategies to inhibit or kill *P. nicotianae*. Unfortunately, the number of chemicals that effectively control *P. nicotianae* or other *Phytophthora* species is limited. Fosetyl-Al, metalaxyl and mefenoxam (a derivative of metalaxyl) have been used extensively; however, strains of *P. nicotianae* resistant to metalaxyl and/or mefenozam have emerged in a number of crops worldwide (Timmer *et al.*, 1998; Hu *et al.*, 2008). Currently, phosphonate (phosphite) is one of the most effective chemical control agents (Erwin and Ribeiro, 1996).

13.4.3 Biological control

It has long been known that some soils suppress the growth of soil-borne pathogens including *Phytophthora* species. The most important elements are microorganisms that actively inhibit pathogen growth. Early studies suggest bacteria and mycorrhizal fungi might be useful biological control agents for *P. nicotianae* (Erwin and Ribeiro, 1996) and recent research has substantiated the early results. Pre-inoculation of tomato roots with the arbuscular mycorrhizal fungi *Glomus intraradices* or *Glomus mosseae* significantly reduces pathogen biomass and the extent of root colonization by *P. nicotianae* (Lioussanne *et al.*, 2009). Although exudates isolated from roots colonized by *G. intraradices* alter *P. nicotianae* zoospore chemotaxis *in vitro*, the root exudates did not affect colonization in soil-grown plants. Analysis of >6000 bacterial strains from the rhizosphere of tobacco roots revealed that 4% were able to inhibit *P. nicotianae* growth (Jin *et al.*, 2011). The antagonistic bacteria were genetically diverse, representing over 33 species from 17 different genera.

13.4.4 Emergence of natural hybrids

The capability of *P. nicotianae* to hybridize with other *Phytophthora* species *in vitro* was demonstrated over 20 years ago and natural hybrids of *P. nicotianae* and *P. cactorum* have now been isolated from a number of plant species in the Netherlands, Peru and Taiwan (Bonants *et al.*, 2000; Hurtado-Gonzales *et al.*, 2009). The hybrids were initially recognized through their atypical morphology, and subsequent examination using isozymes, amplified fragment length polymorphism (AFLP) patterns and rDNA ITS sequences confirmed the presence of genes/proteins from *P. nicotianae* and *P. cactorum*. Hydroponic greenhouse systems are thought to contribute to natural hybridization events (Bonants *et al.*, 2000). Interspecific hybridization is a serious concern because hybrids are often capable of infecting plants that were previously not susceptible to either parent species (see Érsek and Man in 't Veld, Chapter 5, this volume).

13.5 *P. nicotianae* Life Cycle and Infection Strategy

Infection by *P. nicotianae* is usually initiated by motile, biflagellate zoospores produced in multinucleate sporangia formed at hyphal apices during asexual sporulation (Fig. 13.2). The zoospores are attracted to potential hosts through detection of chemical and/or electrical gradients surrounding the plant (van West *et al.*, 2002). At the plant surface the zoospores encyst. During encystment the flagella are detached, adhesive material is secreted from cortical vesicles and a cellulosic cell wall is rapidly formed (Robold and Hardham, 2005) (Fig. 13.2E and F). As discussed below, two adhesive proteins secreted by *P. nicotianae* zoospores during encystment have been identified and characterized, although it is likely that a range of other molecules are also secreted following encystment (V.A. Ludowici, L.M. Blackman and A.R. Hardham, unpublished observations). Recent studies indicate that secreted molecules induce zoospore aggregation and increase the rate of successful infection (Kong *et al.*, 2010).

P. nicotianae cysts usually germinate within 20–30 min after encystment (Fig. 13.2D and G). During penetration of the plant epidermis the tip of the germ tube may differentiate into an appressorium-like structure, which is separated from the rest of the germ tube by a septum (Kebdani *et al.*, 2010) (Fig. 13.2F and G). Penetration and colonization of the plant is facilitated by secretion of cell-wall-degrading enzymes (CWDEs), which degrade the plant cell wall components (Wu *et al.*, 2008; Kebdani *et al.*, 2010).

P. nicotianae, like *P. infestans* and *Phytophthora sojae*, is a hemibiotroph. During early infection it grows biotrophically, developing haustoria that mediate nutrient uptake from the living plant cells. As disease progresses, *P. nicotianae* enters a

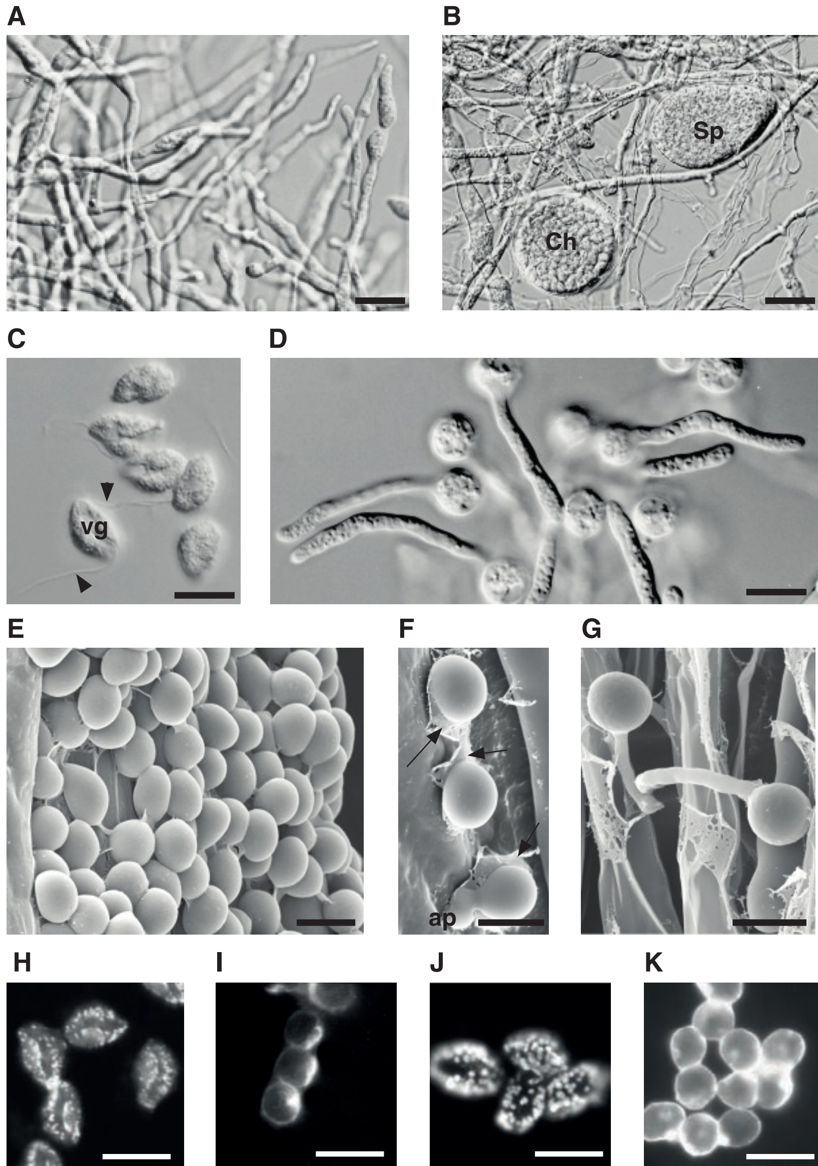

Fig. 13.2. Stages in the asexual life cycle of *P. nicotianae*. **(A–D)** Differential interference contrast light micrographs of: **(A)** vegetative hyphae; **(B)** sporulating hyphae with a sporangium (Sp) and a chlamydospore (Ch); **(C)** zoospores showing the two flagella (arrowheads) emerging from the centre of the ventral groove (vg); and **(D)** germinated cysts. **(E–G)** Cryoscanning electron micrographs of *P. nicotianae* cysts on the surface of tobacco roots. **(E)** Spores encysted in an aggregate on the root surface. **(F)** Mucilage-like material (arrows) has been secreted by the encysting zoospores. One cyst has germinated and developed an appressorium-like swelling (ap) where the germ tube penetrates the plant epidermis. **(G)** Germinated cysts penetrate the tobacco root epidermis. **(H–K)** Immunofluorescence micrographs of *P. nicotianae* zoospores and cysts labelled with antibodies against adhesive proteins. **(H)** Small vesicles on the ventral surface of the zoospores labelled by an antibody directed against the Vsv protein. **(I)** Material secreted onto the ventral surface of young cysts labelled by the Vsv antibody. **(J)** Small vesicles occurring predominantly on the zoospore dorsal surface labelled by an antibody directed against PnCcp. **(K)** Cysts labelled with the antibody against PnCcp. Bars, **(A–B)** = 20 µm; **(C–K)** = 10 µm.

necrotrophic stage, obtaining nutrients from dead or dying plant cells. In susceptible plants infection is rapid, with asexual sporangia developing on the root surface within 3–5 days of infection. *P. nicotianae* also forms asexual chlamydospores, which are important for long-term survival in the soil (Fig. 13.2B). *P. nicotianae* is heterothallic, capable of producing thick-walled oospores when A1 and A2 mating types come together, although the importance of oospores as inoculum in field situations is unknown. In tobacco-growing regions in Virginia, USA, a recent survey found that 6% of *P. nicotianae* isolates belonged to the A1 mating type while 94% of isolates were A2, suggesting that sexual recombination does not play a crucial role in this situation (Parkunan *et al.*, 2010).

13.6 Development of Molecular Resources for *P. nicotianae*

A bacterial artificial chromosome library for *P. nicotianae* has been constructed and used to predict that the *P. nicotianae* genome is about 96 Mb, a size similar to that of *P. sojae* (Shan and Hardham, 2004). The genome of *P. nicotianae* has been sequenced at the Broad Institute using Illumina technology (http://www.broadinstitute.org/annotation/genome/Phytophthora_parasitica/Home.html) and a 71-Mb draft assembly was made available to the public in 2011. RNA-Seq data are currently being released. Seven complementary DNA (cDNA) libraries have been generated from RNA isolated from *P. nicotianae* growing *in vitro* or *in planta*. Analysis indicates the patterns of gene expression at different stages of the life cycle and during plant infection are quite distinct.

13.7 Molecular Biology of *P. nicotianae* Infection Strategies

As outlined above, the key elements of *P. nicotianae* infection are: (i) the active movement of zoospores to suitable infection sites; (ii) rapid adhesion to the plant surface; (iii) secretion of CWDEs to facilitate penetration; and (iv) secretion of effectors to manipulate the host metabolism and suppress plant defence. Recent research is beginning to reveal details of the molecular mechanisms involved in each of these processes.

13.7.1 Zoospore motility

P. nicotianae zoospore motility is achieved through the action of their two flagella (Fig. 13.2C), both of which have a structure typical of eukaryotic flagella. Recently a gene encoding dynein light chain 1 (*PnDLC1*), a component of the flagellar outer dynein arms, was cloned from *P. nicotianae* and its function studied by RNA interference (RNAi)-mediated silencing (Narayan *et al.*, 2010). Outer dynein arms normally facilitate sliding of adjacent microtubule doublets to generate flagellar bending and *PnDLC1*-silenced lines lacked flagella. The non-flagellate zoospores were released from sporangia normally, supporting the idea that zoospore expulsion is driven by the generation of turgor pressure within the sporangium (Narayan *et al.*, 2010). The *PnMas2* gene encodes a component of the tubular shaft of mastigonemes, which line the anterior flagellum and are responsible for forward motility (Blackman *et al.*, 2011). Recent cloning of *PnMas2* will allow molecular characterization of the structure and function of these important flagellar hairs.

13.7.2 Zoospore adhesion

In *Phytophthora* species zoospore adhesive material is believed to be synthesized during sporangium development and stored in cortical secretory vesicles. The adhesive material is rapidly secreted within the first 2 min of encystment and forms an adhesive pad that glues the spores to the plant surface (Robold and Hardham, 2005). High molecular weight (260 kDa) adhesive proteins (named Vsv) secreted from vesicles on the zoospore ventral surface have

been characterized in *Phytophthora cinnamomi* (Robold and Hardham, 2005) and *P. nicotianae* (National Center for Biotechnology Information (NCBI) accession number JF896463) (Fig. 13.2H and I). The adhesins contain approximately 45 thrombospondin type 1 repeats, a motif found in adhesive extracellular matrix proteins in animals and as part of the secreted adhesives in malarial parasites (Robold and Hardham, 2005; L.M. Blackman and A.R. Hardham, unpublished observations).

Another protein secreted during *P. nicotianae* zoospore encystment is PnCcp, which contains a single complement control protein (CCP) module (Škalamera and Hardham, 2006) (Figs. 13.2J and K). CCPs occur widely in animals and belong to key cell signalling and adhesion protein families. PnCcp is stored in large peripheral vesicles together with a glycoprotein, PnLpv, that is not secreted during encystment. Quantitative real-time RT-PCR analysis indicates the *PnCcp* gene is expressed many hours after the expression of *PnLpv* (V.A. Ludowici, W. Zhang, L.M. Blackman and A.R. Hardham, unpublished observations). In addition, immunocytochemical studies indicate PnCcp is deposited in the large peripheral vesicles after the PnLpv protein and that the proteins may be differentially compartmentalized within the vesicles (W. Zhang, V.A. Ludowici, L.M. Blackman and A.R. Hardham, unpublished observations).

13.7.3 Secretion of CWDEs

Analysis of *P. nicotianae* transcriptomes from early and late plant infection stages revealed expression of genes encoding a wide range of CWDEs secreted to break down the plant cell wall and facilitate entry into and through the host (Le Berre *et al.*, 2008; Kebdani *et al.*, 2010). The CWDEs include cellulases (e.g. glucanases), hemicellulases (e.g. xylanases) and pectinases (e.g. polygalacturonases). Pectinases are often the first CWDEs to be secreted by plant pathogens, including *Phytophthora* species. They digest the pectin matrix in the cell wall and middle lamella, leading to the exposure of other wall polymers, cell separation and tissue maceration. A gene family encoding 12 endo-polygalacturonases (endoPG) has been cloned from *P. nicotianae* (Yan and Liou, 2005; Wu *et al.*, 2008). Four of the genes show higher levels of expression at 12 h and 48 h post-inoculation of tomato leaves than in germinated cysts and may play important roles during plant infection. When the *P. nicotianae* endoPGs were expressed in tobacco using agroinfiltration, six of them led to marked phenotypes including dwarfism, necrosis and abnormal leaf morphologies (Wu *et al.*, 2008). At this stage, the cause of these effects is not clear. They may be due to degradation of pectin in the plant cell walls or they may be associated with the induction of plant defence by pectin fragments released by endoPG activity. Analysis of the draft *P. nicotianae* genome has revealed the presence of over 20 endoPG genes and 15 cellulase genes (D. Cullerne, L.M. Blackman, J. Taylor and A.R. Hardham, unpublished observations).

13.7.4 *P. nicotianae* effectors

Phytophthora species produce elicitors and effectors that trigger pathogen recognition and the activation of plant defence responses. Elicitors are conserved across a wide range of related organisms and typically serve fundamental roles in the pathogen and trigger basal defence. Effector proteins are secreted by the pathogen and enhance pathogenicity, often by suppressing plant resistance mechanisms. After their secretion they may remain extracellular (e.g. CWDEs) or they may move into the plant cell cytoplasm. When recognized, effectors trigger the hypersensitive response (HR) in plants.

Until recently a number of molecules produced by *P. nicotianae* were considered to be elicitors but new information on their function suggests they may be better classified as effectors. These molecules include elicitins. Like most other *Phytophthora* species, *P. nicotianae* secretes

conserved 10 kDa protein elicitors called elicitins. Transcriptome studies identified at least ten different elicitin classes (Panabières *et al.*, 2005; Le Berre *et al.*, 2008) but the main secreted elicitin of *P. nicotianae* is parasiticein (Colas *et al.*, 2001). *Phytophthora* elicitins trigger HR and systemic acquired resistance in tobacco, and some *P. nicotianae* isolates virulent on tobacco downregulate the expression of the parasiticein gene and evade recognition (Colas *et al.*, 2001). Elicitins are now considered to be effectors because they play an important role in pathogenicity. *Phytophthora* species are unable to synthesize sterols, and elicitins bind to plant sterols and act as sterol carrier proteins (Osman *et al.*, 2001; Plešková *et al.*, 2011).

Another *P. nicotianae* molecule that may be better classified as an effector is the 34-kDa cell wall glycoprotein cellulose binding elicitor lectin (CBEL) (Gaulin *et al.*, 2002). CBEL binds to cellulose through two cysteine-rich cellulose binding domains, thereby attaching *Phytophthora* hyphae to plant cell walls. Both cellulose binding domains were essential and sufficient to mediate recognition by tobacco and *Arabidopsis*, and to trigger necrosis and defence responses (Gaulin *et al.*, 2006). Silencing of CBEL expression, through the generation of antisense *P. nicotianae* transformants, impaired adhesion and altered hyphal morphology on cellulosic membranes, although it did not significantly affect pathogenicity on tobacco (Gaulin *et al.*, 2002). Future studies of the role of CBEL in *P. nicotianae* will be aided by recently developed methods to express and purify biologically active CBEL in *Pichia pastoris* (Larroque *et al.*, 2011).

There are two main groups of oomycete effectors translocated into the plant cytoplasm where they alter host metabolism and suppress the host's immune system to facilitate infection. The first group contains an N-terminal RXLR tetra-amino acid motif involved in effector uptake into the plant cell (Whisson *et al.*, 2007). RXLR effectors are recognized by host resistance proteins in a gene-for-gene manner. In *P. nicotianae* 13 candidate RXLR genes were identified in the appressorium transcriptome (Kebdani *et al.*, 2010). Seven were not found in other *Phytophthora* genomes and three preferentially accumulated in the appressoria.

The second group of putative cytoplasmic effectors is the crinkling and necrosis (CRN) effectors (Torto *et al.*, 2003). CRN effectors lack the RXLR motif but have a conserved LXLFLAK motif that may function in the delivery of the CRN effectors into plant cells (Schornack *et al.*, 2010). CRN effector homologues were found in *P. nicotianae* early and late infection transcriptomes (Le Berre *et al.*, 2008; Kebdani *et al.*, 2010), but there is little information about these effectors in *P. nicotianae* and their roles during infection is unknown.

13.8 Concluding Remarks

As with most species in this remarkable genus, there is much to be learnt about the cellular and molecular biology of *P. nicotianae* and its infection of host plants. Recent technical advances and the sequencing and on-going annotation of its genome will greatly facilitate future research into *P. nicotianae* biology and pathogenicity. An important component in these advances is the ability to transform *P. nicotianae*, thus allowing localization of GFP-tagged proteins and RNAi-mediated silencing of genes (Bottin *et al.*, 1999; Le Berre *et al.*, 2008; Kebdani *et al.*, 2010; Narayan *et al.*, 2010). These approaches have allowed the identification of key proteins in pathogen adhesion and zoospore development (Gaulin *et al.*, 2002; Narayan *et al.*, 2010) and will greatly aid the functional analysis of *P. nicotianae* proteins. The improved understanding of *P. nicotianae* biology should, in turn, lead to development of novel control measures for *P. nicotianae* diseases.

References

Blackman, L.M., Arikawa, M., Yamada, S., Suzaki, T. and Hardham, A.R. (2011) Identification of a mastigoneme protein from *Phytophthora nicotianae*. *Protist* 162, 100–114.

Blair, J.E., Coffey, M.D., Park, S.-Y., Geiser, D.M. and Kang, S. (2008) A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. *Fungal Genetics and Biology* 45, 266–277.

Bonants, P.J.M., Hagenaar-de Weerdt, M., Man in 't Veld, W.A. and Baayen, R.P. (2000) Molecular characterization of natural hybrids of *Phytophthora nicotianae* and *P. cactorum*. *Phytopathology* 90, 867–874.

Bottin, A., Larche, L., Villalba, F., Gaulin, E., Esquerré-Tugayé, M.-T. and Rickauer, M. (1999) Green fluorescent protein (GFP) as gene expression reporter and vital marker for studying development and microbe–plant interaction in the tobacco pathogen *Phytophthora parasitica* var. *nicotianae*. *FEMS Microbiology Letters* 176, 51–56.

Cahill, D. (1993) Review of Phytophthora diseases in Australia. *Rural Industries Research and Development Corporation Research Paper Series No 93/94.* Department of Primary Industries and Energy Canberra, Australia.

Colas, V., Conrod, S., Venard, P., Keller, H., Ricci, P. and Panabières, F. (2001) Elicitin genes expressed *in vitro* by certain tobacco isolates of *Phytophthora parasitica* are down regulated during compatible interactions. *Molecular Plant–Microbe Interactions* 14, 326–335.

Cooke, D.E.L., Drenth, A., Duncan, J.M., Wagels, G. and Brasier, C.M. (2000) A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* 30, 17–32.

Csinos, A.S. (2005) Relationship of isolate origin to pathogenicity of race 0 and 1 of *Phytophthora parasitica* var. *nicotianae* on tobacco cultivars. *Plant Disease* 89, 332–337.

Drenth, A. and Sendall, B. (2004) Economic impact of Phytophthora diseases in Southeast Asia. In: Drenth, A. and Guest, D.I. (eds) *Diversity and Management of* Phytophthora *in Southeast Asia.* Australian Centre for International Agricultural Research, Canberra, Australia, pp. 10–28.

Erwin, D.C. and Ribeiro, O.K. (1996) *Phytophthora Diseases Worldwide*. APS Press, St Paul, Minnesota, 562 pp.

Gaulin, E., Jauneau, A., Villalba, F., Rickauer, M., Esquerré-Tugayé, M.T. and Bottin, A. (2002) The CBEL glycoprotein of *Phytophthora parasitica* var. *nicotianae* is involved in cell wall deposition and adhesion to cellulosic substrates. *Journal of Cell Science* 115, 4565–4575.

Gaulin, E., Dramé, N., Lafitte, C., Torto-Alalibo, T., Martinez, Y., Ameline-Torregrosa, C., Khatib, M., Mazarguil, H., Villalba-Mateos, F., Kamoun, S., Mazars, C., Dumas, B., Bottin, A., Esquerré-Tugayé, M.T. and Rickauer, M. (2006) Cellulose binding domains of a *Phytophthora* cell wall protein are novel pathogen-associated molecular patterns. *Plant Cell* 18, 1766–1777.

Gautam, Y., Cahill, D.M. and Hardham, A.R. (1999) Development of a quantitative immunodipstick assay for *Phytophthora nicotianae*. *Food and Agricultural Immunology* 11, 229–242.

Graham, J.H. and Menge, J.A. (2000) *Phytophthora*-induced diseases. In: Timmer, L.W., Garnsey, S.M. and Graham, J.H. (eds) *Compendium of Citrus Diseases*, 2nd edition. APS Press, St Paul, Minnesota, pp. 12–15.

Grote, D., Olmos, A., Kofoet, A., Tuset, J.J., Bertolini, E. and Cambra, M. (2002) Specific and sensitive detection of *Phytophthora nicotianae* by simple and nested-PCR. *European Journal of Plant Pathology* 108, 197–207.

Hall, G. (1993) An integrated approach to the analysis of variation in *Phytophthora nicotianae* and a redescription of the species. *Mycological Research* 97, 559–574.

Hu, J.H., Hong, C.X., Stromberg, E.L. and Moorman, G.W. (2008) Mefenoxam sensitivity and fitness analysis of *Phytophthora nicotianae* isolates from nurseries in Virginia, USA. *Plant Pathology* 57, 728–736.

Hurtado-Gonzales, O.P., Aragon-Caballero, L.M., Flores-Torres, J.G., Man in 't Veld, W. and Lamour, K.H. (2009) Molecular comparison of natural hybrids of *Phytophthora nicotianae* and *P. cactorum* infecting loquat trees in Peru and Taiwan. *Mycologia* 101, 496–502.

Jin, F., Ding, Y., Ding, W., Reddy, M.S., Fernando, W.G.D. and Du, B. (2011) Genetic diversity and phylogeny of antagonistic bacteria against *Phytophthora nicotianae* isolated from tobacco rhizosphere. *International Journal of Molecular Sciences* 12, 3055–3071.

Kebdani, N., Pieuchot, L., Deleury, E., Panabières, F., Le Berre, J.Y. and Gourgues, M. (2010) Cellular and molecular characterization of *Phytophthora parasitica* appressorium-mediated penetration. *New Phytologist* 185, 248–257.

Kong, P., Tyler, B., Richardson, P., Lee, B., Zhou, Z. and Hong, C. (2010) Zoospore interspecific signaling promotes plant infection by *Phytophthora*. *BMC Microbiology* 10, 313–321.

Kroon, L.P.N.M., Bakker, F.T., van den Bosch, G.B.M., Bonants, P.J.M. and Flier, W.G. (2004) Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. *Fungal Genetics and Biology* 41, 766–782.

Lacourt, I. and Duncan, J.M. (1997) Specific detection of *Phytophthora nicotianae* using the polymerase chain reaction and primers based on the DNA sequence of its elictin gene *ParA1*. *European Journal of Plant Pathology* 103, 73–83.

Larroque, M., Ramirez, D., Lafitte, C., Borderies, G., Dumas, B. and Gaulin, E. (2011) Expression and purification of a biologically active *Phytophthora parasitica* cellulose binding elicitor lectin in *Pichia pastoris*. *Protein Expression and Purification* 80, 217–223.

Le Berre, J.-Y., Engler, G. and Panabières, F. (2008) Exploration of the late stages of the tomato–*Phytophthora parasitica* interactions through histological analysis and generation of expressed sequence tags. *New Phytologist* 177, 480–492.

Lioussanne, L., Jolicoeur, M. and St Arnaud, M. (2009) Role of the modification in root exudation induced by arbuscular mycorrhizal colonization on the intraradical growth of *Phytophthora nicotianae* in tomato. *Mycorrhiza* 19, 443–448.

Narayan, R.D., Blackman, L.M., Shan, W. and Hardham, A.R. (2010) *Phytophthora nicotianae* transformants lacking dynein light chain 1 produce non-flagellate zoospores. *Fungal Genetics and Biology* 47, 663–671.

Osman, H., Vauthrin, S., Mikes, V., Milat, M.L., Panabières, F., Marais, A., Brunie, S., Maume, B., Ponchet, M. and Blein, J.P. (2001) Mediation of elicitin activity on tobacco is assumed by elicitin-sterol complexes. *Molecular Biology of the Cell* 12, 2825–2834.

Panabières, F., Amselem, J., Galiana, E. and Le Berre, J.-Y. (2005) Gene identification in the oomycete pathogen *Phytophthora parasitica* during *in vitro* vegetative growth through expressed sequence tags. *Fungal Genetics and Biology* 42, 611–623.

Parkunan, V., Johnson, C.S., Bowman, B.C. and Hong, C.X. (2010) Population structure, mating type, and mefenoxam sensitivity of *Phytophthora nicotianae* in Virginia tobacco fields. *Plant Disease* 94, 1361–1365.

Plešková, V., Kašparovský, T., Obořil, M., Ptáčková, N., Chaloupkov, R., Ladislav, D., Damborský, J. and Lochman, J. (2011) Elicitin–membrane interaction is driven by a positive charge on the protein surface: role of Lys13 residue in lipids loading and resistance induction. *Plant Physiology and Biochemistry* 49, 321–328.

Robold, A.V. and Hardham, A.R. (2005) During attachment *Phytophthora* spores secrete proteins containing thrombospondin type 1 repeats. *Current Genetics* 47, 307–315.

Schornack, S., van Damme, M., Bozkurt, T.O., Cano, L.M., Smoker, M., Thines, M., Gaulin, E., Kamoun, S. and Huitema, E. (2010) Ancient class of translocated oomycete effectors targets the host nucleus. *Proceedings of the National Academy of Sciences USA* 107, 17421–17426.

Shan, W. and Hardham, A.R. (2004) Construction of a bacterial artificial chromosome library, determination of genome size, and characterization of an *Hsp70* gene family in *Phytophthora nicotianae*. *Fungal Genetics and Biology* 41, 369–380.

Škalamera, D. and Hardham, A.R. (2006) PnCcp, a *Phytophthora nicotianae* protein containing a single complement control protein module, is sorted into large peripheral vesicles in zoospores. *Australasian Plant Pathology* 35, 593–603.

Sukhada, M., Manjula, R. and Rawal, R.D. (2011) Evaluation of arbuscular mycorrhiza and other biocontrol agents against *Phytophthora parasitica* var. *nicotianae* infecting papaya (*Carica papaya* cv. Surya) and enumeration of pathogen population using immunotechniques. *Biological Control* 58, 22–29.

Timmer, L.W., Graham, J.H. and Zitko, S.E. (1998) Metalaxyl-resistant isolates of *Phytophthora nicotianae*: occurrence, sensitivity, and competitive parasitic ability on citrus. *Plant Disease* 82, 254–261.

Torto, T.A., Li, S., Styer, A., Huitema, E., Testa, A., Gow, N.A.R., van West, P. and Kamoun, S. (2003) EST mining and functional expression assays identify extracellular effector proteins from the plant pathogen *Phytophthora*. *Genome Research* 13, 1675–1685.

van West, P., Morris, B.M., Reid, B., Appiah, A.A., Osborne, M.C., Campbell, T.A., Shepherd, S.J. and Gow, N.A.R. (2002) Oomycete plant pathogens use electric fields to target roots. *Molecular Plant–Microbe Interactions* 15, 790–798.

Wang, Y., Meng, Y., Zhang, M., Tong, X., Wang, Q., Sun, Y., Quan, J., Govers, F. and Shan, W. (2011) Infection of *Arabidopsis thaliana* by *Phytophthora parasitica* and identification of variation in host specificity. *Molecular Plant Pathology* 12, 187–201.

Waterhouse, G.M. (1963) Key to the genus *Phytophthora* de Bary. *Mycological Papers* 92, 1–22.

Whisson, S.C., Boevink, P., Moleleki, L., Avrova, A.O., Morales, J.G., Gilroy, A.M., Armstrong, M.R., Grouffaud, S., van West, P., Chapman, S., Hein, I., Toth, I.K., Pritchard, L. and Birch, P.R.J. (2007) A translocation signal for delivery of oomycete effector proteins into host plant cells. *Nature* 450, 115–119.

Wu, C.-H., Yan, H.-Z., Liu, L.-F. and Liou, R.-F. (2008) Functional characterization of a gene family encoding polygalacturonases in *Phytophthora parasitica*. *Molecular Plant–Microbe Interactions* 21, 480–489.

Yan, H.Z. and Liou, R.F. (2005) Cloning and analysis of *pppg1*, an inducible endopolygalacturonase gene from the oomycete plant pathogen *Phytophthora parasitica*. *Fungal Genetics and Biology* 42, 339–350.

14 *Phytophthora cinnamomi* in Australia

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14.1 Introduction

Phytophthora cinnamomi is one of the world's most devastating pathogens. Its effects on the Australian environment have resulted in its inclusion in a list of key threatening processes under the Environment Protection and Biodiversity Conservation Act 1999 (Cahill *et al*., 2008), and it is the only oomycete that is included in the top 100 of the world's worst invasive alien species as identified by the Invasive Species Specialist Group (ISSG, 2007). The identification of *P. cinnamomi* as a key threatening process in Australia has resulted in the preparation of a Threat Abatement Plan to provide a national strategy to manage the impact of this pathogen on the biodiversity of Australian flora and fauna (Australian Government Department of the Environment and Heritage, 2001). Recent technological advances, including the current sequencing of the *P. cinnamomi* genome (DOE Joint Genome Institute, 2012) and the ability to silence selected genes (Horta *et al*., 2008, 2010), promise to greatly facilitate research that will increase our knowledge of *P. cinnamomi* biology, leading to a better understanding of *P. cinnamomi* pathogenicity and, ultimately, to improved management strategies.

14.2 *P. cinnamomi* Epidemiology and Host Range

P. cinnamomi is one of the most destructive root pathogens and infects a wide range of trees and woody ornamentals in tropical, subtropical and temperate regions of the world (Cahill *et al*., 2008). *P. cinnamomi* causes: (i) mortality and decline of *Quercus suber* (cork oak) and *Quercus ilex* in the Mediterranean (Serrano *et al*., 2010); (ii) white oak decline in southern Ohio (Nagle *et al*., 2010); (iii) root/crown rot and death of Brazilian pine (*Araucaria angustifolia*) in Brazil (Dos Santos *et al*., 2011); and (iv) root rot/trunk cankers and decline of macadamia (*Macadamia integrifolia* and *Macadamia tetraphylla*) in Kenya (Mbaka *et al*., 2009). In South-east Asia, the possible origin of *P. cinnamomi* (Cahill *et al*., 2008), it causes pineapple heart rot in Vietnam, and stem cankers, root rot and dieback in quinine (*Cinchona ledgeriana* and *Cinchona succirubra*) and cinnamon trees (*Cinznamomum verum*) in Indonesia and Malaysia (Cahill *et al*., 2008). *P. cinnamomi* causes avocado root rot in the Philippines (Avenido *et al*., 2009) and limits avocado production in most avocado-producing countries.

These examples illustrate the extent of *P. cinnamomi* diseases in a global context,

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but in terms of the number of species affected and the degree of damage to native vegetation communities, none exceed those found in Australian ecosystems (Cahill *et al.*, 2008). *P. cinnamomi* infects more than 2500 Australian native plant species (Fig. 14.1A and B), as well as important agricultural and horticultural crops and forest species. In Australian agriculture, economic losses were estimated nearly 20 years ago to be over AUS$200 million annually (Irwin *et al.*, 1995). In eucalyptus forests and heathland communities, both within and outside national parks, *P. cinnamomi* causes extensive plant mortality, forest decline and environmental damage (Hardy *et al.*, 2001; Hardham, 2005). It threatens ecosystem stability and floristic biodiversity (Hill *et al.*, 2011). An extinction-risk assessment determined that of 33 taxa of plants in the Stirling Range National Park in Western Australia, 20 are at moderate or greater risk of extinction (Cahill *et al.*, 2008). Reliable detection and control measures are urgently needed (Fig. 14.1C–E).

14.3 Life Cycle and Disease Dissemination

Like other *Phytophthora* species, *P. cinnamomi* has both sexual and asexual phases in its life cycle. The presence of different sexual mating types can result in increased pathogenicity (Birch and Whisson, 2001), but despite the presence of both the A1 and the A2 mating types, there is no evidence to suggest that sexual recombination occurs in Australia (Dobrowolski *et al.*, 2003). Instead, the key feature responsible for successful propagation and dissemination of *P. cinnamomi* is the production of large numbers of asexual, flagellated spores called zoospores (Fig. 14.2). *Phytophthora* zoospores are able to swim over considerable distances to reach potential host plants and are classified as heterokonts because they have two morphologically different flagella (Fig. 14.2D). The anterior (tinsel) flagellum possesses two opposite rows of tripartite, tubular hairs called mastigonemes. Mastigonemes consist of a tapering basal segment, a tubular shaft and two or three terminal filaments. Forward motility is achieved by movement of the mastigonemes as waves propagate along the flagellum (Hardham, 2005). Zoospores develop within multinucleate sporangia that form at hyphal tips after the induction of asexual sporulation (Fig. 14.2B). Sporangia may germinate directly by forming germ tubes or they subdivide into about 30 uni-nucleate zoospores that are released through an apical pore in the sporangium (Fig. 14.2C).

The ability of zoospores to respond to chemical and electrical gradients emanating from potential hosts greatly improves their chance of reaching a suitable infection site (Walker and van West, 2007) (Fig. 14.2A). At the plant surface the zoospore encysts and secretes a number of proteins including substances that attach the cyst to the host (Gaulin *et al.*, 2002; Robold and Hardham, 2005). *PcVsv1*, a gene encoding a 260-kDa component of this adhesive material, has been sequenced from *P. cinnamomi*. The PcVsv1 protein contains 47 copies of the thrombospondin type 1 repeat, a motif that is associated with adhesins of animals and malarial pathogens (Robold and Hardham, 2005). The cysts germinate, often forming appressorium-like swellings to penetrate the plant surface (Fig. 14.2E). *P. cinnamomi* cysts and hyphae synthesize and secrete a range of pathogenicity factors including effector proteins that facilitate plant infection (Hardham and Cahill, 2010).

14.4 Effectors and Elicitors

During the colonization of host plants by *Phytophthora* species, a number of effectors are secreted to facilitate penetration and nutrient absorption and to inhibit defence systems (Hardham and Cahill, 2010). Effectors can be divided into two groups: apoplastic and cytoplasmic effectors. Apoplastic effectors include cell wall degrading enzymes (CWDEs) that function in the penetration of the plant cell wall. For example, polygalacturonases (PGs) and pectin methyl esterases (PMEs) digest

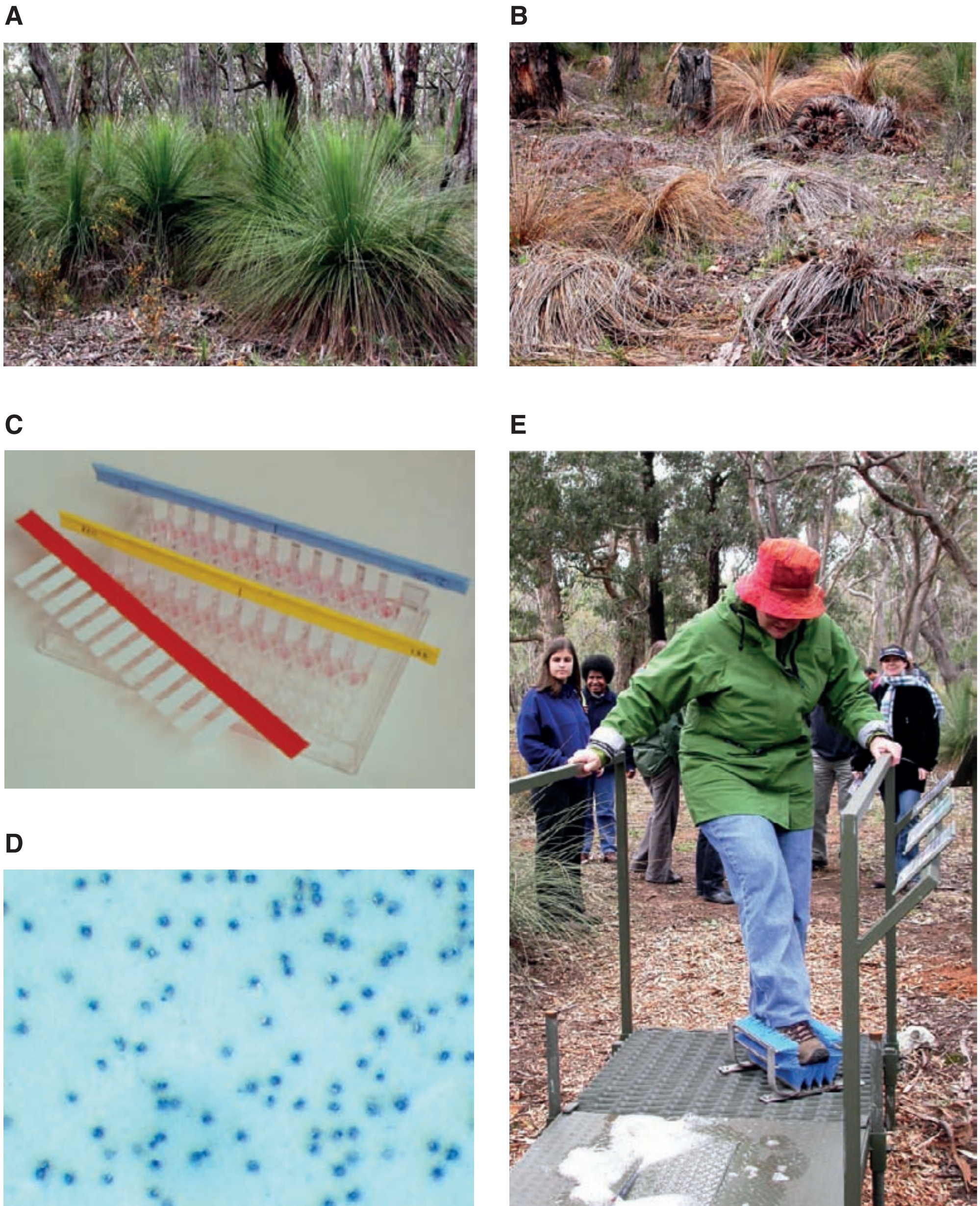

Fig. 14.1. *Phytophthora cinnamomi* occurrence, detection and control in the Brisbane Ranges National Park, Victoria. **(A)** Healthy eucalypt forest with an understorey of grass trees, *Xanthorrhoea australis*. **(B)** Area of forest infected by *P. cinnamomi*. Grass trees are especially susceptible. **(C)** Dipstick array for use in a 96-well ELISA format. **(D)** Dipstick membrane with numerous adherent *P. cinnamomi* cysts stained blue in a specific immunoreaction. **(E)** Cleaning and disinfecting footwear to remove all adhering soil or plant debris before moving between infected and non-infected areas within the Brisbane Ranges National Park.

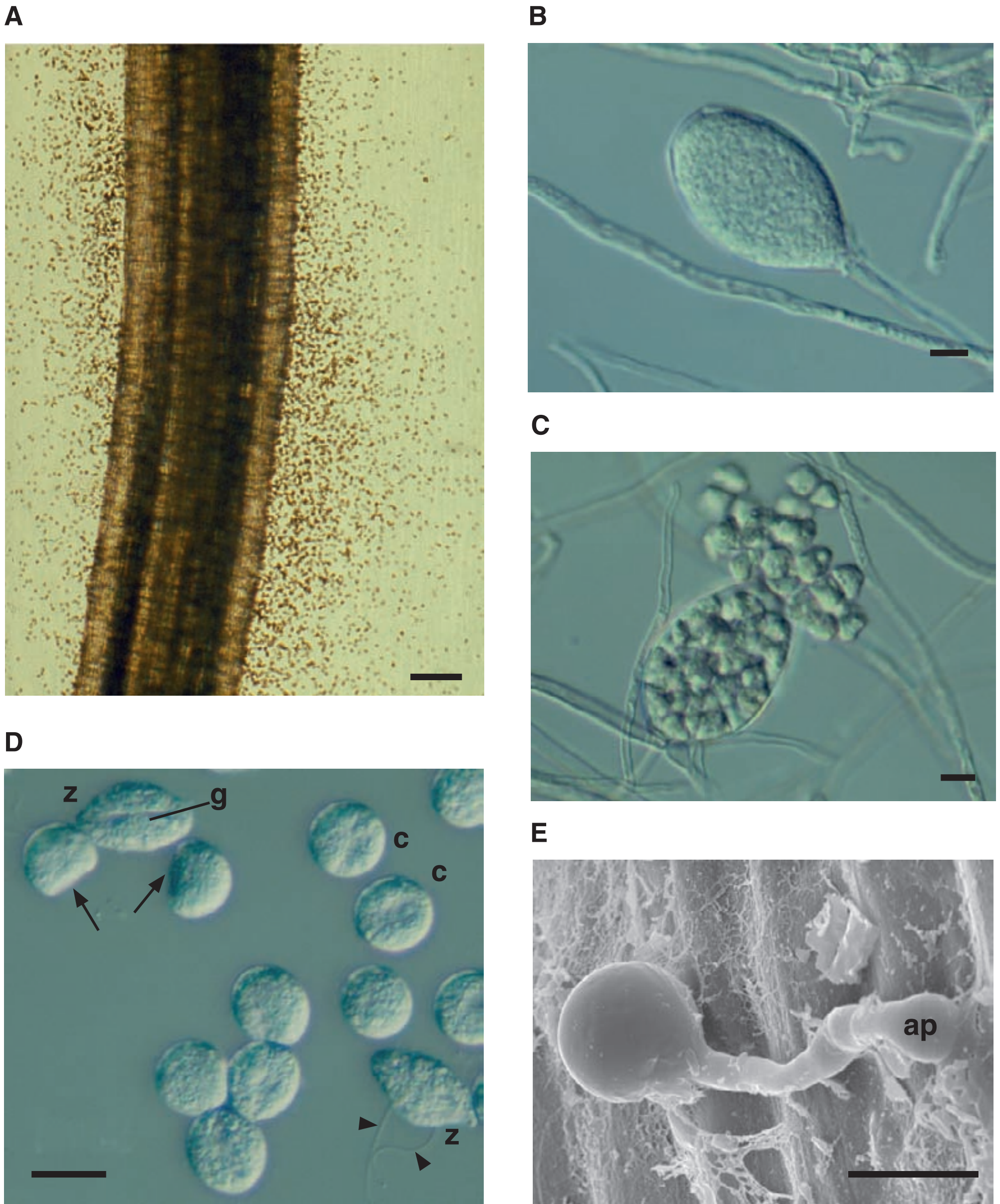

Fig. 14.2. *P. cinnamomi* zoospores, cysts and germinated cysts. **(A)** Elongation zone of an onion root surrounded by encysted spores. **(B)** Hyphae and uncleaved sporangium. **(C)** Sporangium that has cleaved and is in the process of releasing zoospores. **(D)** Zoospores (z) and cysts (c). Two flagella (arrowheads) emerge from a groove (g) along the zoospore ventral surface. The ventral surface remains flat for 1–2 min after encystment (arrows). **(E)** Cryoscanning electron micrograph of germinated cyst on the surface of a lucerne root. An appressorium-like swelling (ap) has formed where the germ tube has penetrated along the anticlinal wall between two epidermal cells. Bars, **(A)** = 200 µm; **(B–E)** = 10 µm.

pectin, thus weakening the network of complex polysaccharides within the wall and facilitating the subsequent action of pathogen-derived cellullases and hemicellulases. The large size of CWDE gene families in *Phytophthora* was first shown in *P. cinnamomi* with the identification of over 17 putatively-active PGs (Götesson *et al.*, 2002). It is now known to include glucanases in *Phytophthora infestans* (McLeod *et al.*, 2003) and PMEs in *Phytophthora nicotianae* (P.S. Torreña, L.M. Blackman and A.R. Hardham, unpublished results). The size of CWDE gene families is thought to reflect the need to digest the wide range of complex polysaccharides within the plant cell wall (D'Ovidio *et al.*, 2004).

Cytoplasmic effectors fall into two categories. Members of one group contain an N-terminal RXLR motif that is responsible for protein uptake into the host cytoplasm; members of the second group contain the amino acid sequence LXLFLAK and cause leaf crinkling and necrosis (Hardham and Cahill, 2010). The sequenced *Phytophthora* genomes contain abundant, highly divergent RXLR effectors that are undergoing rapid evolution (Win *et al.*, 2007). While none of about 1000 *P. cinnamomi* accessions in the National Center for Biotechnology Information (NCBI) contain both a secretion signal (for secretion from the pathogen) and an RXLR motif, a preliminary analysis of short reads in NCBI arising from the *P. cinnamomi* genome sequencing project indicates the genome also contains a large number of RXLR effectors (P.S. Torreña, L.M. Blackman and A.R. Hardham, unpublished results).

Oligosaccharide fragments released by *Phytophthora* CWDEs are only one of many molecules that trigger basal defence responses during plant–*Phytophthora* interactions. These responses are also induced by a class of secreted, 10-kDa *Phytophthora* molecules called elicitins (Kamoun *et al.*, 1998; Vleeshouwers *et al.*, 2006). Elicitins synthesized by *P. cinnamomi* are called cinnamomins and are categorized into two types, α- and β-cinnamomin, according to their pI and necrosis-inducing capacity (Nespoulous *et al.*, 1992). The four members of the cinnamomin gene family are clustered within the *P. cinnamomi* genome (Jiang *et al.*, 2006). β-Cinnamomin is found to be involved in a late stage of infection in cork oak and contributes to the aggressiveness of the pathogen, whereas α-cinnamomin may be involved in the early stage of infection (Horta *et al.*, 2008, 2010). Elicitins induce hypersensitive cell death in solanaceous plants, a process typically seen in response to effectors. This has led elicitins to be considered by some to be effectors (see Hardham and Cahill, 2010). Consistent with this idea, β-cinnamomin forms a stable complex with sterol molecules and acts as a sterol carrier during plant infection (Rodrigues *et al.*, 2006). This function is important because *Phytophthora* species cannot synthesize sterols and must obtain them from the host (Mikes *et al.*, 1998). Recent research by Horta and co-authors (2008, 2010) has shown that a *P. cinnamomi* transgenic line with a downregulated β-cinnamomin gene has a decreased ability to infect cork oak roots indicating that this elicitin has a fundamental role in disease pathogenicity. The successful transformation of *P. cinnamomi* has opened new avenues for fundamental studies of the role of proteins in *P. cinnamomi* biology and pathogenicity.

14.5 Detection and Diagnostics of *P. cinnamomi*

The identification and diagnosis of pathogens is crucial for disease management in agriculture, horticulture and forestry, and for maintaining biodiversity in natural ecosystems. Methods to isolate *Phytophthora* include pathogen baiting with susceptible plant tissues, and pathogen isolation from infected plant tissues and soil water using selective media. Conventional methods for identification of species are largely based on direct microscopic examination. The efficacy of these methods to identify *Phytophthora* species is poor due to a number of factors (Hüberli *et al.*, 2000) including: (i) different isolates

within a single species can vary greatly in their ability to infect a selected bait material; (ii) pathogen propagules may remain dormant or grow very slowly depending on prevalent conditions (Collins *et al.*, 2012); and (iii) the presence of other micro-organisms may inhibit *Phytophthora* growth during pathogen isolation. In general, these conventional methods are labour intensive, time consuming and require taxonomic expertise for reliable identification.

Some of the limitations of conventional approaches can be avoided by using serological (immunological) or DNA-based diagnostic techniques. Serological detection uses polyclonal or monoclonal antibodies that react specifically with molecules in the target organism. If the antibodies are specific a positive immunoreaction can yield rapid and unambiguous diagnosis. Three immunoassay formats have been developed to detect *P. cinnamomi* (Gabor *et al.*, 1993; Cahill and Hardham, 1994). Immunofluorescence and ELISAs require specialized laboratory equipment, but a dipstick assay format (Fig. 14.1C and D) allows the diagnostic test to be conducted in the field. The dipstick assay exploits the chemotactic ability of *P. cinnamomi* zoospores and is rapid and sensitive (Cahill and Hardham, 1994).

DNA-based tests are now a popular diagnostic approach. They have the advantage over antibody-based methods in that targeted genomic DNA is always present while proteins or other molecules targeted by antibodies may occur only under certain conditions or at selected stages of the life cycle. Methods based on PCR have now been developed for the identification of a number of *Phytophthora* species, including *P. cinnamomi* (O'Brien *et al.*, 2009; Langrell *et al.*, 2011). PCR using primers that amplify a *Phytophthora*-specific target can be sensitive and allow high throughput sample processing. Nested PCR, which utilizes two different pairs of primers in two rounds of PCR, is more sensitive for detecting *P. cinnamomi* compared with conventional PCR (Langrell *et al.*, 2011). It can detect as little as 1 pg of DNA. The efficacy of PCR-based methods depends on the specificity of the primers and the abundance of the target DNA sequence. The most commonly targeted sequences are within the ribosomal RNA internal transcribed spacer (ITS), and the high copy number of ITS sequences in genomes contributes to the sensitivity of detection (O'Brien *et al.*, 2009). Both nested PCR and real-time quantitative PCR (qPCR) can be used as multiplex approaches for diagnosis of multiple pathogens in a time- and cost-effective way (Cooke *et al.*, 2007; Langrell *et al.*, 2011). qPCR has also proved to be effective to quantify infection by comparing pathogen genomic DNA (gDNA) to that of the host (Eshraghi *et al.*, 2011). The success of these molecular-based techniques is reliant upon DNA quality, and quantification of necrotrophs such as *P. cinnamomi* can be difficult. However, recent developments, such as the inclusion of plasmid DNA to standardize samples, may aid accurate quantification (Eshraghi *et al.*, 2011).

14.6 Control of *P. cinnamomi*

Although *Phytophthora* and other oomycetes have a similar morphology and life style to filamentous fungal pathogens, they are phylogenetically unrelated and the majority of fungicides useful for fungal diseases are not effective. As a consequence, the development of new control strategies is an important aspect of research on *P. cinnamomi* and other *Phytophthora* pathogens.

The need to integrate a range of procedures for effective and sustainable disease or pest management is widely recognized. In the case of *P. cinnamomi*, a number of management strategies are used in Australia to control its spread in forests, mining sites and national parks (Hardy *et al.*, 2001). Strategies that aim to restrict the spread of disease include: (i) input from trained experts to demarcate infested areas; (ii) restrictions on the movement of vehicles, contaminated plants and soil between diseased and healthy areas (Fig. 14.1E); (iii) prevention of movement of infested water

into disease-free areas; and (iv) increased public awareness of the risks associated with *P. cinnamomi* dispersal. Increased disease resistance through breeding or grafting is also being developed for important crop or horticultural plants. For example, the development of tolerant rootstocks in avocado has proved to be an effective means to control *P. cinnamomi* root rot (Douhan *et al.*, 2004). In addition to these approaches, biological and chemical control measures are applied in infected areas.

14.6.1 Biological control

A number of potentially useful biological control agents have been tested against *P. cinnamomi*. For example, an isolate of the fungal plant pathogen *Myrothecium roridum* is an active antagonist that protected highly susceptible seedlings of *Persea indica* inoculated with *P. cinnamomi* (Gees and Coffey, 1989). In addition, *Pythium nunn* (Fang and Tsao, 1995), a mycoparasitic oomycete, *Penicillium funiculosum* and a number of other soil-borne microorganisms, including *Trichoderma harzianum*, *Epiccocum purpurascens* and *Streptomyces griseoalbus*, inhibit *P. cinnamomi* growth through competition, antibiosis or parasitism (Costa *et al.*, 2000). The growth of *P. cinnamomi* is also inhibited by soil mulching and manure-based compost.

In mulched soils inhibition is due, at least in part, to the activity of cellulytic enzymes secreted by litter-decaying fungi. The inhibitory effect of manure compost is due to a range of bacteria and fungi resident within the manure (Aryantha and Guest, 2006). Suppression of *P. cinnamomi* growth also occurs in proximity to several *Acacia* species (D'Souza *et al.*, 2005). Jiménez *et al.* (2011) also found that ethanol-soluble extracts from the red alga, *Gracillaria chilensis*, contain active anti-fungal compounds that inhibit the growth of *P. cinnamomi* mycelia.

14.6.2 Chemical control

Several chemicals have been tested *in planta* and *in vitro* for their efficacy against *P. cinnamomi*. Avocado trees treated with metalaxyl (Ridomil) recovered well from infection while trees treated with Fosetyl-Al and Ethazole showed little improvement (Darvas *et al.*, 1978). Application of methyl bromide (Meth-O-Gas), dazomet (Basamid) or metalaxyl significantly reduced root rot of grapevines but the pathogen became re-established in the soil (Marais and Hattingh, 1986). Metalaxyl inhibits chlamydospore and sporangium production but it does not affect cyst germination (Coffey *et al.*, 1984). Mefenoxam inhibited *P. cinnamomi* populations from ornamental nurseries (Hu *et al.*, 2010). Borbonol, an antifungal substance isolated from roots and stems of *Persea borbonia*, inhibits *P. cinnamomi* vegetative growth and sporangium production (Zaki *et al.*, 1980). Ca^{2+} fertilizers inhibit the production of sporangia, chlamydospores and zoospores but not mycelial growth (Serrano *et al.*, 2012). The chemical phosphite has proved to be particularly effective in controlling *P. cinnamomi* in agriculture and natural ecosystems (King *et al.*, 2010).

14.6.3 Use of phosphite to control *P. cinnamomi* diseases

Phosphite ($H_2PO_3^-$) is the anionic form of phosphorous acid (H_3PO_3), an analogue or reduced form of phosphate (King *et al.*, 2010). It is used extensively in Australia and increasingly in the USA and Europe to control *P. cinnamomi* diseases. The sensitivity of *Phytophthora* species and isolates to phosphite is highly variable, possibly because of variation in the amount of phosphite taken up by the mycelium (Darakis *et al.*, 1997). Phosphite suppresses *P. cinnamomi* mycelial growth, causes hyphal swelling, hyphal branch deformation, and lyses chlamydospores, sporangia and zoospores (King *et al.*, 2010; P.S.

Torreña, L.M. Blackman and A.R. Hardham, unpublished results). Transcriptome studies have begun to uncover the changes in gene expression induced by phosphite treatment, highlighting effects on genes involved in cell wall synthesis and cytoskeleton function (King *et al.*, 2010). In Western Australia phosphite has proved to be effective when directly injected into the trunks of infected trees or when applied as a foliar or aerial spray (Barrett *et al.*, 2004). Phosphite controls the *P. cinnamomi* diseases, chestnut ink disease (Gentile *et al.*, 2009) and root and heart rot of pineapple (Anderson *et al.*, 2012).

Although phosphite has been an effective control for *P. cinnamomi* diseases for an extended period of time, problems do arise from phytotoxicity in some plant species. When applied to native plants in Western Australia, phosphite produces phytotoxicity or death in many species (Barrett *et al.*, 2004). Phytotoxicity symptoms include: (i) foliar necrosis; (ii) defoliation; (iii) reduced root growth; (iv) reproductive abnormalities; and (v) chlorosis. Phytotoxicity interferes with the defence response in *Eucalyptus marginata* (Pilbeam *et al.*, 2011). Thus, while phosphite is an important component of management strategies for *P. cinnamomi*, there is an urgent need to expand our understanding of the mode of action of phosphite in both *P. cinnamomi* and in plants (Hardy *et al.*, 2001).

14.7 Concluding Remarks

The scale of the devastation of native ecosystems in southern Australia by *P. cinnamomi* is hard to comprehend until viewed first hand. Why are so many Australian native plants susceptible to this pathogen? What does *P. cinnamomi* do to be able to avoid or overcome the defences of so many plant species? These are but two of many important questions that may be fruitfully addressed in the future using a combination of modern genomic, transcriptomic and advanced microscopy approaches. There is a need to identify genes that are significantly up- or down-regulated during plant infection, and to use functional genomics and cell biology to determine the function of the proteins encoded by these genes. The greatly increased understanding of the molecular and cellular basis of *P. cinnamomi* pathogenicity arising from these studies should facilitate the development of effective and sustainable methods to control this species.

References

Anderson, J., Pegg, K., Scott, C. and Drenth, A. (2012) Phosphonate applied as a pre-plant dip controls *Phytophthora cinnamomi* root and heart rot in susceptible pineapple hybrids. *Australasian Plant Pathology* 41, 59–68.

Aryantha, I.N.P. and Guest, D.I. (2006) Mycoparasitic and antagonistic inhibition on *Phytophthora cinnamomi* Rands by microbial agents isolated from manure composts. *Plant Pathology Journal* 5, 291–298.

Australian Government Department of the Environment and Heritage (2001) Threat abatement plan for dieback caused by the root rot fungus *Phytophthora cinnamomi*. Available at: http://www.environment.gov.au/biodiversity/threatened/publications/tap/phytophthora.html (accessed 25 January 2012).

Avenido, R., Galvez, H., Dimaculangan, J., Welgas, J., Frankie, R. and Damasco, O. (2009) Somatic embryogenesis and embryo culture coupled with gamma irradiation for generating avocado (*Persea americana* Miller) mutants in the Philippines. In: *Induced Mutation in Tropical Fruit Trees*. International Atomic Energy Agency, Vienna, Austria, pp. 47–69.

Barrett, S., Shearer, B. and Hardy, G.E.S.J. (2004) Phytotoxicity in relation to *in planta* concentration of the fungicide phosphite in nine Western Australian native species. *Australasian Plant Pathology* 33, 521–528.

Birch, P.R.J. and Whisson, S.C. (2001) *Phytophthora infestans* enters the genomics era. *Molecular Plant Pathology* 2, 257–263.
Cahill, D.M. and Hardham, A.R. (1994) A dipstick immunoassay for the specific detection of *Phytophthora cinnamomi* in soils. *Phytopathology* 84, 1284–1292.
Cahill, D.M., Rookes, J.E., Wilson, B.A., Gibson, L. and McDougall, K.L. (2008) *Phytophthora cinnamomi* and Australia's biodiversity: impacts, predictions and progress towards control. *Australian Journal of Botany* 56, 279–310.
Coffey, M., Klure, L. and Bower, L. (1984) Variability in sensitivity to metalaxyl of isolates of *Phytophthora cinnamomi* and *Phytophthora citricola*. *Phytopathology* 74, 417–422.
Collins, S., McComb, J.A., Howard, K., Shearer, B.L., Colquhoun, I.J. and Hardy, G.E.S.J. (2012) The long-term survival of *Phytophthora cinnamomi* in mature *Banksia grandis* killed by the pathogen. *Forest Pathology* 42, 28–36.
Cooke, D., Schena, L. and Cacciola, S. (2007) Tools to detect, identify and monitor *Phytophthora* species in natural ecosystems. *Journal of Plant Pathology* 89, 13–28.
Costa, J.L.S., Menge, J.A. and Casale, W.L. (2000) Biological control of Phytophthora root rot of avocado with microorganisms grown in organic mulches. *Brazilian Journal of Microbiology* 31, 239–246.
D'Ovidio, R., Mattei, B., Roberti, S. and Bellincampi, D. (2004) Polygalacturonases, polygalacturonase-inhibiting proteins and pectic oligomers in plant–pathogen interactions. *Biochimica et Biophysica Acta* 1696, 237–244.
D'Souza, N., Colquhoun, I., Sheared, B. and Hardy, G.E. (2005) Assessing the potential for biological control of *Phytophthora cinnamomi* by fifteen native Western Australian jarrah-forest legume species. *Australasian Plant Pathology* 34, 533–540.
Darakis, G., Bourbos, V. and Skoudridakis, M. (1997) Phosphonate transport in *Phytophthora capsici*. *Plant Pathology* 46, 762–772.
Darvas, J., Toerien, J. and Kotze, J. (1978) Chemical control of *Phytophthora* root rot on fully grown avocado trees. *South African Avocado Growers' Association Yearbook* 2, 23–24.
Department of Energy (DOE) Joint Genome Institute (2012) Genome Portal. Available at: http://genome.jgi.doe.gov/ (accessed 22 February 2012).
Dobrowolski, M., Tommerup, I., Shearer, B. and O'Brien, P. (2003) Three clonal lineages of *Phytophthora cinnamomi* in Australia revealed by microsatellites. *Phytopathology* 93, 695–704.
Dos Santos, Á.F., Tessmann, D.J., Alves, T.C.A., Vida, J.B. and Harakava, R. (2011) Root and crown rot of Brazilian pine (*Araucaria angustifolia*) caused by *Phytophthora cinnamomi*. *Journal of Phytopathology* 159, 194–196.
Douhan, G., Fuller, E., McKee, B. and Pond, E. (2004) Genetic diversity analysis of avocado (*Persea americana* Miller) rootstocks selected under greenhouse conditions for tolerance to Phytophthora root rot caused by *Phytophthora cinnamomi*. *Euphytica* 182, 209–217.
Eshraghi, L., Aryamanesh, N., Anderson, J.P., Shearer, B., McComb, J.A., Hardy, G.E.S.J. and O'Brien, P.A. (2011) A quantitative PCR assay for accurate *in planta* quantification of the necrotrophic pathogen *Phytophthora cinnamomi*. *European Journal of Plant Pathology* 131, 419–430.
Fang, J. and Tsao, P. (1995) Evaluation of *Pythium nunn* as a potential biocontrol agent against Phytophthora root rots of azalea and sweet orange. *Phytopathology* 85, 29–36.
Gabor, B., O'Gara, E., Philip, B., Horan, D. and Hardham, A. (1993) Specificities of monoclonal antibodies to *Phytophthora cinnamomi* in two rapid diagnostic assays. *Plant Disease* 77, 1189–1197.
Gaulin, E., Jauneau, A., Villalba, F., Rickauer, M., Esquerré-Tugayé, M.T. and Bottin, A. (2002) The CBEL glycoprotein of *Phytophthora parasitica* var. *nicotianae* is involved in cell wall deposition and adhesion to cellulosic substrates. *Journal of Cell Science* 115, 4565–4575.
Gees, R. and Coffey, M. (1989) Evaluation of a strain of *Myrothecium roridum* as a potential biocontrol agent against *Phytophthora cinnamomi*. *Phytopathology* 79, 1079–1084.
Gentile, S., Valentino, D. and Tamietti, G. (2009) Control of ink disease by trunk injection of potassium phosphite. *Journal of Plant Pathology* 91, 565–571.
Götesson, A., Marshall, J.S., Jones, D.A. and Hardham, A.R. (2002) Characterization and evolutionary analysis of a large polygalacturonase gene family in the oomycete plant pathogen *Phytophthora cinnamomi*. *Molecular Plant–Microbe Interactions* 15, 907–921.
Hardham, A.R. (2005) *Phytophthora cinnamomi*. *Molecular Plant Pathology* 6, 589–604.
Hardham, A.R. and Cahill, D.M. (2010) The role of oomycete effectors in plant–pathogen interactions. *Functional Plant Biology* 37, 919–925.
Hardy, G.E.S.J., Barrett, S. and Shearer, B.L. (2001) The future of phosphite as a fungicide to control the

soilborne plant pathogen *Phytophthora cinnamomi* in natural ecosystems. *Australasian Plant Pathology* 30, 133–139.

Hill, R.J., Wilson, B.A., Rookes, J.E. and Cahill, D.M. (2011) Use of high resolution digital multi-spectral imagery to assess the distribution of disease caused by *Phytophthora cinnamomi* on heathland at Anglesea, Victoria. *Australasian Plant Pathology* 38, 120–127.

Horta, M., Sousa, N., Coelho, A.C., Neves, D. and Cravador, A. (2008) *In vitro* and *in vivo* quantification of elicitin expression in *Phytophthora cinnamomi*. *Physiological and Molecular Plant Pathology* 73, 48–57.

Horta, M., Caetano, P., Medeira, C., Maia, I. and Cravador, A. (2010) Involvement of the β-cinnamomin elicitin in infection and colonisation of cork oak roots by *Phytophthora cinnamomi*. *European Journal of Plant Pathology* 127, 427–436.

Hu, J., Hong, C., Stromberg, E.L. and Moorman, G.W. (2010) Mefenoxam sensitivity in *Phytophthora cinnamomi* isolates. *Plant Disease* 94, 39–44.

Hüberli, D., Tommerup, I. and Hardy, G.E.S.J. (2000) False-negative isolations or absence of lesions may cause mis-diagnosis of diseased plants infected with *Phytophthora cinnamomi*. *Australasian Plant Pathology* 29, 164–169.

Invasive Species Specialist Group (ISSG) (2007) Global Invasive Species Database. Available at: http://www.issg.org/database/species/ecology.asp?si=143&fr=1&sts=&lang=EN (accessed 22 January 2012).

Irwin, J.A.G., Cahill, D.M. and Drenth, A. (1995) *Phytophthora* in Australia. *Australian Journal of Agricultural Research* 46, 1311–1338.

Jiang, R.H.Y., Tyler, B.M., Whisson, S.C., Hardham, A.R. and Govers, F. (2006) Ancient origin of elicitin gene clusters in *Phytophthora* genomes. *Molecular Biology and Evolution* 23, 338–351.

Jiménez, E., Dorta, F., Medina, C., Ramírez, A., Ramírez, I. and Peña-Cortés, H. (2011) Anti-phytopathogenic activities of macro-algae extracts. *Marine Drugs* 9, 739–756.

Kamoun, S., van West, P., Vleeshouwers, V.G.A.A., de Groot, K.E. and Govers, F. (1998) Resistance of *Nicotiana benthamiana* to *Phytophthora infestans* is mediated by the recognition of the elicitor protein INF1. *Plant Cell* 10, 1413–1426.

King, M., Reeve, W., Van der Hoek, M.B., Williams, N., McComb, J., O'Brien, P.A. and Hardy, G.E.S.J. (2010) Defining the phosphite-regulated transcriptome of the plant pathogen *Phytophthora cinnamomi*. *Molecular Genetics and Genomics* 284, 425–435.

Langrell, S., Morel, O. and Robin, C. (2011) Touchdown nested multiplex PCR detection of *Phytophthora cinnamomi* and *P. cambivora* from French and English chestnut grove soils. *Fungal Biology* 115, 672–682.

McLeod, A., Smart, C.D. and Fry, W.E. (2003) Characterization of 1,3-β-glucanase and 1,3;1,4-β-glucanase genes from *Phytophthora infestans*. *Fungal Genetics and Biology* 38, 250–263.

Marais, P. and Hattingh, M. (1986) Reduction of root rot caused by *Phytophthora cinnamomi* in grapevines by chemical treatments. *Plant Disease* 70, 109–111.

Mbaka, J., Wamocho, L., Turoop, L. and Waiganjo, M. (2009) The incidence and distribution of *Phytophthora cinnamomi* Rands on macadamia in Kenya. *Journal of Animal and Plant Sciences* 4, 289–297.

Mikes, V., Milat, M.L., Ponchet, M., Panabières, F., Ricci, P. and Blein, J.P. (1998) Elicitins, proteinaceous elicitors of plant defense, are a new class of sterol carrier proteins. *Biochemical and Biophysical Research Communications* 245, 133–139.

Nagle, A.M., Long, R.P., Madden, L.V. and Bonello, P. (2010) Association of *Phytophthora cinnamomi* with white oak decline in southern Ohio. *Plant Disease* 94, 1026–1034.

Nespoulous, C., Huet, J.C. and Pernollet, J.C. (1992) Structure–function relationships of α and β elicitins, signal proteins involved in the plant–*Phytophthora* interaction. *Planta* 186, 551–557.

O'Brien, P.A., Williams, N. and Hardy, G.E.S.J. (2009) Detecting *Phytophthora*. *Critical Reviews in Microbiology* 35, 169–181.

Pilbeam, R., Howard, K., Shearer, B. and Hardy, G. (2011) Phosphite stimulated histological responses of *Eucalyptus marginata* to infection by *Phytophthora cinnamomi*. *Trees – Structure and Function* 25, 1121–1131.

Robold, A.V. and Hardham, A.R. (2005) During attachment *Phytophthora* spores secrete proteins containing thrombospondin type 1 repeats. *Current Genetics* 47, 307–315.

Rodrigues, M.L., Archer, M., Martel, P., Miranda, S., Thomaz, M., Enguita, F.J., Baptista, R.P., Pinho e Melo, E., Sousa, N. and Cravador, A. (2006) Crystal structures of the free and sterol-bound forms of β-cinnamomin. *Biochimica et Biophysica Acta* 1764, 110–121.

Serrano, M.S., Fernández-Rebollo, P., De Vita, P., Carbonero, M.D., Trapero, A. and Sánchez, M.E. (2010) *Lupinus luteus*, a new host of *Phytophthora cinnamomi* in Spanish oak-rangeland ecosystems. *European Journal of Plant Pathology* 128, 149–152.

Serrano, M.S., De Vita, P., Fernández-Rebollo, P. and Sánchez Hernández, M. (2012) Calcium fertilizers induce soil suppressiveness to *Phytophthora cinnamomi* root rot of *Quercus ilex*. *European Journal of Plant Pathology* 132, 271–279.

Vleeshouwers, V., Driesprong, J.D., Kamphuis, L.G., Torto-Alalibo, T., Van't Slot, K.A.E., Govers, F., Visser, R.G.F., Jacobsen, E. and Kamoun, S. (2006) Agroinfection-based high-throughput screening reveals specific recognition of INF elicitins in *Solanum*. *Molecular Plant Pathology* 7, 499–510.

Walker, C.A. and van West, P. (2007) Zoospore development in the oomycetes. *Fungal Biology Reviews* 21, 10–18.

Win, J., Morgan, W., Bos, J., Krasileva, K.V., Cano, L.M., Chaparro-Garcia, A., Ammar, R., Staskawicz, B.J. and Kamoun, S. (2007) Adaptive evolution has targeted the C-terminal domain of the RXLR effectors of plant pathogenic oomycetes. *The Plant Cell* 19, 2349–2369.

Zaki, A., Zentmyer, G., Pettus, J., Sills, J., Keen, N. and Sing, V. (1980) Borbonol from *Persea* spp. – chemical properties and antifungal activity against *Phytophthora cinnamomi*. *Physiological Plant Pathology* 16, 205–208.

15 *Phytophthora* in US Forests

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15.1 Introduction

US forests have experienced major pathogen epidemics resulting in near extinction of some host species such as the American chestnut (*Castanea dentata*). Among the plant pathogens that threaten forest health, *Phytophthora* ranks as one of the most significant. This chapter will focus on three key *Phytophthora* species (*Phytophthora cinnamomi*, *Phytophthora ramorum* and *Phytophthora lateralis*) that currently threaten US forests, causing widespread mortalities and changing the overall forest structure. In addition, other *Phytophthora* species associated with forests will be briefly discussed. *Phytophthora* species found in forests of the USA were previously reviewed by Hansen (2000, 2003), Cline *et al*. (2008), Rizzo and Fichtner (2009) and Frankel and Hansen (2011).

15.2 *P. cinnamomi*

This species was first recognized in the USA in the 1930s after it was isolated from dying American chestnuts and rhododendrons (Crandall *et al*., 1945; Erwin and Ribeiro, 1996). The symptoms resembled those found on European chestnut trees (*Castanea sativa*), also known as ink disease, caused mainly by *Phytophthora cambivora* (see Jung *et al*., Chapter 16, this volume). This caused some confusion among scientists in the 1930s as to whether the causal organism was *P. cambivora* or *P. cinnamomi*. However, based on the large amount of literature that has accumulated over the years, it is clear that *P. cinnamomi* was probably the pathogen involved and not *P. cambivora*. This is also supported by recent surveys of oak forest ecosystems in the eastern USA where *P. cinnamomi* is found frequently and *P. cambivora* rarely (Balci *et al*., 2007, 2010). In addition, only *P. cinnamomi* is continually isolated from dying chestnut seedlings in recent plantings in Appalachian oak forests and in chestnut tree nurseries (Y. Balci, unpublished).

P. cinnamomi's widespread distribution in central and south-eastern US forests was noted in earlier records where great numbers of chestnut trees were dying (Crandall *et al*., 1945). Today the pathogen is found anywhere below the 40°N latitude range in the eastern USA. It is not clear why the pathogen is not found in forests above this latitude range, but it is possible that climatic constraints have a major impact on its northward distribution (Marcais *et al*., 2004; Eggers *et al*., 2012). The distribution in West Coast forests extends from California to Washington State where the pathogen is occasionally isolated from conifer and oak forests in association with root rot and stem cankers. The host list of *P. cinnamomi* is the most extensive among known species of *Phytophthora*. The diversity of plants infected in Western Australia probably best illustrates the broad host range of this pathogen (see Ludowici *et al*., Chapter 14, this volume). Ten per cent of native flora, including woody and herbaceous plants, are classified as moderately or highly susceptible (Cahill *et al*., 2008, and references therein). The situation in the USA is similar and *P. cinnamomi* infects

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most of the major tree species (e.g. Zentmyer, 1980; Erwin and Ribeiro, 1996). However, the impact of *P. cinnamomi* in the eastern USA differs from Western Australia as many plant species remain 'virtually' healthy. This may be due to different levels of host susceptibility or environmental factors in the Appalachian broadleaf forests.

Historical and recent observations indicate American chestnut is one of the most susceptible and most severely affected host species. Other significant forest tree associations include trees primarily found in warmer southern climates. These include: (i) little leaf disease of multiple pine species (*Pinus echinata* and *Pinus taeda*); (ii) root rot and mortality of sand pine (*Pinus clausa*); and (iii) mortality of fir plantations (reviewed in Zentmyer, 1980). On the West Coast its impact has become clearer through extensive surveys to identify *P. ramorum*. It is occasionally found causing bleeding cankers on coast live oak (*Quercus agrifolia*) (Garbelotto *et al*., 2006), but many other hosts are also infected. A noteworthy recent finding from California woodlands indicates that *P. cinnamomi* is causing destruction of the rare plant species Ione manzanita (*Arctostaphylos myrtifolia*), which had previously gone unnoticed (Swiecki *et al*., 2003). This recent finding is indicative of the potential impact of *P. cinnamomi* on additional hosts and vegetation yet to be fully recognized in the USA. The Mediterranean climate type, such as in California, appears to provide the optimum conditions for this pathogen.

Despite the widespread occurrence of *P. cinnamomi* in Appalachian broadleaf forests, its impact on oaks and other dominant tree species such as maple (*Acer* spp.), hickory (*Carya* spp.) and tulip poplar (*Liriodendron tulipifera*) is still not clear. Recent studies looking at the involvement of *P. cinnamomi* in white oak decline provide possible clues to its impact on oak trees (Balci *et al*., 2010; Nagle *et al*., 2010). In these studies *P. cinnamomi*-infected white oaks had 2.5 times fewer fine roots compared with oaks with no *P. cinnamomi* infection. Similarly, several studies in European oak forests (see Jung *et al*., Chapter 16, this volume) indicate the impact of *Phytophthora* species on oak trees is mostly due to their involvement in fine root mortality. Epidemiologically, *P. cinnamomi* appears to be restricted primarily to fine roots in eastern oak forests. Fine root mortality caused by *P. cinnamomi* in oak forests was previously unrecognized and additional research is needed to understand the impact on other tree species that appear to harbour the pathogen. This is in contrast to findings from forests in central Mexico, Western Australia and Mediterranean Europe where the pathogen is capable of causing extensive aboveground damage (bleeding cankers) and crown rot on forest tree species (Brasier *et al*., 1993; Tainter *et al*., 2000; Cahill *et al*., 2008). Although *P. cinnamomi* is readily isolated from cankers, in the Appalachian Mountains no cankers are visible and it is extremely difficult to isolate from fine roots despite being present in the rhizosphere soil.

Due to the widespread distribution of *P. cinnamomi*, no management practices have been employed to target the eradication or spread of this pathogen in the USA. The only management strategy that has been used is breeding for resistance in American chestnut and Christmas trees (*Abies* spp.) (Frampton *et al*., 2005; Jeffers *et al*., 2009). Previous breeding efforts for the American chestnut focused on resistance to the chestnut blight pathogen (*Cryphonectria parasitica*); however, the widespread distribution of *P. cinnamomi* in native chestnut forests and the potential for seedling mortality has prompted the inclusion of *P. cinnamomi* in resistance breeding efforts.

Considering the recent episodes of oak decline in the Mid-Atlantic States, and the possible involvement of this pathogen, it will be necessary to adopt management strategies in forests that incorporate silvicultural practices to establish a diverse stand structure for areas at greater risk. Fuel reduction treatments were not effective for eradicating *P. cinnamomi* in southern Appalachian forests (Meadows *et al*., 2011). However, vegetation destruction combined with fungicide and fumigation

treatments, together with containment barriers, were successful in spot eradication of *P. cinnamomi* in Australia (Dunstan *et al.*, 2010).

15.3 *P. ramorum*, the Sudden Oak Death Pathogen

P. ramorum is a relatively new pathogen first identified in the USA *c.*1995 (literature reviewed in Kliejunas, 2010). It is the first known species that effectively spreads aerially in the forest setting, enabling the rapid expansion of its geographical range. A similar aerial biology is observed with tropical *Phytophthora* species such as *Phytophthora palmivora* and most recently with *Phytophthora pinifolia* on Monterey pine (*Pinus radiata*) in Chile (see Ahumada *et al.*, Chapter 17, this volume). *P. ramorum* is primarily found in northern and central coastal Californian forests and restricted areas of south-western Oregon (the geographic range is updated periodically at www.suddenoakdeath.org).

P. ramorum is associated with very high mortality of trees in wildlands and the urban–wildland interface (Fig. 15.1) and is capable of infecting different parts of plants, resulting in three unique symptoms: (i) stem cankers, referred to as bleeding cankers (Fig. 15.1C); (ii) twig dieback (Fig. 15.2A and B); and (iii) leaf blight or necrosis (Fig. 15.2A–C). As a consequence, plants that are infected on their stems and larger branches (the infections continue downwards and eventually girdle the tree) are killed, whereas most plants with foliar infections and shoot dieback remain alive. Epidemiologically, foliar hosts can lead to the build-up of inoculum, as well as the spread of the pathogen and survival within a stand. A foliar host that plays an important role in inoculum build-up is California bay laurel (*Umbellularia californica*). Other host plants, such as the maidenhair fern (*Adiantum* spp.), may enable the pathogen to survive in the environment with no significant impact on the plant itself (Fig. 15.2C).

As *P. ramorum* spread along coastal Californian oak forests it became evident that not every host is equally susceptible. While some tree species such as coast live oak and tanoak (*Notholithocarpus densiflorus*) were mainly killed, many others remained alive despite the detection of the pathogen from foliage or smaller shoots. Interestingly, most affected plants and many hosts are broadleaf plants. Conifers that become infected remain healthy since in most instances only their foliage or younger shoots are killed (Fig. 15.2A and B). In a few instances, such as with Pacific yew (*Taxus brevifolia*), stem cankers have also been observed (J.C. Bienapfl, unpublished). The host list continues to grow and will probably expand to include many other plants, similar to *P. cinnamomi*. As of February 2012 the host list included 46 regulated hosts and 89 associated hosts (pending Koch's postulates) (USDA Animal and Plant Health Inspection Service, 2012).

A pathogen with such a large host range presents major management challenges. Many of the important plant genera that are hosts of *P. ramorum* are also important as nursery ornamentals. In addition, the pathogen is capable of infecting the root systems of some hosts without causing symptoms, and surviving in soil on dead material (Shishkoff, 2007; Fichtner *et al.*, 2009). Current management practices in the USA include: (i) eradication efforts in newly infested sites; (ii) adoption of best management practices and implementation of regulatory actions including nursery certification protocols to prevent further spread; and (iii) prevention of movement of infected soil and plant material in natural ecosystems using educational tools and alerts. In urban settings fungicidal treatments are suggested for high value trees as a preventive treatment. As with most *Phytophthora* species, phosphonate compounds have been effective against *P. ramorum* infection on stems or foliage to prevent infection (for a review of fungicides tested see Kliejunas, 2010).

In spite of success in limiting the interstate movement of the pathogen through nursery stock, the pathogen has moved to the East Coast via nursery

A

B

C

Fig. 15.1. Impact of *Phytophthora ramorum* on woodlands in California. **(A, B)** Aerial and close-up view of extensive tanoak mortality in Sonoma County, California. **(C)** Bleeding canker on tanoak in Mendocino County, California.

A

B

C

Fig. 15.2. Symptoms of *Phytophthora ramorum* on conifer and broadleaf hosts. **(A)** Dieback on the leader shoot of Douglas fir (*Pseudotsuga menziesii*). **(B)** Foliar necrosis on coast redwood (*Sequoia sempervirens*). **(C)** Leaf lesions on maidenhair fern.

shipments. In isolated locations the pathogen escaped to natural ecosystems (e.g. streams). The source of contamination was traced to infected material in nurseries. It was also possible at some locations to detect *P. ramorum* from the vegetation along the streams. As of 2009 these states included Alabama, Florida, Georgia, Mississippi and Washington (Chastagner *et al.*, 2010). In each case *P. ramorum* was repeatedly isolated over a number of years, up to 2011. It is not clear how the pathogen survives and reproduces such that it can be detected from streams at many time points after its establishment. There is no management practice available to treat the water in natural settings, but treatment of recycled irrigation water in nurseries with currently available fungicides or filtration techniques has been successful in eliminating *P. ramorum*.

15.4 *P. lateralis*

P. lateralis was first recognized in the 1920s causing significant mortality to Port-Orford-cedar (*Chamaecyparis lawsoniana*) (Erwin and Ribeiro, 1996; Hansen, 2000). Its distribution spans the natural range of the Port-Orford-cedar, mainly in south-western Oregon and northern California. The only other reports in the environment outside of the USA are in Brittany, France, and the cloud forests of Taiwan (Brasier *et al.*, 2010; Robin *et al.*, 2011). In France the pathogen was traced back to the nursery trade, whereas in Taiwan it is not clear if *P. lateralis* was introduced, and the possible origin of the pathogen has been the source of speculation.

Compared with the extensive host ranges of *P. cinnamomi* and *P. ramorum*, which include both conifers and broadleaf species, the host range of *P. lateralis* is primarily restricted to conifers, particularly *Chamaecyparis* spp. In Oregon, Pacific yew has been intermittently found to be infected but only when growing along streams and near dead Port-Orford-cedar. Hence, this pathogen is relatively specific to Port-Orford-cedar.

P. lateralis is similar to *P. cinnamomi* in its epidemiology in US forests because both are essentially soil-borne *Phytophthora* species. *P. lateralis* will kill the root systems and cause infections on stems that expand from roots and crowns of the trees. However, recent findings in France and Taiwan suggest an air-borne component to the epidemiology of this pathogen and in these instances *P. lateralis* caused foliar infections with no obvious connection to soil-borne inoculum similar to *P. ramorum* (Brasier *et al.*, 2010; Robin *et al.*, 2011; Webber *et al.*, 2011). These findings illustrate how Phytophthora diseases can manifest differently in different environments.

Various management strategies have been implemented since it was first recognized as a major plant pathogen in the USA (Hansen, 2000). Due to its soil-borne biology, management practices focus on preventing spread through: (i) permanent or seasonal road closures; (ii) harvesting restrictions; and (iii) roadside sanitation of infested vehicles. Resistance breeding is an ongoing research endeavour carried out by the United States Department of Agriculture (USDA) in collaboration with Oregon State University for use in reforestation. Resistant ornamental varieties of Port-Orford-cedar have been available since 2008 at nursery retailers for planting.

15.5 Other *Phytophthora* Associations in Forest Ecosystems

Several other species are known to cause damage on trees in forest settings in the USA. However, most of the findings are incidental and widespread destruction has not been reported. For many, their importance as plant pathogens in nature needs to be determined. For example, *Phytophthora alni* is causing extensive damage in Europe on alder trees (*Alnus* spp.) (see Érsek and Man in ’t Veld, Chapter 5, this volume); however, no such destruction was reported when *P. alni* subsp. *uniformis* was found in Alaska (Adams *et al.*, 2008).

As demonstrated with *P. ramorum*, *Phytophthora* species are often introduced

from nurseries to the forests. However, one important observation is that some species that are frequently found in nurseries are rarely found in forest settings. It is not clear why these discrepancies exist and, more importantly, why they do not incite the same levels of damage as seen in nurseries. For example, *Phytophthora cactorum* has long been known to cause damping-off of seedlings in tree nurseries, as well as root and collar rot, shoot blight and stem necrosis on a number of woody plant species in nurseries. However, its impact and occurrence in forest settings is only incidental and restricted to woody plants in urban settings. Similarly, no *Phytophthora nicotianae* was found in extensive soil surveys in oak forests in the eastern USA or in similar surveys on the West Coast (Balci *et al*., 2007). These pathogens are most frequently associated with agricultural crops, such as citrus, and many other subtropical and tropical plants (Erwin and Ribeiro, 1996). Considering their worldwide distribution and no reports of these pathogens involved in widespread mortality in natural settings, it is possible that they require very specific environmental conditions and are perhaps somewhat host specific, despite being able to kill several plant species.

Some species have only been found in nurseries, such as *Phytophthora foliorum* and *Phytophthora hedraiandra*. On the other hand, there are species that are only reported from forests and have yet to be found in nurseries. These include *Phytophthora quercetorum* and *Phytophthora siskiyouensis*. These species are thought to be endemic since no widespread disease outbreaks have been noted. However, if these species make their way to nursery plants they could spread to new areas and possibly cause disease. For example, *P. siskiyouensis* was first discovered in southwest Oregon from infected native plants (*N. densiflorus* and *Umbellularia californica*), soil and water (Reeser *et al*., 2007). In subsequent years the pathogen was isolated from planted Italian alder (*Alnus cordata*) and white alder (*Alnus rhombifolia*) from southern California (Palmieri *et al*., 2009).

One of the most frequently encountered *Phytophthora* species causing damage in nurseries is *Phytophthora citricola*. For many years isolate variation has been observed for *P. citricola*. Indeed, re-evaluations of diverse sets of isolates have resulted in the delineation of multiple new taxa. These include *Phytophthora pini*, *Phytophthora multivora*, *Phytophthora plurivora*, *Phytophthora capensis* and others that are still awaiting formal description. In European oak forests *P. citricola* populations appear to be dominated by *P. plurivora*, but clarification is still needed for the US *P. citricola* populations (Jung and Burgess, 2009). Although *P. citricola* is one of the most prevalent species in US forests and can infect stems and roots of a diverse set of plants, it is only occasionally found in association with mortality in the USA. In US nurseries, both on the West Coast and East Coast, it is one of the most commonly found species and is mainly associated with blight and root rot of rhododendrons (Donahoo and Lamour, 2008; Warfield *et al*., 2008; Yakabe *et al*., 2009). Damage on forest tree species is primarily seedling root rot, but there are a few reports of trunk cankers (Erwin and Ribeiro, 1996). This finding is in contrast to European forests where *P. plurivora* is involved in European beech decline (*Fagus sylvatica*) (see Jung *et al*., Chapter 16, this volume). Interestingly, *P. plurivora* also causes stem cankers on planted European beech in the state of New York (Jung *et al*., 2005; Nelson and Hudler, 2007). Its importance in US oak forests remains to be determined.

Two other species that are frequently found in *P. ramorum*-infested areas in California are *Phytophthora nemorosa* and *Phytophthora pseudosyringae*. Results from population studies suggest that both species may have been introduced in the USA (Linzer *et al*., 2009). Both species are isolated from multiple plant hosts in *P. ramorum*-infested areas (Wickland *et al*., 2008) and cause similar types of infections and remain virtually indistinguishable from *P. ramorum*, but extensive mortality has not been associated with either species.

P. nemorosa is only found in Oregon and California; however, *P. pseudosyringae* is more widespread and has been found in streams in the eastern USA and oak forest soils in Europe (see Jung *et al.*, Chapter 16, this volume) (Hwang *et al.*, 2008; Y. Balci, unpublished). This species was also detected on nursery material both on the West Coast and on the East Coast (Yakabe *et al.*, 2009; J.C. Bienapfl and Y. Balci, unpublished).

Another species that has become more frequently encountered in the USA is *Phytophthora europaea*. This species was first found in oak forest soils in Europe but was subsequently isolated from eastern oak forest soils, Christmas tree plantation soils in Michigan, and from streams and bay laurel leaves in California (Balci *et al.*, 2007). Based on these findings, this species appears to be more widespread in the USA than in Europe where it was only reported in Central European oak forests. *P. cambivora* is another species found on both the East and the West Coast of the USA and was recently found in a Maryland nursery in association with *Pieris japonica* (J.C. Bienapfl and Y. Balci, unpublished). It is frequently encountered in North Carolina nurseries and has also been found in Californian nurseries (Warfield *et al.*, 2008; Yakabe *et al.*, 2009). Other reports from the USA indicate nut and fruit trees as hosts (Erwin and Ribeiro, 1996). This species has been well studied since 1900 and has possibly spread widely with nursery trade. In Europe *P. cambivora* is associated with chestnut ink disease and causes bleeding cankers on European beech (see Jung *et al.*, Chapter 16, this volume); however, it is rare in US forests. The most recent finding is from Oregon where it was found causing stem cankers on golden chinkapin (*Chrysolepis chrysophylla*) (Saavedra *et al.*, 2007).

15.6 Conclusion and Perspectives

The discovery of *P. ramorum* on the West Coast has prompted a closer look at *Phytophthora* across the USA. This has led to the discovery of 'endemic' and exotic species that were previously not recognized or that have expanded their distribution to areas where no obvious destruction was seen. These surveys have also provided new perspectives on their role as plant pathogens. First, recognition of endemic species raises questions regarding their ecological roles and their possible impact on plant health. Previously, *Phytophthora* species were generally considered to be introduced pathogens because they tend to be studied in connection with devastating plant diseases. However, the roles they play in forest ecosystems and, in particular, aquatic environments are largely unknown. Secondly, we have also started to recognize the widespread occurrence of known exotic pathogens that have been expanding their ranges into areas where no disease is immediately noticeable. In these cases the mounting evidence suggests that *Phytophthora* infection is among the factors that may trigger dieback and decline but may not be the single causal agent.

As more data accumulate, it appears that some *Phytophthora* species found in nurseries have been dispersed over time, giving them the appearance of being cosmopolitan. As with many other plant pathogens, significant damage is only found when there is a susceptible host and a conducive environment. Therefore, many *Phytophthora* species behave as opportunistic pathogens or exist in the environment without causing significant damage. It is also now clear from the epidemiology of *P. ramorum* and *P. lateralis*, and less common species like *P. pseudosyringae* and *Phytophthora europeae*, that their biology is different based on the environments they inhabit, causing air-borne infections in one environment and remaining soil-borne in others. These new findings raise serious questions relating to management of Phytophthora diseases, particularly those related to the level of destruction that may occur when exotic species are introduced to new locations.

References

Adams, G.C., Catal, M., Trummer, L., Hansen, E.M., Reeser, P. and Worrall, J.J. (2008) *Phytophthora alni* ssp. *uniformis* found in Alaska beneath thinleaf alders. *Plant Health Progress*. Available at: http://www.plantmanagementnetwork.org/php/ (accessed 16 February 2012).

Balci, Y., Balci, S., Eggers, J., MacDonald, W.L., Juzwik, J., Long, R.P. and Gottschalk, K.W. (2007) *Phytophthora* spp. associated with forest soils in eastern and north-central US oak ecosystems. *Plant Disease* 91, 705–710.

Balci, Y., Long, R., Mansfield, M. and Balser, D. (2010) Involvement of *Phytophthora* species in white oak (*Q. alba*) decline in southern Ohio. *Forest Pathology* 40, 430–442.

Brasier, C.M., Robredo, F. and Ferraz, J.F.P. (1993) Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline. *Plant Pathology* 42, 140–145.

Brasier, C.M., Vettraino, A.M., Chang, T.T. and Vannini, A. (2010) *Phytophthora lateralis* discovered in a Taiwan old growth *Chamaecyparis* forest. *Plant Pathology* 59, 595–603.

Cahill, D.M., Rookes, J.E., Wilson, B.A., Gibson, L. and McDougall, K.L. (2008) *Phytophthora cinnamomi* and Australia's biodiversity: impacts, predictions and progress towards control. *Australian Journal of Botany* 56, 279–310.

Chastagner, G., Oak, S., Omdal, D., Ramsey-Kroll, A., Coats, K., Valachovic, Y., Lee, C., Jaesoon, H., Jeffers, S. and Elliott, M. (2010) Spread of *P. ramorum* from nurseries into waterways – implications for pathogen establishment in new areas. In: Frankel, S.J., Kliejunas, J.T. and Palmieri, K.M. (technical coordinators) Proceedings of the Sudden Oak Death Fourth Science Symposium, Santa Cruz, California, 15–18 June 2009. General Technical Report PSW-GTR-229. United States Department of Agriculture, Forest Service, Pacific Southwest Research Station, Albany, California, pp. 22–26.

Cline, E.T., Farr, D.F. and Rossman, A.Y. (2008) A synopsis of *Phytophthora* with accurate scientific names, host range, and geographic distribution. *Plant Health Progress*. Available at: http://www.plantmanagementnetwork.org/php/ (accessed 16 February 2012).

Crandall, B.S., Gravatt, G.F. and Ryan, M.M. (1945) Root disease of *Castanea* species and some coniferous and broadleaf nursery stocks, caused by *Phytophthora cinnamomi*. *Phytopathology* 35, 162–180.

Donahoo, R.S. and Lamour, K.H. (2008) Characterization of *Phytophthora* species from leaves of nursery woody ornamentals in Tennessee. *HortScience* 43, 1833–1837.

Dunstan, W.A., Rudman, T., Shearer, B.L., Moore, N.A., Paap, T., Calver, M.C., Dell, B. and Hardy, G. (2010) Containment and spot eradication of a highly destructive, invasive plant pathogen (*Phytophthora cinnamomi*) in natural ecosystems. *Biological Invasions* 12, 913–925.

Eggers, J., Balci, Y. and MacDonald, W.L. (2012) Variation among *Phytophthora cinnamomi* isolates from oak forest soils in the eastern United States. *Plant Disease* 96, 1608–1614.

Erwin, D.C. and Ribeiro, O.K. (1996) *Phytophthora Diseases Worldwide*. APS Press, St Paul, Minnesota, 562 pp.

Fichtner, E.J., Lynch, S.C. and Rizzo, D.M. (2009) Survival, dispersal, and soil-mediated suppression of *Phytophthora ramorum* in a California redwood-tanoak forest. *Phytopathology* 99, 608–619.

Frampton, J., Benson, D.M., Li, J., Brahan, A.M., Hudson, E.E. and Potter, K.M. (2005) Seedling resistance to *Phytophthora cinammomi* in the genus *Abies*. In: Proceedings of the 28th Southern Forest Tree Improvement Conference, Raleigh, North Carolina, 21–23 June 2005. United States Department of Agriculture Forest Service, Washington, DC, pp. 146–147.

Frankel, S.J. and Hansen, E.M. (2011) Forest Phytophthora diseases in the Americas: 2007–2010. Fifth meeting of the International Union of Forest Research Organizations (IUFRO) working party S07-02-09, 'Phytophthora Diseases in Forests and Natural Ecosystems', 7–12 March 2010, Auckland and Rotorua, New Zealand. *New Zealand Journal of Forestry Science* 41S, 159–167.

Garbelotto, M., Huberli, D. and Shaw, D. (2006) First report on an infestation of *Phytophthora cinnamomi* in natural oak woodlands of California and its differential impact on two native oak species. *Plant Disease* 90, 685.

Hansen, E.M. (2000) *Phytophthora* in the Americas. In: Hansen, E.M. and Sutton, W. (eds) Proceedings of the first meeting of the International Union of Forest Research Organizations (IUFRO) working party S07-02-09 'Phytophthoras in Forest and Wildland Ecosystems'. Oregon State University Print, Corvallis, Oregon, pp. 23–27.

Hansen, E.M. (2003) *Phytophthora* in the Americas 2001. In: McComb, J.A., Hardy, G.E.St.J. and Tommerup, I.C. (eds) Proceedings of the second meeting of the International Union of Forest Research Organizations (IUFRO) working party S07-02-09, Albany, Western Australia, 30 September–5 October 2001. Murdoch University Print, Perth, Western Australia, pp. 19–24.

Hwang, J., Oak, S.W. and Jeffers, S.N. (2008) Detecting *Phytophthora ramorum* and other species of *Phytophthora* in streams in natural ecosystems using baiting and filtration methods. In: Frankel, S.J., Kliejunas, J.T. and Palmieri, K.M. (technical coordinators) Proceedings of the Sudden Oak Death Third Science Symposium, Santa Rosa, California, 5–9 March 2007. General Technical Report PSW-GTR-214. United States Department of Agriculture, Forest Service, Pacific Southwest Research Station, Albany, California, pp. 55–58.

Jeffers, S.N., James, J.B. and Sisco, P.H. (2009) Screening for resistance to *Phytophthora cinnamomi* in hybrid seedlings of American chestnut. In: Goheen, E.M. and Frankel, S.J. (technical coordinators) Proceedings of the fourth meeting of the International Union of Forest Research Organizations (IUFRO) working party S07-02-09 ‘Phytophthoras in Forests and Natural Ecosystems’. General Technical Report PSW-GTR-221. United States Department of Agriculture, Forest Service, Pacific Southwest Research Station, Albany, California, pp.188–194.

Jung, T. and Burgess, T.I. (2009) Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. *Persoonia* 22, 95–110.

Jung, T., Hudler, G.W., Griffiths, H.M., Fleischmann, F. and Osswald, W. (2005) Involvement of *Phytophthora* spp. in the decline of European beech in Europe and the USA. *Mycologist* 19, 159–166.

Kliejunas, J.T. (2010) *Sudden Oak Death and* Phytophthora ramorum*: a Summary of the Literature*. General Technical Report PSW-GTR-234. United States Department of Agriculture, Forest Service, Pacific Southwest Research Station, Albany, California, 181 pp.

Linzer, R.E., Rizzo, D.M., Cacciola, S.O. and Garbelotto, M. (2009) AFLPs detect low genetic diversity of *Phytophthora nemorosa* and *P. pseudosyringae* in the US and Europe. *Mycological Research* 113, 298–307.

Marcais, B., Bergot, M., Perarnaud, V., Levy, A. and Desprez-Loustau, M.L. (2004) Prediction and mapping of the impact of winter temperature on the development of *Phytophthora cinnamomi*-induced cankers on red and pedunculate oak in France. *Phytopathology* 94, 826–831.

Meadows, I.M., Zwart, D.C., Jeffers, S.N., Waldrop, T.A. and Bridges, W.C. (2011) Effects of fuel reduction treatments on incidence of *Phytophthora* species in soils of a southern Appalachian mountain forest. *Plant Disease* 95, 811–820.

Nagle, A.M., Long, R.P., Madden, L.V. and Bonello, P. (2010) Association of *Phytophthora cinnamomi* with white oak decline in Southern Ohio. *Plant Disease* 94, 1026–1034.

Nelson, A.H. and Hudler, G.W. (2007) A summary of North American hardwood tree diseases with bleeding canker symptoms. *Arboriculture and Urban Forestry* 33, 122–131.

Palmieri, K., Alexander, J., Lee, C. and Frankel, S.J. (2009) Sudden Oak Death and *Phytophthora ramorum*: 2009 Summary Report. Available at: http://www.suddenoakdeath.org/ (accessed 15 February 2012).

Reeser, P.W., Hansen, E.M. and Sutton, W. (2007) *Phytophthora siskiyouensis*, a new species from soil, water, myrtlewood (*Umbellularia californica*) and tanoak (*Lithocarpus densiflorus*) in southwestern Oregon. *Mycologia* 99, 639–643.

Rizzo, D. and Fichtner, E. (2009) *Phytophthora* in forests and natural ecosystems of the Americas. In: Goheen, E.M. and Frankel, S.J. (technical coordinators) Proceedings of the fourth meeting of the International Union of Forest Research Organizations (IUFRO) working party S07-02-09 ‘Phytophthoras in Forests and Natural Ecosystems’. General Technical Report PSW-GTR-221. United States Department of Agriculture, Forest Service, Pacific Southwest Research Station, Albany, California, pp. 35–44.

Robin, C., Piou, D., Feau, N., Douzon, G., Schenck, N. and Hansen, E.M. (2011) Root and aerial infections of *Chamaecyparis lawsoniana* by *Phytophthora lateralis*: a new threat for European countries. *Forest Pathology* 41, 417–424.

Saavedra, A., Hansen, E.M. and Goheen, D.J. (2007) *Phytophthora cambivora* in Oregon and its pathogenicity to *Chrysolepis chrysophylla*. *Forest Pathology* 37, 409–419.

Shishkoff, N. (2007) Persistence of *Phytophthora ramorum* in soil mix and roots of nursery ornamentals. *Plant Disease* 91, 1245–1249.

Swiecki, T.J., Bernhardt, E.A. and Garbelotto, M. (2003) First report of root and crown rot caused by *Phytophthora cinnamomi* affecting native stands of *Arctostaphylos myrtifolia* and *A. viscida* in California. *Plant Disease* 87, 1395.

Tainter, E., O'Brien, G.J., Hernandez, A., Orozco, F. and Rebolledo, O. (2000) *Phytophthora cinnamomi* as a cause of oak mortality in the state of Colima, Mexico. *Plant Disease* 84, 394–398.

United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (2012) Available at: www.aphis.usda.gov/plant_health/plant_pest_info/pram (accessed 13 February 2012).

Warfield, C.Y., Hwang, J. and Benson, D.M. (2008) Phytophthora blight and dieback in North Carolina nurseries during a 2003 survey. *Plant Disease* 92, 474–481.

Webber, J.F., Vettraino, A.M., Chang, T.T., Bellgard, S.E., Brasier, C.M. and Vannini, A. (2011) Isolation of *Phytophthora lateralis* from *Chamaecyparis* foliage in Taiwan. *Forest Pathology* 42, 136–143.

Wickland, A.C., Jensen, C.E. and Rizzo, D.M. (2008) Geographic distribution, disease symptoms and pathogenicity of *Phytophthora nemorosa* and *Phytophthora pseudosyringae* in California, USA. *Forest Pathology* 38, 288–298.

Yakabe, L.E., Thomas, S.L. and MacDonald, J.D. (2009) Identification and frequency of *Phytophthora* species associated with foliar disease in California ornamental nurseries. *Plant Disease* 93, 883–890.

Zentmyer, G.A. (1980) *Phytophthora cinnamomi* and the diseases it causes. *APS Monograph* no. 10. APS Press, St Paul, Minnesota, 95 pp.

16 The Impact of Invasive *Phytophthora* Species on European Forests

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16.1 Introduction

A number of important broadleaved tree species are currently threatened by *Phytophthora* species causing widespread declines and diebacks. In Europe, besides spruce and eucalypt plantations for timber production, forests in temperate and Mediterranean climates are composed primarily of broadleaved tree species and pines. The European beech (*Fagus sylvatica* L.) is a highly competitive tree species of Western and Central Europe and mountain areas of Eastern and Southern Europe due to high shade tolerance, growth capacity, and wide climatic and geological amplitude. On mesic sites in Atlantic to sub-Atlantic climates, beech trees form pure stands with a sparse understorey. In temperate regions the deciduous oak species *Quercus petraea* and *Quercus robur* are dominant climax trees in diverse mixed forests on drier sites (sandy soils, steep slopes, subcontinental to continental climate) and on nutrient-rich heavy soils with seasonal flooding or high water tables. In Mediterranean lowland and sub-mountainous zones the dominant forest tree species include evergreen oaks (*Quercus ilex* and *Quercus suber*) that are sometimes mixed with several pine species and locally adapted deciduous oak species (*Quercus cerris*, *Quercus faginea*, *Quercus frainetto*, *Quercus pubescens* and *Quercus pyrenaica*). Chestnut forests (*Castanea sativa*) occupy the more acidic mountainous sites. Across Europe *Alnus* species dominate extreme sites such as swamps, waterlogged sites and riverbanks (*Alnus glutinosa*), and dry calcaric slopes and gravelly riverbanks (*Alnus incana*, *Alnus cordata*). This chapter describes the symptoms caused by various *Phytophthora* species on broadleaved tree hosts and overviews disease distributions across Europe. In addition, a historical perspective is provided and potential pathways and triggering factors discussed.

16.2 Ink Disease of Chestnut

Ink disease is one of the most destructive diseases of the genus *Castanea*. It causes root and collar rot, and a high mortality of trees and seedlings in nurseries, plantations and forests. Symptoms on adult trees include: (i) chlorotic small-sized leaves; (ii) thinning and dieback of the crown; and (iii) flame-shaped dark brown necrosis of the inner bark at the collar originating from extensive necrosis of the main roots (Fig. 16.1A). The disease was named after the

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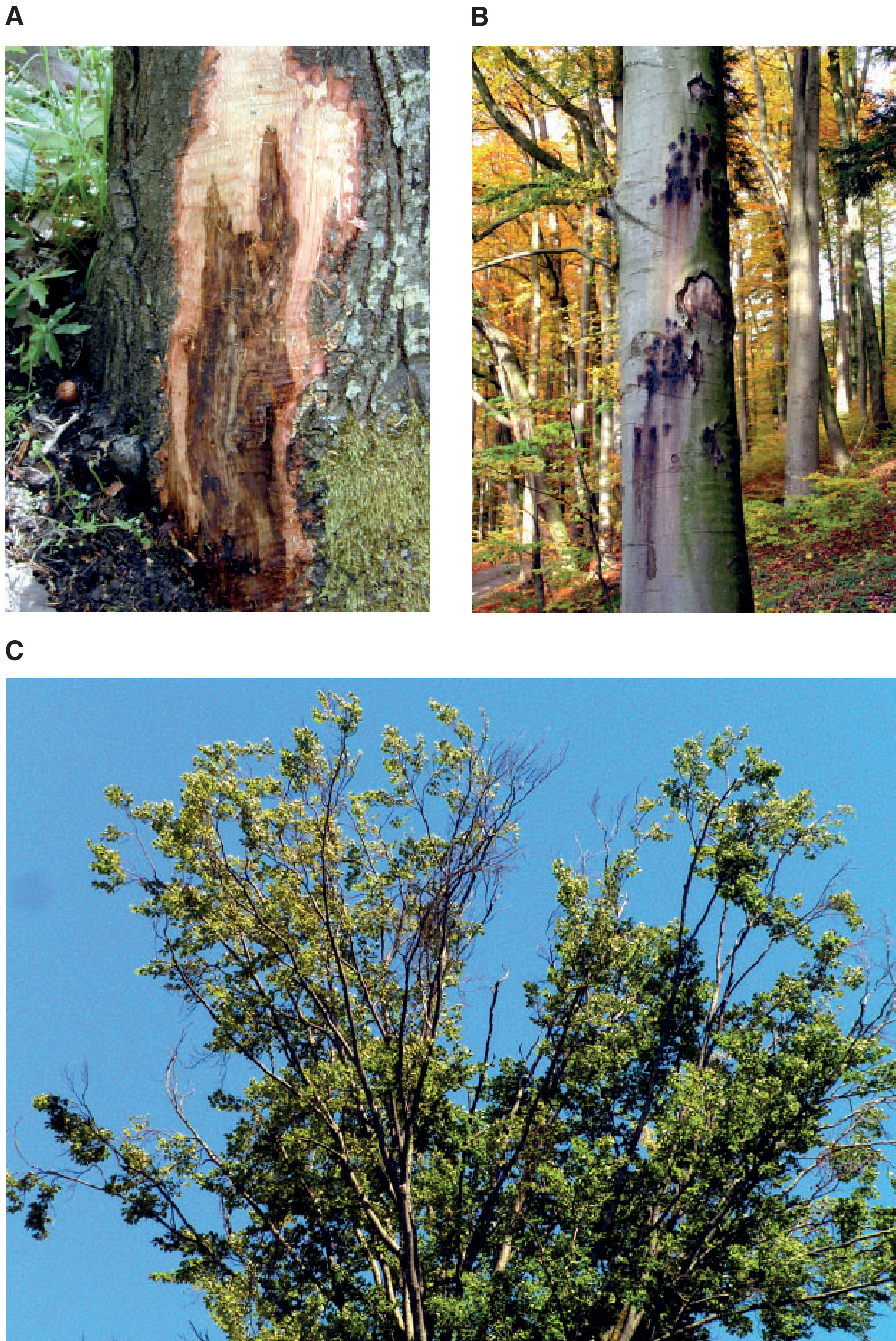

Fig. 16.1. Ink disease of chestnut and beech decline. **(A)** Flame-shaped inner bark lesion caused by *Phytophthora cambivora* at the collar of a mature *Castanea sativa* in Italy. **(B)** Series of aerial bleeding bark cankers caused by *Phytophthora plurivora* on the stem of a mature *Fagus sylvatica* in a mountain forest in Germany. **(C)** Crown of a mature declining *F. sylvatica* in Germany with thinning and dieback due to root and collar rot caused by *P. plurivora*.

black exudates from necrotic roots staining the surrounding soil. Infected seedlings in nurseries or plantations undergo a rapid or gradual wilting.

The disease was first recorded in Portugal in 1838 (Crandall, 1950). At present it occurs across the natural range of *C. sativa* in Europe (Vannini and Vettraino, 2001; Vettraino *et al.*, 2005; Černý *et al.*, 2008). In the USA it was the main problem on *Castanea dentata* before the chestnut blight epidemic (Crandall, 1950). In Asia it causes the decline of *Castanea crenata* and its hybrid with *Castanea mollissima* (Uchida, 1967; Lee *et al.*, 2009). Three *Phytophthora* species are considered the major cause of the disease: (i) *Phytophthora cinnamomi* is dominant in the USA on *C. dentata* (Vettraino *et al.*, 2001) and in Western Europe (Portugal, Spain, France) on *C. sativa*; (ii) *Phytophthora cambivora* is the main cause of ink disease on *C. sativa* in south-east Europe (Czech Republic, Italy, Greece, Hungary, Slovakia, Turkey; Vannini and Vettraino, 2001; Černý *et al.*, 2008); and (iii) *Phytophthora katsurae* causes ink disease in Japan and South Korea on *C. crenata* and *C. crenata* × *C. mollissima* (Uchida, 1967; Lee *et al.*, 2009). In many sites in the UK, continental Europe and the USA, *P. cinnamomi* and *P. cambivora* coexist (Day, 1938; Vannini and Vettraino, 2001). The distribution of *P. cinnamomi* in chestnut forests and plantations is limited by its intolerance to low temperatures. An average minimum soil temperature of 1.4°C has been reported as the threshold determining the presence/absence of *P. cinnamomi* in chestnut soils in Europe (Vettraino *et al.*, 2005). Currently, *P. cinnamomi* is spreading to chestnut areas in Italy at low altitude sites or sites characterized by mild winters. This is consistent with the increase in *P. cinnamomi* activity in Europe predicted by the CLIMEX model for increasing average temperatures (Brasier, 1996). Several other *Phytophthora* species including *Phytophthora cactorum*, *Phytophthora cryptogea*, *Phytophthora gonapodyides*, *Phytophthora megasperma*, *Phytophthora nicotianae*, *Phytophthora plurivora*, *Phytophthora pseudosyringae* and *Phytophthora syringae* have also been found associated with chestnut stands (Vettraino *et al.*, 2005; Scanu *et al.*, 2010). For some of these species the pathogenicity to chestnut has been proven, but their role is confined to fine root damage. Recently, severe root and collar rot of *C. sativa* by *P. cryptogea* has been recorded in Greece (Perlerou *et al.*, 2010).

Ink disease outbreaks are associated with local climatic parameters such as precipitation, temperature, pH (Vettraino *et al.*, 2005) and landscape heterogeneity as well as any factor that facilitates inoculum movement along creeks and natural drainage channels (Vannini *et al.*, 2010). Short- and long-distance movement of contaminated substrates are the main pathways of inoculum dispersal. Tillage practices, vehicle movement along rural roads and planting of infested nursery stock have been associated with disease spread (Fonseca *et al.*, 2004; Vannini *et al.*, 2005; Martins *et al.*, 2007).

16.3 Beech Decline

Since the mid-1990s beech trees across their natural range in Europe and the USA have increasingly declined, showing typical symptoms of *Phytophthora* infection. Symptoms include: (i) small-sized, sparse and often yellowish foliage; (ii) a dieback of the crown; (iii) extensive losses of fine roots and of small woody roots, necrotic lesions on coarse roots and dieback of taproots; (iv) collar rot; and (v) aerial bleeding cankers up to stem heights of >20 m (Fig. 16.1B–C) (Jung *et al.*, 2005; Hartmann *et al.*, 2006; Brown and Brasier, 2007; Jung, 2009; Jung and Burgess, 2009). In the first half of the 20th century a local epidemic with similar symptomatology was recorded from beech forests in the UK and associated to *Phytophthora* infections (Day, 1938).

In infested beech stands aerial cankers and collar rots usually have a scattered or patchy distribution and lead to rapid mortality of affected trees. Extensive fine root losses and root lesions are commonly associated with the widespread chronic

decline of the trees. The root damage predisposes them to increased mortality from droughts, storm damage and attacks by secondary fungal pathogens and bark beetles (Jung, 2009).

In large-scale surveys of beech stands in Germany, Austria and Belgium, and in preliminary studies in 15 other countries, a total of 14 *Phytophthora* species and taxa were isolated from 82% of the 265 mature beech stands examined (Table 16.1). The most common species with the widest distributions were *P. cambivora* and *P. plurivora.* In pathogenicity tests these species were the most aggressive pathogens to the bark and root systems of beech trees

Table 16.1. *Phytophthora* species associated with decline and mortality of *Fagus sylvatica* in Europe.

Country	Number of stands	Stands infested with *Phytophthora* spp.[a]									
		Total	CAC	CAM	GON	KER	PLU	PSE	RAM	SYR	*P.* spp.
Austria[e,f]	27	25	4	15	1		7			1	2[b]
Belgium[g]	49	19		18	1						
Czech Republic[f,h]	2	2	1				1				
Denmark[i]	1	1					1				
Germany[j]	142	127	21	66	10		71	4		6	3[c]
Italy[f,k]	8	8	1	1			1	2			3[d]
The Netherlands[f]	2	2						1	1		
Norway[l]	2	2		1			1				
Poland[m]	10	8		8			3				
Romania[f]	1	1		1							
Serbia[n]	1	1					1				
Slovenia[f,o]	2	2					2				
Switzerland[f]	1	1	1				1				
Sweden[p]	5	5	2	3			2			1	
Turkey[q]	1	1		1							
UK[f,r]	11	11		2	2	6	3	1	1		
Total number of stands	265	216	30	116	14	6	94	8	2	8	8
Proportion (%)		81.5	11.3	43.8	5.3	2.3	35.5	3.0	0.8	3.0	3.0

[a]CAC = *P. cactorum*, CAM = *P. cambivora*, GON = *P. gonapodyides*, KER = *P. kernoviae*, PLU = *P. plurivora*, PSE = *P. pseudosyringae*, RAM = *P. ramorum*, SYR = *P. syringae*, *P.* spp. = other *Phytophthora* spp.
[b]*P. psychrophila* and an unknown taxon related to *P. quercina*.
[c]*P. psychrophila*, *P. uliginosa*, *Phytophthora* taxon 'Pgchlamydo'.
[d]*P. europaea*, *P. katsurae*, *P. psychrophila*, *P. quercina*.
[e]T. Jung and T. Cech (unpublished).
[f]Jung *et al.* (2009).
[g]Schmitz *et al.* (2007).
[h]Černý *et al.* (2009).
[i]K. Thinggard, Ringe, Denmark (2010, personal communication).
[j]Hartmann *et al.* (2006); Jung (2009).
[k]Cacciola *et al.* (2005); Vettraino *et al.* (2008); S. Cacciola, Catania, Italy and B. Ginetti, Florence, Italy (2011, personal communication).
[l]V. Talgø, Ås, Norway (2011, personal communication).
[m]Orlikowski *et al.* (2006); Stępniewska and Dłuszyński (2010).
[n]I. Milenkovic, N. Keca and T. Jung (unpublished).
[o]Munda *et al.* (2007).
[p]T. Jung and J. Witzell (unpublished).
[q]Balci and Halmschlager (2003b).
[r]Brown and Brasier (2007).

(Fleischmann *et al.*, 2002; Brasier and Jung, 2003; Jung *et al.*, 2003a,b). The air-borne *Phytophthora kernoviae* and *Phytophthora ramorum* were geographically restricted to regions in the UK and the Netherlands where a humid climate and widespread occurrence of the susceptible leaf host *Rhododendron ponticum* allowed continuous sporangium production and spread (Brown and Brasier, 2007; Jung *et al.*, 2009).

Phytophthora species were recovered from stands on a wide range of geological substrates with soil pH values ($CaCl_2$) ranging from 3.3 to 7.8. Disease incidences and mortality rates were most severe on base-rich sites and on sites with heavy soils or seasonal waterlogging (Hartmann *et al.*, 2006; Jung, 2009). Significant damage was also found under circumstances that are generally not believed to favour *Phytophthora* infections such as sites with a very low soil pH (<4.0) and sites with good vertical or lateral drainage (Jung, 2009). On these sites *Phytophthora* species most probably act as opportunistic pathogens that survive unfavourable conditions in dormancy or as fine root nibblers at almost undetectable population levels and cause episodic dieback after inoculum quickly builds up during unseasonal, heavy or prolonged rain.

Both soil- and air-borne *Phytophthora* species can infect stem bark via rain splash or wind dispersal of sporangia and/or via root lesions that extend into the collar. Once established in the bark they can spread non-symptomatically in the xylem causing multiple cankers along the stem (Fig. 16.1B; Brown and Brasier, 2007). Due to the resemblance of aerial bleeding lesions caused by *Phytophthora* to those described from 'beech bark disease' (BBD), a primary involvement of *Phytophthora* species in the complex aetiology of BBD in mature stands in Europe has been suggested (Jung, 2009).

In Germany surveys of forest nursery fields and young plantings of beech revealed widespread infestations with *Phytophthora* species, in particular *P. cactorum*, *P. cambivora* and *P. plurivora* (Hartmann *et al.*, 2006; Jung, 2009). An investigation of possible triggering factors for beech decline in Bavaria in Southern Germany revealed the majority of bark cankers found between 2004 and 2007 originated in the year 2002, which was characterized by extremely high precipitation in spring, late summer and autumn, often in the form of heavy rain (Jung, 2009). Unseasonal heavy or extended rainfall also preceded the onset of *Phytophthora*-mediated beech decline in Northern Germany, Austria and southern Sweden (Hartmann *et al.*, 2006; T. Cech and T. Jung, unpublished; T. Jung and J. Witzell, unpublished).

16.4 Oak Decline

Since the beginning of the 20th century several episodes of oak decline have economically and ecologically threatened European forestry (Delatour, 1983). The current oak decline started in the 1980s and occurs in most temperate and Mediterranean regions of Europe. Frost, drought, air pollutants, falling groundwater levels, defoliators, bark beetles, fungal pathogens, bacteria, mycoplasms and viruses have been discussed as causal agents, and there is a general agreement that oak decline is a complex interaction of various abiotic and biotic agents (Delatour, 1983; Thomas *et al.*, 2002). Symptoms include: (i) thinning and dieback of the crown; (ii) formation of epicormic shoots; (iii) yellowing and wilting of leaves; and (iv) tarry exudates from the bark (Brasier *et al.*, 1993; Jung *et al.*, 1996, 2000; Hartmann and Blank, 2002).

Although these symptoms are indicative of water stress and malnutrition, prior to the 1990s surprisingly little attention was paid to the condition of the root systems and the potential role of root pathogens. First reports about a possible involvement of *Phytophthora* species in oak decline came from Iberia where a strong association was found between the presence of *P. cinnamomi* and rapid mortality and decline of *Q. suber* and *Q. ilex* (Brasier *et al.*, 1993). The first finding of fine root losses and root lesions in declining *Q. cerris*, *Q. ilex*, *Q. petraea*, *Q. pubescens* and *Q. robur* trees in Central

European and Italian forests, and the isolation of several *Phytophthora* species from fine roots and rhizosphere soil, caused controversial discussions and stimulated several national and international *Phytophthora* projects (Fig. 16.2A) (Jung *et al.*, 1996). Between 1992 and 2011 a diverse community of ten known *Phytophthora* species and ten previously unknown taxa was found in 61% of the 558 stands surveyed in 14 European countries (Table 16.2). The most frequent and widespread species in temperate regions is the newly described oak-specific *Phytophthora quercina* followed by *P. plurivora* and *P. cambivora*. In the Mediterranean climate *P. cinnamomi* and *P. quercina* prevail (Table 16.2). The distribution and frequency of *P. cinnamomi* in oak stands in Southern Europe shows remarkable variation between regions. This was also reported for chestnut stands and most likely reflects the spread of this exotic pathogen from the supposed original points of introduction in Iberia. *P. cinnamomi* was recovered from 66% of oak stands in Spain and Portugal and from 29 and 28% of the stands in southern France and southern Italy, respectively, whereas it was largely absent in northern and central Italy and Turkey (Robin *et al.*, 1998; Vettraino *et al.*, 2002; Balci and Halmschlager, 2003b).

The fine root systems of mature *Q. robur* and *Q. petraea* trees were analysed in 35 forest stands on a wide range of sites in Bavaria, southern Germany (Jung *et al.*, 2000). On sites with a mean soil pH ($CaCl_2$) above 3.5, and sandy-loamy to clayey soil texture, *P. quercina* and other *Phytophthora* spp. were commonly isolated from rhizosphere soil, and significant correlation was found between crown density and various fine root parameters. Oaks with *P. quercina* or other *Phytophthora* spp. in their rhizospheres had significantly higher losses of fine roots and of small woody roots and a 20% reduced crown density compared with oaks without *Phytophthora*. In contrast, in stands on well-drained sandy to sandy-loamy soils with a mean pH below 3.9, no *Phytophthora* species could be isolated and correlations between crown and root condition, as well as the differences in root parameters between declining and healthy oaks, were less significant and markedly smaller than in the *Phytophthora*-infested stands (Jung *et al.*, 2000). A contingency analysis showed that the presence of *Phytophthora* spp. in the rhizosphere almost triples the risk of an oak declining. A significant association of *P. quercina* with declining trees was also demonstrated for oak stands in Austria, Italy, Turkey and Sweden (Vettraino *et al.*, 2002; Balci and Halmschlager, 2003a,b; Jönsson *et al.*, 2005).

In soil infestation tests, *P. cinnamomi*, *P. quercina*, *P. cambivora* and *P. plurivora* caused necrotic bark and root lesions, substantial losses of fine roots and small woody roots, and eventually mortality of *Q. robur*, *Q. petraea*, *Q. ilex* and *Q. suber* saplings (Jung *et al.*, 1996, 1999, 2003a,b; Robin *et al.*, 1998; Gallego *et al.*, 1999; Sanchez *et al.*, 2002).

Considering the epidemic extent and the long duration of the current oak decline in Europe, possible triggers may include changes of weather patterns such as: (i) a rise of mean winter temperatures; (ii) a seasonal shift of precipitation from summer into wintertime; and (iii) a tendency for heavy rain and prolonged droughts (Brasier *et al.*, 1993; Brasier, 1996; Jung *et al.*, 1996, 2000).

The interaction between *Phytophthora* infections and drought was demonstrated in a soil infestation trial where *P. quercina* and *P. cinnamomi* caused greater root damage on young *Q. robur* trees when severe drought, instead of a mesic water regime, was applied during the periods between subsequent short floodings (Jung *et al.*, 2003b). Based on abundant experimental data and field studies, it is now generally accepted that soil-borne *Phytophthora* species, in particular *P. quercina*, *P. cinnamomi*, *P. cambivora* and *P. plurivora*, are strongly involved in European oak decline by causing progressive fine root losses. Due to the multicyclic nature of Phytophthora fine root diseases it can take decades until the fine root losses exceed the trees' capacities to replace them, causing a chronic

A

B

C

Fig. 16.2. Oak decline, and root and collar rot of alders. **(A)** Small woody roots (diameter 2–5 mm) of a declining mature *Quercus robur* in Germany with severe loss of lateral and fine roots and open lesions caused by *Phytophthora quercina*. **(B)** Crown dieback of riparian *Alnus glutinosa* trees in Germany due to root and collar rot caused by *Phytophthora alni* ssp. *alni*. **(C)** Severe collar rot with tarry spots at the outer bark and tongue-shaped orange-brown necrosis of the inner bark caused by *P. alni* ssp. *alni* on a young planted *A. glutinosa* in Germany.

Table 16.2. *Phytophthora* species recorded between 1992 and 2011 from oak stands in Europe.

Country	Number of stands	Number of stands with *Phytophthora*[a]							
		Total	QUE	CAC	CAM	CIN	GON	PLU	*P.* spp.
Austria[j]	35	17	11				2	7	4[b]
France[k,l]	84	38	13		3	7	6	11	20[c]
Germany[m,n,o]	125	75	48	4	17		6	24	19[d]
Hungary[m]	3	3	1					2	
Italy[m,p]	40	28	12	4	4	6	3	12	9[e]
Poland[o]	7	6	4	2		1		1	1[f]
Portugal[o,q,r]	115	74	3			72			1[f]
Serbia[o]	6	6	6					2	
Slovenia[m]	1	1						1	
Spain[o,q,s]	36	31	2			28	6		9[g]
Sweden[t]	27	11	10	1	1				
Switzerland[o]	4	4				1		3	
Turkey[u]	51	38	29			1	2	4	9[h]
UK[l,o,v]	24	16	8		3	1	5	3	4[i]
Temperate	291	169	101	7	24	2	19	54	48
Infestation (%)		58.1%	34.7%	2.4%	8.3%	0.7%	6.5%	18.6%	16.5%
Mediterranean	267	179	46	4	4	115	11	16	28
Infestation (%)		67.0%	17.2%	1.5%	1.5%	43.1%	4.1%	6.0%	10.5%
All stands	558	348	147	11	28	117	30	70	76
Infestation (%)		62.4%	26.3%	2.0%	5.0%	21.0%	5.4%	12.5%	13.6%

[a]QUE = *P. quercina*, CAC = *P. cactorum*, CAM = *P. cambivora*, CIN = *P. cinnamomi*, GON = *P. gonapodyides*, PLU = *P. plurivora* (in most studies under its previous name *P. citricola*), *P.* spp. = other *Phytophthora* spp.
[b]*P. syringae*, *P. europaea*.
[c]*P. bilorbang*, *P. europaea*, *P. gallica*, *P. megasperma*, *P. pseudosyringae*, *P. psychrophila*, *P. syringae*, *Phytophthora* taxon ‘forestsoil’, *Phytophthora* taxon ‘riversoil’.
[d]*P. europaea*, *P. pseudosyringae*, *P. psychrophila*, *P. syringae*, *P. uliginosa*.
[e]*P. cryptogea*, *P. europaea*, *P. katsurae*, *P. syringae*, two unidentified taxa.
[f]*P. uliginosa*.
[g]*P. drechsleri*, *P. megasperma*, *P. psychrophila*.
[h]*P. cryptogea*, two unidentified taxa.
[i]*P. ramorum*, *P. syringae*.
[j]Balci and Halmschlager (2003a).
[k]Robin *et al.* (1998); Hansen and Delatour (1999); Jung *et al.* (2009).
[l]Delatour (2001).
[m]Jung *et al.* (1996).
[n]Jung *et al.* (2000); Hartmann and Blank (2002).
[o]T. Jung (unpublished).
[p]Vettraino *et al.* (2002); S. Cacciola, Catania, Italy (2012, personal communication); T. Jung and B. Scanu (unpublished).
[q]Brasier *et al.* (1993).
[r]Moreira and Martins (2005); Caetano (2007).
[s]Gallego *et al.* (1999); Sanchez *et al.* (2002); Caetano (2007).
[t]Jönsson *et al.* (2005).
[u]Balci and Halmschlager (2003b).
[v]Brown and Brasier (2007).

decline of the crowns and predisposing the oaks to droughts and opportunistic pathogens and pests. However, the interaction between Phytophthora fine root damage and severe defoliations, and/or one or several abiotic stress factors (such as prolonged droughts, waterlogging, fluctuating water tables, sandy or shallow soils, and unseasonal heavy rain), can accelerate the disease progress, eventually leading to rapid wilting and mortality (Brasier *et al.*, 1993; Jung *et al.*, 2000, 2003b; Vettraino *et al.*, 2002; Balci and Halmschlager, 2003a,b; Jönsson *et al.*, 2005; Moreira and Martins, 2005).

16.5 Root and Collar Rot Epidemic of Alders

Across all countries of Central and Western Europe, the Iberian Peninsula, Italy, Slovenia, Hungary, Sweden and the Baltic countries, riparian and forest stands of *A. glutinosa*, *A. incana* and *A. cordata* are suffering from a severe widespread dieback, which was first reported in the early 1990s from the UK (Gibbs *et al.*, 1999, 2003; Streito *et al.*, 2002; Jung and Blaschke, 2004; Černý and Strnadová, 2010; Solla *et al.*, 2010). Symptoms include: (i) small-sized, sparse and often yellowish foliage; (ii) a dieback of the crown; (iii) early and often excessive fructification; and (iv) formation of adventitious shoots on the stem. These symptoms are caused by root rot and tongue-shaped necrosis of the inner bark, which can extend up to 3 m from the stem base producing tarry or rusty spots on the surface of the bark (Fig. 16.2B–C) (Gibbs *et al.*, 2003; Jung and Blaschke, 2004). The causal organisms were identified as previously unknown interspecific hybrids and subsequently described as *Phytophthora alni* s.l., which contains the following three subspecies: (i) *P. alni* ssp. *alni* (PAA); (ii) *P. alni* ssp. *uniformis* (PAU); and (iii) *P. alni* ssp. *multiformis* (PAM) (Brasier *et al.*, 2004). Molecular analyses of a European collection of isolates of PAA, PAM, PAU, *P. cambivora* and *Phytophthora fragariae* indicates that: (i) PAU is most probably a distinct species; (ii) PAU and PAM were probably the progenitors of PAA; (iii) PAM may also be the result of an ancient hybridization; and (iv) several hybridization events must have occurred (Ioos *et al.*, 2006). Recently, PAU was found to be widespread in streams and alder stands, without notable damage, in remote areas of Alaska (Adams *et al.*, 2008). A microsatellite analysis of PAU isolates from Europe and Alaska demonstrated lower genetic diversity in the European population suggesting a North American origin of PAU (Aguayo *et al.*, 2011). In pathogenicity tests PAA was highly aggressive to the bark of mature *A. glutinosa* logs compared with PAU, PAM and *P. cambivora*. All three subspecies were non-pathogenic to a variety of other tree species indicating their host specificity to *Alnus* species (Brasier and Kirk, 2001; Santini *et al.*, 2003). Interestingly, the North American *Alnus rubra* was significantly more resistant to PAA than *A. incana*, *A. cordata* and *A. glutinosa* (Jung and Blaschke, 2006).

A survey in Bavaria revealed that root and collar rot of *A. glutinosa* and *A. incana* exists in >20,000 km of riparian forests and the majority of the river systems. In most rivers and streams the source of inoculum was traced back to young riparian or forest alder plantations (Jung and Blaschke, 2004). A similar association was found in 26 river systems around Vienna, Austria (Cech, 2008; Jung *et al.*, 2009). The subsequent finding of the disease in more than 800 forest and riparian alder plantings in Bavaria, all of which were younger than 21 years old, and the recovery of PAA and PAU from *A. glutinosa* fields of German nurseries, clearly demonstrates the importance of the nursery pathway for the epidemic spread of this disease (Jung and Blaschke, 2004; Schumacher *et al.*, 2006).

The highest disease incidences, with mortality rates above 50%, are found: (i) in seasonally flooded riparian ecosystems; (ii) in permanently wet swamps; and (iii) on seasonally waterlogged soils (Fig. 16.2B) (Gibbs *et al.*, 1999, 2003; Streito *et al.*, 2002; Jung and Blaschke, 2004). A detailed study along 35 rivers in France indicates components of the watercourse type (width and

slope of the river and speed of water flow, mean summer temperatures of the water, and the soil texture of the riverbanks) were the most important factors affecting disease incidence. Infection rate and mortality of alders increased with increasing river width, the summer temperature of river water, the clay content of the riverbank and with slower water flow rates (Thoirain *et al.*, 2007).

16.6 Conclusions and Perspectives

In Europe *P. cambivora*, *P. cinnamomi*, *P. plurivora*, *P. quercina* and the parents of the hybrid PAA are considered as non-native invasive pathogens (Erwin and Ribeiro, 1996; Jung *et al.*, 1999; Brasier *et al.*, 2004; Cooke *et al.*, 2005; Jung and Burgess, 2009; Aguayo *et al.*, 2011). Research over the past two decades indicates that: (i) invasive *Phytophthora* species are regularly associated with decline and dieback of alder, beech, chestnut and oak stands across Europe; (ii) excessive rainfall and droughts are triggering the epidemics; and (iii) widespread *Phytophthora* infestations of nursery stock probably play a key role in spreading these aggressive pathogens into forests and semi-natural ecosystems. Due to the expected intensification of the triggering climatic trends and widespread nursery infestations in Europe, a proliferation of *Phytophthora* damage may be expected, increasing the instability and vulnerability of forest ecosystems dominated by tree species susceptible to *Phytophthora*.

Preliminary results from trials in mature oak, beech and chestnut stands in Austria, Germany and Italy suggest aerial and stem applications of potassium phosphite is an effective short-term control measure for damaged stands. Long term, resistance screening programmes might be the most promising management approach as indicated by results from recent studies on *A. glutinosa* and *Castanea* spp. (Jung and Blaschke, 2006; Robin *et al.*, 2006; Miranda-Fontaíña *et al.*, 2007).

References

Adams, G.C., Catal, M., Trummer, L., Hansen, E.M., Reeser, P. and Worrall, J.J. (2008) *Phytophthora alni* subsp. *uniformis* found in Alaska beneath thinleaf alders. *Plant Health Progress*. Available at: http://www.plantmanagementnetwork.org/pub/php/brief/2008/alder/ (accessed 27 September 2012).

Aguayo, J., Adams, G.C., Nagy, Z.A., Husson, C., Halkett, F., Frey, P. and Marcais, B. (2011) Genetic structure of American and European populations of *Phytophthora alni* subsp. *uniformis* reveals two divergent populations. Book of abstracts for the 4th meeting of the European Union Cooperation in Science and Technology (COST) Action FP0801, Hungarian Academy of Sciences, Budapest, Hungary, p.10.

Balci, Y. and Halmschlager, E. (2003a) Incidence of *Phytophthora* species in oak forests in Austria and their possible involvement in oak decline. *Forest Pathology* 33, 157–174.

Balci, Y. and Halmschlager, E. (2003b) *Phytophthora* species in oak ecosystems in Turkey and their association with declining oak trees. *Plant Pathology* 52, 694–702.

Brasier, C.M. (1996) *Phytophthora cinnamomi* and oak decline in southern Europe. Environmental constraints including climate change. *Annales des Sciences Forestière* 53, 347–358.

Brasier, C.M. and Jung, T. (2003) Progress in understanding Phytophthora diseases of trees in Europe. In: McComb, J.A., Hardy, G.E.St.J. and Tommerup, I. (eds) Phytophthora *in Forests and Natural Ecosystems*. Murdoch University, Perth, Australia, pp. 4–18.

Brasier, C.M. and Kirk, S.A. (2001) Comparative aggressiveness of standard and variant hybrid alder phytophthoras, *Phytophthora cambivora* and other *Phytophthora* species on bark of *Alnus*, *Quercus* and other woody hosts. *Plant Pathology* 50, 218–229.

Brasier, C.M., Robredo, F. and Ferraz, J.F.P. (1993) Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline. *Plant Pathology* 42, 140–145.

Brasier, C.M., Kirk, S.A., Delcan, J., Cooke, D.E.L., Jung, T. and Man in ’t Veld, W.A. (2004) *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. *Mycological Research* 108, 1172–1184.

Brown, A.V. and Brasier, C.M. (2007) Colonization of tree xylem by *Phytophthora ramorum*, *P. kernoviae* and other *Phytophthora* species. *Plant Pathology* 56, 227–241.

Cacciola, S.O., Motta, E., Raudino, F., Chimento, A., Pane, A. and Magnano di San Lio, G. (2005) *Phytophthora pseudosyringae* the causal agent of bleeding cankers of beech in central Italy. *Journal of Plant Pathology* 87, 289.

Caetano, P.L.C. (2007) *Phytophthora cinnamomi* in the decline of *Quercus suber* and *Q. rotundifolia*: influence of biotic and abiotic factors on the progression of the disease. Attempt of chemical control. PhD thesis, University of Algarve, Faro, Portugal.

Cech, T.L. (2008) Monitoring of alder *Phytophthora* in Vienna. In: Hoyer-Tomiczek, U., Knížek, M., Forster, B. and Grodzki, W. (eds) Proceedings of the International Union of Forest Research Organizations (IUFRO) working party S7-03-10 workshop on 'Methodology of Forest Insect and Disease Survey in Central Europe', 11–14 September 2006, Gmunden, Austria. Federal Research and Training Centre for Forests, Natural Hazards and Landscape, Vienna, Austria, pp. 136–141.

Černý, K. and Strnadová, V. (2010) *Phytophthora* alder decline: disease symptoms, causal agent and its distribution in the Czech Republic. *Plant Protection Science* 46, 12–18.

Černý, K., Gregorová, B., Strnadová, V., Tomšovský, M., Holub, V. and Gabrielová, S. (2008) *Phytophthora cambivora* causing ink disease of sweet chestnut recorded in the Czech Republic. *Czech Mycology* 60, 265–274.

Černý, K., Strnadová, V., Gregorova, B., Holub, V., Tomšovský, M., Mrazkova, M. and Gabrielová, S. (2009) *Phytophthora cactorum* causing bleeding canker of common beech, horse chestnut, and white poplar in the Czech Republic. *Plant Pathology* 58, 394.

Cooke, D.E.L., Jung, T., Williams, N.A., Schubert, R., Oßwald, W. and Duncan, J. (2005) Genetic diversity of European populations of the oak fine-root pathogen *Phytophthora quercina*. *Forest Pathology* 35, 1–14.

Crandall, B.S. (1950) The distribution and significance of the chestnut root rot Phytophthoras, *P. cinnamomi* and *P. cambivora*. *Plant Disease Reporter* 34 (6), 194–196.

Day, W.R. (1938) Root-rot of sweet chestnut and beech caused by species of *Phytophthora*. I. Cause and symptoms of disease: its relation to soil conditions. *Forestry* 12, 101–116.

Delatour, C. (1983) Les dépérissements de chênes en Europe. *Revue forestière francaise* 15, 265–282.

Delatour, C. (ed.) (2001) *Long-term Dynamics of Oak Ecosystems: Assessment of the Role of Root Pathogens and Environmental Constraints as Interacting Decline Inducing Factors*. Final report of the EU project PATHOAK (FAIR CT 97-3926). Institut National de la Recherche Agronomique (INRA), Nancy, France.

Erwin, D.C. and Ribeiro, O.K. (1996) *Phytophthora Diseases Worldwide*. APS Press, St Paul, Minnesota, 562 pp.

Fleischmann, F., Schneider, D., Matyssek, R. and Oßwald, W.F. (2002) Investigations on Net CO_2 assimilation, transpiration and root growth of *Fagus sylvatica* infested with four different *Phytophthora* species. *Plant Biology* 4, 144–152.

Fonseca, T.F., Abreu, C.G. and Parresol, B.R. (2004) Soil compaction and chestnut ink disease. *Forest Pathology* 34, 173–183.

Gallego, F.J., Perez de Algaba, A. and Fernandez-Escobar, R. (1999) Etiology of oak decline in Spain. *European Journal of Forest Pathology* 29, 17–27.

Gibbs, J.N., Lipscombe, M.A. and Peace, A.J. (1999) The impact of *Phytophthora* disease on riparian populations of common alder (*Alnus glutinosa*) in southern Britain. *European Journal of Forest Pathology* 29, 39–50.

Gibbs, J.N., Van Dijk, C. and Webber, J.F. (eds) (2003) *Phytophthora* disease of alder in Europe. *Forestry Commission Bulletin* 126, Edinburgh, UK.

Hansen, E. and Delatour, C. (1999) *Phytophthora* species in oak forests of north-east France. *Annals of Forest Science* 56, 539–547.

Hartmann, G. and Blank, R. (2002) Vorkommen und standortbezüge von *Phytophthora* – arten in geschädigten eichenbeständen in Nordwestdeutschland [Occurrence and site relations of *Phytophthora* species in damaged oak stands in north-western Germany]. *Forst und Holz* 57, 539–545.

Hartmann, G., Blank, R. and Kunca, A. (2006) Collar rot of *Fagus sylvatica* caused by *Phytophthora cambivora*: damage, site relations and susceptibility of broadleaf hosts. In: Brasier, C.M., Jung, T. and Oßwald, W. (eds) *Progress in Research on Phytophthora Diseases of Forest Trees*. Forest Research, Farnham, Hampshire, UK, pp. 135–138.

Ioos, R., Andrieux, A., Marçais, B. and Frey, P. (2006) Genetic characterization of the natural hybrid species *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses. *Fungal Genetics and Biology* 43, 511–529.

Jönsson, U., Jung, T., Sonesson, K. and Rosengren, U. (2005) Relationships between *Quercus robur* health, occurrence of *Phytophthora* species and site conditions in southern Sweden. *Plant Pathology* 54, 502–511.

Jung, T. (2009) Beech decline in Central Europe driven by the interaction between *Phytophthora* infections and climatic extremes. *Forest Pathology* 39, 73–94.

Jung, T. and Blaschke, M. (2004) Phytophthora root and collar rot of alders in Bavaria: distribution, modes of spread, and possible management strategies. *Plant Pathology* 53, 197–208.

Jung, T. and Blaschke, M. (2006) Phytophthora dieback of alders in Bavaria: distribution, pathways and management strategies. In: Brasier, C.M., Jung, T. and Oßwald, W. (eds) *Progress in Research on Phytophthora Diseases of Forest Trees*, Forest Research, Farnham, Hampshire, UK, pp. 61–66.

Jung, T. and Burgess, T.I. (2009) Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. *Persoonia* 22, 95–110.

Jung, T., Blaschke, H. and Neumann, P. (1996) Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. *European Journal of Forest Pathology* 26, 253–272.

Jung, T., Cooke, D.E.L., Blaschke, H., Duncan, J.M. and Oßwald, W. (1999) *Phytophthora quercina* sp. nov., causing root rot of European oaks. *Mycological Research* 103, 785–798.

Jung, T., Blaschke, H. and Oßwald, W. (2000) Involvement of *Phytophthora* species in Central European oak decline and the effect of site factors on the disease. *Plant Pathology* 49, 706–718.

Jung, T., Blaschke, H. and Oßwald, W. (2003a) Effect of environmental constraints on *Phytophthora*-mediated oak decline in Central Europe. In: McComb, J.A., Hardy G.E.St.J. and Tommerup, I. (eds) Phytophthora *in Forests and Natural Ecosystems*. Murdoch University, Perth, Australia, pp. 89–98.

Jung, T., Nechwatal, J., Cooke, D.E.L., Hartmann, G., Blaschke, M., Oßwald, W.F., Duncan, J.M. and Delatour, C. (2003b) *Phytophthora pseudosyringae* sp. nov., a new species causing root and collar rot of deciduous tree species in Europe. *Mycological Research* 107, 772–789.

Jung, T., Hudler, G.W., Jensen-Tracy, S.L., Griffiths, H.M., Fleischmann, F. and Oßwald, W. (2005) Involvement of *Phytophthora* spp. in the decline of European beech in Europe and the USA. *Mycologist* 19, 159–166.

Jung, T., Vannini, A. and Brasier, C.M. (2009) Progress in understanding Phytophthora diseases of trees in Europe 2004–2007. In: Goheen, E.M. and Frankel, S.J. (eds) *Phytophthoras in Forests and Natural Ecosystems*, General Technical Report PSW-GTR-221. United States Department of Agriculture Forest Service, Albany, California, pp. 3–24.

Lee, J.K., Jo, J.W., Shin, K.C., Lee, S.H. and Lee, S.Y. (2009) Isolation, identification and characterization of *Phytophthora katsurae*, causing chestnut ink disease in Korea. *The Plant Pathology Journal* 25, 121–127.

Martins, L., Castro, J., Macedo, W., Marques, C. and Abreu, C. (2007) Assessment of the spread of chestnut ink disease using remote sensing and geostatistical methods. *European Journal of Plant Pathology* 119, 159–164.

Miranda-Fontaíña, M.E., Fernández-López, J., Vettraino, A.M. and Vannini, A. (2007) Resistance of *Castanea* clones to *Phytophthora cinnamomi*: testing and genetic control. *Silvae Genetica* 56, 11–21.

Moreira, A.C. and Martins, J.M.S. (2005) Influence of site factors on the impact of *Phytophthora cinnamomi* in cork oak stands in Portugal. *Forest Pathology* 35, 145–162.

Munda, A., Zerjav, M. and Schroers, H.-J. (2007) First report of *Phytophthora citricola* occurring on *Fagus sylvatica* in Slovenia. *Plant Disease* 91, 907.

Orlikowski, B.L., Oszako, T. and Szkuta, G. (2006) First record of *Phytophthora* spp. associated with the decline of European beech stand in southwest Poland. *Phytopathologia Polonica* 42, 37–46.

Perlerou, C., Tziros, G., Vettraino, A.M. and Diamandis, S. (2010) *Phytophthora cryptogea* causing ink disease of *Castanea sativa* newly reported in Greece. *Plant Pathology* 59, 799.

Robin, C., Desprez-Loustau, M.L., Capron, G. and Delatour, C. (1998) First record of *Phytophthora cinnamomi* on cork and holm oaks in France and evidence of pathogenicity. *Annales des Sciences Forestière* 55, 869–883.

Robin, C., Morel, O., Vettraino, A.M., Vannini, A., Perlerou, C. and Diamandis, S. (2006) Genetic variation in susceptibility to *Phytophthora cambivora* in European chestnut (*Castanea sativa*). *Forest, Ecology and Management* 226, 199–207.

Sanchez, M.E., Caetano, P., Ferraz, J. and Trapero, A. (2002) Phytophthora disease of *Quercus ilex* in southwestern Spain. *Forest Pathology* 32, 5–18.

Santini, A., Barzanti, G.P. and Capretti, P. (2003) Susceptibility of some mesophylic hardwoods to alder *Phytophthora*. *Journal of Phytopathology* 151, 406–410.

Scanu, B., Linaldeddu, B.T. and Franceschini, A. (2010) First report of *Phytophthora pseudosyringae* associated with ink disease of *Castanea sativa* in Italy. *Plant Disease* 94, 1068.

Schmitz, S., Zini, J. and Chandelier, A. (2007) Involvement of *Phytophthora* species in the decline of beech (*Fagus sylvatica*) in the southern part of Belgium. In: Goheen, E.M. and Frankel, S.J. (eds) *Phytophthoras in Forests and Natural Ecosystems*, General Technical Report PSW-GTR-221. United States Department of Agriculture Forest Service, Albany, California, pp. 320–323.

Schumacher, J., Leonhard, S., Grundmann, B.M. and Roloff, A. (2006) New alder disease in Spreewald biosphere reserve – causes and incidental factors of an epidemic. *Nachrichtenblatt Deutscher Pflanzenschutzdienst* 58, 141–147.

Solla, A., Pérez-Sierra, A., Corcobado, T., Haque, M.M., Diez, J.J. and Jung, T. (2010) *Phytophthora alni* on *Alnus glutinosa* reported for the first time in Spain. *Plant Pathology* 59, 798.

Stępniewska, H. and Dłuszyński, J. (2010) Incidence of *Phytophthora cambivora* in bleeding lesions on beech stems in selected forest stands in south-eastern Poland. *Phytopathologia* 56, 39–51.

Streito, J.C., Legrand, P., Tabary, F. and Jarnouen de Villartay, G. (2002) Phytophthora disease of alder (*Alnus glutinosa*) in France: investigations between 1995 and 1999. *Forest Pathology* 32, 179–191.

Thoirain, B., Husson, C. and Marçais, B. (2007) Risk factors for the *Phytophthora*-induced decline of alder in north-eastern France. *Phytopathology* 97, 99–105.

Thomas, F.M., Blank, R. and Hartmann, G. (2002) Abiotic and biotic factors and their interactions as causes of oak decline in Central Europe. *Forest Pathology* 32, 277–307.

Uchida, K. (1967) *Phytophthora* disease of chestnut. *Plant Protection* 21, 383–387.

Vannini, A. and Vettraino, A.M. (2001) Ink disease of chestnut: impact on European chestnut. *Forest, Snow and Landscape Research* 76, 345–350.

Vannini, A., Vettraino, A.M., Fabi, A., Montaghi, A., Valentini, R. and Belli, C. (2005) Monitoring ink disease of chestnut with the airborne multispectral system A.S.P.I.S. *Acta Horticulturae*, 693, 529–533.

Vannini, A., Natili, G., Anselmi, N., Montaghi, A. and Vettraino, A.M. (2010) Distribution and gradient analysis of ink disease in chestnut forests. *Forest Pathology* 40, 73–86.

Vettraino, A.M., Natili, G., Anselmi, N. and Vanini, A. (2001) Recovery and pathogenicity of *Phytophthora* species associated with resurgence of ink disease on *Castanea sativa* in Italy. *Plant Pathology* 50, 90–96.

Vettraino, A.M., Barzanti, G.P., Bianco, M.C., Ragazzi, A., Capretti, P., Paoletti, E., Luisi, N., Anselmi, N. and Vannini, A. (2002) Occurrence of *Phytophthora* species in oak stands in Italy and their association with declining oak trees. *Forest Pathology* 32, 19–28.

Vettraino, A.M., Morel, O., Perlerou, C., Robin, C., Diamandis, S. and Vannini, A. (2005) Occurrence and distribution of *Phytophthora* species in European chestnut stands, and their association with ink disease and crown decline. *European Journal of Plant Pathology* 111, 169–180.

Vettraino, A.M., Jung, T. and Vannini, A. (2008) First report of *Phytophthora cactorum* associated with beech decline in Italy. *Plant Disease* 92, 1708.

17 *Phytophthora pinifolia*: the Cause of Daño Foliar del Pino on *Pinus radiata* in Chile

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17.1 Introduction

Phytophthora pinifolia was first observed in *Pinus radiata* plantations in 2004 in the coastal areas of the Arauco province of Chile. *P. pinifolia* causes a disease known locally as 'daño foliar del pino (DFP)', which translates as pine foliar damage. *P. pinifolia* is unusual because it is the only known *Phytophthora* infecting the needles and succulent tissue of any *Pinus* spp. (Durán *et al.*, 2008). *P. pinifolia* was formally described in 2008 and the origin is unknown (Durán *et al.*, 2008; Wingfield, 2008).

Symptoms of DFP were first observed in July of 2004, and by October of 2004 there was mortality of 1- and 2-year-old plantations (Wingfield, 2008). Currently, DFP-infected trees are present in plantations in the coastal Bío Bío and Los Ríos regions and in some young plantations in the Maule region. Annual monitoring indicates affected areas increased from 3300 ha in 2004 to 54,000 ha in 2006; by 2007 they had decreased to about 2000 ha and have remained at this level through to 2011 (R. Ahumada, unpublished). The overall reduction is probably the result of many factors including: (i) non-conducive environmental conditions; (ii) removal of infected trees over large areas; and (iii) implementation of research-based strategies to reduce the impact of DFP. Research-based strategies include: (i) the use of models to define risk levels at different sites; (ii) methods to select tolerant planting stock; and (iii) appropriately timed chemical treatments.

17.2 Phylogeny

P. pinifolia has unbranched sporangiophores and non-papillate, sub-globose to ovoid sporangia. Sequence analysis of the internal transcribed spacer (ITS) region places it into Group 6 of the phylogeny-based classification of Cooke *et al.* (2000), and its closest relatives are *Phytophthora megasperma*, *Phytophthora gonapodyides*, *Phytophthora thermophile* and *Phytophthora litoralis*. Other Group 6 species include *Phytophthora humicola*, *Phytophthora inundata*, *Phytophthora gibbosa*, *Phytophthora gregata*, *Phytophthora rosacearum* and several undescribed species (Cooke *et al.*, 2000; Jung *et al.*, 2011). Group 6 species are either sterile or inbreeding and occur primarily in forest or riparian ecosystems (Brasier *et al.*, 2003; Kroon *et al.*, 2004). *P. pinifolia* is ecologically and morphologically

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distinct because other Group 6 species are soil-borne and *P. pinifolia* is found above ground. In addition, *P. pinifolia* occasionally produces caducous sporangia that do not proliferate internally or externally; a trait distinct from *P. gonapodyides*, *P. megasperma*, *P. inundata* and *P. humicola*.

17.3 Disease Characteristics and Spatial Distribution

P. pinifolia attacks the needles and the cambium of succulent tissues. Infected needles die relatively quickly (Fig. 17.1A) and may lead to defoliation (Fig. 17.1B) (Durán *et al.*, 2008). Chile has a Mediterranean climate, and symptoms are generally observed from autumn (May) to late spring (November) during the rainy season. Infected needles often have black bands (Fig. 17.2A), which are resinous, translucent areas (Fig. 17.2B). The tops of infected trees have a greyish appearance and turn brown at the end of spring. Infected trees in 1- and 2-year-old plantations typically die, while trees in 3–6-year-old plantations suffer needle damage and defoliation and survive. In adult plantations (older than 6 years) DFP is limited to the needles and the succulent tissue of young branches and the trees do not die (Durán *et al.*, 2008). *P. pinifolia* does not survive in the felled green timber of infected trees and wood from infected trees is not a risk for subsequent infections. Since 2008 ongoing investigations by Bioforest (a research company of the Arauco Group) indicate that *P. pinifolia* is not transported with timber from Chile (Ahumada *et al.*, 2012).

In Chile *P. pinifolia* is found primarily in coastal areas from Constitución to Valdivia, with the highest incidence in the Arauco province. Areas with high levels of infection have high humidity for most of the year due to their proximity to the Pacific coast. Environmental conditions substantially influence disease severity and disease is most severe in plantations with a southern exposure where the trees remain cooler and moister for longer periods (Fig. 17.1C). The spread of DFP in trees older than 2 years has a clear pattern, where disease starts in the lower canopy, which is cool and wet due to less sunlight, and then progresses upwards. The highest levels of infection are observed from June to September, followed by defoliation from July to November.

17.4 Isolation and Identification

P. pinifolia can be isolated on CARP media (cornmeal agar specific medium with 0.01 g benomyl, 0.01 g pimaricin, 0.2 g ampicillin, 0.01 g rifampicin), and recovery from freshly infected needles, particularly those with resinous bands, is relatively simple and mycelium is visible in 5–7 days. In addition, sporangia and zoospores can be visualized by placing infected needles on a glass slide in calcofluor fluorescent brightener (0.001%) for 30 s and examining them microscopically under a fluorescent light (Fig. 17.2C). Isolates grown on V8 |(354 ml V8 juice buffered with 5 g of $CaCO_3$, 15 g agar and 1 l distilled water; Erwin and Ribeiro, 1996) and CA (corn meal agar) media are characterized by fluffy mycelium with regular to rosaceous or petallate margins and growth is optimal at 25°C (Erwin and Ribeiro, 1996). The sporangia are occasionally detached from the medium-length pedicels (Durán *et al.*, 2008). Oogonia and antheridia have not been observed, and *P. pinifolia* is thought to be sterile (Durán *et al.*, 2008).

Molecular identification of *P. pinifolia* can be accomplished using species-specific PCR primers or a PCR-restriction fragment length polymorphism (RFLP) assay (Durán *et al.*, 2009) (see Martin, Chapter 3, this volume). There are two *P. pinifolia*-specific primer sets. One is based on the ITS region of the ribosomal RNA (rRNA) gene and the other is based on the ras-related protein gene *Ypt*1 (Durán *et al.*, 2009). The PCR-RFLP assay was developed according to Drenth *et al.* (2006) and results in a unique profile compared with other *Phytophthora* spp. In Chile *P. pinifolia* is routinely identified using genomic DNA from infected plants using either species-specific primers or the PCR-RFLP assay.

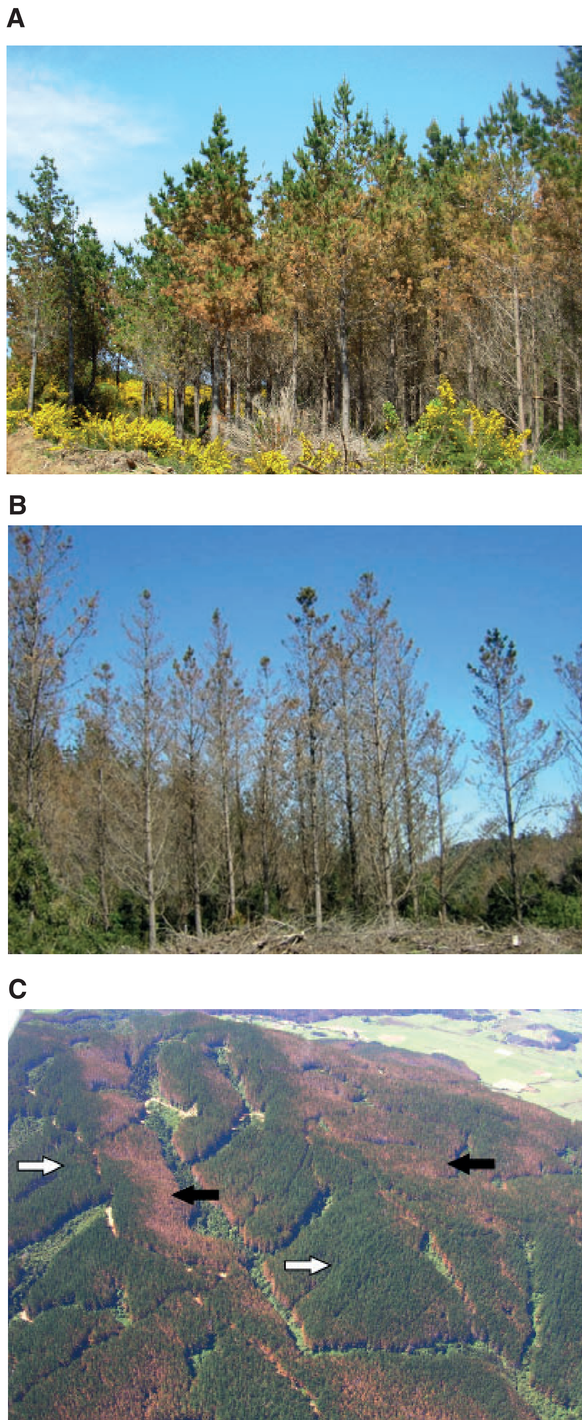

Fig. 17.1. Symptoms of *Phytophthora pinifolia* on *Pinus radiata*. **(A)** Infected adult trees, **(B)** defoliated trees, and **(C)** aerial photo of infected trees with a southern exposure (white arrows) and adjacent healthy trees with a northern exposure (black arrows).

A

B

C

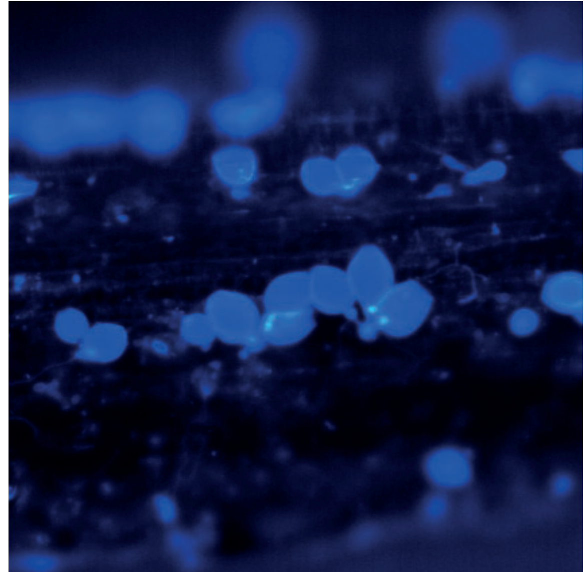

Fig. 17.2. *Phytophthora pinifolia* on needles of *Pinus radiata*. **(A)** Infected needle with black bands (arrows), **(B)** close-up of the resinous translucent band and **(C)** sporangia on the needle surface observed by fluorescent light microscopy.

A large number of isolates of *P. pinifolia* have been collected in Chile and analysed using amplified fragment length polymorphism (AFLP) markers. Results indicate a single clonal lineage is dominant and the overall population structure is clonal (Durán *et al.*, 2010). *P. pinifolia* appeared suddenly in Chile in 2004 across a very limited area, and it is probably an introduced pathogen. Interestingly, other *Pinus* species and conifers (e.g. *Pinus pinaster* and *Pseudotsuga menziesii*) found in areas with DFP are not infected, and it appears that *P. pinifolia* is host specific. While the origin of *P. pinifolia* is currently unknown, it may originate from areas where *Pinus radiata* or related species are native. Future studies are needed to determine the origin of *P. pinifolia* as this might prevent its accidental movement to new environments.

17.5 Epidemiology

P. pinifolia is spread via sporangia. Sporangia are produced on green needles and on dead needles in the trees or that fall to the forest floor. The majority of sporangia are produced during the winter months and needle wetness and temperature are key factors for infection and the development of DFP symptoms. Along the coast of the Arauco province the frequent mist and rain and the temperatures experienced are conducive to the development of DFP symptoms (Del Pozo and Del Canto, 1999).

The incidence of new infections has been monitored using healthy trap plants, and new infections occur throughout the year with the highest incidence during the wet winter and spring months. The area affected by DFP on the Arauco coast province has varied considerably between 2006 and 2011, from a high of 54,000 ha to the current low of 2000 ha. The number of days that are favourable for *Phytophthora* development has been estimated using the Hyre model (Hyre, 1954), and there were 141 favourable days in 2006 and between 51 and 60 favourable days between 2007 and 2011. Clearly, wet weather favours disease development.

17.6 Management of the Disease

The management of DFP in *P. radiata* stands includes: (i) selection of clones tolerant to the infection; (ii) selection of sites that do not have the conditions for disease development; and (iii) use of specific fungicides. Tolerant clones are identified by placing young clonal plants under the canopies of trees with high levels of infection. Clones exhibit a range of tolerance and are ranked to select tolerant clones for planting in high risk areas. The selection of tolerant clones is a long-term programme, but it is already providing promising results. In addition, rapid laboratory and greenhouse screening techniques are being developed to improve the efficiency of selecting tolerant clones.

Low risk sites are identified based on knowledge gained from monitoring areas with severe disease incidence (roughly 120,000 ha from 2005 to 2009). The monitoring includes annual aerial photography of the same areas when symptoms are the most severe (October). Monthly climatic data is used to estimate the dew point and relative humidity, and a model has been developed to estimate the amount of time each month where the temperature is above 7°C, the minimum temperature where *P. pinifolia* can grow. Another model estimates the length of time with >90% relative humidity (assumed to be a condition favourable for *P. pinifolia* infection). The models confirm that the coastal area of the Arauco province (also called the Arauco Gulf) is the most favourable for infection by *P. pinifolia*, and aerial surveys in 2006 confirmed a direct relationship between the number of days favourable for infection and the amount of damage observed.

Several fungicides have been tested for DFP control. Systemic products such as metalaxyl and mefenoxam (phenylamides), propamocarb HCL (carbamate), dimetomorph and mandipropamid (carboxylic acid amides) and other fungicides including fosetyl-aluminium (ethyl phosphonate) and salts of phosphorous acid (phosphite) have been considered for their efficacy to reduce infection by *P. pinifolia*. The use of

chemicals in young plantations consistently reduced the damage of DFP. Several trials carried out between 2008 and 2011 indicate spraying with phenylamides and mefenoxam two to four times/year reduced symptoms of DFP by up to 90% and reduced plant mortality to less than 5%. Other formulations of mefenoxam with chlorotalonil and mancozeb, sprayed four times during the year, reduced DFP by 77% and 96% and mortality to 2% and 14%, respectively.

Phosphites sprayed alone or alternated with phenylamides showed promising results for the management of *P. pinifolia* in the field. Phosphites sprayed four times/year alone gave a control of between 73% and 99% with 700 cc/hL (equivalent to 2.9 g P_2O_5/l and 1.9 g K_2O/l) and 1400 cc/hL (equivalent to 5.8 g P_2O_5/l and 3.8 g K_2O/l), respectively. The variation in results could be a consequence of the different chemical formulations. The use of phosphites in rotation with other fungicides could reduce the emergence of resistant strains of *P. pinifolia* to phenylamides. An important advantage of phosphites is the translocation between both phloem and xylem (Guest and Grant, 1991). Fosetyl-Al and phosphite are less effective than metalaxyl-Mz (metalaxyl) and metalaxyl-M (mefenoxam). In addition, phosphites are treated as fertilizers and have a low environmental impact.

To achieve the best control, application of fungicides should occur before the resinous bands on the needles are first observed. On the Arauco coast, applications are made from April to July in plantations older than 1 year. For establishment of plantations, plants are treated in the nursery before they are planted. For areas with high risk of infection, plants are treated every 3 months with phosphites to prevent the development of DFP. Adult plantations are only treated with phosphite if located in the highest risk areas.

17.7 Conclusions and Future Research

P. pinifolia has been studied intensely since it was first recognized. Damage was severe in commercial plantations and much of the work has occurred in the private sector. A better understanding of the climatic conditions favouring disease has made it possible to develop strategies for management, which, thus far, appear to be working. The clonal nature of the pathogen in Chile and its apparent specificity to *Pinus* spp. suggests that it was accidentally introduced into the country. Studies to determine its origin may be helpful to identify pathways of introduction and reduce the risk of *P. pinifolia* being introduced into other areas of the world.

P. pinifolia is one of the most important pathogens to affect plantation-grown pines in the last decade and it may be a threat to *Pinus radiata* in other parts of the world. This includes areas where *P. radiata* is planted as a non-native and where the species is native. Very little is known regarding the susceptibility of other *Pinus* spp., but preliminary studies suggest that relatives of *P. radiata* are susceptible to infection (R. Ahumada, unpublished data). Clearly, continued research is needed to prevent the spread of *P. pinifolia* to new areas.

References

Ahumada, R., Rotella, A., Slippers, B. and Wingfield, M.J. (2012) Potential of *Phytophthora pinifolia* to spread via sawn green lumber: a preliminary investigation. *Southern Forests* 74 (1), 1–6.

Brasier, C.M., Sanchez-Hernandez, E. and Kirk, S.A. (2003) *Phytophthora inundata* sp. nov., a part heterothallic pathogen of trees and shrubs in wet or flooded soils. *Mycological Research* 107, 477–484.

Cooke, D.E.L., Drenth, A., Duncan, J.M., Wagels, G. and Brasier, C.M. (2000) Molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* 30, 17–32.

Del Pozo, A. and Del Canto, P. (1999) Características del Valle Central. In: Á*reas Agroclimáticas y Sistemas Productivos en las VII y VIII Regiones*. Instituto de Investigaciones Agropecuarias, Centro Regional de Investigación Quilamapu, Chillán, Chile, p. 115.

Drenth, A., Wagels, G., Smith, B., Sendall, B., O'Dwyer, C., Irvine, G. and Irwin, J.A.G. (2006) Development of a DNA-based method for detection and identification of *Phytophthora* species. *Australasian Plant Pathology* 35, 147–159.

Durán, A., Gryzenhout, M., Slippers, B., Ahumada, R., Rotella, A., Flores, F., Wingfield, B.D. and Wingfield, M.J. (2008) *Phytophthora pinifolia* sp. nov. associated with a serious needle disease of *Pinus radiata* in Chile. *Plant Pathology* 57, 715–727.

Durán, A., Slippers, B., Gryzenhout, M., Ahumada, R., Drenth, A., Wingfield, B.D. and Wingfield, M.J. (2009) DNA-based method for rapid identification of the pine pathogen, *Phytophthora pinifolia*. *FEMS Microbiology Letters* 298, 99–104.

Durán, A., Gryzenhout, M., Drenth, A., Slippers, B., Ahumada, R., Wingfield, B.D. and Wingfield, M.J. (2010) AFLP analysis reveals a clonal population of *Phytophthora pinifolia* in Chile. *Fungal Biology* 114, 746–752.

Erwin, D.C. and Ribeiro, O.K. (1996) *Phytophthora Diseases Worldwide*. APS Press, St Paul, Minnesota, 562 pp.

Guest, D. and Grant, B. (1991) The complex action of phosphonates as antifungal agents. *Biological Review* 66, 159–187.

Hyre, R.A. (1954) Progress in forecasting late blight of potato and tomato. *Plant Disease Reporter* 38, 245–253.

Jung, T., Stukely, M.J.C., Hardy, G.E.St.J., White, D., Paap, T., Dunstan, W.A. and Burgess, T.I. (2011) Multiple new *Phytophthora* species from ITS Clade 6 associated with natural ecosystems in Australia: evolutionary and ecological implications. *Persoonia* 26, 13–39.

Kroon, L.P.N.M., Bakker, F.T., van den Bosch, G.B.M., Bonants, P.J.M. and Flier, W.G. (2004) Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. *Fungal Genetic and Biology* 41, 766–782.

Wingfield, M.J. (2008) A new species of *Phytophthora* associated with dying pine needles in Chile. Forestry and Agricultural Biotechnology Institute University of Pretoria. Available at: www.fabinet.up.ac.za/tpcp/pinifolia (accessed 23 March 2012).

18 *Phytophthora* in Woody Ornamental Nurseries

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18.1 Introduction

The global horticultural industry has grown rapidly in recent decades, especially as landscaping and gardening are increasingly fashionable. New technologies have improved plant breeding and propagation, and production facilities operate at large scales to meet consumer demand for novel and exotic plants and 'instant landscapes'. Also, online marketing and trading, new packaging and shipment technologies, and efficient transport systems greatly extend the range of plants available (Drew *et al.*, 2010). Unfortunately, increased plant trade facilitates movement of invasive plants (Reichard and White, 2001) and exotic pathogens (Jones and Baker, 2007; Brasier, 2008). Organisms harmless in their centres of origin often alter population dynamics and the structure of invaded communities (Mooney and Cleland, 2001; Bohlen, 2006). In this context *Phytophthora* species are of particular importance.

The root pathogen *Phytophthora cinnamomi* is a good example. It is able to infect more than 3000 woody and herbaceous plant species (Hardham, 2005) (see Hee *et al.*, Chapter 14, this volume). It has caused large-scale devastation of: (i) eucalypt forests, *Banksia* woodlands and diverse heathland ecosystems in Australia; (ii) oak forests in Iberia; and (iii) chestnut, oak and pine forests in the south-eastern USA (Zentmyer, 1980; Shearer and Tippett, 1989; Brasier *et al.*, 1993; Hardham, 2005; Balci and Bienapfl, Chapter 15, this volume). The primary pathway was most probably infested planting stock and soil. *Phytophthora lateralis* is another good example that was first detected in ornamental nurseries in Washington State in the 1920s before it spread across the native range of *Chamaecyparis lawsoniana* in Oregon and California, causing a devastating root and collar rot epidemic (Hansen *et al.*, 2000). Recently, the *P. lateralis* eastern-Asian origin was elucidated, and it is now considered a major threat to ornamental *C. lawsoniana* in Europe (Brasier *et al.*, 2010; Robin *et al.*, 2010). Also, the parental species of the hybrid *Phytophthora alni* ssp. *alni*, causal agent of root and collar rot epidemics of *Alnus* species across Europe, were probably introduced to Europe with infested nursery stock (Jung and Blaschke, 2004; Brasier, 2008; Jung *et al.*, 2011; Érsek and Man in 't Veld, Chapter 5, this volume).

In ornamental nurseries *Phytophthora* species are ubiquitous and cause severe economic losses. Once *Phytophthora* is established in nurseries woody ornamental plants act as vectors to gardens and urban landscapes and to natural ecosystems. Despite efforts to regulate the free movement of soil-borne pathogens in the nursery trade, there are still many avenues for spread and the consequences include: (i) movement to new regions (commercial and natural); (ii) expansion of species host ranges through

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encounters with new hosts; and (iii) the development of interspecific hybrids (Brasier *et al*., 2004; Ioos *et al*., 2006; Brasier, 2008) (see Érsek and Man in ’t Veld, Chapter 5, this volume).

18.2 Phytophthora Diseases in Woody Ornamental Nurseries

Nursery conditions favour plant growth and diseases caused by *Phytophthora*. Ornamental woody plants are especially vulnerable to disease problems because of: (i) the production system (outlined below); (ii) the often fragile nature of plants in the nursery setting; and (iii) bad nursery practices (Hong *et al*., 2008). The production of nursery stock is highly intense: plants are grown in dense stands and, in particular, container-grown plants may pass through different locations within the same nursery or between different nurseries during their production. This movement provides numerous opportunities for both dispersal and acquisition of new pathogens. Ornamental nurseries often produce a wide range of plants from different families, species and cultivars including deciduous and evergreen trees, woody ornamentals, shrubs, foliage plants, bedding plants and flowering potted plants. This enables cross-infections and new host–*Phytophthora* encounters that may never occur in nature. The intense trading of diverse ornamentals within the nursery sector is a major pathway for the introduction and dissemination of *Phytophthora*.

The irrigation system and the type of irrigation water used is another major avenue for *Phytophthora* into and within nursery facilities. Also, the development of diseases caused by *Phytophthora* spp. and other pathogens is encouraged by bad nursery practices such as: (i) re-using compost or plastic containers; (ii) recirculation of irrigation water; (iii) storing containerized nursery stock on poorly drained surfaces; and (iv) collection of plant debris near the production area.

In many ornamental nurseries one or more of the following *Phytophthora* symptoms can be observed: (i) root lesions; (ii) root rot; (iii) root loss; (iv) collar rot; (v) stalk rot; (vi) stem lesions; (vii) wilt, shoot and leaf blight; (viii) chlorosis; (ix) discoloration of leaves; (x) defoliation; (xi) leaf spots; and (xii) collapse of the entire plant. Dark-brown, orange or gum-like exudations may be present on stems (Figs 18.1 and 18.2).

Diseases caused by *Phytophthora* species in ornamental nurseries are a global problem. Ever since nursery pathways were shown to be important for the introduction and spread of the exotic pathogen *Phytophthora ramorum*, the causal agent of sudden oak death in North America, many studies have focused on woody ornamentals. Characteristically, *P. ramorum*, which is responsible for the mortality of native oaks (*Quercus agrifolia* and *Quercus kelloggii*) and tanoak (*Lithocarpus densiflorus*) in northern California and southern Oregon, was first described associated with twig and foliar blights of *Rhododendron* and *Viburnum* plants in ornamental nurseries in Germany and the Netherlands (Werres *et al*., 2001). *P. ramorum* has since been detected in nurseries of almost every European country, the USA and Canada, and the number of new susceptible hosts is steadily increasing in both continents. Currently, the host list includes more than 130 plant species in 75 genera and 37 families, among them many important shrubs and trees of ornamental or environmental significance. As a result, woody ornamental nurseries are subject to regular surveys and inspections to prevent the introduction and further spread of *P. ramorum.* However, despite emergency phytosanitary measures and regulations, *P. ramorum* has been introduced on at least three separate occasions in the USA. Furthermore, movement to Canada occurred through infested nursery stock imported from the USA (Goss *et al*., 2011). In Europe, where *P. ramorum* is mainly occurring on ornamental *Rhododendron*, *Viburnum* and *Camellia* plants, the threat of *P. ramorum* to woodlands and the natural environment is a major concern. This concern was warranted, as has been shown in the UK, where an

A

B

C

Fig. 18.1. (A) Leaf necrosis, wilting and dieback of *Azalea* sp. caused by *Phytophthora plurivora*. **(B)** Wilting of *Lavandula* sp. caused by *Phytophthora nicotianae*. **(C)** Chlorosis and wilting of *Syringa vulgaris* due to root rot caused by *Phytophthora citrophthora*.

A

B

C

Fig. 18.2. **(A)** *Thuja occidentalis* killed by root rot caused *by Phytophthora cinnamomi.* **(B)** Gum exudation on a stem lesion of ornamental *Prunus* sp. caused by *Phytophthora nicotianae*. **(C)** Root rot of *Agave* sp. caused by *Phytophthora cryptogea.*

extensive dieback and mortality of mature and juvenile plantations of Japanese larch (*Larix kaempferi*) caused by *P. ramorum* has been observed since 2009 (Webber *et al.*, 2010).

As a by-product of the *P. ramorum* surveys, extensive information on the occurrence of other *Phytophthora* spp. in woody ornamental nurseries has been generated. Since the late 1980s more than 30 *Phytophthora* species have been reported worldwide on nursery-grown ornamentals. In Western Australia seven different *Phytophthora* species were found causing root rot on 37 different plant genera: (i) *Phytophthora cactorum*; (ii) *P. cinnamomi*; (iii) *Phytophthora citricola*; (iv) *Phytophthora cryptogea*; (v) *Phytophthora drechsleri*; (vi) *Phytophthora megasperma*; and (vii) *Phytophthora nicotianae* (Hardy and Sivasithamparam, 1988). The high risk of unintentional *Phytophthora* introductions via infected nursery stock was also demonstrated in Western Australia where *P. cinnamomi* var. *parvispora*, *Phytophthora citrophthora*, *P. drechsleri*, *P. nicotianae*, *Phytophthora niederhauserii*, *Phytophthora palmivora* and an unidentified *Phytophthora* sp. were intercepted from potted nursery stock imported from other states of Australia (Davison *et al.*, 2006). Several US studies confirmed the ubiquitous occurrence of *Phytophthora* spp. in ornamental nurseries. At 11 locations in the eastern USA *P. nicotianae* and *P. drechsleri* were recovered from dark collar lesions and discolored softened roots of eight floricultural crops (Lamour *et al.*, 2003). *P. cactorum*, *Phytophthora cambivora* and *P. citricola* were found causing blight and dieback on different *Rhododendron* and *Pieris* cultivars in 14 nurseries in North Carolina (Warfield *et al.*, 2008). Furthermore, *P. cryptogea*, *P. drechsleri*, *P. nicotianae* and *Phytophthora tropicalis* were isolated from diverse floriculture crops at 11 locations (Olson and Benson, 2011). In Ohio *Phytophthora heveae*, *Phytophthora inflata* and *Phytophthora insolita* were recorded on *Rhododendron* causing foliar necrotic lesions and dieback (Testa *et al.*, 2005; Linderman and Davis, 2006). Eight *Phytophthora* species (*P. cactorum*, *P. cambivora*, *P. citricola*, *P. citrophthora*, *Phytophthora hedraiandra*, *P. megasperma*, *P. nicotianae* and *P.* taxon 'Pgchlamydo') were isolated from diseased leaves, stems and roots of a wide range of hosts in 15 ornamental nurseries in Minnesota (Schwingle *et al.*, 2007). In Tennessee seven *Phytophthora* species (*P. cactorum*, *P. citricola*, *P. citrophthora*, *Phytophthora foliorum*, *P. nicotianae*, *P. palmivora* and *P. tropicalis*) were recovered from leaves of nursery-grown ericaceous hosts, with *P. citricola* and *P. citrophthora* being the most common (Donahoo and Lamour, 2008). In addition, Hulvey *et al.* (2010) also detected *Phytophthora hydropathica* on *Pieris japonica*. In California ornamental nurseries from 32 counties were surveyed for leaf spots as part of the mandatory surveys for *P. ramorum* and 14 *Phytophthora* species were reported (*P. cactorum*, *P. cambivora*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, *P. foliorum*, *Phytophthora gonapodyides*, *Phytophthora hibernalis*, *Phytophthora nemorosa*, *P.* taxon 'Pgchlamydo', *Phytophthora pseudosyringae*, *P. ramorum*, *Phytophthora syringae* and *P. tropicalis*) (Yakabe *et al.*, 2009).

In Europe the situation of ornamental nurseries is comparable to the USA. However, knowledge is often limited to first reports or new disease records of *Phytophthora* spp. on ornamental plants. Also, some studies have been published in national journals and, hence, are often difficult to access. In Italy the most common species isolated from ornamental plants in commercial nurseries were *P. nicotianae*, *P. palmivora*, *P. cryptogea* and *P. citricola*. Less frequent were *Phytophthora asparagi*, *P. cactorum*, *P. cinnamomi*, *P. drechsleri*, *P. gonapodyides*, *P. hedraiandra*, *Phytophthora inundata*, *P. niederhauserii*, *Phytophthora tentaculata* and unidentified *Phytophthora* spp. (Cacciola *et al.*, 2008). In the UK the most common affected hosts were *Taxus*, *Rhododendron*, *Rubus*, *Prunus* and *Viburnum*, and the *Phytophthora* species detected using baiting methods were *P. cactorum*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, *P. gonapodyides* and

P. niederhauserii. Using molecular methods a wider range of species were detected that included *P. alni*, *P. cambivora*, *P. hibernalis*, *P. megasperma*, *Phytophthora porri*, *Phytophthora quercina* and *P. syringae* (Denton *et al.*, 2008). In Spain Moralejo *et al.* (2009) surveyed nurseries, garden centres and botanic gardens and recorded 17 *Phytophthora* species/taxa (i.e. *P. cactorum*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, *P. drechsleri*, *P. hedraiandra*, *P. hibernalis*, *P.* taxon 'kelmania', *P. nicotianae*, *P. niederhauserii*, *P. palmivora*, *P.* taxon 'Pgchlamydo', *P. ramorum*, *P. syringae*, *P. tentaculata* and *P. tropicalis*) on 37 ornamental species. Thirty-seven host–pathogen combinations were new to Spain and 28 were previously unknown to science. In the Czech Republic *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. citrophthora*, *P. megasperma*, *Phytophthora multivora*, *Phytophthora plurivora*, *P.* taxon 'raspberry' and unidentified *Phytophthora* spp. were detected causing root and collar rot and dieback on *Pieris* spp., *Rhododendron* spp. and *Vaccinium corymbosum* (Černý *et al.*, 2011).

Phytophthora records from diseased ornamentals in nurseries of other continents include *P. nicotianae* on *Euphorbia pulcherrima* in Puerto Rico (Estevez de Jensen *et al.*, 2006), on *Zamioculcas zamiifolia* in Taiwan (Feng *et al.*, 2006), on *Peperomia tetraphylla* (Zeng *et al.*, 2010) and on *Lilium* spp. (Yang *et al.*, 2010) in China, and *P. cryptogea* on *Osteospermum* spp. in South Africa (McLeod and Coertze, 2007). Results from preliminary *Phytophthora* surveys in *Alnus* and *Fagus* fields in Germany indicate that the situation in forest nurseries is comparable to ornamental nurseries (Jung and Blaschke, 2004; Schumacher *et al.*, 2006; Jung, 2009).

Sixteen of the *Phytophthora* taxa found in ornamental nurseries were unknown to science prior to 2000: *P. asparagi* (Granke *et al.*, 2012), *P. foliorum* (Donahoo *et al.*, 2006), *P. hedraiandra* (de Cock and Lévesque, 2004), *P. hydropathica* (Hong *et al.*, 2010), *P. inundata* (Brasier *et al.*, 2003a), *Phytophthora kernoviae* (Brasier *et al.*, 2005), *P. niederhauserii* (Abad *et al.*, 2012), *P. multivora* (Scott *et al.*, 2009), *P. nemorosa* (Hansen *et al.*, 2003), *P. plurivora* (Jung and Burgess, 2009), *P. pseudosyringae* (Jung *et al.*, 2003), *P. ramorum* (Werres *et al.*, 2001), *P. tropicalis* (Aragaki and Uchida, 2001) and the informally designated taxa *P.* taxon 'Pgchlamydo' and *P.* taxon 'raspberry' (Brasier *et al.*, 2003b), and *P.* taxon 'kelmania' (G. Abad, United States Department of Agriculture (USDA), Beltsville, Maryland, 2006, personal communication). Obviously, our knowledge of this genus is partial and more unknown species remain to be found (Brasier, 2009).

Symptoms vary depending on hosts. On ornamentals:

- *P. cinnamomi*, *P. cryptogea*, *P. drechsleri*, *P. gonapodyides*, *P. megasperma*, *P. palmivora*, *P. plurivora* and *P.* taxon 'raspberry' cause root rot.
- *P. citricola*, *P. citrophthora*, *P. nicotianae*, *P. plurivora*, *P. ramorum*, *P. syringae* and *P. tropicalis* have been reported causing twig blight.
- *P. cactorum*, *P. citricola*, *P. foliorum*, *P. hibernalis*, *P. nicotianae* and *P. ramorum* have been recovered from leaf spots.

However, under nursery conditions the same species might show different behaviour in different locations or on different hosts. Air-borne species with caducous sporangia such as *P. cactorum* and *P. palmivora* can also cause root rot, while soil-borne species with persistent sporangia such as *P. plurivora*, *P. syringae* and *P. tentaculata* can be spread to above-ground parts by rain splash causing stalk, twig or leaf blight.

18.3 *Phytophthora* Introduction to and Spread in Nurseries

The most important routes of *Phytophthora* into nurseries are through infested plant material, substrate, amendments and irrigation water. Infected plant material can originate from breeders, producers, distributors, wholesale growers and even retailers. Purchased plants are often re-sold immediately and may pass through several

nurseries before delivery to an end-user (Drew *et al*., 2010). This facilitates the movement of pathogens through the nursery sector. Infected plants held for extended periods act as a continuous source of inoculum. Another source of infection is container mixes as shown by Ferguson and Jeffers (1999) who detected *P. cactorum*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. cryptogea* and *P. nicotianae* in naturally infested container mixes from South Carolina nurseries. Furthermore, the soil adjacent to infected nursery stock can contain *Phytophthora* inoculum. In Washington State retail nurseries, Dart *et al.* (2007) found *P. citricola* and *P. drechsleri* evenly distributed through the soil profile while *P. ramorum* was mainly recovered from the upper 5 cm, suggesting recent introduction of the latter species. *P. ramorum* can survive for many months in potting mixes and compost from containerized nursery stock, either embedded in plant leaf tissue or as free chlamydospores, and was shown to persist in soil inside of root debris for up to 11 months (Linderman and Davis, 2006; Shishkoff, 2007). Tjosvold *et al.* (2009) also proved the long-term survival of *P. ramorum* in infested potting media and showed that the latter served as primary inoculum for new foliar infections. In addition, they observed that infected leaves could be a source of inoculum for root infections of nursery plants.

Recycling irrigation water is common in nurseries and an excellent way to disperse *Phytophthora*. The use of *Phytophthora*-contaminated recycled water for irrigation is a serious threat to ornamental crops. MacDonald *et al.* (1994) detected seven different *Phytophthora* species in Californian nursery irrigation effluents with *P. citrophthora* being most common followed by *P. citricola*, *P. cinnamomi*, *P. cryptogea*, *P. nicotianae*, *P. megasperma* and *P. syringae*. Similar results were found at container nurseries in Virginia and Germany. In Virginia *P. cryptogea* and *P. drechsleri* were recovered from the surface of the irrigation reservoir while *P. cactorum*, *Phytophthora capsici*, *P. citricola*, *P. citrophthora*, *P. cryptogea* and *P. drechsleri* were isolated at depths of 1 and 1.5 m (Bush *et al*., 2003). In Germany water and sediments of nursery reservoirs were infested with at least ten known *Phytophthora* spp., including *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. cryptogea*, *P. drechsleri*, *P. gonapodyides*, *Phytophthora richardiae* and *P. syringae*, and multiple unidentified *Phytophthora* isolates (Themann *et al*., 2002). The results of the latter study demonstrated that infected plants were the most important source of water contamination in commercial nurseries. Decontamination of irrigation water is used to reduce losses caused by *Phytophthora* spp. especially during the growing season, but in some nurseries untreated water is still used during the winter months. The long-term implications were recently demonstrated by the finding of ten different *Phytophthora* species (i.e. *P. citrophthora*, *P. gonapodyides*, *P. hydropathica*, *P. inundata*, *Phytophthora irrigata*, *P. megasperma*, *Phytophthora pini*, *Phytophthora polonica*, *P. syringae* and *P. tropicalis*) in an irrigation reservoir of a commercial nursery in Virginia during the winter months. It is most likely that these species will cause latent infections in the cold season, which will escalate during the following growing season (Ghimire *et al*., 2011).

The type of irrigation system can also favour *Phytophthora* dissemination. In particular, overhead irrigation can splash propagules from the soil surface, plant debris or infected plants on to above-ground plant parts (Yakabe *et al*., 2009).

18.4 Control of Phytophthora Diseases in Nurseries

The most effective way to avoid *Phytophthora* infections is to prevent pathogen entry. As mentioned, this is difficult because *Phytophthora* can move rather freely through the nursery distribution chain and the risk of buying infested plants, particularly those with latent infections, is high. Training nursery staff to identify early symptoms minimizes risks, although visual

inspections will not detect latent infections or *Phytophthora* spp. travelling as passive hitchhikers on non-host plants. Thermo-sterilization of potting media and the filtering or disinfestation of irrigation water can help reduce the risk of *Phytophthora* introductions.

Fungicides are commonly used in nurseries. This rapidly selects for resistant strains, as shown by the high proportion of metalaxyl-resistant *P. ramorum* isolates in European nurseries (Webber, 2011). In addition, these chemicals can mask infection at the nursery production site. Once planted the healthy-looking plants succumb to disease with a high risk of infecting other hosts or contaminating new areas.

Different physical, chemical and biological methods are used to disinfect irrigation water in nurseries. These include: (i) barriers that can inhibit or reduce the spread of pathogenic propagules; (ii) filtration (slow media, sand, lava grains, pumice); (iii) heat (95°C for 30 s); (iv) ultraviolet light; (v) high pressure (may play a role in reducing the viability of propagules); (vi) ultrasonic energy; (vii) electrostatic precipitation; and (viii) chlorine, chlorine dioxide, chlorine-bromine, ozone or surfactants (Stewart-Wade, 2011).

Poor nursery hygiene increases *Phytophthora* problems, and strategies to limit infection include: (i) the use of disease-free plant material, non-contaminated compost, soil or water; (ii) lifting containers and keeping them on well-drained surfaces; (iii) avoiding re-using containers, trays or green waste; (iv) avoiding rotation of crops on infected beds; (v) avoiding sprinkle irrigation; (vi) removing all leaf and plant debris; (vii) composting; and (viii) disinfecting tools, footwear, plant beds and capillary matting.

18.5 Implications and Conclusions

The easier it is for woody ornamentals to be shipped between or within countries, the easier it is for *Phytophthora* species to invade new areas (Dehnen-Schmutz *et al.*, 2010). This has been the case for *P. alni*, *P. cinnamomi*, *P. lateralis* and *P. ramorum*. Other species that have probably been introduced through infected nursery stock include *Phytophthora ilicis*, *P. kernoviae* and *P. plurivora* (Brasier, 2008; Jung and Burgess, 2009). Movement of *Phytophthora* through ornamental nurseries may threaten agricultural crops, forest plantations or natural ecosystems. Hong *et al.* (2008) tested the pathogenicity of ten *Phytophthora* species from nursery irrigation water to six ornamental and four vegetable species. Their study demonstrated the potential linkage between ornamental *Phytophthora* infestations and vegetable crop health. Another link between agricultural crops and ornamentals is demonstrated by *P. asparagi*. It was initially detected on asparagus in New Zealand (Boesewinkel, 1974) and Australia (McGeary *et al.*, 1985) and a few years later was found killing *Agave*, *Aloe*, *Furcraea* and *Yucca* plants at the Royal Botanic Gardens, Melbourne (Cunnington *et al.*, 2005), and *Banksia* spp. and *Lomandra sonderi* in natural ecosystems in Western Australia (Burgess *et al.*, 2009; Jung *et al.*, 2011). The same pathogen was reported causing an epidemic on asparagus in Michigan (Saude *et al.*, 2008). Another example is *P. niederhauserii*, which was first reported on ornamental *Thuja occidentalis* and *Hedera helix* in North Carolina (Abad and Abad, 2003). In Western Australia this pathogen was isolated from potted nursery stock in 2001/2002 (Davison *et al.*, 2006), and more recently it was detected in natural *Banksia* woodlands (Burgess *et al.*, 2009). Also in Europe *P. niederhauserii* was detected on container-grown ornamental hosts before it was found on seriously infected almond trees in Spain (Pérez-Sierra *et al.*, 2010).

For plants moving through the nursery trade a phytosanitary certificate confirming that they are free of pathogens is mandatory. However, this system has major flaws that reduce the probability of detecting *Phytophthora* infestations including: (i) inspections are based on known harmful species while it is likely that many important species are unknown to science

and, hence, not included in those lists; (ii) most alien *Phytophthora* species that are considered to be well established are not included in the quarantine lists, despite their proven aggressiveness to endemic host plants; (iii) fungicidal and fungistatic chemicals suppress disease symptoms and mask disease; and (iv) *Phytophthora* species can colonize a host without showing any symptoms or can passively hitchhike on non-host plants.

In conclusion, strategies to limit *Phytophthora* distribution in the nursery trade must consider the complexity of the system. Due to the failing of self-regulation of the industry, an international hygiene code requiring non-infested nursery stock is urgently required. Since even partial code compliance can result in complete failure, regulatory controls of compliance are necessary. In addition, campaigns to develop consumer awareness of invasive pathogens will be a strong incentive for the nursery industry to build up a positive reputation by distributing only clean stock.

References

Abad, Z.G. and Abad, J.A. (2003) Advances in the integration of morphological and molecular characterization in the genus *Phytophthora*: the case of *P. niederhauserii* sp. nov. *Phytopathology* 93, S1.

Abad, Z.G., Abad, J.A., Cunnington, J.H., Smith, I.W., Blomquist, C., Balci, Y., Moralejo, E., Pérez-Sierra, A., Abad-Campos, P., Alvarez-Bernaola, L.A., Henricot, B., Denton, G., Herrero, M.L., Spies, C., McLeod, A., Cacciola, S.O., Pane, A., Faedda, R., Bakonyi, J., Józsa, A., Belbahri, L., Cooke, D., Kageyama, K., Uematsu, S., Kurbetli, I. and Degirmenci, K. (2012) *Phytophthora niederhauserii* sp. nov., a new polyphagous species associated with ornamentals, fruit trees and native plants in thirteen countries. *Mycologia* (in press).

Aragaki, M. and Uchida, J.Y. (2001) Morphological distinctions between *Phytophthora capsici* and *P. tropicalis* sp. nov. *Mycologia* 93, 137–145.

Boesewinkel, H.J. (1974) *Phytophthora* on asparagus in New Zealand. *Plant Disease Reporter* 58, 525–529.

Bohlen, P.J. (2006) Biological invasions: linking the above ground and below ground consequences. *Applied Soil Ecology* 32, 1–5.

Brasier, C.M. (2008) The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathology* 57, 792–806.

Brasier, C.M. (2009) *Phytophthora* biodiversity: how many *Phytophthora* species are there? In: Goheen, E.M. and Frankel, S.J. (eds) *Phytophthoras in Forests and Natural Ecosystems*. General Technical Report PSW-GTR-221. United States Department of Agriculture (USDA) Forest Service, Albany, California, pp. 101–115.

Brasier, C.M., Robredo, F. and Ferraz, J.F.P. (1993) Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline. *Plant Pathology* 42, 140–145.

Brasier, C.M., Sánchez-Hernández, E. and Kirk, S.A. (2003a) *Phytophthora inundata* sp. nov., a part heterothallic pathogen of trees and shrubs in wet or flooded soils. *Mycological Research* 107, 477–484.

Brasier, C.M., Cooke, D.E.L., Duncan, J.M. and Hansen, E.M. (2003b) Multiple new phenotypic taxa from trees and riparian ecosystems in *Phytophthora gonapodyides–P. megasperma* ITS Clade 6, which tend to be high-temperature tolerant and either inbreeding or sterile. *Mycological Research* 107, 277–290.

Brasier, C.M., Kirk, S.A., Delcan, J., Cooke, D.E.L. and Jung, T. (2004) *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. *Mycological Research* 108, 1172–1184.

Brasier, C.M., Beales P.A., Kirk, S.A., Denman, S. and Rose, J. (2005) *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. *Mycological Research* 109, 853–859.

Brasier, C.M., Vettraino, A.M., Chang, T.T. and Vannini, A. (2010) *Phytophthora lateralis* discovered in an old growth *Chamaecyparis* forest in Taiwan. *Plant Pathology* 59, 595–603.
Burgess, T.I., Webster, J.L., Ciampini, J.A., White, D., Hardy, G.E.St.J. and Stukely, M.J.C. (2009) Re-evaluation of *Phytophthora* species isolated during 30 years of vegetation health surveys in Western Australia using molecular techniques. *Plant Disease* 93, 215–223.
Bush, E.A., Hong, C.X. and Stromberg, E.L. (2003) Fluctuations of *Phytophthora* and *Pythium* spp. in components of a recycling irrigation system. *Plant Disease* 87, 1500–1506.
Cacciola, S.O., Pane, A., Martini, P., Agosteo, G.E., Raudino, F. and Magnano di San Lio, G. (2008) Recovery of *Phytophthora* species from potted ornamentals in commercial nurseries in Italy. *Journal of Plant Pathology* 90, S2.185.
Černý, K., Tomšovský, M., Mrázková, M. and Strnadová, V. (2011) The present state of knowledge on *Phytophthora* spp. diversity in forest and ornamental woody plants in the Czech Republic. *New Zealand Journal of Forestry Science* 41 (Supplement), S75–S82.
Cunnington, J.H., Alwis, S., Pascoe, I. and Symes, P. (2005) The 'asparagus' *Phytophthora* infecting members of the *Agavaceae* at the Royal Botanic Gardens, Melbourne. *Australasian Plant Pathology* 34, 413–414.
Dart, N.L., Chastagner, G.A, Rugarber, E.F. and Riley, K.L. (2007) Recovery frequency of *Phytophthora ramorum* and other *Phytophthora* spp. in the soil profile of ornamental retail nurseries. *Plant Disease* 91, 1419–1422.
Davison, E.M., Drenth, A., Kumar, S., Mack, S., Mackie, A.E. and McKirdy, S. (2006) Pathogens associated with nursery plants imported into Western Australia. *Australasian Plant Pathology* 35, 473–475.
de Cock, A.W.A.M. and Lévesque, C.A. (2004) New species of *Pythium* and *Phytophthora*. *Studies in Mycology* 50, 481–487.
Dehnen-Schmutz, K., Holdenrieder, O., Jeger, M.J. and Pautasso, M. (2010) Structural change in the international horticultural industry: some implications for plant health. *Scientia Horticulturae* 125, 1–15.
Denton, G., Denton, J., Waghorn, I. and Henricot, B. (2008) *Phytophthora* diversity in UK gardens. *Journal of Plant Pathology* 90, S2.186.
Donahoo, R., Blomquist, C.L., Thomas, S.L., Moulton, J.K., Cooke, D.E.L. and Lamour, K.H. (2006) *Phytophthora foliorum* sp. nov., a new species causing leaf blight of azalea. *Mycological Research* 110, 1309–1322.
Donahoo, R.S. and Lamour, K.H. (2008) Characterization of *Phytophthora* species from leaves of nursery woody ornamentals in Tennessee. *HortScience* 43, 1833–1837.
Drew, J., Anderson, N. and Andow, D. (2010) Conundrums of a complex vector for invasive species control: a detailed examination of the horticultural industry. *Biological Invasions* 12, 2837–2851.
Estevez de Jensen, C., Abad, G., Roberts, P. and Rosa, E. (2006) First report of wilt and stem canker of poinsettia (*Euphorbia pulcherrima*) caused by *Phytophthora nicotianae* in Puerto Rico. *Plant Disease* 90, 1459.
Feng, C.T., Ho, W.C. and Chao, Y.C. (2006) Basal petiole rot and plant kill of *Zamioculcas zamiifolia* caused by *Phytophthora nicotianae*. *Plant Disease* 90, 1107.
Ferguson, A.J. and Jeffers, S.N. (1999) Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. *Plant Disease* 83, 1129–1136.
Ghimire, S.R., Richardson, P.A., Kong, P., Hu, J., Lea-Cox, J.D., Ross, D.S., Moorman, G.W. and Hong, C. (2011) Distribution and diversity of *Phytophthora* species in nursery irrigation reservoir adopting water recycling system during winter months. *Journal of Phytopathology* 159, 713–719.
Goss, E.M., Larsen, M., Vercauteren, A., Werres, S., Heungens, K. and Grünwald, N.J. (2011) *Phytophthora ramorum* in Canada: evidence for migration within North America and from Europe. *Phytopathology* 101, 166–171.
Granke, L.L., Saude, C., Windstam, S.T., Webster, B.J. and Hausbeck, M.K. (2012) *Phytophthora asparagi* Saude & Hausbeck, sp. nov. Fungal Planet 107. *Persoonia* 28, 147–148.
Hansen, E.M., Goheen, D.J., Jules, E.S. and Ullian, B. (2000) Managing Port-Orford-cedar and the introduced pathogen *Phytophthora lateralis*. *Plant Disease* 84, 4–14.
Hansen, E.M., Reeser, P.W., Davidson, J.M., Garbelotto, M., Ivors, K., Douhan, L. and Rizzo, D.M. (2003) *Phytophthora nemorosa,* a new species causing cankers and leaf blight of forest trees in California and Oregon, USA. *Mycotaxon* 88, 129–138.
Hardham, A.R (2005) *Phytophthora cinnamomi*. *Molecular Plant Pathology* 6, 589–604.

Hardy, G.E. and Sivasithamparam, K. (1988) *Phytophthora* spp. associated with container-grown plants in nurseries in Western Australia. *Plant Disease* 72, 432–437.

Hong, C., Richardson, P.A. and Kong, P. (2008) Pathogenicity to ornamental plants of some existing species and new taxa of *Phytophthora* from irrigation water. *Plant Disease* 92, 1201–1207.

Hong, C., Gallegly, M.E., Richardson, P.A., Kong, P., Moorman, G.W., Lea-Cox, J.D. and Ross, D.S. (2010) *Phytophthora hydropathica*, a new pathogen identified from irrigation water, *Rhododendron catawbiense* and *Kalmia latifolia*. *Plant Pathology* 59, 913–921.

Hulvey, J., Gobena, D., Finley, L. and Lamour, K.H. (2010) Co-occurrence and genotypic distribution of *Phytophthora* species recovered from watersheds and plant nurseries of eastern Tennessee. *Mycologia* 102, 1127–1133.

Ioos, R., Andrieux, A., Marçais, B. and Frey, P. (2006) Genetic characterization of the natural hybrid species *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses. *Fungal Genetics and Biology* 43, 511–529.

Jones, D.R. and Baker, R.H.A. (2007) Introductions of non-native plant pathogens into Great Britain, 1970–2004. *Plant Pathology* 56, 891–910.

Jung, T. (2009) Beech decline in Central Europe driven by the interaction between *Phytophthora* infections and climatic extremes. *Forest Pathology* 39, 73–94.

Jung, T. and Blaschke, M. (2004) *Phytophthora* root and collar rot of alders in Bavaria: distribution, modes of spread, and possible management strategies. *Plant Pathology* 53, 197–208.

Jung, T. and Burgess, T.I. (2009) Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. *Persoonia* 22, 95–110.

Jung, T., Nechwatal, J., Cooke, D.E.L., Hartmann, G., Blaschke, M., Oßwald, W.F., Duncan, J.M. and Delatour, C. (2003) *Phytophthora pseudosyringae* sp. nov., a new species causing root and collar rot of deciduous tree species in Europe. *Mycological Research* 107, 772–789.

Jung, T., Stukely, M.J.C., Hardy, G.E.St.J., White, D., Paap, T., Dunstan, W.A. and Burgess, T.I. (2011) Multiple new *Phytophthora* species from ITS Clade 6 associated with natural ecosystems in Australia: evolutionary and ecological implications. *Persoonia* 26, 13–39.

Lamour, K.H., Daughtrey, M.L., Benson, D.M., Hwang, J. and Hausbeck, M.K. (2003) Etiology of *Phytophthora drechsleri* and *P. nicotianae* (=*P. parasitica*) diseases affecting floriculture crops. *Plant Disease* 87, 854–858.

Linderman, R.G. and Davis, E.A. (2006) Survival of *Phytophthora ramorum* compared to other *Phytophthora* in potting media components, compost, and soil. *HortTechnology* 16, 502–507.

MacDonald, J.D., Ali-Shtayeh, M.S., Kabashima, J. and Stites, J. (1994) Occurrence of *Phytophthora* in recirculated nursery irrigation effluents. *Plant Disease* 78, 607–611.

McGeary, D.J., Ward, P. and Gardner, R.K. (1985) Trends in asparagus production and marketing. Australian Department of Agriculture and Rural Affairs Technical Report 115, Canberra, Australia.

McLeod, A. and Coertze, S. (2007) First report of *Phytophthora cryptogea* on *Osteospermum* spp. in South Africa. *Plant Disease* 91, 322.

Mooney, H.A. and Cleland, E.E (2001) The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences USA* 98, 5446–5451.

Moralejo, E., Pérez-Sierra, A., Álvarez, L.A., Belbahri, L., Lefort, F. and Descals, E. (2009) Multiple alien *Phytophthora* taxa discovered on diseased ornamental plants in Spain. *Plant Pathology* 58, 100–110.

Olson, H.A. and Benson, D.M. (2011) Characterization of *Phytophthora* spp. on floriculture crops in North Carolina. *Plant Disease* 95, 1013–1020.

Pérez-Sierra, A., León, M., Álvarez, L.A., Alaniz, S., Berbegal, M., García-Jiménez, J. and Abad-Campos, P. (2010) Outbreak of a new *Phytophthora* sp. associated with severe decline of almond trees in eastern Spain. *Plant Disease* 94, 534–541.

Reichard, S.H. and White, P. (2001) Horticulture as a pathway of invasive plant introductions in the United States. *Bioscience* 51, 103–113.

Robin, C., Piou, D., Feau, N., Douzon, G., Schenck, N. and Hansen, E.M. (2010) Root and aerial infections of *Chamaecyparis lawsoniana* by *Phytophthora lateralis*: a new threat for European countries. *Forest Pathology* 41, 417–424.

Saude, C., Hurtado-Gonzales, O.P., Lamour, K.H. and Hausbeck, M.K. (2008) Occurrence and characterization of a *Phytophthora* sp. pathogenic to asparagus (*Asparagus officinalis*) in Michigan. *Phytopathology* 98, 1075–1083.

Schumacher, J., Leonhard, S., Grundmann, B.M. and Roloff, A. (2006) New alder disease in Spreewald biosphere reserve – causes and incidental factors of an epidemic. *Nachrichtenblatt Deutscher Pflanzenschutzdienst* 58, 141–147.

Schwingle, B.W., Smith, J.A. and Blanchette, R.A. (2007) *Phytophthora* species associated with diseased woody ornamentals in Minnesota nurseries. *Plant Disease* 91, 97–102.

Scott, P.M., Burgess, T.I., Barber, P.A., Shearer, B.L., Stukely, M.J.C., Hardy, G.E.St.J. and Jung, T. (2009) *Phytophthora multivora* sp. nov., a new species recovered from declining *Eucalyptus*, *Banksia*, *Agonis* and other plant species in Western Australia. *Persoonia* 22, 1–13.

Shearer, B.L. and Tippett, J.T. (1989) *Jarrah Dieback: the Dynamics and Management of* Phytophthora cinnamomi *in the Jarrah (*Eucalyptus marginata*) Forests of South-western Australia.* Department of Conservation and Land Management, Perth, Western Australia.

Shishkoff, N. (2007) Persistence of *Phytophthora ramorum* in soil mix and roots of nursery ornamentals. *Plant Disease* 91, 1245–1249.

Stewart-Wade, S.M. (2011) Plant pathogens in recycled irrigation water in commercial plant nurseries and greenhouses: their detection and management. *Irrigation Science* 29, 267–297.

Testa, A., Schilb, M., Lehman, J.S., Cristinzio, G. and Bonello, P. (2005) First report of *Phytophthora insolita* and *P. inflata* on *Rhododendron* in Ohio. *Plant Disease* 89, 1128.

Themann, K., Werres, S., Lüttmann, R. and Diener, H.A. (2002) Observations of *Phytophthora* spp. in water recirculation systems in commercial hardy ornamental nursery stock. *European Journal of Plant Pathology* 108, 337–343.

Tjosvold, S.A., Chambers, D.L., Fichtner, E.J., Koike, S.T. and Mori, S.R. (2009) Disease risk of potting media infested with *Phytophthora ramorum* under nursery conditions. *Plant Disease* 93, 371–376.

Warfield, C.Y., Hwang, J. and Benson, D.M. (2008) Phytophthora blight and dieback in North Carolina nurseries during a 2003 survey. *Plant Disease* 92, 474–481.

Webber, J.F. (ed.) (2011) Final Report – RAPRA – Risk analysis for *Phytophthora ramorum*, a newly recognised pathogen threat to Europe and the cause of Sudden Oak Death in the USA. Forestry Commission Research Agency, Farnham, Hampshire, UK.

Webber, J.F., Mullett, M. and Brasier, C.M. (2010) Dieback and mortality of plantation Japanese larch (*Larix kaempferi*) associated with infection by *Phytophthora ramorum*. *New Disease Reports* 22, 19.

Werres, S., Marwitz, R., Man in ’t Veld, A.W.A.M., Bonants, P.J.M., De Weerdt, M., Themann, K., Ilieva, E. and Baayen, R.P (2001) *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. *Mycological Research* 105, 1155–1165.

Yakabe, L.E., Blomquist, C.L., Thomas, S.L. and MacDonald, J.D. (2009) Identification and frequency of *Phytophthora* species associated with foliar diseases in California ornamental nurseries. *Plant Disease* 93, 883–890.

Yang, X.M., Wang, J.H., Qu, S.P., Wang, L.H. and Peng, L.C. (2010) First report of Phytophthora blight of lily caused by *Phytophthora nicotianae* in China. *Plant Disease* 94, 782.

Zeng, D.X., Wu, X.L. and Li, Y.H. (2010) First report of root and stem rot caused by *Phytophthora nicotianae* on *Peperomia tetraphylla* in China. *Plant Disease* 94, 1171.

Zentmyer, G.A. (1980) *Phytophthora cinnamomi* and the diseases it causes. *American Phytopathological Society (APS) Monograph* No.10. APS, St Paul, Minnesota.

19 Distribution and Biology of *Phytophthora tropicalis*

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19.1 Introduction

Phytophthora tropicalis attacks hosts in 23 plant families and is often recovered from plant nurseries and economically important trees. Described formally in 2001, *P. tropicalis* has long been considered an evolutionarily distinct species (Aragaki and Uchida, 2001). The earliest placement was into the *Phytophthora palmivora* provisional morphological form 4 (MF4) group. Later the MF4 isolates were placed into a revised version of *Phytophthora capsici* (Uchida and Aragaki, 1989). Phenotypic characters (primarily spore morphology) were used to describe *P. tropicalis* as a distinct species (Aragaki and Uchida, 2001). Other important characteristics include the production of chlamydospores (unlike most *P. capsici*) and a host range that includes many ornamental and woody hosts. In the USA both species are distributed widely: *P. tropicalis* in the nursery trade and *P. capsici* in essentially all areas that vegetables are grown intensively. In many cases their host ranges overlap (e.g. a nursery is near or adjacent to a vegetable production site). In the USA, despite intensive field and nursery sampling during the past decade (and susceptibility of vegetable hosts to *P. tropicalis* under controlled conditions), *P. tropicalis* is not found on pepper, tomato or cucurbits (hosts commonly attacked by *P. capsici*), and *P. capsici* is not found on nursery hosts commonly infected by *P. tropicalis*. Laboratory crosses between *P. tropicalis* and *P. capsici* showed very low fecundity and/or abnormal progeny, although oospores were produced abundantly (Donahoo and Lamour, 2008). Single and multi-gene phylogenies indicate *P. tropicalis* is closely related and slightly ancestral to *P. capsici* (Zhang *et al.*, 2004; Bush *et al.*, 2006; Donahoo and Lamour, 2008). This chapter provides an overview of the biology and common hosts for *P. tropicalis* and recommendations for managing disease.

Sporangia of *P. tropicalis* are similar in shape to *P. capsici* but are generally more narrow (Fig. 19.1A). For many isolates, thick-walled, spherical chlamydospores form promptly in broth culture and on agar plates. *P. tropicalis* is heterothallic but some isolates appear to be sexually sterile, possibly requiring unique conditions for oospore formation (Uchida and Aragaki, 1980). Most isolates grow poorly or not at all at 35°C. Gallegly and Hong (2008) provide excellent molecular data confirming the separation of *P. palmivora* MF4 (= *P. tropicalis*) as a new species, and other studies comparing *P. tropicalis* with *P. capsici* have been published (Zhang *et al.*, 2004) along with studies identifying *P. tropicalis* with morphological and molecular data (Gerlach and Schubert, 2001; Pane *et al.*, 2008; Ann *et al.*, 2010).

Hosts presented in the species description included ten plants and seven families (Aragaki and Uchida, 2001). Since then, *P. tropicalis* has been isolated from pothos (Kadooka *et al.*, 1998), palm, giant taro, breadfruit (Cerquiera *et al.*, 2006; Redfern, 2010), apricots, *Catharanthus*, *Cuphea*, *Vanilla* and other hosts (Table 19.1).

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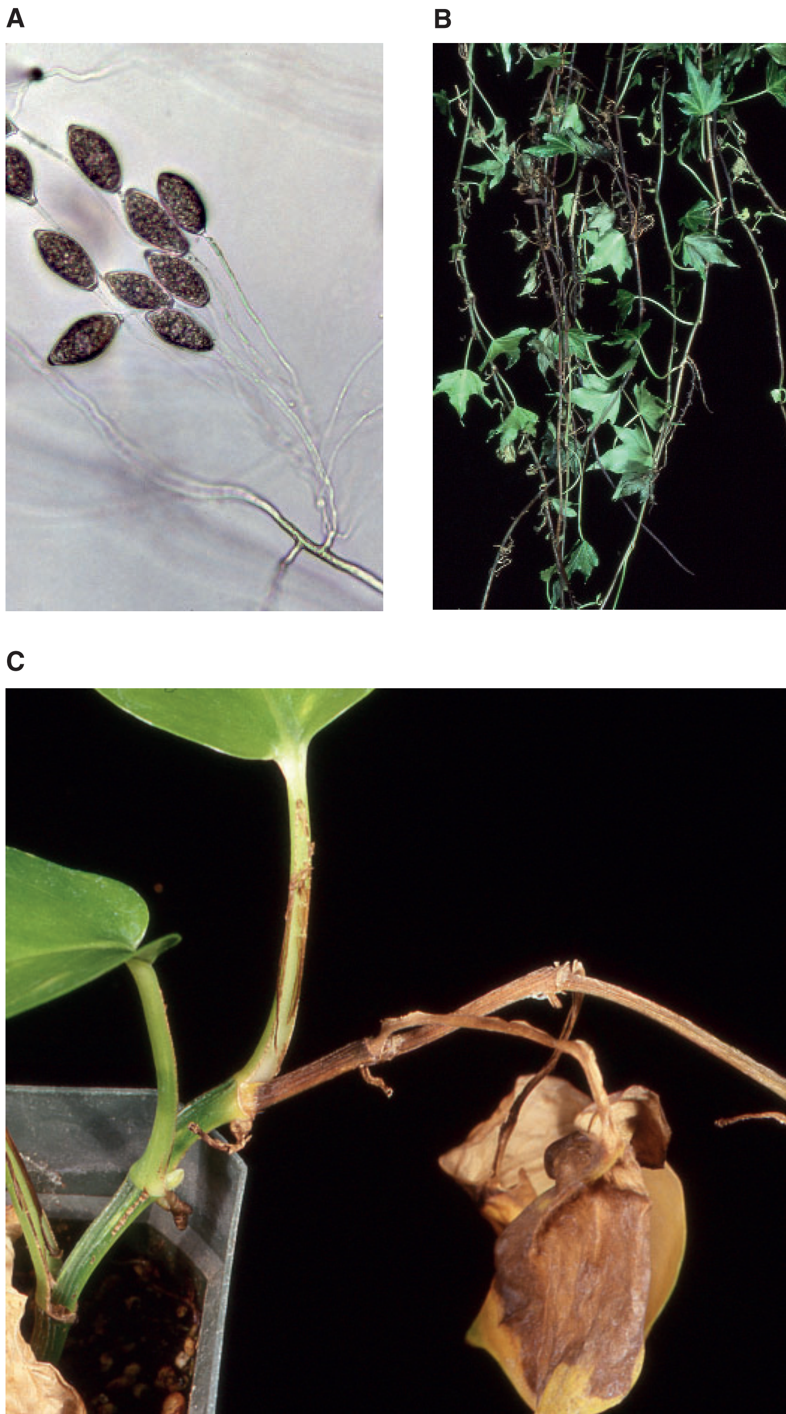


Fig. 19.1. (A) Close monoclasial sporangia formation typical of *Phytophthora tropicalis*. **(B)** Severe vine and leaf blight of English ivy caused by *P. tropicalis*. **(C)** *Epipremnum* (pothos) blights developing 5 days following inoculation with zoospores of *P. tropicalis*.

Table 19.1. Naturally occurring hosts reported for *Phytophthora tropicalis.*

Host	Family	Report (location[a])
Cherimoya (*Annoa cherimola*)	*Annonaceae*	Aragaki and Uchida (2001) (HI)
Rose periwinkle (*Catharanthus roseus*)	*Apocynaceae*	Hao *et al*. (2010) (VA)
Anthurium (*Anthurium andraeanum*)	*Araceae*	Aragaki and Uchida (2001) (HI)
Pothos (*Epipremnum aureum*)	*Araceae*	Kadooka *et al*. (1998) (HI), Leahy (2006) (FL)
Ivy (*Hedera helix*)	*Araliaceae*	Uchida and Aragaki (1989) (HI), Ann *et al*. (2010) (Taiwan)
China doll (*Radermachera sinica*)	*Bignoniaceae*	Aragaki and Uchida (2001) (HI)
Bowervine (*Pandorea jasminoides*)	*Bignoniaceae*	Pane *et al*. (2008) (Italy)
Papaya (*Carica papaya*)	*Caricaceae*	Aragaki and Uchida (2001) (HI)
Carnation (*Dianthus caryophyllus*)	*Caryophyllaceae*	Aragaki and Uchida (2001) (HI), Ann *et al*. (2010) (Taiwan)
Chayote (*Sechium edule*)	*Cucurbitaceae*	Aragaki and Uchida (2001) (HI)
Pieris (*Pieris japonica*)	*Ericaceae*	Hong *et al*. (2008) (VA)
Strawberry tree (*Arbutus unedo*)	*Ericaceae*	Moralejo *et al*. (2008) (Spain)
Rhododendron (*Rhododendron catawbiense*)	*Ericaceae*	Hong *et al*. (2008) (VA)
Rubber (*Hevea brasiliensis*)	*Euphorbiaceae*	Sdoodee, R. (2004) (Brazil)
Rosemary (*Rosmarinus officinalis*)	*Lamiaceae*	Ann *et al*. (2010) (Taiwan)
Gloxinia (*Sinningia speciosa*)	*Gesneriaceae*	Farr and Rossman (2012) (USA)
Cigar flower (*Cuphea ignea*)	*Lythraceae*	Pane *et al*. (2005) (Italy)
Breadfruit (*Artocarpus altilis*)	*Moraceae*	Cerquiera *et al*. (2006) (Brazil), Redfern, (2010) (HI)
Cyclamen (*Cyclamen persicum*)	*Myrsinaceae*	Gerlach and Schubert (2001) (Germany, the Netherlands)
Vanilla (*Vanilla* sp.)	*Orchidaceae*	Hong *et al*. (2008) (VA)
Black pepper (*Piper nigrum*)	*Piperaceae*	Manohara *et al*. (2004) (Indonesia)
Macadamia (*Macadamia integrifolia*)	*Proteaceae*	Aragaki and Uchida (2001) (HI), Ko (2009) (HI)
Leucospermum (*Leucospermum* sp.)	*Proteaceae*	Aragaki and Uchida (2001) (HI)
Apricot (*Prunus armeniaca*)	*Rosaceae*	Pane *et al*. (2009) (Italy)
Aubergine (*Solanum melongena*)	*Solanaceae*	Aragaki and Uchida (2001) (HI)
Cacao (*Theobroma cacao*)	*Sterculiaceae*	Aragaki and Uchida (2001) (HI)
Camellia (*Camellia* sp.)	*Theaceae*	Yakabe *et al*. (2009) (CA)
Verbena × hybrid	*Verbenaceae*	Farr and Rossman (2012) (USA)

[a]CA, California; FL, Florida; HI, Hawai'i; VA, Virginia.

Table 19.2. Hosts susceptible to *Phytophthora tropicalis* following controlled inoculations.

Host[a]	Latin binomial	Family	Isolate origin
Rose periwinkle	*Catharanthus roseus* 'Little Bright Eyes'	*Apocynaceae*	Irrigation water and vanilla
Anthurium[a]	*Anthurium andraeanum*	*Araceae*	Cacao
Dusty-miller	*Senecio cineraria* A.P. de Candolle	*Asteraceae*	Irrigation water and vanilla
Begonia	Cultivar 'Nonstop'	*Begoniaceae*	Vanilla
Cucumber	*Cucumis sativus* L. 'Orient Express'	*Cucurbitaceae*	Irrigation water
Azalea	*Rhododendron* × *Kurume* 'Hershey's Red'	*Ericaceae*	Irrigation water and vanilla
Lupin	*Lupinus albus* L. 'Russell mixed'	*Fabaceae*	Vanilla
Sweet pepper	*Capsicum annuum* L. 'California Wonder'	*Solanaceae*	Irrigation water
Aubergine	*Solanum melongena* 'Black Bell'	*Solanaceae*	Irrigation water
Tomato	*Solanum lycopersicum* L.	*Solanaceae*	Irrigation water

[a]All hosts were reported in Hong *et al.* (2008), except for anthurium, which was reported by Paim *et al.* (2006).

P. tropicalis (referred to as *P. palmivora* MF4) has also been recovered from *Theobroma cacao*, *Hevea brasiliensis* and *Piper nigrum* and the US National Fungus Collection Database lists *Sinningia speciosa* and a *Verbena* × hybrid as hosts, adding two additional plant families. *P. tropicalis* has also been identified in soil samples obtained from mixed-hardwood forests in Virginia, and it is likely the host list will continue to expand. Table 19.2 lists hosts found to be susceptible to *P. tropicalis* under controlled conditions.

19.2 Nursery Hosts

Ornamental plants such as ivy (*Hedera* spp.) are quickly killed by leaf and stem rot from *P. tropicalis* (Fig. 19.1B). For other hosts, such as palms, it may take years to succumb to the pathogen. On rose periwinkle or *Catharanthus roseus*, leaf infections begin with tiny dark green to black lesions on the leaves and increase in size until the leaves become brown, wilted and then completely necrotic. Infections easily enter the stem causing typical dark blights of the foliage and shoots (Hao *et al.*, 2010). Overall spread and development of the disease is rapid, as long as the environment is moist. Cyclamen wilt caused by *P. tropicalis* in Germany and the Netherlands (Gerlach and Schubert, 2001) manifests as a change in leaf colour from dark green to olive green, followed by flagging and yellowing of the entire crown. In this scenario the plants eventually died and *P. tropicalis* was isolated from the corms. Disease was produced when cyclamen, *Epipremnum aureum*, *Dianthus caryophyllus* and *Hedera helix* were inoculated. Hong *et al.* (2008) reported that spraying inoculum on the foliage of pepper and tomato caused no infection, whereas adding inoculum to the root system caused seven of ten pepper plants and nine of ten tomato plants to be infected. In addition, 18% of cucumber plants became infected and aubergine, pepper and tomato fruits were susceptible when inoculum was inserted into wounds (Table 19.2) (Hong *et al.*, 2008).

In Florida the causal agent of *Epipremnum* blight was originally misidentified as *P. capsici*, but, based on the report from Germany, the identification was re-evaluated (Leahy, 2006).

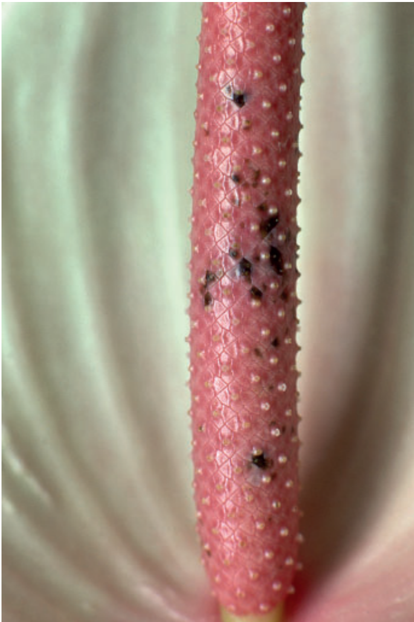
Pothos or *Epipremnum* includes several cultivars popular for hanging baskets, potted indoor plants and ground covers. In recent years pothos has been badly infected with *P. tropicalis* (Fig. 19.1C) or *Phytophthora nicotianae*, and many US nurseries find it more economical to purchase rooted cuttings from foreign countries. Infection often begins in the propagation nurseries where root rots and node infections can go undetected. These infected rooted cuttings are used by many nurseries, and the resulting hanging baskets and landscape ground covers are diseased (Fig. 19.2A). Once on the foliage, sporangia form and are splashed to other leaves or petioles, causing extensive disease (Kadooka *et al.*, 1998). For ornamental plants such as anthurium, even small black blemishes on the flower (spadix) prevent sale of the diseased flowers, leading to large economic losses (Fig. 19.2B).

A study of 1619 nurseries in California revealed 16 species of *Phytophthora* from foliar lesions, including *P. tropicalis* from *Camellia* leaves (Yakabe *et al.*, 2009). The leaf lesions are generally dark, often brown to black, and are not specific to any one species. A similar study of ornamental nurseries in northern and southern Italy found seven species of *Phytophthora*, including *P. tropicalis*, with more than 17 types of popular landscape and ornamental plants infected with crown and root rots (Pane *et al.*, 2005). Other studies also show that *Phytophthora* at ornamental nurseries and landscape nurseries is common (Bush *et al.*, 2006; Olsen and Benson, 2011).

Movement of infected pothos has introduced *P. tropicalis* to vast areas in the USA including Florida, Hawai'i, North Carolina and undoubtedly many other areas. Pothos have a reputation for being vigorous, hardy and easy to maintain, making them highly popular, resulting in a wide distribution of these infected plants. Leahy (2006) determined that *P. tropicalis*

A

B



C

Fig. 19.2. (A) Diseased pothos in the landscape. **(B)** *Anthurium spadix* infected with *Phytophthora tropicalis*. The spadix is composed of many flowers with the ovaries protruding and triangular tepals surround the ovary. **(C)** Macadamia raceme blight caused by *P. tropicalis.*

was found on *Epipremnum* plants propagated in Costa Rica while *P. nicotianae* was found on diseased plants from Guatemala. The common occurrence of *P. nicotianae* can prevent discovery of *P. tropicalis* as the former is assumed to be the pathogen. In Italy 54% of the plants infected with *Phytophthora* were diseased with *P. nicotianae* (Pane *et al.*, 2005), and in North Carolina 56% of the isolates recovered over 2 years from floricultural nurseries were *P. nicotianae* (Olson and Benson, 2011). *P. tropicalis* was found on severely diseased imported carnation and English ivy in Hawai'i and Taiwan, and there is ample evidence that many *Phytophthora* species are moving internationally on ornamental plants (Ann *et al.*, 2010).

19.3 Trees

On macadamia, raceme and leaf blight caused by *Phytophthora* has been known for many years (Fig. 19.2C) (Aragaki and Uchida, 1980). The pathogen survives on twigs or in stem cankers until the following season when new leaves and buds are formed. Macadamia quick decline, caused by *P. tropicalis*, caused serious problems in a large orchard at Keaau on the Island of Hawai'i (Ko, 2009). In 1986 mature macadamia trees had canopies that changed from dark green to light or dull green and eventually became yellow to brown before the trees died within a month or two. Trunks with sap exudate (bleeding) also occurred. The disease spread to other parts of the island and to another island, Maui, and in 1998 about 5000 trees were lost to this disease (Ko, 2009).

On trees it is common for the flower, fruits and young leaves to be infected. This can lead to the production of trunk cankers, which can kill the tree. This has been observed for breadfruit and apricot trees (Pane *et al.*, 2009; Redfern, 2010). In Italy apricot trees were infected by four *Phytophthora* species, with *P. tropicalis* recovered from 20% of the diseased trees. On papaya, fruits are diseased and seedlings can be killed. Mature trees are generally not killed, although stem cankers on the succulent trunk can cause the tree to crack. However, *P. palmivora* is very common in papaya fields and it is difficult to separate effects from *P. palmivora* and *P. tropicalis*. Root rot of palm is a chronic problem and palm trees slowly decline and eventually die when the collar is infected. Seedlings are generally killed a few months after infection.

Black pod rot of cacao is a serious problem (see Drenth and Guest, Chapter 20, this volume), and several *Phytophthora* species are responsible including *P. tropicalis*, *Phytophthora citrophthora*, *P. palmivora* (MF1) and *Phytophthora megakarya*. In Brazil *P. tropicalis* is the most significant species on cacao and has been reported from other Central and South American countries, the West Indies, Indonesia and India (Bowers *et al.*, 2001).

19.4 Disease Management

The best way to limit disease in a nursery setting is to limit the introduction of infected plant material. This can be challenging and requires close monitoring of new plant material through careful inspection and quarantine prior to movement into the main production areas. Overall hygiene is important. As with all the *Phytophthora* species, proper irrigation and drainage is crucial and should be planned when orchards and fields are first designed. Unless the source of water is close to an underground spring, water purification is generally needed before surface water can be used. In general, it is best to avoid using water from streams or rivers as they are frequently contaminated with many plant pathogens, especially *Phytophthora* and *Pythium*. In a recent survey of a commercial nursery in Virginia, ten species of *Phytophthora*, including *P. tropicalis*, were detected in the irrigation reservoir (Ghimire *et al.*, 2011).

Common conditions promoting disease incidence include: (i) excessive moisture; (ii) temperatures of 20–30°C; (iii) monoculture; (iv) absence of sanitation practices;

and (v) movement and mixing of diseased plants and/or planting material. Cultural practices that can help limit disease include:

- increasing air movement in the greenhouse;
- increasing spacing between plants;
- moisture control;
- sanitation practices;
- employee education; and
- preventing pathogen movement by identifying infected plants.

Often, employee education is needed to aid recognition of the early symptoms of disease. In wet weather, or if there is too much irrigation, growth of *Phytophthora* is rapid and succulent plants are easily infected. Irrigation and fertility levels must be controlled and the ground or floor at the nursery kept clean with no weeds, algae, moss, fern growth or rubbish. Controlling slugs and snails is important as they can move the pathogen.

For known diseases of trees grown in the USA or in natural habitats, quarantine is important. Once the disease arrives and is established, it is often impossible to eradicate. Since *P. tropicalis* has not been found on cacao in Hawai'i, seeds or plants should not be imported from locations with this disease. The same is true for strawberry trees (*Arbutus unedo*), which are a known host for *P. tropicalis* and four other *Phytophthora* species, some of which have not been found in Hawai'i (Moralejo *et al.*, 2008). For nurseries and stock plants, diseased plants should be trimmed and placed on benches with good air circulation. In the field, air movement between plants must be sufficient and trees should be trimmed or planted to reduce the overall canopy. Good sanitation reduces spore levels and can help break the disease cycle. If any *Phytophthora* disease occurs in stock plant beds, the entire bed must be replaced. When starting from vegetative cuttings it is important to avoid cuttings with roots in contact with the original medium and to prevent all old media from transferring to new pots or beds. Only completely healthy plants should be transplanted and transplants need to be monitored daily. Any suspicious plants should be immediately removed, along with the surrounding soil or media.

19.5 Distribution and Future Prospects

Losses can be very high for ornamental and landscape plants grown in moist tropical or subtropical environments. Without diagnosis and cultural controls 50–90% of the crop can be infected and many plants discarded. Unfortunately, landscapers often put diseased plants into the environment (Fig. 19.2A). For some crops such as pothos more than 75% of the plants may become diseased and need to be replaced. These plants, that are essentially vines, are easily killed. Similarly when English ivy is diseased large sections of the hanging baskets are killed. The lush growth is rapidly infected and the tip section dies. Baskets are commonly hung at the nursery and this also spreads disease to the crops below. Thousands of dollars are lost to *P. tropicalis* yearly through *Phytophthora* infections of rose periwinkle, English ivy, pothos, anthurium, carnation, palms, *Radermachera*, cigar flowers and cyclamen. A major problem with *P. tropicalis* on ornamentals is movement into the environment on nursery plants used to landscape commercial areas and residential properties. Many sites that require ornamental plants become contaminated, making replacement with healthy crops difficult. Movement of zoospores in streams, ditches and rivers is well known. In a state-wide survey of recycled irrigation water for *Phytophthora* species in Virginia, nine species were recovered including *P. tropicalis* (Bush *et al.*, 2006). Two other surveys documented *Phytophthora cinnamomi*, *Phytophthora citricola*, *P. citrophthora*, *Phytophthora cryptogea*, *Phytophthora megasperma*, *Phytophthora syringae* and *P. nicotianae* (MacDonald *et al.*, 1994), with the species recovered varying by year (Bush *et al.*, 2003).

For some crops, identifying resistant cultivars is proceeding (e.g. cacao, rubber

and breadfruit) and others have improved cultural and chemical controls (e.g. papaya, nursery and landscape crops). In general, it is likely that the distribution of *P. tropicalis* will increase as it travels internationally with ornamental plants. Although less prevalent than some other *Phytophthora* spp. commonly found in nurseries (e.g. *P. nicotianae*), *P. tropicalis* is devastating once it is introduced.

References

Ann, P.-J., Wong, I.-T. and Tsai, J.-N. (2010) New records of Phytophthora diseases of aromatic crops in Taiwan. *Plant Pathology Bulletin* 19, 53–68.

Aragaki, M. and Uchida, J.Y. (1980) Foliar stage of Phytophthora blight of macadamia. *Plant Disease* 64, 483–484.

Aragaki, M. and Uchida, J.Y. (2001) Morphological distinction between *Phytophthora capsici* and *P. tropicalis* sp. nov. *Mycologia* 93, 137–145.

Bowers, J.H., Bailey, B.A., Hebbar, P.K., Sanogo, S. and Lumsden, R.D. (2001) The impact of plant disease on world chocolate production. *Plant Health Progress*. Available at: http://www.apsnet.org/publications/apsnetfeatures/Pages/WorldChocolateProduction.aspx (accessed 10 October 2012).

Bush, E.A., Hong, C. and Stromberg, E.L. (2003) Fluctuations of *Phytophthora* and *Pythium* in components of recycling irrigation system. *Plant Disease* 87, 1500–1506.

Bush, E.A., Stromberg, E.L., Hong, C., Richardson, P.A. and Kong, P. (2006) Illustration of key morphological characteristics of *Phytophthora* species identified in Virginia nursery irrigation water. Online. *Plant Health Progress.* Available at: http://www.plantmanagementnetwork.org/pub/php/diagnosticguide/2006/va/ (accessed 10 October 2012).

Cerquiera, A.O., Luz, E.D.M.N. and De Souza, J.T. (2006) First record of *Phytophthora tropicalis* causing leaf blight and fruit rot on breadfruit in Brazil. *Plant Pathology* 55, 296.

Donahoo, R. and Lamour, K. (2008) Interspecific hybridization and apomixis between *Phytophthora capsici* and *Phytophthora tropicalis*. *Mycologia* 100, 911–920.

Farr, D.F. and Rossman, A.Y. (2012) Fungal databases, systematic mycology and microbiology laboratory, United States Department of Agriculture Agricultural Research Service (USDA-ARS). Available at: http://nt.ars-grin.gov/fungaldatabases/ (accessed 29 February 2012).

Gallegly, M.E. and Hong, C. (2008) Phytophthora*: Identifying Species by Morphology and DNA Fingerprints*. APS Press, St Paul, Minnesota.

Gerlach, W.W.P. and Schubert, R. (2001) A new wilt of cyclamen caused by *Phytophthora tropicalis* in Germany and the Netherlands. *Plant Disease* 85, 334.

Ghimire, S.R., Richardson, P.A., Kong, P., Hu, J., Lea-Cox, J.D., Ross, D.S., Moorman, G.W. and Hong, C. (2011) Distribution and diversity of *Phytophthora* species in nursery irrigation reservoir adopting water recycling system during winter months. *Journal of Phytopathology* 159, 713–719.

Hao, W., Richardson, P.A. and Hong, C. (2010) Foliar blight of annual vinca (*Catharanthus roseus*) caused by *Phytophthora tropicalis* in Virginia. *Plant Disease* 94, 274.

Hong, C., Richardson, P.A. and Kong, P. (2008) Pathogenicity to ornamental plants of some existing species and new taxa of *Phytophthora* from irrigation water. *Plant Disease* 92, 1201–1207.

Kadooka, C.Y., Uchida, J.Y. and Aragaki, M. (1998) New disease of *Epipremnum aureum* caused by *Phytophthora tropicalis*. *Phytopathology* 88, S45 (abstract).

Ko, W.-H. (2009) Nature of slow and quick decline of macadamia trees. *Botanical Studies* 50, 1–10.

Leahy, R.M. (2006) Phytophthora blight of pothos. *Plant Pathology Circular* No. 401. Florida Department of Agriculture and Conservation Service, Division of Plant Industry, Gainsville, Florida.

MacDonald, J.D., Ali-Shtayeh, M.S., Kabashima, J. and Stites, J. (1994) Occurrence of *Phytophthora* species in recirculated nursery irrigation effluents. *Plant Disease* 78, 607–611.

Manohara, D., Mulya, K., Purwantara, A. and Wahyuno, D. (2004) *Phytophthora capsici* [now *P. tropicalis*] on black pepper in Indonesia. In: Drenth, A. and Guest, D.I. (eds) *Diversity and Management of* Phytophthora *in Southeast Asia*. Australian Centre for International Agricultural Research (ACIAR) Monograph 114.

Moralejo, E., Belbahri, L., Calmin, G., Garcia-Munoz, J.A., Lefort, F. and Descals, E. (2008) Strawberry tree blight in Spain, a new disease caused by various *Phytophthora* species. *Journal of Phytopathology* 156, 577–587.

Olsen, H.A. and Benson, D.M. (2011) Characterization of *Phytophthora* spp. on floriculture crops in North Carolina. *Plant Disease* 95, 1013–1020.
Paim, M.C.A., Luz, E.D., De Souza, J.T., Cerqueira, A.O. and Lopes, J.R. (2006) Pathogenicity of *Phytophthora* species to *Anthurium andraeanum* in Brazil. *Australasian Plant Pathology* 35, 275–277.
Pane, A., Martini, P., Cimento, A., Rapetti, S., Savona, S., Grasso, F.M. and Cacciola, S.O. (2005) *Phytophthora* species on ornamental plants in Italy. *Journal of Plant Pathology* 87, 301.
Pane, A., Cacciola, S.O., Chimento, A., Allatta, C., Scibetta, S. and Magnano di San Lio, G. (2008) First report of *Phytophthora* spp. as pathogens of *Pandorea jasminoides* in Italy. *Plant Disease* 92, 313.
Pane, A., Cacciola, S.O., Scibetta, S., Bentivenga, G. and Magnano di San Lio, G. (2009) Four *Phytophthora* species causing foot and root rot of apricot in Italy. *Plant Disease* 93, 844.
Redfern, T.M. (2010) Etiological study of breadfruit diseases in Hawai'i. MSc thesis, Tropical Plant Pathology Program at the University of Hawai'i, Honolulu, Hawai'i.
Sdoodee, R. (2004) Phytophthora diseases of rubber. In: Drenth, A. and Guest, G.I. (eds) *Diversity and Management of* Phytophthora *in Southeast Asia*. Australian Centre for International Agricultural Research (ACIAR) Monograph 114.
Uchida, J.Y. and Aragaki, M. (1980) Chemical stimulation of oospore formation in *Phytophthora capsici*. *Mycologia* 72, 1103–1108.
Uchida, J.Y. and Aragaki, M. (1989) Comparison of pepper isolates of *Phytophthora capsici* from New Mexico to other solanaceous and non-solanaceous isolates. Paper presented at the 1989 Annual Meeting of the American Psychological Society, Richmond, Virginia. *Phytopathology* 79, 1212 (abstract).
Yakabe, L.E., Blomquist, C.L., Thomas, S.L. and MacDonald, J.D. (2009) Identification and frequency of *Phytophthora* species associated with foliar diseases in California ornamental nurseries. *Plant Disease* 93, 883–890.
Zhang, Z.G., Zhang, J.Y., Zheng, X.B., Yang, Y.W. and Ko, W.H. (2004) Molecular distinction between *Phytophthora capsici* and *Ph. tropicalis* based on ITS sequences of ribosomal DNA. *Journal of Phytopathology* 152, 358–364.

20 *Phytophthora palmivora* in Tropical Tree Crops

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20.1 Introduction

Although most Phytophthora diseases were first described in temperate regions, it is likely that many originated in the tropics where they cause devastating losses to a variety of hosts, including tropical tree crops. Losses to *Phytophthora* in the tropics are significant but not well documented. Annual global losses to the cocoa industry are estimated to be around 450,000 t, valued at over US$1 billion (Drenth and Guest, 2004; IPARC, 2012). In South-east Asia (Thailand, Malaysia, Indonesia, Vietnam, the Philippines) the economic impact of *Phytophthora* on cocoa, durian, rubber, coconut, pepper, citrus and potato was estimated to be at least US$2.3 billion (Drenth and Sendall, 2004). Losses due to *Phytophthora* ranged from 5–10% for coconut and black pepper, to 15–25% for rubber, durian and cocoa.

This chapter provides an overview of the key factors driving the epidemiology of *Phytophthora* on tropical tree crops including the fundamental biology, relevant environmental factors and agronomic practices unique to these systems. The example of a small cocoa farmer, Pak Aryadi, is used as a case study to put the tropics situation in perspective and to suggest disease management strategies appropriate for smallholder farmers.

20.2 Cocoa Black Pod Disease

Pak Aryadi is a cocoa farmer in Sulawesi, a large tropical Indonesian island that produces about 20% of the world's cocoa. Like most cocoa-growing areas, Sulawesi has constant warm temperatures and a wet season from November to April when most of the annual 3000 mm of rain falls. Pak Aryadi's cocoa trees flower throughout the year. Five months after flowering, the pods ripen and are harvested. The harvest peaks between May and July with a second peak between November and December. Harvesters scoop out the cocoa beans and discard the empty pod cases in piles among the trees (Fig. 20.1A and Fig. 20.2A). Each year at the start of the wet season, black lesions appear on Aryadi's crop of ripening cocoa pods and within a few days these pods are rotten (Fig. 20.1B). Symptomatic pods can be processed during the early stages of infection, which leads to infected pod cases being spread among the trees. Pods in the later stages of infection rot internally and, being worthless, are often left on the tree.

The disease, known as black pod, is caused by *Phytophthora palmivora*. In an average year *P. palmivora* will reduce the yield of pods by 30% (Erwin and Ribeiro, 1996; Guest, 2007). In addition to pod rot, *P. palmivora* also devastates nurseries

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A

B

C

Fig. 20.1. (A) Splitting cocoa pods. **(B)** Phytophthora pod rot of cocoa. **(C)** Seedling blight in the nursery.

A

B

Fig. 20.2. **(A)** Piles of empty pod cases become breeding sites for flying beetles and sources of *Phytophthora* inoculum. **(B)** Stem canker, with the bark removed to show the underlying lesion.

of young seedlings (Fig. 20.1C), causes debilitating cankers (Fig. 20.2B), and causes leaf blight and chupon wilt on mature trees. Collectively, these significantly reduce Pak Aryadi's annual income. *Phytophthora* species also cause significant damage to nearby crops including durian, rubber, coconut, oil palm, black pepper, citrus, papaya and pineapple. Small farmers like Aryadi want to know why *Phytophthora* is such a devastating pathogen of tropical tree crops and what they can do to protect their crops.

20.3 *Phytophthora* in the Tropics

Phytophthora is a water-borne heterokont straminipile related to the chlorophyll c-containing algae (Gunderson *et al.*, 1987) in the Kingdom *Chromista* (Dick, 2001; Beakes *et al.*, 2012). Although many species are soil-borne, *Phytophthora* species on tropical tree crops are predominantly aerial, causing leaf blights, bark cankers, chupon wilts and fruit rots. Chupons are new, upright-growing shoots that grow on the tree stem or the larger branches. Many factors contribute to the success of *Phytophthora* in the tropics. These include: (i) constantly warm temperatures; (ii) cloud cover; (iii) extended periods of high humidity; (iv) frequent rainfall; and (v) occasional cyclonic weather events that promote the production and dissemination of sporangia. Susceptible host tissue (pods, leaves, flowers and bark) are always present and the disease cycle continues throughout the year. Within 5 days an infected cocoa pod can produce up to four million sporangia, each containing 30–40 infective zoospores (Medeiros, 1976).

In the wet tropics *Phytophthora* species do not need thick-walled chlamydospores or oospores to survive dry or fallow periods, as is common during the winter in temperate parts of the world. Nevertheless, these propagules aid survival during the dry season. Chlamydospores in mummified pods and cankers germinate and mass-produce sporangia once the rains start following a dry period (Brasier *et al.*, 1981). Most cocoa farmers do not harvest mummified pods in their cocoa plantations, leaving a huge reservoir of primary inoculum for the next wet season.

Although several of the *Phytophthora* species common in the tropics, including *P. palmivora*, are heterothallic and both mating types are present (Zentmyer *et al.*, 1973), oospores are rarely reported and their role as a source of inoculum is poorly understood. Plantation agriculture using endemic as well as exotic tree crop species may place a unique selection pressure to favour a limited range of genotypes able to 'jump' into the canopy and establish parasitic infection and cause epidemics. Despite limited pathogen diversity in the canopy, there may be wide genetic diversity present in the soil where root infection and saprophytic growth provide the major sources of nutrition for *Phytophthora*. In South-east Asia, monoculture plantations of South American cocoa provide a strong selection pressure for virulent genotypes adapted to aerial dispersal. Although damage to cocoa roots has been reported (Turner, 1965), and *P. palmivora* survives as a saprophyte in the soil of cocoa plantations (Konam and Guest, 2002), infected roots are merely a platform for individual virulent genotypes to migrate into the tree canopy where most damage occurs. Once the jump from the soil to the canopy has occurred, clonal reproduction occurs at a very high level. Thus, most studies of pathogenic *Phytophthora* populations in the tropics reveal scant evidence of sexual recombination (Arentz, 1986; Truong *et al.*, 2010).

The centre of origin for most species of *Phytophthora* is unknown but we do know that the spread of plantation crops such as cocoa has allowed some *Phytophthora* species (e.g. *P. palmivora*) to follow. This has occurred since colonial times and large areas of the tropics are infected (CABI, 2007). Smallholders like Aryadi have limited cultivatable land and have no option but to replant back into *Phytophthora*-infested soils. Compounding Aryadi's problem is the fact that his planting material is usually diseased prior to planting. This is due to a variety of factors including: (i) poor

nursery hygiene; (ii) the use of soil from orchards as potting mix; (iii) placement of pots on the ground; (iv) irrigation with infested surface water; (v) dense spacing; and (vi) failure to remove infected plants. Each of these can lead to infected plant material and a poor start for the tree crop.

In addition to an ideal setting for disease where there is an aggressive pathogen, susceptible hosts and conducive environment, there are several agronomic practices that further exacerbate *Phytophthora* disease problems. Shade crops are planted to protect young foliage and fruit quality from the high light intensity – increasing the humidity in the canopy. To compensate for disease losses, trees are sometimes spaced closer together, creating humid conditions and further exacerbating the problem. Often, tree crops are grown in areas with poor drainage where surface water ponds during heavy rainfall. This allows spores (sporangia and zoospores) to splash into the lower canopy. Crops like black pepper, citrus and durian trees are sometimes planted into a slight hollow to facilitate irrigation during the dry season. This depression holds the irrigation water but also allows the bare soil to be baked hard under the tropical sun. During the wet season the hollow retains water at the surface, stressing trees and providing ideal conditions for sporangial production, splash dispersal and zoospore release.

20.4 Disease Cycle of *Phytophthora* in the Tropics

To effectively manage *Phytophthora* on tropical tree crops it is crucial to understand the epidemiology. At the start of the wet season inoculum is produced on stem cankers (Fig. 20.2B) and unharvested mummified fruit in the canopy (Fig. 20.1B). During high humidity and rainfall chlamydospores germinate to form sporangia on the exposed surfaces (Maddison and Griffin, 1981). A large International Cocoa Black Pod Research Project found that *Phytophthora* moves from the soil into the canopy through: (i) rain splash; (ii) tent-building ants; (iii) insects; (iv) rodents; and (v) cultural practices (Gregory and Maddison, 1981).

Rain splash is important to move sporangia or zoospores from the soil level into the canopy. Zoospores in particular are easily splashed and will rapidly encyst and stick to the plant surface, and then germinate and infect the host plant (Sing and Bartnicki-Garcia, 1975). Rain splash is often correlated with infection of fruit and leaves that are low in the canopy, typically up to about 70 cm above soil level (Gregory *et al.*, 1985). Once fruit and leaves in the lower canopy are infected they produce sporangia that are spread further up the canopy through wind and wind-driven aerosol.

In tropical tree crops there appears to be a link between insect activity and the occurrence of *Phytophthora*. Although poorly understood, insects can carry inoculum from the soil and from sporulating lesions on fruit hanging in the trees or lying discarded on the ground (Fig. 20.2A). Several different species of ants (e.g. *Crematogaster*, *Iridomyrex* and *Soleopsis*) build tents of mud when they move up the trunk and frequently incorporate soil infected with *Phytophthora* into their construction material (Taylor and Griffin, 1981; McGregor and Moxon, 1985). Fruit and stem borers create *Phytophthora*-contaminated frass that is readily rain splashed and blown by the wind (Konam and Guest, 2004). A strong association has been reported between larval channels made by *Panthorhytes* weevils and cankers on cocoa. Borers and nematodes may also provide entry points that facilitate penetration of the bark by *Phytophthora* (Prior and Smith, 1981).

20.5 Management

Unfortunately, there is not a single solution or simple remedy to manage *Phytophthora* on tropical tree crops. The situation is complex due to: (i) the biology of the pathosystem; (ii) economics; (iii) politics; and (iv) human behaviour. At the minimum, improved control will require

significant changes in cultural practices and a serious commitment to improved crop management.

20.5.1 Hygiene

New plantations must be started with pathogen-free planting material. Potting mixes should be pathogen-free, either through the process of sterilization or composting to reach temperatures high enough to kill *Phytophthora* (Hoitink and Fahy, 1986). Growers need to plant disease-free cuttings or seeds from disease-free fruit into clean potting mix and then continue to keep it clean by placing the pots at least 80 cm off the ground to prevent infection from rain splash. Infected plant material should be removed immediately from the nursery to prevent any further infection. The application of systemic fungicides in *Phytophthora*-free nurseries is simply not needed. In fact, the danger of using systemic fungicides is that they mask the presence of the pathogen. Once the temporary suppression wears off, *Phytophthora* rapidly increases after the trees are transplanted into the field (Graham and Timmer, 1994). The use of systemic fungicides in nurseries with poor hygiene practices is a major factor in the local and global spread of many *Phytophthora* pathogens.

Pathogen-free planting material must be placed into a properly prepared planting site. The site should be well drained, both vertically and horizontally, to avoid ponding and the subsequent anaerobic stress that allows *Phytophthora* to proliferate (McDonald *et al.*, 2002). Ideally the soil should be drained to a depth of 1.5 m. Drainage can be improved through the construction of ditches, and/or planting the trees on raised beds or rows. To reduce the inoculum load and the presence of secondary inoculum it is important to harvest cocoa pods frequently and remove all pods that show visible signs of disease. Once cocoa pods are infected it takes about 4 days to become completely colonized by *P. palmivora* and produce abundant inoculum. Piles of rotting fruit are a breeding ground for flying beetles able to vector *Phytophthora* (Konam and Guest, 2004), and disposed fruit and pod cases should be buried or composted.

Effective management is based on clean planting material, soil, water and nutrient management, regular pruning, harvesting, sanitation and hygiene. Clean planting material must be propagated in sterilized substrates, grown on raised benches, and include strict hygiene with rigorous removal and destruction of diseased plants. Drainage should be improved in the orchard before planting and mounds built so that newly planted seedlings are not flooded during rain or irrigation. Growing trees in the plantation and shade trees need to be pruned, especially before the wet season, to allow adequate ventilation, and soil organic matter should be supplemented with manures and composts. Cankers should be scraped and treated through application of copper and/or phosphonate to the affected area, and any dead trees removed and burnt. Harvesters should not leave diseased fruit on the trees, as these mummified fruit provide primary inoculum for future epidemics. Unsaleable fruit should be composted or fermented and then used as a soil amendment in the orchard. Curiously, while sanitation is taken for granted in the management of human disease epidemics, the same understanding is rarely applied in tropical horticulture.

20.5.2 Soils

Soils containing high levels of salt should be avoided as salinity is a major predisposing factor to disease from *Phytophthora* (Blaker and MacDonald, 1986). To stimulate plant root growth, organic matter is important. Ammonia and other volatile organic acids released by decomposing organic matter can kill *Phytophthora* spores and stimulate competitive and antagonistic microorganisms in the soil (Lazavorits, 2001). The addition of composted chicken manure has been reported to significantly reduce the survival of *Phytophthora* and the development of disease (Aryantha *et al.*, 2000).

After planting, it is important to mulch trees to: (i) stimulate root growth; (ii) increase nutrient uptake; (iii) increase the soil water-holding capacity; (iv) regulate soil temperature; and (v) decrease evaporation from the soil surface. Companion or cover cropping should be considered to reduce the impact of *Phytophthora* disease. The companion crop should not compete too much with the cocoa but it is important to: (i) keep the soil covered; (ii) increase the amount of organic material in the soil; (iii) encourage the growth of microbes that suppress *Phytophthora*; and (iv) prevent the splashing of *Phytophthora* propagules in the canopy. Fertilization of tropical tree crops must be done carefully. The role of fertilization and nutrients in controlling or suppressing Phytophthora diseases is unclear, but high levels of inorganic nitrate appear to accentuate *Phytophthora* damage (Lee and Zentmyer, 1982; Schmitthenner and Canaday, 1983, Sugimoto *et al.*, 2007). A good example of how the combined measures of careful planting, mulching and fertilization can help control *Phytophthora* was initially shown by the Ashburner system developed in Australia and detailed in Cook and Baker (1983).

20.5.3 Host plants

The choice of planting material is also very important. With tree crops this often includes choosing an appropriate rootstock and an appropriate scion. Phytophthora diseases, notably root diseases, can sometimes be effectively controlled using resistant rootstocks (e.g. citrus and avocado) (Wheaton *et al.*, 1991; Whiley *et al.*, 2002).

Resistance is often touted as the ultimate solution to control Phytophthora diseases. For tree crops like cocoa, which are susceptible to a range of different Phytophthora diseases on multiple plant parts, the search for resistance is challenging. The germplasm of fruit trees has often been domesticated from limited wild genetic material carefully selected to produce high yields of desirable fruit. Quality in fruit is not easily defined, and selecting for quality fruit and for resistance to *Phytophthora* is challenging. Disease is often considered an ‘externality’ by breeders and resistance usually comes at the expense of yield or quality. Modern breeding has had a strong focus on yield under low disease pressure or yield attained with the help of agrochemicals. Many such varieties do not yield well when the protective shield of agrochemicals is removed.

Another factor limiting tree breeding is that a newly planted seedling may take 5 years to produce fruit. This means growers must be prepared, to a much larger degree than a producer of annual crops, to manage the Phytophthora diseases in their orchards. Aryadi has to protect this year’s cocoa harvest and maintain the productive capacity of his trees for years to come.

Since *Phytophthora* in cocoa is mainly an aerial pathogen, the emphasis for breeding needs to be on resistance in the scion. Many small farmers know that most of their trees (planted as a range of different clones on seedling rootstock) are quite susceptible. But, at the same time, it is common to observe trees with much lower levels of disease. One inexpensive option is to cut back the canopy of his most susceptible trees and graft on to these the bud wood from his most resistant trees. This way the farmer can improve the overall resistance in a small orchard over a relatively short time and reduce his losses (Pinto *et al.*, 1996).

20.5.4 Chemical and biological control

Fungicides such as metalaxyl, phosphonate and copper can provide good control of *P. palmivora* on cocoa (Guest *et al.*, 1994; Opuku *et al.*, 2004); however, chemicals are not widely used in managing Phytophthora diseases of tropical tree crops. In addition to cost, trees have to be sprayed when disease pressure is highest, typically during periods of high rainfall when plants are growing rapidly and the canopy is dense. In addition, the farmers often use older, less-efficient equipment and there is poor coverage of the

overall plant canopy. Many smallholder farmers lack training or access to advice on pesticide use, and may also lack the resources to implement effective management using agrochemicals. Spraying one farm is of limited value when neighbouring farms are not sprayed, as sporangia of *Phytophthora* effectively move around in rain splash (Hunter and Kunimoto, 1974; Timmer *et al.*, 2000). Regional spraying programmes, such as that implemented in cocoa-growing areas of Ghana, are most effective when part of an overall strategy that includes training farmers in integrated farm and crop management.

In practice, biocontrol using selected isolates of hyperparasites as biopesticides has not been successful. *Phytophthora* epidemics are explosive during wet weather and therefore always outrun the biocontrol agent. Although many biocontrol agents are touted as silver bullets, few, if any, significantly reduce the impact of *Phytophthora* when tested in rigorous independent scientific field trials. Far more promising results have come when agronomic practices are changed to encourage disease-suppressive soils that are rich in a diverse range of antagonists while at the same time promoting root growth. Improved drainage, compost and manure applications, and careful nutrient management stimulate soil microbial activity and soil health (Cook and Baker, 1983).

20.6 Future Perspectives

Understanding the complex interplay of the pathogen, the environment, the host and the current cultural practices in plantations is crucial to manage *Phytophthora* in the tropics. To be successful, Pak Aryadi, and other smallholder farmers, need to consider the agricultural production system as a whole and must pay close attention to detail during every stage of the farming process. If tropical fruit farmers are educated about the factors above, and are willing to invest the time and effort to carefully address each stage of fruit production, they can reduce the impact of *Phytophthora* and produce more fruit on their small plots without costly external inputs.

References

Arentz, F. (1986) A key to *Phytophthora* species found in Papua New Guinea, with notes on their distribution and morphology. *Papua New Guinea Journal of Agriculture, Forests and Fisheries* 34, 9–18.

Aryantha, I.P., Cross, R. and Guest, D.I. (2000) Suppression of *Phytophthora cinnamomi* in potting mixes amended with uncomposted and composted animal manures. *Phytopathology* 90, 775–782.

Beakes, G.W., Glockling, S.L. and Sekimoto, S. (2012) The evolutionary phylogeny of the oomycete 'fungi'. *Protoplasma* 249, 3–19.

Blaker, N.S. and MacDonald, J.D. (1986) The role of salinity in the development of Phytophthora root rot of citrus. *Phytopathology* 76, 970–975.

Brasier, C.M., Griffin, M.J. and Maddison, A.C. (1981) The cocoa black pod Phytophthoras. In: Gregory, P.H. and Maddison, A.C. (eds) *Epidemiology of* Phytophthora *on Cocoa in Nigeria. Phytopathological Paper* No. 25. Commonwealth Mycological Institute, Kew, Surrey, UK, pp. 18–30.

CABI (2007) *Crop Protection Compendium, Global Module*, 2nd edition. CAB International, Wallingford, Oxon, UK.

Cook, J.R. and Baker, K.F. (1983) *The Nature and Practice of Biological Control of Plant Pathogens*. APS Press, St Paul, Minnesota, 539 pp.

Dick, M.W. (2001) *Straminipilous Fungi*. Kluwer Academic Publishers, Dordrecht, the Netherlands, 670 pp.

Drenth, A. and Guest, D.I. (2004) *Phytophthora* in the tropics. In: Drenth, A. and Guest, D.I. (eds) *Diversity and Management of* Phytophthora *in Southeast Asia. Australian Centre for International Agricultural Research (ACIAR) Monograph* 114. ACIAR, Canberra, pp. 30–41.

Drenth, A. and Sendall, B. (2004) Economic impact of Phytophthora diseases in Southeast Asia. In: Drenth, A., Guest, D.I. (eds) *Diversity and Management of* Phytophthora *in Southeast Asia. Australian Centre for International Agricultural Research (ACIAR) Monograph* 114. ACIAR, Canberra, pp. 10–28.

Erwin, D.C. and Ribeiro, O.K. (1996) *Phytophthora Diseases Worldwide*. APS Press, St Paul, Minnesota, 562 pp.

Graham, J.H. and Timmer, L.W. (1994) *Phytophthora Diseases of Citrus*. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Florida, USA. Available at: http://edis.ifas.ufl.edu (accessed 28 February 2012).

Gregory, P.H. and Maddison, A.C. (eds) (1981) *Epidemiology of* Phytophthora *on Cocoa in Nigeria*. *Phytopathological Paper* No. 25. Commonwealth Mycological Institute, Kew, Surrey, UK.

Gregory, P.H., Griffin, M.J., Maddison, A.C. and Ward, M.R. (1985) Cocoa black pod: a reinterpretation. *The Planter* 61, 195–197.

Guest, D.I. (2007) Black pod: diverse pathogens and the greatest global impact on cocoa yield. *Phytopathology* 97, 1650–1653.

Guest, D.I., Anderson, R.D., Ford, H.J., Phillips, D., Worboys, S. and Middleton, R.M. (1994) Long-term control of Phytophthora diseases of cocoa using trunk-injected phosphonate. *Plant Pathology* 43, 479–492.

Gunderson, J.H., Elwood, H., Ingold, A., Kindle, K. and Sogin, M.L (1987) Phylogenetic relationships between chlorophytes, chrysophytes, and oomycetes. *Proceedings of the National Academy of Sciences USA* 84, 5823–5827.

Hoitink, H.A.J. and Fahy, P.C. (1986) Basis for the control of soilborne plant pathogens with composts. *Annual Review of Phytopathology* 24, 93–114.

Hunter, J.E. and Kunimoto, R.K. (1974) Dispersal of *Phytophthora palmivora* sporangia by wind-blown rain. *Phytopathology* 64, 202–206.

International Pesticide Application Research Consortium (IPARC) (2012) The world's worst cocoa problems. IPARC, Imperial College, Ascot, Berks, UK. Available at: www.dropdata.org/cocoa/cocoa_prob.htm#Phytophthora (accessed 4 October 2012).

Konam, J.K. and Guest, D.I. (2002) Leaf litter mulch reduces the survival of *Phytophthora palmivora* under cocoa trees in Papua New Guinea. *Australasian Plant Pathology* 31, 381–383.

Konam, J.K. and Guest, D.I. (2004) Role of flying beetles (Coleoptera: Scolytidae and Nitidulae) in the spread of Phytophthora pod rot of cocoa in Papua New Guinea. *Australasian Plant Pathology* 33, 55–59.

Lazarovits, G. (2001) Management of soil-borne plant pathogens with organic soil amendments: a disease control strategy salvaged from the past. *Canadian Journal of Plant Pathology* 23, 1–7.

Lee, B.S. and Zentmyer, G.A. (1982) Influence of calcium nitrate and ammonium sulfate on Phytophthora root rot of *Persea indica*. *Phytopathology* 72, 1558–1564.

Maddison, A.C. and Griffin, M.J. (1981) Detection and movement of inoculum. In: Gregory, P.H. and Maddison, A.C. (eds) *Epidemiology of* Phytophthora *on Cocoa in Nigeria*. *Phytopathological Paper* No. 25. Commonwealth Mycological Institute, Kew, Surrey, UK, pp. 31–50.

McDonald, K.L., Sutherland, M.W. and Guest, D.I. (2002) Temporary hypoxia suppresses the oxidative burst and subsequent hypersensitive cell death in cells of tobacco and soybean challenged with zoospores of incompatible isolates of *Phytophthora* species. *Physiological and Molecular Plant Pathology* 61, 133–140.

McGregor, A.J. and Moxon, J.E. (1985) Potential for biological control of tent-building species of ants associated with *Phytophthora palmivora* pod rot of cocoa in Papua New Guinea. *Annals of Applied Biology* 107, 271–277.

Medeiros, A.G. (1976) Sporulation of *Phytophthora palmivora* (Butl.) Butl. in relation to epidemiology and chemical control of cacao black pod disease. Comissão Executiva do Plano da Lavoura Cacaueira (CEPLAC), Centro de Pesquisas do Cacao, Setor sudoeste, Campus do Inmet, Cruzeiro, Brasil.

Opuku, I.Y., Akrofi, A.Y., Holderness, M. and Holmes, K.A. (2004) Phosphonic acid: an alternative approach to the control of black pod disease of cocoa caused by *Phytophthora megakarya*. In: Akrofi, A.Y., Ackonor, J.B. and Ollenu, L.A.A. (eds) *Proceedings of the 4th International Permanent Working Group for Cocoa Pests and Diseases (INCOPED) Seminar 'Dealing with Pressing Crop Protection Problems'*, Accra, Ghana, 19–21 October 2003. Ghana Cocoa Board, Accra, Ghana, pp. 59–69.

Pinto, L.R.M., Lopes, U.V., Monteiro, W.R. and Luz, E.D.M.N. (1996) Selection of cacao genotypes resistant to witches' broom under field conditions. In: Proceedings of 12th International Cocoa Research Conference, Salvador-Bahia, Brazil, 17–23 November 1996, Abstract p. 469.

Prior, C. and Smith, E.S.C. (1981) Association of *Phytophthora palmivora* bark canker and insect damage in cocoa in Papua New Guinea. *Annals of Applied Biology* 97, 27–30.

Schmitthenner, A.F. and Canaday, C.H. (1983) Role of chemical facts in the development of Phytophthora diseases. In: Erwin, D.C., Bartnicki-Garcia, S. and Tsao, P.H. (eds) Phytophthora: *Its Biology, Taxonomy, Ecology, and Pathology*. American Phytopathological Society Press, St Paul, Minnesota, pp. 189–196.

Sing, V.O. and Bartnicki-Garcia, S. (1975) Adhesion of *Phytophthora palmivora* zoospores: detection and ultrastructural visualization of concanavalin A-receptor sites appearing during encystment. *Journal of Cell Science* 19, 11–20.

Sugimoto, T., Watanabe, K., Yoshida, S., Aino, M., Matsuyama, M., Maekawa, K. and Irie, K. (2007) The effects of inorganic elements on the reduction of Phytophthora stem rot disease of soybean, the growth rate and zoospore release of *Phytophthora sojae*. *Journal of Phytopathology* 155, 97–107.

Taylor, B. and Griffin, M.J. (1981) The role and relative importance of different ant species in the dissemination of black pod disease of cocoa. In: Gregory, P.H. and Maddison, A.C. (eds) *Epidemiology of* Phytophthora *on Cocoa in Nigeria*. *Phytopathological Paper* No. 25. Commonwealth Mycological Institute, Kew, Surrey, UK, pp. 114–131.

Timmer, L.W., Zitko, S.E., Gottwald, T.R. and Graham, J.H. (2000) Phytophthora brown rot of citrus: temperature and moisture effects on infection, sporangium production, and dispersal. *Plant Disease* 84, 157–163.

Truong, N.V., Liew, E.C. and Burgess, L.W. (2010) Characterisation of *Phytophthora capsici* isolates from black pepper in Vietnam. *Fungal Biology* 114, 160–170.

Turner, P.D. (1965) Behaviour of *Phytophthora palmivora* in soil. *Plant Disease Reporter* 49, 135–137.

Wheaton, T.A., Castle, W.S., Whitney, J.D. and Tucker, D.P.H. (1991) Performance of citrus scion cultivars and rootstock in a high-density planting. *HortScience* 26, 837–840.

Whiley, A.W., Schaffer, B. and Wolstenholme, B.N. (2002) *The Avocado: Botany, Production and Uses*. CAB International, Wallingford, Oxon, UK, 416 pp.

Zentmyer, G.A., Mitchell, D.J., Jefferson, L., Roheim, J. and Carnes, D. (1973) Distribution of mating types of *Phytophthora palmivora*. *Phytopathology* 63, 663–667.

21 Phytophthora Root Rot of Avocado

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21.1 Introduction

Avocado, *Persea americana* Miller, is a nutritious and economically important fruit crop in the subtropics and tropics. About 3.84 million metric t (MMT) were produced worldwide in 2010 and, in descending order, Mexico, Chile, the Dominican Republic, Indonesia, Colombia, Peru, Brazil, the USA, Kenya and China were the top ten producers (FAO, 2012). Production in Mexico alone was valued at US$645 million.

Three botanical races of avocado are recognized (Knight, 2002). The Mexican (M) (*P. americana* var. *drymifolia*) and Guatemalan (G) (*P. americana* var. *guatemalensis*) races originated in the respective highlands of those countries, whereas the West Indian (WI) or Antillean race (*P. americana* var. *americana*) arose on the Pacific coast of Central America. Due to environmental adaptations and marketing histories, different cultivars are grown in different regions. The world export trade is dominated by a single M×G cultivar, 'Hass', but diverse M, G, WI and interracial hybrids are locally or regionally important. Although fruit are produced on trees that were established as seedlings worldwide, commercial production relies on clonal scions of the various cultivars grafted on seedlings and, increasingly, clonal rootstocks.

Phytophthora root rot (PRR), caused by *Phytophthora cinnamomi* Rands, is the most important constraint to avocado production worldwide (Zentmyer, 1980; Coffey, 1992; Menge and Ploetz, 2003; Dann *et al.*, 2012). It was first reported in Puerto Rico in 1927 (Tucker, 1929) and is now found in all regions in which this crop is grown. The disease has eliminated commercial avocado production in many areas in tropical America and is a primary limiting factor in other producing areas (Ploetz *et al.*, 2002). Coffey (1992) estimated annual losses due to PRR in California in excess of US$40 million. The disease also affects production of plants in the nursery and has decimated repositories of avocado germplasm; for example, important collections have been lost at the United States Department of Agriculture - Agricultural Research Service (USDA-ARS) station in Isabella, Puerto Rico, and the Pan American School of Agriculture in Zamorano, Honduras.

21.2 Symptoms

The pathogen infects root tips and wounded portions of avocado feeder roots, causing discrete brownish-black lesions (Zentmyer, 1980; Menge and Ploetz, 2003; Dann *et al.*, 2012). As the disease progresses, much of the feeder root system becomes black and brittle. On individual roots, lesions extend throughout the root cortex but do not extend past the stele. In advanced stages, feeder roots are scarce and soil underneath affected trees becomes wet. PRR reduces tree vigour, as trees without feeder roots are unable to absorb water and nutrients, and because

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affected trees expend increasingly scarce carbohydrate reserves to replace lost roots. Eventually, a critical threshold is reached where the roots and leaves needed to sustain the tree cannot be regenerated. Large, woody roots usually remain unaffected and functional until the tree dies.

Foliar symptoms include small leaves that wilt frequently and have brown necrotic tips (Fig. 21.1). During periods of water stress or increased hydraulic demand (e.g. drought or during flowering and fruit set) large portions of the canopy may defoliate. New vegetative growth in severely affected trees is uncommon. Eventually, entire limbs die and trees are sparsely foliated. Fruit yield declines, although high numbers of small, unmarketable fruit may be set in some trees, and trunk cankers can form at the root collar. In flooded soils PRR-affected trees may wilt suddenly, with leaves remaining attached for several weeks after the trees are killed (Ploetz and Schaffer, 1989). The profound interaction between PRR and flooding is discussed below.

21.3 Causal Agent(s)

Although *P. cinnamomi* is the primary cause of root rot on avocado, other species in the genus can cause minor to moderate damage to roots and/or the root collar, including *Phytophthora boehmeriae*, *Phytophthora heveae*, *Phytophthora mengei* (formerly *citricola*) and *Phytophthora palmivora* (Menge and Ploetz, 2003). *P. cinnamomi* is in clade 7b of the genus

Fig. 21.1. An avocado tree in Queensland, Australia, severely affected by Phytopthora root rot (PRR). Note the sparsely foliated canopy and wilted, small leaves, and in the upper portions of the canopy the prevalence of tip-burned leaves, another aboveground symptom of PRR.

(Kroon *et al.*, 2012). It is thought to have originated in New Guinea (Martin and Coffey, 2012), although an alternative hypothesis indicates a South African origin (Erwin and Ribeiro, 1996). Clearly, the pathogen has been disseminated widely by man and is not native to most areas in which it is now found, including the Meso-American centres of origin for avocado; thus, high levels of co-evolved resistance to PRR are not available in this crop (Ploetz, 2007).

In 1922 Rands described *P. cinnamomi* on *Cinnamomum burmanii* in Sumatra (Zentmyer, 1980). The pathogen is now known to affect more than 3000 other plant species, including economically significant forest, agricultural and horticultural hosts such as azalea, chestnut, macadamia, pineapple and rhododendron (Hardham 2005; Dann *et al.*, 2012). However, its greatest impact has been in the jarrah forests of Australia in which eucalyptus-dominated landscapes and unrelated understorey plants have been devastated over the last several decades (Zentmyer, 1980; Hee *et al.*, Chapter 14, this volume).

Cardinal growth temperatures are 5–15°C, 20–32.5°C and 30–36°C, and it produces distinctive corraloid mycelium (Zentmyer, 1980; Menge and Ploetz, 2003; Dann *et al.*, 2012). Its non-papillate, non-caducous sporangia are elliptical to ovoid but are rarely formed in culture; they range from 43–75 μm long to 24–47 μm wide, with a length:breadth ratio of 1.54. Terminal and intercalary chlamydospores, 31–50 μm (mean 40 μm) in diameter, are abundant in culture and form in botryose clusters. The chlamydospore cell walls are much thinner than those produced by many other species of *Phytophthora*. Hyphal swellings can also be abundant. *P. cinnamomi* is heterothallic. Oogonia are 21–58 μm (mean 40 μm) in diameter, antheridia are amphigynous and oospores are plerotic. *P. cinnamomi* can be recovered on semi-selective media, such as PARPH (pimaricin, ampicillin, rifamycin, pentachlorornitrobenzene and hymexazol added to a cornmeal agar base), or by baiting with green avocado fruit, lupin seedlings or other vegetative baits (Zentmyer, 1980; Menge and Ploetz, 2003; Dann *et al.*, 2012). Recently, more rapid and specific tests have been developed for detection and identification (Dann *et al.*, 2012; Kroon *et al.*, 2012).

21.4 Epidemiology

PRR develops between 30°C and 12°C, and is most severe in cooler temperatures where *P. cinnamomi* grows better than avocado (Zentmyer, 1980; Menge and Ploetz, 2003; Dann *et al.*, 2012). Disease develops in diverse soils, under a wide range of nutritional conditions, and between pH 3.5 and 8.0. However, PRR development can be inhibited in soils with high levels of organic matter, ammonia or calcium. In virtually all soils in which *P. cinnamomi* is now found it has been introduced by humans. Although the pathogen can move in infested soil, tools and irrigation water, it is most commonly moved to new production areas in nursery stock. Chlamydospores are the primary propagule in decaying roots and soil (Dann *et al.*, 2012). They germinate directly with germ tubes or indirectly to produce sporangia above 15°C in saturated soil. In general, oospores appear to play a minimal role in the life cycle of this pathogen (Martin and Coffey, 2012).

Soil moisture is the major environmental factor influencing the development of PRR on avocado (Menge and Ploetz, 2003). Sporangia are formed at matric potentials of –2 to –300 kPa. At soil matric potentials of 0 to –1000 kPa, mycelium, chlamydospores and oospores are able to survive and grow in roots. The formation of chlamydospores is stimulated by dry conditions, and they can survive for years in these situations. Wet soils are needed for the release and movement of *P. cinnamomi* zoospores (Zentmyer, 1980; Menge and Ploetz, 2003). Zoospores are released at matric potentials of 0 to –40 kPa but are unable to swim once water drains from soil pores (less than –25 kPa). Likewise, the diffusion of exudates from roots, which attract zoospores, is limited under these conditions. Although zoospore movement is impeded in clay

soils, due to the small pore size, they can move for long distances in surface runoff on these poorly drained soils.

Soil moisture also affects avocado. Flooding for as little as 3 days predisposes roots to infection by *P. cinnamomi* due to the release of exudates, whereas drought stress injures roots and predisposes them to infection the next time the soil is saturated. The notion that *P. cinnamomi* favours flooded soils arises from its ability to survive, grow and cause disease after inundation and under decreased O_2 and increased CO_2 levels that are toxic to avocado roots (Menge and Ploetz, 2003). Although periodically wet soils may increase populations of this pathogen, they are reduced when flooded soil becomes anaerobic. Air in well-drained soil may contain 15% O_2 and 0.03% CO_2, whereas poorly drained soils may approach 1% O_2 and 16% CO_2. Unfortunately, avocado is even more sensitive to anaerobiosis than is *P. cinnamomi*, and 1–5% O_2 can damage or kill avocado roots. Although sporangium production, chlamydospore germination and zoospore activity of *P. cinnamomi* require 2.5–15% O_2 and 0–2% CO_2, zoospore germination, hyphal growth and chlamydospore formation are not affected greatly at O_2 levels of 0.1% and CO_2 levels as high as 20%. Chlamydospore production is actually increased by high CO_2 levels.

The impact of PRR on avocado is exacerbated by flooding. Ploetz and Schaffer (1989) examined the PRR × flooding interaction in pathogen-free (microwaved) soil and soil that was artificially infested with *P. cinnamomi*. Net CO_2 assimilation (photosynthesis), transpiration and stomatal conductance were significantly reduced in plants with PRR after 3 days of flooding, and CO_2 assimilation declined to non-detectable levels within 1 week; in plants without PRR (pathogen-free soil) this occurred only 4 weeks after flooding. In general, PRR affected the above gas exchange parameters in avocado in the absence of flooding only when severe disease was induced in these and subsequent experiments (high levels of inoculum). In combination, PRR and flooding reduced root and shoot biomass, and dramatically impaired photosynthesis and normal stomatal function in avocado.

Saline soils can be an issue where high quality irrigation water is expensive or unavailable (e.g. California and Israel). Avocado is sensitive to saline conditions, and root damage can occur when soil salinity reaches 4 dS/m (Menge and Ploetz, 2003). Roots of avocado become more susceptible to PRR presumably due to increased exudation that occurs in salt-damaged roots. Infected roots are unable to exclude salt and leaf margins often develop the brown, necrotic symptoms of salt damage (Fig. 21.1).

21.5 Management

Despite intense research, no completely effective approach for managing PRR has been developed. However, several measures reduce its impact and are effective when used in an integrated fashion (Coffey, 1992; Dann *et al.*, 2012).

21.5.1 Cultural control

The movement of diseased nursery stock has played a major role in the worldwide distribution of *P. cinnamomi*. Sanitation is the single most important tool for preventing PRR in the nursery (Zentmyer, 1980). Clean seeds are needed for propagation. Seeds should not be collected from the ground and must be treated with 49–50°C water for 30 min. Well-drained growth media must be used and should be disinfested with either steam (100°C for 30 min) or aerated steam (60°C for 30 min) before use (Zentmyer, 1980). Solarized and composted materials are also useful, but temperature must be monitored to ensure that ≥45°C is reached and maintained for several hours. Plants need to be elevated on screen or wire supports 1 m above the nursery floor, which should be covered in concrete, gravel or crushed rock (Dann *et al.*, 2012). Ideally, irrigation water should be from wells and if only surface water is available it must be decontaminated with ozone, copper sulfate

(20 µg/ml), chlorine (0.5 µg/ml) or by other means. The use of chemicals to manage PRR in the nursery is discouraged as it does not eliminate *P. cinnamomi* and facilitates the production of infested/infected materials that are ultimately planted in the field.

In the field, host vigour must be maintained via optimum nutrition. With regard to PRR, calcium is an important nutrient and applications of calcium carbonate, calcium nitrate and calcium sulfate reduce PRR (Zentmyer, 1980), and applications of 1500–3000 kg of gypsum/ha are recommended to manage disease (Menge and Ploetz, 2003). Calcium may reduce the size and number of sporangia that are produced by *P. cinnamomi* and improve soil drainage. Manure can reduce populations of *P. cinnamomi* in soil, presumably due to the release of ammonia, which is toxic to the pathogen (Menge and Ploetz, 2003). However, these materials should be used judiciously as ammonia damages avocado roots, increasing their susceptibility to PRR.

Some rainforest soils in Queensland, Australia are free of *P. cinnamomi*-induced disease, even though the pathogen is present (Zentmyer, 1980). These 'suppressive soils' are thought to result from high resident populations of microbes, high levels of organic matter (>7%), and high exchangeable magnesium, calcium and nitrogen. These conditions were reproduced with the 'Ashburner method' (Ashburner was an avocado producer in Australia), wherein coarse organic mulches, chicken manure and dolomite were added to soil (Zentmyer, 1980). Based on subsequent experience, this method has been modified to incorporate the most effective components. Currently, only organic mulches and gypsum are added to the soil surface, which in turn stimulate cellulose- and lignin-degrading microorganisms that affect *P. cinnamomi* (Menge and Ploetz, 2003). The mulches are 15–30 cm thick and can be composed of yard and avocado trimmings, maize and sorghum stubble, wheat and lucerne straw, and pine bark with a C:N ratio between 25:1 and 100:1. Regardless of the materials, the mulches should drain freely and grass clippings or other materials that can become compacted or which retain excessive water should be avoided.

When selecting sites for new production fields, poorly drained, saline and *P. cinnamomi*-infested soils should be avoided (Zentmyer, 1980). Soils should have good internal and surface drainage and be at least 1.5 m deep. Soils with hardpans can be improved by deep ploughing and with subsurface drains. In poorly drained soils establishing trees on mounds or ridges is beneficial (Menge and Ploetz, 2003). Eradicating *P. cinnamomi* via fumigation, solarization or other soil treatments is not possible (Menge and Ploetz, 2003). In fact, since *P. cinnamomi* can re-infest fumigated soil (it is a moderately good saprobe), PRR can become worse following fumigation. Soil solarization has been used to treat *P. cinnamomi*-infested soil after tree removal in Arizona, California, the Canary Islands and in the Mediterranean (Erwin and Ribiero, 1996). It is most effective in areas with hot, sunny weather, where it can reduce but not eliminate the threat posed by PRR. Field irrigation should be based on data from tensiometers or soil capacitance probes, or on local evaporative demands; matric potentials in the root zone should be maintained between –10 kPa and –50 kPa.

Accurate soil moisture data are crucial when trees are mulched. Irrigation management is difficult when PRR is present, as affected trees have reduced capacities to absorb water and can be overwatered if an irrigation schedule for healthy trees is used. In these situations reduced irrigation is indicated, either by reducing the numbers of emitters beneath affected trees or by replacing them with those of lower output. Given the pathogen's wide host range, care should be taken when selecting and planting ornamentals and wind breaks to ensure that they do not carry *P. cinnamomi*.

21.5.2 Chemical control

Fungicides used for most avocado root pathogens are ineffective against *Phytophthora* spp. Interestingly, before the idea that

Phytophthora spp. are not fungi was widely understood, different classes of 'fungicides' were developed to manage diseases that are caused by oomycetes (Cohen and Coffey, 1986). In general, two of these, the acylalanines and phosphonates, are still widely used against these diseases.

Two acylalanines have been widely used against PRR and other oomycete-induced diseases. Metalaxyl (Ridomil®), which is a racemic mix, and mefanoxam (Ridomil Gold®), which is the specific, effective enantiomer found in metalaxyl, inhibit pathogen growth and sporulation (Dann *et al.*, 2012). They are water soluble and when used in soil applications (granular and water soluble formulations are available) move readily to roots and into the xylem. The acylalanines can be highly effective against PRR but have some important deficiencies. Unfortunately, with repeated use biodegradation can occur in soil resulting in rapid reductions of effective concentrations of these products. With continued use resistant populations are selected over time (the phenylamide group in which the acylalanines are found is classified as 'high risk' for resistance by the Fungicide Resistance Action Committee (FRAC) (FRAC, 2012). Furthermore, once moderate to severe PRR develops in the field, these products are ineffective since insufficient, healthy roots remain to absorb therapeutic dosages. In practice the acylalanines are effective on bearing trees in the field that have low levels of PRR and to protect nursery plants used to replace trees in the field that have succumbed to PRR.

Fosetyl-Al (Aliette®), the first commercialized phosphonate, was released by Rhone Poulenc in 1977 (Cohen and Coffey, 1986). Since fosetyl-Al and related phosphonates are both xylem and phloem mobile, they can be applied as soil drenches, foliar sprays, trunk paints or trunk injections (Dann *et al.*, 2012). If soil or trunk applications are made, these compounds move in the xylem to the canopy where they are remobilized to the root system in the phloem. The phosphonates have a direct impact on the pathogen and an indirect impact via the induction of host defences, together inhibiting root colonization but not killing the pathogen. In bearing avocado trees the phosphonates are most often and effectively applied via trunk injections (Dann *et al.*, 2012). Even trees that are severely affected by PRR can be rejuvenated with this approach. In the early 1980s Joe Darvas was the first to inject a phosphonate into avocado trees. His work in South Africa was conducted with solutions of a wettable powder formulation of fosetyl-Al in which the carrier fraction was allowed to settle out of solution overnight. The clarified portion of the suspension was then injected into trees with modified veterinary syringes. Darvas' work was followed up and refined by Ken Pegg and Tony Whiley in Australia in the late 1980s (Dann *et al.*, 2012). Their work, conducted primarily with potassium phosphonate, determined therapeutic dosages and application frequency. Importantly, they determined that developing fruit and vegetative flushes were powerful sinks for the phosphonate moiety and that much of the injected product was immobilized in the canopy (and did not reach the roots) if injections were made at those times. Thus, they indicated that injections should avoid these phenologic events and precede root growth. Once tree health has been re-established with trunk injections, PRR can be kept in check with high volume foliar sprays (e.g. >2500 l/ha, 0.5% active ingredient mono-dipotassium phosphonate, pH 7.2), and four to six strategically timed sprays may be required. Information from Pegg and Whiley's work in the 1980s is used currently worldwide when managing PRR on avocado.

21.5.3 Host resistance

PRR-tolerant avocado rootstocks were first developed in the 1950s by George Zentmyer (Zentmyer, 1980; Douhan *et al.*, 2011). His work focused initially on collecting seed from diverse sources in tropical America and screening them for PRR response in *P. cinnamomi*-infested potting mix in greenhouses at the University of California at Riverside (UCR). Although he found PRR

tolerance in different *Persea* spp., those in the subgenus *Eriodaphne* were graft incompatible with *Persea americana* (subgenus *Persea*) and unusable as rootstocks. Within *P. americana*, Zentmyer found PRR tolerance in two seedlings from the 'Duke' cultivar, 'Duke 6' and 'Duke 7', the latter of which became the first commercially successful rootstock for this crop in the 1970s. Since then the UCR programme (Zentmyer and his successors) has developed many additional rootstocks with PRR tolerance, some of which have been commercialized. In addition, other, similar programmes have been established in Australia, the Canary Islands, Florida, Israel and South Africa (Douhan *et al*., 2011). Currently a selection from South Africa, 'Dusa', is the industry standard. Since *P. americana* is an outcrossing, somewhat self-incompatible species, seedlings of it are hybrids. Thus, for a complex trait like PRR tolerance, clonal, rather than seedling, progeny of a given selection must be used to maintain the tolerant phenotype. Clonal propagation of avocado rootstocks requires an involved series of grafting on nurse seeds, etiolation and hormone treatment, and variations of the original Froelich technique are used for this purpose worldwide.

References

Coffey, M.D. (1992) Phytophthora root rot of avocado. In: Kumar, J., Chaube, H.S., Singh, U.S. and Mukhopadhyay, A.N. (eds) *Plant Diseases of International Importance Volume III. Diseases of Fruit Crops*. Prentice Hall, Englewood Cliffs, New Jersey, pp. 423–444.

Cohen, Y. and Coffey, M.D. (1986) Systemic fungicides and the control of oomycetes. *Annual Review of Phytopathology* 24, 311–338.

Dann, E., Ploetz, R.C., Coates, L. and Pegg, K.G. (2012) Foliar, fruit and root diseases. In: Schaffer, B., Wolstenholme, B.N. and Whiley, A.W. (eds) *The Avocado: Botany, Production and Uses*, 2nd edn. CABI, Wallingford, Oxon, UK.

Douhan, G.W., Fuller, E., McKee, B. and Pond, E. (2011) Genetic diversity analysis of avocado (*Persea americana* Miller) rootstocks selected under greenhouse conditions for tolerance to Phytophthora root rot (PRR) caused by *Phytophthora cinnamomi*. *Euphytica* 182, 209–217.

Erwin, D.C. and Ribeiro, O.K. (1996) *Phytophthora Diseases Worldwide*. APS Press, St Paul, Minnesota, 562 pp.

Food and Agriculture Organization of the United Nations (FAO) (2012) FAOSTAT online database. Available at: http://www.fao.org/default.htm (accessed 15 June 2012).

Fungicide Resistance Action Committee (FRAC) (2012) FRAC Code List 2012. Fungicides sorted by mode of action (including FRAC Code numbering). Available at: http://www.frac.info/frac/publication/anhang/FRAC-Code-List2011-final.pdf (accessed 3 October 2012).

Hardham, A.R. (2005) *Phytophthora cinnamomi*. *Molecular Plant Pathology* 6, 589–604.

Knight, R.L., Jr (2002) History, distribution and uses. In: Whiley, A.W., Schaffer, B. and Wolstenholme, B.N. (eds) *The Avocado: Botany, Production and Uses*, 1st edn. CABI, Wallingford, Oxon, UK, pp. 1–14.

Kroon, L.P.N.M., Brouwer, H., de Cock, A.W.A.M. and Govers, F. (2012) The genus *Phytophthora* anno 2012. *Phytopathology* 102, 348–364.

Martin, F.N. and Coffey, M. (2012) Mitochondrial haplotype analysis for differentiation of isolates of *Phytophthora cinnamomi*. *Phytopathology* 102, 229–239.

Menge, J.A. and Ploetz, R.C. (2003) Diseases of avocado. In: Ploetz, R.C. (ed.) *Diseases of Tropical Fruit Crops*. CABI Publishing, Wallingford, Oxon, UK, pp. 35–71.

Ploetz, R.C. (2007) Diseases of tropical perennial crops: challenging problems in diverse environments. *Plant Disease* 91, 644–663.

Ploetz, R.C. and Schaffer, B. (1989) Effects of flooding and Phytophthora root rot on net gas exchange and growth of avocado. *Phytopathology* 79, 204–208.

Ploetz, R., Schnell, R.J. and Haynes, J. (2002) Variable response of open-pollinated seedling progeny of avocado to Phytophthora root rot. *Phytoparasitica* 30, 262–268.

Tucker, C.M. (1929) Report of the plant pathologist. *Report of the Puerto Rico Agricultural Experiment Station* 1928, 29–35.

Zentmyer, G.A. (1980) *Phytophthora cinnamomi* and the diseases it causes. *American Phytopathological Society (APS) Monograph* 10. APS, St Paul, Minnesota.

22 The Occurrence and Impact of *Phytophthora* on the African Continent

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22. 1 Introduction

Surprisingly little is known about *Phytophthora* on the African continent, and most of what is known is limited to South Africa. To date 23 species of *Phytophthora* have been reported, of which 20 are known from South Africa and only ten from the rest of Africa (Table 22.1). As elsewhere, *Phytophthora* attacks several cultivated plant species, particularly agronomic crop plants, and occurs in native ecosystems. Here we review the occurrence, importance and potential threats of *Phytophthora* to agriculture and natural ecosystems in Africa.

Table 22.1. *Phytophthora* species occurrence in Africa.

Species	Host	Country
Agriculture		
Phytophthora cactorum	*Citrus* spp., *Malus domestica*, *Vitis vinifera*	South Africa
	Malus domestica	Tunisia
Phytophthora capsici	*Capsicum* spp., *Cumus melo*, *Curcurbita* spp., *Solanum lycopersicum*	South Africa
	Capsicum sp.	Nigeria
Phytophthora cinnamomi	*Annanas comosus*, *Macadamia* spp., *Persea americana*, *Pyrus communis*, *Vitis* spp.	South Africa
	Persea americana	Various countries
	Macadamia spp.	Kenya
Phytophthora citricola	*Citrus* spp.	South Africa
Phytophthora citrophthora	*Citrus* spp.	South Africa
	Citrus spp.	Liberia
Phytophthora cryptogea	*Citrus* spp., *Vitis vinifera*	South Africa
Phytophthora drechsleri	*Brassica oleracea*, *Medicago sativa*	South Africa
Phytophthora infestans	Solanaceous crops	Various countries
Phytophthora medicaginis	*Medicago sativa*	South Africa
Phytophthora megakarya	*Theobroma cacao*	Various countries
Phytophthora megasperma	*Vitis vinifera*	South Africa

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Species	Host	Country
Phytophthora multivora	*Medicago sativa*	South Africa
Phytophthora nicotianae	*Chamaecytisus palmensis*, *Citrus* spp., *Musa* sp., *Nicotiana tabacum*, *Rheum rhaponticum*, *Solanum lycopersicum*, *Vitis vinifera*	South Africa
	Nicotiana tabacum, *Solanum betacae*	Ghana
	Passiflora edulis	Kenya
	Agave spp., *Citrus* sp., *Hibuscus cannabinus*	Tanzania
	Capsicum annuum, *Malus domestica*	Tunisia
	Nicotiana tabacum	Zimbabwe
Phytophthora niederhauserii	*Vitis* spp.	South Africa
Phytophthora palmivora	*Agave* spp., *Cocos nucifera*, *Hevea brasiliensis*, *Musa* sp., *Persea americana*, *Theobroma cacao*	Various countries
Phytophthora porri	*Allium cepa*	South Africa
Phytophthora syringae	*Citrus* sp.	South Africa
	Citrus sp.	Libya
Forestry		
Phytophthora alticola	*Eucalyptus* spp.	South Africa
Phytophthora boehmeriae	*Acacia mearnsii*, *Eucalyptus* spp.	South Africa
P. cinnamomi	*Eucalyptus* spp., *Pinus* spp.	South Africa
Phytophthora frigida	*Eucalyptus* spp.	South Africa
Phytophthora meadii	*Acacia mearnsii*	South Africa
P. nicotianae	*Acacia mearnsii*, *Eucalyptus* spp.	South Africa
Ornamental/exotic		
P. cactorum	*Antirrhinum majus*, *Centaurium moschata*, *Dianthus caryophyllus*, *Verbena* sp.	South Africa
P. cinnamomi	*Araucaria angustifolia*, *Banksia* spp., *Casuarina cunninghamiana*, *Cedrus deodara*, *Centaurium* sp., *Chamaecyparis lawsoniana*, *Cryptomeria japonica*, *Rhododendron* sp., *Telopea speciosissima*, *Thuja* sp.	South Africa
P. cryptogea	*Godetia* sp.	South Africa
P. infestans	*Petunia* × *hybrida*	South Africa
P. nicotianae	*Delphinium* sp., *Gypsophila paniculata*, *Trichocaulon* sp.	South Africa
P. palmivora	*Mimusops elengi*	Ghana
Native plants and habitats		
Phytophthora capensis	*Curtisia dentata*, *Olea capensis*, rivers	South Africa
P. cinnamomi	*Bruniaceae*, *Ericaceae* and *Proteacae*, *Agasthoma* spp., *Cliffortia* spp., *Curtisia dentata*, *Nymania capensis*, *Ocotea bullata*, *Priestleya* sp., *Widdringtonia* spp., rivers	South Africa
P. citricola	Rivers	South Africa
P. cryptogea	*Agathosma* spp., *Osteospermum* sp., rivers	South Africa
P. drechsleri	*Agathosma* spp., rivers	South Africa
Phytophthora heveae	Unknown	Cameroon
P. infestans	*Aspalia africa*, *Solanecio biafrae*, *Solanum* spp.	Cameroon, Kenya
P. megakarya	*Dracaena mannii*, *Funtumia elastica*, *Irvingia* spp, *Ricinodendron heudelotii*, *Sterculia tragacantha*	Cameroon, Ghana
P. multivora	*Agathosma* spp., *Ocotea bullata*	South Africa
P. nicotianae	*Agathosma* spp., *Cotyledon* sp.	South Africa
Phytophthora taxon 'emzansi'	*Agathosma* spp.	South Africa

22.2 *Phytophthora* in Agriculture

Africans depend heavily on agriculture, and plant diseases and insect pests are crucially important to the livelihood of many people. Seventeen *Phytophthora* spp. are known to cause diseases of agricultural crops but research has focused primarily on *Phytophthora infestans*, *Phytophthora megakarya*, *Phytophthora palmivora* and *Phytophthora cinnamomi*. For other species (e.g. *Phytophthora cryptogea*, *Phytophthora drechsleri*, *Phytophthora medicaginis*, *Phytophthora megasperma*, *Phytophthora porri* and *Phytophthora syringae*) there are often only first reports of the pathogen. Follow-up studies are rare and exceptions include *Phytophthora capsici*, *P. cinnamomi* and *P. infestans* where genetic diversity has been characterized in South African populations.

In most cases *Phytophthora* is found on the same plant hosts as on other continents. Although yet to be tested experimentally, the non-native crop plants cultivated in Africa are probably affected by non-native *Phytophthora* spp., for instance: (i) *P. cactorum* is recovered from *Malus domestica* (apple) from America, the UK and Switzerland; (ii) *Phytophthora citrophthora* from *Citrus* spp. from Egypt, Israel, Spain and various Mediterranean islands; and (iii) *P. porri* from *Allium cepa* (onion) from Japan and the Netherlands. The only exception is *P. megakarya*, one of the species involved in cacao black pod disease, which occurs exclusively in Central to Western Africa (Guest, 2007).

There are a few cases where new plant associations have been reported. One example is the proposed (but not formally described) *Phytophthora niederhauserii*. This species causes disease on several ornamental plants in Europe including *Banksia* spp., *Calistemon citrinus*, *Cistus* spp., *Laurus nobilis* and *Pistacia lentiscus* (Cacciola *et al.*, 2009; Scanu *et al.*, 2011), and almond (*Prunus dulcis*) decline in Spain (Pérez-Sierra *et al.*, 2010). *P. niederhauserii* was also reported in Western Australia from a *Banksia* sp. as well as from soil associated with nursery plants imported from the Northern Territory of Australia (Davison *et al.*, 2006). Recently it was isolated from the crowns of healthy grapevines from South Africa, which is the first report of *P. niederhauserii* on this host (Spies *et al.*, 2011).

While *Phytophthora* is associated with many crop diseases in Africa, only a few have a significant impact. These include blight caused by *P. infestans* on potato and tomato, black pod of cacao caused by *P. megakarya* and *P. palmivora*, and a range of important hosts that are attacked by *P. cinnamomi*. Each of these major disease systems is discussed individually and an attempt is made to highlight their importance in Africa.

22.2.1 Late blight of potato and tomato caused by *P. infestans*

Africa produces 12–18 million t/year of potato and 13–16 million t/year of tomato. Most of Africa's potato production is consumed locally; however, a small amount is exported. African countries, on average, import slightly more (470,970 t) potatoes than they export (386,240 t). On the other hand, African countries export almost ten times more tomatoes than they import (216,251 t versus 24,926 t, respectively) (FAO, 2011). These crops are economically very important in Africa.

Although late blight caused by *P. infestans* is one of the most important diseases (both socially and economically), the impact is difficult to quantify. Yield losses result in loss of sustenance and/or loss of income for farmers, and chemical control agents are a necessity. However, chemicals are costly and decrease profit. In Africa late blight can result in total crop losses of potato and tomato if no chemical control measures are taken, although disease severity varies considerably. This variation can be attributed to variable weather conditions and to the degree of resistance in the cultivars being planted (Mukalazi *et al.*, 2001; Olanya *et al.*, 2001).

The centre of origin of *P. infestans* is believed to be Central Mexico. The initial dissemination of *P. infestans* occurred in the 1840s, when the A1 mating type and a few genotypes spread to the USA on potatoes. The subsequent spread to Europe and the rest of the world was made by a single clonal lineage of the A1 mating type and has led to the presence of the A1 mating type in every potato- and tomato-growing country in Africa. Thereafter, additional migrations of *P. infestans* introduced the A2 mating type and new genotypes into the USA and the rest of the world on several occasions. The A2 mating type of *P. infestans* was first reported from Africa from infected potato tubers originating from Egypt. However, a later study did not yield the A2 mating type from Egyptian *P. infestans* isolates. The A2 mating type and new genotypes have also recently been observed in Morocco, where the A1 mating type is also present. Other than Egypt and Morocco, there have been no other reports of the A2 mating type or new genotypes in Africa. If the A2 type is introduced to Africa and outcrossing with the A1 genotypes already present is possible, then the resulting genetically diverse populations may be able to evolve more rapidly, allowing them to develop fungicide resistance or to overcome host defences of resistant varieties of potato in a shorter time compared with an asexually reproducing population. In addition, the oospores may allow the pathogen to survive for longer periods outside of host tissue, compared with asexual populations.

P. infestans can also infect other plant species and causes late blight of huckleberry (*Solanum scabrum*), which is native to Africa, in Cameroon, where its leaves and shoots are widely used as a vegetable for subsistence and commercial production. *P. infestans* has also been reported from other solanaceous and asteraceous plants from Cameroon and Kenya (Table 22.1) (Fontem *et al.*, 2004). These alternative hosts complicate control measures as they can act as an overwintering cache and a continuous source of inoculum.

22.2.2 Black pod disease caused by *P. palmivora* and *P. megakarya*

Africa is the world's largest cacao grower, producing on average 70% of the world's crop, of which 38% is produced by Côte d'Ivoire and 19% by Ghana (ICCO, 2007). When this is contrasted with the production of the Americas (12%), Asia and Oceania (17%), it is clear that cacao production is one of Africa's most significant agricultural industries. Pod rot is the most important symptom of *Phytophthora* infection of cacao because it results in direct loss of crop yield. Infection can occur on mature or immature pods (cherelles) and is readily visible as spreading brown to black lesions (see Drenth and Guest, Chapter 20, this volume) (Guest, 2007). Initially the infection does not affect the cacao beans, but as the disease progresses the beans are infected, which renders them unusable. *Phytophthora* spp. can infect other parts of cacao trees besides the pods, such as the main stems, leaves, flower cushions and roots. The importance of these types of infection has been underestimated as they influence cacao tree health and thus indirectly decrease cacao yield (Appiah *et al.*, 2004). Diseased pods and other infections such as stem cankers contribute to the spread, establishment and severity of further infections as they are a source of secondary inoculum (Bowers *et al.*, 2001).

In cacao-producing regions of the world several *Phytophthora* spp., including *P. capsici*, *P. citrophthora*, *Phytophthora hevea*, *P. megasperma* and *Phytophthora nicotianae*, cause cacao black pod disease. However, in Africa only two species cause disease of cacao. Initially *P. palmivora* was implicated as the sole cause of black pod, but later a second species, *P. megakarya*, was also shown to be involved (Brasier and Griffin, 1979). *P. palmivora* occurs globally, whereas *P. megakarya* is restricted to parts of Central and Western Africa. Initially *P. megakarya* was known only to occur in Nigeria and Cameroon, but later its range was expanded to Gabon, Ghana, Equatorial Guinea, Côte d'Ivoire and Togo (Guest,

2007). Both mating types of *P. megakarya* and *P. palmivora* are present in Africa. Most isolates of *P. megakarya* encountered on cacao are A1, but both mating types are found in Equatorial Guinea, Cameroon, Ghana and Nigeria (Appiah *et al.*, 2003). In contrast, *P. palmivora* is mostly A2 in Africa, with both mating types found only in Ghana and Togo (Appiah *et al.*, 2003). The dominance of one mating type limits the opportunity for sexual reproduction within populations of the pathogen.

Although the two *Phytophthora* spp. occurring on cacao in Africa cause the same disease, they have slightly different disease cycles and attributes. *P. megakarya* is much more virulent than *P. palmivora* and consequently results in higher yield losses of up to 100% compared with the 20–30% yield loss caused by *P. palmivora*. *P. megakarya* has a propensity for earlier and more profuse release of zoospores than *P. palmivora* allowing it to spread faster (Brasier *et al.*, 1981). *P. palmivora* is better at surviving in mummified pods and *P. megakarya* is unable to endure the dry season in shrivelled pods. They also differ in their ability to cause cankers. *P. megakarya* has a higher incidence of tree girdling cankers, whereas *P. palmivora* causes cankers higher up on the stems (Appiah *et al.*, 2004).

Cacao is a major cash crop in the African countries, with many smallholder farmers reliant on cacao as an important source of income. Black pod disease is a serious constraint of cacao production due to the high yield losses, especially when caused by *P. megakarya*. *Phytophthora*-infested cacao plantations incur additional expenses as chemical control agents are necessary to reduce yield loss. The high cost associated with fungicides and their application decreases the farmer's profit per unit of cacao sold. Simply planting more cacao is not an option because of the limited land available for production (see Drenth and Guest, Chapter 20, this volume).

P. megakarya and *P. palmivora* occur on several tree species other than cacao. In Ghana *P. megakarya* infects *Funtumia elastica*, *Sterculia tragacantha*, *Dracaena mannii* and *Ricinodendron heudelotii* (Table 22.1). These native trees are often retained in cacao plantations to provide shade. In Cameroon *P. megakarya* was also reported from the fallen fruit of a native *Irvingia* sp. occurring in a native forest habitat. Although *P. palmivora* has a very broad host range and occurs globally, in Africa it has only been reported from a small number of hosts other than cacao. These include *Hevea brasiliensis* and a *Mimusops* sp. in Ghana, *Musa* sp. in Nigeria, and *Cocos nucifera* in Tanzania (Table 22.1). All the above-mentioned trees, with the exception of the *Mimusops* sp., are commonly intercropped together with cacao trees. The effect that these alternative hosts have on the disease incidence is not clear, but they could act as reservoirs for *P. megakarya* and *P. palmivora* (Opoku *et al.*, 2002).

22.2.3 Avocado root root caused by *P. cinnamomi*

Avocado (*Persea americana*) trees are widely planted in Africa and the fruit are consumed locally and exported. Annually Africa produces ≈14% of the world's avocados, with Kenya, South Africa, Democratic Republic of the Congo, Ethiopia and Cameroon having the largest production (FAO, 2011). The most important disease of avocado in Africa is root rot and dieback caused by *P. cinnamomi*. The first African reports misidentified *P. cinnamomi* as *Phytophthora cambivora* in South Africa (Wager, 1941). Root rot is particularly severe in South Africa due to the high soil temperatures and excessive moisture it receives via summer rainfall. In South Africa losses due to *P. cinnamomi* are estimated at 10% of the annual gross value of avocados (Bekker, 2007). Although estimates of the impact are scarce, root rot is widespread and is known to be a serious disease of avocados in other African countries including Cameroon, Ethiopia and Kenya.

P. cinnamomi is heterothallic, with the A2 mating type the more common globally.

Both mating types occur in South Africa in agricultural and native environments but the overall distribution is not known for most areas. The A1 mating type is restricted to the South Western Cape while the A2 mating type occurs in both the South Western Cape as well as the Mpumalanga province of South Africa (Linde *et al.*, 1997). In Kenya both mating types are also present on *Macadamia* spp. (Mbaka *et al.*, 2010).

Although *P. cinnamomi* is best known from avocado, it causes disease on several other important crops that receive much less attention. In the Western Cape of South Africa it caused crown and root rot of grapevines during the 1970s (van der Merwe *et al.*, 1972). Although other *Phytophthora* species were involved, *P. cinnamomi* was the most virulent (Table 22.1). More recently *Phytophthora* has become much less common on grapevines in South Africa, probably due to widespread use of chemical control measures. *P. cinnamomi* also occurs on *Macadamia* spp. in South Africa and Kenya, as well as on pineapple in South Africa and Ghana, but little research has been done on these important hosts. *P. cinnamomi* has also been recovered from various other countries, including the Republic of Congo, Democratic Republic of Congo, Republic of Guinea, Côte d'Ivoire, Morocco, Uganda, Zambia and Zimbabwe (Zentmyer *et al.*, 1976) but the hosts were not mentioned.

22.2.4 *Phytophthora* spp. in plantation forestry

In Africa forests are a valuable natural resource and important source of timber and non-timber forest products. Of Africa's total forest area only 2.3% consists of planted forests and 38% of these plantations contain non-native tree species. Thirty per cent of Africa's forest area is primarily for production of timber and non-timber forest products. The majority of timber harvested in Africa, be it from natural or planted forests, is used as fuel wood and only about 10% for industrial purposes. Countries with planted forests produce a significantly larger proportion of industrial wood. Plantations of non-native trees are uncommon and irregularly distributed, but where they do occur, countries rely almost exclusively on them for industrial wood (FAO, 2010). Of these the most prominent plantation species are *Pinus*, *Eucalyptus*, *Acacia* and *Cupressus*.

Black wattle (*Acacia mearnsii*) plantations make up approximately 8% of the total forestry land usage in South Africa, and the trees are mainly used for tannin production and pulpwood. In the 1960s a serious disease, known as black butt disease (Fig. 22.1B and C), appeared in South African plantations. The disease is characterized by blackened bases of the tree trunks, copious gum exudation as well as mottled lesions occurring on the stems of young trees (Zeiljemaker, 1971). Initially, *P. nicotianae* was shown to be the cause of this disease, but two additional species, *Phytophthora boehmeriae* and *Phytophthora meadii*, were later shown to be associated with the same symptoms (Roux and Wingfield, 1997). Black butt disease is also present in Kenya and Tanzania, but the *Phytophthora* sp. involved has not been identified (Roux *et al.*, 2005).

Pines and eucalypts provide the backbone of the South African forestry industry and make up about 50% of the total forestry land area planted. Several *Phytophthora* species attack these trees (Table 22.1). In plantations *P. cinnamomi* causes root and collar rot of both pines and eucalypts (Fig. 22.1A). In forestry nurseries *P. cinnamomi* causes damping off of pine and eucalypt seedlings and has resulted in nursery quarantine and the destruction of countless seedlings. Until 20 years ago *P. cinnamomi* was the only species known to cause disease in pine and eucalypt plantations. Thereafter two species, *P. boehmeriae* and *P. nicotianae*, were identified on several *Eucalyptus* species (Linde *et al.*, 1994). *P. nicotianae*, in particular, became so prevalent that it was more consistently recovered from dying eucalypts than *P. cinnamomi.* More recently two new species, *Phytophthora alticola* and

A

B

C

Fig. 22.1. Plantation trees affected by Phytophthora diseases. **(A)** Dead, defoliated *Eucalyptus* spp. caused by *Phytophthora cinnamomi* infection. **(B, C)** Basal stem canker of *Acacia mearnsii* suffering from black butt disease with exposed stem lesion **(B)** and bleeding stem cankers **(C)** (photographs courtesy of Jolanda Roux).

Phytophthora frigida, were found to cause root and collar rot of cold-tolerant eucalypts in the Kwazulu-Natal province (Maseko *et al.*, 2007).

22.3 *Phytophthora* spp. in Native Environments

There has been very little research on *Phytophthora* in native environments in Africa. This is evident when comparing the number of *Phytophthora* species from crops to those occurring on indigenous plants or in native environments (Table 22.1). Even among *Phytophthora* spp. identified from indigenous plants, many have been isolated from plants cultivated as food crops, medicines, cosmetics or for the flower trade.

Recently, there has been an increased international focus on *Phytophthora* associated with native ecosystems because a number of very serious Phytophthora diseases have emerged in native woody ecosystems. But, surveys for these organisms in native African environments have yet to be made. The Cape Floristic Region (CFR), and especially the sclerophyllous shrubland known as fynbos in South Africa, has received more attention relating to *Phytophthora* spp. than any other native habitat in Africa. This was initially motivated by the dramatic death of the iconic silver trees (*Leucodendron argenteum*) (Fig. 22.2) and other *Proteaceae* in this megadiverse flora. Multiple *Phytophthora* species are now known to occur in CFR rivers and their catchments including *Phytophthora capensis*, *P. cinnamomi*, *Phytophthora citricola*, *P. cryptogea* and *Phytophthora dreschleri* (Von Broembsen, 1984). Multiple *Phytophthora* species are involved in disease of buchu (*Agathosma* spp.), including a yet-to-be described taxon in the *P. citricola* complex informally referred to as *Phytophthora* 'emzansi'. Additionally, *P. capensis* was described as a new species after re-examining isolates from buchu previously classified as *P. citricola* (Bezuidenhout *et al.*, 2010). Apart from *P. capensis* and the newly discovered *Phytophthora* 'emzansi', the other species have a cosmopolitan distribution and diverse host range.

P. cinnamomi is the most commonly encountered *Phytophthora* in the CFR. It is associated with root rot and decline of several native species in the *Bruniaceae*, *Ericaceae* and *Proteaceae* families in the fynbos. *P. cinnamomi* is also a serious problem for the cut flower industry, where it causes root rot of cultivated members of the *Proteaceae*. Additionally, *P. cinnamomi* is associated with the decline of stinkwood (*Ocotea bullata*) trees in native forests of the Eastern Cape of South Africa (Table 22.1).

22.4 Conclusions

Phytophthora has a major socio-economic impact on the countries and people of Africa. The most important are losses in agriculture to pathogens such as *P. infestans*, which severely affects potato and tomato yields. Black pod disease of cacao is responsible for substantial losses of cacao, of which Africa is the largest producer. Black pod disease directly impacts resource-poor farmers who are dependent on cacao as a source of income (see Drenth and Guest, Chapter 20, this volume). Avocado production is also severely affected by *P. cinnamomi* and can result in extreme economic losses.

Although forestry is an important industry, little is known about the impact of *Phytophthora* on native or non-native trees. Several of the most commonly planted trees, including non-native acacias, eucalypts and pines, are affected by various *Phytophthora* species. *P. cinnamomi* is a significant threat to commercial forestry and also surrounding native ecosystems. In addition to *P. cinnamomi*, *P. nicotianae* has emerged as a serious pathogen disease of eucalypts and pines and other *Phytophthora* species also appear to be involved. Vigilance is required for the early detection of possible new *Phytophthora* species or known pathogenic species from elsewhere in the world.

Compared with the research on agronomic crops, *Phytophthora* in native

A

B

Fig. 22.2. Dieback of *Leucodendron argenteum* (*Proteaceae*) caused by *Phytophthora cinnamomi*. **(A)** Cracking of the bark, covering affected areas of the tree stem and early signs of wilting visible from the leaves. **(B)** A severely wilted tree (left) with grey discoloured leaves.

habitats has received limited attention. Although this bias is understandable, there is much room for further investigation. Africa has several regions of plant mega-diversity and endemism and probably harbours several new *Phytophthora* species. Thus far the majority of *Phytophthora* species on native and indigenous plant hosts have been found in the CFR of South Africa. In just the last decade the number of described *Phytophthora* species has more than doubled, but only three of these new species descriptions originated from Africa. This lack of species discovery is due to: (i) the limited number of studies investigating species diversity; (ii) a focus on economically important species; and (iii) a lack of funding and trained researchers in the field. There is consequently a great need for comprehensive studies on *Phytophthora* species diversity, especially in native habitats in Africa. This is especially important because novel *Phytophthora* species may be a threat to important non-native or native plants elsewhere in the world.

References

Appiah, A.A., Flood, J., Bridge, P.D. and Archer, S.A. (2003) Inter and intraspecific morphometric variation and characterization of *Phytophthora* isolates from cocoa. *Plant Pathology* 52, 168–180.

Appiah, A.A., Opoku, I.Y. and Akrofi, A.Y. (2004) Natural occurrence and distribution of stem cankers caused by *Phytophthora megakarya* and *Phytophthora palmivora* on cocoa. *European Journal of Plant Pathology* 110, 983–990.

Bekker, T.F. (2007) Efficacy of water soluble silicon for control of *Phytophthora cinnamomi* root rot of avocado. MSc thesis, University of Pretoria, Pretoria, South Africa.

Bezuidenhout, C.M., Denman, S., Kirk, S.A., Botha, W.J., Mostert, L. and McLeod, A. (2010) *Phytophthora* taxa associated with cultivated *Agathosma*, with emphasis on the *P. citricola* complex and *P. capensis* sp. nov. *Persoonia* 25, 32.

Bowers, J.H., Bailey, B.A., Hebbar, P.K., Sanogo, S. and Lumsden, R.D. (2001) The impact of plant diseases on world chocolate production. *Plant Health Progress*. Available at: http://www.plantmanagementnetwork.org/pub/php/ (accessed 3 October 2012).

Brasier, C.M. and Griffin, M.J. (1979) Taxonomy of *Phytophthora palmivora* on cocoa. *Transactions of the British Mycological Society* 72, 111–143.

Brasier, C.M., Griffin, M.J. and Maddison, A.C. (1981) The cocoa black pod Phytophthoras. In: Gregory, P.H. and Maddison, A.C. (eds) *Epidemiology of* Phytophthora *on Cocoa in Nigeria*. *Phytopathological Paper* No. 25. Commonwealth Mycological Institute, Kew, Surrey, UK, pp. 18–30.

Cacciola, S.O., Scibetta, S., Pane, A., Faedda, R. and Rizza, C. (2009) *Callistemon citrinus* and *Cistus salvifolius*, two new hosts of *Phytophthora* taxon niederhauserii in Italy. *Plant Disease* 93, 1075–1075.

Davison, E.M., Drenth, A., Kumar, S., Mack, S., Mackie, A.E. and McKirdy, S. (2006) Pathogens associated with nursery plants imported into Western Australia. *Australasian Plant Pathology* 35, 473–475.

Food and Agriculture Organization of the United Nations (FAO) (2010) *Global Forest Resources Assessment 2010: Main Report.* FAO, Forestry Department, Rome.

Food and Agriculture Organization of the United Nations (FAO) (2011) FAOSTAT statistics database. Available at: http://faostat.fao.org (accessed 15 January 2011).

Fontem, D.A., Olanya, O.M. and Njualem, B.F. (2004) Reaction of certain solanaceous and asteraceous plant species to inoculation with *Phytophthora infestans* in Cameroon. *Journal of Phytopathology* 152, 331–336.

Guest, D. (2007) Black pod: diverse pathogens with a global impact on cocoa yield. *Phytopathology* 97, 1650–1653.

International Cocoa Organization (ICCO) (2007) International Cocoa Organization Annual Report 2006/2007. ICCO, London.

Linde, C., Kemp, G.H.J. and Wingfield, M.J. (1994) *Pythium* and *Phytophthora* species associated with eucalypts and pines in South Africa. *European Journal of Forest Pathology* 24, 345–356.

Linde, C., Drenth, A., Kemp, G.H.J., Wingfield, M.J. and Von Broembsen, S.L. (1997) Population structure of *Phytophthora cinnamomi* in South Africa. *Phytopathology* 87, 822–827.

Maseko, B., Burgess, T.I., Coutinho, T.A. and Wingfield, M.J. (2007) Two new *Phytophthora* species from South African *Eucalyptus* plantations. *Mycological Research* 111, 1321–1338.

Mbaka, J.N., Losenge, T., Waiganjo, M.M. and Wamocho, L.S. (2010) Phenotypic variation in three *Phytophthora cinnamomi* populations from macadamia growing areas in Kenya. *Journal of Animal and Plant Sciences* 8, 900–911.

Mukalazi, J., Adipala, E., Sengooba, T., Hakiza, J.J., Olanya, M. and Kidanemariam, H.M. (2001) Variability in potato late blight severity and its effect on tuber yield in Uganda. *African Crop Science Journal* 9, 195–201.

Olanya, O.M., Adipala, E., Hakiza, J.J., Kedera, J.C., Ojiambo, P., Mukalazi, J.M., Forbes, G. and Nelson, R. (2001) Epidemiology and population dynamics of *Phytophthora infestans* in sub-Saharan Africa: progress and constraints. *African Crop Science Journal* 9, 185–194.

Opoku, I.Y., Akrofi, A.Y. and Appiah, A.A. (2002) Shade trees are alternative hosts of the cocoa pathogen *Phytophthora megakarya*. *Crop Protection* 21, 629–634.

Pérez-Sierra, A., León, M., Álvarez, L.A., Alaniz, S., Berbegal, M., García-Jiménez, J. and Abad-Campos, P. (2010) Outbreak of a new *Phytophthora* sp. associated with severe decline of almond trees in eastern Spain. *Plant Disease* 94, 534–541.

Roux, J. and Wingfield, M.J. (1997) Survey and virulence of fungi occurring on diseased *Acacia mearnsii* in South Africa. *Forest Ecology and Management* 99, 327–336.

Roux, J., Meke, G., Kanyi, B., Mwangi, L., Mbaga, A., Hunter, G.C., Nakabonge, G., Heath, R.N. and Wingfield, M.J. (2005) Diseases of plantation forestry trees in eastern and southern Africa. *South African Journal of Science* 101, 409.

Scanu, B., Linaldeddu, B.T. and Franceschini, A. (2011) A new *Phytophthora* sp. causing root and collar rot on *Pistacia lentiscus* in Italy. *Plant Disease* 95, 618.

Spies, C.F.J., Mazzola, M. and McLeod, A. (2011) Characterisation and detection of *Pythium* and *Phytophthora* species associated with grapevines in South Africa. *European Journal of Plant Pathology* 131, 103–119.

van der Merwe, J.J.H., Joubert, D.J. and Matthee, F.N. (1972) *Phytophthora cinnamomi* root rot of grapevines in the western Cape. *Phytophylactica* 4, 133–136.

Von Broembsen, S.L. (1984) Distribution of *Phytophthora cinnamomi* in rivers of the South-Western Cape Province. *Phytophylactica* 16, 227–229.

Wager, V.A. (1941) Descriptions of the South African Pythiaceae with records of their occurrence. *Bothalia* 4, 3–35.

Zeiljemaker, F.C.J. (1971) Black-butt disease of black wattle caused by *Phytophthora nicotianae* var. *parasitica*. *Phytopathology* 61, 144–145.

Zentmyer, G.A., Leary, J.V., Klure, L.J. and Grantham, G.L. (1976) Variability in growth of *Phytophthora cinnamomi* in relation to temperature. *Phytopathology* 66, 982–986.

23 *Phytophthora* in Mexico

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23.1 Introduction

Although *Phytophthora* has been studied in Mexico since the 1950s, only 17 *Phytophthora* species are reported in the literature. These species are *Phytophthora cactorum*, *Phytophthora capsici*, *Phytophthora cinnamomi*, *Phytophthora citricola*, *Phytophthora citrophthora*, *Phytophthora drechsleri*, *Phytophthora fragariae*, *Phytophthora heveae*, *Phytophthora infestans*, *Phytophthora ipomoeae*, *Phytophthora medicaginis*, *Phytophthora megasperma*, *Phytophthora mexicana*, *Phytophthora mirabilis*, *Phytophthora nicotianae*, *Phytophthora palmivora* and *Phytophthora phaseoli* (Table 23.1). Other reports simply mention the genus *Phytophthora* and the actual number of species is likely to be much higher. For example, in a recent study of *Phytophthora* in ornamental nurseries in the state of Michoacan, seven different *Phytophthora* species were isolated from wilting plants. Three were a first report for Mexico, including a hybrid of two *Phytophthora* species (S.P. Fernández-Pavía, M. Díaz-Celaya and G. Rodríguez-Alvarado, unpublished data). The most studied species in Mexico are *P. capsici*, *P. cinnamomi* and *P. infestans*. Avocado root rot caused by *P. cinnamomi* receives the most attention as Mexico is the main producer of avocado worldwide. In this chapter the biology, impact and control of these three *Phytophthora* species in Mexico will be discussed.

23.2 *P. capsici*

23.2.1 Economic importance and hosts

Chili pepper (*Capsicum annuum* L.) is one of the most important horticultural crops in Mexico. During 2010, 148,759 ha were planted, which produced 2,335,560 t (SIAP, 2010). The chilli pepper industry is important because it is one of the most important ingredients in Mexican food. Chilli pepper wilt caused by *P. capsici* is the most damaging disease causing severe yield losses in many production areas. This disease was first reported in chilli pepper fields located in central Mexico in 1956, by J. Galindo Alonso (Romero-Cova, 1988). Thereafter, *P. capsici* has been recovered from infected *Capsicum frutescens*, *Cucumis sativus*, *Cucurbita* sp., *Solanum lycopersicum* and *Fragaria* × *ananassa*. Recently *P. capsici* was isolated from ornamental nursery-produced pepper plants (S.P. Fernández-Pavía, M. Díaz-Celaya and G. Rodríguez-Alvarado, unpublished data).

23.2.2 Epidemiology

The morphological characteristics for *P. capsici* in Mexico are typical, with the one exception that isolates from ornamental pepper plants often have terminal or intercalary chlamydospores. Most investigations have used morphologic characterization for identification purposes but

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Table 23.1. List of *Phytophthora* species and associated hosts reported in Mexico.

Species	Host
Phytophthora cactorum	*Cocos nucifera*, *Fragaria chiloensis*, *Fragaria* × *ananassa*, *Malus domestica*, *Prunus persica*
Phytophthora capsici	*Capsicum annuum*, *Capsicum frutescens*, *Cucumis sativus*, *Cucurbita* sp., *Fragaria chiloensis*, *Solanum lycopersicum*
Phytophthora cinnamomi	*Byrsonima crassifolia*, *Mangifera indica*, *Persea americana*, *Pseudotsuga macrolepis*, *Quercus elliptica*, *Quercus glaucoides*, *Quercus magnoliifolia*, *Quercus peduncularis*, *Quercus salicifolia*
Phytophthora citricola	*Persea americana*
Phytophthora citrophthora	*Citrus limetta*, *Citrus limon*, *Citrus maxima*, *Citrus sinensis*, *Theobroma cacao*
Phytophthora drechsleri	*Capsicum frutescens*, *Carthamus tintoreus*, *Euphorbia pulcherrima*, *Lactuca sativa*
Phytophthora fragariae	*Fragaria chiloensis*
Phytophthora heveae	*Persea americana*
Phytophthora infestans	*Capsicum frutescens*, *Physalis ixocarpa*, *Solanum antipoviczii*, *Solanum boreale*, *Solanum brachycarpum*, *Solanum cardiophyllum*, *Solanum demissum*, *Solanum dulcamara*, *Solanum ehrenbergii*, *Solanum iopetalum*, *Solanum lycopersicum*, *Solanum pinnatisectum*, *Solanum sambucinum*, *Solanum stoloniferum*, *Solanum tuberosum*, *Solanum verrucosum*
Phytophthora ipomoeae	*Ipomea orizabensis*, *Ipomea purpurea*
Phytophthora medicaginis	*Medicago sativa*
Phytophthora megasperma	*Medicago sativa*
Phytophthora mexicana	*Solanum lycopersicum*
Phytophthora mirabilis	*Mirabilis jalapa*
Phytophthora nicotianae	*Ananas comosus*, *Ananas sativus*, *Carica papaya*, *Citrus sinensis*, *Cucurbita pepo*, *Hibiscus sabdariffa*, *Persea americana*, *Phaseolus vulgaris*, *Zea mays*
Phytophthora palmivora	*Carica papaya*, *Cocos nucifera*, *Sorghum halepense*, *Theobroma cacao*
Phytophthora phaseoli	*Phaseolus vulgaris*
Phytophthora spp.	*Abies religiosa*, *Achras zapota*, *Allium cepa*, *Allium sativum*, *Annona muricata*, *Camellia japonica*, *Carya illinoensis*, *Castanea sativa*, *Cedrela odorata*, *Cedrus* spp., *Citrullus vulgaris*, *Cucumis melo*, *Cydonia oblonga*, *Cymbidium* sp., *Juglans regia*, *Dianthus caryophyllus*, *Linum usitatissimum*, *Olianthes tuberosa*, *Psidium guajava*, *Punica granatum*, *Sesamum indicum*, *Solanum melongena*

in recent years the internal transcribed spacer (ITS) region has been used more frequently to identify field isolates (Silva-Rojas *et al.*, 2009). *P. capsici* is heterothallic, producing oospores when the A1 and A2 mating types are found in the same field. Studies by Ramírez and Romero-Cova (1967) showed that oospores are able to survive in soil under field conditions and act as the primary inocula in subsequent crops (Pérez-Moreno *et al.*, 2003; Silva-Rojas *et al.*, 2009). Interestingly, there are claims that *P. capsici* can survive on seeds, most notably in Leonian's 1922 species description, but this has yet to be proven.

On pepper *P. capsici* infects roots and crown causing severe wilting. It can also attack aerial organs, such as leaves and

fruits, causing foliar blight and fruit rot; but, in Mexico infection of aerial parts is rare, probably because peppers are grown in areas with low relative humidity. When it is warm (25–28°C) and wet *P. capsici* is highly aggressive, causing severe damage in a short period of time (Romero-Cova, 1988). Spores from infected plants are transported by irrigation water to other plants (Silva-Rojas *et al*., 2009). The greatest damage occurs during the rainy season (August and September) and crop loss can reach 100% in some regions particularly in fields with poor water drainage (Guigón-López and González-González, 2001; Rodríguez-Moreno *et al*., 2004).

23.2.3 Management of chilli wilt

Recommendations for managing chilli pepper wilt in Mexico include crop rotation and the levelling of fields to help with water drainage. Although these are sound recommendations, few growers use them and prefer the use of pesticides to control the disease. Several fungicides are used extensively even though their ability to control disease is very low. One chemical used frequently is metalaxyl and resistance has been documented in field populations (Pérez-Moreno *et al*., 2003). Some researchers have reported the use of antagonistic bacteria in biological control assays. *Bacillus licheniformis*, *Bacillus subtilis* and *Bacillus amyloliquefaciens* reduce the degree of root rot and necrosis in chilli pepper under field conditions (Guillén-Cruz *et al*., 2006). Other studies show that solarization in combination with chicken manure can reduce the amount of inocula in chilli pepper fields infested with *P. capsici* (Yáñez-Juárez *et al*., 2001). *Glomus intraradices*, an arbuscular mycorrhizal fungus, has been shown to decrease the severity of root necrosis *in vitro* (Espinosa-Victoria *et al*., 2004).

In several regions in Mexico the search for new sources of genetic resistance in wild populations of chilli pepper is ongoing (Morán-Bañuelos *et al*., 2010). Although the Mexican chilli pepper landrace known as CM-334 has promising characteristics for resistance against *P. capsici*, durable resistance has yet to be transferred to commercial varieties. More recently the use of CM-334 plants as rootstock is being considered, as the use of disease-resistant rootstock has been successful in tomato crops against some fungal root pathogens.

23.3 *P. cinnamomi*

23.3.1 Economic importance and hosts

Mexico is the top producer of avocado (*Persea americana*) worldwide. During 2009, 1,107,135 t were produced on 123,403.69 ha. The state of Michoacan generates 86% of the fruit (SIAP, 2010). The avocado industry benefits a great number of families. Although there are several diseases affecting avocado in Mexico, root rot caused by *P. cinnamomi* is the most important. In some regions disease is severe and affects 50–90% of the trees (Téliz and Mora, 2008). Root rot in avocado was first reported in Mexico in 1951 by Zentmyer (1951), and additional host plants include *Byrsonima crassifolia*, *Mangifera indica*, *Pseudotsuga macrolepis* and several species of *Quercus*. Recently it has been found in nursery-produced ornamental plants (S.P. Fernández-Pavía, M. Díaz-Celaya and G. Rodríguez-Alvarado, unpublished data).

23.3.2 Morphological characteristics

Sporangia can be difficult to produce in culture, but with *P. cinnamomi* isolates obtained from avocado in Mexico sporangia form abundantly in non-sterile soil extract. Another feature is the spherical to ovoid chlamydospores in typical grapelike clusters that are produced abundantly in culture (Romero-Cova, 1988). *P. cinnamomi* is heterothallic, forming oospores when A1 and A2 mating type isolates are paired in culture. In Mexico the mating type A1 has not been found and oospores have not been detected.

23.3.3 Epidemiology

P. cinnamomi can survive as chlamydospores in plant debris for several years. These thick-walled asexual resting spores tolerate low moisture and temperature. When high humidity and optimal temperature conditions are present, chlamydospores germinate to produce sporangia and, if there is free water, swimming zoospores are released. The zoospores are disseminated by water to the roots of healthy hosts. Infected plants eventually exhibit wilting and chlorotic foliage and the more severely affected plants die. Once the tree has died the residual chlamydospores withstand unfavourable conditions allowing the disease cycle to continue until another susceptible host is planted (Téliz and Mora, 2008). The pathogen has been detected on avocado seedlings in several commercial nurseries, and the lack of a disease-free certification programme is considered an important route for dissemination of *P. cinnamomi* to new and established orchards.

23.3.4 Management

In Mexico an integrated management programme for avocado has been proposed that includes: (i) the use of ovine manure, foliage and soil chemical fertilizers; (ii) pruning of diseased trees; (iii) appropriate irrigation practices; (v) pest control; and (vi) addressing problems caused by excessive soil salinity and low soil pH. Implementation in several Mexican states has been encouraging, with reduced damage in affected avocado orchards, increased yields and reduced production costs due to the decreased use of pesticides. An important part of this management programme is the use of pathogen-free materials including planting substrates, planting containers, irrigation water and seeds.

The use of resistant rootstocks could be a low-cost way to manage *P. cinnamomi*, but in Mexico resistant rootstocks are not commercially available. It is an old practice among avocado nursery growers to collect their own rootstock seeds directly from field-grown avocado trees of the type 'criollo' (*Persea americana* var. *drymifolia*). This may explain the variable resistance to *P. cinnamomi* observed among the different rootstocks available in nurseries. Currently, several research groups in Mexico are looking for rootstocks with high resistance levels. Resistant lines used in the USA (e.g. Duke 7) are not useful in Mexico. Metalaxyl is frequently used to control avocado root rot. Much like the situation with other crops, extended use has led to *P. cinnamomi* isolates with resistance and there is reduced efficacy in some avocado orchards (Téliz and Mora, 2008).

23.4 *P. infestans*

23.4.1 Economic importance and hosts

During the 1960s potato (*Solanum tuberosum* L.) was cultivated in a few regions in the central part of Mexico. The average annual yield was approximately 300,000 t. Later, in the early 1980s, farmers in the western and northern regions of the country began cultivating potatoes and in some cases employed irrigation. This resulted in a threefold annual yield increase and potato is now one of the most important horticultural crops in Mexico with annual production of 1,536,617.37 t (SIAP, 2010). Late blight caused by *P. infestans* is the most important disease and causes up to 100% loss in some regions. Late blight was first reported in Mexico in 1930 on *Solanum antipoviczii* (Reddick, 1932). Later, it was found on *Physalis ixocarpa* and other plants in the family *Solanaceae* including *Capsicum frutescens* and 13 *Solanum* species.

Populations of *P. infestans* in the Toluca Valley, in central Mexico, are thought to be more diverse than any other populations worldwide, a feature which has interested researchers in Mexico and abroad for many years. Around 25 articles in international journals describe different aspects of *P. infestans* in this region. In addition to the diversity recorded in the Toluca Valley,

recent studies using isozymes and compatibility type showed the presence of 52 multi-locus genotypes in the western state of Michoacan, indicating that other regions in Mexico may harbour *P. infestans* populations with even more genetic variation than the populations in the Toluca Valley.

23.4.2 Morphological characteristics

P. infestans presents smooth mycelium that is hyaline and coenocytic, with few branches. Sporangia are obovoid to ellipsoid, semi-papillated, deciduous, 21–38 µm in length and 12–23 µm in width. Sporangiophores are branched in a simple sympodia. Oogonia are smooth, spherical and 38–50 µm in diameter. It has amphigynous antheridia and aplerotic oospores with thick and smooth walls that are 25–35 µm in diameter (Romero-Cova, 1988). A low frequency of homothallic populations has been detected on potato and tomato in the states of México and Michoacan, respectively. Populations of *P. infestans* recovered from tomato plants from Michoacan are compatible with potato isolates from the same state; in crosses performed between the two, in our laboratory, oospores were observed on V-8 media. These results, as well as the fact that cross-infection among the two hosts was detected, shows that there is no host specificity (M.R. Gregorio-Cipriano, S.P. Fernández-Pavía and G. Rodríguez-Alvarado, unpublished data).

23.4.3 Epidemiology

The main sources of primary inoculum for *P. infestans* on potato are cull piles and volunteer plants. In addition, seed tubers can be infected or contaminated superficially, and in this case *P. infestans* usually survives as mycelia, although oospores have also been found (Fernández-Pavía *et al.*, 2002). Oospore survival and infectivity has been shown in the Toluca Valley (Fernández-Pavía *et al.*, 2004). Both the A1 and A2 compatibility types have been detected in potato leaves with multiple blight lesions, and in some cases up to 3000 oospores have been found in a single leaf. These leaves fall to the ground where the oospores are able to survive between growing seasons (Díaz-Valasis *et al.*, 2004). It is possible that oospores are an important source of primary inoculum in the majority of the Mexican states where potato is grown since both compatibility types have been detected. During the rainy season sporangia produced on leaves and stems of infected potato plants are disseminated to healthy plants by water and wind (Romero-Cova, 1988). Sporangia are an important source of secondary inoculum if fungicides are not applied in a timely manner. An entire crop can be decimated in a week under favourable conditions. Once sporangia are washed into the soil, the potato tubers can be infected (Romero-Cova, 1988); however, this type of infection is only seen in some northern production areas. This appears to be due to the presence of suppressive soils from which different microorganisms like *Pseudomonas* sp., *Burkholderia* sp., *Streptomyces* sp. and the fungus *Trichoderma* sp. have been isolated and appear to have antagonistic effects against *P. infestans* (Lozoya-Saldaña *et al.*, 2006).

23.4.5 Management

In the 1950s Dr John S. Niederhauser initiated a programme in Mexico to develop potato varieties resistant to *P. infestans*. Later, the National Potato Program of Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) has continued developing varieties with resistance to late blight at the Toluca Research Station in the Toluca Valley. The severe disease pressure and the high genetic diversity of *P. infestans* makes this site optimal for screening candidate potato lines, and potato breeders from around the world test potato lines here.

Some of the potato lines developed by the National Potato Program are highly resistant; however, growers prefer the susceptible varieties because these have better commercial qualities; less than 10%

of the total potato-growing areas cultivate resistant varieties. Growers planting susceptible varieties must spray contact and systemic fungicides to prevent blight and, if they have the resources, may use as many as 20–28 spray applications on a single crop in areas with severe late blight problems. Some low-income growers prefer resistant varieties to reduce the number of fungicide applications. Metalaxyl is often used in an attempt to control late blight but, not surprisingly, heavy use in several states has led to resistant populations and decreased disease control (Matuszak *et al.*, 1994; Jaime-García *et al.*, 2000).

23.5 Conclusions and Perspectives

Phytophthora species severely impact the most important agricultural crops in Mexico. Due to the cost of conducting research, most work has focused on only a few economically important hosts. Our current research on wilting diseases in ornamental nursery plants indicates there are species not previously reported, and additional studies using morphological and molecular tools are needed. The movement of plants and plant parts worldwide means that better understanding of the species diversity in Mexico is important.

References

Díaz-Valasis, M., Cadena-Hinojosa, M.A., Fernández-Pavía, S., Guzmán-Plazola, R. and Grünwald, N.J. (2004) Detección y cuantificación de oosporas de *Phytophthora infestans* (Mont.) de Bary en suelos de papas (*Solanum tuberosum* L.) cultivadas y silvestres de la región central de México. *Revista Mexicana de Fitopatología* 22, 52–60.

Espinosa-Victoria, D., González-Mendoza, D., Placencia-de la Parra, J. and García Espinosa, R. (2004) Reducción de la incidencia de *Phytophthora capsici* Leo. en el sistema radical de plántulas de chile pre-micorrizadas con *Glomus intraradices*. *Terra Latinoamericana* 22, 317–326.

Fernández-Pavía, S.P., Grünwald, N.J. and Fry, W.E. (2002) Formation of *Phytophthora infestans* oospores in nature on tubers in central Mexico. *Plant Disease* 86, 73.

Fernández-Pavía, S.P., Grünwald, N.J., Díaz-Valasis, M., Cadena-Hinojosa, M. and Fry, W.E. (2004) Soilborne oospores of *Phytophthora infestans* in central Mexico survive winter fallow and infect potato plants in the field. *Plant Disease* 88, 29–33.

Guigón-López, C. and González-González, P.A. (2001) Estudio regional de las enfermedades del chile (*Capsicum annuum*, L.) y su comportamiento temporal en el Sur de Chihuahua, México. *Revista Mexicana de Fitopatología* 19, 49–56.

Guillén-Cruz, R., Hernández Castillo, F.D., Gallegos-Morales, G., Rodríguez-Herrera, R., Aguilar-González, C.N., Padrón-Corral, E. and Reyes-Valdés, M.H. (2006) *Bacillus* spp. como biocontrol en un suelo infestado con *Fusarium* spp., *Rhizoctonia solani* Kühn y *Phytophthora capsici* Leonian y su efecto en el desarrollo y rendimiento del cultivo de chile (*Capsicum annuum* L.). *Revista Mexicana de Fitopatología* 24, 105–114.

Jaime-García, R., Trinidad-Correa, R., Felix-Gastelum, R., Orum, T.V., Wasmann, C.C. and Nelson, M.R. (2000) Temporal and spatial patterns of genetic structure of *Phytophthora infestans* from tomato and potato in Del Fuerte Valley. *Phytopathology* 90, 1188–1195.

Leonian, L.H. (1922) Stem and fruit blight of peppers caused by *Phytophthora capsici* sp. nov. *Phytopathology* 12, 401–408.

Lozoya-Saldaña, H., Coyote-Palma, M.H., Ferrera-Cerrato, R. and Lara-Hernández, M.E. (2006) Antagonismo microbiano contra *Phytophthora infestans* (Mont) de Bary. *Agrociencia* 40, 491–499.

Matuszak, J.M., Fernandez-Elquezabal, J., Gu, W.K., Villareal-Gonzalez, M. and Fry, W.E. (1994) Sensitivity of *Phytophthora infestans* populations to metalaxyl in Mexico: distribution and dynamics. *Plant Disease* 78, 911–916.

Morán-Bañuelos, S.H., Aguilar-Rincón, V.H., Corona-Torres, T. and Zavaleta-Mejía, E. (2010) Resistencia a *Phytophthora capsici* Leo. de chiles nativos del Sur de Puebla, México. *Revista Fitotecnia Mexicana* 33, 21–26.

Pérez-Moreno, L., Durán-Ortiz, L.J., Ramírez-Malagón, R., Sánchez-Pale, J.R. and Olalde-Portugal, V. (2003) Compatibilidad fisiológica y sensibilidad a fungicidas de aislamientos de *Phytophthora capsici* Leo. *Revista Mexicana de Fitopatología* 21, 19–25.

Ramírez, V.J. and Romero-Cova, S. (1967) Supervivencia de *Phytophthora capsici* Leo., agente causal de la marchitez del chile. *Agrociencia* 2, 9–18.
Reddick, D. (1932) Some diseases of wild potatoes in Mexico. *Phytopathology* 22, 609–612.
Rodríguez-Moreno, V.M., Luna-Ruiz, J.J., Valle-García, P., Triscareño-López, M. and Ruiz-Corral, J.A. (2004) Caracterización patogénica y sexual de *Phytophthora capsici* Leonian y análisis de su distribución espacial en el Centro-Norte de México mediante un sistema de información geográfica. *Revista Mexicana de Fitopatología* 22, 72–81.
Romero-Cova, S. (1988) *Hongos Fitopatógenos*, 1st edn. Universidad Autónoma Chapingo, México, Mexico.
Servicio de Información Agroalimentaria and Pesquera (SIAP) (2010) Available at: http://www.siap.gob.mx (accessed 7 February 2012).
Silva-Rojas, H.V., Fernández-Pavía, S.P., Góngora-Canul, C., Macías-López, B.C. and Ávila-Quezada, G.D. (2009) Distribución espacio temporal de la marchitez del chile (*Capsicum annuum* L.) en Chihuahua e identificación del agente causal *Phytophthora capsici* Leo. *Revista Mexicana de Fitopatología* 27, 134–147.
Téliz, O.D. and Mora, A.A. (2008) *El Aguacate y su Manejo Integrado*, 2nd edn. Mundi Prensa México, México, Mexico.
Yáñez-Juárez, G.M., Zavaleta-Mejía, E., Flores-Revilla, C., Chávez-Alfaro, J.J. and Valdivia-Alcalá, R. (2001) Management of wilting (*Phytophthora capsici* Leo.), root galling (*Nacobbus aberrans* Thorne and Allen), and virosis in pepper (*Capsicum annuum* L.). *Revista Mexicana de Fitopatología* 19, 40–48.
Zentmyer, G.A. (1951) Avocado diseases. *California Avocado Society Yearbook* 7, 103–106.

24 *Phytophthora* in China

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24.1 Introduction

Agriculture in China faces significant challenges presented by *Phytophthora*. The most important species and hosts are similar to other countries, with *Phytophthora infestans* causing significant losses to potato and tomato, *Phytophthora sojae* causing significant losses to soybean and *Phytophthora capsici* limiting the production of diverse vegetables. In addition, more than 30 other *Phytophthora* species have been reported in China, but most have not yet been associated with large-scale losses and are not discussed as there is limited information available. Here we provide an overview of the biology and management of the three most common Phytophthora diseases in China.

24.2 *P. infestans*

Late blight caused by *P. infestans* is one of the most devastating diseases of potato and tomato in China. Potato is the third most important food crop in the country, providing a primary source of food for about 13% of the Chinese population. Six provinces including Inner Mongolia (north China), Gansu (north-east China), Yunnan, Sichuan, Guizhou and Guangxi (south-west China) make up the main potato-producing areas. The climate in most of these areas is favourable for *P. infestans* and late blight is one of the most serious threats to production. In the past two decades blight has occurred more frequently and has been reported from nearly all potato and tomato production areas. Recent studies report field strains with increasingly complex virulence phenotypes and higher tolerance to the fungicide metalaxyl – factors that may contribute to the higher incidence of disease (Li *et al.*, 2009).

A variety of markers and phenotypes have been used to characterize *P. infestans* populations in China. These include: (i) mating type; (ii) sensitivity to metalaxyl; (iii) virulence on potato differentials; (iv) allozyme profiles for the glucose-6-phosphate isomerase (Gpi) and peptidase A (Pep) enzymes; (v) mitochondrial DNA (mtDNA) haplotypes; and (vi) various DNA-based fingerprinting techniques. Two Gpi loci (Gpi 100/100/111 and 100/100) are common, although the Gpi86/100 and 100/111 genotypes have also been identified. A high frequency of the Gpi 100 allele is typical and the Pep 100/100 is typically found. Four mitochondrial haplotypes (Ia, Ib, IIa, IIb) were found in Chinese isolates with the majority having the IIb haplotype (Li *et al.*, 2009; Guo *et al.*, 2010). Genotype analyses indicate that clonal lineages are restricted in their total distribution, but this could change dramatically if infected seed potatoes are transported between different regions (Guo *et al.*, 2010).

The A1 mating type of *P. infestans* has been the most common in China, although a recent introduction of the A2 mating type is associated with an overall increase in disease incidence and severity. The A2 type was first detected in Northern China in 1996 (Zhang *et al.*, 1996) and has since spread as

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far south as Fujian and Yunnan (Zhao and Zhang, 1999; Li *et al.*, 2009). There is no evidence that A1 and A2 types have sexually recombined to produce more adapted or more aggressive isolates, but the possibility of sexual reproduction exists, and growers and scientists need to be alert to this development. An assessment of virulence phenotypes indicates the number of races in China is increasing and race type was not associated with geographic origin, sample type, mating type or metalaxyl sensitivity (Li *et al.*, 2009; Guo *et al.*, 2009, 2010; Han *et al.*, 2010).

Successful management of late blight relies on an integrated approach where growers use: (i) resistant cultivars (when possible); (ii) weather forecasting to guide fungicide applications; (iii) scouting activity; (iv) removal of cull potatoes and volunteers; (v) planting of healthy seed tubers; and (vi) appropriate protective or systemic fungicides. In China fungicides are normally only used in a preventative manner and in some cases in conjunction with disease forecasting.

24.3 *P. sojae*

Stem and root rot of soybean caused by *P. sojae* is one of the most important factors limiting soybean production in China. It appears to be a relatively new disease as it was first reported in 1989 (Shen and Su, 1991) but did not cause serious losses in the 1990s. However, in the past two decades the incidence and severity of disease has increased annually. Disease incidence is often 3–5% but can reach 75% and total crop loss (Li *et al.*, 1999). In the north-eastern soybean production regions, *P. sojae* causes serious economic losses to soybean production each year. Since *P. sojae* has only recently become a problem in China, it is listed as one of the most important plant quarantine pests by the Chinese government. It has caused significant soybean yield lost in Heilongjiang and Fujian provinces where many soybeans were previously imported from foreign countries. Comparison of random amplified polymorphic DNA (RAPD) fingerprint profiles between isolates recovered from Chinese and US *P. sojae* populations indicates the populations may be closely related (Wang *et al.*, 2006).

An assessment of virulence phenotypes (race typing) for 75 isolates collected from Heilongjiang and Fujian revealed 35 unique race types. Overall it appears that the number of unique races is increasing (Cui *et al.*, 2010). Because soybean originated in China, the disease resistance of soybean cultivars has been studied in several laboratories. A number of soybean cultivars have been shown to have high levels of resistance, especially cultivars from the central region of China (Xia *et al.*, 2011). Studies of the virulence composition of *P. sojae* from China revealed that less than 5% of the Chinese isolates assessed could overcome *Rps* genes 1a, 1c and 1k, indicating that soybean cultivars with these genes can be more widely grown to control Phytophthora stem and root rot (Cui *et al.*, 2010).

Several strategies are useful to control *P. sojae* on soybeans in China, although the most important component is the deployment of resistant hosts. In addition to the host, it is important to plant on well-drained sites that employ crop rotation. Due to the longevity of the oospore, crop rotation alone is not sufficient to control disease. Another useful strategy is the use of a seed treatment such as metalaxyl or mefenoxam to limit seedling disease. Although it is not always possible, the best approach is to plant treated seeds of cultivars with high levels of partial resistance in well-drained fields where crop rotation is practised.

24.4 *P. capsici*

P. capsici infects many vegetables from the families *Solanaceae* and *Cucurbitaceae*. In China *P. capsici* has traditionally caused blight and fruit rot of peppers and chillies, but it is now found attacking pumpkin, tomato, aubergine, cucumber, melons and squash. Similar to *P. infestans* and *P. sojae*, the incidence and severity of epidemics has increased dramatically in recent decades. Once susceptible crops become infected,

crop loss can easily reach 100%. Compared with Northern China, Southern China has more problems due to the warmer and wetter climate.

Three different *P. capsici* mating types have been found in China: A1, A2 and A1A2 (self-fertile). Both the A1 and A2 mating types have been recovered from all of the vegetable production areas and in several instances A1 and A2 mating types were found in the same field, suggesting the potential for sexual reproduction (Zhang *et al.*, 2008; Yang *et al.*, 2009). The contribution to disease epidemics is uncertain. Especially in the Yunnan province of south-west China, the mating types of *P. capsici* on pepper were diverse, indicating self-fertile isolates may play an important role in the epidemiology (Yang *et al.*, 2009).

Populations of *P. capsici* characterized using DNA fingerprints (RAPD) had high levels of diversity, but the diversity was not associated with differing levels of virulence (Sun and Zhang, 2008). There was no significant correlation between mating type and pathogenicity of the isolates of *P. capsici* (Li *et al.*, 2007, 2011). In most parts of China growers rely on a limited number of fungicides to control Phytophthora root, crown and fruit rot. Many growers use metalaxyl and mefenoxam, and as early as 1999 researchers found insensitive isolates (Luo *et al.*, 1999; Qi *et al.*, 2008; Yang *et al.*, 2009; Zhang *et al.*, 2011). Highly resistant, moderately resistant and sensitive strains were detected within the same areas and from different Chinese provinces.

Planting resistant plants is considered one of the most important methods to manage *P. capsici*. Some Chinese local pepper cultivars are more resistant to *P. capsici* and are being used as material for breeding. No fungicide has been shown to be sufficiently effective to manage *P. capsici*; however, some are a valuable tool when used in conjunction with cultural practices. Rotation with non-host crops will reduce the accumulation of *P. capsici* spores that survive in the soil. And finally, in China farmers constantly monitor plant health and remove diseased plants as soon as they are found.

References

Cui, L., Yin, W., Tang, Q., Dong, S., Zheng, X., Zhang, Z. and Wang, Y. (2010) Distribution, pathotypes, and metalaxyl sensitivity of *Phytophthora sojae* from Heilongjiang and Fujian provinces in China. *Plant Disease* 94, 881–884.

Guo, J., van der Lee, T., Qu, D.Y., Yao, Y.Q., Gong, X.F., Liang, D.L., Xie, K.Y., Wang, X.W. and Govers, F. (2009) *Phytophthora infestans* isolates from Northern China show high virulence diversity but low genotypic diversity. *Plant Biology* 11, 57–67.

Guo, L., Zhu, X., Hu, C. and Ristaino, J.B. (2010) Genetic structure of *Phytophthora infestans* populations in China indicates multiple migration events, *Phytopathology* 100, 997–1006.

Han, Y., Qin, Y. and Zhu, J. (2010) Distribution of physiological races of *Phytophthora infestans* on potato collected in the investigated areas of China from 2006 to 2008. *Scientia Agricultura Sinica* 43, 3684–3690.

Li, B., Ma, S. and Ding, J. (1999) Investigations of soybean Phytophthora root rot disease and its determinant factors. *Plant Protection – Beijing* 25, 8–10.

Li, B., Chen, Q., Lu, X., Lan, C., Zhao, J. and Weng, Q. (2009) Phenotypic and genotypic characterization of isolates of *Phytophthora infestans* from China. *Journal of Phytopathology* 157, 558–567.

Li, P., Qi, R., Yang, G., Xu, Z. and Gao, Z. (2011) Preliminary studies on relationship between mating type and pathogenicity of *Phytophthora capsici*. *Journal of Anhui Agricultural University* 38, 319–322.

Li, Z., Long, W., Zheng, J. and Lei, J. (2007) Isolation and identification of *Phytophthora capsici* in Guangdong province and measurement of their pathogenicity and physiological race differentiation. *Journal of South China Agricultural University* 28, 50–54.

Luo, H., Xie, B., Ma, F. and Zhu, G. (1999) A study on *Phytophthora capsici* genetic resistance to metalaxyl and cymoxianil. *Journal of Hunan Agricultural University* 25, 52–56.

Qi, R., Ding, J., Gao, Z., Ni, C., Jiang, J. and Li, P. (2008) Resistance of *Phytophthora capsici* isolates to metalaxyl in Anhui Province. *Acta Phytopathologica Sinica* 35, 245–250.

Shen, C.Y. and Su, Y.C. (1991) Discovery and preliminary studies of *Phytophthora megasperma* on soybean in China. *Acta Phytopathologica Sinica* 21, 298.

Sun, W. and Zhang, X. (2008) Pathogenicity and DNA genetics of *Phytophthora capsici* from different areas. *Journal of Yangtze University (Natural Sciences Edition)* 5, 41–47.

Wang, Z., Wang, Y., Zhang, Z. and Zheng, X. (2006) Genetic relationships among Chinese and American isolates of *Phytophthora sojae* assessed by RAPD markers. *Chinese Science Bulletin* 51, 2095–2102.

Xia, C.J., Zhang, J.Q., Wang, X.M., Wu, X.F., Liu, Z.X. and Zhu, Z.D. (2011) Analyses of resistance genes to Phytophthora root rot in soybean germplasm. *Chinese Journal of Oil Crop Sciences* 33, 396–401.

Yang, M., Cao, J., Li, X., Sun, D., Wang, Y. and Zhao, Z. (2009) Molecular diagnosis and characterization of blight disease pathogen on pepper in Yunnan. *Acta Phytopathologica Sinica* 39, 297–303.

Zhang, H., Liu, Y., Lu, H., Guo, J. and Song, S. (2008) Mating types and distribution of causal organism of pepper in Gansu province. *Acta Agriculturae Boreali-occidentalis Sinica* 17, 91–93.

Zhang, H., Ma, H., Li, X. and Jiang, J. (2011) A study on sensitivity of *Phytophthora capsici* to metalaxyl. *Acta Agriculture Universitatis Jiangxiensis* 33, 270–274.

Zhang, Z.M., Li, Y.Q. and Tian, S.M. (1996) The occurrence of potato late blight pathogen (*Phytophthora infestans*) A2 mating type in China. *Journal of Agricultural University of Hebei* 19, 65–69.

Zhao, Z. and Zhang, Z. (1999) Occurrence of the A2 mating type of *Phytophthora infestans* on potato in Yunnan. *Southwest China Journal of Agricultural Science* 12, 1–3.

25 Globalization and *Phytophthora*

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25.1 Introduction

As far back as the 1920s patches of dead trees were visible in the hills surrounding Perth, Western Australia (Dell *et al.*, 2005.). By 1964, when the causal agent was identified as *Phytophthora cinnamomi*, the disease had spread and was causing widespread decline of the dominant forest species *Eucalyptus marginata* (jarrah) (see Hee *et al.*, Chapter 14, this volume). The disease is known as 'jarrah dieback'; a particularly misleading title for a disease that has decimated extensive regions in this fragile biodiversity hotspot. In Western Australia *P. cinnamomi* is known as a biological bulldozer and 2284 of the 5710 described plant species are susceptible or highly susceptible (Shearer *et al.*, 2004) (see Hee *et al.*, Chapter 14, this volume). This is just one example of the impact caused by invasive *Phytophthora* species and there are many additional examples from natural ecosystems, agriculture and agroforestry worldwide. The common thread is human-mediated movement, and the origin of many species remains a mystery.

25.2 Molecular Tools

The development of molecular tools for detection and identification has revolutionized plant pathology. In the seminal paper of Cooke *et al.* (2000) *Phytophthora* species were classified into ten phylogenetic clades based on the internal transcribed spacer (ITS) region (see Thines, Chapter 2, this volume). This led to the synonymizing of some species (including *Phytophthora nicotianae* and *Phytophthora parasitica*) (Ho and Jong, 1989) and the identification of cryptic species (including *Phytophthora citricola*, *Phytophthora plurivora* and *Phytophthora multivora*). Molecular tools are being used to re-evaluate existing collections, such as the world *Phytophthora* collection (Park *et al.*, 2008) and the Vegetation Health Service collection in Western Australia (Burgess *et al.*, 2009), and are allowing rapid evaluation of species from environmental surveys (Reeser *et al.*, 2011). Since 2000 there has been an increase in the discovery of new species (Kroon *et al.*, 2011) due to increased research and survey activity and reclassification of isolates originally characterized on moprhology alone (Fig. 25.1). Population studies using molecular markers (e.g. *Phytophthora infestans* (Cooke and Lees, 2004) and *Phytophthora ramorum* (Grünwald *et al.*, 2009)) have illuminated the phylogeography for some species and may help elucidate pathways of spread and micro-evolutionary changes.

25.3 Biology and Dispersal

Oomycetes are protists belonging to the Chromeaveolata with the closest relationship to brown algae. The earliest oomycete genera were predominantly marine organisms and lack the sexual stage (Beakes and Sekimoto, 2009). Crown oomycetes are oogamous and the thick-walled oospores are resistant to desiccation. Most oomycetes are parasites, and it appears that they were

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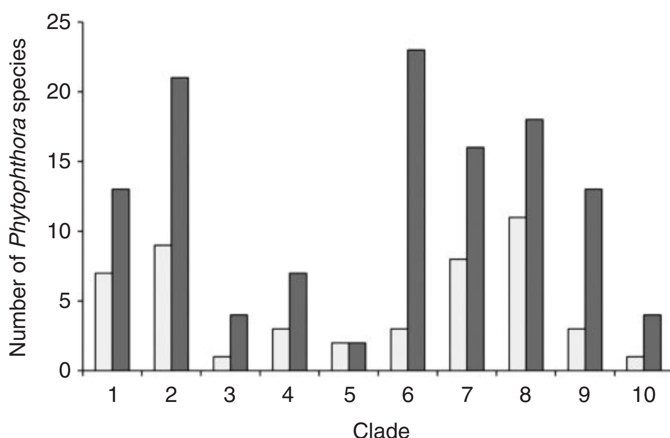

Fig. 25.1. Number of *Phytophthora* species described within each of the currently recognized molecular phylogenetic clades discovered before (pale grey) and after (dark grey) the year 2000.

hard wired for parasitism since their inception (Beakes and Sekimoto, 2009). The most advanced oomycetes (downy mildews) are obligate parasites, which cannot be cultured apart from their hosts. There are some unculturable *Phytophthora* species but most can grow as saprophytes and are readily grown in culture.

Phytophthora species often have a large host range and can impact on a wide variety of species when dispersed into new regions. Examples include *P. cinnamomi* (Shearer *et al.*, 2004) and *P. ramorum* (Rizzo *et al.*, 2002), and, under predicted models of climatic change, the overall impact may increase for some species (*P. cinnamomi*; Brasier, 1996). Genetic recombination may result in more virulent genotypes either through intraspecific mating, which is common for the vegetable pathogens *Phytophthora capsici* and *P. infestans* in some locations (Grünwald and Flier, 2005) (see Granke *et al.*, Chapter 11, this volume), or interspecific mating to produce novel species hybrids (Brasier *et al.*, 2004) (see Érsek and Man in 't Veld, Chapter 5, this volume).

Hybrid *Phytophthora* species have been created in the laboratory, and naturally generated within greenhouses and natural ecosystems, and may lead to rapid generation of new pathogens (Érsek and Nagy, 2008; Kroon *et al.*, 2011) (see Érsek and Man in 't Veld, Chapter 5, this volume). *Phytophthora alni* and variants (Brasier *et al.*, 2004) are thought to have developed when species that had evolved apart were brought together in nurseries. Because original speciation had been allopatric, it appears that reproductive boundaries between the different species were not fully formed and consequently when bought together they could hybridize through sexual reproduction (Ioos *et al.*, 2006; Érsek and Nagy, 2008). Similarly, a hybrid *Phytophthora* has been reported in the Netherlands between the resident species *Phytophthora cactorum* and the introduced species *P. nicotianae* on a *Cyclamen* species, which is not known to be a host of either parental species (Bonants *et al.*, 2000; Érsek and Nagy, 2008).

Phytophthora species have evolved various mechanisms for dispersal including the ability to swim, as zoospores, towards plants in the soil and in surface water, and aerial dispersal from lesions on above-ground plant parts (Ristaino and Gumpertz, 2000). They can also be spread by insects (Konam and Guest, 2004), snails (Alvarez *et al.*, 2009) and feral pigs (G. Hardy, unpublished data).

25.4 Pathways for Global Dispersal

As mentioned above, *Phytophthora* species are readily spread through movement of live plants, organic matter, soils and irrigation water. Species often produce environmentally resistant, thick-walled resting structures and have adapted widely, occupying niches ranging from aquatic to arid environments. Extreme examples include the aquatic species *Phytophthora gemini* that attacks *Zostera marina* (seagrass) (Man in 't Veld *et al.*, 2011) and *Phytophthora arenaria*, which is found in the arid northern sand plains of Western Australia (Rea *et al.*, 2011).

An important dispersal route is in the soil of potted plants. Historically, a great deal of movement may have occurred during the 'exploration, dispersal and colonization' of the world. New plants (food and ornamental) were transported from Europe to the colonies and, in the case of Australia and New Zealand, may have taken up to 3 months to reach their destination. It is thought they were re-potted in harbours such as Singapore, Capetown and Jakarta and may have acquired hitch-hiking *Phytophthora* species along the way. In addition, plants collected from colonies and newly discovered lands were transported back to European gardens, especially during the era of the great plant hunters (Reichard and White, 2001). Many *Phytophthora* species are fine-root feeders and take a considerable time to kill their hosts allowing ample time for movement to new locations. Aerial *Phytophthora* species may also be transported during their quiescent phase or on non-symptomatic hosts.

It is now generally accepted that humans are the primary movers of exotic pathogens and pests between biogeographical zones (Brasier, 2008). It is often impractical to screen live plants, organic matter, soil and irrigation water for *Phytophthora* species. Making matters worse, *Phytophthora* infections may be asymptomatic. Examples include: *P. ramorum* and *Phytophthora kernoviae*, species that can actively sporulate on symptomless foliage and fruit (Denman *et al.*, 2009); *P. cinnamomi*, which has been isolated from symptomless roots of a range of resistant legume species (D'Souza *et al.*, 2005); and *P. infestans*, which has been associated with asymptomatic potato seed tubers during cold storage (Johnson and Cummings, 2009). *Phytophthora* species also produce non-specific symptoms commonly caused by other pathogens. An example is *P. ramorum*, which causes leaf blight, shoot dieback and bole canker on diverse hosts (Hansen *et al.*, 2005). Chemical controls (e.g. phosphite), especially within the nursery trade, can mask disease symptoms on *Phytophthora*-infected plants (Linderman and Davis, 2006), and the verification of disease-free plants from exporting countries cannot effectively control the movement of *Phytophthora* species (Brasier, 2008).

Soil attached to the boots of hikers and ecotourists can spread *P. cinnamomi* within Western Australia (Kelly *et al.*, 2003) – the irony being that those most interested in nature may inadvertently be spreading devastating pathogens into the environments they love. Currently, globalization has led to a huge increase in trade to all corners of the planet and the most dangerous pathway for the movement of *Phytophthora* is 'plants for planting'. Some countries (e.g. Australia) do not allow soil to cross the borders but many other countries still receive plants in soil. Even if plants are inspected, fungicides can mask symptoms and there are no visible symptoms on some hosts, despite active infections.

25.5 Global Host Distributions of *Phytophthora* Species

As of 2012 there are 121 described *Phytophthora* species with 4384 distinct host–pathogen associations distributed in 138 countries. Since the year 2000 there has been a substantial increase in the number of new *Phytophthora* species, with 51 species recorded before, and 70 species

recorded after, the year 2000. Many of the newly described species are from clades 2 and 6 (Fig. 25.1). Regarding known impacts, 41 species impact only agriculture, 31 species affect agriculture and forestry or natural ecosystems, 41 species impact only forestry or natural ecosystems, and the impacts of eight species are unknown.

Phytophthora species have been recorded in all continents except Antarctica (Table 25.1). The USA has the greatest number of recorded *Phytophthora* species,

Table 25.1. Number of *Phytophthora* species reported by country.

Country	Species	Country	Species	Country	Species
USA	69	Sri Lanka	7	Brunei	2
Australia	39	Virgin Islands	7	Cambodia	2
UK	35	Sweden	7	Guadeloupe	2
France	27	Thailand	7	Liberia	2
Germany	27	Turkey	7	Micronesia	2
Japan	26	Austria	6	Nepal	2
Italy	26	Côte d'Ivoire	6	Pacific Islands	2
China	26	Denmark	6	Reunion	2
Netherlands	24	Fiji	6	Samoa	2
New Zealand	24	Israel	6	Sierra Leone	2
Canada	22	Mauritius	6	Solomon Islands	2
South Africa	22	Myanmar	6	Yugoslavia	2
Brazil	21	Pakistan	6	Poland	2
Spain	21	Panama	6	Albania	1
Taiwan	20	Portugal	6	Bangladesh	1
Greece	19	Wales	6	Benin	1
Korea	19	Cameroon	5	Cook Islands	1
India	18	Egypt	5	Equatorial Guinea	1
Mexico	18	Jamaica	5	French Guiana	1
Argentina	15	Nigeria	5	Gabon	1
Iran	14	Bolivia	5	Guam	1
Papua New Guinea	14	Bermuda	4	Guinea	1
Chile	13	Colombia	4	Guyana	1
Ireland	12	Dominican Republic	4	Iceland	1
Malaysia	12	East Africa	4	Lebanon	1
Bulgaria	11	Ecuador	4	Libya	1
Indonesia	11	Finland	4	Luxembourg	1
Peru	11	Ghana	4	Martinique	1
Philippines	10	Honduras	4	Mongolia	1
Guatemala	10	Nicaragua	4	Mozambique	1
Hungary	9	Romania	4	Paraguay	1
Puerto Rico	9	Tanzania	4	Rwanda	1
Switzerland	9	West Indies	4	Sao Tome and Principe	1
Vietnam	9	Barbados	3	Seychelles	1
Russia	9	Cyprus	3	Somalia	1
Congo	8	Ethiopia	3	South Korea	1
Costa Rica	8	French Polynesia	3	South-east Asia	1
El Salvador	8	Kenya	3	Southern Africa	1
Europe	8	Madagascar	3	Sudan	1
Norway	8	Malawi	3	Syria	1
Venezuela	8	New Caledonia	3	Togo	1
Zimbabwe	8	Serbia	3	Tonga	1
Belgium	7	Slovenia	3	Tunisia	1
Cuba	7	South America	3	Uganda	1
Czech Republic	7	Trinidad and Tobago	3	Vanuatu	1
Morocco	7	Zambia	3		

followed by Australia, the UK and France and Germany. Thirty countries report ten or more *Phytophthora* species, while 108 countries had less than ten species and 33 countries had only one species. Wealthy countries fund plant pathology research and are clearly more likely to identify and report *Phytophthora* species. All of the countries with over 20 known *Phytophthora* species are economically developed (Table 25.1). It is highly unlikely that less developed countries have fewer *Phytophthora* species than developed countries. *Phytophthora* species may have a larger impact on less developed countries that are more dependent on agriculture and subsistence living and have fewer resources to manage plant diseases.

Country distribution does not clearly correspond to geographic distribution and may under- or over-represent the impacts of a *Phytophthora* species. For example, Australia covers a range of biological zones including arid and temperate zones. Some *Phytophthora* species have been found in a range of climatic conditions affecting a wide host range, including *P. multivora* (Scott *et al.*, 2009), while others are comparatively restricted and only known from a few hosts (e.g. *Phytophthora constricta*) (Rea *et al.*, 2011). Similarly *Phytophthora pinifolia* is only known from *Pinus radiata* in Chile where it has had a devastating impact on pine (Durán *et al.*, 2008) (see Ahumada *et al.*, Chapter 17, this volume).

Phytophthora species have also been incorrectly identified, especially when originally identified using morphological techniques. For example, species related to *P. citricola* (*P. multivora* (Scott *et al.*, 2009) and *P. plurivora* (Jung and Burgess, 2009)) were only correctly assigned using molecular phylogeny. In addition, it is impossible to confirm the host range for *Phytophthora* isolates that no longer exist in culture collections.

25.6 Conclusions

The current number of known *Phytophthora* species and observed rates of discovery are approaching 200 of the 600 potential *Phytophthora* species suggested by Brasier (2009). A large number of *Phytophthora* species are yet to be discovered and adequate quarantine methods are required to control their potential threat. When considering globalization and *Phytophthora* it is hard to envisage any good news. Quarantine best practices in Australia and New Zealand prevent the importation of any plant material with attached soil and mandatory inspection of all travellers and their luggage. One can assume these practices greatly reduce the chance introduction of new *Phytophthora* species (although there are still new species to be described that were introduced prior to quarantine practices). However, countries and regions with land borders have a much greater battle. To block spread via soil the only real solution is to exclude any plants that have soil attached (Kelly *et al.*, 2003). It is also important to educate the public to examine machinery and boots for soil when moving within and between countries. Globally, more enforcement is required if movement of *Phytophthora* species is to be halted.

To date, countries with the most recorded *Phytophthora* species are those where resources are readily available for research, and the full extent of globalization is not known. As molecular techniques for detection become increasingly simple and cost effective one can imagine an ‘explosion’ of data on diversity and distribution. Of particular interest are ecosystems where *Phytophthora* is present but not causing damage. In this way, the origin and dispersal mechanisms of some of our most devastating *Phytophthora* species may be determined and measures implemented to block pathways of movement.

References

Alvarez, L.A., Gramaje, D., Abad-Campos, P. and García-Jiménez, J. (2009) Role of the *Helix aspersa* snail as a vector of *Phytophthora citrophthora* causing branch cankers on clementine trees in Spain. *Plant Pathology* 58, 956–963.

Beakes, G.W. and Sekimoto, S. (2009) The evolutionary phylogeny of oomycetes – insights gained from studies of holocarpic parasites of algae and invertebrates. In: Lamour, K. and Kamoun, S. (eds) *Oomycete Genetics and Genomics: Diversity, Interactions, and Research Tools*. John Wiley and Sons, New Jersey, pp. 1–24.

Bonants, P.J.M., Hagenaar-de Weerdt, M., Man in 't Veld, W.A. and Baayen, R.P. (2000) Molecular characterization of natural hybrids of *Phytophthora nicotianae* and *P. cactorum*. *Phytopathology* 90, 867–874.

Brasier, C.M. (1996) *Phytophthora cinnamomi* and oak decline in southern Europe. Environmental constraints including climate change. *Annals of Forest Science* 53, 347–358.

Brasier, C.M. (2008) The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathology* 57, 792–808.

Brasier, C.M. (2009) *Phytophthora* biodiversity: how many *Phytophthora* species are there? In: Goheen, E.M. and Frankel, S.J. (eds) *Phytophthoras in Forests and Natural Ecosystems*. General Technical Report PSW-GTR-221. United States Department of Agriculture (USDA) Forest Service, Albany, California, pp. 101–115.

Brasier, C.M., Kirk, S.A., Delcan, J., Cooke, D.E.L., Jung, T. and Man in 't Veld, W.A. (2004) *Phytophthora alni* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. *Mycological Research* 108, 1172–1184.

Burgess, T.I., Webster, J.L., Ciampini, J.A., White, D., Hardy, G.E.S.J. and Stukely, M.J.C. (2009) Re-evaluation of *Phytophthora* species isolated during 30 years of vegetation health surveys in Western Australia using molecular techniques. *Plant Disease* 93, 215–223.

Cooke, D.E.L. and Lees, A.K. (2004) Markers, old and new, for examining *Phytophthora infestans* diversity. *Plant Pathology* 53, 692–704.

Cooke, D.E.L., Drenth, A., Duncan, J.M., Wagels, G. and Brasier, C.M. (2000) A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology* 30, 17–32.

Dell, B., Hardy, G.E.S.J. and Vear, K. (2005) History of *Phytophthora cinnamomi* management in Western Australia. In: Calver, M.C., Bigler-Cole, H., Bolton, G., Dargavel, J., Gaynor, A., Horwitz, P., Mills, J. and Wardell-Johnson, G. (eds) *A Forest Consciousness: Proceedings of the 6th National Conference of the Australian Forest History Society*. Millpress Science Publishers, Rotterdam, the Netherlands, pp. 391–406.

Denman, S., Kirk, S.A., Moralejo, E. and Webber, J.F. (2009) *Phytophthora ramorum* and *Phytophthora kernoviae* on naturally infected asymptomatic foliage. *EPPO Bulletin* 39, 105–111.

D'Souza, N., Colquhoun, I.J., Shearer, B.L. and Hardy, G.E.S.J. (2005) Assessing the potential for biological control of *Phytophthora cinnamomi* by fifteen native Western Australian jarrah-forest legume species. *Australasian Plant Pathology* 34, 533–540.

Durán, A., Gryzenhout, M., Slippers, B., Ahumada, R., Rotella, A., Flores, F., Wingfield, B.D. and Wingfield, M.J. (2008) *Phytophthora pinifolia* sp. nov. associated with a serious needle disease of *Pinus radiata* in Chile. *Plant Pathology* 57, 715–727.

Érsek, T. and Nagy, Z. (2008) Species hybrids in the genus *Phytophthora* with emphasis on the alder pathogen *Phytophthora alni*: a review. *European Journal of Plant Pathology* 122, 31–39.

Grünwald, N.J. and Flier, W.G. (2005) The biology of *Phytophthora infestans* at its center of origin. *Annual Review of Phytopathology* 43, 171–190.

Grünwald, N.J., Goss, E.M., Ivors, K., Garbelotto, M., Martin, F.N., Prospero, S., Hansen, E., Bonants, P.J.M., Hamelin, R.C., Chastagner, G., Werres, S., Rizzo, D.M., Abad, G., Beales, P., Bilodeau, G.J., Blomquist, C.L., Brasier, C., Brière, S.C., Chandelier, A., Davidson, J.M., Denman, S., Elliott, M., Frankel, S.J., Goheen, E.M., de Gruyter, H., Heungens, K., James, D., Kanaskie, A., McWilliams, M.G., Man in 't Veld, W., Moralejo, E., Osterbauer, N.K., Palm, M.E., Parke, J.L., Sierra, A.M.P., Shamoun, S.F., Shishkoff, N., Tooley, P.W., Vettraino, A.M., Webber, J. and Widmer, T.L. (2009) Standardizing the nomenclature for clonal lineages of the sudden oak death pathogen, *Phytophthora ramorum*. *Phytopathology* 99, 792–795.

Hansen, E.M., Parke, J.L. and Sutton, W. (2005) Susceptibility of Oregon forest trees and shrubs to *Phytophthora ramorum*: a comparison of artificial inoculation and natural infection. *Plant Disease* 89, 63–70.

Ho, H.H. and Jong, S.C. (1989) *Phytophthora nicotianae* (*P. parasitica*). *Mycotaxon* 35, 243–276.

Ioos, R., Andrieux, A., Marçais, B. and Frey, P. (2006) Genetic characterization of the natural hybrid species *Phytophthora alni* as inferred from nuclear and mitochondrial DNA analyses. *Fungal Genetics and Biology* 43, 511–529.

Johnson, D.A. and Cummings, T.F. (2009) Latent infection of potato seed tubers by *Phytophthora infestans* during long-term cold storage. *Plant Disease* 93, 940–946.

Jung, T. and Burgess, T. (2009) Re-evaluation of *Phytophthora citricola* isolates from multiple woody hosts in Europe and North America reveals a new species, *Phytophthora plurivora* sp. nov. *Persoonia* 22, 95–110.

Kelly, C.L., Pickering, C.M. and Buckley, R.C. (2003) Impacts of tourism on threatened plant taxa and communities in Australia. *Ecological Management and Restoration* 4, 37–44.

Konam, J.K. and Guest, D.I. (2004) Role of beetles (Coleoptera: Scolytidae and Nitidulidae) in the spread of *Phytophthora palmivora* pod rot of cocoa in Papua New Guinea. *Australasian Plant Pathology* 33, 55–59.

Kroon, L.P.N.M., Henk, B., de Cock, A.W.A.M. and Govers, F. (2011) The *Phytophthora* genus anno 2012. *Phytopathology* 102, 348–364.

Linderman, R.G. and Davis, E.A. (2006) Evaluation of chemical and biological agents for control of *Phytophthora* species on intact plants or detached leaves of rhododendron and lilac. In: Frankel, S.J., Shea, P.J. and Haverty, M.I. (eds) *Proceedings of the Sudden Oak Death Second Science Symposium: The State of Our Knowledge*. General Technical Report PSW-GTR-196. United States Department of Agriculture (USDA) Forest Service, Pacific Southwest Research Station, Albany, California, pp. 265–268.

Man in ’t Veld, W.A., Rosendahl, K.C., Brouwer, H. and de Cock, A.W. (2011) *Phytophthora gemini* sp. nov., a new species isolated from the halophilic plant *Zostera marina* in the Netherlands. *Fungal Biology* 115, 724–732.

Park, J., Park, B., Veeraraghavan, N., Jung, K., Lee, Y.-H., Blair, J.E., Geiser, D.M., Isard, S., Mansfield, M.A., Nikolaeva, E., Park, S.-Y., Russo, J., Kim, S.H., Greene, M., Ivors, K.L., Balci, Y., Peiman, M., Erwin, D.C., Coffey, M.D., Rossman, A., Farr, D., Cline, E., Grünwald, N.J., Luster, D.G., Schrandt, J., Martin, F., Ribeiro, O.K., Makalowska, I. and Kang, S. (2008) *Phytophthora* database: a forensic database supporting the identification and monitoring of *Phytophthora*. *Plant Disease* 92, 966–972.

Rea, A.J., Burgess, T.I., Hardy, G.E.S.J., Stukely, M.J.C. and Jung, T. (2011) Two novel and potentially endemic species of *Phytophthora* associated with episodic dieback of Kwongan vegetation in the south-west of Western Australia. *Plant Pathology* 60, 1055–1068.

Reeser, P.W., Sutton, W., Hansen, E.M., Remigi, P. and Adams, G.C. (2011) *Phytophthora* species in forest streams in Oregon and Alaska. *Mycologia* 103, 22–35.

Reichard, S.H. and White, P. (2001) Horticulture as a pathway of invasive plant introductions in the United States. *BioScience* 51, 103–113.

Ristaino, J.B. and Gumpertz, M.L. (2000) New frontiers in the study of dispersal and spatial analysis of epidemics caused by species of the genus *Phytophthora*. *Annual Review of Phytopathology* 38, 541–576.

Rizzo, D.M., Garbelotto, M., Davidson, J.M., Slaughter, G.W. and Koike, T. (2002) *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. *Plant Disease* 86, 205–214.

Scott, P.M., Burgess, T.I., Barber, P.A., Shearer, B.L., Stukely, M.J.C., Hardy, G.E.S.J. and Jung, T. (2009) *Phytophthora multivora* sp. nov., a new species recovered from declining *Eucalyptus*, *Banksia*, *Agonis* and other plant species in Western Australia. *Persoonia* 22, 1–13.

Shearer, B.L., Crane, C.E. and Cochrane, A. (2004) Quantification of the susceptibility of the native flora of the South-West Botanical Province, Western Australia, to *Phytophthora cinnamomi*. *Australian Journal of Botany* 52, 435–443.

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