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Pathogen profile update

Phytophthora cinnamomi

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SUMMARY

Phytophthora cinnamomi is one of the most devastating plant pathogens in the world. It infects close to 5000 species of plants, including many of importance in agriculture, forestry and horticulture. The inadvertent introduction of *P. cinnamomi* into natural ecosystems, including a number of recognized Global Biodiversity Hotspots, has had disastrous consequences for the environment and the biodiversity of flora and fauna.

The genus *Phytophthora* belongs to the Class Oomycetes, a group of fungus-like organisms that initiate plant disease through the production of motile zoospores. Disease control is difficult in agricultural and forestry situations and even more challenging in natural ecosystems as a result of the scale of the problem and the limited range of effective chemical inhibitors. The development of sustainable control measures for the future management of *P. cinnamomi* requires a comprehensive understanding of the cellular and molecular basis of pathogen development and pathogenicity. The application of next-generation sequencing technologies to generate genomic and transcriptomic data promises to underpin a new era in *P. cinnamomi* research and discovery. The aim of this review is to integrate bioinformatic analyses of *P. cinnamomi* sequence data with current knowledge of the cellular and molecular basis of *P. cinnamomi* growth, development and plant infection. The goal is to provide a framework for future research by highlighting potential pathogenicity genes, shedding light on their possible functions and identifying suitable targets for future control measures.

Taxonomy: *Phytophthora cinnamomi* Rands; Kingdom Chromista; Phylum Oomycota or Pseudofungi; Class Oomycetes; Order Peronosporales; Family Peronosporaceae; genus *Phytophthora*.

Host range: Infects about 5000 species of plants, including 4000 Australian native species. Host plants important for agriculture and forestry include avocado, chestnut, macadamia, oak, peach and pineapple.

Disease symptoms: A root pathogen which causes rotting of fine and fibrous roots, but which can also cause stem cankers. Root damage may inhibit water movement from roots to shoots, leading to dieback of young shoots.

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Useful websites: http://fungidb.org/fungidb/; http://genome.jgi.doe.gov/Phyci1/Phyci1.home.html; http://www.ncbi.nlm.nih.gov/assembly/GCA_001314365.1; http://www.ncbi.nlm.nih.gov/assembly/GCA_001314505.1

Keywords: dieback disease, Oomycetes, *Phytophthora cinnamomi*, root pathogen, soil-borne pathogen.

INTRODUCTION

Phytophthora cinnamomi is one of the most devastating plant pathogens known. It has a world-wide distribution and a host range approaching 5000 species (Cahill *et al.*, 2008; Jung *et al.*, 2013). In addition to causing substantial economic losses in agriculture, forestry and horticulture, the inadvertent introduction of *P. cinnamomi* has had disastrous consequences for natural ecosystems and biodiversity. Prime examples include the impact of *P. cinnamomi* on chestnut and holm oak forests in Europe (Serrazina *et al.*, 2015; Sghaier-Hammami *et al.*, 2013), on avocado and macadamia worldwide (Akinsanmi *et al.*, 2017; Reeksting *et al.*, 2016) and on natural vegetation in south-east and south-west Australia (Cahill *et al.*, 2008; Jung *et al.*, 2013) (Fig. 1). About 4000 Australian native species, sometimes close to 50% of species present, are susceptible in forests and heathlands in Western Australia (Shearer *et al.*, 2004; Weste, 2003). The recognition of the magnitude of the environmental problems caused by *P. cinnamomi* has led to its inclusion in the list of Key Threatening Processes in the Commonwealth Environmental Protection & Biodiversity Conservation Act 1999 and to the development of a National Threat Abatement Plan aimed at the management and control of *P. cinnamomi* diseases (Australian Government, 2014).

Phytophthora is a genus in the Oomycetes, a Class in Phylum Pseudofungi within the Kingdom Chromista (Beakes *et al.*, 2012; Cavalier-Smith and Chao, 2006). The defining character of the Chromista is the production of motile asexual spores possessing a flagellum adorned by tubular hairs which are responsible for forward movement (Beakes *et al.*, 2012; Cahill *et al.*, 1996). For many species of *Phytophthora*, the motile zoospores are the main infective agent that initiates plant disease. *Phytophthora cinnamomi* has been ranked in the Top 10 Oomycete plant pathogens based on scientific and economic importance (Kamoun *et al.*,

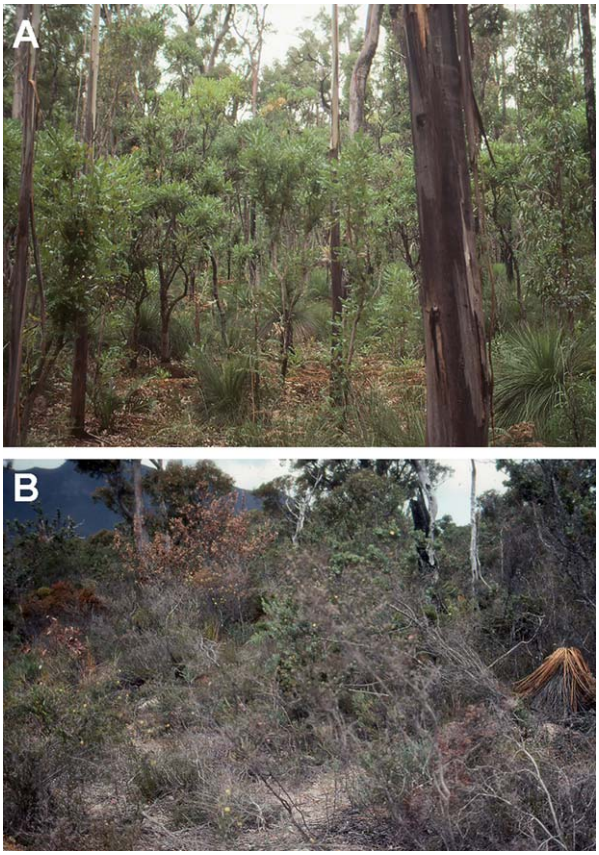

Fig. 1 *Phytophthora cinnamomi* kills thousands of plant species in natural ecosystems in Western Australia, threatening the environment and biodiversity. (A) An uninfected area within a Eucalypt forest south of Perth dominated by *Eucalyptus marginata* (Jarrah), *Banksia grandis* and *Xanthorea* species (grasstrees). (B) *Xanthorea* species and many proteaceous plants in Western Australia are highly susceptible to *P. cinnamomi*. When present, *P. cinnamomi* dramatically changes the floral composition of the region, with more resistant species, such as acacias, rushes and sedges, replacing the plants that have been killed.

2015). It has been estimated that, in California, losses in avocado crops caused by *P. cinnamomi* exceed US$40 million annually (Ploetz, 2013).

A number of key questions relating to the management of *P. cinnamomi* diseases urgently need answers. What factors are responsible for *P. cinnamomi*'s extremely extensive host range? Many of these factors must be species specific because other *Phytophthora* species, such as *P. infestans* and *P. sojae*, have narrow host ranges (Cooke and Andersson, 2013; Dorrance, 2013). Is *P. cinnamomi*'s success in establishing disease a result of its ability to avoid triggering host defence and/or to suppress or overcome host defences? What aspects of *P. cinnamomi* molecular or cellular make-up would be good targets for novel, specific and sustainable control measures? The answers to these questions will be informed by a better understanding of the cellular and molecular biology of *P. cinnamomi* development and infection strategies.

The advent and application of next-generation sequencing technologies herald a new era in *P. cinnamomi* research and discovery. The genomes (78 Mb) of three isolates of *P. cinnamomi* have now been sequenced and are publicly available (http://fungidb.org/fungidb/; http://genome.jgi.doe.gov/Phyci1/Phyci1.home.html; http://www.ncbi.nlm.nih.gov/assembly/GCA_001314365.1; http://www.ncbi.nlm.nih.gov/assembly/GCA_001314505.1). The first sequencing project was conducted at the Joint Genome Institute (JGI). The sequence data are in draft form, with only preliminary annotation, and are accessible on JGI and FungiDB websites. Sequence data for two further isolates were released in 2016 [available on the National Center for Biotechnology Information (NCBI) website]. These unannotated data have been assembled into about 4000 contigs. At the time of writing, there were two published analyses of *P. cinnamomi* transcriptomes available (Meyer *et al.*, 2016; Reitmann *et al.*, 2016). The first reports data from *Eucalyptus nitens*, 5 days after inoculation with *P. cinnamomi;* the second reports data from a library of cysts and germinated cysts.

This present Pathogen Profile focuses on information that has become available since the last Pathogen Profile on *P. cinnamomi* was published in 2005 (Hardham, 2005). The goal of the article is to integrate bioinformatic analyses of *P. cinnamomi* sequence data with our current understanding of the cellular and molecular basis of growth, development and plant infection strategies employed by *P. cinnamomi* and other *Phytophthora* species. It is hoped that this will provide a framework for future research by highlighting pathogenicity genes, shedding light on their possible functions and identifying potential targets for future control measures.

THE *P. CINNAMOMI* LIFECYCLE

Phytophthora cinnamomi is a soil-borne pathogen with sexual and asexual phases in its lifecycle (Zentmyer, 1980). It can grow saprophytically on dead organic matter or parasitically on susceptible hosts. Typically, *P. cinnamomi* infects fine, feeder roots, but it can also invade woody stems, especially through wounds or natural breaks in the peridermal layer (O'Gara *et al.*, 2015). Growth within the root system causes root rotting and interferes with water uptake and transport to the shoot, resulting in wilting and chlorosis of the foliage. Plants may die rapidly, or may survive, often without showing disease symptoms, for many years. The ability of *P. cinnamomi* to grow saprophytically in the soil or symptomlessly in infected plants is a major contributing factor to the long-term survival of the pathogen. Sexual oospores, asexual chlamydospores and intracellular hyphal aggregates are thought to enable the pathogen to survive for long periods under adverse conditions and make complete eradication of the disease extremely difficult (Jung *et al.*, 2013).

Gene silencing experiments in *P. sojae* have shown that the signalling pathways involved in oospore development include PsGK5, a G-protein-coupled receptor (GPCR) that has a phosphatidylinositol

Table 1 *Phytophthora cinnamomi* proteins involved in sporulation.

P. cinnamomi gene name*	Homologues used for BLAST query	Proposed protein function	References
Oospore development			
PHYCI_277748	*P. sojae PsGK5* PHYSO_335695	G-protein-coupled receptor with C-terminal phosphatidylinositol phosphate (PIP) kinase domain Silencing > inhibits oospore development	Yang *et al.* (2013)
PHYCI_112968	*P. sojae PsMPK7* PHYSO_355777	Stress-associated mitogen-activated protein kinase (MAPK). Gene expression is up-regulated in zoospores, cysts and germinating cysts Silencing > inhibits oospore development; reduces virulence	Gao *et al.* (2015)
Asexual sporulation and zoosporogenesis			
PHYCI_89449 PHYCI_233998 PHYCI_111102† PHYCI_199460†	*P. cinnamomi* α-tubulin	α-tubulin subunit of microtubule. Microtubules are required for shape and spacing of nuclei within sporangia and form the framework of the flagella axoneme	Hardham (1987); Hyde and Hardham (1993)
PHYCI_88710	*P. cinnamomi* β-tubulin	β-tubulin subunit of microtubule. Microtubules are required for shape and spacing of nuclei within sporangia and form the framework of the flagella axoneme	Weerakoon *et al.* (1998)
PHYCI_93292	*P. infestans Pigpb1* PITG_06376	G-protein β-subunit Silencing > inhibits sporangium formation	Latijnhouwers and Govers (2003)
PHYCI_90010	*P. sojae PsMPK1* ACJ09359	MAPK. Gene expression is up-regulated in sporulating hyphae and early infection Silencing > inhibits sporangium formation; reduces virulence	Li *et al.* (2014)
PHYCI_80900	*P. cinnamomi PcLpv* AF315065	Small family of high-molecular-weight glycoproteins contained in zoospore large peripheral vesicles. Gene expression peaks 6–8 h after induction of sporulation	Marshall *et al.* (2001b)
PHYCI_92681	*P. infestans PiCdc14* PITG_18578	Phosphatase involved in cell cycle regulation and localized near the basal bodies at the nuclear apex Silencing > inhibits sporangium formation Overexpression > inhibits sporangial cleavage	Ah-Fong and Judelson (2011)
PHYCI_97894 PHYCI_290439	*P. infestans* actin PITG_15078	Actin microfilaments are reorganized during sporangial cleavage	Jackson and Hardham (1998)
PHYCI_91218	*P. infestans PiGK4* PITG_05519	G-protein-coupled receptor with a C-terminal PIP kinase domain Silencing > inhibits sporangial cleavage	Hua *et al.* (2013)
PHYCI_243332	*P. sojae PsMYB1* PHYSO_351786	Myb transcription factor. Transcription increases in sporulating hyphae, germinating cysts and early infection Silencing > inhibits sporangial cleavage and zoospore release; causes direct germination of sporangia; reduces virulence	Zhang *et al.* (2012)
PHYCI_95800	*P. sojae PsGPR11* PHYSO_352568	G-protein-coupled receptor which does not interact with PsGPA1 Silencing > cleavage is normal, but zoospore release from sporangia is inhibited; reduces virulence	Wang *et al.* (2010)

*Accession number in FungiDB.

†Genome assembly shows these as truncated α-tubulin sequences, but this is likely to be an assembly error.

phosphate (PIP) kinase domain at its C-terminus (Yang *et al.*, 2013), and PsMPK7, a stress-associated mitogen-activated protein kinase (MAPK) (Gao *et al.*, 2015). The *P. cinnamomi* genome contains a homologue for both of these genes (Table 1).

ASEXUAL SPORULATION AND ZOOSPOROGENESIS

Sporangiogenesis: sporangium development

Reduction in nutrient availability triggers the development of asexual, multinucleate sporangia (Fig. 2) (Hardham, 2005). The shape and distribution of 20–30 nuclei within the sporangia are maintained by arrays of microtubules (Hyde and Hardham, 1992, 1993). Microtubules are composed of tubulin dimers. The *P. cinnamomi* genome is likely to contain four α-tubulin genes, although the current assembly shows only truncated versions for two of them (Table 1). There is a single β-tubulin gene in the *P. cinnamomi* genome, as reported previously (Weerakoon *et al.*, 1998).

The analysis of *P. cinnamomi* transcriptomes during sporangiogenesis has revealed that changes in gene expression begin rapidly after the induction of sporulation. Screening of 5280 cDNA clones from a library constructed from mycelial mRNA isolated 4 h after the induction of sporulation identified 226 genes whose expression was up-regulated compared with levels in vegetative hyphae (Narayan, 2004). Many of the proteins encoded by these

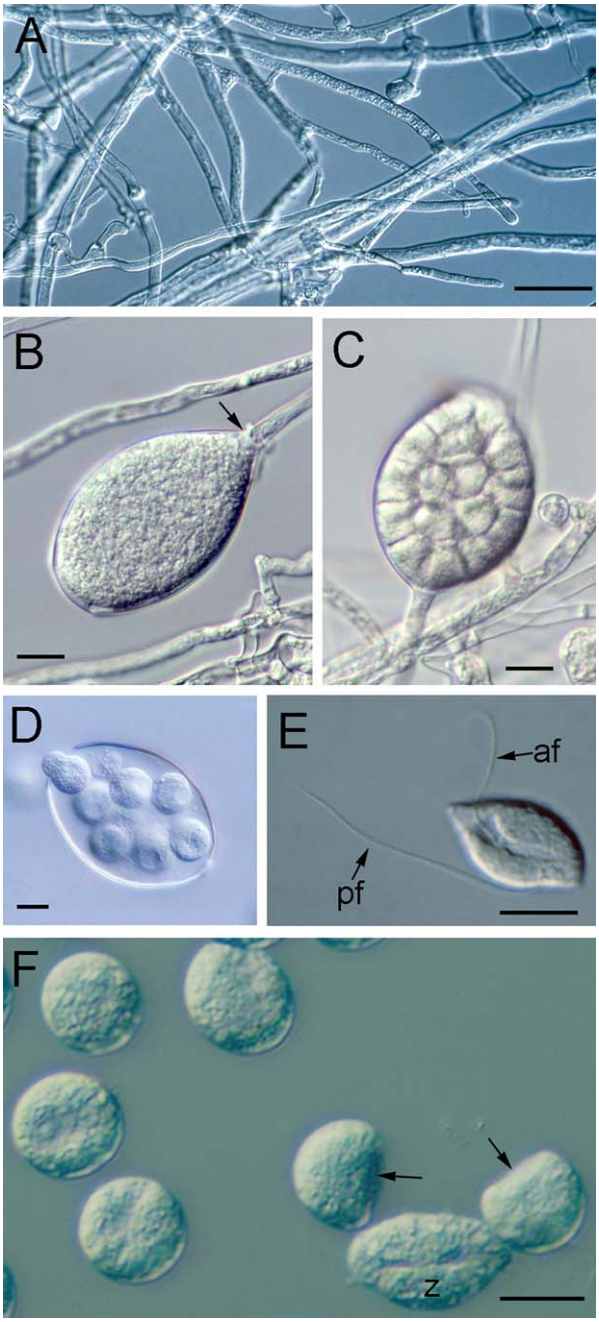

Fig. 2 The asexual lifecycle of *Phytophthora cinnamomi*. (A) Vegetative hyphae. (B) Mature, uncleaved sporangium which is sealed off from the subtending hypha by a septum (arrow). (C) Cleaved sporangium in which uninucleate domains have been formed through the development of a system of cleavage membranes that will become the plasma membranes of the future zoospores. (D) Zoospore release from a sporangium through an apical pore. (E) A biflagellate zoospore. The two flagella emerge from the groove along the ventral surface of the zoospore. The anterior flagellum (af) projects forward and pulls the cell along. The posterior flagellum (pf) trails behind the zoospore and functions in changing the direction of swimming. (F) A zoospore (z) and six young cysts, two of which still retain the initially flat region (arrows) corresponding to the zoospore ventral surface. Bars, 10 μm. (B) and (F) Reproduced with permission from Hee *et al.* (2013).

genes are involved in protein synthesis, signal transduction, general metabolism and cell structure. The quantification of RNA blots reveals a range of distinct expression patterns during sporangiogenesis. Transcript levels for genes encoding translation elongation factor 1α (PcEF1a), calmodulin (PcCaM), *S*-adenosylhomocysteinase (PcSAc), annexin VII (PcAnx) and the SET1 transcription factor, PcSet1, rise transiently 2–6 h after the induction of sporulation (Fig. S1, see Supporting Information) (Marshall *et al.*, 2001a,b; Narayan, 2004). The expression of genes encoding glyceraldehyde-3-phosphate dehydrogenase (PcGDH), pyruvate phosphate dikinase (PcPdk) and lactate dehydrogenase (PcLDH) decreases initially, before transcript levels peak 4 h after induction. The expression of the gene encoding ubiquitin, PcUbq, increases within the first 30 min and elevated transcript levels are maintained for the ensuing 6 h. By contrast, the expression of *PcCen*, a *P. cinnamomi* centrin gene, changes little over the 24-h time course. Transient elevation of gene expression has also been documented during *P. infestans* sporangiogenesis from microarrays (Judelson *et al.*, 2009). In *P. infestans* and *P. sojae*, sporangial development is inhibited by silencing the expression of genes encoding the G-protein β-subunit Pigpb1, the MAPK PsMPK1 and the cell cycle phosphatase PiCdc14 (Ah-Fong and Judelson, 2011; Latijnhouwers and Govers, 2003; Li *et al.*, 2014). *Phytophthora cinnamomi* has homologues to each of these three proteins (Table 1). Future studies using RNA-sequencing (RNA-Seq) to analyse sporulation transcriptomes will provide a comprehensive understanding of the global changes in gene expression associated with sporangiogenesis.

Zoosporogenesis: sporangial cleavage and zoospore release

Subdivision of the multinucleate sporangia into uninucleate zoospores (Figs (2 and 3)) is induced by a decrease in temperature, and a 'cold box' motif involved in the ensuing changes in gene expression has been identified in the promoters of zoosporogenesis-specific genes in *P. infestans* (Tani and Judelson, 2006). During sporangial cytokinesis (zoosporogenesis), organelles are apportioned and arranged within the cytoplasm of the future zoospores (Hardham, 2005). Three types of storage vesicle, for example, become distributed in specific zones of the zoospore cortical cytoplasm, mitochondria adopt a subcortical localization, actin microfilaments associate with the zoospore plasma membrane and the flagella are assembled. Actin microfilaments play a range of essential roles in eukaryotic cells and actin genes are expressed throughout the *Phytophthora* lifecycle (Yan and Liou, 2006). The *P. cinnamomi* genome includes two actin genes (Table 1).

Increases in the concentration of cytoplasmic Ca^{2+} and pH are required for the induction of sporangial cleavage in *P. cinnamomi* (Jackson and Hardham, 1996; Suzaki *et al.*, 1996), and the involvement of Ca^{2+} in signal transduction during zoosporogenesis has

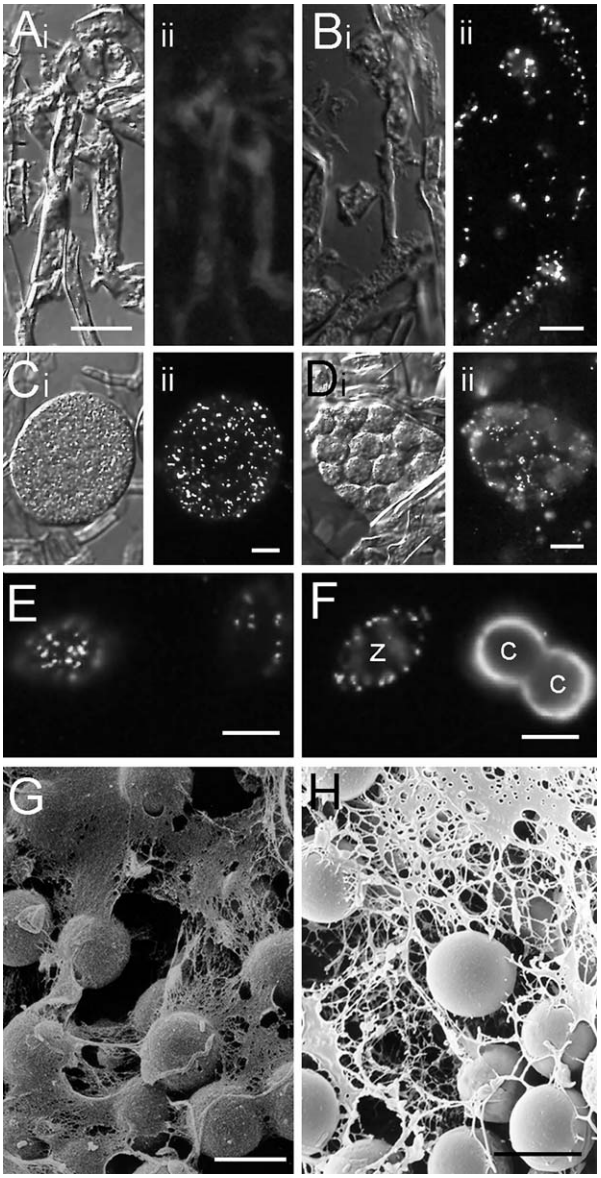

Fig. 3 Synthesis of secretory proteins during *Phytophthora cinnamomi* sporulation and their secretion during encystment. These processes are exemplified in (A)–(F) by immunolabelling with monoclonal antibody (mAb) PcCpa2 and in (G) and (H) by mucin-like material visualized by scanning electron microscopy (SEM). Micrographs in (Ai)–(Di) are bright field images of the same field of view as shown in the fluorescence images in (Aii)–(Dii). (A) Cryosection of vegetative hyphae. No components in vegetative hyphae react with PcCpa2 mAb after immunofluorescence labelling. (B) Cryosection of sporulating hyphae immunolabelled with PcCpa2 mAb. PcCpa2 reacts with three high-molecular-weight polypeptides that are synthesized after the induction of sporulation and packaged into zoospore dorsal vesicles (Gubler and Hardham, 1988). (C) Mature sporangium immunolabelled with PcCpa2 mAb. Dorsal vesicles containing PcCpa2 proteins are randomly distributed throughout the sporangial cytoplasm. (D) During sporangial cleavage, the dorsal vesicles labelled by PcCpa2 mAb become distributed near cleavage membranes that will form the dorsal surface of the future zoospores. (E) PcCpa2-containing vesicles next to the zoospore dorsal surface. (F) PcCpa2-containing vesicles in the zoospore (z) cortical cytoplasm and on the surface of two young cysts (c). The absence of immunolabelling in the region of contact between the two cysts may be because this was the ventral surface of both cells or because the antibody did not have access to this region. (G) Mucin-like material secreted during zoospore encystment on a root surface visualized by SEM after critical point drying. (H) Mucin-like material secreted during zoospore encystment on a root surface visualized by cryo-SEM. Images in (A)–(F) are courtesy of Dr Michele Cope. The image in (H) is reproduced with permission from Hardham *et al.* (1994). Bars, 10 μm.

been substantiated by experimental studies using inhibitors of phospholipase C and Ca^{2+} channels (Tani *et al.*, 2004). In *P. infestans* and *P. sojae*, silencing of the expression of genes encoding a GPCR, PiGK4 and the Myb transcription factor, PsMYB1, also inhibits sporangial cleavage (Hua *et al.*, 2013; Zhang *et al.*, 2012). Silencing of PsMYB1 in *P. sojae* causes a switch from indirect to direct sporangial germination. *Phytophthora cinnamomi* possesses homologues of both of these genes (Table 1).

After sporangial cytokinesis, zoospores are released into an evanescent vesicle originating from material in the apical papilla of the sporangium. Zoospore expulsion is thought to be driven by hydrostatic pressure within the sporangium because the process still occurs in a *P. parasitica* transformant that makes flagella-less zoospores (Narayan *et al.*, 2010). However, silencing of the gene encoding the PsGPR11 GPCR in *P. sojae* inhibits zoospore release, although sporangial cleavage apparently occurs normally (Wang *et al.*, 2010).

CELLULAR AND MOLECULAR BASIS OF *P. CINNAMOMI* INFECTION STRATEGIES

Active movement to a potential host plant

Dissemination of *P. cinnamomi* via hyphal growth may be especially important for the uphill spread of the pathogen (O'Gara *et al.*, 2005) but, in most cases, infection is initiated through the active movement of biflagellate zoospores. *Phytophthora* flagella have the 9 + 2 microtubular substructure typical of eukaryotic flagella (Hardham, 1987) and the *P. cinnamomi* genome contains genes encoding eukaryotic flagellar proteins. Two of these, namely dynein light chain 1 (*PcDLC1*) and radial spoke protein 6 (*PcRSP6*) (Table 2), have been cloned (Narayan, 2004; Narayan *et al.*, 2010). Their expression increases soon after the induction of asexual sporulation, consistent with flagella assembly during zoosporogenesis (Cope and Hardham, 1994). Silencing of the expression of a *PcDLC1* homologue in *P. parasitica* (*PnDLC1*) leads to the formation of zoospores that lack flagella, suggesting that interference in the production of this outer dynein arm component aborts flagellar assembly (Narayan *et al.*, 2010).

Table 2 *Phytophthora cinnamomi* proteins involved in the initiation of plant infection.

P. cinnamomi gene name*	Homologues used for BLAST query	Protein description and proposed function	References
Zoospore motility, chemotaxis and encystment			
PHYCI_551329	*P. cinnamomi PcDLC1* ADI77080.1	Dynein light chain 1 is a leucine-rich repeat protein that is a component of the flagellar outer dynein arm Silencing > inhibits flagella assembly	Narayan *et al.* (2010)
PHYCI_78591	*P. cinnamomi PcRSP6*	Flagellar radial spoke protein 6. Occurs along the length of the flagella	Narayan (2004)
PHYCI_79928 PHYCI_71401	*P. parasitica PnCen1* PPTG_05358 *PnCen2* PPTG_12273	Centrin is a 20-kDa protein that occurs within the flagella and a connecting band between the two basal bodies and in an anterior flagellar root	Harper *et al.* (1995)
PHYCI_207361	*P. infestans Pigpa1* PITG_03162 *P. sojae PsGPA1* PHYSO_323786	G-protein α-subunit Silencing > disrupts zoospore motility, inhibits chemotaxis, negative geotaxis and auto-aggregation and induces encystment; reduces virulence	Hua *et al.* (2008); Latijnhouwers *et al.* (2004)
PHYCI_232701	*P. sojae PsHint1* PHYSO_494520	Histidine triad domain-containing protein that interacts with PsGPA1 Silencing > inhibits chemotaxis and induces encystment; reduces virulence	Zhang *et al.* (2016)
PHYCI_91218†	*P. infestans PiGK4* PITG_05519	G-protein-coupled receptor with a C-terminal phosphatidyl-inositol phosphate (PIP) kinase domain Silencing of *P. sojae* PsGK4 (PHYSO_286453) > inhibits chemotaxis and induces encystment	Yang *et al.* (2013)
PHYCI_95800	*P. sojae PsGPR11* PHYSO_352568	G-protein-coupled receptor which does not interact with PsGPA1 Silencing > no effect on chemotaxis, but induces encystment; reduces virulence	Wang *et al.* (2010)
PHYCI_92931	*P. sojae PsSAK1* PHYSO_545105	Mitogen-activated protein kinase (MAPK) Silencing > induces encystment; reduces virulence	Li *et al.* (2010)
Cyst adhesion, protection and germination; germling growth; appressorium formation			
PHYCI_1255 PHYCI_208606	*P. cinnamomi PcVsv1* AAX84973.1	220-kDa protein stored in small ventral vesicles in zoospores and secreted during encystment. PcVsv1 contains 47 copies of a thrombospondin Type I repeat motif, a domain occurring in adhesive proteins. *PcVsv1* expression is induced during asexual sporulation	Robold and Hardham (2005)
PHYCI_310810 PHYCI_80900 PHYCI_7831 PHYCI_300556 PHYCI_85845	*P. cinnamomi 1PcLpv*, partial AAK27342.1 AAK27345.1	DNA, RNA and immunoblots show the presence of three *PcLpv* genes that encode three 11–14-kb transcripts and three 500–600-kDa proteins. Five annotated genes in the *P. cinnamomi* genome with homology to cloned *PcLpv* genes encode proteins <54 kDa; the two largest contain long regions of undetermined sequence. *PcLpv* expression is up-regulated during sporulation. PcLpv is stored in large peripheral vesicles, is not secreted during encystment and is degraded during germling growth	Gubler and Hardham (1988, 1990); Marshall *et al.* (2001a)
3PHYCI_95662	*P. parasitica PnCcp* PPTG_01661	12-kDa protein stored within large peripheral vesicles in zoospores and secreted during encystment. Gene expression is up-regulated in sporulating hyphae and zoospores	Škalamera and Hardham (2006); Zhang *et al.* (2013)
PHYCI_91218 PHYCI_481282 PHYCI_376712 PHYCI_277748 PHYCI_269807 PHYCI_212799 PHYCI_105008 PHYCI_285878 PHYCI_291554 PHYCI_260267 PHYCI_97820 PHYCI_3455 PHYCI_105811	*P. infestans PiGK4* PITG_05519 *P. sojae PsGK4* PHYSO_286453	G-protein-coupled receptor with C-terminal PIP kinase domain Silencing of PsGK4 and PiGK4 > inhibits cyst germination	Hua *et al.* (2013); Yang *et al.* (2013)

Table 2 *Continued*

P. cinnamomi gene name*	Homologues used for BLAST query	Protein description and proposed function	References
PHYCI_95800	*P. sojae PsGPR11*	G-protein-coupled receptor which does not interact with PsGPA1	Wang *et al.* (2010)
PHYCI_89253	PHYSO_352568	Silencing > inhibits cyst germination; reduces virulence	
PHYCI_91364			
PHYCI_207361	*P. sojae PsGPA1*	G-protein α-subunit. Single copy gene	Hua *et al.* (2008)
	PHYSO_323786	Silencing > inhibits cyst germination; reduces virulence	
PHYCI_232701	*P. sojae PsHint1*	Histidine triad domain-containing protein that interacts with PsGPA1	Zhang *et al.* (2016)
	PHYSO_494520	Silencing > inhibits cyst germination; causes branched germ tubes; inhibits hyphal extension	
PHYCI_92931	*P. sojae PsSAK1*	MAPK	Li *et al.* (2010)
	ACJ09358	Silencing > inhibits cyst germination and appressorium formation; reduces virulence	
PHYCI_243332	*P. sojae PsMYB1*	Myb transcription factor whose transcript levels are decreased in PsSAK1-silenced mutants. Transcription of PsMYB1 increased in germinating cysts and early infection	Zhang *et al.* (2012)
	PHYSO_351786	Silencing > inhibits cyst germination; reduces virulence	
PHYCI_257808	*P. infestans PiNIFC1–3*	NIF proteins interact with nuclear LIM transcription factors to regulate gene expression. Three of four PiNIF genes are expressed during zoosporogenesis	Judelson and Tani (2007); Tani *et al.* (2005)
PHYCI_461512	PITG_11238, PITG_11237, PITG_11239	Silencing > inhibits cyst germination by 60%	
PHYCI_112968	*P. sojae PsMPK7*	Stress-associated MAPK which is up-regulated in zoospores, cysts and germinating cysts	Gao *et al.* (2015)
	PHYSO_355777	Silencing > abnormal germ tubes; swelling of the hyphal apex; reduces virulence	
PHYCI_91649	*P. sojae PsVPS1*	Dynamin-related vacuolar sorting protein that mediates budding of clathrin-coated vesicles from the late Golgi apparatus	Li *et al.* (2013)
	PHYSO_562318	Silencing > induces apical swelling and branching of germ tubes and reduces hyphal growth; reduces virulence	
PHYCI_327508	*P. parasitica PPMUCL1*	Mucin-like proteins. High-molecular-weight secreted glycoproteins found in biofilms produced by germinated cysts. Biofilm mucins may provide protection against desiccation	Larousse *et al.* (2014)
PHYCI_253325	PPTG_17796		
PHYCI_253304‡			
PHYCI_196387§	*P. infestans* PITG_23049	*P. infestans* Car90 (cyst germination-specific acidic repeat) protein which is expressed in germinating cysts and during appressorium formation. Car proteins contain an octapeptide tandem repeat found in mammalian mucins	Görnhardt *et al.* (2000)
	P. parasitica PPTG_13017		
PHYCI_232811	*P. infestans Pi-ts1*	Threonine synthase. Increased gene expression in germinated cysts with appressoria	Grenville-Briggs *et al.* (2005)
	PITG_13139		
PHYCI_306811	*P. infestans Pi-met1*	Methionine synthase. Increased gene expression in germinated cysts with appressoria	Grenville-Briggs *et al.* (2005)
	PITG_01072		
PHYCI_96428	*P. infestans Pi-kari1*	Ketol-acid reductoisomerase. Increased gene expression in germinated cysts with appressoria	Grenville-Briggs *et al.* (2005)
	PITG_02925		
PHYCI_111395	*P. infestans Pi-als1*	Acetolactate synthase. Increased gene expression in germinated cysts with appressoria	Grenville-Briggs *et al.* (2005)
	PITG_03410		
PHYCI_95137	*P. infestans Pi-trp1*	Tryptophan synthase. Increased gene expression in germinated cysts with appressoria	Grenville-Briggs *et al.* (2005)
	PITG_00221		
Plant colonization – hyphal growth, haustoria formation			
PHYCI_137521	*P. ramorum* PSURA_75613.1	Mucin or mucin-like protein isolated from *P. ramorum* mycelial cell walls. It has homology to the *P. infestans* Car mucins. PSURA_75613.1 has a transmembrane (TM) domain	Larousse *et al.* (2014); Meijer *et al.* (2006)
	P. parasitica PPTG_16290.1		
PHYCI_111888	*P. ramorum* PSURA_83136	Mucin or mucin-like protein isolated from *P. ramorum* mycelial cell walls with homology to the *P. infestans* Car mucins	Larousse *et al.* (2014); Meijer *et al.* (2006)
PHYCI_202815	*P. parasitica* PPTG_17896.1		
PHYCI_115477	*P. ramorum* PSURA_80868.1		Larousse *et al.* (2014); Meijer *et al.* (2006)
	P. parasitica PPTG_13138.1		

Table 2 *Continued*

P. cinnamomi gene name*	Homologues used for BLAST query	Protein description and proposed function	References
		Mucin or mucin-like protein isolated from *P. ramorum* mycelial cell walls with homology to the *P. infestans* Car mucins	
PHYCI_218680	*P. ramorum* PSURA_75750	Mucin or mucin-like protein isolated from *P. ramorum*	Larousse *et al.* (2014);
PHYCI_271751	*P. parasitica* PPTG_01865.1	mycelial cell walls. It has homology to the *P. infestans* Car mucins. PSURA_75750.1 has a TM domain	Meijer *et al.* (2006); Reitmann *et al.* (2016)
PHYCI_88279	*P. parasitica PpPDI1* PPTG_18309	Secreted protein disulfide isomerase that induces cell death Over-expression of GFP-PpPDI1 > enhances haustoria formation; increases virulence	Meng *et al.* (2015)

*Accession number in FungiDB.

†Additional *PiGK4* homologues are listed in the cyst germination section.

‡These are the top three *P. cinnamomi* genes homologous to PPMUCL1 in table S3 in Larousse *et al.* (2014).

§The *P. cinnamomi* gene sequence with the closest homology to PITG_23049. The sequence is only 88 amino acids in length and the comparison yields an E-value of $2e^{-34}$. PHYCI_196387 is not included in the list of 60 *P. cinnamomi* mucin genes with homology to PPMUCL1 in table S3 in Larousse *et al.* (2014).

Zoospore chemotaxis is a vital aspect of disease initiation and the *P. cinnamomi* genome contains homologues of three genes implicated in this process in *P. infestans* and *P. sojae*, namely a G-protein α-subunit (*GPA1*), a GPA1-interacting protein (*Hint1*) and a GPCR (*GK4*) (Table 2). Silencing of these three genes in *P. infestans* or *P. sojae* reduces the period of zoospore motility before encystment and inhibits chemotaxis, negative geotaxis and auto-aggregation (Hua *et al.*, 2008; Latijnhouwers *et al.*, 2004; Yang *et al.*, 2013). By contrast, silencing of two other GPCRs, *PsGPR11* and *PsSAK1*, in *P. sojae* does not inhibit zoospore chemotaxis, although it does induces encystment (Li *et al.*, 2010; Wang *et al.*, 2010; Yang *et al.*, 2013). There are homologues of these two genes in the *P. cinnamomi* genome (Table 2).

GPCRs involved in zoospore motility are thought to reside in the zoospore plasma membrane, but, as yet, there is no evidence for this. However, antibody binding to a flagellar membrane protein in *P. cinnamomi* rapidly induces zoospore encystment (Hardham and Suzaki, 1986). This flagella surface antigen is thus conserved across the *Phytophthora* genus and it would be fascinating to determine whether it is a GPCR involved in the regulation of zoospore motility.

Attachment and protection at the plant surface

Phytophthora cinnamomi zoospores swim towards the elongation zone of plant roots, often settling preferentially in the grooves above epidermal anticlinal walls (Hardham, 2005). At high densities, they may exhibit auto-aggregation and cluster at certain sites on the plant root surface (Fig. 4), a phenomenon requiring both chemotaxis and bioconvection (Savory *et al.*, 2014). Encysting zoospores attach to the root surface through the secretion of a 250-kDa adhesin protein, PcVsv1, stored in small ventrally located vesicles (Hardham and Gubler, 1990; Robold and Hardham, 2005). Expression of the *PcVsv1* gene is up-regulated during asexual sporulation, coincident with the appearance of ventral vesicles. Homologues of the Vsv1 adhesin occur throughout the Oomycetes.

Studies in *P. parasitica* have shown that a 12-kDa complement control protein is also secreted during zoospore encystment (Škalamera and Hardham, 2006). There are single *Ccp* genes in both *P. parasitica* and *P. cinnamomi* genomes (Table 2). Ccp proteins are stored in an outer shell surrounding an inner core of Lpv glycoproteins within large peripheral vesicles (Gubler and Hardham, 1990; Zhang *et al.*, 2013). Remarkably, during encystment, the small Ccp proteins are secreted, whereas the 500–600-kDa Lpv proteins are retained, an example of selective protein secretion. In mammals, multiple complement control domains facilitate a function in cell adhesion but, as *Phytophthora* Ccp proteins contain only a single complement control module, an adhesive role seems unlikely. In *P. cinnamomi*, Lpv proteins apparently serve as a protein store utilized during germling growth (Gubler and Hardham, 1990). DNA and RNA blots show the presence of three *PcLpv* genes which give rise to three transcripts of 11–14 kb in size (Marshall *et al.*, 2001b). None of the *Phytophthora* genomes in FungiDB include *Lpv* genes that would give rise to transcripts of this length. Of the five predicted *P. cinnamomi* transcripts with homology to cloned partial *Lpv* genes, the two largest include long regions of undetermined nucleotides (Table 2). Sequencing of the cloned, partial *PcLpv* genes has shown that the C-terminal half of the Lpv proteins consists of 12–18 copies of almost identical 178-amino-acid repeats (Marshall *et al.*, 2001b), a factor that has so far confounded the identification and sequence determination of the complete length of an *Lpv* gene.

A set of three large PcCpa glycoproteins of >330 kDa in molecular weight is secreted from small dorsal vesicles to form a mucilage-like coating over the cyst surface (Gubler and Hardham, 1988; Hardham, 2005). The appearance of the secreted PcCpa

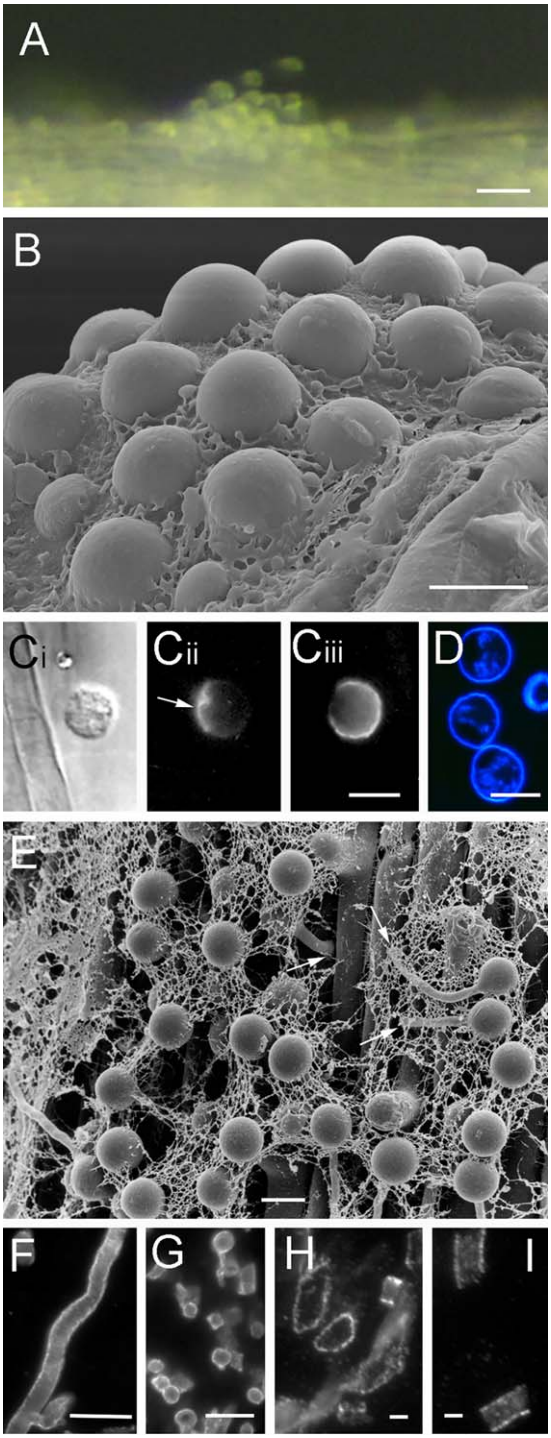

Fig. 4 Adhesion and penetration by *Phytophthora cinnamomi* on the plant surface. (A) Aggregation of motile zoospores on the surface of a plant root. (B) A cluster of cysts embedded in mucin-like material secreted during encystment on a root surface. (C) A cyst on the surface of a plant root labelled with PcVsv1 monoclonal antibody (mAb) (Cii) or soybean agglutinin (SBA) (Ciii). The same cell is shown in bright field in (Ci). Proteins secreted from zoospore ventral vesicles form an adhesive pad between the cyst and the root (arrow in Cii). SBA binds to *N*-acetylgalactosyl and galactosyl residues in PcCpa2 glycoproteins that are secreted from dorsal vesicles onto the zoospore dorsal surface which faces away from the root (Ciii). (D) The cell wall that is rapidly formed on the surface of young cysts is stained by calcofluor. (E) Cysts on the surface of a plant root germinate and the germ tube often penetrates the root along the periclinal wall between adjacent epidermal cells (arrows). (F–I) Secreted cell wall-degrading enzymes coat the surface of *P. cinnamomi* hyphae. Polygalacturonases (F–H) and endoglucanases (I) are immunolabelled by polyclonal antibodies raised against *Sclerotinia sclerotiorum* (Martel *et al.*, 1996), *Fusarium moniliforme* (De Lorenzo *et al.*, 1987), *Colletotrichum lindemuthianum* (Hugouvieux *et al.*, 1995) and *Macrophomina phaseolina* (Jones and Wang, 1997) enzymes, respectively. Bars: (A) 50 μm; (B–G) 10 μm; (H, I) 2 μm.

material (Fig. 4) is similar to that of the biofilm formed by mucin-like (MUCL) proteins secreted by *P. parasitica* germinated cysts onto the host surface (Larousse *et al.*, 2014). According to Larousse *et al.* (2014, Table S3), the *P. cinnamomi* genome contains 60 genes with similarity to *P. parasitica PPMUCL1*. Other *P. cinnamomi* genes are homologous to *P. infestans* cyst-germination-specific acidic repeat (Car) mucins (Görnhardt *et al.*, 2000) and to six cell wall mucins in *P. ramorum* (Meijer *et al.*, 2006) (Table 2). In the absence of PcCpa sequence information, there are currently no molecular data linking these proteins with the cyst or hyphal mucins described in these other *Phytophthora* species. Not all *P. parasitica PPMUCL* genes are expressed during biofilm formation (Larousse *et al.*, 2014), and thus it is possible that some mucin gene homologues in *P. cinnamomi* might encode the PcCpa glycoproteins synthesized during sporulation, stored in dorsal vesicles and secreted during zoospore encystment. The confirmation or negation of this hypothesis awaits the identification of the *PcCpa* genes, but immunoblots show that secreted PPMUCL glycoproteins are of a similar size to the PcCpa glycoproteins. Mucins secreted by epithelial cells in animals form a highly hydrated barrier that protects the underlying cells and tissues against pathogen invasion. Biofilms formed by pathogenic organisms are thought to enhance virulence by contributing to host attachment and protecting against desiccation. It is thus possible that mucins secreted by *Phytophthora* zoospores and germinated cysts serve similar protective functions during the establishment of plant infection.

Plant penetration and colonization

Cyst germination and chemotropic growth

Phytophthora cinnamomi cysts typically germinate 20–30 min after zoospore encystment. Germination can occur in distilled water, suggesting that it might be programmed to follow zoospore encystment in the absence of external chemical signals. Consistent with the expectation that signalling proteins will be involved in directing changes in gene expression, protein synthesis and the switch from cell motility to secretion as the dominant cellular activity, silencing of the *GPCRs*, *PiGK4* and *PsGPR11*, not

only curtails zoospore motility, but also inhibits cyst germination (Hua *et al.*, 2013; Wang *et al.*, 2010; Yang *et al.*, 2013). *Phytophthora cinnamomi* has 13 *GPCR* genes with homology to *PiGK4* and three with homology to *PsGPR11* (Table 2). *Phytophthora cinnamomi* also has close homologues to three other classes of genes implicated by gene silencing studies in cyst germination, namely a MAPK *PsSAK1* (Li *et al.*, 2010), a Myb transcription factor *PsMYB1* (Zhang *et al.*, 2012) and three NIF proteins that interact with nuclear LIM transcription factors (Judelson and Tani, 2007; Tani *et al.*, 2005) (Table 2). Silencing of the expression of *PsSAK1* also inhibits appressorium formation and leads to the production of longer germ tubes (Li *et al.*, 2010). *Phytophthora cinnamomi* also has homologues to two *P. sojae* proteins, the MAPK PsMPK7 and a dynamin-related vacuolar sorting protein PsVPS1, which function in regulating the polarity of germ tube extension (Gao *et al.*, 2015; Li *et al.*, 2013), and to five genes encoding enzymes involved in amino acid synthesis whose expression is upregulated during appressorium formation (Grenville-Briggs *et al.*, 2005) (Table 2).

Cell wall-degrading enzymes (CWDEs)

Initial penetration and subsequent colonization of the plant are made possible by the action of a wide range of pathogen degradative enzymes that digest components of the plant cell wall. *Phytophthor*a genomes contain large multigene families encoding CWDEs that contain one or more Carbohydrate-Active enZyme (CAZyme) modules (Blackman *et al.*, 2014; Götesson *et al.*, 2002; Larroque *et al.*, 2012; Ospina-Giraldo *et al.*, 2010).

Gene-by-gene analysis of predicted *P. cinnamomi* transcripts included in the FungiDB database using dbCAN (http://csbl.bmb.uga.edu/dbCAN/) indicates that there are 438 CWDE genes in the *P. cinnamomi* genome (Tables (3–5)), a number similar to the 431 and 423 CWDE genes identified in *P. parasitica* and *P. infestans*, respectively, using the same approach (Blackman *et al.*, 2014) (Table S1, see Supporting Information). The 438 CWDEs in *P. cinnamomi* include 271 proteins containing a glycoside hydrolase (GH) module (Table 3), 17 proteins containing an auxiliary activity (AA) module (Table 4), 53 proteins containing a carbohydrate esterase (CE) module (Table 4), 42 proteins containing a polysaccharide lyase (PL) module (Table 4) and 64 proteins containing only a non-catalytic carbohydrate-binding module (CBM) (Table 5). Proteins from these five classes of CAZymes degrade the main polysaccharides in the plant cell wall, namely cellulose, hemicelluloses, pectins and β-1,3-glucans (Tables (3–5)). Cellulose is attacked by CWDEs containing modules from nine GH, five CBM and three AA families. Hemicelluloses are attacked by CWDEs containing modules from 12 GH, four CE and six CBM families. Pectins are attacked by CWDEs containing modules from 10 GH, three PL, three CE and one CBM family. The identification of 26 GH28 polygalacturonase genes in FungiDB (15 full length and 11 partial) confirms the earlier report of more than 17 *P. cinnamomi* polygalacturonase genes based on Southern DNA blots (Götesson *et al.*, 2002).

Phytophthora cinnamomi polygalacturonases are synthesized and secreted during an early phase of growth after subculturing *in vitro*. Although the total protein concentration in the culture medium continues to increase over a 15-day growth period, polygalacturonase activity peaks 5–6 days after inoculation of the medium (J. Schick *et al.*, unpublished observations). Secreted polygalacturonase proteins can be immunolocalized on the hyphal surface (Fig. 4) and recombinant, single-chain, anti-polygalacturonase antibodies can inhibit the activity of polygalacturonases secreted by *P. cinnamomi* (Manatunga *et al.*, 2005).

The measurement of *P. cinnamomi* polygalacturonase transcript levels using quantitative real-time polymerase chain reaction (qPCR) shows that the GH28 genes are differentially expressed both *in vitro* and *in planta* (E. Landgren *et al.*, unpublished observations). In *in vitro* assays using 5% V8 broth or Ribeiro's minimal medium (Ribeiro, 1978) supplemented with defined carbon sources, some *P. cinnamomi* GH28 genes are expressed regardless of the type of carbohydrate present, some are expressed most highly in the presence of glucose and others are expressed most highly in the presence of pectin (Fig. S2, see Supporting Information). These results provide evidence of the operation of carbon catabolite repression mechanisms in the regulation of GH28 gene expression. qPCR measurement of *P. cinnamomi* GH28 transcript levels during the infection of lupin roots by *P. cinnamomi* also indicates that, as in the lupin–*P. parasitica* interaction (Blackman *et al.*, 2015), some GH28 genes are expressed early in plant infection, with transcript levels decreasing as infection proceeds (Fig. S3, see Supporting Information).

Hyphal growth

Having penetrated the plant surface, *P. cinnamomi* hyphae grow intracellularly or intercellularly through the root cortex and into the central vascular bundle (Fig. 5). Blockage of the xylem through hyphal obstruction and deposition of material by the plant inhibits water movement from the roots to the shoots, resulting in water stress (Ruiz Gómez *et al.*, 2015). Necrosis of infected fine feeder roots exacerbates the problem and can lead to rapid plant death (McConnell and Balci, 2015; Oßwald *et al.*, 2014).

Phytophthora cinnamomi hyphae, like other tip-growing cells, extend through the fusion of small transport vesicles at the hyphal apex and the secretion of new membrane and wall material. *Phytophthora* and other Oomycete cell walls are based on a cellulosic, rather than chitinous, microfibrillar framework. According to a recent study, *P. cinnamomi* and other *Phytophthora* and Peronosporales species have Type I cell walls, distinguished mainly by their lack of *N*-acetylglucosamine and their cellulose content (Mélida *et al.*, 2013).

Table 3 *Phytophthora cinnamomi* proteins involved in plant cell wall degradation: enzymes containing glycosyl hydrolase (GH) modules.

CAZyme family	Potential substrates	Potential enzyme activities	Number of genes
GH1	Cellulose, hemicellulose (XG), pectin (RGI)	β-Glucosidase, β-galactosidase, β-mannosidase, exo-β-1,4-glucanase	14
GH2	Hemicellulose (mannans), glycoproteins	β-Mannosidase	1
GH3	Cellulose, hemicellulose (XG), pectin (RGI), AGPs	β-Glucosidase, exo-β-1,4-glucosidase, β-1,4-xylosidase, β-1,3-glucosidase, α-L-arabinofuranosidase	26
GH5	Cellulose, hemicellulose (xylans, galactomannans), β-1,3-glucans	Endo-β-1,4-glucanase, β-1,4-cellobiosidase, endo-β-1,4-xylanase, endo-β-1,4-mannosidase, β-1,3-glucosidase	30
GH6	Cellulose	Endo-β-1,4-glucanase, cellobiohydrolase	7
GH7	Cellulose	Endo-β-1,4-glucanase, cellobiohydrolase	7
GH10	Hemicellulose (xylans)	Endo-β-1,4-β-xylanase	6
GH12	Cellulose, hemicellulose (XG)	Endo-β-1,4-glucanase, XG endo-β-1,4-glucanase	10
GH13	Starch	α-Amylase, α-glucosidase	2
GH16	Hemicellulose (XG), β-1,3-glucans	XG endo-β-1,4-glucanase, β-1,3-glucosidase	21
GH17	β-1,3-Glucans	Endo-β-1,3-glucosidase	21
GH18	*N*-linked oligosaccharides	Endo-β-*N*-acetylglucosaminidase	3
GH19	*N*-linked oligosaccharides	Endo-β-*N*-acetylglucosaminidase	1
GH28	Pectin (HG)	Polygalacturonase	26
GH30	Cellulose, hemicellulose (XG), pectin (RGI), AGPs	β-Glucosidase, endo-β-1,4-xylanase, xylan β-1,4-xylosidase, endo-β-1,6-galactanase, β-1,6-glucanase	17
GH31	Starch, hemicellulose (XG)	α-Glucosidase, α-xylosidase	6
GH32	Sucrose	Invertase	3
GH35	Hemicellulose (XG), pectin (HG), AGPs	β-Galactosidase, exo-β-1,4-galactanase	1
GH37	Trehalose (α,α-1,1-glucans)	α,α-Trehalase	2
GH38	*N*-linked oligosaccharides	α-Mannosidase	1
GH43	Hemicellulose (xylans), pectin (RGI), AGPs	α-L-Arabinofuranosidase	7
GH45	Cellulose, β-glucans	Endo-glucanase	1
GH47	*N*-linked oligosaccharides	α-Mannosidase	5
GH53	Pectin (RGI)	Endo-β-1,4-galactanase	6
GH54	Pectin (RGI)	α-L-Arabinofuranosidase	2
GH63	*N*-linked oligosaccharides	α-1,3-Glucosidase, α-glucosidase	1
GH72	β-1,3-Glucans	β-1,3-Glucanosyl-transglycosylase	10
GH78	Pectin (RGI)	α-L-Rhamnosidase	6
GH81	β-1,3-Glucans	Endo-β-1,3-glucanase	12
GH85	AGPs	Endo-β-*N*-acetylglucosaminidase	1
GH89	*N*-linked oligosaccharides	α-*N*-Acetylglucosaminidase	1
GH105	Pectin (RGI)	Unsaturated rhamnogalacturonyl hydrolase	1
GH109	*O*-linked oligosaccharides	α-*N*-Acetylgalactosaminidase	8
GH114	Glycoproteins	Endo-α-1,4-polygalactosaminidase	1
GH123	*O*-linked oligosaccharides	Glycosphingolipid β-*N*-acetylgalactosaminidase	1
GH131	Cellulose, hemicellulose (β-1,4-glucans)	Exo-β-1,3/1,6-glucanase, endo-β-1,4-glucanase	3
Total			271

AGP, arabinogalactan protein; CAZyme, *C*arbohydrate-*A*ctive en*Z*yme; HG, homogalacturonan; RGI, rhamnogalacturonan I; XG, xyloglucan.

Pathogen CWDEs are also secreted through vesicle fusion at the hyphal apex, and the consequent hydrolysis of plant cell walls is indicated by changes in wall morphology, cell separation and tissue maceration (Redondo *et al.*, 2015; Ruiz Gómez *et al.*, 2015). There is some evidence that these changes are accompanied by de-esterification of plant cell wall pectins during the infection of holm oak (*Quercus ilex*) by *P. cinnamomi* (Ruiz Gómez *et al.*, 2015); however, the causal relationship between these features and details of the molecular mechanisms underlying plant cell wall degradation await future research.

Haustoria. Although previously considered to be a necrotrophic pathogen, recent observations of putative *P. cinnamomi* haustoria within cortical and vascular tissues in a number of host plants, including *Lupinus augustifolius* (Fig. 5A), have suggested that *P. cinnamomi* can grow as a hemibiotroph (Crone *et al.*, 2013b; Redondo *et al.*, 2015). In *Arabidopsis*, the extrahaustorial membrane surrounding haustoria of the Oomycete, *Hyaloperonospora arabidopsidis*, contains the plasmodesmata-located protein1 (PDL1) (Caillaud *et al.*, 2014). The demonstration of the presence of homologues of PDL1 around the *P. cinnamomi* haustoria-like structures could confirm their identification. Molecular details of

Table 4 *Phytophthora cinnamomi* proteins involved in plant cell wall degradation: enzymes containing auxiliary activity (AA), carbohydrate esterase (CE) and polysaccharide lyase (PL) modules.

CAZyme family	Potential substrates	Potential enzyme activities	Number of genes
Auxiliary activity modules			
AA1	Benzenediol, catechol	Laccase, oxidoreductase, ferroxidase	1
AA7	Cellobiose, chitin, glycoproteins	Glucooligosaccharide oxidase, chitooligosaccharide oxidase	8
AA8	Cellulose	Iron reductase domain	5
AA10	Cellulose	Copper-dependent monooxygenase	3
Total			17
Carbohydrate esterase modules			
CE1	Hemicellulose	Feruloyl esterase	5
CE2	Hemicellulose	Acetyl xylan esterase	1
CE4	Hemicellulose, *N*-linked oligosaccharides	Acetyl xylan esterase, peptidoglycan GlcNAc deacetylase	2
CE5	Hemicellulose	Acetyl xylan esterase, cutinase	9
CE8	Pectin (HG)	Pectin methylesterase	17
CE12	Pectin (HG, RGI)	Pectin and RGI acetylesterase, acetyl xylan esterase	12
CE13	Pectin (HG)	Pectin acetylesterase	7
Total			53
Polysaccharide lyase modules			
PL1	Pectin (HG)	Pectate lyase, pectin lyase	21
PL3	Pectin (HG, RGI)	Pectate lyase	22
PL4	Pectin (RGI)	Rhamnogalacturonan lyase	6
Total			42

CAZyme, *C*arbohydrate-*A*ctive en*Z*yme; GlcNAc, *N*-acetylglucosamine; HG, homogalacturonan; RGI, rhamnogalacturonan I.

Table 5 *Phytophthora cinnamomi* proteins involved in plant cell wall degradation: enzymes containing carbohydrate-binding (CBM) modules.

CAZyme family	Potential substrates	Number of genes
CBM1	Cellulose	14
CBM9	Hemicellulose (xylans)	1
CBM13	Hemicellulose (xylans)	9
CBM14	Chitin	1
CBM16	Cellulose, hemicellulose (glucomannans)	1
CBM17	Cellulose	2
CBM20	Starch, fucose	3
CBM23	Hemicellulose (mannans)	1
CBM25	Starch	1
CBM32	Pectin (galactose, PGA and β-galactosyl-β-1,4-GlcNAc)	2
CBM37	Cellulose, hemicellulose (xylans)	2
CBM38	Inulin	1
CBM40	Sialides	1
CBM47	Fucose	4
CBM48	Glycogen (starch)	1
CBM50	Chitin, glycoproteins	2
CBM60	Hemicellulose (xylans)	1
CBM63	Cellulose	16
Total		63

CAZyme, *C*arbohydrate-*A*ctive en*Z*yme; GlcNAc, *N*-acetylglucosamine; PGA, polygalacturonic acid.

Phytophthora haustorial function are yet to be elucidated, although a protein disulfide isomerase PpPDI1 has been associated with haustoria and enhanced pathogenicity in *P. parasitica* (Meng *et al.*, 2015). The *P. cinnamomi* genome contains a homologue of the *PpPDI1* gene (Table 2) and characterization of its encoded protein may provide important information on *P. cinnamomi* haustorium function.

The timing of the transition from an initially biotrophic to a subsequent necrotrophic phase of plant infection by *P. cinnamomi* is influenced by the plant species and environmental conditions. Maintenance of the biotrophic phase means that *P. cinnamomi* can be present in infected plants in the absence of obvious disease symptoms (Crone *et al.*, 2013a,b). Long-term survival in infected plants can be achieved through the formation of chlamydospores, stromata, hyphal aggregates within lignitubers and oospores (Crone *et al.*, 2013b; Jung *et al.*, 2013).

PLANT–PATHOGEN INTERACTIONS

Effectors and elicitors

During infection, *P. cinnamomi*, like other plant pathogens, secretes a diverse range of effector molecules into the plant apoplast. From the pathogen's point of view, the intended function of these effectors is to facilitate the establishment of disease. From the plant's point of view, the goal is to recognize the effectors and trigger a defence response that will inhibit the development of disease. When an intended effector is recognized by the plant and elicits a defence response, it is termed an avirulence factor or elicitor.

Effector proteins produce metabolic or structural changes in host cells that aid pathogen growth and favour the development of disease. They are secreted by the pathogen and act either in

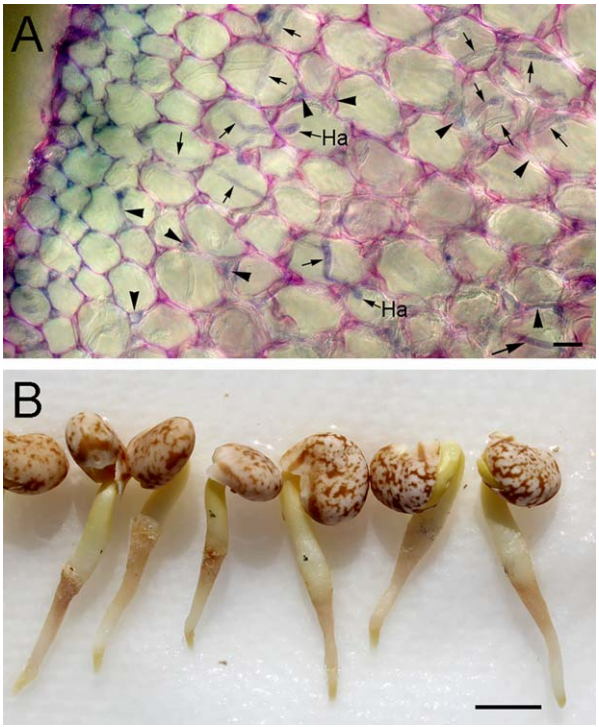

Fig. 5 Colonization and lesion development in lupin (*Lupinus augustifolius*) roots 20 h after inoculation with *Phytophthora cinnamomi* zoospores. (A) A transverse hand-section of an infected lupin root. During initial colonization of the root, hyphae grow from the epidermis, through the cortex and into the vascular cylinder. Hyphal growth may be intracellular (arrows) or intercellular (arrowheads). Two putative haustoria (Ha) are indicated. (B) Lesions develop on the lupin roots just below the surface of the zoospore suspension whose approximate position is marked by black ink spots. Bars, 10 μm.

the plant apoplast or symplast. Apoplastic effectors include CWDEs, elicitins, toxins and inhibitors of plant enzymes. Symplastic effectors are translocated across the plant plasma membrane by an as yet unknown mechanism and act within the plant cell cytoplasm. The function of the majority of known or putative cytoplasmic effectors is still unclear, but some have been shown to suppress host defence responses, such as callose deposition or hypersensitive cell death (Dou *et al.*, 2008a; Du *et al.*, 2015). Effectors have sequence variants that may or may not be recognized by a potential host plant. In many cases, it was the recognized avirulence protein form that triggers host defence that was first identified.

A great diversity of avirulence proteins and other elicitors have been described and studied. Elicitors may be proteins, carbohydrates or lipids, and may be of pathogen or plant origin. In the latter case, an elicitor can arise, for example, as a result of the digestion of a plant cell wall component by a pathogen enzyme. Many elicitor molecules are highly conserved across broad taxonomic groups and are often referred to as pathogen- or microbe-associated molecular patterns (PAMPS or MAMPs). PAMPs trigger basal defence responses, also referred to as PAMP-triggered immunity (PTI), including localized cell wall reinforcement and callose deposition, production of antimicrobial compounds, such as phytoalexins and reactive oxygen species, and, in some cases, cell death (Chang *et al.*, 2015). Some elicitors are conserved, but occur in only one or a few genera. Small cysteine-rich proteins, called elicitins, produced by species of *Phytophthora*, including *P. cinnamomi*, are examples of this latter class of elicitor. Elicitins function in plant sterol uptake, and thus, when they are not recognized by the plant, *Phytophthora* elicitins are likely to serve as pathogenicity factors and can be considered to be effectors.

The potential role of many *P. cinnamomi* effectors in pathogenicity has been presented already as part of the discussion of the infection strategies employed, especially during the early stages of disease establishment, such as adhesion and penetration. However, full disease development involves a great diversity of complex plant–pathogen interactions and will depend on many putative effectors not yet discussed. Some effectors will facilitate attack and colonization so that the pathogen can access the nutrients it needs for growth and reproduction. Other effectors will be central to pathogen defence mechanisms that provide protection against plant defences.

Putative effectors of pathogen attack strategies

P. cinnamomi elicitins

Elicitins are 10-kDa globular proteins produced by all *Phytophthora* and some *Pythium* species (Duclos *et al.*, 1998; Jiang *et al.*, 2006). They are not recognized by most plants, but, in some solanaceous species (notably *Nicotiana* species) and some cultivars of radish and turnip, they trigger plant hypersensitive cell death and can induce plant resistance to a variety of bacterial and fungal pathogens (Oßwald *et al.*, 2014). *Phytophthora* species typically contain 10–20 elicitin genes (Jiang *et al.*, 2006). BLAST analysis of the *P. cinnamomi* genome, using the PHYCI_98389 β-cinnamomin gene as query, resulted in the identification of 32 putative elicitin sequences (Table S2, see Supporting Information), considerably more than the single α-cinnamomin and β-cinnamomin and two highly acidic elicitin (HAE) genes previously reported in *P. cinnamomi* (Duclos *et al.*, 1998).

Phytophthora elicitins bind dehydroergosterol and, because *Phytophthora* species cannot synthesize sterols, they are believed to serve an essential role in *Phytophthora* development and pathogenicity through their ability to transfer sterols from plant membranes to the pathogen (Osman *et al.*, 2001; Rodrigues *et al.*, 2006). qPCR measurements have indicated that all four previously reported cinnamomin genes are expressed *in vitro* (Horta *et al.*, 2008). In cork oak (*Quercus suber*) roots, transcript levels of α-cinnamomin increased about three-fold, whereas those for β-cinnamomin and α-*HAE* decreased by about 20% and 90%, respectively, during a 24-h period after inoculation with *P.*

cinnamomi. Expression of the *β-HAE* gene was detected only at 36 h post-inoculation (hpi). These results may indicate that β-cinnamomin mainly functions during the initial establishment of infection, whereas α-cinnamomin functions throughout colonization and sporulation (Horta *et al*., 2008). The reported silencing of the expression of the β-cinnamomin gene reduces the pathogen's ability to penetrate and colonize the roots of *Q. suber* and chestnut (*Castanea sativa*) seedlings compared with wild-type controls, leading to decreased symptom severity in the inoculated plants (Horta *et al*., 2010; Maia *et al*., 2012). As these studies are based on only one silenced transformant line, they must be viewed as being preliminary; nevertheless, they represent an exciting advance in the demonstration of *P. cinnamomi* elicitin function. The establishment of a reproducible and stable transformation strategy for *P. cinnamomi* would greatly facilitate the elucidation of *P. cinnamomi* gene function and the molecular basis of plant infection.

Plant recognition of cinnamomin contributes to the induction of host defence responses. Pretreatment of oak (*Q. suber* or *Q. ilex*) or chestnut (*C. sativa*) roots with α- or β-cinnamomin results in a significant reduction in root colonization by *P. cinnamomi* (Ebadzad *et al*., 2015; Maia *et al*., 2008; Medeira *et al*., 2012). Further studies of the role of cinnamomins in *Phytophthora*–plant interactions may be aided by the development of an improved method for the production and purification of β-cinnamomin (Hofzumahaus and Schallmey, 2013).

Small cysteine-rich (SCR) proteins

Phytophthora species produce a second category of small cysteine-rich (SCR) effector proteins. The first representative, *P. cactorum* PcF, induces the expression of plant pathogenicity genes and programmed cell death (Orsomando *et al*., 2001). Previously analysed *Phytophthora* species contain 3–19 *SCR* genes (Chen *et al*., 2015; Orsomando *et al*., 2011). A search using *P. cactorum SCR* genes has revealed that the *P. cinnamomi* genome contains one homologue of *PcF*, eight homologues of *Scr96*, 15 homologues of *Scr99* and one homologue of *Scr121* (Table 6), and thus appears to have the largest *PcF/SCR* gene family reported so far. Silencing of *P. cactorum Scr96* reduces pathogen virulence on *Nicotiana benthamiana* leaves and makes it more sensitive to oxidative stress (Chen *et al*., 2015), indicating that *Phytophthora* SCR proteins are effectors that function as virulence factors under the same circumstances in which they trigger plant cell death.

GP42 transglutaminase

A 42-kDa transglutaminase, GP42, was initially identified in *P. sojae* because of its elicitor activity (Nürnberger *et al*., 1994). Transglutaminases occur in multiple *Phytophthora* species and catalyse an acyl transfer reaction that renders peptide bonds more resistant to proteolytic degradation. A single *P. cinnamomi* transglutaminase sequence has been described (Martins *et al*., 2014b), but BLAST analysis with the two *P. ramorum GP42* genes shows that there are 21 putative *P. cinnamomi* sequences (Table 6). *GP42* transglutaminase homologues are expressed during early infection, suggesting that they may function during the establishment of *Phytophthora* disease (Brunner *et al*., 2002).

CRN crinklers

Members of the Crinkler (CRN) group of effectors were first identified amongst *P. infestans* genes that caused crinkling and necrosis when transiently expressed in *N. benthamiana* cells (Torto *et al*., 2003), although more recent studies have shown that the majority of CRN effectors suppress plant defence (Chen *et al*., 2013; Haas *et al*., 2009; Stam *et al*., 2013). CRN proteins have a conserved N-terminal domain that includes LFLAK and DWL motifs that function in directing translocation of the CRN proteins from the apoplast into the plant cytoplasm (Schornack *et al*., 2010). Variable C-terminal domains endow CRN proteins with a range of functions, including nuclear localization required for effector function (Liu *et al*., 2011; Mafurah *et al*., 2015; Van Damme *et al*., 2012).

All *Phytophthora* species examined so far have large multigene families of *CRN* genes (Haas *et al*., 2009; Tyler *et al*., 2006). PBLAST searches of the *P. cinnamomi* genome with 45 representative *P. infestans* CRN proteins from the list in Table S10 in Haas *et al*. (2009) identified 280 *P. cinnamomi* homologous sequences. Of these, 42 contain conserved LFLAK and DWL domains (Tables 6 and S3, see Supporting Information). Of the 42 proteins, 14 have an N-terminal secretion signal and 11 have sequences associated with non-classical secretion pathways. If the N-terminal truncation of four additional proteins is caused by assembly errors, these proteins may also be secreted, giving a total of 29 putative *P. cinnamomi* CRN effectors. A *P. cinnamomi CRN* homologue, shown in the current genome assembly to be truncated at the N-terminus, is the most highly expressed *P. cinnamomi* gene 5 days after inoculation of *Eucalyptus nitens* (Meyer *et al*., 2016). Thirteen of the 42 *P. cinnamomi CRN* homologues have a nuclear localization signal (NLS) in the C-terminal region (Table S3). Further characterization of the cohort of 29 putative *P. cinnamomi* CRN proteins is likely to be an important area for future research. The CRN proteins may be critical factors in *P. cinnamomi*'s success as a pathogen and its ability to infect such a wide range of plant species.

NLPs: Nep1-like protein

A second group of secreted effectors believed to act within the plant cell cytoplasm and to cause plant cell necrosis is the Nep1-like proteins (NLPs). NLPs occur widely among bacteria, fungi and Oomycetes (Gijzen and Nürnberger, 2006; Oome and Van Den Ackerveken, 2014). They contain a conserved NPP1 (necrosis-

Table 6 *Phytophthora cinnamomi* proteins involved in plant–pathogen interactions: putative effectors involved in plant colonization.

P. cinnamomi gene name (in FungiDB)	Homologues used for BLAST query	Proposed protein function	References
Elicitins*			
PHYCI_127817	*P. cinnamomi* α-cinnamomin	Sterol carrier protein believed to be required for virulence	Duclos *et al.* (1998); Horta *et al.* (2008)
PHYCI_98389	*P. cinnamomi* β-cinnamomin	Sterol carrier protein Silencing > reduces virulence	Duclos *et al.* (1998); Horta *et al.* (2010); Maia *et al.* (2012)
PHYCI_251414	*P. cinnamomi* Highly acidic elicitin-α (*HAE*-α)	Sterol carrier protein believed to be required for virulence	Duclos *et al.* (1998); Osman *et al.* (2001)
PHYCI_98390	*P. cinnamomi* *HAE*-β	Sterol carrier protein believed to be required for virulence	Duclos *et al.* (1998); Osman *et al.* (2001)
Small cysteine-rich (SCR) toxins			
PHYCI_83830	*P. cactorum PcF* AAK63068	PcF and PcF-like toxin proteins, small cysteine-rich proteins from *P. cactorum*	Nicastro *et al.* (2009); Orsomando *et al.* (2001)
PHYCI_93258 PHYCI_93260 PHYCI_93259 PHYCI_92597 PHYCI_323321 PHYCI_85664 PHYCI_85660 PHYCI_97296	*P. cactorum Scr96* ALC04448	PcF toxin family of SCR proteins in *P. infestans, P. sojae, P. ramorum* and *P. cactorum* *In planta, P. cactorum* Scr96 transcript levels are high in early infection (12 hpi) and then decline. Silencing > reduces virulence and increases sensitivity to oxidative stress	Chen *et al.* (2015)
PHYCI_90211 PHYCI_96480 PHYCI_20145 PHYCI_97226 PHYCI_330581 PHYCI_234114 PHYCI_90122 PHYCI_259880 PHYCI_254679 PHYCI_252576 PHYCI_269098 PHYCI_237327 PHYCI_194590 PHYCI_256642 PHYCI_241899	*P. cactorum Scr99* ALC04449	PcF toxin family of SCR proteins *In planta*, Scr99 transcript levels increase during the first 48 hpi	Chen *et al.* (2015)
PHYCI_97299	*P. cactorum Scr121* ALC04450	PcF toxin family of SCR proteins *In planta*, Scr121 transcript levels increase during the first 48 hpi	Chen *et al.* (2015)
Transglutaminases			
PHYCI_89523 PHYCI_208557 PHYCI_471842 PHYCI_89525 PHYCI_89524 PHYCI_209063 PHYCI_471855 PHYCI_288438 PHYCI_1375 PHYCI_227786 PHYCI_240555 PHYCI_1278 PHYCI_15457 PHYCI_261219 PHYCI_181539 PHYCI_13742 PHYCI_12440 PHYCI_178520	*P. ramorum GP42* PSURA_53744, PSURA_83169	Two GP42 transglutaminases have been found in hyphal cell wall proteomes in *P. infestans* and *P. ramorum*. They catalyse peptide bond formation that can increase protein stability. GP42 transglutaminases induce plant defence responses. They are expressed early in infection, but their function in pathogenicity is unknown	Brunner *et al.* (2002); Grenville-Briggs *et al.* (2010); Martins *et al.* (2014b); Meijer *et al.* (2006)

Table 6 *Continued*

P. cinnamomi gene name (in FungiDB)	Homologues used for BLAST query	Proposed protein function	References
PHYCI_134840			
PHYCI_198929			
PHYCI_97108			
Crinkler (CRN) effectors			
PHYCI_99711†	*P. infestans*	CRN effectors are secreted proteins that may cause crinkling and necrosis. The conserved N-terminus includes LFLAK and DWL motifs that function in protein uptake into the plant cytoplasm. Variable C-terminal regions are associated with a range of effector functions, including translocation into the plant nucleus. Many CRN proteins suppress plant defence, including hypersensitive cell death, possibly through interaction with plant catalases	Chen *et al.* (2013); Haas *et al.* (2009); Liu *et al.* (2011); Mafurah *et al.* (2015); Stam *et al.* (2013); Van Damme *et al.* (2012); Zhang *et al.* (2015)
PHYCI_98916	45 CRN proteins representative of those reported in table S10 in Haas *et al.* (2009)		
PHYCI_93895			
PHYCI_82897			
PHYCI_75608			
PHYCI_68121			
PHYCI_557374			
PHYCI_251494			
PHYCI_213501			
PHYCI_148758			
PHYCI_115716		In *P. sojae*, silencing > reduces virulence	
PHYCI_141767			
PHYCI_105597			
PHYCI_111648			
NLPs (Nep1-like proteins)			
72 *NLP* homologues in *P. cinnamomi* [see Table S4 (Supporting Information) for full list]	*P. sojae NLPs* PHYSO_562453 PHYSO_509399 PHYSO_249691	NLPs are Nep1-like proteins that have an NPP necrosis-inducing *Phytophthora* protein (PFAM PF05630) domain. Secreted and believed to act within the plant cytoplasm. Induce a range of defence responses, including changes in gene expression, callose deposition, production of ethylene, nitric oxide, reactive oxygen species (ROS) and phytoalexins, and cell death. They promote pathogen growth and pathogenicity	Bailey *et al.* (2005); Feng and Li (2013); Kanneganti *et al.* (2006); Oome and Van Den Ackerveken (2014); Qutob *et al.* (2006); Santhanam *et al.* (2013)
RXLR effectors			
171 *P. cinnamomi* proteins have an RxLR or RxL(E/D/Q) motif and an N-terminal SP. Most also have the EER motif	*P. infestans* RxLR 122 RxLR-containing genes described in Haas *et al.* (2009)	Small secreted proteins containing an RxLR motif believed to be responsible for protein translocation across the plant plasma membrane RxLR effectors have diverse sequences and are likely to have diverse functions	Haas *et al.* (2009)
PHYCI_87160	*P. sojae Avr3b* PHYSO_286971 *P. infestans* Avr3b PITG_15732 PITG_15679	Effector containing RxLR, EER and Nudix domains. Nudix hydrolases are ADP-ribose/NADH pyrophosphorylases *In planta* expression increases susceptibility to *Phytophthora*	Dong *et al.* (2011)
PHYCI_324245‡ PHYCI_129006 PHYCI_568683	*P. sojae PsPSR2* PHYSO_290752	RxLR effectors that suppress gene silencing in plants and promote infection	Qiao *et al.* (2013); Xiong *et al.* (2014)
PHYCI_24296 PHYCI_297058	*P. infestans PiSNE1* PITG_13157	An RxLx effector that is targeted to the plant nucleus and that suppresses plant cell necrosis	Kelley *et al.* (2010)

*Accession numbers of the two elicitin and two HAE genes described by Duclos *et al.* (1998) are listed. There are 32 homologous sequences in the *P. cinnamomi* genome with E-values of <E-05.

†The 14 *P. cinnamomi* sequences that contain an N-terminal secretion signal and conserved LFLAK and DWL domains. These sequences are a subset of 280 *P. cinnamomi* sequences with homology to 45 representative *P. infestans* CRN proteins listed in Haas *et al.* (2009). The full list of 42 genes containing LFLAK and DWL domains is presented in Table S2 (see Supporting Information) with information on secretion motifs and possible N-terminal truncation.

‡The top three sequences identified in the BLAST analysis.

inducing *Phytophthora* protein) domain. The *NLP* gene family is especially large in the Oomycetes. *Phytophthora sojae* and *P. ramorum* have been reported to possess 50–60 members, although more than one-half are predicted to be pseudogenes (Gijzen and Nürnberger, 2006). *Phytophthora cinnamomi* has 72 putative *NLP* genes (Tables 6 and S4, see Supporting Information). NLPs may contribute to pathogen virulence by eliciting a range of plant defence responses or by acting as toxins (Bailey *et al.*, 2005;

Oome and Van Den Ackerveken, 2014; Qutob *et al.*, 2002; Santhanam *et al.*, 2013).

RxLR proteins

Phytophthora genomes include hundreds of genes that encode putative effectors containing an RxLR motif, believed to direct the passage of the effector from the plant apoplast into the plant cell cytoplasm (Dou *et al.*, 2008b). Analysis of the genomes of *P. infestans*, *P. ramorum* and *P. sojae* has shown the presence of approximately 560, 370 and 390 RxLR-containing, small secreted proteins, respectively (Haas *et al.*, 2009; Jiang *et al.*, 2008; Wang *et al.*, 2011). The use of 122 of the *P. infestans* RxLR genes in pBLAST searches of the *P. cinnamomi* genome produced hits with 340 proteins which also have an N-terminal signal peptide. Manual screening for the presence of RxLR and EER motifs resulted in the identification of 171 RxLR genes in the *P. cinnamomi* genome (Table 6). Despite the potential importance of RxLR proteins in the infection of plants by *Phytophthora* pathogens, to date, the molecular function of only a few RxLR effectors has been determined or their precise role in plant pathogenicity established. Of 10 *Phytophthora* RxLR effectors that have been functionally characterized, only three have homologues in the *P. cinnamomi* genome (Table 6).

Avr3b Nudix hydrolase. Seven RxLR genes in the *Avr3b* family in *P. sojae* contain a C-terminal Nudix motif found in Nudix hydrolases, which catalyse the hydrolysis of nucleoside diphosphate derivatives (Dong *et al.*, 2011). pBLAST searches showed that the *P. cinnamomi* genome contains a number of potential *Avr3b* homologues, but, in the current assembly, only sequence PHYCI_87160 has all four domains typical of Nudix hydrolases, i.e. a signal peptide, RxLR, EER and Nudix domains (Table 6). Three (PHYCI_257012, PHYCI_95983 and PHYCI_305326) of six other homologous *P. cinnamomi* sequences are missing 5′ data which might have included secretion signal and RxLR domains; one (PHYCI_194603) of the six sequences is missing 3′ data which might have included a Nudix domain; and two (PHYCI_130927 and PHYCI_552641) of the six sequences are missing 5′ and 3′ data. It seems likely that the absence of domains characteristic of Avr3b effectors from some or all of these six sequences is a result of assembly errors, meaning that there could be up to seven *Avr3b* homologues in *P. cinnamomi*.

In *Arabidopsis*, synthesis of a Nudix hydrolase, AtNUDT7, is induced by pathogen attack and acts as a negative regulator of plant defence against bacterial and Oomycete pathogens (Bartsch *et al.*, 2006). It is suggested that *Phytophthora* Avr3b effectors may down-regulate plant defence by mimicking endogenous plant Nudix hydrolases (Dong *et al.*, 2011).

PSR suppressors of RNA silencing. A study of *P. sojae* RxLR effectors has identified two genes whose encoded proteins are able to suppress RNA silencing in plants (Xiong *et al.*, 2014). The gene family, designated *PSR* (*Phytophthora* suppressor of RNA silencing), is reported to be conserved, with at least one gene present in a number of *Phytophthora* species, including *P. cinnamomi* (Xiong *et al.*, 2014). pBLAST analysis in FungiDB, comparing one of the *P. sojae* PsPSR2 proteins (PHYSO_290752) with sequences in *P. cinnamomi* genomes, yielded a number of proteins with 30%–40% identity. It is thus surprising that the *P. cinnamomi* (var. *robiniae*) sequence listed in Table S1 in Xiong *et al.* (2014) is 94% identical to the *P. sojae* PHYSO_290752 sequence, but only 41% identical to the top *P. cinnamomi* (var. *cinnamomi*) hit. Nevertheless, there is evidence of a family of PSR2-like proteins in *P. cinnamomi* (Table 6). Transient expression of *PSR* or *PSR*-like genes in *N. benthamiana* increases their susceptibility to *P. infestans* infection, although details of the mechanism(s) involved are yet to be elucidated (Qiao *et al.*, 2013).

PiSNE1 suppressor. A *P. infestans* protein with an RxLR-like motif, *PiSNE1* (*P. infestans*, suppressor of necrosis 1), is transcribed during the biotrophic phase of infection in tomato and suppresses the induction of plant cell necrosis and programmed cell death in response to pathogen attack (Kelley *et al.*, 2010). The PiSNE1 protein has an NLS and is translocated to the plant nucleus. There are two putative homologues of *PiSNE1* in the *P. cinnamomi* genome, both of which have an NLS according to the Plant-PLoc algorithm (Table 6).

Putative effectors of pathogen defence strategies

Pathogens may attempt to avoid the triggering of host defences by masking or changing the sequence of elicitors recognized by the plant (Dodds *et al.*, 2006; Fujikawa *et al.*, 2012). If these strategies are unsuccessful, the pathogen will need to deal with multifaceted plant defences, possibly through a variety of mechanisms, including suppression, deactivation and tolerance.

Protection against reactive oxygen species (ROS)

To establish disease, pathogens must be able to survive an oxidative burst that may occur as part of the plant's defence response. The oxidative burst involves the production of reactive oxygen species, such as the superoxide anion (O_2^-) and H_2O_2. To detoxify these molecules, phytopathogens produce superoxide dismutases (SODs) which convert O_2^- to H_2O_2, and catalases and peroxidases that catalyse the breakdown of H_2O_2 to water and oxygen.

The *P. cinnamomi* genome contains three *SOD* genes (Table 7). Two SODs use manganese as a cofactor and the third uses copper/zinc ions. During plant–pathogen interactions, pathogen *SOD* gene expression may be up-regulated, and encoded SODs act as pathogenicity factors (Rolke *et al.*, 2004; Veluchamy *et al.*, 2012). Plants, in turn, may be able to inhibit the function of pathogen SODs, as the activity of *P. cinnamomi* MnSODs has been reported to be lower in the presence of avocado root or cell wall components (Guzmán-Deara *et al.*, 2013).

Table 7 *Phytophthora cinnamomi* proteins involved in plant–pathogen interactions: putative effectors involved in combating plant defences.

P. cinnamomi gene name (in FungiDB)	Homologues used for BLAST query	Proposed protein function	References
Defence against reactive oxygen species (ROS)			
PHYCI_89802	*P. parasitica PnMnSOD2* PPTG_04295	Mitochondrial manganese superoxide dismutase	Blackman *et al.* (2005)
PHYCI_213407	*P. parasitica PnMnSOD1a* PPTG_04112	Cytosolic manganese superoxide dismutase	Blackman *et al.* (2005)
PHYCI_74368	*P. parasitica* PPTG_06915	Copper/zinc superoxide dismutase	Blackman *et al.* (2005)
PHYCI_316206	*P. parasitica PnCat1* PPTG_06664	Catalase that is predicted to be cytosolic	Blackman and Hardham (2008)
PHYCI_94911	*P. parasitica PnCat2* PPTG_06713	Catalase that is predicted to be targeted to peroxisomes	Blackman and Hardham (2008)
PHYCI_97994	*P. parasitica PnCat3* PPTG_06866	Catalase that is predicted to be cytosolic	Blackman and Hardham (2008)
PHYCI_238395	*P. parasitica* PPTG_02738 PPTG_04280	Catalase-peroxidases that are predicted to be secreted	Blackman and Hardham (2008)
PHYCI_112968	*P. sojae PsMPK7* PHYSO_355777	Stress-associated mitogen-activated protein kinase (MAPK) Silencing > reduces ability to suppress plant ROS; reduces virulence	Gao *et al.* (2015); Li *et al.* (2010)
Glucanase inhibitor proteins (GIPs)			
PHYCI_99638 PHYCI_585554 PHYCI_245084 PHYCI_322876 PHYCI_430555 PHYCI_99743 PHYCI_217079	*P. parasitica GIP*s PPTG_10494 PPTG_13057 PPTG_10494 PPTG_13156 PPTG_13062	Glucanase inhibitor proteins (GIPs). Chymotrypsin serine protease homologues that lack the catalytic triad of amino acids, His-Asp-Ser, required for protease activity. Differential expression during plant infection and evidence for co-evolution with host endoglucanases	Damasceno *et al.* (2008); Martins *et al.* (2014a); Rose *et al.* (2002)
Kazal-like protease inhibitors: EPI1–4			
PHYCI_116215 PHYCI_313058 PHYCI_237404 PHYCI_150461	*P. infestans EPI1* PITG_22681	Kazal-like protease inhibitor During infection, *P. infestans* EPI1 inhibits the tomato P69B subtilisin (PR7). EPI1 and P69B genes have similar expression profiles	Sels *et al.* (2008); Tian *et al.* (2004)
PHYCI_15239 PHYCI_230761 PHYCI_247340 PHYCI_279459 PHYCI_296195	*P. infestans EpI10* PITG_12129	Kazal-like protease inhibitor that inhibits plant subtilisin A proteases	Chinnapun *et al.* (2009); Tian *et al.* (2005)
Cystatin-like protease inhibitors: EPIC1–4			
PHYCI_95628 PHYCI_313378 PHYCI_16414 PHYCI_247204 PHYCI_13396 PHYCI_97582	*P. infestans EPIC2A* PITG_09175 *P. infestans EPIC1* PITG_09169	Cystatin-like protease inhibitors. Inhibit tomato papain-like protease Rcr3 and potato papain-like cysteine protease, C14	Kaschani and van der Hoorn (2011); Song *et al.* (2009); Tian *et al.* (2007)

The *P. cinnamomi* genome contains three catalase genes, each homologous to one of three *PnCat* genes in *P. parasitica* (Blackman and Hardham, 2008) (Table 7). In the current version of the *P. cinnamomi* genome, there are also one full-length (PHYCI_238395) (Table 7) and two truncated (PHYCI_12861 and PHYCI_12862) catalase-peroxidase genes, all of which have signal peptides directing their secretion.

The sensing of oxidative stress is typically followed by the activation of signal transduction pathways involving MAPKs. The expression of genes encoding two *P. sojae* MAPKs, PsSAK1 and PsMPK7, is induced by H_2O_2 (Gao *et al.*, 2015; Li *et al.*, 2010), and *P. cinnamomi* has homologues of both genes (Tables 1 and 7). Silencing of *PsSAK1* impairs plant colonization (Li *et al.*, 2010). Silencing of *PsMPK7* leads to increased sensitivity to oxidative stress and reduced pathogenicity (Gao *et al.*, 2015).

Inhibitors of plant endoglucanases (GIPs)

β-1,3-Glucans are key components of cell walls of Oomycetes and fungi. As part of their defence response, plants may produce endoglucanases that target pathogen wall β-1,3-glucans,

not only weakening the cell wall, but also releasing glucan fragments that are potent elicitors of the defence response (Sels *et al.*, 2008). *Phytophthora* species have evolved a counter-defence strategy by producing β-1,3-glucanase inhibitor proteins (GIPs) that inhibit the activity of plant endo-β-1,3-glucanases (York *et al.*, 2004).

Interrogation of the *P. cinnamomi* genome with five *P. parasitica GIP* genes indicates that *P. cinnamomi* contains seven putative full-length *GIP* genes (Table 7), as well as one partial sequence (PHYCI_92737). All lack the catalytic triad His–Asp–Ser. The full-length genes have a secretion signal peptide at the N-terminus. The partial gene is truncated at the N-terminus, possibly as a result of sequencing or assembly errors. The expression of one of the full-length genes, PHYCI_217079, increases during the first 24 h after inoculation of roots of chestnut before decreasing over the next 12 h (Martins *et al.*, 2014a).

Protease inhibitors

Plant proteases play important roles in plant immunity. They are involved in pathogen detection, activation of defence responses and degradation of pathogen proteases (Bae *et al.*, 2013; Figueiredo *et al.*, 2014). Early recognition and proteolysis of pathogen proteins can release elicitors and induce other defence strategies. However, as part of the continuing arms race between hosts and pathogens, *Phytophthora* and other microorganisms have evolved proteinaceous inhibitors of plant proteases.

The first identified extracellular protease inhibitor (EPI) produced by a plant-pathogenic microorganism was *P. infestans* EPI1 (Tian *et al.*, 2004). EPI1 contains two Kazal-like domains found in some serine protease inhibitor proteins. EPI1 specifically inhibits members of the subtilisin A class of serine proteases, including the tomato pathogenesis-related protein P69B subtilase (Sels *et al.*, 2008). The *P. cinnamomi* genome contains three full-length (Table 7) and one truncated (PHYCI_150461) *EPI1* homologue. The protein encoded by a second member of the *P. infestans EPI* multigene family, *EPI10*, also inhibits P69B activity (Tian *et al.*, 2005). A homologue in *P. palmivora*, Ppepi10, inhibits subtilisin A and an uncharacterized rubber plant (*Hevea brasiliensis*) protease (Chinnapun *et al.*, 2009). The *P. cinnamomi* genome contains five *EPI10* homologues (Table 7).

A second family of *P. infestans* inhibitors, the cystatin-like protease inhibitors (EPIC), contain cystatin-like protease inhibitor domains (Song *et al.*, 2009; Tian *et al.*, 2007). *Phytophthora infestans* EPIC1 and EPIC2B inhibit the papain-like tomato proteases PIP1 and Rcr3, and the potato papain-like cysteine protease, C14 (Kaschani and van der Hoorn, 2011; Song *et al.*, 2009). *Phytophthora cinnamomi* contains six full-length (Table 7) and three truncated (PHYCI_237355, PHYCI_126736 and PHYCI_237330) *EPIC* homologues.

GEOGRAPHICAL AND HOST RANGE

Improvements in the sensitivity and accuracy of detection and in identification techniques have confirmed that *P. cinnamomi* has spread throughout the world from its centre of origin in South-east Asia, with the number of susceptible plant species now approaching 5000 (Jung *et al.*, 2013). *Phytophthora cinnamomi* has been detected in 15 biodiversity hotspots, regions in which 'exceptional concentrations of endemic species are undergoing exceptional loss of habitat' (Myers *et al.*, 2000), including the Mediterranean Basin, south-west Western Australia and the fynbos of South Africa (Bezuidenhout *et al.*, 2010).

The Mediterranean Basin contains more than 25 000 species of plant, about 50% of which are endemic to the region (Myers *et al.*, 2000). Much of the area is populated by the evergreen oaks, *Quercus ilex* (holm oak) and *Q. suber* (cork oak), which are highly susceptible to infection by *P. cinnamomi*. Dehesa forests of *Q. ilex* and *Q. suber* within herbaceous pastures are the most widespread agroforestry land-use system in Europe (Martín-García *et al.*, 2015), and dense *Q. ilex* forests comprise much of the maquis vegetation of the Mediterranean islands and archipelagos (Scanu *et al.*, 2015). Over the last 30 years, extensive Mediterranean oak decline has been attributed primarily to *P. cinnamomi* (Camilo-Alves *et al.*, 2013; Linaldeddu *et al.*, 2014; Martín-García *et al.*, 2015). *P. cinnamomi* is also one of two *Phytophthora* species responsible for the destructive ink disease of sweet chestnut (*C. sativa*), with serious economic consequences in Europe for timber and nut production and soil stability (Dal Maso and Montecchio, 2015). In south-west Western Australia, over 3500 plant species, many of which are endemic, are susceptible to *P. cinnamomi*. *P. cinnamomi* is responsible for dramatic changes to the composition of plant communities over vast areas (Shearer *et al.*, 2007) and threatens macrofungal and bird biodiversity in this region (Anderson *et al.*, 2010; Davis *et al.*, 2014). In South Africa, sclerophyllous shrubs in the *Agathosma* genus are part of the Cape Floral Kingdom, a small region in terms of land area, but an area which has the greatest known species richness (Cowling *et al.*, 1996). *Agathosma* species are also grown for commercial use and their decline has been associated with infection by a number of soil-borne *Phytophthora* species, including *P. cinnamomi* (Bezuidenhout *et al.*, 2010).

It is predicted that global warming and climate change will increase *P. cinnamomi* disease problems in agriculture and forestry, and will exacerbate problems in biodiversity hotspots, including increasing the rate of extinction of endemic species (Burgess *et al.*, 2017; Malcolm *et al.*, 2006). The Mediterranean Basin and south-west Western Australia are considered to be especially vulnerable. Experimental simulations of the effects of potential weather extremes on infection of holm oak by *P. cinnamomi* indicate that periods of drought following waterlogging will accelerate disease development and the rate of oak seedling mortality

(Corcobado *et al.*, 2014). Increased temperatures and soil water content will favour *P. cinnamomi* growth and dispersal, but accurate predictions of the effects of changing climatic conditions on disease development also need to take into account a range of other factors, such as effects on plant hosts. Mathematical models based on data from *P. cinnamomi* that utilize multiple parameters associated with climatic change and pathogen and plant responses are being developed (Dal Maso and Montecchio, 2015; Thompson *et al.*, 2013). Globalization and increased movement of plant material throughout the world also provide enhanced opportunities, via genetic recombination, for the development of novel isolates with altered virulence and/or host range.

INTEGRATED DISEASE MANAGEMENT STRATEGIES

Key requirements for successful prediction, control and management of diseases include a thorough knowledge of the cellular and molecular basis of pathogen biology and pathogenicity; methods for rapid and sensitive detection and identification; multifaceted control procedures; and access to resistant plant cultivars. Our current understanding of *P. cinnamomi* biology and infection strategies has been described above. An overview of detection and control methods follows.

Detection and identification

The development of immunodiagnostic techniques based on antibodies that recognize pathogen molecules (antigens) is a major improvement over traditional methods which assess morphological and physiological characteristics (Hardham, 2005). However, the fact that the targeted antigen may not be present in all viable samples can lead to false negative results. DNA, by contrast, is present throughout an organism's lifecycle, and DNA-based assays utilizing qPCR to amplify DNA have a number of advantages over immunodiagnostic techniques (Elliot *et al.*, 2015; Engelbrecht *et al.*, 2013; Eshraghi *et al.*, 2011b; Vincelli and Tisserat, 2008). It should, however, be remembered that assays targeting DNA do not discriminate between viable and non-viable material (O'Brien *et al.*, 2009).

The sensitivity and specificity of qPCR-based assays are influenced by the selection of the DNA sequence that is targeted and the design of the oligonucleotide primers that are used (O'Brien *et al.*, 2009). Nested PCR assays which incorporate two rounds of amplification utilizing different primers may improve taxonomic discrimination and increase assay sensitivity (Engelbrecht *et al.*, 2013; Schena *et al.*, 2008). In multiplex assays, differentially tagged primer pairs have been used to simultaneously test for the presence of *P. cinnamomi* and other pathogen species in the same soil, water or plant samples (Kostov *et al.*, 2016). Specific recognition of DNA sequences has also been used to detect *P. cinnamomi* *in planta* through the application of fluorescence *in situ* hybridization (Li *et al.*, 2014).

Control strategies

As a result of the phylogenetic distance between *Phytophthora* and true fungi, most fungicides are ineffective and the choice of chemicals available for *Phytophthora* management is limited. The two chemicals most frequently used to control *P. cinnamomi* are metalaxyl (a phenylamide) and phosphite (a phosphonate and the active ingredient in fosetyl-Al); however, their prolonged use has led to decreased sensitivity and the development of resistance (Dobrowolski *et al.*, 2008; Hu *et al.*, 2010).

Phosphite can control *P. cinnamomi* diseases directly through the inhibition of pathogen growth or indirectly through enhancement of plant defence. *In vitro*, phosphite induces cell wall lysis and hyphal abnormalities, and reduces hyphal growth and spore production or germination (King *et al.*, 2010; McCarren *et al.*, 2009a,b). Initial investigations have documented the differential expression of 44 *P. cinnamomi* genes after phosphite treatment, including the down-regulation of nine genes encoding putative cellulose synthases, a feature consistent with increased hyphal wall lysis (King *et al.*, 2010; Wong *et al.*, 2009).

Phosphite induction of plant defence varies depending on the plant species, but often allows normally susceptible plants to resist infection (Anderson *et al.*, 2012; Daniel and Guest, 2006; Eshraghi *et al.*, 2011a; Lim *et al.*, 2013; Pilbeam *et al.*, 2011; Shearer and Crane, 2012; Suddaby *et al.*, 2008). Phosphite can be phytotoxic, and the mode and rate of its application need to be monitored and the most suitable methods used (Akinsanmi and Drenth, 2013; Anderson *et al.*, 2012; Crane and Shearer, 2014; Gentile *et al.*, 2009; Groves *et al.*, 2015; Pilbeam *et al.*, 2011; Scott *et al.*, 2015; Shearer and Fairman, 2007; Stasikowski *et al.*, 2014). Changes in the plant transcriptome and proteome induced by phosphite are now beginning to be explored (Burra *et al.*, 2014; Eshraghi *et al.*, 2011a, 2014; Lim *et al.*, 2013).

The identification of biological control agents for *P. cinnamomi* has been limited (but see Bosso *et al.*, 2016), although the application of mulches, composts or animal manure can inhibit *P. cinnamomi* growth, perhaps through the secretion of cellulase enzymes by microorganisms proliferating in the mulch (Aryantha and Guest, 2006; Evidente *et al.*, 2009; Richter *et al.*, 2011). The scale of *P. cinnamomi* distribution, its ability to survive for years in soil or symptomless plants, and the extent of plant susceptibility make the management of *P. cinnamomi* diseases challenging and difficult. The benefits of implementing integrated strategies have been demonstrated for the management of avocado root rot, the most important disease of avocado worldwide (Ramírez-Gil *et al.*, 2017). A combination of drenching in metalaxyl, mancozeb and silicate, with phosphite injection, addition of compost to the soil and

mulching with organic material reduce disease occurrence and increase the production of first class fruit by almost 70% and 44%, respectively, relative to untreated controls.

CONCLUDING REMARKS

In the years leading up to the publication of the previous Pathogen Profile on *P. cinnamomi* in *Molecular Plant Pathology* in 2005, a major contributor to the then current state of knowledge was the application of immunological techniques to studies of *P. cinnamomi* cellular and molecular biology and the development of novel diagnostic assays. Immunocytochemistry has continued to expand our understanding but, over the last decade, additional approaches have helped to elucidate key aspects of *P. cinnamomi* biology and pathogenicity, such as those addressed by the questions posed at the beginning of this review article, relating to *P. cinnamomi*'s extensive host range, infection strategies and potential targets for novel controls.

In the years leading up to the present Pathogen Profile on *P. cinnamomi*, a major component of our increased knowledge has been the release of the *P. cinnamomi* genome sequence. A central goal of this review article has been to place new molecular data obtained through bioinformatic analysis of the genomic information within the context of our understanding of the cellular biology of *P. cinnamomi* growth and infection. In so doing, a number of potentially important candidates for future research have been identified. For example, *P. cinnamomi* has the largest family of genes encoding SCR virulence factors reported to date. How critical is the role of SCR proteins in plant infection? Do they contribute to *P. cinnamomi*'s wide host range? It is also clear that *P. cinnamomi* is well equipped to deal with induced plant defence, containing, as it does, abundant CRN, Avr3b and PsSNE1 homologues, all of which have been shown to suppress host defence in other *Phytophthora* species.

In the next phase of research on *P. cinnamomi*, it will be crucial to identify key pathogenicity genes and to determine the role of their encoded proteins in plant infection. One approach that will be central to this endeavour will be the application of next-generation sequencing technologies, such as RNA-Seq, to obtain comprehensive information on *P. cinnamomi* transcriptomes during development and plant infection.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. S1 Relative expression of selected *Phytophthora cinnamomi* genes before and after the induction of sporulation by transfer of mycelia to mineral salts solution. The graphs show data derived by the quantification of bands on RNA blots (Narayan, 2004) and are indicative of a number of different expression profiles during sporulation. *Anx*, annexin; *CaM*, calmodulin; *Cen*, centrin; *EF1α*, elongation factor 1α; *GDH*, glyceraldehyde-3-phosphate dehydrogenase; *LDH*, D-lactate dehydrogenase; *Pc*, *P. cinnamomi*; *Pdk*, pyruvate phosphate dikinase; *SAc*, *S*-adenosylhomocysteinase; *Set1*, SET1 transcription factor; *Ubq*, ubiquitin; *STk*, serine/threonine protein kinase.

Fig. S2 Transcript levels of three *Phytophthora cinnamomi* polygalacturonase genes in cultures growing in 5% V8 broth and defined media. Mycelia were bulked in media containing 2% glucose as the sole carbon source and then transferred to 5% V8 broth or one of six defined media. Transcript levels were measured using quantitative polymerase chain reaction (qPCR) (Dandipat, 2009).

Fig. S3 Transcript levels of three *Phytophthora cinnamomi* polygalacturonase genes in roots of lupin (*Lupinus augustifolius*) after inoculation. Transcript levels were measured using quantitative polymerase chain reaction (qPCR) (Dandipat, 2009).

Table S1 The number of genes in *C*arbohydrate-*A*ctive en*Z*yme (CAZyme) families in the genomes of *Phytophthora cinnamomi* (Pcin) and *P. parasitica* (Ppar).

Table S2 Putative *Phytophthora cinnamomi* elicitin genes. An initial group of 32 *P. cinnamomi* sequences with E-values of <E-05 was obtained by BLAST analysis of the *P. cinnamomi* genome in FungiDB with the β-cinnamomin gene, PHYCI_98389. Subsequently, two additional *P. cinnamomi* sequences with high homology to PITG_06908 and PHYSO_30815 were identified. The table shows the *P. infestans* (or, in one case, *P. sojae*) elicitins to which the 34 *P. cinnamomi* sequences are most similar.

Table S3 Putative Crinkler (CRN) homologues in the *Phytophthora cinnamomi* genome. A PBLAST search using 45 CRN proteins representative of those reported in table S10 in Haas *et al.* (2009) resulted in the identification of 280 homologous sequences. These 280 sequences were analysed for the presence of conserved LFLAK and DWE motifs. This yielded a list of 42 putative *P. cinnamomi* CRN genes. These sequences were then analysed for the presence of a classical secretion signal (SP) or for evidence of secretion via a non-classical pathway, as indicated by an NN score of >0.6. The list of 42 putative CRN genes includes six genes that are truncated at the N-terminus in the current *P. cinnamomi* genome assembly. It also includes 13 genes that are not truncated at the N-terminus, but for which there is no evidence for secretion. Genes were also analysed for the presence of a nuclear localization signal (NLS).

Table S4 Putative *Phytophthora cinnamomi* Nep1-like protein (NLP) genes. Three *P. sojae* NLP genes, PHYSO_562453, PHYSO_509399 and PHYSO_249691, were used to BLAST the *P. cinnamomi* genome in FungiDB. This resulted in the identification of the 72 putative *P. cinnamomi* NLP genes listed here. The degree of homology of each of the *P. cinnamomi* genes to each of the three *P. sojae* genes is indicated by the scores and E-values in the table. Genes shown with light or dark blue shading lack homology to one or two, respectively, of the three *P. sojae* genes. Only sequences with E-values of <E-05 are included.