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New insights into the survival strategy of the invasive soilborne pathogen *Phytophthora cinnamomi* in different natural ecosystems in Western Australia

By T. Jung[1,2,3,5], I. J. Colquhoun[4] and G. E. St. J. Hardy[1]

[1]Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, 90 South Street, Murdoch, WA 6150, Australia; [2]Phytophthora Research and Consultancy, Thomastrasse 75, Brannenburg, 83098, Germany; [3]Institute for Biotechnology and Bioengineering, Centre of Genomics and Biotechnology (IBB/CGB), Laboratório de Biotecnologia Molecular e Fitopatologia, University of Algarve, Campus de Gambelas, Faro, 8005-139, Portugal; [4]Alcoa of Australia, Ltd, Huntley Mine Site, Pinjarrah, WA, 6208 Australia; [5]E-mail: trjung@ualg.pt (for correspondence)

Summary

Despite its importance as one of the most notorious, globally distributed, multihost plant pathogens, knowledge on the survival strategy of *Phytophthora cinnamomi* in seasonally dry climates is limited. Soil and fine roots were collected from the rhizosphere of severely declining or recently dead specimens of 13 woody species at 11 dieback sites and two dieback spots and from healthy specimens of five woody species at four dieback-free sites in native forests, woodlands and heathlands of the south-west of Western Australia (WA). *Phytophthora cinnamomi* was recovered from 80.4, 78.1 and 100% of tested soil, fine root and soil–debris slurry samples at the 11 dieback sites, in some cases even after 18-month storage under air-dry conditions, but not from the small dieback spots and the healthy sites. Direct isolations from soil–debris slurry showed that *P. cinnamomi* colonies exclusively originated from fine roots and root fragments not from free propagules in the soil. Microscopic investigation of *P. cinnamomi*-infected fine and small woody roots and root fragments demonstrated in 68.8, 81.3 and 93.8% of samples from nine woody species the presence of thick-walled oospores, stromata-like hyphal aggregations and intracellular hyphae encased by lignitubers, respectively, while thin-walled putative chlamydospores were found in only 21.2% of samples from five woody species. These findings were confirmed by microscopic examination of fine roots from artificially inoculated young trees of 10 woody species. It is suggested that (i) the main function of chlamydospores is the survival in moderately dry conditions between consecutive rain events and (ii) selfed oospores, hyphal aggregations, and encased hyphae and vesicles in infected root tissue of both host and non-host species are the major long-term survival propagules of *P. cinnamomi* during the extremely dry summer conditions in WA.

1 Introduction

Phytophthora cinnamomi is a highly aggressive soilborne pathogen with an extremely wide host range, which includes almost 5000 woody plant species in more than 70 countries (Zentmyer 1980; Shearer and Dillon 1995, 1996; Erwin and Ribeiro 1996; Shearer et al. 2004; Cahill et al. 2008). Of the 5710 endemic plant species in the south-west Botanical Province of Western Australia (WA), 2284 species (40%) in 39 families are susceptible and 800 species (14%) are highly susceptible to *P. cinnamomi* (Shearer et al. 2004). Most likely native to South-East Asia, *P. cinnamomi* has been introduced to Europe, North America, Africa and Australia where it has become invasive causing some of the most destructive diseases of woody plants and natural ecosystems known to date, that is, ink disease of chestnuts and oak decline in southern Europe, the south-eastern US and Mexico (Crandall 1950; Brasier et al. 1993; Gallego et al. 1999; Tainter et al. 2000; Vettraino et al. 2002, 2005; Balci et al. 2007; Jung et al. 2013); little leaf disease of pine species in the south-eastern US and the Caribbean (Tainter and Baker 1996; Jung and Dobler 2002); avocado root and collar rot in California, South Africa and Australia (Zentmyer 1980; Erwin and Ribeiro 1996); dieback of Fynbos heathlands in the Cape province of South Africa (Von Broembsen and Kruger 1985); and dieback of eucalypt forests, Banksia woodlands and heathlands in WA (Shearer and Tippett 1989; Shearer and Dillon 1995, 1996; Shearer et al. 2004) and Victoria (Weste and Marks 1987; Marks and Smith 1991; Weste 2003; Cahill et al. 2008). All these diseases are thriving under Mediterranean climatic conditions, which are characterized by mild humid winters and hot dry summers. During summer, soil temperatures in the south-west of WA reach more than 30°C, and soil moisture potentials and soil moisture contents drop below –6 Mpa (−60 bar) and 1%, respectively (Shearer and Tippett 1989; Lamont and Bergl 1991; Enright and Lamont 1992; Collins et al. 2011). How does an oomycete pathogen that is dependent on the presence of free soil water for spread and infection survive such extreme droughts?

Dieback caused by *Phytophthora cinnamomi* Rands is the most important and destructive disease of the jarrah (*Eucalyptus marginata*) forest of WA (Shearer and Tippett 1989). The introduced invasive pathogen has become renowned for its unparalleled impact on flora diversity. About 20% of the jarrah forest area is currently infested. In *E. marginata, P. cinnamomi* regularly infects fine roots and large woody roots but the collar region is only rarely invaded (Podger 1972; Dell and Wallace 1981; Shea and Dell 1981; Shea et al. 1982; Davison and Shearer 1989; Shearer and Tippett 1989). As with other *Phytophthora*-related root diseases (Erwin and Ribeiro 1996; Tainter and Baker 1996; Jung et al. 2000; Jung 2009), development of the first visible crown symptoms in *E. marginata* usually takes more than 10 years, symptoms include chronic crown decline or wilting, dieback and death of scattered individuals or groups of trees extending over several hectares (Podger 1972; Davison and Shearer 1989; Shearer and Tippett 1989; Hardy 2000; Hardy et al.

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2001). Mass deaths of *E. marginata* only occur on sites where a concreted lateritic layer is within 1 m of the soil surface, causing temporary water logging and enabling *P. cinnamomi* to invade and kill large vertical roots just above the lateritic layer (Shea et al. 1983). In contrast, in highly susceptible species such as *Xanthorrhoea* spp., *Banksia* spp. and many other proteaceous species, the pathogen often infects and girdles the root collars, leading to rapid death (Shea 1979; Shearer and Tippett 1989). A few years after its introduction to a site, *P. cinnamomi* causes dieback and mortality of many susceptible midstorey and understorey plant species, and highly susceptible hosts can be eliminated from an infested area.

Apart from truly aquatic members of the genus, most known *Phytophthora* species are primary parasites with a poor ability to compete with other microorganisms, and in particular with true fungi, for dead organic substrates and survive saprophytically. Therefore, soilborne *Phytophthora* species have developed a specific life cycle that is characterized by relatively short active phases of spread and infection via zoospores during wet soil conditions and subsequent primary invasion of healthy tissue alternating with longer phases of inactivity caused by dry soil conditions and presence of antagonistic fungi. To survive these unfavourable periods, *Phytophthora* species need successful survival strategies and resilient long-term resting structures. In homothallic *Phytophthora* species as well as in heterothallic species when partners of both mating types (A1 and A2) are present, sexually derived oospores are formed within oogonia in infected tissues. Due to the thickness and the chemical composition of their walls, oospores are highly resistant to both drought and microbial destruction and enable *Phytophthora* species to survive unfavourable (dry, hot and cold) periods for several years (Blackwell 1949; Hickman 1958; Erwin and Ribeiro 1996; Jung et al. 1999; Turkensteen et al. 2000). In addition, many *Phytophthora* species including *P. cinnamomi* produce chlamydospores vegetatively by expansion of the hyphal wall, flow of cytoplasm into the structure, delimitation from the hypha by septa and secondary thickening of the wall (Blackwell 1949; Hemmes and Wong 1975; Erwin and Ribeiro 1996; McCarren et al. 2005). Blackwell (1949) considered chlamydospores of *Phytophthora* species to be resistant structures enabling the pathogens to survive adverse conditions.

Due to the rare occurrence of the A1 mating type of *P. cinnamomi* in Australia and based on studies of *P. cinnamomi* populations in the soil using soil sieving and plating techniques, Weste and Vithanage (1978, 1979) considered chlamydospores to be the major survival structure and inoculum source of the pathogen in Australia. This view was also generally accepted for the jarrah forest (Old et al. 1984a; Shearer and Tippett 1989). However, in most studies, the identity of the colony-forming units has not been examined (Marks et al. 1975; Weste and Vithanage 1978, 1979; Shearer and Shea 1987) and it cannot be excluded that the colony-forming units belonged to another morphological structure. This suggestion is supported by Shea et al. (1980) who reported that *P. cinnamomi* colonies developing from plated soil suspension on selective agar were exclusively originating from small pieces of organic matter but not from free chlamydospores. In a microscopic examination of the organic pieces, no chlamydospores could be found.

Recently, McCarren et al. (2005), in their comprehensive literature review, critically appraised the role of chlamydospores as the main long-term survival propagules of *P. cinnamomi*. They concluded that despite many studies, there is only limited evidence that chlamydospores are an important survival propagule of *P. cinnamomi* in Australia. In fact, the evidence that *P. cinnamomi* produces chlamydospores in the soil is sparse. Several studies demonstrated that *P. cinnamomi* is able to produce the commonly known, thin-walled chlamydospores on nylon discs colonized by mycelium and buried in non-sterile soil within 5–15 days and could be re-isolated after up to 18 months (Kuhlman 1964; Mircetich and Zentmyer 1966, 1967; Reeves 1975; Weste 1983). However, only in the study of Weste (1983), which lasted only 28 days, the identity and viability of the spores were confirmed by the use of a fluorescent brightener and germination of the chlamydospores. Unfortunately, there is no information on whether or not there were any non-germinated, viable dormant chlamydospores, which could have enabled survival longer than the experimental period of 28 days. In another study, Weste and Vithanage (1979) buried nylon mesh discs inoculated with chlamydospore suspension in six different non-sterile soils. Depending on the organic content and the water potential of the soils, *P. cinnamomi* was able to survive between <2 and 10 months. There were no survivors after 12 months. Survival was favoured by decreasing soil water potential and increasing organic content. However, again the identity of the colony-forming units was not examined in detail neither was the occurrence of viable potentially dormant chlamydospores. In the experiments of Mircetich and Zentmyer (1966, 1967), *P. cinnamomi* could not be recovered from soil after 6 weeks when moisture had dropped from 21.6 to 2% although numerous chlamydospores and oospores were observed. In addition, no disease developed when the soils were re-moistened and planted with susceptible avocado seedlings.

Apparently, root tissue could provide a buffered environment to protect chlamydospores from adverse conditions in the surrounding soil. Therefore, the reservoir of chlamydospores in infected roots may be more important for long-term survival of *P. cinnamomi* than free chlamydospores in the soil. This is supported by the experiments of Mackay et al. (1985) who buried artificially inoculated roots of *E. sieberi* seedlings in three conducive and one suppressive soil and incubated them at three different matric potentials (−0.33, −5 and −10 bar). It was shown that *P. cinnamomi* was able to survive over a period of 100 days in all soil type–matric potential combinations and that survival was solely due to chlamydospores in the root tissue. However, after 200 days, matric potential had dropped below −10 bar and *P. cinnamomi* could no longer be recovered. After artificial inoculation, chlamydospores have also been observed in roots of both susceptible species like *E. marginata*, *E. sieberi*, *Lupinus angustifolius*, *Xanthorrhoea australis*, *Xanthorrhoea resinosa* and *Acacia melonoxylon*, and field-resistant species like *Corymbia calophylla*, *Corymbia maculata*, *Gahnia radula*, *Juncus bufonius* and *Themeda australis* (Mackay et al. 1985; Cahill et al. 1989; McCarren 2006). However, conclusions on the survival of *P. cinnamomi* in the field drawn from experiments using *in vitro* produced chlamydospores must be treated with caution. In a field trial, only a few chlamydospores were produced in field-inoculated roots and stems of *E. marginata* over a 12-month period (Tippett et al. 1983). Mircetich and Zentmyer (1966, 1967) and Hwang and Ko (1978) observed chlamydospores of

P. cinnamomi also in naturally infected avocado roots in California. Still there is no clear evidence of the formation of chlamydospores in naturally infected roots in the jarrah forest.

As McCarren et al. (2005) mentioned 'there is a conceptual link between thick-walled spores, dormancy and survival' although there is no evidence that wall thickness is the primary factor of resistance against desiccation and microbial destruction (Hemmes and Wong 1975). According to some authors, chlamydospores of *P. cinnamomi* may either be thin walled (wall diam. <1 μm) or thick walled (wall diam. >1 μm; Mircetich and Zentmyer 1967; Marks et al. 1975; Weste and Vithanage 1979; Erwin and Ribeiro 1996). However, there is only limited evidence for the existence of thick-walled chlamydospores (McCarren et al. 2005). This is mainly due to the difficulties of germinating thick-walled structures and distinguishing morphologically between thick-walled chlamydospores and oospores.

Surprisingly, little attention has yet been paid to other possible survival structures of *P. cinnamomi* such as hyphae inside root cells, stromata-like hyphal aggregations or oospores produced by selfing.

The objectives of this study were the following questions:

1 Does *P. cinnamomi* survive the hot dry summer of WA as free propagules in the soil or within infected root tissue?
2 Which morphological structures enable the long-term survival of *P. cinnamomi*?

In brief, the approach used was characterized by the collection of soil and fine root samples from various widespread woody species at dieback sites and in dieback-free areas of different ecosystems in WA, confirmation of the presence of *P. cinnamomi* and other *Phytophthora* species in these samples using sensitive baiting tests and studying the identity of the survival propagules and their location in the infested soil samples by direct plating of fine roots, small woody roots and soil–debris slurry onto selective agar, baiting from fine roots and small woody roots and by microscopic examination of infected debris and roots.

2 Material and methods

2.1 Sampling sites and sampling procedure

Sampling sites 1–12 were located in the jarrah forest south of Dwellingup (between 32.8–32.9°S and 116.0–116.1°E). At sites 1–8, *E. marginata*, *Banksia grandis*, *Xanthorrhoea preissii* and other susceptible species showed severe dieback (Fig. 1 c,d), while at sites 9–10, dieback was patchy. At sites 11–12, no dieback was noticed (Fig. 1a). Site no. 13 was chosen in a Kwongan heathland on poor mineral sands with patch dieback of *Banksia* spp. (Fig. 1f) near Eneabba, 230 km north of Perth (29.8°S, 115.3°E), while site no. 14 was located in a Banksia woodland with severe dieback (Fig. 1e) at Whiteman Park 22 km north of Perth (31.9°S, 116.0°E). Site no. 15 was selected at a dieback front in the tingle forest (*Eucalyptus jacksonii*) near Walpole in the south-west of WA (34.6°S, 116.5°E). The occurrence of crown dieback in the eucalypt overstorey at sites 1–10 and site 15 indicated that *P. cinnamomi* must have been present for at least 10 years (Fig. 1c). The presence of recently killed *Banksia* spp. trees (<6 months dead with leaves still attached; Collins et al. 2011) at all 13 dieback sites indicated recent activity of the pathogen (Fig. 1d–f).

At the 11 dieback sites and in the dieback patches of sites 9–10, rhizosphere soil containing fine roots had been collected in different seasons from 130 specimens of 13 woody species including 34 declining or recently killed *E. marginata* trees at eight sites (Fig. 1c) and one long-dead (>1 year dead, without leaves, bark dry and cracking; Collins et al. 2011) and 42 recently killed *B. grandis* trees (Fig. 1d) at eight sites. Furthermore, recently killed trees of *Banksia hookeriana* (Fig. 1e), *Banksia attenuata* (Fig. 1f), *Banksia sessilis*, *X. preissii*, *Macrozamia riedlei* and *Acacia blakelyi*, declining trees of *Corymbia calophylla*, *E. jacksonii* and *Persoonia longifolia* and healthy trees of the resistant *Eucalyptus megacarpa* (Fig. 1b) were sampled at different sites.

At the two dieback-free sites 11–12 and in large dieback-free areas of sites 9–10, rhizosphere soil was sampled from 40 healthy specimens of five woody species: *B. grandis*, *E. marginata*, *C. calophylla*, *P. longifolia* and *M. riedlei*.

From each plant, *c.* 3 to 5 l soil containing fine roots were sampled at two to three points within 1 m distance from the stem to a depth of *c.* 30 cm and bulked. To reduce the number of individual soil samples to be processed, the samples of 4–5 trees of the same plant species (*B. attenuata*, *B. grandis*, *E. marginata*, *E. megacarpa*, *C. calophylla*, *B. sessilis*, *P. longifolia* and *X. preissii*) at sites 3–10 and at site 14 were bulked to give a total of 50 soil samples from dieback sites and 10 soil samples from the two dieback-free areas.

In addition, at sites 1 and 8, dry bark samples including the cambium were taken from the upper 20 cm of collar rot lesions of two recently killed and two long-dead *B. grandis* trees. At site 13, collar rot lesions of two recently killed *B. hookeriana* trees were sampled in the same way. The bark samples were placed in distilled water and transported to the laboratory in cool boxes (Jung 2009).

2.2 Isolation methods

2.2.1 Isolation from soil

In the laboratory, each of the soil samples was carefully mixed. Then, a modified baiting technique of Jung et al. (1996, 2000) was used to isolate *Phytophthora* species from the soil samples. Subsamples of *c.* 200 ml were filled in 18 × 12 cm tubs, moistened with distilled water and incubated at *c.* 20°C to stimulate germination of *Phytophthora* resting structures. The remaining 3–5 l of soil from each sample tree was stored air-dry at 20°C. After 24 h, all tubs were flooded with

Fig. 1. Natural ecosystems in Western Australia sampled during this study; (a) healthy jarrah (*Eucalyptus marginata*) forest at site 11 with dense understorey of *Banksia grandis* and *Xanthorrhoea preissii*; (b) healthy bullich (*Eucalyptus megacarpa*) trees at dieback site 7; (c) extensive dieback and mortality of *E. marginata* and most understorey species at dieback site 6; (d) recently killed young *B. grandis* trees at jarrah forest dieback site 2; (e) recently killed *Banksia hookeriana* in a Kwongan heathland at dieback site 13; and (f) severe mortality of Banksia woodland at dieback site 14 with recently killed and long-dead *Banksia attenuata* trees.

distilled water to 3–4 cm in depth. Floating organic material was moved to the side of the baiting tray with flyscreen meshing, and any remaining organic material floating on the surface of the baiting water was removed with paper towelling. In a preliminary isolation test with the 19 soil samples from sites 1 and 2, juvenile leaves of *Quercus ilex*, *Quercus suber*, *Pittosporum undulatum* and *Pittosporum tenuifolium* as well as *Eucalyptus sieberi* cotyledons (Marks et al. 1975) and *Lupinus angustifolius* radicles (Chee and Newhook 1965) were floated on the water as baits in a comparative assay (Fig. 2 a). As *Q. ilex* and *Q. suber* leaves proved to be the most sensitive baits, they were used in all further baiting tests.

Leaves with brownish lesions, usually appearing after 3–7 days, were examined for the presence of *Phytophthora* sporangia using a light microscope. Positive leaflets were blotted dry, cut into small segments and plated onto Phytophthora-selective PARPNH agar [V8 agar (16 g agar, BBL; Becton, Dickinson and Co, Sparks, MD, USA), 3 g $CaCO_3$, 100 ml Campbell' s V8 juice clarified and 900 ml distilled water) amended with 10 mg l^{-1} pimaricin, 200 mg l^{-1} ampicillin, 10 mg l^{-1} rifampicin, 100 mg l^{-1} pentachloronitrobenzene, 50 mg l^{-1} nystatin and 25 mg l^{-1} hymexazol; Jung et al. 2000]. Antibiotics were dissolved in 2–3 ml of 75% ethanol and added to the agar after autoclaving and cooling down to *c.* 45°C. Petri dishes were incubated at 20°C in the dark and examined for *Phytophthora*-like hyphae using a stereomicroscope.

2.2.2 Isolation from bark

Bark samples were incubated for 2 days in distilled water at 20°C, and the water was replaced four times per day to remove excess polyphenols. Then, 20–50 small pieces (*c.* 8 × 3 × 3 mm) were cut from different parts and depths of the

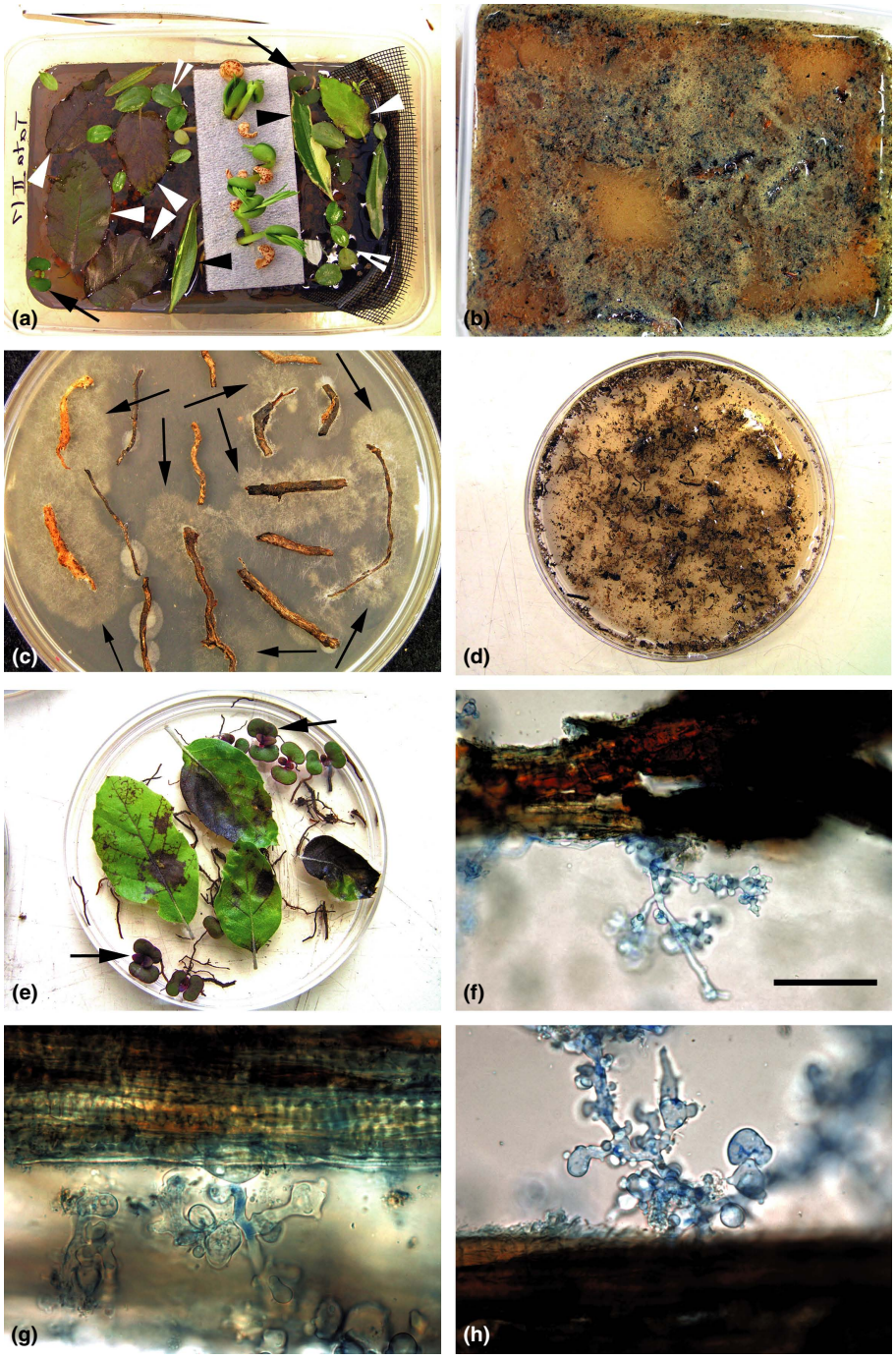

Fig. 2. Techniques for isolating *Phytophthora cinnamomi* from soil and root samples; (a) comparative baiting assay from a soil sample: infected brownish leaves of *Quercus ilex* and *Q. suber* (white-filled arrow heads), uninfected leaves of *Pittosporum undulatum* (black-filled arrow heads) and *P. tenuifolium* (white split arrow heads), uninfected cotyledons of *Eucalyptus sieberi* (arrows), uninfected seedlings of *Lupinus angustifolius* (on the raft); (b) feeder roots, organic debris and soil particles floating on a flooded soil sample after stirring; (c) colonies of *P. cinnamomi* (arrows) growing from fine roots and small suberized roots of *B. grandis* on selective PARPHN agar; (d) soil–debris slurry collected from surface of a flooded soil sample and plated on PARPHN agar; (e) infected *Q. suber* leaves and *E. sieberi* cotyledons (arrows) floating on submersed fine roots of *Banksia grandis*; and (f–h) coralloid hyphae and vesicles of *P. cinnamomi* growing from root debris particles on PARPHN agar. Scale bar = 25 μm for (g) and (h), and 40 μm for (f).

lesions, blotted on filter paper and plated onto selective PARPNH agar. The remaining pieces of necrotic tissue were flooded with distilled water and baited with young leaflets of *Q. ilex* and *Q. suber* (Jung 2009). The water was replaced daily to remove excess polyphenols and decrease bacterial populations.

2.2.3 Isolation from roots and soil–root debris slurry

Only the 32 soil samples from dieback sites 1–8 and 13–15 from which *P. cinnamomi* but no other oomycetes had been isolated in the baiting test and the 10 soil samples from the dieback-free sites 9–12 were used for this investigation. Subsamples of *c.* 200 ml were taken from the remaining 3–5 l samples, flooded in 18 × 12 cm tubs with distilled water, stirred and incubated for 48 h at 20°C (Fig. 2b) to stimulate germination of resting structures.

Then, 40–50 fine long and short roots (<2 mm diam., 3–20 mm length) and a 10-ml slurry of soil particles and organic material floating on the surface were removed from each tub with a pair of tweezers and a pipette, respectively. Some root sections of small woody roots and some lateral fine roots were also cut from immersed roots. The organic material mainly consisted of detached fine roots and disintegrated root debris in different stages of decay.

The roots were washed thoroughly, surface disinfested for 10 s in 70% ethanol, blotted dry, plated onto selective PARPNH agar and examined for developing colonies of *P. cinnamomi* after 48- and 72-h incubation at 20°C in the dark (Fig. 2c).

The 10 ml soil–debris slurry was diluted with distilled water at a ratio of 1 : 1, and each 5 ml of the suspension poured onto 90-mm-diam. Petri dishes with selective PARPNH agar (Fig. 2d; 4 Petri dishes per sample). After 48-h incubation at 20°C in the dark, the water was discarded from the plates and the Petri dishes were incubated for another 24 h (Shearer and Shea 1987). Then, the Petri dishes were thoroughly examined under the stereomicroscope for developing colonies of *P. cinnamomi*. All colonies were examined under the light microscope whether they originated from free chlamydospores, sporangia, zoospores or from debris.

2.2.4 Survival in air-dry soil and roots

The survival time of *P. cinnamomi* in air-dry soil and roots was examined using the 13 soil samples of *B. grandis* and *E. marginata* from dieback sites 1 and 2 collected in February 2008 and nine soil samples of *B. grandis*, *C. calophylla*, *E. marginata*, *E. megacarpa* and *X. preissii* from dieback sites 3, 4, 7 and 8 collected in October 2009. The samples were stored air-dry at 20–35°C in the dark, and after 6, 12, 18 and 24 months, *c.* 200 ml subsamples of soil were placed in 18 × 12 × 10 cm tubs, moistened with distilled water for 2 days and then baited as described before. In addition, just before flooding, 40–50 fine long and short roots were randomly collected from the moistened soil samples, washed thoroughly, surface disinfested for 10 s in 70% ethanol and blotted dry. Then, 15–20 roots were plated onto PARPNH agar and incubated at 20°C in the dark, while the remaining roots were baited with *Q. suber* leaves and *E. sieberi* cotyledons in 90-mm Petri dishes filled with distilled water at 20°C and natural daylight (Fig. 2e). The survival rate after 24 months was only tested with the 13 soil and root samples from dieback sites 1 and 2.

2.3 Microscopic examination of fine roots and root debris

Fine roots and small woody roots from which *P. cinnamomi* had been recovered by direct plating or by baiting and small pieces of debris from which *P. cinnamomi* colonies grew from were used for microscopic examination. In addition, other fine roots and feeder roots floating on flooded soil or cut from suberized roots immersed in the flooding water of samples tested positive for *P. cinnamomi* were also used.

Young plants of *B. attenuata*, *B. chamaephyton*, *B. occidentalis*, *E. marginata*, *C. calophylla*, *Eremaea pauciflora*, *Hakea eneabba*, *Lambertia multiflora*, *Kunzea acuminata* and *X. preissii* are routinely used in pathogenicity trials with artificially infested soils at the CPSM. Fine roots and small woody roots of plants from which *P. cinnamomi* had been reisolated were included as a comparison.

The roots and the pieces of debris were cleared, stained and destained using a slightly modified protocol of Brundrett et al. (1996). For clearing, cheesecloth bags containing 1–2 g subsamples of debris and fine roots were flooded with 10% KOH, autoclaved for 20 min at 121°C and rinsed under running distilled water. The samples were stained by immersing the cheesecloth bags in lactoglycerol trypan blue (88% lactic acid, glycerol and distilled water 1 : 1 : 1 + 0.05% w/v trypan blue) and incubating for 4 h at 70°C. Afterwards, the organic particles and roots were removed from the cheesecloth bags and destained for 12 h in lactoglycerol (1 : 1 of 88% lactic acid, glycerol). Storage was also in lactoglycerol.

From small woody roots (2–4 mm diam), thin longitudinal sections were cut using a razorblade. These sections, the fine roots (<2 mm diam) and the debris pieces, were placed on microscope slides, covered with lactoglycerol and gently squeezed with cover slips. Samples were examined for the presence of morphological structures typical of *P. cinnamomi* under a light microscope at ×200 and ×400 magnifications, respectively.

2.4 Microscopic examination of *P. cinnamomi* isolates in culture

Ten isolates of *P. cinnamomi* collected during this study in WA grown on V8A at 20°C in the dark were examined after 2 and 4 weeks under a light microscope at ×200 and ×400 magnifications for the viability of the chlamydospores and for the presence of alternative survival structures. All isolates belonged to the A2 mating type. Five of the 10 isolates were paired on V8A with a strain of *Trichoderma viride* (Brasier 1971, 1978). Inoculum plugs (5 mm diam) of the isolate to be tested and the tester isolate were placed on opposite sides of a 9-cm Petri dish, 2 cm from the edge. The plates were incubated at 20°C in darkness and examined for oogonial formation 30 days after the two colonies had met.

3 Results

3.1 Isolation tests

3.1.1 Soil samples

In the comparative assay of the first isolation test with 19 soil samples of *B. grandis* and *E. marginata* from sites 1 and 2, the efficacy of the different baits was significantly different. In all 13 *P. cinnamomi*-infested soil samples, leaves of *Q. ilex* and *Q. suber* developed brownish necroses within 2–5 days (Fig. 2a), and microscopic examination showed large numbers of non-papillate sporangia of *P. cinnamomi* formed on their surface. With five positive samples, the second best baits were cotyledons of *E. sieberi,* which after 3–7 days developed greenish discolorations of the naturally purple-coloured lower surface. *Phytophthora cinnamomi* could be easily isolated from all plated infected oak leaves and eucalypt cotyledons. In contrast, almost all leaves of the two *Pittosporum* species and all lupin radicles remained uninfected for 10 days (Fig. 2a) despite the formation of high numbers of secondary sporangia on the oak leaves floating around them. Therefore, only leaves of *Q. ilex* and *Q. suber* and in some cases also *E. sieberi* cotyledons were used in the other baiting tests.

Phytophthora cinnamomi could be isolated from 37 of the 46 soil samples from dieback sites 1–8 and 13–15 (80.4%) but not from the four soil samples from the small dieback spots at the largely dieback-free sites 9 and 10 (Table 1). In addition, two *P. cinnamomi*-positive soil samples, one from a recently dead *B. grandis* tree at site 1 and one from a declining *E. jacksonii* tree at site 15, also yielded *P. multivora* and *P. cryptogea*, respectively. An unidentified *Globisporangium* sp. was recovered from three *P. cinnamomi*-positive samples from sites 2 and 3, while an unidentified *Phytopythium* sp. was present in two *P. cinnamomi*-positive samples from sites 1 and 14. The 13 soil samples from which *P. cinnamomi* could not be isolated and the six *P. cinnamomi*-positive soil samples that also contained other oomycete species were excluded from the isolation tests from fine roots and root debris. No *Phytophthora* sp. could be recovered from the 10 soil samples collected at the dieback-free sites.

3.1.2 Bark samples

Phytophthora cinnamomi was recovered from the collar rot lesions of three of the four recently killed trees of *B. grandis* from sites 1 and 8 and from the collar rot lesion of one of the two recently killed trees of *B. hookeriana* from site 13 by baiting with *Q. suber* leaves and in two cases also by direct plating onto PARPNH. Again, young leaves of *P. undulatum* and lupin radicles remained uninfected in the baiting tests. From one *B. hookeriana* tree, *Phytophthora arenaria* was isolated by baiting. In contrast, no *Phytophthora* was isolated from necrotic collars of *B. grandis* trees that were dead for more than 1 year.

3.1.3 Fine roots and soil–debris slurry

Phytophthora cinnamomi could be isolated from plated soil–root debris slurry of all 32 samples from dieback sites 1–8 and 13–15 included in this test. The number of *P. cinnamomi* colonies per Petri dish = per 2.5 ml soil–debris slurry) ranged between 2 and 15. Thorough examinations of all plates under the light microscope at $\times 80$ and $\times 200$ magnifications showed that all colonies exclusively originated from debris pieces (Fig. 2f–h). No evidence could be found for free viable chlamydospores in the soil. Initial confirmation of *P. cinnamomi* was by its typical irregular hyphae and coralloid hyphal swellings growing from plated long and short roots and root debris (Fig. 2f–h). After 96 h, chlamydospores began to form either individually or in clusters. In some cases, *P. cinnamomi* also formed sporangia on the surface of root debris pieces.

No *Phytophthora* sp. was recovered from soil–root debris slurry of the 10 samples from the four dieback-free sites. In all samples from both dieback sites and dieback-free areas, several unidentified zygomycetes grew vigorously from most debris pieces, indicating that the 48-h flooding had leached the antibiotics from the selective agar. In some cases, hyphae of unidentified *Pythium* species were also observed.

In 25 of the 32 samples (78.1%) from dieback sites 1–8 and 13–15, *P. cinnamomi* was also isolated from plated long and short fine roots and small woody roots (Fig. 2c), which like the organic debris had been floating on the surface of the flooded soils or been attached to immersed roots. Isolation frequencies ranged from 2 to 11%. No *Phytophthora* sp. was isolated from roots of the 10 samples from the four dieback-free sites.

3.1.4 Survival time of P. cinnamomi in air-dry soil and roots

Phytophthora cinnamomi was isolated from all 22 soil samples and from 17 of the 22 root samples (77.3%) of *B. grandis*, *C. calophylla*, *E. marginata*, *E. megacarpa* and *X. preissii* after 6 months of air-dry storage (Table 2). After 12 months, the isolation rate dropped to 16 soil (72.7%) and 13 root samples (59.1%). After 18 months, the pathogen could only be isolated from each six soil and root samples (27.3%) of *B. grandis*, *E. megacarpa* and *X. preissii*. The survival rate after 24 months of air-dry storage was only tested with 11 soil and root samples of *B. grandis* and two soil and root samples of *E. marginata* from sites 1 and 2. *Phytophthora cinnamomi* could no longer be isolated from any of the 13 soil and root samples (Table 2). Root isolations were generally more successful with oak leaf baiting (Fig. 2e) as compared to direct plating.

Table 1. Recovery of *Phytophthora cinnamomi* from soil samples, fine roots and organic debris sampled from 11 dieback sites, four dieback-free sites and two dieback spots at largely dieback-free sites in jarrah forest (sites 1–12), Kwongan heathland (site 13), Banksia woodland (site 14) and tingle forest (site 15) in Western Australia.

		No. of		No. of samples with recovery of *P. cinnamomi*		
Site no.	Source	Samples	Plants	Soil	Roots	Debris
Dieback sites						
1	*Banksia grandis*	8	8	5[1,2]	3[1]	4[1]
	Eucalyptus marginata	2	2	1	1	1
2	*B. grandis*	7	7	6[3]	4	5
	E. marginata	2	2	1	1	1
3	*B. grandis*	2	2	1	1	2
	E. marginata	1	1	1	1	1
	Macrozamia riedlei	1	1	1[3]	n.t.	n.t.
4	*E. marginata*	1	4	1	1	1
	Banksia sessilis	1	4	–	n.t.	n.t.
5	*B. grandis*	1	4	1	1	1
6	*B. grandis*	2	9	2	1	2
	E. marginata	1	4	1	–	1
	Persoonia longifolia	1	4	1	–	1
	Xanthorrea preissii	1	4	1	1	1
7	*C. calophylla*	1	4	1	–	1
	E. marginata	2	9	2	1	1
	E. megacarpa	1	4	1	1	1
	X. preissii	1	4	1	1	1
8	*B. grandis*	1	4	1	1	1
	C. calophylla	1	4	–	n.t.	n.t.
	E. marginata	2	4	2	1	1
9	*B. grandis*	1	4	–	n.t.	n.t.
	C. calophylla	1	4	–	n.t.	n.t.
	E. marginata	1	4	–	n.t.	n.t.
10	*B. grandis*	1	4	–	n.t.	n.t.
13	*Acacia blakeley*	1	1	1	1	1
	B. hookeriana	1	1	1	1	1
14	*B. attenuata*	2	8	2	2	2
15	*E. jacksonii*	2	2	2[4]	1	1
Total	13 species	50	130	37	25	32
Dieback-free sites						
9	*E. marginata*	1	4	–	–	–
10	*B. grandis*	1	4	–	–	–
	E. marginata	1	4	–	–	–
	P. longifolia	1	4	–	–	–
11	*B. grandis*	1	4	–	–	–
	E. marginata	1	4	–	–	–
	M. riedlei	1	4	–	–	–
12	*B. grandis*	1	4	–	–	–
	C. calophylla	1	4	–	–	–
	E. marginata	1	4	–	–	–
Total	5 species	10	40	0	0	0

n.t., not tested.
[1] *Phytophthora multivora* isolated from one sample.
[2] *Phytopythium* sp. isolated from one sample.
[3] *Globisporangium* sp. isolated from one sample.
[4] *Phytophthora cryptogea* isolated from one sample.

3.2 Microscopic examination of fine roots, small woody roots and root debris from dieback sites, dieback-free areas and young plants in artificially infested soil

All observations refer to fine roots, small woody roots and root debris pieces from which *P. cinnamomi* had been isolated by direct plating or by baiting (Figs. 2c,e) and additional fine long and short roots floating on flooded soil or cut from woody roots immersed in the flooding water of soil samples, which tested positive for *P. cinnamomi*.

After comparing the colour and anatomical structure of the examined roots or their mother roots with roots of the sampled tree species, the species identity of the examined roots from the field sites was usually unambiguous. As on almost all sites the ground flora underneath the sampled trees was relatively sparse and mainly consisted of sedges, ferns or Ericaceae, Fabaceae and *Acacia* species with a different root anatomy than *Banksia* spp., *Eucalyptus* spp., *C. calophylla*, *P. longifolia* and *X. preissii*, it can be assumed that most roots belonged to the sampled tree species. Many of the less decayed root debris pieces could also be identified by comparing their anatomy with known roots of the sampled species.

Table 2. Recovery of *Phytophthora cinnamomi* from soil samples and fine roots of *Banksia grandis*, *Corymbia calophylla*, *Eucalyptus marginata*, *Eucalyptus megacarpa* and *Xanthorrhoea preissii* from six dieback sites in the jarrah forest of Western Australia after 6- , 12- , 18- and 24-month storage under air-dry conditions at 20–35°C.

			No. of samples with recovery of *P. cinnamomi* from							
			Soil after months				Roots after months			
Site no.	Source	No. of samples	6	12	18	24	6	12	18	24
1	*Banksia grandis*	5	5	3	1	0	3	2	1	0
	Eucalyptus marginata	1	1	1	0	0	1	1	0	0
2	*B. grandis*	6	6	4	2	0	4	3	2	0
	E. marginata	1	1	0	0	0	1	0	0	0
3	*B. grandis*	1	1	1	1	n.t.	1	1	1	n.t.
	E. marginata	1	1	1	0	n.t.	1	0	0	n.t.
4	*E. marginata*	1	1	1	0	n.t.	1	1	0	n.t.
7	*Corymbia calophylla*	1	1	1	0	n.t.	1	1	0	n.t.
	E. marginata	2	2	1	0	n.t.	1	1	0	n.t.
	E. megacarpa	1	1	1	1	n.t.	1	1	1	n.t.
	Xanthorrhoea preissii	1	1	1	1	n.t.	1	1	1	n.t.
8	*B. grandis*	1	1	1	0	n.t.	1	1	0	n.t.
Total		22	22	16	6	0	17	13	6	0

n.t., not tested.

Five morphological structures that might play a role in survival of *P. cinnamomi* were identified in both naturally infected roots and roots of young plants in artificially infested soil (Table 3).

3.2.1 Irregular, inter- and intracellular hyphae

In 29 of the 32 samples (90.6%) from *A. blakelyi*, *B. attenuata*, *B. grandis*, *B. hookeriana*, *C. calophylla*, *E. jacksonii*, *E. marginata*, *E. megacarpa*, *P. longifolia* and *X. preissii*, inter- and intracellular hyphae, sometimes irregular and coralloid, could be observed in parenchyma cortex cells and sometimes also in vascular tissue of most examined fine roots and root pieces infected with *P. cinnamomi* (Table 3; Figs 4b,c and 5c). In 17 samples from *B. grandis*, *E. marginata*, *P. longifolia* and *X. preissii,* intracellular hyphae were also present in extremely thick-walled sclerenchyma cells (Fig. 4k). Root cells with intracellular hyphae were sometimes reddish- to dark-brown (Figs 3k and 4d) due to the accumulation of phenolic substances. Furthermore, in some fine roots of *B. grandis,* irregular non-septate hyphae could also be observed in root hairs (Fig. 5h).

Inter- and intracellular hyphae, sometimes irregular and coralloid, were regularly observed in thin-walled parenchyma cells (100% of samples; Fig. 5o–q) and partly also in thick-walled sclerenchyma cells (24%; Fig. 4k) of fine roots from young plants of *B. attenuata*, *B. chamaephyton*, *B. occidentalis*, *C. calophylla*, *E. pauciflora*, *E. marginata*, *H. eneabba*, *K. acuminata*, *L. multiflora* and *X. preissii* grown in artificially infested soil (Table 3).

Irregular, coralloid, inter- and intracellular hyphae were not found in roots and root debris from dieback-free areas (Table. 3).

3.2.2 Chlamydospores

In nine samples (28.1%) of *A. blakelyi, B. grandis*, *C. calophylla*, *E. megacarpa* and *X. preissii,* thin-walled globose to subglobose structures were infrequently observed in cortex cells of fine roots and debris pieces from which *P. cinnamomi* had been recovered (Table 3; Fig. 3a–c). The thin walls (diam. <1 μm) of these structures, the lack of a surrounding oogonial wall and the observation that they were sometimes formed in groups (Fig. 3a) or clusters suggest that they might be chlamydospores of *P. cinnamomi*. This is also supported by the absence of such thin-walled structures in the fine roots and debris from dieback-free areas. Diameters of these putative chlamydospores ranged from 13.2 to 26.5 μm.

Thin-walled and in one case also thicker-walled (1–1.5 μm diam; Fig. 3d) putative chlamydospores were also formed on the surface of *B. grandis* fine roots floating for 7 days in a flooded *P. cinnamomi*-infested soil sample from site 2. No chlamydospore-like structures could be observed in roots of *B. grandis* and *E. marginata* from which *P. cinnamomi* could be isolated after 6- , 12- and 18 (only *B. grandis*)-month storage under air-dry conditions, suggesting they might either not have been formed or have already been degraded in these roots.

In fine roots of young plants grown in artificially infested soil, thin-walled putative chlamydospores were only found in *C. calophylla* and *L. multiflora* (Table 3). No chlamydospore-like structures were present in fine roots and debris from dieback-free areas.

3.2.3 Oospores

In 22 samples (68.8%) from *A. blakelyi, B. grandis*, *B. hookeriana*, *C. calophylla*, *E. jacksonii*, *E. marginata*, *E. megacarpa*, *P. longifolia* and *X. preissii,* thick-walled globose to subglobose oospores surrounded by oogonia with thin walls and

Table 3. Phytophthora structures and histopathological reactions observed in fine roots and root debris from which *P. cinnamomi* had been isolated.

			No. of samples with						
						Irregular coralloid hyphae in			
Site no.	Plant species	No. of root/debris samples	Oospores	Chlamydo-spores	Stromata-like hyphal aggregations	Thick-walled sclerenchyma cells	Thin-walled cells	Papillae and short, unbranched lignitubers	Long, some times branched and complex lignitubers
1	*Banksia grandis*	3	1	1	1	3	3	2	1
	Eucalyptus marginata	1	1			1	1	1	
2	*B. grandis*	5	3	3[1]	3	3	5	5	5
	E. marginata	1	1		1		1	1	
3	*B. grandis*	1	1		1			1	1
	E. marginata	1	1			1	1	1	
4	*E. marginata*	1	1		1	1	1	1[2]	1
5	*B. grandis*	1	1		1		1	1	1[3]
6	*B. grandis*	2	1	1	1	1	2	2	2
	E. marginata	1	1		1	1	1	1	1
	Xanthorrhoea preissii	1	1	1	1	1	1	1	1
	Persoonia longifolia	1	1		1	1	1	1	1
7	*Corymbia calophylla*	1	1		1		1	1	1
	E. marginata	2	1		1	2	2	2	2
	Eucalyptus megacarpa	1	1	1	1		1	1	1
	X. preissii	1	1		1		1	1	1
8	*C. calophylla*	1		1	1		1	1	1[2]
	E. marginata	2	1		1	1	1	2[2]	2
15	*E. jacksonii*	1	1		1	1	1	1	1
13	*Acacia blakeley*	1	1	1	1		1	1	1
	B. hookeriana	1	1				1	1	1
14	*B. attenuata*	2			2		2	2	2
Total	10 plant species	32	22	9	22	17	30	31	27
Dieback-free sites									
9	*E. marginata*	1						(1)	
10	*B. grandis*	1						(1)	
	E. marginata	1						(1)	
	P. longifolia	1							
11	*B. grandis*	1						(1)	
	E. marginata	1							
12	*B. grandis*	1						(1)	
	C. calophylla	1						(1)	
	E. marginata	1						(1)	
Total	4 plant species	9						(7)	
Young plants grown in artificially infested soil									
	B. attenuata	2					2	2	2
	B. chamaephyton	2			2		2	2	2
	B. occidentalis	3	2				3	2	
	C. calophylla	2	1	2[4]	2		2	2	1
	Eremaea pauciflora	2	1				2	2	2
	E. marginata	3	2		3	1	3	3	3
	Hakea eneabba	3	1		2		3	3	2
	Kunzea acuminata	2			1	1	2	2	2
	Lambertia multiflora	4	4	1	4	4	4	4	4
	X. preissii	2					2	2	2
Total	10 plant species	25	11	3	14	6	25	24	20

Lignituber, sheath of cell wall material (mainly callose) apposited by a root cell around an invading intracellular hypha; papilla, cell wall apposition in the vicinity of intercellular hyphae.
[1]Chlamydospores formed on root surface after 14 days flooding.
[2]Lignitubers and papillae also found in sclerenchyma cells.
[3]Lignitubers and papillae also found in root hairs.
[4]Also some thick-walled putative chlamydospores.

sometimes tapering bases were observed in fine roots and debris pieces from which only *P. cinnamomi* had been recovered (Table 3; Fig. 3g–k). Especially in roots of *B. grandis* and *E. marginata,* oospores were formed regularly. In some *B. grandis* fine roots, oospore formation was abundant (Fig. 3g–i) but usually their distribution was scattered. Oospores were also

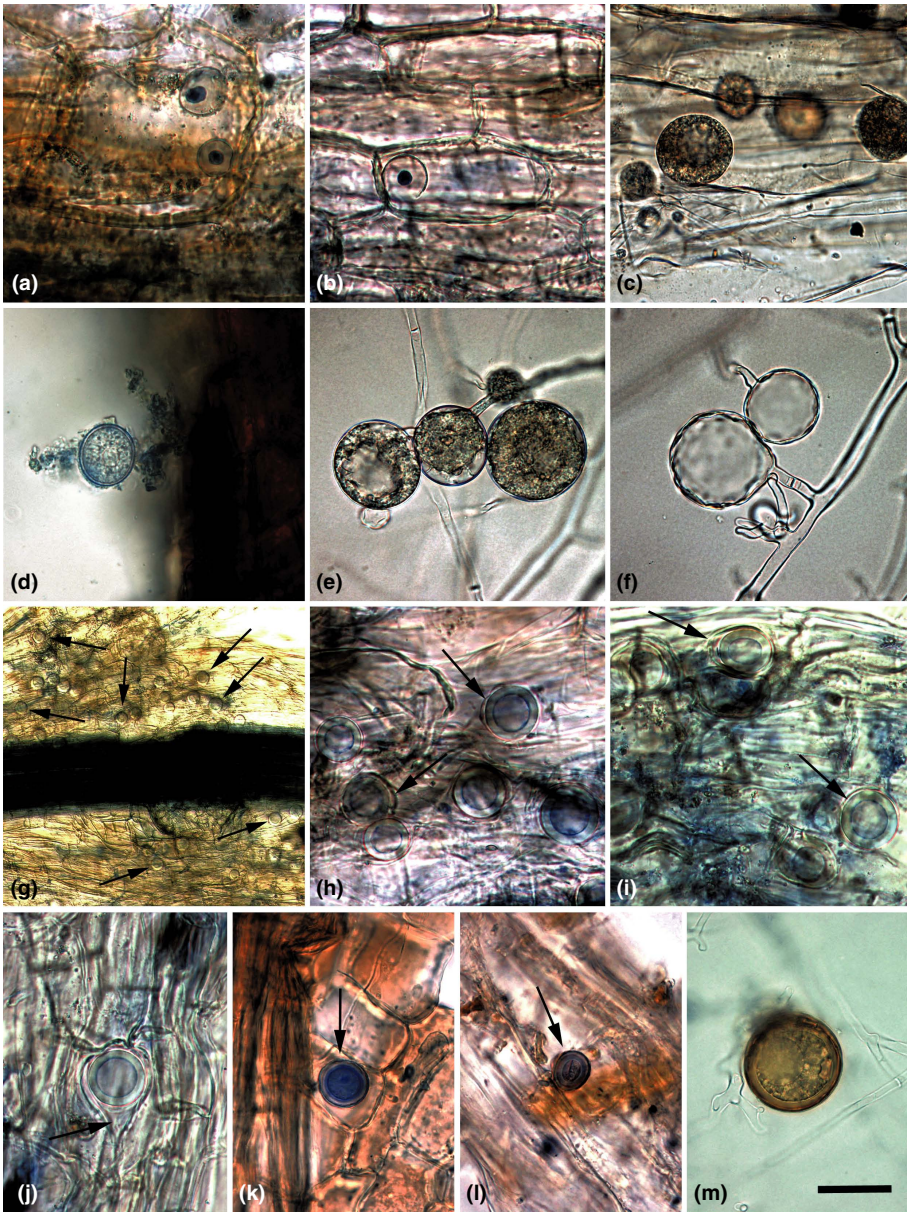

Fig. 3. Putative chlamydospores and oospores of *P. cinnamomi* in roots and in culture; (a–c) thin-walled putative chlamydospores in cortex cells of *P. cinnamomi*-infected *B. grandis* fine roots from jarrah forest dieback sites 1 and 6; (d) thick-walled putative chlamydospore formed on surface of a *P. cinnamomi*-infected *B. grandis* fine root after flooding soil for 1 week; (e) *P. cinnamomi* chlamydospores on V8 agar starting to abort after 2 weeks at 20°C; (f) aborted *P. cinnamomi* chlamydospores on V8 agar after 4 weeks at 20°C; (g–j) longitudinal sections through *P. cinnamomi*-infected fine roots of a recently killed *B. grandis* from jarrah forest dieback site 2; (g) abundant formation of thick-walled oospores (arrows) in the cortex around the vascular cylinder; (h,i) thick-walled oospores in parenchyma cortex cells, some still surrounded by thin oogonial wall (arrows); (j) oogonium with thick-walled oospore and tapering base (arrow) in parenchyma cortex cell; (k) oogonium with thick-walled oospore in root cortex cell of a dying mature *Eucalyptus marginata* from dieback site 7; (l) oogonium and thick-walled oospore formed by *P. cinnamomi* A2 in a fine root of *Lambertia multiflora* in a soil infestation test; (m) oogonium with thick-walled oospore formed by an A2 isolate of *P. cinnamomi* in paired culture with *Trichoderma viride*. All photographs except (c,e,f) and (m) taken after clearing, staining and destaining of samples. Scale bar = 25 μm for all pictures except of (g) where it is 100 μm.

present in roots of *B. grandis* and *E. marginata* from which *P. cinnamomi* had been isolated after 6- , 12- and 18-month storage under air-dry conditions. The oospores had diameters ranging from 13 to 30.9 μm and thick walls of 2.0 to >6.0 μm (Fig. 3h–k).

Oospores were also formed in fine roots of 11 samples (44%) from young plants of *B. occidentalis*, *C. calophylla*, *Eremaea pauciflora*, *E. marginata*, *H. eneabba* and *L. multiflora* grown in artificially infested soil (Table 3; Fig. 3l).

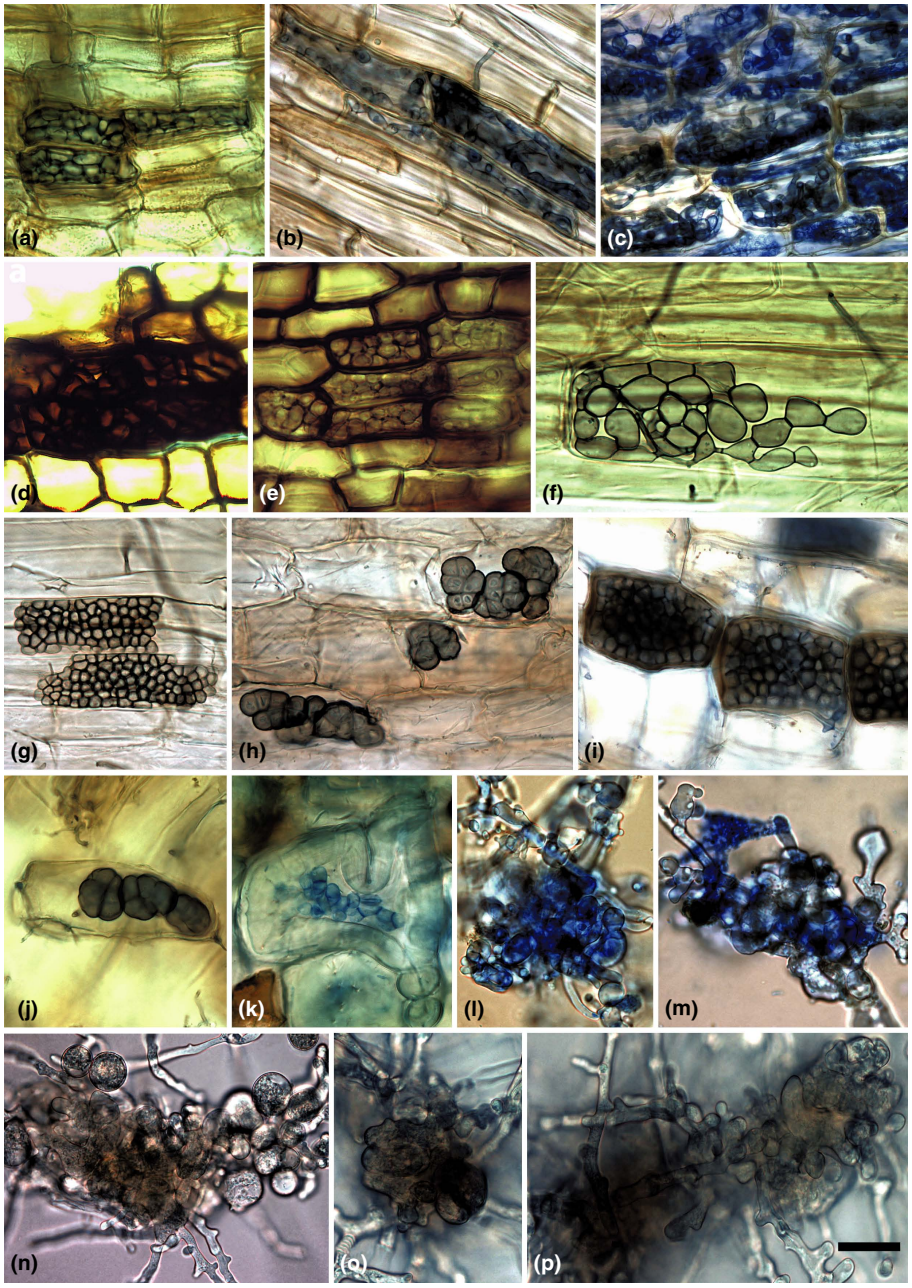

Fig. 4. Stromata-like hyphal aggregations formed by *Phytophthora cinnamomi* in culture and on the surface of naturally infected roots, and putative aggregations of *P. cinnamomi* in naturally and artificially infected roots; (a–c) hyphal aggregations in cortex cells of fine roots from a dying mature *Eucalyptus marginata* from dieback site 4; (d,e) hyphal aggregations in cortex cells of small woody roots from a recently killed *Xanthorrhoea preissii* (d) and from a dying mature *Corymbia calophylla* (e) from dieback site 7; (f–h) dense hyphal aggregations in parenchyma cortex cells of fine roots from dead *E. marginata* plants in a soil infestation test; (i–k) dense hyphal aggregations in parenchyma (i,j) and thick-walled sclerenchyma (k) cortex cells of fine roots from dead *Lambertia multiflora* plants in a soil infestation test; (l,m) dense hyphal aggregations formed by *P. cinnamomi* on the surface of naturally infected fine roots of a recently killed *Banksia grandis* tree from dieback site 3 after 1 week in water; (n–p) dense hyphal aggregations formed by *P. cinnamomi* A2 isolate DCE60 from Western Australia in 4-week-old V8A cultures. Scale bar = 25 μm for all pictures.

Sometimes oogonia and oospores were elongated (Fig. 3i) as though assuming the shape of a host cell. In contrast, no oospores could be observed in fine roots and root debris from dieback-free areas.

3.2.4 Stromata-like hyphal aggregations

In 26 samples (81.3%) from *A. blakelyi, B. grandis*, *B. hookeriana*, *C. calophylla*, *E. jacksonii*, *E. marginata*, *E. megacarpa*, *P. longifolia* and *X. preissii,* intracellular aggregations of hyphae and hyphal swellings were found in parenchyma cells of

Fig. 5. Papillae and lignitubers formed in fine roots and small woody roots infected by *Phytophthora cinnamomi*; arrows, split and filled arrow heads pointing at hyphae, papillae and lignitubers, respectively; (a–f) longitudinal sections through fine roots of recently killed *Banksia grandis* trees from jarrah forest dieback site 2; (a) abundant production of lignitubers in cortex cells around the vascular cylinder; (b) lignitubers and hemispherical papillae; (c) lignituber and intracellular hyphae; (d) lignitubers and thick flat papillae; (e) irregular coralloid lignitubers; (f) hypha growing out of a lignituber and forming a chlamydospore; (g) individual cortex cell after squashing of a *P. cinnamomi*-infected fine root from a recently killed *B. grandis* tree of site 2, filled with intact lignitubers; (h) root hair from a recently killed *B. grandis* tree with nonseptate hyphae, papillae and lignitubers; (i,j) lignitubers in cortex cells of *P. cinnamomi*-infected fine roots from a dying mature *Eucalyptus marginata* at dieback site 7; (k–n) longitudinal sections through *P. cinnamomi*-infected fine roots of a healthy mature *Eucalyptus megacarpa* tree from dieback site 7; many cortex cells are criss-crossed with coralloid, irregularly branched lignitubers; (o–r) structures observed in *P. cinnamomi*-infected fine roots of young plants growing in artificially infested soils; (o) root cortex cells of *Banksia attenuata* showing an intracellular hypha, which is contained in the adjacent cells by lignituber sheaths; (p) intracellular hypha that penetrated a papilla formed by a cortex cell of *E. marginata*; (q) intercellular hypha that is covered by lignituber sheaths after trying to penetrate into the lumen of a *E. marginata* cortex cell; (r) extensive papillae formation in cortex cells of *Xanthorrhoea preissii*. Scale bar = 25 μm for all pictures except of (a) and (k) where it is 40 μm.

fine roots, small woody roots and root debris from which *P. cinnamomi* had been isolated (Table 3). These aggregations often formed dense stromata-like structures and sometimes filled individual root cells completely (Fig. 4a–e). In most aggregations, short hyphal sections and swellings were delimited by septae. The dimensions of aggregations were limited

by the dimensions of the root cells and could reach 119 × 50 μm. In some roots, dense aggregations were also observed in thick-walled sclerenchyma cells. Hyphal aggregations were also present in roots of *B. grandis* and *E. marginata* from which *P. cinnamomi* could be isolated after 6- , 12- and 18-month air-dry storage (Fig. 4a–c). Hyphal aggregations were also formed on the surface of *B. grandis* fine roots after floating for 7 days in a flooded *P. cinnamomi*-infested soil sample from site 2 (Fig. 4l–m). These aggregations measured up to 84 × 50 μm.

Intracellular hyphal aggregations were also observed in parenchyma cells of fine roots and small woody roots of 14 samples (56%) from young plants of *B. attenuata*, *B. chamaephyton*, *B. occidentalis*, *C. calophylla*, *E. pauciflora*, *E. marginata*, *H. eneabba*, *K. acuminata*, *L. multiflora* and *X. preissii* grown in artificially infested soil (Table 3). In *E. marginata* and *L. multiflora,* root cells often contained particularly thick-walled dense aggregations, which sometimes filled the cells completely (Fig. 4f–k). In some roots, dense aggregations were also observed in thick-walled sclerenchyma cells (Fig. 4k). In contrast, no aggregations were found in root and debris samples from non-infested dieback-free areas.

3.2.5 Papillae and lignitubers

Papillae and short unbranched lignitubers were frequently found in parenchyma cortex cells of fine roots, small woody roots and root debris pieces of 30 samples (93.8%) from *A. blakelyi, B. grandis*, *B. hookeriana*, *C. calophylla*, *E. jacksonii*, *E. marginata*, *E. megacarpa*, *P. longifolia* and *X. preissii* from which *P. cinnamomi* had been isolated (Table 3). Papillae were either formed as hemispherical pads or as elongated layers along the root cell walls (Fig. 5b,d,h). Hyphae that had penetrated root cell walls and grew intracellularly were frequently encased by appositions of cell wall material forming lignitubers (Fig. 5a–n). The hyphae inside the lignitubers were often visible as faint lines (from the side) or as central dots (in cross sections). The callose depositions followed the branching patterns of *Phytophthora* hyphae in the root cells, and often the lignitubers clearly reflected the typical coralloid hyphae and vesicles of *P. cinnamomi* (Fig. 5e,k–n). In some fine roots of the susceptible host species *B. grandis*, *E. marginata* and *X. preissii* but also of the resistant species *E. megacarpa,* most cortex cells contained lignitubers (Fig. 5a,k), and many cells were crisscrossed by a 3-dimensional system of tube-like (Fig. 5a,g) or coralloid irregular lignitubers (Fig. 5k–n). Such long, often branched and complex lignitubers were present in fine roots and in root debris pieces of 26 samples from dieback sites (81.3%; Table 3). Papillae and lignitubers often had a brownish or yellowish colour, which was most likely due to the incorporation of phenolic and/or lignin compounds. The affected cortex cells were also often reddish-brown to dark-brown due to the accumulation of polyphenols. In some cases, lignitubers were also formed in thick-walled sclerenchyma cells. Lignitubers together with irregular non-septate hyphae could also be observed in root hairs of some *B. grandis* fine roots (Fig. 5h). Papillae and lignitubers were also present in roots of *B. grandis* and *E. marginata* from which *P. cinnamomi* had been isolated after 6- , 12- and 18-month air-dry storage. In one fine root of *B. grandis* from site 2 floating in flooded soil for 1 week, a lignituber was observed germinating and forming a chlamydospore (Fig. 5f). When fine roots were squeezed between a glass slide and cover slide, individual cortex cells filled with lignitubers and even individual intact lignitubers were observed floating in lactoglycerol (Fig. 5g).

Papillae and short unbranched lignitubers were also found in 24 of the 25 samples (96%) from 10 species grown in artificially infested soil, that is, *B. attenuata*, *B. chamaephyton*, *B. occidentalis*, *C. calophylla*, *E. pauciflora*, *E. marginata*, *H. eneabba*, *K. acuminata*, *L. multiflora* and *X. preissii* (Table 3; Fig. 5o–r). In *X. preissii,* the thickness of the wall appositions could reach 5.7 μm (Fig. 5r). Long sometimes branched lignitubers were observed in roots of 20 samples (80%) from nine species (Table 3).

In fine roots and debris from dieback-free areas, thin papillae and short lignitubers were only rarely found, while long complex lignitubers were completely absent (Table 3).

3.3 Microscopic examination of *P. cinnamomi* cultures

In cultures of all 10 A2 isolates of *P. cinnamomi* from WA abortion of thin-walled chlamydospores started after 2 weeks (Fig. 3e). In 4- to 6-week-old cultures, most chlamydospores were aborted (Fig. 3f). Thick-walled chlamydospores were not formed by any isolate. No oogonia were observed in single cultures of any isolate after 4 weeks but were formed by three of the tested five A2 isolates in paired cultures with a strain of *T. viride* (Fig. 3m). Oospore wall diameters ranged from 2.1 to 4.0 μm. Interestingly, all isolates produced dense stromata-like hyphal aggregations (50–155 μm diameter) resulting from multiple lateral branching of short sections of main hyphae or at the end of short lateral hyphae, and subsequent twisting and dense intermingling of the irregular coralloid lateral hyphae, which often contained hyphal swellings, too (Fig. 4n–p). In 4- to 6-week-old cultures, most aggregations were still viable.

4 Discussion

Having its centre of origin in Hainan, south China, Taiwan or New Guinea (Crandall and Gravatt 1967; Ko et al. 1978; Zentmyer 1980; Arentz and Simpson 1986; Chang 1993; Zeng et al. 2009), *P. cinnamomi* evolved in a humid tropical climate. This is reflected by its phenotype comprising a heterothallic outcrossing breeding system and a wide host range, ensuring adaptability and survival in ecosystems with high species diversity. The relatively low oospore wall index and the formation of thin-walled chlamydospores are also adaptations to a humid climate without prolonged drought. Nevertheless, following its global clonal spread by man, the A2 mating type of *P. cinnamomi* was able to establish, become invasive and cause devastating epidemics of forests, semi-natural ecosystems and plantations particularly in Mediterranean climatic zones with prolonged severe summer droughts. Despite its importance as one of the most notorious and aggressive plant pathogens

with a known host range of *c.* 5000 plant species (Zentmyer 1980; Shearer and Dillon 1995, 1996; Erwin and Ribeiro 1996; Shearer et al. 2004; Cahill et al. 2008), knowledge of the survival strategy of the A2 mating type of *P. cinnamomi* in dry climates in the absence of the A1 mating type has previously been based largely on assumptions rather than evidence.

Shea (1979) demonstrated that *P. cinnamomi* is able to survive for at least 1 year in infected collars of dead *B. grandis* trees and suggested that infected major roots and collars of *B. grandis* might be the long-term reservoir of the pathogen (Shea et al. 1982). In the study of Collins et al. (2011), re-isolations of *P. cinnamomi* A2 from woody roots and collars of artificially inoculated mature *B. grandis* trees at a jarrah forest site declined over time from 60% at 3 months to 0.1% at 34 months after death with a sharp decrease after 10–12 months when temperatures increased in late spring to early summer. There was no recovery from above-ground collar tissue at 18 months after death. In the present study, *P. cinnamomi* could be isolated from dry collars of several recently dead (<6 months) *B. grandis* and *B. hookeriana* trees but not from collars of *B. grandis* trees that were dead for over 1 year. In contrast, *P. cinnamomi* was readily recovered from rhizosphere soil samples and from surface disinfested fine roots after 18 months storage in air-dry conditions, demonstrating the existence of a soilborne reservoir for long-term survival of the pathogen. In the following, the potential role of different morphological structures for long-term survival of *P. cinnamomi* will be discussed.

4.1 The role of chlamydospores for the survival of *P. cinnamomi*

Based on results from soil sieving and plating experiments, Weste and Vithanage (1978, 1979) and Weste (1983) considered chlamydospores as the major survival structure and inoculum source of the pathogen in Australia. This might be true for forests in Victoria where rainfall and soil matric potentials in summer are higher than in the south-west of WA, although the origin of the *P. cinnamomi* colonies in the Victorian experiments has never been examined. However, cumulative evidence casts doubt on a possible role of chlamydospores for long-term survival of *P. cinnamomi* in natural ecosystems in WA.

First, the longest recorded survival period of chlamydospores of *P. cinnamomi* in roots buried in different non-sterile soils was 8 months (Mackay et al. 1985). However, after 200 days when matric potential had dropped to less than −10 bar, *P. cinnamomi* could no longer be recovered. In the experiments of Weste and Vithanage (1979), some chlamydospores on nylon mesh discs buried in non-sterile soil survived up to 10 months at soil water potentials of −3000 kPa. However, no chlamydospores were viable after 12 months. Similarly, Mircetich and Zentmyer (1967) reported that *P. cinnamomi* could not be recovered from infested soil or infected avocado roots after 6 weeks and 2 months, respectively, when moisture has dropped from 21.6 to 2%, although numerous chlamydospores were present. In addition, no disease developed when the soils were re-moistened and planted with avocado seedlings. Mircetich and Zentmyer (1967) concluded that chlamydospores and oospores are not capable of remaining viable under conditions of extreme drought. Unfortunately, they did not consider the possibility of dormancy, which is proven for oospores of many *Phytophthora* spp. (Erwin and Ribeiro 1996). In the jarrah forest of WA, soils become extremely dry during the long hot summers and soil water potentials may drop below −6000 kPa (=−60 bar; Shea 1975; Weste and Vithanage 1979). In the Northern sandplains of WA, soil water potential and soil moisture content may drop below −7000 kPa (=−70 bar) and 1%, respectively (Lamont and Bergl 1991; Enright and Lamont 1992). Therefore, from the experimental results of Mircetich and Zentmyer (1967), Weste and Vithanage (1979) and Mackay et al. (1985), it seems unlikely that chlamydospores either in soil or in infected root tissue are capable of surviving the long period of extremely dry soil conditions in natural ecosystems in WA. Furthermore, *P. cinnamomi* was shown to survive in soil in the absence of a living host for 6 years (Zentmyer and Mircetich 1966). This is more than 5 years longer than any demonstrated survival period of chlamydospores even under optimum conditions. Also the survival of *P. cinnamomi* in air-dry fine roots and small woody roots of *B. grandis* and *E. marginata* for 18 months in the present study clearly exceeded the known survival time of chlamydospores under dry conditions. In oak forests in Extremadura, south-west Spain, *P. cinnamomi* survived several years of severe drought and could readily be recovered from soils after rewetting by a rainfall event (Brasier et al. 1993). Another important source of evidence against a major role of chlamydospores in long-term survival of *P. cinnamomi* comes from studies on the origin of *P. cinnamomi* cultures in the soil and root plating experiments by Shea et al. (1980) and the present study. Shea et al. (1980) investigated population levels of *P. cinnamomi* in soil of diseased jarrah forest on freely drained sites over a 14-month period using direct plating and soil baiting techniques. One major objective of their study was to clarify the survival strategy of *P. cinnamomi* in the jarrah forest. Before the present work, this was the only study where the origin of colonies developing from plated soil suspensions had been determined. In samples from non-irrigated dry sample plots, *c.* 40–70% of the colonies originated from small pieces of organic matter. In some cases, sporangia could be observed on the cultures. The origin of the remaining colonies was unclear. Also in the present study, all *P. cinnamomi* colonies originated from fine root fragments and pieces of root debris. In both studies, no free chlamydospores were present on the culture media. Shea et al. (1980) could not find chlamydospores in microscopic examinations of roots and organic matter particles, from which *P. cinnamomi* colonies had grown. Likewise, in the present study, thin-walled globose to sub-globose structures that might be putative chlamydospores were only occasionally observed during extensive microscopic examinations of fine roots, small woody roots and root debris pieces from which *P. cinnamomi* had been recovered by direct plating or baiting. The same was true for fine roots and feeder roots floating on flooded soil or cut from woody roots immersed in the flooding water of samples, which tested positive for *P. cinnamomi*. In addition, no evidence for the production of thick-walled chlamydospores in infected roots could be found in either study.

In accordance with the results of Shea et al. (1980) and the experimental results on chlamydospore survival in dry soils (Mircetich and Zentmyer 1967; Weste and Vithanage 1979; Mackay et al. 1985), the present study indicated that

chlamydospores are not important for long-term survival of *P. cinnamomi* under the extreme conditions on freely drained sites in natural ecosystems in WA. This conclusion is also supported by the high abortion rates of chlamydospores observed in 6-week-old cultures of multiple *P. cinnamomi* isolates examined in this study. The production of thin-walled chlamydospores on the surface of *B. grandis* roots in flooded soil during a baiting test of this work indicated that their ecological role in the life cycle of *P. cinnamomi* might be the short-term survival between consecutive rain events during the more or less humid autumn and spring periods of WA. As chlamydospores experience no dormancy they can immediately germinate by forming sporangia after re-moistening of soil. The formation of thin-walled chlamydospores by most aquatic and all soilborne *Phytophthora* spp. thriving under moist conditions further accentuates their role as short-term survival structures under humid rather than extremely dry conditions (Erwin and Ribeiro 1996; Jung et al. 2011). In contrast, in aerial *Phytophthora* species including *P. palmivora*, *P. ramorum* and *P. tropicalis,* chlamydospores are predominantly thick walled (Erwin and Ribeiro 1996; Aragaki and Uchida 2001; Werres et al. 2001) most likely as an adaption to the dry conditions in necrotic leaves. In the present study, putative chlamydospores of *P. cinnamomi* with slightly thicker than normal wall diameter could only be observed in one case on the surface of *B. grandis* roots after 1 week of flooding, suggesting that they are not a regular part of the life history of this pathogen.

With a diameter range of 13.2–26.5 μm, the putative chlamydospores found in fine roots during this study were on average *c.* 50% smaller than chlamydospores formed in culture [10–56 (70) μm, Gerretson-Cornell 1983] and *c.* 50% larger than chlamydospores formed by *P. cinnamomi* in lupin roots (5.2–14.4 μm; Cother and Griffin 1973). Cother and Griffin (1973) suggested the inimical and low nutritional environment in roots being responsible for the markedly smaller sizes of chlamydospores in roots as compared to culture media.

4.2 The role of selfed oospores for the survival of *P. cinnamomi*

Due to the heterothallic breeding behaviour of *P. cinnamomi* and the rare occurrence of the A1 mating type, it has always been assumed that oospores cannot be an integral part of the life cycle of *P. cinnamomi* in Australia in general. This view was adopted for WA, and in particular the jarrah forest, and supported by Dobrowolski et al. (2003) who, using microsatellite markers, found no genetic evidence for crossings between A1 and A2 isolates of *P. cinnamomi* in Banksia woodlands where both mating types co-occur in the same rhizosphere. Similarly, in a molecular RAPD analysis of A1 and A2 isolates from a small area in Taiwan and in two extensive isozyme studies of A1 and A2 isolates from South Africa and Australia, respectively, no genetic evidence for sexual recombination in *P. cinnamomi* was found (Old et al. 1984b; Chang et al. 1996; Linde et al. 1997). However, in the present study in samples from *A. blakelyi, B. grandis*, *B. hookeriana*, *C. calophylla*, *E. jacksonii*, *E. marginata*, *E. megacarpa*, *P. longifolia* and *X. preissii*, thick-walled typical oospores, often still surrounded by the oogonial wall, were found in fine roots, small woody roots and root debris from which *P. cinnamomi* had been exclusively isolated. Their size ranged from 13 to 30.9 μm, which is on average *c.* 40% smaller compared to oospores formed in culture (19–54 μm in Erwin and Ribeiro 1996; 24–45 μm in Gerretson-Cornell 1983). Oogonia and oospores were sometimes elongated as though assuming the shape of a host cell, a feature also known from *P. quercina* and *P. plurivora* in fine roots of oaks, beech and other tree species in Europe (Jung 1998; Jung et al. 1999). In the experiments of Jayasekera et al. (2007), some of the selfed oospores and oogonia produced by A2 isolates of *P. cinnamomi* in lupin roots in the presence of *A. pulchella* also had an elongated ellipsoid shape.

This is the first report of oospores in naturally infected fine roots from woody plants in the jarrah forest and other natural ecosystems in WA and it is beyond reasonable doubt that these oospores were from *P. cinnamomi*. This conclusion is supported by the recent finding of oospores in naturally infected roots of annual and perennial herbaceous plants which, after germination on V8 agar, produced cultures of *P. cinnamomi* A2 mating type as confirmed by sequencing the ITS region (M. Crone and G.E.St.J. Hardy, unpublished results). Due to the scattered rare distribution of the A1 mating type of *P. cinnamomi* in WA and the apparent incompatibility of both mating types in nature, the oogonia and oospores observed here must have been formed by selfing. This view is supported by the observation of oospores in *P. cinnamomi*-infected roots of young plants of *B. occidentalis*, *C. calophylla*, *Eremaea pauciflora*, *E. marginata*, *H. eneabba* and *L. multiflora* grown in artificially infested soil in this study. Mircetich and Zentmyer (1966, 1967) also found oospores of *P. cinnamomi* in fine roots of mature declining avocado trees in orchards where only the A2 mating type of *P. cinnamomi* was present, and in fine roots of avocado seedlings artificially infected by A2 isolates of *P. cinnamomi*.

There are convincing demonstrations that the A2 mating type of *P. cinnamomi* is able to self under different conditions of stress. Brasier (1971, 1978), Zentmyer (1980) and the present study showed that the presence of the fungi *Trichoderma harzianum*, *T. koningii* and *T. viride*, respectively, all strong antagonists of *P. cinnamomi* can induce selfing in the A2 mating type of *P. cinnamomi* in culture. In the experiments of Reeves (1975), the A2 mating type of *P. cinnamomi* was able to produce oospores in infected fine roots of *Castanea sativa* only if *T. viride* was present. It is likely that a variety of other antagonistic fungi could have a similar effect. A stimulation of selfing in A2 isolates of *P. cinnamomi* was also demonstrated for root exudates from avocado, a highly susceptible host of *P. cinnamomi* (Ho and Zentmyer 1977; Zentmyer 1979) and from *Acacia pulchella*, a highly resistant tree species, which is widespread in the jarrah forest (Jayasekera et al. 2007). In the latter study, selfed oospores were also abundantly formed in infected *Lupinus angustifolius* roots buried in natural soil taken from under *A. pulchella* trees in the jarrah forest and in rhizosphere soil of potted *A. pulchella* plants. Also mechanical damage to the mycelium using a scalpel or the addition of H_2O_2 or diethyl ether can induce selfing (Reeves and Jackson 1974). McCarren (2006) reported regular selfing of four A2 isolates of *P. cinnamomi* grown on Ribeiro's minimal medium in the absence of any stress factors. Using the tetrazolium viable staining test, viability of at least 27% of the oospores produced by selfing was demonstrated. Some oospores germinated during the experimental period but most remained dormant. In

Kenya, homothallic strains of *P. cinnamomi* were isolated from soil, roots and stem cankers of *Macadamia tetraphylla,* which showed faster growth in culture and higher virulence than co-occurring A1 and A2 isolates (Mbaka et al. 2010).

In conclusion, despite having a heterothallic breeding system, the A2 mating type appears to be functionally homothallic (Zentmyer 1979, 1980). Selfing has also been shown for other heterothallic *Phytophthora* species including *P. cambivora*, *P. nicotianae* and *P. palmivora* (Brasier and Sansome 1975; Erwin and Ribeiro 1996; T. Jung, unpublished results). Recently, Judelson et al. (2010) demonstrated secondary homothallism caused by an RNA symbiont in both A1 and A2 isolates of *P. infestans*.

Due to the thickness and chemical composition of their walls, oospores are highly resistant to damage by microbial decomposition, drought and fungicides and enable *Phytophthora* species to survive in a dormant state for many years (Erwin and Ribeiro 1996). The ability of isolates of the A2 mating type to produce oospores by selfing, therefore, provides an efficient mechanism for long-term survival of *P. cinnamomi* (Zentmyer 1980). In the present study, the oospores formed by selfing in the fine and small woody roots had much thicker walls than oospores produced in mating tests of A1 and A2 isolates in culture (Erwin and Ribeiro 1996). Interestingly, the majority of selfed oospores produced in single cultures in the experiments of McCarren (2006) and in paired cultures with *T. viride* in the present study also had thick walls. This striking difference between selfed oospores and oospores derived from outcrossing might reflect their different major functions as long-term survival structures (selfed oospores) and source of new genetic variation (oospores from matings), respectively.

4.3 The role of hyphae for the survival of *P. cinnamomi*

Shea et al. (1980) stated that in soil, '*P. cinnamomi* occurs primarily as mycelium in small pieces of organic matter which are relatively shortlived'. The present study also demonstrated the presence of irregular coralloid hyphae typical of *P. cinnamomi* in parenchyma cortex cells and less frequently also in vascular tissue of fine roots, small woody roots and root debris pieces from 10 woody plant species on all sampled dieback sites and from young plants of 11 woody plant species grown in artificially infested soil. The results confirm Shea and Dell (1981) who showed that *P. cinnamomi* infects both the short lateral fine roots of *E. marginata* and the long fine roots and small woody roots that form the perennial framework from which the next flush of short lateral fine roots would originate.

In some fine roots of *B. grandis,* irregular coralloid hyphae could also be observed in root hairs. Infection of root hairs by *P. cinnamomi* was previously shown in artificially inoculated *E. marginata* seedlings (Malajczuk et al. 1977).

In several species, including the widespread *B. grandis*, *E. marginata* and *X. preissii*, hyphae had also colonized thick-walled sclerenchyma cells, and it is likely that in these cells, the buffering effect against dry soil conditions will be even more pronounced than in thin-walled parenchyma cells. Due to their sensitivity to dry conditions, hyphae may only play a role in long-term survival of *P. cinnamomi* in the form of dense stromata-like aggregations, inside thick-walled sclerenchyma cells or covered by lignituber sheaths.

4.4 The role of stromata-like hyphal aggregations for the survival of *P. cinnamomi*

In the excellent compilation of Blackwell (1949) on terminology in *Phytophthora,* hyphal aggregations were not mentioned and it was only recently that evidence for the importance of such structures for *Phytophthora* survival began to accumulate. Hyphal aggregations are regularly formed by many aquatic sterile *Phytophthora* species and their role as survival structures for dry periods in ephemeral streams has recently been proposed (Jung et al. 2011; T. Jung, unpublished results). Brasier et al. (2010) also reported the formation of stromata in agar cultures of *P. lateralis* isolates from Taiwan. Interestingly, *P. gregata*, a homothallic species from *Phytophthora* ITS Clade 6 with a largely corrupted breeding system, also regularly produces dense and large hyphal aggregations (Jung et al. 2011). The consistent finding of dense intermingled hyphal aggregations in cortex cells of *P. cinnamomi*-infected fine roots and small woody roots from nine woody plant species on all sampled dieback sites, and from young plants of 11 woody plant species grown in artificially infested soil, and their apparent absence in roots from dieback-free areas strongly suggests a common identity of these structures with the hyphal aggregations formed by all examined isolates of *P. cinnamomi* in agar cultures. In roots of the commonly occurring *B. grandis*, *E. marginata* and *L. multiflora,* particularly dense and often quite large hyphal aggregations were observed that sometimes filled root cells entirely. In most aggregations, short hyphal sections and swellings were delimited by septae, a phenomenon that likely provides additional protection against desiccation and has previously been reported by Gerretson-Cornell (1983) from *P. cinnamomi* cultures. The hyphal aggregations resembled stromata formed by *P. ramorum*, a species with a partial incompatibility between isolates of the A1 and A2 mating types (Brasier and Kirk 2004; Boutet et al. 2009), beneath the cuticle of infected leaves (Moralejo et al. 2006), and it is likely that they play an integral part in the life cycle of *P. cinnamomi* as a long-term survival structure.

4.5 Possible role of papillae and lignitubers for the survival of *P. cinnamomi*

Papilla formation on host cell walls and lignituber formation around invading intracellular hyphae have repeatedly been reported as a non-specific defence response to hyphal invasion (De Bary 1863; Aist 1976; Heath 1980; Cahill and Weste 1983; Cahill et al. 1989; Blaschke 1994a,b; Jung et al. 1996; Jung 1998). Papillae are callose deposits formed between the inner cell wall and the plasmalemma adjacent to an invading hypha in the intercellular space to prevent penetration of the cell wall (Aist 1976; Heath 1980; Cahill and Weste 1983). They can either be hemispherical pads, which are the most

common shape or elongated layers along the cell wall (Heath 1980). After a hypha has penetrated the host cell wall, it may remain enveloped by a host-derived membrane, which appears as an invagination from the plasma membrane, and callose sheaths may be apposited onto this wall to encapsulate the invading hypha within the host cell (Aist 1976; Heath 1980; Lipka et al. 2005; Lu et al. 2012). These callose sheaths are called lignitubers (Manners 1993; Blaschke 1994a,b; Jung et al. 1996; Jung 1998). The callose appositions may continue until the intracellular structure is completely encased (Heath 1980). Such a lignituber formed around haustoria or hyphae of *Phytophthora* spp. has been described as a wall apposition, which is continuous with the host cell wall at the penetration site and resembles a papilla, which has not been penetrated (Ehrlich and Ehrlich 1966; Bracker and Littlefield 1973). Some authors do not distinguish between papillae and lignitubers and generally use the terms callose deposits and papillae interchangeably (Cahill and Weste 1983; Cahill et al. 1989). Callose layers are almost impermeable and, due to the incorporation of suberin, lignin and polyphenols, highly resistant against enzymatic maceration (Aist 1976; Heath 1980). There have been various studies on histopathological reactions of artificially infected fine roots of *E. marginata* and other plants from the jarrah forest to infection by *P. cinnamomi* (Malajczuk et al. 1977; Cahill and Weste 1983; Cahill et al. 1989). However, this is the first study of histopathological reactions of naturally infected fine roots from dieback sites in the jarrah forest and other natural ecosystems in WA. For the first time, regular production of papillae and lignituber sheaths around intracellular hyphae and vesicles of *P. cinnamomi* could be demonstrated in cortex cells of fine and small woody roots of 10 common plant species from dieback sites in different ecosystems of WA. In some fine roots, most cortex cells contained lignitubers, and many cells were filled with a 3-dimensional system of tube-like or coralloid irregular lignitubers. These findings are similar to results from studies on European oak decline (Blaschke 1994a,b; Jung et al. 1996; Jung 1998). Papillae and lignitubers were also commonly found as a response of oak fine roots to infection by a range of *Phytophthora* species, including *P. plurivora*, *P. quercina* and *P. gonapodyides* (Blaschke 1994a,b; Jung et al. 1996; Jung 1998). The formation of papillae and lignitubers in root cells as a response to infection by *P. cinnamomi* was experimentally demonstrated by several Australian research groups. Cahill and Weste (1983) inoculated fine roots of seedlings from 13 plant species with *P. cinnamomi* and found an obvious association between the formation of papillae and host resistance. Callose deposits were produced by seven tolerant or resistant species including *E. calophylla*, *Acacia melanoxylon*, *A. pulchella*, *Gahnia radulata*, *Juncus bufonius*, *Zea mays* and *Triticum aestivum*. Callose pads or plugs along the inner cell wall adjacent to intercellular hyphae of *P. cinnamomi* were formed by all seven host species; callose collars or sheaths around intracellular hyphae or haustoria were only observed in the resistant hypersensitive species *A. pulchella* and in *Z. mays*. In contrast, papillae were not formed by any of the susceptible species including *E. sieberi*, *E. marginata*, *Xanthorrhoea australis*, *Xanthorrhoea resinosa* and *Themeda australis*. However, in a later study, Cahill et al. (1989) demonstrated that papillae can also be formed by susceptible species if conditions temporarily restrict the growth of *P. cinnamomi*. Papillae were frequently produced as a response to *P. cinnamomi* infection in the epidermis and cortex, rarely in the vascular parenchyma, of *E. marginata* roots at 14°C but not at 24°C. In the present study, the formation of papillae and lignituber sheaths around invading hyphae could also be demonstrated for seedlings of *C. calophylla* and the nine susceptible species *B. attenuata*, *B. chamaephyton*, *B. occidentalis*, *E. pauciflora*, *E. marginata*, *H. eneabba*, *K. acuminata*, *L. multiflora* and *X. preissii* grown at 20°C in artificially infested soil. Similar results were reported from soil infestation trials with *Quercus robur* seedlings artificially infected by *P. cambivora*, *P. plurivora* and *P. quercina* (Jung et al. 1996; Jung 1998).

In many plant–fungal endophyte associations, papilla formation and encasement of intracellular hyphae are an integral part of the fine-tuned balance between host defence and fungal invasive ability to achieve asymptomatic or mutualistic symbioses rather than parasitism (Suske and Acker 1990; Currah et al. 1993; Yu et al. 2001; Schulz and Boyle 2005; Tsuneda et al. 2009; Tellenbach 2011). In the present study, many of the affected cortex cells examined in living fine roots of the susceptible hosts *B. grandis*, *E. marginata* and *X. preissii* and in particular of the resistant species *E. megacarpa* and *C. calophylla* were apparently still alive even though they were partly filled with a 3-dimensional tube-like system of lignitubers. It appears that in resistant plants and in susceptible host plants under unfavourable conditions, such as temperatures below 15°C (Cahill et al. 1989), *P. cinnamomi* can show behaviour similar to a root endophyte. The encapsulating of hyphae and vesicles by lignitubers might provide advantages for the fine root, because the infection is halted and the damage is restricted, and for the pathogen, because it is buffered against extreme summer droughts and protected against microbial decomposition; provided that the encapsuled hyphae and vesicles remain viable, which is likely as the callose material is not deposited directly onto the hyphal wall but is covered to both sides by a membrane continuous with the plant cell plasmalemma (Heath 1980; Lu et al. 2012).

In biotrophic and hemibiotrophic fungal and oomycete pathogens, for example *Colletotrichum* spp., *P. infestans*, *Albugo* spp., *Bremia* spp. and *Peronospora* spp., haustoria, specialized intracellular hyphae for the absorption of nutrients from the host cytoplasm are formed after penetration of the host cell wall. During growth in the host cell, haustoria become enveloped by the host-derived extrahaustorial membrane (EHM), which constitutes a highly active interface between the haustorium and the host cell across which the haustorium absorbs nutrients from and releases effector proteins into the host cell (Donofrio and Delaney 2001; Micali et al. 2011; Lu et al. 2012; Underwood 2012). In incompatible interactions or in later stages of compatible interactions, the EHM becomes encased with callose appositions to constrain the haustoria and decrease the efficiency of nutrient transfer from the host cell (Allen and Friend 1983; Donofrio and Delaney 2001; Lu et al. 2012). In incompatible potato–*P. infestans* reactions, cessation of hyphal growth is most likely caused by starvation as a result of the encasement of haustoria (Allen and Friend 1983). However, the viability of the encased haustoria was not examined in their study. In the wheat–*Puccinia striiformis* f. sp. *tritici* pathosystem, haustorium encasement leads to malformation and sometimes death of haustoria (Zhang et al. 2012). In contrast, in the *Pinus silvestris–Melampsora pinitorqua* pathosystem, a high frequency of encased haustoria contained intact cell structures (Walles 1974). Also in immune

cowpea cultivars infected by the rust fungus, *Uromyces phaseoli* var. *vignae* encasement of haustoria did not result in their immediate death (Heath 1971). Considering the resemblance between encased haustoria and the lignitubers observed in naturally and artificially infected roots in the present study, it seems possible that *P. cinnamomi* hyphae and vesicles are able to survive inside the lignitubers. This conclusion is also supported by the observation of a germinating lignituber forming a chlamydospore in a *B. grandis* root after 1-week incubation in flooded soil.

Unfortunately, neither in the microscopic studies of the defence responses triggered by *P. cinnamomi* in roots of various susceptible and resistant Australian plant species (Cahill and Weste 1983; Cahill et al. 1989), in the microscopic studies of naturally and artificially infected roots in the present study nor in the studies on European oak decline (Jung et al. 1996; Jung 1998) has the viability of the encapsuled hyphae been investigated. Therefore, experiments are required to clarify whether the hyphae and vesicles inside the lignitubers are viable (using tetrazolium staining) and whether they may eventually be able to restart growth, penetrate through the callose sheath and form sporangia and chlamydospores on the surface of roots when conditions are favourable (i.e. high moisture, temperature >12°C). In this case, lignitubers, first in living fine roots surrounded by liquid cytoplasm, and subsequently, following death and decomposition of the fine roots in root debris and finally in the soil, would function as resting structures for long-term survival of *P. cinnamomi*.

Oospores, thin-walled chlamydospores, hyphal aggregations and hyphae inside lignitubers were found in roots of both susceptible and field-resistant species and it appears that the latter also play an important role as a reservoir for survival propagules of *P. cinnamomi*. This suggestion is supported by the findings of *P. cinnamomi* survival in contained root necroses of the field-resistant species *C. calophylla*, *G. radulata*, *Lepidosperma laterale* and *Poa sieberana* (Grant and Byrt 1984; Phillips and Weste 1984). Interestingly, sporulation capacity of *P. cinnamomi* was higher in roots of the resistant avocado cultivar Duke 7 than in roots of the susceptible avocado cultivar Topa Topa (Kellam and Coffey 1985). In mature forests in Victoria, higher population densities of *P. cinnamomi* were found in the rhizosphere of the tolerant species *E. bicostata* and *C. maculata* as compared to the susceptible species *E. sieberi* and *E. obliqua* (Marks et al. 1975). These results indicate that in tolerant or resistant species containment of *P. cinnamomi* within necrotic areas of living roots and possibly also more extensive and thicker encasement of intracellular hyphae in lignitubers provides a comparable if not superior shelter for the pathogen than necrotic dead roots of susceptible species. Accordingly, it was shown recently that *P. cinnamomi* can cause asymptomatic root infections in 10 annual and perennial herbaceous species, which might also act as a reservoir for *P. cinnamomi* (Crone et al. 2012).

5 Conclusions

Despite the genetic bottleneck experienced during its clonal global spread, the A2 mating type of *P. cinnamomi* has a highly plastic survival strategy. Marks et al. (1975) concluded from their experiments and field studies that '…the short-term survival of *P. cinnamomi* depends on exogenously dormant, antagonist-sensitive, fast-germinating and readily inactivated structures produced in large numbers in parasitized and decaying roots, while the long-term survival may be by oospores and thick-walled chlamydospores'. They also stated that '…such a two-tiered system could account for both the rapid seasonal fluctuations in population density and the persistence of the fungus (at low inoculum levels) for long periods'.

From the results of the present study and the results from extensive field studies (Shea et al. 1980) and experiments on *P. cinnamomi* survival in soil and infected tissue (Mircetich and Zentmyer 1966, 1967; Weste and Vithanage 1979; Mackay et al. 1985; Collins et al. 2011), papillae and lignituber formation in roots of non-host and host plants after artificial inoculation (Cahill and Weste 1983; Cahill et al. 1989), and on oospore formation of A2 isolates of *P. cinnamomi* by selfing (Mircetich and Zentmyer 1966, 1967; Brasier 1971, 1978; Zentmyer 1980; McCarren 2006; Jayasekera et al. 2007) and recent knowledge on the role of stromata-like hyphal aggregations as survival structures of *Phytophthora* species with a corrupted breeding system (Brasier and Kirk 2004; Moralejo et al. 2006; Boutet et al. 2009; Brasier et al. 2010; Jung et al. 2011), the following two-tiered system for the survival of the A2 mating type of *P. cinnamomi* is proposed:

Chlamydospores on the surface of host roots and thin-walled intracellular chlamydospores, vesicles and hyphae in roots and collars of infected woody and herbaceous species are responsible for short-term survival (weeks to several months) in moderately dry conditions between consecutive rainfall events enabling rapid propagation via the formation of sporangia after remoistening of soil. Long-term survival of *P. cinnamomi* in the soil is most likely via the following structures produced in cortex cells of infected fine roots and woody roots: (i) thick-walled dormant oospores produced by selfing as a response to different stress factors such as drought and antagonistic fungi or bacteria, and stimulating root exudates from both host and non-host species, (ii) dense stromata-like hyphal aggregations and (iii) hyphae and vesicles buffered against dry soil conditions and protected against microbial decomposition by thick host cell walls, papillae and lignituber sheaths.

Due to the consistent association of the oospores, hyphal aggregations and lignitubers with roots and root debris from which *P. cinnamomi* was isolated in samples from both dieback sites and artificially inoculated plants, it is beyond reasonable doubt that these structures belonged to the pathogen. Nevertheless, additional experiments are required to verify the postulated hypothesis on the role of different structures for short- and long-term survival of *P. cinnamomi*. For this the viability of oospores, putative chlamydospores, hyphal aggregations, and the hyphae and vesicles inside the lignitubers in fine roots from both field sites and soil infestation tests should be tested using the tetrazolium viable staining test (Thiozolyl Blue Tetrazolium Bromide; Delcan and Brasier 2001). Furthermore, the different structures should be germinated and pure cultures isolated to confirm their identity as *P. cinnamomi*. In work related to the present study, oospores in naturally infected roots of annual and perennial herbaceous plants could be germinated to produce cultures of *P. cinnamomi* A2, and these were further confirmed as *P. cinnamomi* through sequencing of the ITS region (M. Crone and G.E.St.J. Hardy, unpublished results.

5.1 Recommendations for eradication of *P. cinnamomi* from infested natural ecosystems

Phytophthora cinnamomi is a major threat to the unique diverse flora of WA. Therefore, the Department of Environment and Conservation and private companies are obliged to develop management strategies and control measures for preventing and controlling Phytophthora dieback during operations in natural ecosystems such as logging, mining and road construction. Since the 1990s, there has been an increasing focus on the early detection and eradication of new *P. cinnamomi* spot infestations. For eradication measures to be successful, knowledge of the survival strategy and the lifespan of the various different survival structures are crucial.

The results of Shea et al. (1980) and the present study leave no doubt that survival structures in fine roots, small woody roots and root debris, rather than free propagules in the soil, are mainly responsible for long-term survival of *P. cinnamomi* in the jarrah forest and other natural ecosystems of WA. Due to the hydrophobic character of organic debris and especially of the lignituber sheaths (Heath 1980), the use of liquid fungicides might not be suitable for eradicating *P. cinnamomi* from infested soil. The experiments of Dunstan et al. (2009) demonstrated that *P. cinnamomi* can be effectively eradicated by the use of the fumigant metham sodium. In these experiments, *P. cinnamomi* was killed to a soil depth of at least 80 cm when metham sodium was released at 60 cm soil depth. However, as metham sodium is a non-specific highly toxic chemical killing all organisms in the treated soil, large-scale application is not feasible. In the present study, *P. cinnamomi* could be isolated from air-dry soil and roots after 18 months but not after 24 months. Therefore, a fallow period of 3 years is recommended as an environmentally less deleterious measure for the eradication of *P. cinnamomi* from infested soil under the dry conditions of WA; subject to the infested area being kept free from vegetation by the use of herbicides and fenced-off to prevent *P. cinnamomi* spread from and into these spots on the paws and hooves of animals.

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References

Aist, J. R., 1976: Papillae and related wound plugs of plant cells. Annu. Rev. Phytopathol. **14**, 145–163.

Allen, F. H. E.; Friend, J., 1983: Resistance of potato tubers to infection by *Phytophthora infestans*: a structural study of haustorial encasement. Physiol. Plant Pathol. **22**, 285–292.

Aragaki, M.; Uchida, J. Y., 2001: Morphological distinctions between *Phytophthora capsici* and *P. tropicalis* sp. nov. Mycologia **93**, 137–145.

Arentz, F.; Simpson, J. A., 1986: Distribution of *Phytophthora cinnamomi* in Papua New Guinea and notes on its origin. Trans. Br. Mycol. Soc. **87**, 289–295.

Balci, Y.; Balci, S.; Eggers, J.; MacDonald, W. L.; Juzwik, J.; Long, R. P.; Gottschalk, K. W., 2007: *Phytophthora* species associated with forest soils in Eastern and North-central U.S. oak ecosystems. Plant Dis. **91**, 705–710.

Blackwell, E., 1949: Terminology in *Phytophthora*. Mycolog. Pap. **30**, 24 pp.

Blaschke, H., 1994a: Veränderungen durch Pilzbefall an Wurzeln geschädigter Stieleichen. Allg. Forstz. **37**, 775–777.

Blaschke, H., 1994b: Decline symptoms on roots of *Quercus robur*. Eur. J. For. Pathol. **24**, 386–398.

Boutet, X.; Laurent, F.; Chandelier, A., 2009: Influence of the medium-solidifying agent, the nutrient, and the genotype on the production of gametangia by *Phytophthora ramorum in vitro*. Mycol. Res. **113**, 110–116.

Bracker, C. E.; Littlefield, L. J., 1973: Structural concepts of host-pathogen interfaces. In: Fungal Pathogenicity and the Plant's Responses. Ed. by Byrde, R. J. W.; Cutting, C. V. UK: Academic Press, London, pp. 159–318.

Brasier, C. M., 1971: Induction of sexual reproduction in single A2 isolates of *Phytophthora* species by *Trichoderma viride*. Nature **231**, 283.

Brasier, C. M., 1978: Stimulation of oospore formation in *Phytophthora* by antagonistic species of *Trichoderma* and its ecological implications. Ann. Appl. Biol. **89**, 135–139.

Brasier, C. M.; Kirk, S., 2004: Production of gametangia by *Phytophthora ramorum in vitro*. Mycol. Res. **108**, 823–827.

Brasier, C. M.; Sansome, E., 1975: Diploidy and gametangial meiosis in *Phytophthora cinnamomi*, *P. infestans* and *P. drechsleri*. Trans. Br. Mycol. Soc. **65**, 49–65.

Brasier, C. M.; Robredo, F.; Ferraz, J. F. P., 1993: Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline. Plant. Pathol. **42**, 140–145.

Brasier, C. M.; Vettraino, A. M.; Chang, T. T.; Vannini, A., 2010: *Phytophthora lateralis* discovered in an old growth *Chamaecyparis* forest in Taiwan. Plant. Pathol. **59**, 595–603.

Brundrett, M.; Bougher, N.; Dell, B.; Grove, T.; Malajczuk, N., 1996: Working with Mycorrhizas in Forestry and Agriculture. Canberra: Australian Centre for International Agricultural Research, ACIAR Monograph 32, 374 pp.

Cahill, D.; Weste, G., 1983: Formation of callose deposits as a response to infection with *Phytophthora cinnamomi*. Trans. Br. Mycol. Soc. **80**, 23–29.

Cahill, D.; Legge, B.; Grant, B.; Weste, G., 1989: Cellular and histopathological changes induced by *Phytophthora cinnamomi* in a group of plant species ranging from fully susceptible to fully resistant. Phytopathology **79**, 417–424.

Cahill, D. M.; Rookes, J. E.; Wilson, B. A.; Gibson, L.; McDougall, K. L., 2008: *Phytophthora cinnamomi* and Australia's biodiversity: impacts, predictions and progress towards control. Aust. J. Bot. **56**, 279–310.

Chang, T. T., 1993: Decline of two *Cinnamomum* species associated with *Phytophthora cinnamomi* in Taiwan. Plant Pathol. Bull. **2**, 1–6.

Chang, T. T.; Wang, W. W.; Wang, W. Y., 1996: Use of random amplified polymorphic DNA markers for the detection of genetic variation in *Phytophthora cinnamomi* in Taiwan. Bot. Bull. Acad. Sin. **37**, 165–171.

Chee, K. H.; Newhook, F. J., 1965: Improved methods for use in studies on *Phytophthora cinnamomi* Rands and other *Phytophthora* species. N. Z. J. Agr. Res. **8**, 88–95.

Collins, S.; McComb, J. A.; Howard, K.; Shearer, B. L.; Colquhoun, I. J.; Hardy, G. E. St. J., 2011: The long term survival of *Phytophthora cinnamomi* in mature *Banksia grandis* killed by the pathogen. Forest Pathol. **42**, 28–36.

Cother, E. J.; Griffin, D. M., 1973: Formation of chlamydospores by *Phytophthora drechsleri*. Trans. Br. Mycol. Soc. **61**, 379–383.
Crandall, B. S., 1950: The distribution and significance of the chestnut root rot Phytophthoras, *P. cinnamomi* and *P. cambivora*. Plant Dis. Rep. **34**, 194–196.
Crandall, B. A.; Gravatt, G. F., 1967: The distribution of *Phytophthora cinnamomi*. Ceiba **13**, 43–58.
Crone, M.; McComb, J. A.; O'Brien, P. A.; Hardy, G. E. St. J., 2012: Annual and herbaceous perennial native Australian plant species are symptomless hosts of *Phytophthora cinnamomi* in the *Eucalyptus marginata* jarrah forest of Western Australia. Plant. Pathol., in press. Doi: 10.1111/ppa.12016 (Published online).
Currah, R. S.; Tsuneda, A.; Murakami, S., 1993: Morphology and ecology of *Phialocephala fortinii i*n roots of *Rhododendron brachycarpum*. Can. J. Bot. **65**, 2473–2482.
Davison, E. M.; Shearer, B. L., 1989: *Phytophthora* spp. in indigenous forests in Australia. N. Z. J. For. Sci. **19**, 277–289.
De Bary, A., 1863: Recherche sur le developpement des quelques champignons parasites. Ann. Sci. Nat. Bot. Biol. Veg. Ser. 4 **20**, 32–67.
Delcan, J.; Brasier, C. M., 2001: Oospore viability and variation in zoospore and hyphal tip derivatives of the hybrid alder phytophthoras. Forest Pathol. **31**, 65–83.
Dell, B.; Wallace, I. M., 1981: Recovery of *Phytophthora cinnamomi* from naturally infected jarrah roots. Australas. Plant Pathol. **10**, 1–2.
Dobrowolski, M. P.; Tommerup, I. C.; Blakeman, H. D., 2003: Non-mendelian inheritance revealed in a genetic analysis of sexual progeny of *Phytophthora cinnamomi* with microsatellite markers. Fungal Genet. Biol. **35**, 197–212.
Donofrio, N. M.; Delaney, T. P., 2001: Abnormal callose response phenotype and hypersusceptibility to *Peronospora parasitica* in defense-compromised Arabidopsis *nim1-1* and salicylate hydroxylase-expressing plants. Mol. Plant Microbe In. **14**, 439–450.
Dunstan, W. A.; Rudman, T.; Shearer, B. L.; Moore, N. A.; Paap, T.; Calver, M. C.; Dell, B.; Hardy, G. E. St. J., 2009: Containment and spot eradication of a highly destructive, invasive plant pathogen (*Phytophthora cinnamomi*) in natural ecosystems. Biol. Invasions **12**, 913–925.
Ehrlich, M. A.; Ehrlich, H. G., 1966: Ultrastructure of the hyphae and haustoria of *Phytophthora infestans* and hyphae of *P. parasitica*. Can. J. Bot. **44**, 1495–1503.
Enright, N. J.; Lamont, B. B., 1992: Survival, growth and water relations of *Banksia* seedlings on a sand mine rehabilitation site and adjacent scrub-heath sites. J. Appl. Ecol. **29**, 663–671.
Erwin, D. C.; Ribeiro, O. K., 1996: Phytophthora Diseases Worldwide. St Paul, MN: American Phytopathological Society Press, pp. 562.
Gallego, F. J.; Perez de Algaba, A.; Fernandez-Escobar, R., 1999: Etiology of oak decline in Spain. Eur. J. For. Pathol. **29**, 17–27.
Gerretson-Cornell, L., 1983: A compendium of the morphology of *Phytophthora cinnamomi* Rands from Australia. Acta Bot. Hung. **29**, 91–105.
Grant, B. R.; Byrt, P., 1984: Root temperature effects on the growth of *Phytophthora cinnamomi* in the roots of *Eucalyptus marginata* and *E. calophylla*. Phytopathology **74**, 179–184.
Hardy, G. E. St. J., 2000: Phosphite and its potential to control *P. cinnamomi* in natural plant communities and adjacent rehabilitated mine-sites in Western Australia. In: Phytophthora Diseases of Forest Trees. Ed. by Hansen, E. M.; Sutton, W. Proceedings of the 1st International IUFRO Working Party 7.02.09 Meeting, 1999, Grants Pass, OR: Forest Research Laboratory, Oregon State University Press, pp. 82–86.
Hardy, G. E. St. J.; Barrett, S.; Shearer, B. L., 2001: The future of phosphite as a fungicide to control the soilborne plant pathogen *Phytophthora cinnamomi* in natural ecosystems. Australas. Plant Pathol. **30**, 133–139.
Heath, M. C., 1971: Haustorial sheath formation in cowpea leaves immune to rust infection. Phytopathology **61**, 383–388.
Heath, M. C., 1980: Reactions of non-suscepts to fungal pathogens. Annu. Rev. Phytopathol. **18**, 211–236.
Hemmes, D. E.; Wong, L. D. S., 1975: Ultrastructure of chlamydospores of *Phytophthora cinnamomi* during development and germination. Can. J. Bot. **53**, 2945–2957.
Hickman, C. J., 1958: *Phytophthora* – plant destroyer. Trans. Br. Mycol. Soc. **41**, 1–13.
Ho, H. H.; Zentmyer, G. A., 1977: Morphology of *Phytophthora cinnamomi*. Mycologia **69**, 701–713.
Hwang, S. C.; Ko, W. H., 1978: Biology of chlamydospores, sporangia and zoospores of *Phytophthora cinnamomi* in soil. Phytopathology **68**, 726–731.
Jayasekera, A. U.; McComb, J. A.; Shearer, B. L.; Hardy, G. E. St. J., 2007: *In planta* selfing and oospore production of *Phytophthora cinnamomi* in the presence of *Acacia pulchella*. Mycol. Res. **111**, 355–362.
Judelson, H. S.; Ah-Fong, A. M. V.; Fabritius, A.-L., 2010: An RNA symbiont enhances heat tolerance and secondary homothallism in the oomycete *Phytophthora infestans*. Microbiology **156**, 2026–2034.
Jung, T., 1998: Die Phytophthora –Erkrankung der Europäischen Eichenarten – wurzelzerstörende Pilze als Ursache des Eichensterbens The Phytophthora Disease of European Oak Species – Root Destroying Fungi as Cause of Oak Decline. Munich: Lincom Europe, pp. 143.
Jung, T., 2009: Beech decline in Central Europe driven by the interaction between *Phytophthora* infections and climatic extremes. Forest Pathol. **39**, 73–94.
Jung, T.; Dobler, G., 2002: First report of littleleaf disease caused by *Phytophthora cinnamomi* on *Pinus occidentalis* in the Dominican Republic. Plant Dis. **86**, 1275.
Jung, T.; Blaschke, H.; Neumann, P., 1996: Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. Eur. J. For. Pathol. **26**, 253–272.
Jung, T.; Cooke, D. E. L.; Blaschke, H.; Duncan, J. M.; Osswald, W., 1999: *Phytophthora quercina* sp. nov., causing root rot of European oaks. Mycol. Res. **103**, 785–798.
Jung, T.; Blaschke, H.; Oßwald, W., 2000: Involvement of *Phytophthora* species in Central European oak decline and the effect of site factors on the disease. Plant. Pathol. **49**, 706–718.
Jung, T.; Stukely, M. J. C.; Hardy, G. E. St. J.; White, D.; Paap, T.; Dunstan, W. A.; Burgess, T. I., 2011: Multiple new *Phytophthora* species from ITS Clade 6 associated with natural ecosystems in Australia: evolutionary and ecological implications. Persoonia **26**, 13–39.
Jung, T.; Vettraino, A. M.; Cech, T. L.; Vannini, A., 2013: The impact of invasive *Phytophthora* species on European forests. In: Phytophthora: A Global Perspective. Ed. by Lamour, K. Wallingford, UK: CABI, in press.
Kellam, M. K.; Coffey, M. D., 1985: Quantitative comparison of the resistance to Phytophthora root rot in three avocado rootstocks. Phytopathology **75**, 230–234.
Ko, W. H.; Chang, H. S.; Su, H. J., 1978: Isolates of *Phytophthora cinnamomi* from Taiwan as evidence for an Asian origin of the species. Trans. Br. Mycol. Soc. **71**, 496–499.
Kuhlman, E. G., 1964: Survival and pathogenicity of *Phytophthora cinnamomi* in several south western Oregon soils. For. Sci. **10**, 151–158.
Lamont, B. B.; Bergl, S. M., 1991: Water relations, shoot and root architecture, and phenology of three co-occurring *Banksia* species: no evidence for niche differentiation in the pattern of water use. Oikos **60**, 291–298.
Linde, C.; Drenth, A.; Kemp, G. H. J.; Wingfield, M. J.; von Broembsen, S. L., 1997: Population structure of *Phytophthora cinnamomi* in South Africa. Phytopathology **87**, 822–827.

Lipka, V.; Dittgen, J.; Bednarek, P.; Bhat, R.; Wiermer, M.; Stein, M.; Landtag, J.; Brandt, W.; Rosahl, S.; Scheel, D.; Llorente, F.; Molina, A.; Parker, J.; Somerville, S.; Schulze-Lefert, P., 2005: Pre- and postinvasion defenses both contribute to nonhost resistance in Arabidopsis. Science **310**, 1180–1183.

Lu, Y.-J.; Schornack, S.; Spallek, T.; Geldner, N.; Chory, J.; Schellmann, S.; Schumacher, K.; Kamoun, S.; Robatzek, S., 2012: Patterns of plant subcellular responses to successful oomycete infections reveal differences in host cell reprogramming and endocytic trafficking. Cell. Microbiol. **14**, 682–697.

Mackay, A.; Weste, G.; Sharpe, K., 1985: Survival of *Phytophthora cinnamomi* in buried *Eucalyptus* roots. Phytopathol. Z. **114**, 214–223.

Malajczuk, N.; McComb, A. J.; Parker, C. A., 1977: Infection by *Phytophthora cinnamomi* Rands of roots of *Eucalyptus calophylla* R.Br. and *Eucalyptus marginata* Donn. Ex. Sm. Aust. J. Bot. **25**, 483–500.

Manners, J. G., 1993: Principles of Plant Pathology. Cambridge, NY: Cambridge University Press, p 343.

Marks, G. C.; Smith, I. W., 1991: The Cinnamon Fungus in Victorian forests. History, Distribution, Management and Control. Melbourne, Australia: Department of Conservation and Environment. Lands and Forests Bulletin No. 31: 33 pp.

Marks, G. C.; Kassaby, F. Y.; Fagg, P. C., 1975: Variation in population levels of *Phytophthora cinnamomi* in eucalyptus forest soils of eastern Victoria. Aust. J. Bot. **23**, 435–449.

Mbaka, J. N.; Losenge, T.; Waiganjo, M. M.; Wamocho, L. S., 2010: Phenotypic variation in three *Phytophthora cinnamomi* populations from macadamia growing areas in Kenya. J. Anim. Plant Sci. **8**, 900–911.

McCarren, K. L., 2006: Saprophytic ability and the contribution of chlamydospores and oospores to the survival of *Phytophthora cinnamomi*. PhD Thesis. Murdoch University, Perth, Australia, 201 pp.

McCarren, K. L.; McComb, J. A.; Shearer, B. L.; Hardy, G. E. St. J., 2005: The role of chlamydospores of *Phytophthora cinnamomi* – a review. Australas. Plant Pathol. **34**, 333–338.

Micali, C. O.; Neumann, U.; Grunewald, D.; Panstruga, R.; O'Connell, R., 2011: Biogenesis of a specialized plant fungal interface during host cell internalization of *Golovinomyces orontii* haustoria. Cell. Microbiol. **13**, 210–226.

Mircetich, S. M.; Zentmyer, G. A., 1966: Production of oospores and chlamydospores of *Phytophthora cinnamomi* in roots and soil. Phytopathology **56**, 1076–1078.

Mircetich, S. M.; Zentmyer, G. A., 1967: Existence of *Phytophthora cinnamomi* as chlamydospores and oospores in roots and soil. Calif. Avoc. Soc. Yearbook **51**, 117–124.

Moralejo, E.; Puig, M.; García, J. A.; Descals, E., 2006: Stromata, sporangiomata and chlamydosori of *Phytophthora ramorum* on inoculated Mediterranean woody plants. Mycol. Res. **110**, 1323–1332.

Old, K. M.; Oros, J. M.; Malafant, K. W., 1984a: Survival of *Phytophthora cinnamomi* in root fragments in Australian forest soils. Trans. Br. Mycol. Soc. **82**, 605–613.

Old, K. M.; Moran, G. F.; Bell, J. C., 1984b: Isozyme variability among isolates of *Phytophthora cinnamomi* from Australia and Papua New Guinea. Can. J. Bot. **62**, 2016–2022.

Phillips, D.; Weste, G., 1984: Field resistance in three native monocotyledon species that colonize indigenous sclerophyll forest after invasion by *Phytophthora cinnamomi*. Aust. J. Bot. **32**, 339–352.

Podger, F. D., 1972: *Phytophthora cinnamomi*, a cause of lethal disease in indigenous plant communities in Western Australia. Phytopathology **62**, 972–981.

Reeves, R. J., 1975: Behaviour of *Phytophthora cinnamomi* Rands in different soils and water regimes. Soil Biol. Biochem. **7**, 19–24.

Reeves, R. J.; Jackson, R. M., 1974: Stimulation of sexual reproduction in *Phytophthora* by damage. Trans. Br. Mycol. Soc. **84**, 303–310.

Schulz, B.; Boyle, C., 2005: The endophytic continuum. Mycol. Res. **109**, 661–686.

Shea, S. R., 1975: Environmental Factors of the Northern Jarrah Forest in Relation to Pathogenicity and Survival of *Phytophthora cinnamomi*. Western Australia: Forests Department. Bulletin 85, 83 pp.

Shea, S. R., 1979: *Phytophthora cinnamomi* – a collar rot pathogen of *Banksia grandis*. Australas. Plant Pathol. **8**, 32–34.

Shea, S. R.; Dell, B., 1981: Structure of the surface root system of *Eucalyptus marginata* Sm. and its infection by *Phytophthora cinnamomi* Rands. Aust. J. Bot. **29**, 49–58.

Shea, S. R.; Gillen, K. J.; Leppard, W. I., 1980: Seasonal variation in population levels of *Phytophthora cinnamomi* Rands in soil in diseased freely-drained *Eucalyptus marginata* Sm sites in the northern jarrah forests of south-western Australia. Prot. Ecol. **2**, 135–156.

Shea, S. R.; Shearer, B. L.; Tippett, J. T., 1982: Recovery of *Phytophthora cinnamomi* Rands from vertical roots of jarrah *Eucalyptus marginata* Sm. Australas. Plant Pathol. **11**, 25–27.

Shea, S. R.; Shearer, B. L.; Tippett, J. T.; Deegan, P. M., 1983: Distribution, reproduction, and movement of *Phytophthora cinnamomi* on sites highly conducive to Jarrah dieback in south western Australia. Plant Dis. **67**, 970–973.

Shearer, B. L.; Dillon, M., 1995: Susceptibility of plant species in *Eucalyptus marginata* forest to infection by *Phytophthora cinnamomi*. Aust. J. Bot. **43**, 113–134.

Shearer, B. L.; Dillon, M., 1996: Susceptibility of plant species in *Banksia* woodlands on the Swan Coastal Plain, Western Australia, to infection by *Phytophthora cinnamomi*. Aust. J. Bot. **44**, 433–445.

Shearer, B. L.; Shea, S. R., 1987: Variation in seasonal population fluctuations of *Phytophthora cinnamomi* within and between infected *Eucalyptus marginata* sites of southwestern Australia. For. Ecol. Manage. **21**, 209–230.

Shearer, B. L.; Tippett, J. T., 1989: Jarrah Dieback: The Dynamics and Management of *Phytophthora cinnamomi* in the Jarrah *Eucalyptus marginata* Forest of South-Western Australia. Como, Western Australia: Department of Conservation and Land Management, Research Bulletin 3, 76 pp.

Shearer, B. L.; Crane, C. E.; Cochrane, A., 2004: Quantification of the susceptibility of the native flora of the South-West Botanical Province, Western Australia, to *Phytophthora cinnamomi*. Aust. J. Bot. **52**, 435–443.

Suske, J.; Acker, G., 1990: Host-endophyte interaction between *Lophodermium piceae* and *Picea abies:* cultural, ultrastuctural and immunocytochemical studies. Sydowia **42**, 211–217.

Tainter, F. H.; Baker, F. A., 1996: Principles of Forest Pathology. New York, NY: John Wiley & Sons, Inc., pp. 805.

Tainter, F. H.; O'Brien, J. G.; Hernández, A.; Orozco, F.; Rebolledo, O., 2000: *Phytophthora cinnamomi* as a cause of oak mortality in the state of Colima. Mexico. Plant Dis. **84**, 394–398.

Tellenbach, C. U., 2011: Natural disease control by root endophytes in a changing climate. PhD Thesis. ETH Zurich, Switzerland, 104 pp.

Tippett, J. T.; Shea, R. S.; Hill, T. C.; Shearer, B. L., 1983: Development of lesions caused by *Phytophthora cinnamomi* in the secondary phloem of *Eucalyptus marginata*. Aust. J. Bot. **31**, 197–210.

Tsuneda, A.; Wang, W.; Tsuneda, I.; Currah, R. S., 2009: Endomembrane system of aspen root cells plays a key role in defense against a common fungal root endophyte, *Cryptosporiopsis radicicola*. Mycologia **101**, 182–189.

Turkensteen, L. J.; Flier, W. G.; Wanningen, R.; Mulder, A., 2000: Production, survival and infectivity of oospores of *Phytophthora infestans*. Plant. Pathol. **49**, 688–696.

Underwood, W., 2012: The plant cell wall: a dynamic barrier against pathogen invasion. Front. Plant Sci. **3**, 85.
Vettraino, A. M.; Barzanti, G. P.; Bianco, M. C.; Ragazzi, A.; Capretti, P.; Paoletti, E.; Luisi, N.; Anselmi, N.; Vannini, A., 2002: Occurrence of *Phytophthora* species in oak stands in Italy and their association with declining oak trees. Forest Pathol. **32**, 19–28.
Vettraino, A. M.; Morel, O.; Perlerou, C.; Robin, C.; Diamandis, S.; Vannini, A., 2005: Occurrence and distribution of *Phytophthora* species associated with Ink Disease of chestnut in Europe. Eur. J. Plant Pathol. **111**, 169–180.
Von Broembsen, S. L.; Kruger, F. J., 1985: *Phytophthora cinnamomi* associated with mortality of native vegetation in South Africa. Plant Dis. **69**, 715–717.
Walles, B., 1974: Ultrastructure of the rust fungus *Peridermium pini* Pers. Lev. Studia Forestalia Suecica **122**, 1–30.
Werres, S.; Marwitz, R.; Veld Man In't, W. A.; De Cock, W. A. M.; Bonants, P. J. M.; De Weert, M.; Themann, K.; Ilieva, E.; Baayen, R. P., 2001: *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum*. Mycol. Res. **105**, 1155–1165.
Weste, G., 1983: *Phytophthora cinnamomi*: the dynamics of chlamydospore formation and survival. Phytopathol. Z. **106**, 163–176.
Weste, G., 2003: The dieback cycle in Victorian forests: a 30-year study of changes caused by *Phytophthora cinnamomi* in Victorian forests, woodlands and heathlands. Australas. Plant Pathol. **32**, 247–256.
Weste, G.; Marks, G. C., 1987: The biology of *Phytophthora cinnamomi* in Australasian forests. Annu. Rev. Phytopathol. **25**, 207–229.
Weste, G.; Vithanage, K., 1978: Seasonal variation in numbers of chlamydospores. Aust. J. Bot. **26**, 657–662.
Weste, G.; Vithanage, K., 1979: Survival of chlamydospores of *Phytophthora cinnamomi* in several non-sterile, host-free forest soils and gravels at different soil water potentials. Aust. J. Bot. **27**, 1–9.
Yu, T.; Nassuth, A.; Peterson, R. L., 2001: Characterization of the interaction between the dark septate fungus *Phialocephala fortinii* and *Asparagus officinalis* roots. Can. J. Microbiol. **47**, 741–753.
Zeng, H. C.; Ho, H. H.; Zeng, F. C., 2009: A survey of *Phytophthora* species on Hainan island of South China. J. Phytopathol. **157**, 33–39.
Zentmyer, G. A., 1979: Stimulation of sexual reproduction in the A2 mating type of *Phytophthora cinnamomi* by a substance in avocado roots. Phytopathology **69**, 1129–1131.
Zentmyer, G. A., 1980: *Phytophthora cinnamomi* and the Diseases it Causes. St Paul, MN: The American Phytopathological Society. Monograph No. 10, 96 pp.
Zentmyer, G. A.; Mircetich, S. M., 1966: Saprophytism and persistence in soil by *Phytophthora cinnamomi*. Phytopathology **56**, 710–712.
Zhang, H.; Wang, C.; Cheng, Y.; Chen, X.; Han, Q.; Huang, L.; wie, G.; Kang, Z., 2012: Histological and cytological characterization of adult plant resistance to wheat stripe rust. Plant Cell Rep. **31**, 2121–2137.