



Survival of *phytophthora cinnamomi* in plant material under different soil and moisture conditions

S. Collins^A, B. Shearer^{AB}, J. McComb^A, I. Colquhoun^C, G. Hardy^{AD}

^A Biological sciences, Murdoch University, Murdoch, 6150, Western Australia.

^B Dept. of Conservation and Land Management, 50 Hayman Rd, Perth, 6152, Western Australia.

^C Alcoa World Alumina, GPO 252, Perth, 6153, Western Australia.

^D Corresponding author: G. Hardy (G-Hardy@murdoch.edu.au)

Abstract. Soil moisture and the type of organic matter colonised by *Phytophthora cinnamomi* significantly affected long-term survival of the pathogen. *Banksia grandis* stem pieces, and root tips of *Eucalyptus marginata* (jarrah) colonised with *P. cinnamomi* were placed into pots filled with soil from the jarrah forest or an adjacent rehabilitated bauxite mine site in the south west of Western Australia. The soil was either maintained at container capacity, or allowed to dry-out slowly from container capacity. Samples were harvested over a 210-day period and assessed for *P. cinnamomi* survival. *P. cinnamomi* was recovered after 210 days from banksia stems (98% colonisation) and eucalypt root tips (45% colonisation) from both soil types when the soil was maintained at container capacity. However when the soils were allowed to dry, the pathogen was not recovered after 112 days from either banksia stems or eucalypt roots. Soil origin did not influence *P. cinnamomi* survival for either inoculum type. These findings indicate that under moist conditions the pathogen can survive in small pieces of organic matter for extended periods of time.

Introduction

The *Eucalyptus marginata* (jarrah) forest extends throughout the southwestern corner of Western Australia and is characterised by a Mediterranean climate, typified by long dry summers and cool wet winters (Dell and Havel 1989). The prolonged dry conditions over summer are not favourable to the soil borne plant pathogen *Phytophthora cinnamomi*, which requires a warm, moist environment for optimal growth (Shea 1975; Zentmyer 1980; Shearer and Tippett 1989). Although *P. cinnamomi* may not survive freely in soil for long periods under adverse summer conditions it can survive such conditions saprophytically within dead organic matter (Zentmyer and Mircetich 1966; Shea 1979; Weste and Vithanage 1979; Old *et al.* 1984; Schild 1995). Soil moisture, chemistry, texture, aeration and microbial composition significantly influence the pathogen's survival (Weste and Vithanage 1979; Halsall 1982).

In a modified ecosystem such as the rehabilitated bauxite minesites in the jarrah forest it is likely that, although the rehabilitation processes aim to reproduce the botanical diversity of the surrounding jarrah forest, *P. cinnamomi* may respond differently in the rehabilitated soils compared to the original undisturbed soils (Colquhoun and Hardy 2000). Indeed, *P. cinnamomi* affects floral diversity less in rehabilitated bauxite pits than in infested neighbouring jarrah forest (Colquhoun and Hardy 2000). The current study compared the

survival capacity of *P. cinnamomi* in jarrah forest and rehabilitated bauxite mine soils. The ability of *P. cinnamomi* to survive in fine roots of *Eucalyptus marginata* and in woody stems of *Banksia grandis* as well as the effect of different moisture conditions on the inoculum sources were examined.

Material and methods

Experimental Design

A single *P. cinnamomi* colonised *B. grandis* stem plug or five *P. cinnamomi* colonised *E. marginata* root tips were placed into pots of either jarrah forest or mine site soil. Soil moisture treatments were imposed where the soils were either maintained at container capacity or allowed to dry out naturally from container capacity. The pots were positioned at ambient temperature ($23 \pm 4^\circ\text{C}$) in a complete randomised block design where each block contained 108 pots, consisting of three replicates for each treatment for each harvest. Samples from each treatment were harvested periodically up to 210 days after inoculation and tested for survival of the pathogen.

Soil collection and preparation

Soil was collected from a jarrah forest Havel 'S' vegetation type (Havel 1975) and an adjacent two-year-old rehabilitated mine site. The Havel 'S' type is characterised by lateritic gravel with a sandy loam to loam matrix and is considered conducive to *P. cinnamomi* (Havel 1975). Soil was collected below the litter layer

to a depth of approximately 30 cm from both sites. The soils were sieved using a 2 mm-diameter sieve. Chemical properties were analysed (SoilWorx, Bibra Lake WA). The soils were baited to confirm that they were *P. cinnamomi* free using the technique described by Hüberli *et al.* (2000).

To determine 'container capacity' for each soil type, three 500 mL pots were filled with 400 g of sieved dry soil and weighed. Water was added until each pot was waterlogged and excess water was allowed to drain (2 mm diameter holes). When the water had stopped draining, the pots were re-weighed and the additional weight indicated 'container capacity'. This was 32% of soil weight for mine soil and 26% for forest soil.

Collection and preparation of tissue types

B. grandis stem plugs

Young *B. grandis* stems of 1-2 cm diameter were cut into 2 cm long plugs. Plugs were rinsed, soaked in distilled water overnight then 100 were placed into each of four 2 L flasks with 200 mL of de-ionised water. The plugs were sterilised for 20 minutes at 121°C on three consecutive days. Once sterilised, the remaining water was drained from the flasks and ten 1 cm² blocks of an actively growing culture of *P. cinnamomi* (Isolate MU 97-16) grown on V8 agar (Ribeiro 1978) were distributed uniformly amongst the plugs. The flasks were incubated in the dark at 23°C for six weeks and shaken periodically to ensure uniform colonisation of the plugs.

E. marginata root tips

A clonal line of *Eucalyptus marginata* (SS402) susceptible to *P. cinnamomi* was grown for six months in aeroponics chambers (Burgess *et al.* 1998). A zoospore solution, produced following the methods of O'Gara *et al.* (1996) was used to inoculate actively growing roots that were approximately 1 mm in diameter and 200 mm in length. The roots were dipped into the solution for one minute. This inoculation process was repeated after seven days to ensure that the roots were infected. Three weeks after the initial inoculation, the roots were harvested by cutting each root at the root ball. To identify jarrah root sections colonised with *P. cinnamomi* prior to burial in soil, each root was cut into alternating 2 cm and 0.5 cm lengths. The 0.5 cm pieces were plated onto the NARPH agar plates (Huberli *et al.* 2000), incubated at 23°C and

monitored over three days for *P. cinnamomi* growth. Where *P. cinnamomi* grew from a root section, it was assumed that the adjoining 2 cm section of root was also colonised (moving from the root tip toward the stem) and these pieces were used in the experiment. During the 3-day incubation period to test for colonisation, the reserved 2 cm root lengths were kept in moist paper towels at 23 ± 4°C.

Inoculation of soil with roots and woody plugs

Single *P. cinnamomi* colonised *B. grandis* plugs were placed in plastic mesh bags (10 cm x 7 cm with 1 mm diameter mesh) and sets of five *E. marginata* roots were sandwiched between 25 mm x 35 mm pieces of the plastic mesh and held in place using plastic slide frames (50 mm² plastic frame). The plugs or roots were then placed into the pots containing 300 g of air-dried soil and covered with a further 100 g of dry soil. De-ionised water was added to the pots over a 12 hour period to bring them to container capacity and pots incubated at 23° ± 4° C. The pots maintained at container capacity were watered to weight once a week and were enclosed with plastic bags to minimise evaporation.

Phytophthora cinnamomi recovery and assessment

Treatments were harvested at 0, 7, 14, 28, 42, 70, 112, 154 and 210 days after soil inoculation. Three replicate samples from each treatment were assessed at each harvest for *P. cinnamomi* survival and soil moisture content.

At harvest, the plugs were cut longitudinally and then split into ten pieces and plated onto NARPH selective medium to assess the percentage *P. cinnamomi* recovery from each plug. Similarly, each of the five roots in each replicate was cut transversely into half transversely, to give 10 sections that were placed onto NARPH to assess the percentage of *P. cinnamomi* recovery. Where *P. cinnamomi* was not isolated from the plated samples after five days, the samples were removed from the agar, placed in distilled water and baited using *Pimelia ferruginea* leaves (Hüberli *et al.* 2000). The baiting did not yield any further *P. cinnamomi* recoveries during the trial.

Soil moisture content was determined at each harvest by collecting approximately 150 - 200 g of soil surrounding each sample. The soil was weighed, then dried at 102°C and re-

weighed daily until the weight had not changed for 24 hours (usually two days).

Statistical analysis

Data were analysed using the ANOVA module of Statistica (1999 edition, Statsoft Inc., USA). Percentages were arc-sin transformed for analysis. Data were assessed for homogeneity, variation of the mean from the variance and fit to a normal distribution.

Results

Soil type did not significantly ($P=0.35$) affect the recovery of *P. cinnamomi* from either inoculum type for the duration of the trial despite significant differences in the water content and drying profiles of the soils (Figs. 1 and 2). Since there were no differences in *P. cinnamomi* recovery between the two soil types, the data were combined. Soils held at container capacity maintained 25 – 35 % moisture while soils allowed to dry fell from 30 – 1.5 % moisture (Fig. 1). Moisture significantly ($P < 0.01$) affected the survival of *P. cinnamomi* in the two soils assessed over 210 days. Soil moisture and pathogen survival were also highly correlated ($r = 0.80$). Within both of the moisture treatments (pots drying or maintained at container capacity), the percentage of soil moisture proved to be consistent between the two inoculum types as they were not significantly different ($P = 0.79$). In the soil maintained at container capacity, *P. cinnamomi* was recovered from 98%, and 45% of the woody plugs and roots after 210 days, respectively (Fig. 2). In contrast, when the soils were allowed to dry out, *P. cinnamomi* was not

recovered from either inoculum type after 112 days (Fig. 2). In drying soils recovery declined markedly for both plant tissue types after 42 days (Fig. 2).

The source of inoculum (tissue type) had a significant ($P < 0.0001$) effect on the recovery of *P. cinnamomi* with greater survival in plugs than roots. A large degree of variation in *P. cinnamomi* recovery was evident in the root samples compared to the stem plugs (Fig. 2).

The physical and chemical composition of the two soils varied (Table 1). Mine site soil had lower mineral levels for all of the elements tested, particularly potassium (Table 1). The gravel content for mine soils was also lower than forest soils. Soil pH was within the range for growth and sporelation of *P. cinnamomi* (Zentmyer 1980).

Discussion

The ability of *P. cinnamomi* to survive declined rapidly in drying soils. In contrast, in moist soils it survived for up to 210 days in both fine jarrah roots and woody banksia stems. Similarly Weste and Vithanage (1979) found that *P. cinnamomi* survived for ten months in wet soils compared to less than two months in dry soils. This result is supported by other studies which indicate that *P. cinnamomi* is able to survive in moist conditions in jarrah forest soil for extended periods (Old *et al.* 1984; Shearer and Shea 1987).

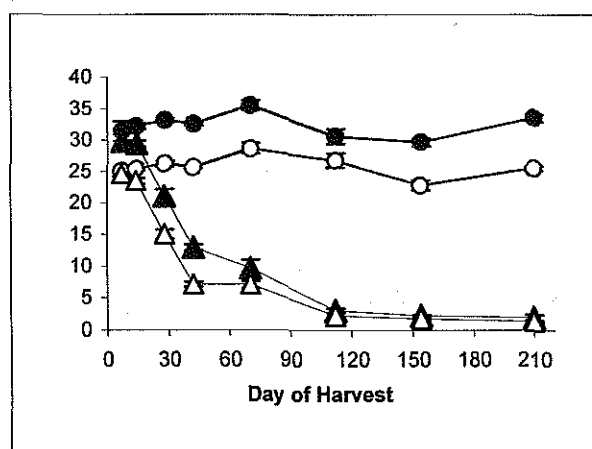


Fig. 1. Water content (%) of soil surrounding *Phytophthora cinnamomi* colonized *Eucalyptus marginata* root tips and *Banksia grandis* woody plugs harvested 0 - 210 days after burial in (●) forest and (○) mine soils maintained at container capacity and (▲) forest and (△) mine soils allowed to dry out over 210 days. Bars represent standard error of the mean.

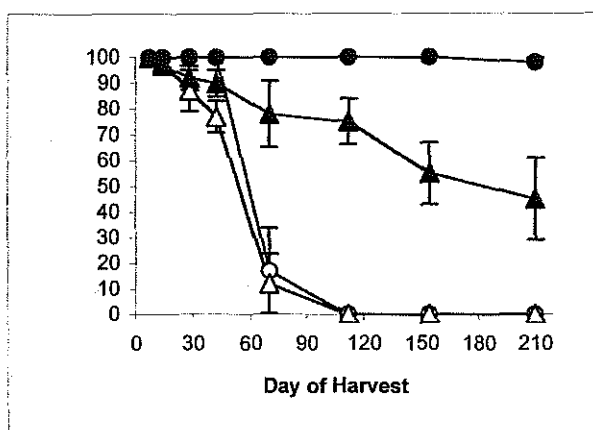


Fig. 2. Recovery of *Phytophthora cinnamomi* (%) from colonised *Banksia grandis* woody plugs in soils maintained at container capacity (●) or allowed to dry out over time (○) and *Eucalyptus marginata* roots in soils maintained at container capacity (▲) or allowed to dry out over time (△) harvested 0 - 210 days after burial. Data from mine and forest soils have been combined. Bars represent standard error of the mean.

Table 1. Physical and chemical Soil properties for the mine and jarrah forest soils

Soil Origin	Nitrate (ppm)	Ammonium (ppm)	Phosphorus (Colwell) (ppm)	Colwell potassium (ppm)	Electrical conductivity	pH in water	pH in calcium chloride
Mine site	1	4	5	28	0.02	6.8	5.7
Jarrah Forest	13	8	7	67	0.06	6.2	5.2

The pathogen responded in the same manner in the two soil types even though they differed in physical and chemical make-up. Soils in rehabilitated bauxite minesites, though taken from areas infested with *P. cinnamomi* before mining, are not highly conducive to *P. cinnamomi* infection (Hardy *et al.* 1996; Colquhoun and Hardy 2000). *P. cinnamomi* commonly causes deaths in areas of impeded drainage in the jarrah forest (Shearer and Tippett 1989). In the rehabilitated areas, improved drainage due to removal of the duricrust during bauxite mining (Colquhoun and Hardy 2000). *P. cinnamomi* commonly causes deaths in areas of impeded drainage in the jarrah forest (Shearer and Tippett 1989). In the rehabilitated areas, improved drainage due to removal of the duricrust during bauxite mining (Colquhoun and Hardy 2000), may be responsible for the reduction in *P. cinnamomi* infestation.

There was more variation in the recovery of the pathogen from the fine roots than

the woody stem plugs. Natural colonisation of the roots may have been more 'patchy' than in the woody plugs, which were colonised more uniformly under sterile and constant environmental conditions in the presence of a large amount of inoculum over an extended period.

The size of the two forms of inoculum may also have affected *P. cinnamomi* survival. The stem plugs were significantly larger than the roots and provided better buffering from changes in soil moisture, temperature, and soil microbial activities. Shea *et al.* (1980) also found that isolation of *P. cinnamomi* after host death was higher and more consistent in larger roots than in fine roots.

This study showed that *P. cinnamomi* was able to survive in forest and rehabilitated bauxite mine soils for at least 210 days, particularly in small pieces of woody tissue. Survival was similar in the two soil types, so the

physical and chemical differences between them were not responsible for the differences in severity of *P. cinnamomi* infestation observed between forest and mined areas. However survival was significantly reduced when soil moisture fell below 10 %. Landscaping practices during mine site rehabilitation that minimise waterlogging are therefore possibly the key to the management and control of *P. cinnamomi* in these areas.

References

- Burgess T, McComb JA, Hardy G, Colquhoun IJ (1998) Influence of low oxygen levels in aeroponics chambers on eucalypt roots infected with *Phytophthora cinnamomi*. *Plant Disease* **82**, 368-373.
- Colquhoun IJ, Hardy G (2000) Managing the risks of *Phytophthora* root and collar rot during bauxite mining in the *Eucalyptus marginata* (Jarrah) forest of Western Australia. *Plant Disease* **84**, 116-127.
- Dell B, Havel JJ (1989) The jarrah forest: an introduction. In 'The jarrah forest: a complex mediterranean ecosystem'. (Eds B Dell, JJ Havel and N Malajczuk). (Kluwer Academic Publishers: Dordrecht, The Netherlands)
- Halsall DM (1982) A forest soil suppressive to *Phytophthora cinnamomi* and conducive to *Phytophthora cryptogea*. I Survival, germination infectivity of mycelium and chlamydospores. *Australian Journal of Botany* **30**, 11-25.
- The early development of disease caused by *Phytophthora cinnamomi* in *Eucalyptus marginata* and *Eucalyptus calophylla* growing in rehabilitated bauxite mined areas. *Plant Pathology* **45**, 944-954.
- Havel JJ (1975) 'Site-vegetation mapping in the northern jarrah forest (Darling range). 1. Definition of site-vegetation types.' Forests Department of Western Australia, Perth.
- Hüberli D, Tommerup IC, Hardy GESJ (2000) False-isolations or absence of lesions may cause mis-diagnosis of diseased plants infected with *Phytophthora cinnamomi*. *Australasian Plant Pathology* **29**, 164-169.
- O'Gara E, Hardy GESJ, McComb JA (1996) The ability of *Phytophthora cinnamomi* to infect through unwounded and wounded periderm tissue of *Eucalyptus marginata*. *Plant Pathology* **45**, 955-963.
- Old KM, Oros JM, Malafant KW (1984) Survival of *Phytophthora cinnamomi* in root fragments in Australian forest soils. *Transactions of the British Mycological Society* **82**, 605-613.
- Ribeiro OF (1978) 'A source book of the genus *Phytophthora*.' (Strauss & Cramer: Hirschberg)
- Schild DE (1995) The survival of *Phytophthora cinnamomi* Rands in the northern jarrah (*Eucalyptus marginata*) forest of Western Australia. PhD thesis, Murdoch University.
- Shea SR (1975) 'Environmental factors of the northern jarrah forest in relation to pathogenecity and survival of *Phytophthora cinnamomi*.' Bulletin 85 Forest Department, Perth.
- Shea SR (1979) *Phytophthora cinnamomi* Rands A collar rot pathogen of *Banksia grandis* Willd. *Australasian Plant Pathology* **8**, 32-34.
- Shea SR, Gillen KJ, Leppard WI (1980) Seasonal variation in population levels of *Phytophthora cinnamomi* Rands in soil in diseased, freely-drained *Eucalyptus marginata* SM sites in the northern jarrah forest of South-western Australia. *Protection Ecology* **2**, 135 - 156.
- Shearer BL, Shea SR (1987) Variation of seasonal population fluctuations of *Phytophthora cinnamomi* within and between infected *Eucalyptus marginata* sites of southwestern Australia. *Forest Ecology and Management* **21**, 209-230.
- Shearer BL, Tippett JT (1989) 'Jarrah dieback: The dynamics and management of *Phytophthora cinnamomi* in the Jarrah (*Eucalyptus marginata*) forest of South-western Australia.' Bulletin 3 (Department of Conservation and Land Management)
- 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. *Australian Journal of Botany* **27**, 1-9.
- Zentmyer GA (1980) '*Phytophthora cinnamomi* and the diseases it causes.' Monograph 10 (The American Phytopathological Society: Minnesota)
- Zentmyer GA, Mircetich SM (1966) Saprophytism and persistence in soil by *Phytophthora cinnamomi*. *Phytopathology* **56**, 710-712.