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Assessing the potential for biological control of *Phytophthora cinnamomi* by fifteen native Western Australian jarrah-forest legume species

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Abstract. Fifteen native Western Australian legumes were assessed for their potential to biologically control *Phytophthora cinnamomi*. Biological control was assessed in a controlled situation, conducive to *P. cinnamomi*, and was based on susceptibility to the pathogen, ability to reduce soil inoculum, amount of asymptomatic root infection and ability for *P. cinnamomi* to effectively sporulate from asymptomatically infected roots. *Acacia extensa*, *Acacia stenoptera* and *Acacia alata* along with *Acacia pulchella*, were identified as species with the highest potential for biological control of *P. cinnamomi*. *Acacia urophylla* and *Viminaria juncea* exhibited the least potential for biological control; these are more likely to harbour the pathogen and provide a source of inoculum when conditions become conducive for *P. cinnamomi* growth and development. These findings have important implications for managing the rehabilitation of bauxite-mined *P. cinnamomi*-infested areas and severely disease-affected forest. By manipulating rehabilitation seed mix ratios, the density of legume species that suppress *P. cinnamomi* inoculum in the soil can be increased and the density of those that harbour the pathogen can be reduced. This could potentially contain the activity of *P. cinnamomi* soil inoculum in infested areas to protect susceptible species and enhance species diversity. Further research is required to ascertain the action of suppression before implementing control measures.

Additional keyword: Oomycetes.

Introduction

The South-West Botanical Province of Western Australia is high in natural diversity and plant species richness with 5710 recorded plant species (Shearer *et al*. 2004). Of these, 2284 are susceptible and 800 are highly susceptible to *Phytophthora cinnamomi*, an introduced soil pathogen that has severely affected the diversity of the region (Shearer *et al*. 2004).

Chemical control is impractical and uneconomic for effective management in native eucalypt forests, except where conservation of endangered species that are susceptible to *P. cinnamomi* is a priority (Komorek *et al*. 1997). Currently, the main method of control in native environments is to minimise the spread and effect of the pathogen. A comprehensive hygiene management program has become standard practice in the jarrah forest. It involves the mapping of disease boundaries, quarantine and hygiene procedures to control the movement of soil and water between infested and non-infested areas and extensive forest-industry operator training (Shearer and Bailey 1989; Shearer *et al*. 1991; Colquhoun and Hardy 2000).

The possibility of biological control using *Acacia* species had promising broad scale potential when it was observed that stands of *Acacia pulchella* that germinated after uncontrolled forest fires rendered the soil less favourable to *P. cinnamomi* (Shea *et al*. 1976). Sporulation by the pathogen was significantly suppressed in forest soil beneath *A. pulchella* (Shea *et al*. 1978). However, regeneration of the legume understorey requires high intensity fires to break the dormancy of *Acacia* seed in the soil (Burrows 1985). Fires of high intensity are hampered by infrequent occurrence of suitable weather conditions and are difficult to control over large areas, causing extensive damage to economically important eucalypt species (Burrows 1985, 1987).

Bauxite mining in the jarrah forest of Western Australia involves an extensive rehabilitation program that aims to minimise the risk of spreading *P. cinnamomi* and to control the effect of disease on botanical diversity (Colquhoun and

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Hardy 2000). The ability to modify the understorey seed mix ratio to promote a range of vegetation densities and species compositions in rehabilitated areas could establish vegetation less favourable to *P. cinnamomi* and alleviate the problems associated with high intensity fire.

Acacia pulchella is highly resistant to *P. cinnamomi* infection (Tippett and Malajczuk 1979), but not all legumes are resistant. Shearer and Dillon (1995) found that many species within the Papilionaceae family tended to die frequently in disease centres and *P. cinnamomi* was readily isolated from some of these species. Asymptomatic infection of some resistant species might also occur, still allowing the pathogen to survive and reproduce (Weste and Cahill 1982; Phillips and Weste 1984; Shearer and Dillon 1995). *A. pulchella* is the only legume that has been shown to control *P. cinnamomi* in a natural environment by suppressing sporulation in forest sites where it dominates, compared with forest sites dominated by Proteaceae (Shea *et al*. 1978). Successful practices that encourage stands of other legume species as well as *A. pulchella* for control of the pathogen are dependent on whether other legume species also act against *P. cinnamomi*. If legume species are susceptible or asymptomatic, they can harbour *P. cinnamomi* and encourage succession of the pathogen so that suppression, and hence control, is not obtained.

Implementation of control measures first requires knowledge of legume species resistance. Resistant species must then be assessed for their ability to control *P. cinnamomi* by reducing soil inoculum or whether they provide a source of further inoculum as a result of asymptomatic infection of roots.

A glasshouse trial was performed to determine the susceptibility to *P. cinnamomi* of legumes commonly used in rehabilitation of bauxite mined areas and to assess the potential of any resistant species to be a biological control agent against the pathogen.

Methods

Experimental design

Five seedlings of each species were planted per pot in eight pots. A randomised block design was used, with one pot each of the 15 legume species and one pot of *Banksia grandis* within each of eight blocks. There were six blocks for inoculation and two for non-inoculated controls. Seedling mortality was assessed as the dependent variable, with species and replicates as the independent variables.

Plants

Fifteen common legume species were identified from Alcoa World Alumina Australia's rehabilitation seed mix to assess for biological control (Table 1). Seedlings of these species were grown by Alcoa's Marrinup Nursery (Dwellingup, Western Australia) in Wynelle pots (Plastic Injection Moulding, Ballarat, Victoria) with internal ribs to minimise root coiling (dimensions 50 × 50 mm top rim, 40 × 40 mm bottom and 122 mm depth). The plants were approximately 6 months old at the time of planting.

Table 1. Legume species assessed for potential biological control of *Phytophthora cinnamomi* in a glasshouse trial

Genus	Species and authority
Acacia	*A. alata* R.Br.
	A. drummondii Lindl.
	A. extensa Lindl.
	A. lateriticola Maslin
	A. pulchella R.Br.
	A. stenoptera Benth.
	A. urophylla Lindl.
Bossiaea	*B. aquifolium* Benth.
Daviesia	*D. decurrens* Meisn.
Hovea	*H. chorizemifolia* (Sweet) DC.
Kennedia	*K. coccinea* Vent.
	K. prostrata R.Br.
Labichea	*L. punctata* Benth.
Mirbelia	*M. dilatata* R.Br.
Viminaria	*V. juncea* (Schrad.et J.C.Wendl.) Hoffmanns

Plants were established in a glasshouse into 255-mm-diameter free-draining black PVC pots, filled with 2-year-old stockpiled, screened, *P. cinnamomi*-free topsoil supplied from Alcoa's Huntly mine. The screening process concentrates the topsoil by removing large debris and gravel before spreading the soil back onto mined pits during rehabilitation. Once in the pots the plants were fertilised with approximately 50 g of diammonium phosphate (DAP) fertiliser per pot and were hand watered approximately every second day to maintain soil moisture.

Inoculation

The soil was inoculated in spring (mid-November) using 1 to 2-cm-diameter *B. grandis* stems cut into plugs 2.5 cm long that were infested with four known pathogenic isolates of *P. cinnamomi* (D'Souza *et al*. 2004); MP97-12 and MP94 are highly pathogenic in jarrah and MP127 and MP97-7 are moderately pathogenic in jarrah (Hüberli *et al*. 2001). Briefly, plugs were prepared by cutting young green stems of *B. grandis* into 2.5-cm long plugs and soaking them in distilled water overnight before autoclaving them in 2-L conical flasks at 138 kPa for 30 min at 121°C and again 24 and 96 h later for 20 min. Plugs were inoculated with 1-week-old *P. cinnamomi* hyphae growing on Pea Agar (10% w/v frozen green peas, Difco Bacto Agar) (D'Souza *et al*. 2004).

At planting, 12-cm-long, 1-cm-diameter screw capped 10-mL plastic tubes (Sarstedt South Australia Item No. 60 9921 819) were pushed into the soil at each inoculation point. This enabled inoculum plugs to be placed into the holes when the tubes were removed without damaging roots. The seedlings were allowed to establish for 3 months before inoculation. Each pot was inoculated with one plug of each of the four isolates at equidistant points. Soil thermistors (Unidata Starlog Data Logging System Version 2.21) within the pots showed that average daily soil temperatures remained within a range of 15–25°C throughout the experiment, which was conducive to *P. cinnamomi* infection.

Susceptibility determination

After inoculation, the number of dead or dying seedlings was assessed three times a week for 8 weeks. Roots of dead and dying plants were surface sterilised in 70% ethanol for 30 s, then washed in three rinses of distilled water and dried on blotting paper. Root and collar segments were aseptically cut longitudinally to expose the cortex. The exposed tissue was directly plated using sterile

forceps onto NARPH *Phytophthora* selective agar (Hüberli *et al.* 2000). The plates were incubated in constant light at 24°C for 72 h and then examined under a light microscope for the presence of *P. cinnamomi* hyphae.

Screening for asymptomatic infection of roots

At the end of the trial (12 weeks), healthy plants in inoculated pots were assessed for *P. cinnamomi* root infection. The free-draining pots were watered to container capacity 48 h before sample collection. From inoculated pots containing healthy plants, four soil cores (10 cm long × 3.5 cm diameter) were taken from between plants at the closest position to the inoculation point. Each core was divided vertically and bulked into two plastic bags. One bag was stored at 4°C for later root mass analysis. The other bag was mixed thoroughly then sifted through a 2-mm sieve to separate as many roots from the soil as possible. The roots were then washed in two rinses of distilled water to remove soil particles and blotted on a paper towel. Approximately 40 5-cm-long pieces of root were separated further. These were rinsed again in fresh distilled water to ensure no soil particles were present. The roots were mostly fine, healthy, lateral roots that ranged in diameter from 0.5–3 mm.

The separated roots were surface sterilised in 70% ethanol for 30 s then immediately rinsed in three washes of distilled water. The roots were blotted dry on paper towel and 25 pieces, 2-cm long, were cut with a sterile scalpel. Fifteen pieces were plated directly onto NARPH *Phytophthora* selective agar and incubated for 72 h at 24°C in constant light. The roots were then examined for the presence of *P. cinnamomi* hyphae under a light microscope (Olympus × 100 magnification). The remaining 10 pieces of sterilised roots were placed into a 90-mm Petri dish and flooded with soil extract (200 g/L mine soil in distilled water, shaken then soaked for 2 h and filtered through paper towel). These were incubated at 24°C in an incubator with a glass door so they were influenced by daylight and darkness. Roots were examined daily for 4 days under a light microscope (Olympus ×100 and ×400 magnification) for the presence of *P. cinnamomi* sporangia and release of zoospores. To ensure the soil extract induced *P. cinnamomi* sporangial formation and sporulation, five 1-cm-diameter discs were cut from the hyphal margin of *P. cinnamomi* growing on V8 agar. These were flooded with soil extract and incubated at 24°C along with the root pieces under observation. In all cases, >50 sporangia per agar disc were observed within 4 days and sporulation occurred.

Soil inoculum potential

After removal of roots from each core sample, the sieved soil from pots of resistant species was analysed for *P. cinnamomi* inoculum potential. The inoculum potential was determined using a direct plating method (D'Souza *et al*. 2004). Inoculum potential was expressed as colony forming units per gram dry weight of soil (c.f.u./g dry weight). Soil cores were also taken from inoculated pots that contained *B. grandis* seedlings after they were harvested and assessed for inoculum potential. The soil inoculum levels were assessed as the dependent variables, with species and replicates as the independent variables.

Relative root mass and length analysis

The soil cores stored at 4°C for determination of the root mass of each species in a pot were air-dried for 1 week to allow ease of sieving. Once dry, each sample was sieved through a 2-mm sieve to remove roots. This was repeated with a 0.85-mm sieve to remove smaller roots until the majority of roots were removed from the soil sample.

The length of a sub-sample of the roots (approximately 10% where possible) was measured by lining up individual root pieces next to a ruler. Subsamples and whole samples were placed separately into paper envelopes and oven-dried for 24 h at 105°C. Each subsample and whole sample was then weighed. Root mass was expressed as grams dry weight of roots per 100 cm^3 of soil. An estimate of the length of roots in a soil core from the subsample weight was also calculated (total length per 100 cm^3), which was sufficient to compare differences between species.

Data analysis

All observations were compared between treatments by analysis of variance (ANOVA) of angular transformed data using Statistical Analysis System Version 6.12 (SAS 1989). To test that the assumptions of ANOVA (equal variances and normality) were met, the residuals were subject to standard tests: stem-leaf plots and plots of residuals *v.* fitted values.

Results

Susceptibility determination

The species considered resistant (0–10%) mortality to *P. cinnamomi* in conditions conducive to the pathogen are *Acacia pulchella*, *Acacia alata*, *Acacia drummondii*, *Acacia extensa*, *Acacia lateriticola*, *Acacia stenoptera*, *Acacia urophylla*, *Kennedia coccinea*, *Kennedia prostrata* and *Viminaria juncea* (Fig. 1). Mortality of *Hovea chorizemifolia* due to *P. cinnamomi* infection was also low, but these seedlings were not vigorous at the time of planting and many did not establish or died before inoculation. Assessment of survival on few individuals makes it difficult to conclude that this species is resistant.

Bossiaea aquifolium (47% mortality) and *Mirbelia dilatata* (33% mortality) were considered moderately susceptible in conditions conducive to the pathogen. *Daviesia decurrens* and *Labichea punctata* with 90 and 85% mean mortality, respectively, were not significantly different ($P > 0.05$) from infection of *B. grandis* (93%) and were susceptible to *P. cinnamomi* in conditions conducive to the pathogen.

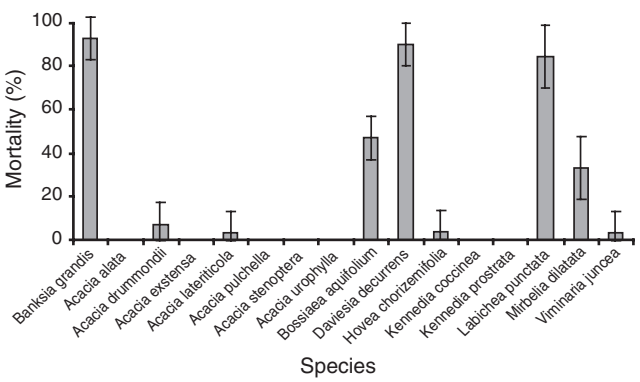

Fig. 1. Mean mortality of legume species tested for susceptibility to *Phytophthora cinnamomi* compared with mortality of *Banksia grandis* following soil inoculation in a glasshouse environment. Bars represent s.e.m.

Screening for asymptomatic infection of roots

None of the roots of *A. pulchella*, *A. extensa* or *A. stenoptera* were asymptomatically infected by *P. cinnamomi* (Fig. 2). Of the species that were infected, *V. juncea* and *A. urophylla* had the highest proportion of root infection, 37 and 33%, respectively. *A. alata* (2%) had the least root infection. All other species screened had between 10–25% of roots infected (Fig. 2).

Sporangial formation was observed on 24% of *V. juncea* root pieces, 13% of *K. coccinea* root pieces and 4% of *A. urophylla* root pieces when placed into soil extract (Fig. 2). Observations of sporangial formation were of less than 10 sporangia on any one 2-cm root piece. All sporangia sporulated.

Soil inoculum potential

Soil inoculum of *P. cinnamomi* was significantly ($P < 0.05$) less in soil planted with *A. pulchella*, *A. alata*, *A. extensa*, *A. lateriticola*, *A. stenoptera*, *K. coccinea* and *K. prostrata* than in soil planted with *B. grandis* (Fig. 3). In contrast, *A. drummondii*, *A. urophylla* and *V. juncea* did not significantly ($P > 0.05$) affect the soil inoculum compared with *B. grandis* (Fig. 3).

Relative root mass and length analysis

Acacia urophylla, *A. pulchella* and *A. stenoptera* had the largest root dry weights and largest length of roots after growing in pots inoculated with *P. cinnamomi* for 6 months (Table 2). *K. prostrata*, *V. juncea* and *K. coccinea* had the least root dry weights and the shortest root systems after growing in pots inoculated with *P. cinnamomi* for 6 months (Table 2).

Discussion

Acacia pulchella was resistant to *P. cinnamomi* infection in this trial, as shown previously (Tippett and Malajczuk 1979).

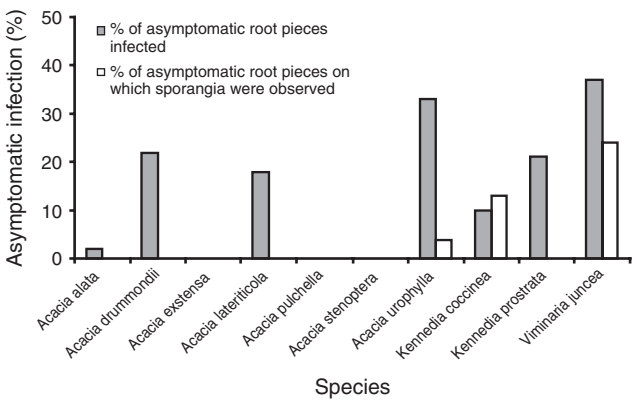

Fig. 2. Asymptomatic root infection by *Phytophthora cinnamomi* of resistant legume species following soil inoculation in a glasshouse environment.

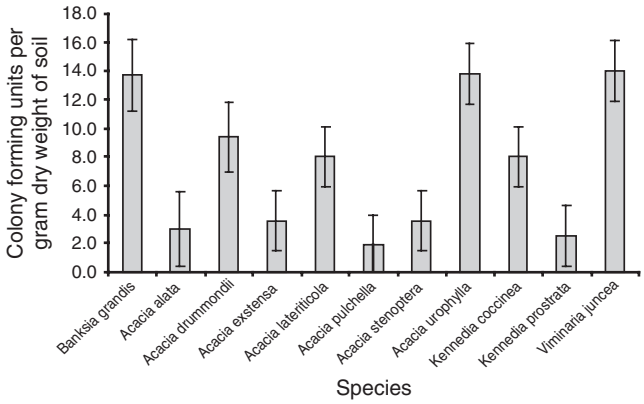

Fig. 3. Inoculum potential of *Phytophthora cinnamomi* (c.f.u./g dry weight of soil) in pots of soil planted with either legume species or *Banksia grandis* in a glasshouse environment. Bars represent the s.e.m.

Table 2. Mean root dry weight and root length (in 100 cm^3 of soil) of legume species resistant to *Phytophthora cinnamomi* and grown in pots inoculated with the pathogen, in a glasshouse environment for 6 months

Legume species	Mean dry weight of roots (g/100 cm^3)	Mean length of roots (m/100 cm^3)
Kennedia prostrata	0.1	3.6
Kennedia coccinea	0.2	4.2
Acacia drummondii	0.4	6.1
Viminaria juncea	0.5	3.8
Acacia extensa	0.7	15.6
Acacia alata	0.7	19.3
Acacia lateriticola	1.0	13.8
Acacia stenoptera	1.1	30.9
Acacia pulchella	1.3	28.7
Acacia urophylla	1.5	30.8

Except for *M. dilatata* and *A. stenoptera*, disease ratings of all other legume species tested in this trial concurred with previous disease ratings in active disease centres of either jarrah forest or banksia woodlands (Shearer and Dillon 1995, 1996).

Mirbelia dilatata was considered resistant in the jarrah forest (Shearer and Dillon 1995), but in the current trial it rated as moderately susceptible. *A. stenoptera* was found to be resistant in this trial with no mortality occurring after inoculation. Previously, *P. cinnamomi* was readily isolated from roots of dead *A. stenoptera* in banksia woodlands even though the percentage of areas in which it died was relatively low (Shearer and Dillon 1996).

The level of susceptibility of *Hovea chorizemifolia* was unable to be determined in the present trial because most of the plants died before inoculation. *P. cinnamomi* was isolated from <10% of those that died after inoculation. Shearer and Dillon (1995) considered this species to be resistant in the jarrah forest.

There are difficulties in assigning different levels of susceptibility in both the uncontrolled forest situation and controlled glasshouse situation. Variation of factors, such as site characteristics, soil type, temperature, moisture, soil microbiology and *P. cinnamomi* inoculum levels, will affect the level of susceptibility of a species in a forest situation, especially those with variable responses to infection (Erwin *et al*. 1983; Dell and Malajczuk 1989; Shearer and Tippett 1989). Alternatively, determining whether a host response is accurate in a controlled situation without these variations is also a restriction to assigning a susceptibility level (Shearer and Dillon 1995). Ultimately, a general level of susceptibility can be assigned by a combination of natural ecosystem observations and controlled conditions that have a consistent response (Shearer and Dillon 1996). This is an appropriate strategy to reduce the number of species for further testing of potential biological control ability.

Further investigation of the 10 resistant species in this trial showed that other legumes can reduce the soil inoculum of *P. cinnamomi*, as shown previously for *Acacia pulchella*, *A. extensa*, *A. lateriticola* and *A. drummondii* (Shea *et al*. 1978; D'Souza *et al*. 2004). *A. stenoptera* reduced soil inoculum levels as much as *A. pulchella* and no asymptomatic infection of roots of this species was observed. *A. stenoptera* also produced an equivalent amount of roots in 6 months in pots as *A. pulchella.* On the basis of these results, *A. stenoptera* has a high potential for control of *P. cinnamomi*.

The ability of *A. extensa* to reduce soil inoculum levels of *P. cinnamomi* in both this trial and the previous trial (D'Souza *et al*. 2004) confirms its high potential for control of *P. cinnamomi*. Also, in the previous glasshouse trial, mortality of *B. grandis* planted with *A. extensa* was delayed as much as that by *A. pulchella* despite neither species being able to ultimately protect *B. grandis* in such a pathogen-conducive environment (D'Souza *et al*. 2004). In the previous field trial, it could not be shown that *A. extensa* protected *B. grandis* from *P. cinnamomi* infection in a bauxite-mined rehabilitated area. The efficacy of this Acacia was compromised by low plant density as a result of poor establishment, so protection was unable to be determined (D'Souza *et al*. 2004).

Although 2% of fine root pieces of *A. alata* were infected, this occurred in conditions conducive to *P. cinnamomi*. The same might not occur in a forest situation. *A. alata* also reduced *P. cinnamomi* soil inoculum as much as *A. pulchella* and produced approximately two-thirds the amount of roots as *A. pulchella* did in pots in 6 months. *A. alata* has potential for biological control of the pathogen.

The reduction in soil inoculum by *Kennedia prostrata* indicates some effect on *P. cinnamomi*. However, this species could be less effective as the mass of roots produced was the lowest of the species tested and might not be sufficient to have an impact in the forest. Also, even though no sporulation was observed on *K. prostrata* roots, the ability of *P. cinnamomi* to infect roots of this species might still allow the pathogen to be maintained in infested areas.

Despite *K. coccinea*, *A. lateriticola* and *A. drummondii* moderately reducing the soil inoculum of *P. cinnamomi*, these species do not have the potential for biological control of the pathogen. *P. cinnamomi* was able to asymptomatically infect the roots of each of these species and none of them reduced the soil inoculum to a level as significant as *A. pulchella*. Sporangial formation and sporulation was also observed from roots of *K. coccinea*. *A. drummondii* and *A. lateriticola* were less effective at reducing the level of *P. cinnamomi* soil inoculum compared with that shown in the previous study (D'Souza *et al*. 2004). This indicates an inconsistent response to the pathogen in a more controlled environment that might be transferred to the uncontrolled field environment, rendering them unreliable for biological control. This conclusion is supported by the inability of *A. drummondii* and *A. lateriticola* to protect *B. grandis* from *P. cinnamomi* infection in the previous rehabilitated pit trial (D'Souza *et al*. 2004).

Observations of plant species that harbour *P. cinnamomi* were reported by Phillips and Weste (1984). They showed that *P. cinnamomi* persisted within contained lesions of resistant sedge and grass species for up to 10 days. *P. cinnamomi* was also able to produce sporangia on the root surfaces of the resistant rush species *Juncus bufonius* without exhibiting disease symptoms (Weste and Cahill 1982). Zoospores are chemotactically attracted to the roots of both susceptible and resistant species (Tippett *et al*. 1976; Malajczuk *et al*. 1977; Halsall 1978; Tippett and Malajczuk 1979); therefore, the roots of some resistant species can potentially provide a long-term source of continued inoculum after susceptible species have died. This makes it difficult for the regeneration of susceptible species.

Significant asymptomatic infection and observation of sporulation from the roots of *V. juncea* and in particular *A. urophylla*, which had the most extensive root system, might provide a source of inoculum in a forest situation when conditions become conducive to the pathogen. *A. urophylla* and *V. juncea* are, consequently, not effective for biological control of *P. cinnamomi*.

It has been proposed that suppression of *P. cinnamomi* that resulted in protection of susceptible plant species was due to either microbial antagonism (Broadbent *et al*. 1971; Shea and Malajczuk 1977; Murray *et al*. 1985; Murray 1987), or a direct or indirect action of plant root exudates on the pathogen (Krupa and Nylund 1972; Whitfield *et al*. 1981). The possibility that *A. pulchella* might have had an influence on the physical or chemical environment that resulted in suppression of *P. cinnamomi* (Shea and Malajczuk 1977; Nesbitt *et al*. 1981; Smith and Marks 1983; Smith *et al*. 1989) has largely been disproved (D'Souza *et al*. 2004).

Microorganisms associated with roots of *A. pulchella* have been shown to cause hyphal lysis and abortive sporangia of *P. cinnamomi*, resulting in reduced sporulation (Shea and Malajczuk 1977). Forty five per cent of bacteria isolated from the rhizosphere of *A. pulchella* were antagonistic to *P. cinnamomi*, compared with 18% of bacterial isolates from rhizospheres of *B. grandis* (Shea and Malajczuk 1977). *Bacillus* species and actinomycetes are associated with suppression of *P. cinnamomi* disease of avocado (Broadbent *et al*. 1971; Malajczuk 1979; Yin *et al*. 2004).

Often the suppression results from soil enzyme activity or antibiotic production that directly affects *Phytophthora*. Downer *et al*. (2001) find that the decline of cellulase and laminarinase, produced by litter decay fungi, with distance away from mulched areas with higher microbial activity, is associated with an increase in the prevalence of *Phytophthora* spp. The cell walls of *Phytophthora* contain cellulose and laminarin and are, therefore, degraded during the mulch decomposition process (Menge and McDonald 2004). Cellulase-producing *Micromonospora carbonacea* in conjunction with an antibiotic-producing *Streptomyces violascens* was able to suppress *P. cinnamomi* and promote growth of the highly susceptible *B. grandis* in glasshouse trials (El-Tarabily *et al*. 1996).

Suppression can also be attributed to exploitation of a niche similar to that occupied by *Phytophthora*, with nutrient competition leading to exclusion of the pathogen (Yin *et al*. 2004) and to reduced disease severity. In such cases, although disease is suppressed, the pathogen is not eliminated from the soil and is still able to rapidly multiply and cause disease when conditions become favourable. In a study by Marks and Cerra (1991), when continual herbicide treatment inadvertently enhanced bacteria that stimulated *P. cinnamomi* sporangial production in a *Pinus radiata* nursery, *P. cinnamomi* root rot disease rapidly increased in intensity in soils that were not believed to be conducive to the pathogen.

The direct action of *A. pulchella* root exudates on *P. cinnamomi* is demonstrated by Whitfield *et al*. (1981), who show that root exudates consist of volatile organic compounds that inhibit hyphal growth, suppress production of sporangia and reduce germination of zoospores. More recently Bais *et al*. (2002) showed that the production of rosmarinic acid is enhanced 2.67-fold in sweet basil (*Ocimum basilicum*) roots, when challenged with fungal cell wall elicitors of *P. cinnamomi* compared with an untreated control. This compound showed direct antimicrobial activity against a range of soil borne microorganisms, and Bais *et al*. (2002) suggest that this compound is released into the surrounding rhizosphere for protection against challenging microbes.

The association of a larger percentage of antagonistic microorganisms with the roots of *A. pulchella* than with *B. grandis* (Shea and Malajczuk 1977) implies their attraction to the rhizosphere by the plant's root exudates. Volatile and non-volatile root exudates are probably produced by many plants, including native legumes, and might play a part by direct chemical interference and by the indirect attraction of antagonistic microorganisms to the rhizosphere (Murray *et al*. 1985).

Yin *et al*. (2004) suggest that large amounts of root exudates are required to produce a change in the microbial composition of the soil, but only normal levels of exudates are required to maintain these populations. This would be supported by the early observations of soil that was less favourable to *P. cinnamomi* beneath stands of *A. pulchella* that germinated after uncontrolled forest fires (Shea *et al*. 1976). The extensive regeneration of *A. pulchella* and the subsequent root exudates emitted into the soil possibly provided the opportunity for a shift in soil microbial populations, antagonistic to *P. cinnamomi*, which suppressed sporulation.

Conclusion

The findings of this study have important management implications for the rehabilitation of bauxite-mined pits in *P. cinnamomi*-infested areas and for severely disease-affected forest. By changing the seed ratio of legumes in understorey seed mixes used for revegetation of these areas, the density and composition of certain species can be influenced to affect biological control. They could be manipulated to increase the density of plant species that suppress *P. cinnamomi* and to reduce the density of those that harbour the pathogen. This could potentially create a protective environment for plant species susceptible to the pathogen, enabling them to establish and survive in infested areas and to restore the species composition.

Acacia pulchella, *A. stenoptera*, *A. extensa* and *A. alata* are species that have a high potential for biological control of *P. cinnamomi*. *K. prostrata* also has some potential for controlling the pathogen. Even though it produced the same effect as *A. pulchella* on *P. cinnamomi* soil inoculum with the smallest mass of roots, *P. cinnamomi* was able to colonise these roots. In a non-contained natural environment, the root systems might not be extensive enough for this colonisation to be important, but this also means they might not have a significant effect on suppressing *P. cinnamomi*. *K. prostrata* requires further investigation.

Kennedia coccinea, *A. lateriticola* and *A. drummondii* do not have the potential for biological control. However, although these three species allowed some root colonisation by *P. cinnamomi* in the glasshouse, in a non-conducive environment the amount of root colonisation might not enhance the activity of *P. cinnamomi* in the soil. *V. juncea* and *A. urophylla* do not have any biological control effect on *P. cinnamomi* and have a high potential for encouraging spread of the pathogen by providing a long-term source of continued inoculum after susceptible species have died.

Before aspects of rehabilitation management practices are adjusted, it is recommended that other legumes be assessed for their potential to control *P. cinnamomi* and, along with those screened here, be established in forest trials to validate the findings of the present study in natural ecosystems. It is also recommended that the legume species density and composition required for suppression of *P. cinnamomi* be determined.

Understanding the action of suppression is also an important factor before implementing control measures in a forest environment. It is necessary to determine how these plant species suppress *P. cinnamomi* soil inoculum and whether or not this suppression results in protection of susceptible species in a natural environment. The role of microbiological activity against *P. cinnamomi* in the soil and the influence of root exudates on the pathogen or on microorganisms antagonistic to the pathogen require further investigation.

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