Suppression of *Phytophthora cinnamomi* by the root exudates of *Acacia pulchella*

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Introduction

The native Western Australian legume, *Acacia pulchella,* is resistant to *P. cinnamomi* attack (Tippet and Malajczuk 1979) and its presence in the understorey has been shown to reduce *P. cinnamomi* inoculum in jarrah forest (*Eucalyptus marginata*) soil (Shea *et al.,* 1978). Evidence suggests that the reduced inoculum is due mainly to the decreased sporangial production of *P. cinnamomi* under *A. pulchella* (Cary 1982; Murray *et al.,* 1985).

Several legumes including *A. pulchella* have a high proportion of soil microflora that are antagonistic towards *P. cinnamomi* (Malajczuk, 1979; Murray, 1987). Also the soil physical conditions under the legumes discourage proliferation of *P. cinnamomi* (Shea *et al.,* 1978). In addition to these factors the roots of *A. pulchella* plants contain saponins (Alexander *et al.,* 1978) and volatiles (Whitfield *et al.,* 1981). These can be extracted from the roots and bioassays have confirmed their suppressiveness toward sporangial production of *P. cinnamomi.* However, it is not known whether these substances are exuded into the soil surrounding the roots of *A. pulchella* plants and have a direct effect on inoculum reduction, or whether the soil microbes are more important in their contribution towards the reduction of *P. cinnamomi* inoculum.

Experiments in aseptic environments were designed to determine the role root exudates play on the suppression of *P. cinnamomi* using *A. pulchella* and *A. urophylla,* a species that shows no suppression of *P. cinnamomi* (D'Souza, 2001). This chapter reports the preliminary findings of the laboratory bioassays conducted to determine the role of root exudates of *A. pulchella* on the mycelium of *P. cinnamomi.*

Materials and methods

Seeds of *A. pulchella* var. *goadbyi* and *A. urophylla* were surface sterilized (2% sodium hypochlorite for 10 min followed by three washes in sterile distilled water) then treated in boiling water (1 min in boiling water followed by a soak in cold water) and germinated on 0.7% water agar. After 10 days germinated seeds were transferred to polycarbonate tubs containing half strength Murashige and Skoog medium (Murashige and Skoog, 1962) with 2% sucrose and placed under light at 25°C for a further 4 weeks. Seedlings with true leaves were removed from the tubs and transplanted in 150 g of the sterile *in vitro* soil (IVS) mix which comprised sphagnum peat, coarse river sand and perlite at a ratio of 0.5:2:2 (Newell *et al.,* 2003) under aseptic conditions. Prior to transplanting, the polyurethane punnets (13 x 7 x 5 cm) containing the IVS mix were autoclaved twice at 2-day intervals. The punnets with five seedlings in each and controls with only IVS mix were placed in sterile polypropylene containers (1000 ml, Plastic product of Bonson Industries Co. Ltd) with a layer of sterilized plastic beads in the bottom. A second container of similar size was placed over the first one as a lid and sealed with plastic wrap (Glad Products of Australia, Padstow, NSW, Australia) (Figure 1). Punnets were placed at 24°C with a 14/10 h light/dark regime and watered weekly with half MS basal mineral solution (Murashige and Skoog, 1962). Samples of 1 g IVS medium from all the punnets were plated on half PDA to detect any contamination.

Although *Aspergillus* and *Pencillium* spp. were present during the experiment they were not regarded to have caused any alteration to the results as these organisms were observed in all the treatments. Leachates in the bottom of the containers were collected weekly with a 20 ml syringe and filter sterilized using a 0.22 Millipore filter. These leachates contained nutrient solution and root exudates.

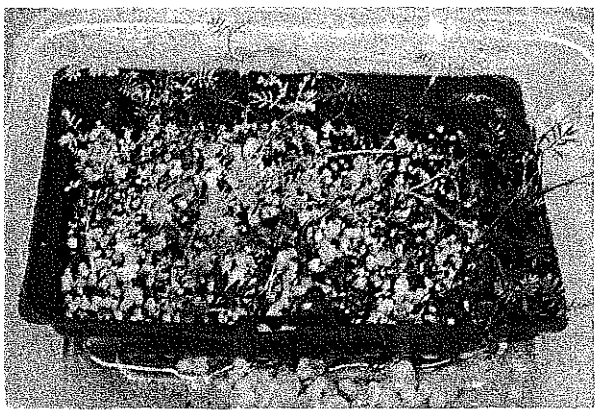

Figure 1 *In vitro* acacias.

Bioassay with mycelial disks

A series of solutions were made by incorporating the filter sterilized leachates from controls without plants, *A. pulchella* and *A. urophylla* plants into Ribeiro's basal medium in Petri dishes (5 cm in diameter). The concentrations ranged from 10–50% leachates in 10 ml of solution. There were two replicates for each concentration. A series of solutions were also made with 10–50% of sterile distilled water and the liquid medium. A mycelial disk of *P. cinnamomi* was placed in each dish and incubated at 22°C in the dark for 5 days. After 5 days the solution in each dish was drained through a pre-weighed Whatman No 1 filter paper (9 cm in diameter). The filter papers, each with a mycelial mat, were oven dried at 60°C for 24 h then weighed.

Preparation of mycelial disks

Miracloth disks (6 mm in diameter) were autoclaved three times at 121°C then plated on V8 juice agar at equal distance from the centre. A square (1 cm^2) of *P. cinnamomi* (isolate 97-16 from Murdoch *Phytophthora* collection) was cut from the edge of a growing colony and placed in the middle. The plates were incubated at 22°C in the dark for 7 days until the colony grew over the disks.

Statistical analysis

A two way ANOVA between subjects was performed using SPSS 12.0.1 for windows (SPSS Inc., USA).

Results

Mycelial growth of *P. cinnamomi* was suppressed by the presence of *A. pulchella* root exudates in the liquid medium. The dry mass of the *P. cinnamomi* colonies were significantly ($P < 0.05$) less (at $f = 173.131$, $df = 3$ and $P = 0.01$) when *A. pulchella* soil leachates were incorporated into the medium compared to that of *A. urophylla* or the control without plants. Total inhibition of the colony growth was observed when the medium contained 50% *A. pulchella* soil leachates (Figure 2). Dilution of the medium with sterile distilled water gave similar results to dilution with soil leachates from *A. urophylla* and the control without plants indicating that the inhibitory effect of adding soil leachate from *A. pulchella* to the medium was more than simply a dilution of nutrients in the medium.

Discussion

It was shown that the antifungal compound(s) produced by the aseptic roots of *A. pulchella* are washed out with soil leachate and effective against mycelial growth of *P. cinnamomi*. These results confirm the suggestions made by previous investigators (Shea, 1979; Shearer and Tippet, 1989) that the root exudates of *A. pulchella* suppress *P. cinnamomi*, evidence for

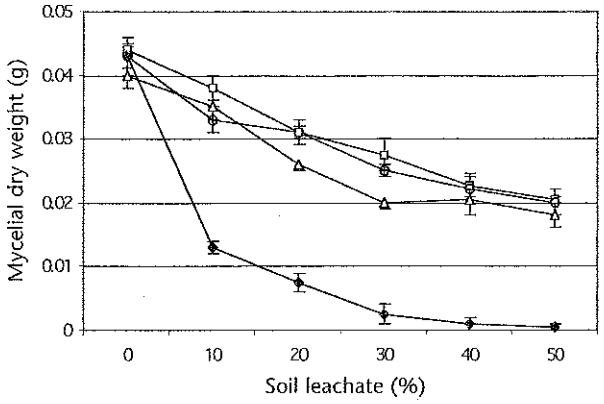

Figure 2 Mycelial growth of *P. cinnamomi* incubated in Ribeiro's liquid medium with soil leachate from *A. pulchella* ◆, *A. urophylla* ○, no plants △ and sterile distilled water □ at different concentrations.

which was lacking at that time. Alexander *et al.* (1978) and Whitfield *et al.* (1981) reported that suppressive saponins and volatiles could be extracted from *A. pulchella* roots. Whether the suppressive compounds from aseptic roots are the same as those found in root extract, e.g. saponins (Alexander *et al.*, 1978) and volatiles (Whitfield *et al.*, 1981), remains to be determined. It is clear that suppression of *P. cinnamomi* in the field by *A. pulchella* may be due to metabolites from *A. pulchella per se* – the contribution of the soil microbes also remains to be evaluated.

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