Interactions between *Phytophthora cinnamomi* and *Acacia pulchella*: consequences on ecology and epidemiology of the pathogen

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Declaration

I declare that this thesis is my own account of my research and contains as its main content work which has not been previously submitted for a degree at any tertiary education institution. To the best of my knowledge, the work performed by others, has been duly acknowledged.

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Abstract

Phytophthora cinnamomi is an important pathogen of many plant species in natural ecosystems and horticulture industries around the world. In Western Australia, a high proportion of native plant species are susceptible to *P. cinnamomi* attack. *Acacia pulchella*, a resistant legume species native to Western Australia has been considered as a potential biological control tool against *P. cinnamomi*. To develop effective control methods, it is important to understand the interactions between the control agent and the different life forms of the pathogen. In this thesis the interactions are investigated between *P. cinnamomi* and varieties of *A. pulchella* which occur in jarrah (*Eucalyptus marginata*) forest and sand plain ecosystems.

The soil inoculum of *P. cinnamomi* was compared under the potted plants of the three common varieties of *A. pulchella*, var. *pulchella*, var. *glaberrima* and var. *goadbyi*. These were grown in infected jarrah forest soil in the glasshouse and *in vitro* in a sterilised soil-less mix aseptically. *Acacia urophylla* (a species non suppressive towards *P. cinnamomi*) was also included as a control. An isolate of the most commonly found clonal lineage of *P. cinnamomi* in the jarrah forest, A2 type 1 was selected for use in experiments after testing showed it reliably produced zoospores and chlamydospores both axenically and in non-sterile conditions, in comparison to several other isolates. The lowest survival of *P. cinnamomi* inoculum was found under *A. pulchella* var. *goadbyi* plants grown both in non sterile soil and in aseptic soil-less mix.

All the life forms of *P. cinnamomi* were affected by *A. pulchella* (Chapters 2, 3, 4 and 5). The soil leachates from potted plants of *A. pulchella* var. *goadbyi* reduced sporangial production (Chapter 2) and caused cytoplasm collapse of chlamydospores (Chapter 3). The confirmation was obtained that soil under *A. pulchella* was inhibitory to sporangial stage of *P. cinnamomi* and new evidence was obtained on chlamydospore inactivation. Cytoplasm collapse in the chlamydospores was observed both for chlamydospores on mycelial discs on Mira cloth exposed to the soil leachate and within infected roots buried in soils under the three varieties of *A. pulchella* plants. The effect was strongest under the plants of *A. pulchella* var. *goadbyi* and indicated that the chlamydospores of *P. cinnamomi* are unlikely to act as persistent structures under *A. pulchella* var. *goadbyi* plants.

In Chapter 4, bioassays were conducted with axenically produced mycelia, chlamydospores and zoospores to test the inhibitory effect of the root exudates collected from aseptically grown *A. pulchella* var. *goadbyi* plants. The zoospores of the same isolate used in the soil leachate tests were immobilised (became sluggish and encysted) within one to two minutes. When incubated for 24 h, zoospores predominantly clumped and germ tubes were observed only from the clumped ones. Chlamydospores produced by four isolates of the common A2 type 1 strain and the only one A2 type 2 strain available at the time were tested. A higher percentage of chlamydospores collapsed and a very low percentage germinated after 24 h. Chlamydospores of all the A2 type 1 isolates were inhibited by the root exudates whilst the A2 type 2 isolate remained viable. The findings showed that the suppressive effect must be due at least in part to substances exuded by the *A. pulchella* plants. However, it appeared that the A2 type 1 isolates were more vulnerable to this effect than the single A2 type 2 isolate.

In Chapter 5, the effect of season on sporangial suppression of *P. cinnamomi* was shown using field soils collected from three jarrah forest soil vegetation types and a *Banksia* woodland on Bassendean sand, collected in winter and summer. The effect of age of *A. pulchella* plants was demonstrated using the soils collected from rehabilitated bauxite mine pits. In all the locations soils were collected under *A. pulchella* plants and 5 m away from the nearest *A. pulchella*. An effect of soil type was evident as whilst the soil leachates made from the three lateritic jarrah forest soil types where *A. pulchella* is common in the understorey were suppressive to the sporangial stage of *P. cinnamomi*, this effect was not evident in the Bassendean sand under *A. pulchella*. *A. pulchella* soils collected in winter were less suppressive towards sporangial production than soils collected in summer. An effect of plant age was demonstrated as soil leachates from four year-old *A. pulchella* stands in rehabilitated bauxite mine sites were more suppressive for sporangia than leachates from one year-old stands.

Further information on the behaviour of the pathogen in soil and in potting mix with and without *A. pulchella* was obtained by infecting lupin radicles with an isolate of each A2 type, 1 and 2 strains of *P. cinnamomi* and burying them in the soil under the three varieties of *A. pulchella* plants. After a week, the chlamydospores were mostly collapsed and hyphae deteriorated. Oospores were observed and in significant numbers under the potted plants of *A. pulchella* var. *glaberrima*.

Isolates of all three clonal lineages of *P. cinnamomi* found in Australian soil were tested for the ability to produce oospores. Two isolates of the A1 and A2 type 2 and three isolates of the common A2 type 1 were screened. The two isozyme types of the A2

clonal lineage isolated in Australia varied in ability to self and produce oospores *in planta* in several soils from the jarrah forest. The isozyme type 2 of the A2 clonal lineage of *P. cinnamomi* produced oospores under these experimental conditions. This stimulation was not effective for most of the tested isolates of the A2 type 1 and the A1 clonal lineage. The *in planta* oospores were viable but dormant and the oogonial-antheridial associations were amphigynous both *in vitro* and *in vivo*. For the first time it was established that, the stimulus for selfing and oospore formation in the A2 type 2 of *P. cinnamomi* is available in some jarrah forest soils, with and without *A. pulchella* and also in the potting mix used. This raises important questions for the management of the pathogen.

Several factors were identified as potential stimuli for selfing. Among them, soil nutrient levels and essentially enhanced sulphur presence were found important. Temperature also played a key role. Oospores were produced abundantly at 21 – 25 ºC but not over 28 ºC.

The biology of *P. cinnamomi* has been studied for several decades but some important aspects remain un-researched. This thesis pioneers research into the *in planta* selfing aspect of the pathogen in soil. It also improved the understanding of the interactions between *P. cinnamomi* and *A. pulchella* which to some extent supports use of *A. pulchella* as a biological control tool against *P. cinnamomi*. However, attention is drawn to the natural mechanisms of this complex pathogen to survive *in planta* by producing oospores, the most persistent form of its life cycle.

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Publications and presentations

Publications

Jayasekera AU, McComb JA, Shearer BL, Hardy GE St J (2006) *In planta* selfing and oospore production of *Phytophthora cinnamomi* in the presence of *Acacia pulchella. Mycological Research* (Under review).

Jayasekera AU, McComb JA, Shearer BL, Hardy GE St J (2004) Suppression of *Phytophthora cinnamomi* by the root exudates of *Acacia pulchella.* In *Proceedings of the 3rd International (IUFRO) Conference on* Phytophthora *in Forests and Natural Ecosystems*, Freising, Germany, September, 2004. (In press).

Poster presentations

Jayasekera A, McComb J, Hardy G, Shearer BL (2005) Inhibition of *Phytophthora cinnamomi* chlamydospores by *Acacia pulchella. Proceedings of the 15th Australasian Plant Pathology Society Conference*, Geelong, Victoria. September 2005.

Aruni Jayasekera, Jen McComb, Giles Hardy, Bryan Shearer (2004) Behaviour of the asexual spores of *Phytophthora cinnamomi* in the root exudates of *Acacia pulchella. In Proceedings of the 3rd International (IUFRO) Conference on* Phytophthora *in Forests and Natural Ecosystems*, Freising, Germany, September, 2004.

Oral presentations

Aruni Jayasekera, Jen McComb, Giles Hardy, Bryan Shearer (2004) Suppression of *Phytophthora cinnamomi* by the root exudates of *Acacia pulchella*. The 3rd International (IUFRO) Conference on *Phytophthora* in Forests and Natural Ecosystems, Freising, Germany, September, 2004.

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