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The role of Pythiaceous soil-borne micro-organisms in the tuart decline at Yalgorup

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Pythiaceous soil borne micro-organism, encompassing *Phytophthora* species, include a diverse group of pathogens that have been identified as contributing and inciting significant forest declines throughout the world, including the south-west of Western Australia and the world. Continuing research indicates that *Phytophthora* species may have significant, yet unclear, roles in forest declines. Improved methods of molecular species identification indicate a greater diversity of species than once evident from strictly morphological identification, and the ongoing evolution and divergence of new species.

The historical and ongoing movement of *Pythiaceous* organisms, specifically through human transport of infected plant material and soils, is of specific concern for species and ecosystem conservation. Host plants are often exposed to pathogens they have insufficiently evolved defence mechanisms to combat.

Pythiaceous soil pathogens may have a role in the tuart decline as the nature of the canopy dieback indicates a decrease in vascular activity, typical of root disease often caused by soil pathogens, and the expansion of the forest area with symptoms of decline may correlate to the progressive spread of a soil borne pathogen. The aim of this study is to identify the role of *Pythiaceous* soil pathogens in the decline of tuart occurring at Yalgorup.

Isolation of Pythiaceous pathogens

To isolate relevant *Pythiaceous* pathogens, root and rhizosphere soil was harvested from declining trees, exposed with the Air-Spade® (Figure 1). Organisms were isolated by baiting rhizosphere soil and symptomatic roots onto selective media, and by direct plating of harvested roots from

declining trees and tuart seedlings grown adjacent to diseased roots (Figure 2) and in harvested rhizosphere soil grown in an *in vitro* soil bioassay.

Assessment of root characteristics of declining tuart

Fine root proliferation, health and mycorrhizal association was measured as a component of the air spading. Declining trees had a lower numbers of fine roots and mycorrhizae associated with exposed root systems than healthy trees. Exposed fine roots from declining trees also showed characteristics of callus formation over the root tips, and



Figure 1: Fine roots and rhizosphere soil was exposed with the air-spade.



Figure 2: Seedlings planted adjacent to fine roots exposed with the air-spade.

proliferation of fine roots posterior to the callused tip. This typical root morphology has been observed as a histological response of Avocado roots to *Phytophthora cinnamomi* infection (Phillips *et al.* 1987) as the host, walls of the pathogen.

Soil bioassays

The presence of soil borne micro-organisms associated with the decline was deciphered through two *in vitro* soil bioassays assessing the difference in growth of seedlings grown in non-pasteurised soil, containing soil micro-organisms from declining sites, and pasteurised soil from the same sites. An additional *in situ* soil bioassay has been conducted with tuart seedlings planted adjacent to the fine roots of declining and non-declining trees (Figure 2). *Pythiaceous* organisms associated with declining seedlings will be isolated through direct plating onto selective and non-selective media.

Three-month growth data from the first *in vitro* soil bioassay indicates no significant variation between growth rates in pasteurised and non-pasteurised soils from healthy and declining sites.



A statistically significant variation was observed between the growth rates of seedlings grown in organic and nutrient layer soils (Figure 3). All seedlings after three months growth exhibited symptoms of nutrient deficiency.

Injection trial

A preliminary cross-classified injection trial was conducted assessing the application of phosphite and Medicap MD®: complete nutrient (nitrogen, phosphorous, potassium, iron, manganese and zinc), zinc, iron and insecticide as a control method for managing the tuart decline.

The role of *Pythiaceous* pathogens associated with the decline was interpreted through response of the declining trees to the Phosphite application. Phosphite application has been shown to control some *Phytophthora* declines by inducing defence mechanisms in the host at concentrations not directly toxic to the pathogen. The first year of canopy data, indicating the influence of the treatment minus seasonal variation, shows that phosphite applied at 75 g/L induced a clear improvement in canopy condition (Figure 4). A clear decrease in canopy condition was also observed when phosphite was applied at 50 g/L. A clear increase in canopy condition was also observed when phosphite was applied in combination with the complete nutrient and zinc treatment (Figure 5). The validity of all findings is being further verified through continuing trials. An additional large phosphite application trial was conducted to confirm the uptake efficiency and impact of phosphite

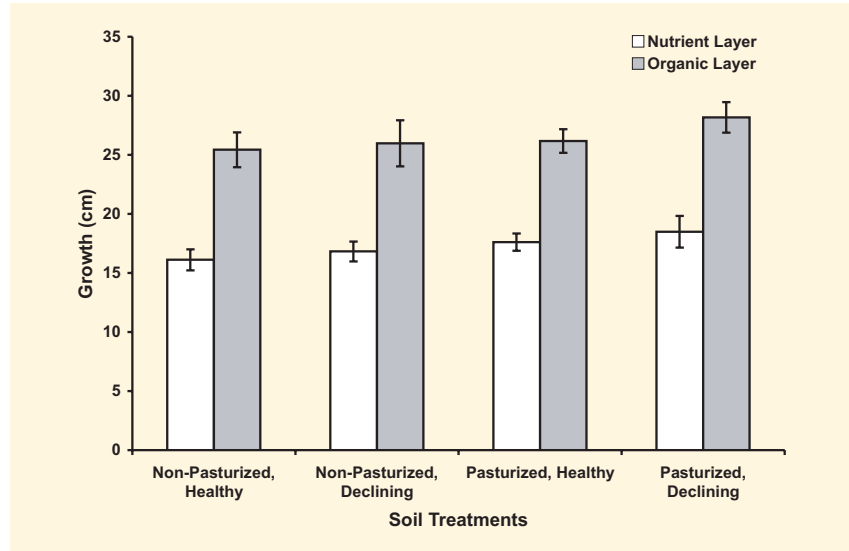


Figure 3: Three-month growth data from the first in vitro soil bioassay. No significant variation between growth rates in pasteurised and non-pasteurised soils from healthy and declining sites.

application at different concentration rates. The first year results from the second phosphite application trial will be available in February 2007.

Ongoing research

- Isolation and identification of *Pythiaceous* microorganisms from the continuing soil bioassay.
- Assessment of the impact of the different soil treatments on seedling growth on the continuing soil bioassays.
- Assessment of the impact of the phosphite, nutrient and insecticide treatments from the initial preliminary injection trial, and the different phosphite applications from the second phosphite application trial, will be continued for the duration of the project.

- Three pathogenicity trials will be conducted to test the pathogenicity of: *Pythiaceous* microorganisms isolated from declining sites and all *Phytophthora* species identified from the south-west of WA.

References

Grimes R. J. (1978) Crown Assessment of Natural Spotted Gum (*Eucalyptus maculata*) Ironbark (*E. fibrosa*, *E. drepanophylla*) Forest. Technical Paper no. 7. Queensland Forest Service, Brisbane, Australia.

Phillips D., Grant B.R., Weste G. (1987) Histological changes in the roots of Avocado cultivar, Duke 7, infected with *Phytophthora cinnamomi*. *Phytopathology* 77, 691-698.

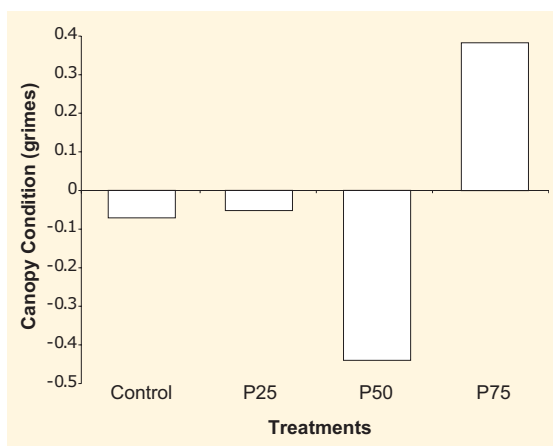


Figure 4: Change in canopy condition (Grimes index) of trees as a result of phosphite application.

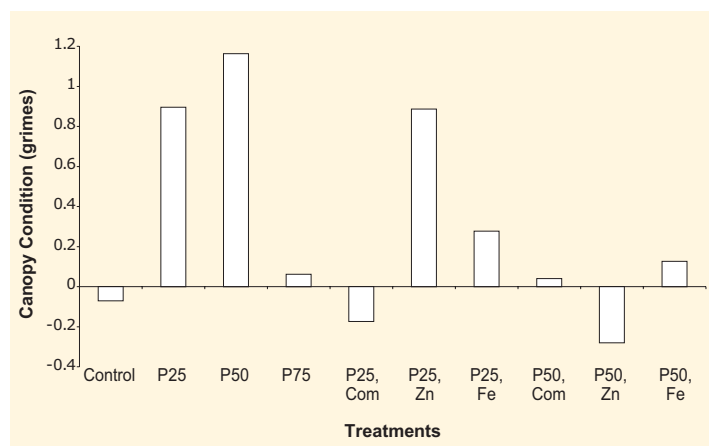


Figure 5: Change in canopy condition (Grimes index) of trees as a result of phosphite and complete nutrient application.