Colonisation of *Phytophthora* taxon Agathis in mycorrhizal root nodules of kauri (*Agathis australis*) – development of a specificity assay

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INTRODUCTION

Kauri trees (*Agathis australis*, Araucariaceae) northern New Zealand possess root nodules (Figs 1 & 2) that house a mycorrhizal endophyte. To-date no knowledge is available on the aetiology or structure of the endophytic association. *Phytophthora* taxon Agathis (PTA) has been identified as a causal agent of kauri dieback (Fig 3) in these culturally and ecologically significant trees (Beever et al. 2009), threatening their conservation. Visualising interactions between kauri, the mycorrhizal endophyte and PTA will help our understanding of the potential for biocontrol of the disease.

Aims:

- Characterise the kauri mycorrhizal association
- Study the infective biology of PTA
- Develop specificity assay using Fluorescent in situ Hybridisation (FISH) to visualise PTA infection.

Fig 1 Kauri root with nodules

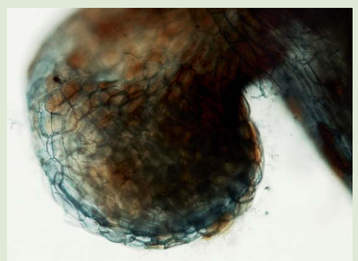


Fig 2 Light micrograph of individual kauri nodule

Fig 3(a) Characteristic lower trunk gummosis associated with PTA infection (b) Chronic crown decline associated with parasitism of cork cambium by PTA

METHODS

Study site: Roots of regenerating kauri growing at Lower Huia Dam in Waitakere Ranges Regional Park (37°00' S; 174°33' E) were collected for study from the top 15 cm of soil.

Clearing and staining: Roots were fixed in 70% ethanol, cleared with 10% KOH, then bleached in H_2O_2. Roots were acidified and stained with Trypan Blue in lactic acid/glycerol, then de-stained in lactic acid glycerol solution (Brundrett et al. 1996) and observed under a light microscope.

SEM/TEM: Roots were fixed in 2.5% glutaraldehyde in 0.1 M mixed phosphate buffer of pH 7 overnight at 4°C. Incubation for 90 min in 1% (w/v) osmium tetroxide in 0.05 M buffer was followed by dehydration in a graded ethanol series. Nodules were dissected with a razor blade under ethanol in a glass Petri dish and transferred into a porous capsule for critical point drying. The nodules were arranged cut face uppermost on adhesive tabs that were attached to SEM stubs. The stubs were stored in a desiccator, then coated with platinum in a sputter coater, and viewed with an FEI XL30 SEM.

FISH: A double-layer plating method (Kleven & McLaughlin 1989) with PDA nutrient agar, enriched with V8-juice was used to grow PTA cultures on glass microscope slides. PTA, *P. cinnamomi*, and a combination of PTA and *P. cinnamomi* were applied to the PDA-V8 layer and incubated for 4 days at 20°C.

KAURI MYCORRHIZAE - SEM

All but a few of the short roots in kauri are converted into nodular structures (Fig. 4). The formation of nodules does not require mycorrhizal infection nor are they dependent upon mycorrhizal infection for their development (Morrison & English 1967). The nodule cortex is colonised by a fungus with structures typical of an arbuscular mycorrhizal fungus. In Fig. 5, the truncated hyphae of a peloton is apparent in the cortical cell, while in Figs 6 and 7 the formation of a small, highly branched arbuscule can be clearly seen.

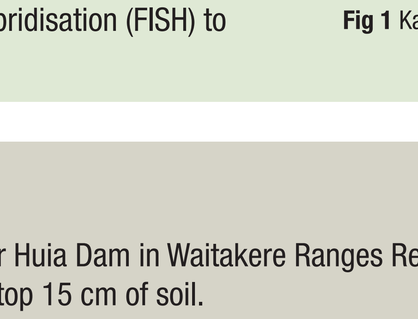


Fig 4 SEM cross-sectional view of kauri nodule developed along root

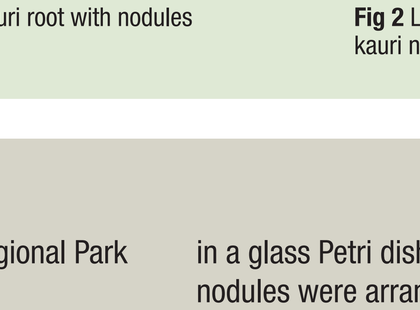
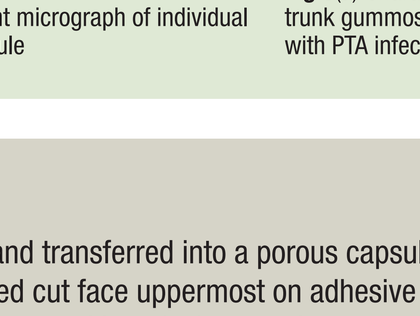
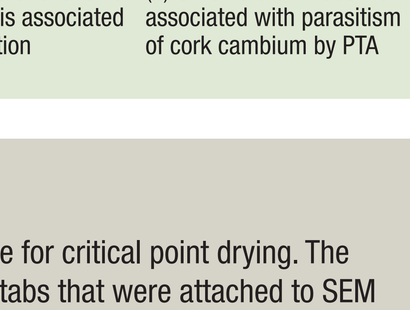


Fig 5 SEM view of internal peloton-structure in cortex





Figs 6 & 7 SEM view of arbuscule-like structure in thickened cortical cell

FUNGAL STRUCTURES - LIGHT MICROSCOPY/TEM

Longitudinal sections (Figs 8 & 9), show each nodule to consist of an outer epidermis and surrounding a cortex. The vascular strand of the developing nodule is connected to that of the main root, and traverses only half the length of the nodule.

Our studies with light microscopy show clearly that the nodule cortex is colonised by a fungus with structures typical of AMF. Figure 10 shows a peloton in the cortical cells. The fungus appears to colonise the root cells as intracellular hyphae (Fig. 11). The presence of tannins does not deter fungal entry into the exodermis (Fig. 12).

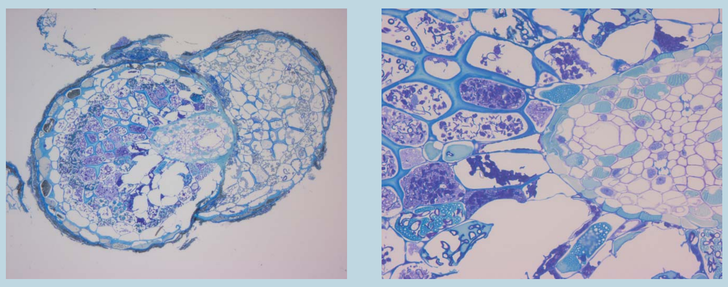


Fig 8 & 9 Longitudinal section showing central vascular bundle. Extensive development of fungal hyphae in cortical cells. Intense staining of arbuscules

Fig 10 Light micrograph of outer cortical cell with peloton/ hyphal coil

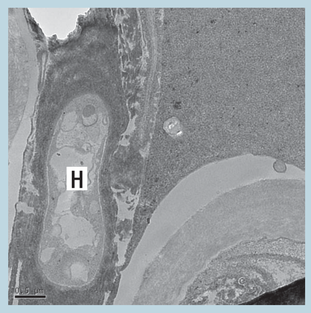


Fig 11 TEM of intracellular hyphae H adjacent to two cortical cells

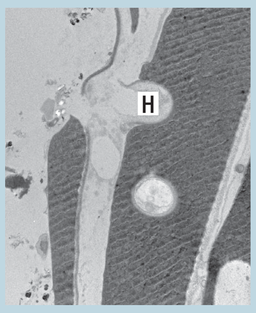


Fig 12 TEM of intracellular hyphae "burrowing" through tannin deposits

FISH PTA SPECIFITY ASSAY

The glass petri dish moist chamber incubator was very useful for the aseptic growth of dual *Phytophthora* cultures (Fig. 13). Characteristic oospores of PTA can be seen growing through the V8-juice enriched media (Fig. 14).

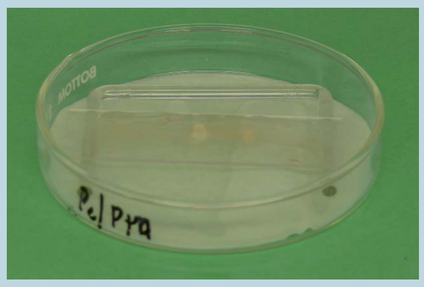


Fig 13 Glass petri dish moist incubation chamber showing filter paper, glass rod, and slide with two agar plugs

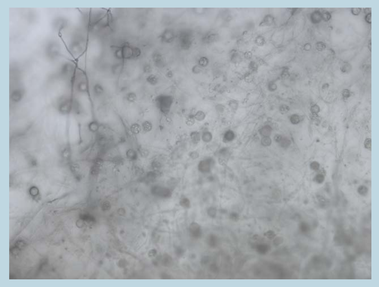


Fig 14 Typical oospores of PTA growing in V8-juice enriched media

NEXT STEPS...

The hybridisation procedure needs to be performed in a darkened room. A hybridisation mix will be prepared by mixing 2 µl of the probe (20 µm) to 125 µL of hybridisation buffer (SET buffer, 0.1% [v/v] Igepal-CA630) and 25 g/ml polyA potassium salt. The hybridisation mix will be added to air-dried slides and incubated for 1.5 hours in the dark (after Li 2011). Epifluorescence microscopy settings will be as follows: 460–500 nm (excitation), 510 nm (emission), with a dichroic long-phase beam splitter = 505 nm (after Vandersea et al. 2006).

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