Assessing the Effect of Phosphite on Arbuscular Mycorrhizal Fungi in the Roots of Kauri (*Agathis australis*)

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BACKGROUND

Kauri dieback, caused by *Phytophthora agathidicida,* is a key threat to the longevity of the native kauri tree.
Phosphite is currently used to treat kauri dieback.
Arbuscular mycorrhizal fungi (AMF) inhabit the roots of kauri and aid in nutrient uptake, water retention and possibly pathogen resistance (1). The effect of phosphite treatment on the AMF inhabiting kauri roots is unclear.

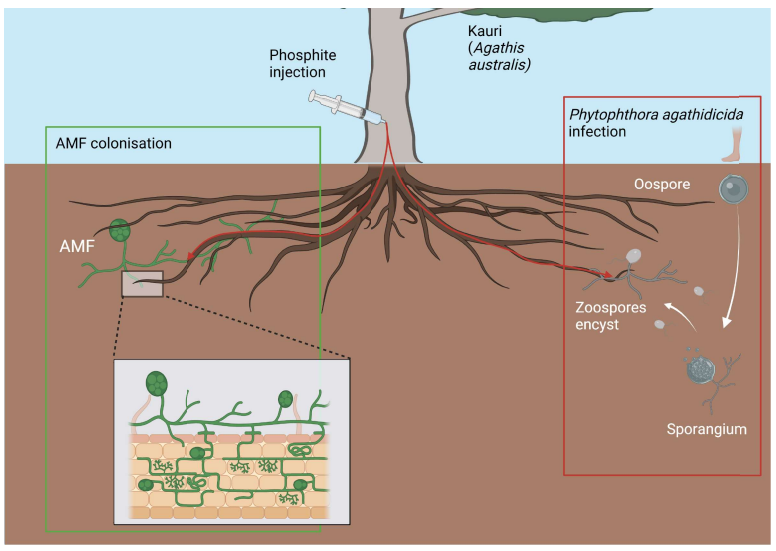


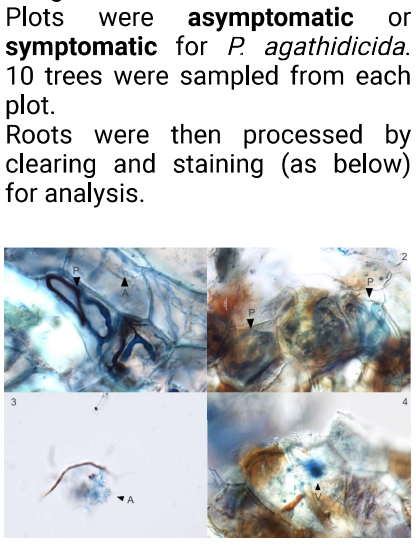
Illustration depicting the presence of both colonising AMF (green box) and *P. agathidicida* (red box) in the roots of a kauri tree . Phosphite treatment illustrated using red arrows.

OBJECTIVES

1. Trial and optimise AMF colonisation quantification in kauri roots.
2. Confirm the presence of and quantify AMF colonisation in selected phosphite-treated trees.
3. Investigate the relationship between soil properties and AMF species diversity and colonisation at each site.

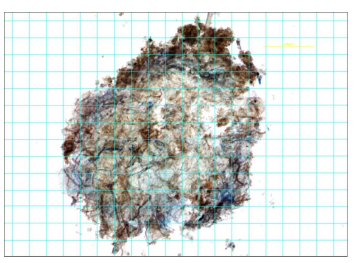
METHODS

Soil and root samples were taken at two sites within the Waitākere Ranges near Piha.
Plots were **asymptomatic** or **symptomatic** for *P. agathidicida*. 10 trees were sampled from each plot.
Roots were then processed by clearing and staining (as below) for analysis.



Peloton (P) coils, fine arbuscule (A) structures and AMF vesicle (V). Scale bar 10 µm.

1 Methods for Quantifying Colonisation (Fig. 3)
3 methods of quantifying colonisation were trialled, including hand sectioning and microtome sectioning. The sections were then manually counted for presence/absence of fungal structures using a grid overlay in ImageJ (below).

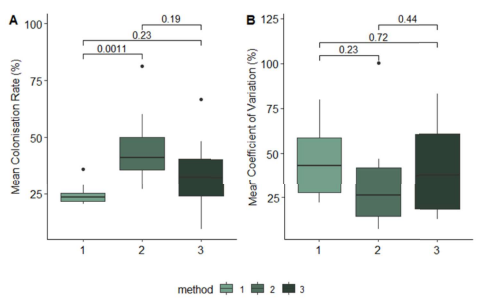


2 AMF Colonisation (Fig. 1, Fig. 2)
Following from Objective 1, the optimised method was used to quantify colonisation rates in eight selected trees (four from each plot).

3 Soil Analysis (Fig. 4)
Soil was sampled at each tree and dried for analysis. Soil was analysed for pH, carbon (C), and nitrogen (N).
Correlation analysis **(Fig. 5)** was done in RStudio to investigate any relationships between soil properties and colonisation.

RESULTS

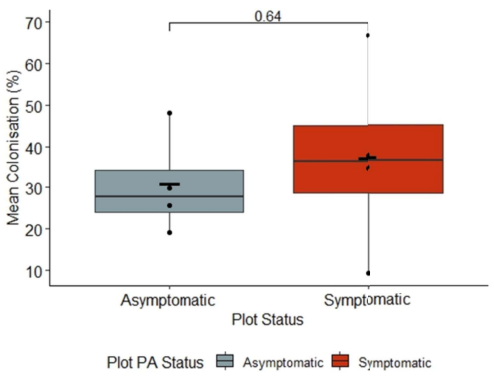
Objective 1
Fig. 3 Statistical Differences in Colonisation Quantification Methods



Box and whisker graph of statistics observed with each method of quantifying colonisation. A) differences in mean colonisation rates observed between methods, B) differences in mean coefficient of variation of the three methods tested.

Three methods were trialled for sectioning and quantifying colonisation in kauri root nodules, including hand and microtome sectioning techniques. The mean of method 1 was significantly different. Coefficient of variation difered but was not significantl. Method 3 was selected for use in colonisation quantification.

Objective 2
Fig. 2 Colonisation Rates in Kauri Root Nodules

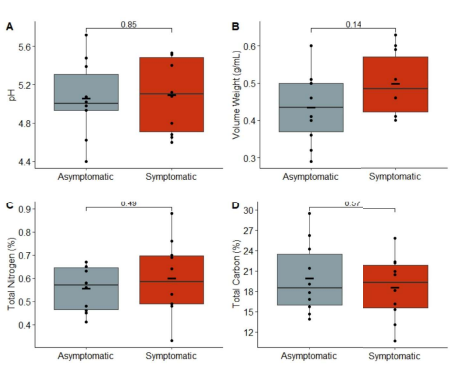


Box and whisker of AMF colonisation rates in roots of eight selected trees. The mean is indicated by the crossbar within each box.

Mean colonisation was 34.0% with a range of 9.4% – 66.8%. There was no significant difference observed between asymptomatic and symptomatic plots.
Colonisation rates at 3 years post-treatment are in line with reported pre-phosphite colonisation rates (2).

Objective 3

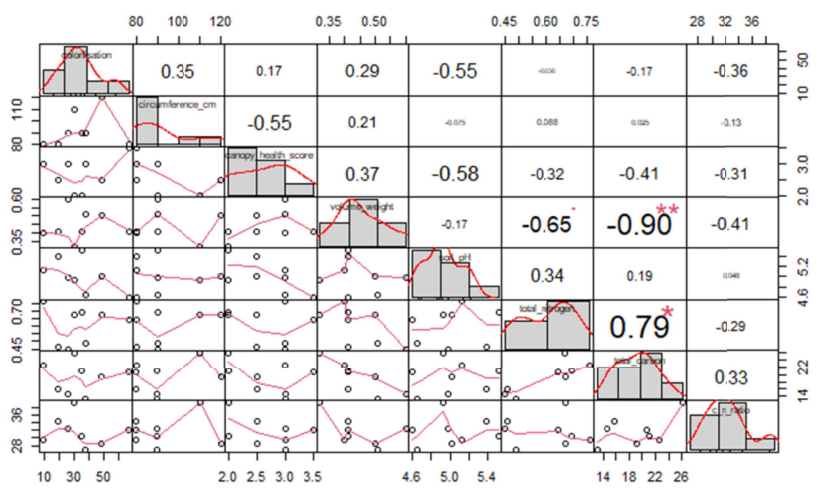
Fig. 4 Soil Properties of Asymptomatic and Symptomatic Kauri Plots



Box and whisker plot of soil properties across the asymptomatic and symptomatic plots

The soil properties observed in each plot were in line with previous data for kauri forests.
Soil pH ranged between 4.4 – 5.7 as expected for kauri forests which produce large amounts of acidic litter (1).
The C/N ratio differed significantly between the asymptomatic (mean = 35.6) and symptomatic (mean = 31.3) plots (p = 0.0046).
Additionally, the canopy health score was better for the asymptomatic plot (not shown here).

Fig. 5 Correlation Plot of Colonisation Rate and Soil Properties



Correlation plot of colonisation rates in eight selected trees with soil and plot characteristics. From left to right the variables are colonisation, tree circumference (cm), canopy health score, soil volume weight (g/mL), pH, total nitrogen (%), total carbon (%), and C/N ratio.

There was a weak negative correlation (correlation coefficient = -0.55, p = 0.17) between soil pH and colonisation rate.

CONCLUSION AND FUTURE DIRECTIONS

Three years after treatment with phosphite, colonisation rates of AMF in the nodules of kauri roots appear comparable to pre-phosphite colonisation levels (2). My data indicates that phosphite may not damage AMF long-term. However, we cannot quantify the effects immediately after treatment and future studies would benefit from comparison to an untreated control group.
Quantifying colonisation in the roots of kauri was a particular challenge during this project due to their dark and recalcitrant tannins. Different methods of hand sectioning and microtome sectoning were trialled. Microtome sectioning provided the best resolution for counting, however it may introduce additional variance. Further optimisation is necessary to explore high-throughput counting methods.
Soil charateristics did not correlate highly with colonisation rate in this study, although soil pH was weakly negatively correlated with colonisation rate.
Future directions include sequencing to ascertain the diversity of AMF colonising phosphite-treated trees.

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(1) Padamsee, M., Johansen, R. B., Stuckey, S. A., Williams, S. E., Hooker, J. E., Burns, B. R., & Bellgard, S. E. (2016). The arbuscular mycorrhizal fungi colonising roots and root nodules of New Zealand kauri Agathis australis. *Fungal biology*, *120*(5), 807-817.
(2) Han, V. (2016). Metagenomic characterisation of AMF and other mycorrhizal communities associated with healthy versus diseased Agathis australis (kauri) roots. University of Auckland .

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