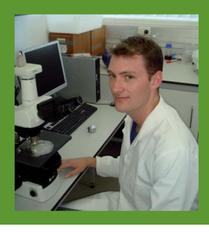
Fishing for *Phytophthora* in the Waitakere Ranges, Auckland, New Zealand



Landcare Research Manaaki Whenua

THE UNIVERSITY OF AUCKLAND NEW ZEALAND Te Whare Wānanga o Tāmaki Makaurau

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Introduction

Kauri (*Agathis australis*) is a dominant tree in forests in northern New Zealand (Wardle 1991). A number of species of *Phytophthora* have been detected in these forests including *Phytophthora* 'taxon Agathis' (PTA) and *P. cinnamomi*, both known pathogens of kauri (Gadgil 1974, Beever et al. 2009).

Stream-based sampling has been employed to detect various *Phytophthora* species at a catchment scale in the US and Australia (Murphy et al. 2009, Smith et al. 2009), suggesting that this method may also be effective in detecting *Phytophthora* spp. in kauri forest.

We aim to extend this approach and determine its applicability for detecting the presence of *Phytophthora* species in kauri forest by:

- surveying six sub-catchments in the Waitakere Ranges for the presence of *Phytophthora* species using different baits and isolation techniques
- identifying correlations between particular species and environmental and temporal variables.

We will determine whether this approach could be used as a passive surveillance method to detect PTA and *P. cinnamomi* at the catchment level.

Methods

Sample sites

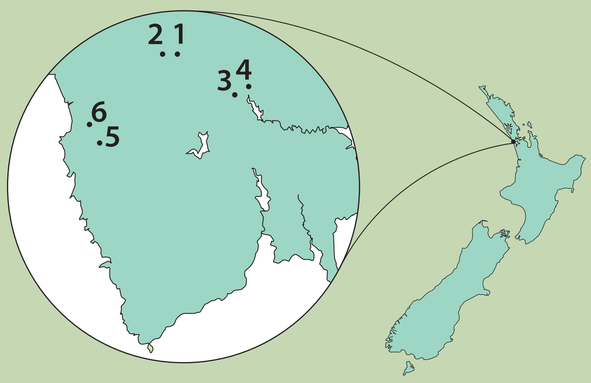

Figure 1. Location of the research sites

Photo: R Stoney

Figure 2. Leaf baiting apparatus in situ at the Cascades B site.

Table 1. Sample sites and incidence of kauri ill-health within the associated sub-catchment based on on-track surveys (N Waipara & A Davis pers. comm.)

Site	Sub-catchment	Kauri ill-heath score
1	Cascades A	High
2	Cascades B	High
3	Nihotupu A	Low
4	Nihotupu B	Low
5	Piha A	High
6	Piha B	High

Baits

Five baits (ten replicates) are being used at each site: 3-day-old germinated lupin seedlings (*Lupinus angustifolius* sourced from Rockfield Pty Ltd, Sassafras, Tasmania), and leaves from locally cultivated Himalayan cedar (*Cedrus deodara*), kauri, kohuhu (*Pittosporum tenuifolium*), and rhododendron (*Rhododendron arboreum*).

Culture and Isolation

After leaving out for 2 weeks, the baits are rinsed in reverse osmosis water and plated to selective agar P_5ARP and/or P_5ARPH (containing hymexazol to inhibit Pythium spp.) (Erwin & Ribeiro 1996) and incubated at 18°C.

After 3–4 days, oomycete-like colonies are subcultured and sorted into morphotype based on:

- colony morphology on PDA at 20°C after 5 days
- sporangial features after transfer to V8 Agar, and subsequent immersion in non sterile soil extract (NSSE)
- oospores presence after a week on V8 Agar, and features if formed.

Isolates representative of the morphotype identified are being examined by ITS sequencing (Cooke et al. 2000).

Results

Catchment studies

Sample 1 baits taken Oct/Nov (spring) 2009 were plated to P_5ARP (Table 2). *Pythium* spp. were prevalent in this sample, hampering the detection and potential recovery of *Phytophthora* spp. isolates. Thus for Sample 2, baits were plated to P_5ARP and P_5ARPH, and recovery compared (Fig. 4).

Table 2. Comparison of the different morpho-species isolated from the first two samplings.

Morphotype \ Site	Sample 1 (Oct/Nov '09)						Sample 2 (Dec/Jan '10)					
	1	2	3	4	5	6	1	2	3	4	5	6
I (ITS Clade 2)						•	•	•	•	•		•
II (ITS Clade 6)		•				•			•		•	
III (*P. kernoviae*)		•			•	•		•			•	
IV (Unknown *sp 1*)		•						•			•	

The *Phytophthora* species recovered were classified into four groups, based on morphological characteristics and ITS sequencing. Preliminary results indicate Groups I and II could be placed in ITS Clades 2 and 6 respectively. Group III has been determined as *P. kernoviae*. Group IV was not readily placed in the recognised clades. PTA and *P. cinnamomi* were not detected in these samples, although these species are known to be present within at least some of these catchments.

Bait type

Numerous oomycete-like isolates were recovered from all baits. The majority of *Phytophthora* isolates were from rhododendron midrib (Fig. 3). This may be because softer or damaged baits such as lupin radicles and injured Himalayan cedar needles were more prone to infection by *Pythium* spp. These *Pythium* spp. are likely to have prevented *Phytophthora* from being isolated unless the baits processing methods utilized P_5ARPH.

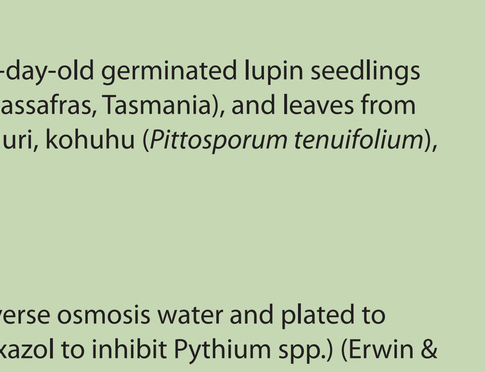

Figure 3. Comparison of the number of *Pythium* spp. versus *Phytophthora* spp.for each bait type, across both samples.

Evaluation of hymexazol

While not all *Phytophthora* spp. are resistant to hymexazol, the two main target species PTA and *P. cinnamomi* are. Therefore we have chosen to use P_5ARPH as part of our routine analysis. Initial analysis shows that hymexazol was effective, given that when used it resulted in a greater number of isolates of *Phytophthora* spp., and comparatively fewer *Pythium* spp., being retrieved.

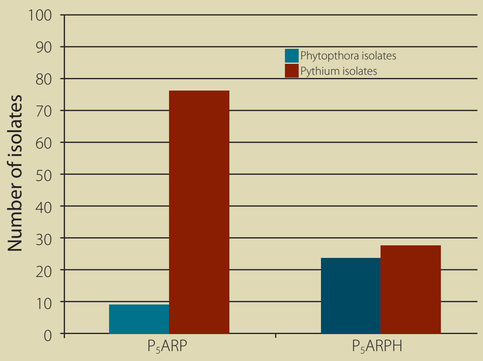

Figure 4. Comparison of the number of isolates that were *Phytophthora* spp. and *Pythium* spp. in the presence or absence of hymexazol.

Future work

- Complete full year of bi-monthly sampling
- Compare stream baiting with direct water filtration
- Examine long-term storage options for isolates.

Conclusions

- The sampling thus far demonstrates proof of concept for the use of this method to satisfy the two stated aims, despite PTA not yet being retrieved
- Variation seen in the two samples shows this method will be able to identify correlations between species detected and temporal and spatial variation.

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