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Kauri Killer on the Loose? – study of human vectors and PTA hygiene treatments

ALVINA PAU'UVALE¹, CHANDAR DEWAN¹, HELEN MORA², NICK WAIPARA³, STAN BELLGARD⁴

BACKGROUND

Kauri Dieback has been identified as an increasing problem affecting kauri (*Agathis australis*) across the Auckland and Northland region. *Phytophthora* taxon Agathis (PTA) has been identified as a causal agent of Kauri Dieback (Beever et al. 2008).

In the Waitakere Ranges Regional Park hygiene prescriptions have been instigated by Auckland Council. These include the installation of boot wash stations at the start and end of the Auckland City Walk (Fig. 1).

The role that humans play in the local-, landscape- and regional-scale spread of *Phytophthora* diseases has been demonstrated for the sudden oak death pathogen *P. ramorum*. (Cushman and Meentemeyer 2005). The present study aimed to examine: 1) What *Phytophthora* species are collected in the boot-wash soil grates? 2) Is PTA able to be transferred via footwear to neighbouring Kauri trees? 3) Is Trigen II Advance able to kill/suppress other *Phytophthora* species found in the kauri forest?



Figure 1: Boot Wash Station – City Walk, Cascades. The soil collected under the grate was removed for study.

Bioassay of boot wash station – Kitekite (Piha) and Auckland City Walk

Soil samples were collected from the Boot Wash Station at the Kitekite track in February, 2010 by Auckland Council Regional Staff (so-called Piha 8). Another sample was obtained from the Auckland City Walk Boot wash station in June, 2011. Two sub-samples were taken and bioassayed using the established standard operating protocol for PTA detection (Beever et al. 2010) (Fig. 2).

A total of seven oomycete fungi were recovered from the soil collected in the Boot wash station at Piha 8 (Table 1).

Table 1: List of Isolates from the Piha 8 Boot wash station (sampled February, 2010)

Sample Number	<i>Phytophthora</i> spp.	Growth morphology (PDA)	Oospores in single culture
AL 01	<i>P. multivora</i>	Stellate	Yes
AL 02	<i>Pythium</i> sp.	Cottony petaloid	Sterile
AL 03	<i>P. gonapodyides</i>	Cottony petaloid	Sterile
AL 04	<i>P. gonapodyides</i>	Cottony petaloid	Sterile
AL 05	<i>Phytophthora</i> taxon PgChlamydo	Cottony petaloid	Sterile
AL 06	<i>P. citrophthora</i>	Stellate	Sterile
AL 07	<i>Pythium</i> sp.	Rosette	yes

P. multivora and *P. citrophthora* were also recovered from the Auckland City Boot wash station soil sample collected in June, 2011.

P. multivora is considered to be an introduced threat to kawakawa (e.g. Ho et al. 2010). *P. citrophthora* is a known pathogen of citrus (Erwin and Ribeiro 1996). These isolates were used in the TriGene II Advance efficacy experiment.

Bioassay of soil/mud from boots

In order to assess the potential for humans to vector *Phytophthora* species in wet-soil conditions, soil was collected from five pairs of boots belonging to; Chandar Dewan, Lee Hill (AC), Geraldine Lee-Kum, Kenny Williams, and Alvina Pau'uvle (project leader). Two sampling efforts were made as we walked around the City Walk, at the Cascades. The first sample was carried out half way along the walk, and the second sample upon returning to the starting point.

Sample 19, 21, 23, and 24 represented the soils scraped from three pairs of boots mid-way along the Auckland City Walk. The recoveries of *Phytophthora* species from boot-samples, is the first report of its kind in New Zealand from the conservation estate. And to our knowledge, this represents the first demonstration that wet-weather tracks (i.e. in winter) contain *Phytophthora* species.

Of particular significance, Sample 23 (i.e. Kenny's boots) contained three different *Phytophthora* species. Although we didn't find PTA in these boot samples, we have demonstrated that boots and the mud/soil they carry do contain *Phytophthora* species that are known to pose a threat to New Zealand's flora of the kauri forest. For example, Sample 24 contained *P. cinnamomi* - *P. cinnamomi* is a known pathogen of kauri (Podger and Newhook 1971).

TriGene II Advance efficacy experiment on other *Phytophthora* species

To assess the efficacy of Trigen Advance II (TriGene) we transferred 6 mm plugs from the advancing edge of the *Phytophthora* species (AL01-07) from PDA agar onto 2% Trigen incorporated into PDA agar. We observed the plated plugs after 14-days under a microscope and recorded the growth (if any) of the *Phytophthora* species mycelium from the agar plugs. In order to assess the viability of the plugs after treatment with TriGene, the hyphal plugs were removed from 2% TriGene Kill plates and transferred to fresh PDA plates and hyphal outgrowths (Fig. 3).



Figure 2: Soil bioassay containers showing lupins floating on water surface prior to being plated to *Phytophthora*-selective agar

Table 2: Boot soil sample isolates recovered from bioassay (sampled June, 2011)

Bioassay samples 20/06/11	Source of sample	Original Isolate number	DNA sequencing Isolate label	PCR Seq data
19	Site 1, Mr Dewans' Boots	19.3	23	<i>P. cryptogea</i>
21	Site 2, Alvina's Boots	21.5	1	<i>Pythium</i> sp.
21	Site 2, Alvina's Boots	21.4	24	<i>P. citrophthora</i>
23	Site 2, Kenny's Boots	23.2	17	<i>P. cinnamomi</i>
23	Site 2, Kenny's Boots	23.6	18	<i>P. gonapodyides</i>
23	Site 2, Kenny's Boots	23.7	8	<i>P. multivora</i>
24	Site 2, Mr Dewans' Boots	24.1	2	<i>P. cinnamomi</i>
28	Twin Peaks Track	28.1	13	<i>P. gonapodyides</i>



Figure 3: Plating of *Phytophthora* plugs from TriGene "Kill plates" to fresh PDA plates

CONCLUSIONS

Piha 8 and Auckland City Walk Boot wash stations

- The *Phytophthora* in the grate soils is still viable, even after one year
- It would be important to remove the contaminated soil (containing *Phytophthora* species) from the grates to a secure, contained land-fill, so that the *Phytophthoras* are not spread

Humans as vectors of *Phytophthora* species

- During winter, in wet soil conditions, a number of *Phytophthora* species were picked up by boots from the muddy track.
- Boot wash stations are essential, to capture the mud and soil hikers have accumulated from the track
- If they do not wash down their boots, then the *Phytophthora* species could be moved accidentally to other kauri forest areas.

Efficacy of TriGene Advance II to suppress/kill other *Phytophthora* species

- TriGene Advance II (at 2%) completely suppresses *Phytophthora* species and is enough to kill the *Phytophthora* species in the laboratory demonstration.
- Hygiene treatments are necessary to control the movement of *Phytophthoras* through the Waitakere Ranges, especially as part of human recreation (Fig. 4)



Figure 4: Example of runner passing-over foot mat soaked with TriGene Advance II

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