Comparative efficacy of disinfectants against *Phytophthora* Taxon *Agathis* (PTA)

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Introduction

Phytophthora taxon *Agathis* (PTA) has been recognized as posing a threat to the health of kauri (*Agathis australis*) in northern New Zealand (Beever et al 2009). Affected trees show a collar rot associated with unusual gummosis leading to tree death. While trees showing such symptoms have been reported widely, they are not universally present. In order to reduce further movement of PTA, Auckland Regional Council (ARC) has instigated various precautionary policies, including the use of footwear hygiene procedures for park visitors (Fig 1).

This project has investigated the efficacy of various disinfectants against PTA:

1.	***Trigene II Advance*** A mixture of halogenated tertiary amines — Active against fungi, bacteria and viruses — Recommended label rate is 2%
2.	***Phytoclean™*** — A quaternary ammonium compound registered in Australia — For the treatment of *Phytophthora cinnamomi* — Recommended label rate is 10%
3.	***Virkon® S*** — A dipotassium peroxodisulphate product — Registered in New Zealand for emergency infectious disease control (for use in cleaning and disinfecting Industrial, Animal and Agricultural Facilities) and is efficacious against a range of viruses, bacteria and fungi — Recommended label rate is 1%.
4.	***Janola®*** — A household formulation of sodium hypochlorite — Widely used disinfectant and bleach — Its recommended label rate is 5% to disinfect gardening tools and equipment.
5.	***Citricidal®***— A product based upon grapefruit seed and pulp extracts — Recommended as a bactericide and fungicide in both pre- and post-harvest treatment of fruits — Range between 0.005–0.025%

Photo Nick Waipara

Figure 1: A runner passing-over foot mat soaked with disinfectant, Auckland Regional Park

Methods and Materials

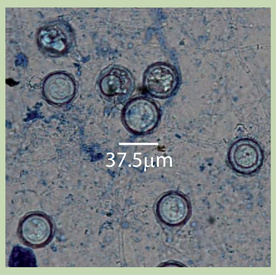
Mycelial inhibition

Actively growing 6.5 mm diameter plugs of PTA were placed on PDA amended with the five disinfectant treatments. The plates were incubated at 20ºC and colony growth scored at 4 days. To check on viability plugs were removed after 8-days, plated to fresh PDA plates and assessed for growth 3–5 days.

Oospore inhibition

Oospore suspensions (containing c. 250 oospores from 56-day old clarified V8 liquid culture) were added to plates containing 0.6% water agar amended with each of the five disinfectant treatments at levels identified as lethal to mycelium After 10 days (at 20°), viability was assessed using tetrazolium staining (Jiang & Erwin 1990) as follows: pink=dormant; red=activated; black=non-viable.

Characteristic colony of PTA on *Phytophthora-selective P_5ARP* media growing from a trimmed leaf-bait of Himalayan Cedar (*Cedrus deodara*)



PTA Oospore preparation

Semi-papillate zoosporangium of PTA

Zoospore inhibition

Zoospore suspensions produced by incubating PTA colonies growing on V8 juice agar in non-sterile soils extract, were added to disinfectant solutions to give the recommended label rates. The contents were plated to P_5ARP selective medium for Phytophthora species and colony forming units (CFU's) per ml were counted after 3 days.

Inhibiting PTA in soil

Two methods were used. In the first, 20g soil samples spiked with 1500 oospores/g were placed in mesh bags and soaked in the disinfectants at their label rates. The treated soil was assayed using the extended bioassay method (Stack & Millar 1985) and the number of CFUs/ml was also determined.
In the second, boot were surface sterilised and pressed into soil spiked with oospores (Fig 2). The boots were then cleaned by spraying the boot to run-off using hand-held, commercial pump-packs sprayer containing the disinfectants at label rates (Fig 3). The treated soil left adhering to the boot was scraped off after the spray treatment and bioassayed for PTA using the extended bioassay method.

Figure 2 & 3: Rubber gumboot being pressed into soil "spiked" with PTA oospores (1,500 /g) and then sprayed with disinfectant to run-off

Results

Mycyelial inhibition (Fig 4)

- TriGene and Phytoclean completely suppressed growth of PTA mycelium at all concentrations and no mycelium grew from plugs after 8-days exposure.
- Virkon (at 0.2 and 0.1% a.i.) completely suppressed growth of PTA and no mycelium grew after 8-days exposure.
- Janola (at 0.2, 0.1, 0.05% a.i.) completely suppressed PTA. No mycelium grew after 8-days exposure.
- Citricidal inhibited PTA growth at all concentrations, but mycelial plugs grew out after 8-days exposure.

Oospore inhibition (Fig 5)

- The majority (c. 80%) of the oospores in the unamended control were dormant, about 10% were activated and the remainder (c. 10%) were non-viable (Fig 5).
- Virkon and Janola significantly reduced oospore viability, whereas Trigene Phytoclean and Citricidal had little effect (Fig 5).

Zoospore inhibition

- Trigene (2%), Phytoclean (10%), Virkon (1%) and Janola (5%) all proved lethal to zoospores.
- Many of the zoospores placed in the Citricidal and Control (i.e. RO water) survived.

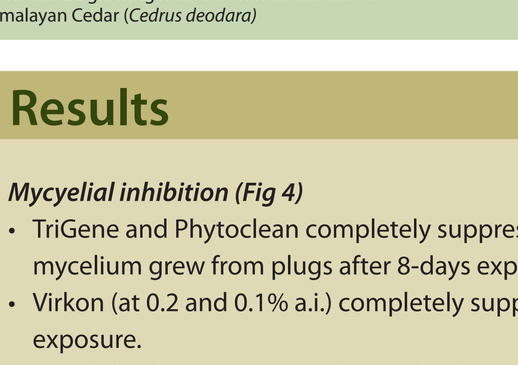


Figure 4: Growth rates of PTA (mean of five replicates) on agar amended with 5 disinfectants (Tri = TriGene; Q = Phytoclean; V = Virkon; NaOCl = Janola; Cit = Citricidal; Cont = unamended control) after 4 days (absence of columns equals zero).

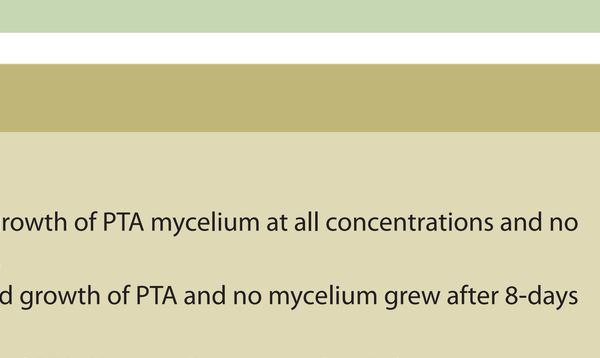


Figure 5: Oospore viability counts after 10-days incubation in the 5 disinfectants. Bars with the same letter are not significantly different (P = 0.05).

Inhibiting PTA in soil (Table 1)

- TriGene and Phytoclean, completely suppressed PTA and all soil fungi/bacteria (Table 1).
- Virkon and Janola also completely suppressed PTA (Table 1). However, Virkon- and Janola-treated soil did not suppress all soil fungi and bacteria (Table 1). *Pythium* sp. were found in the soils treated with Virkon and Janola respectively and bacteria were also associated with the Virkon treated soil.
- Citricidal and the RO water (control) did not suppress PTA.

Table 1: Ability of disinfectants to kill PTA in soil. Data represent total number of leaf baits colonised out of 30 and mean number of colonies formed on P_5ARP after 3 days (n = 3).

Soil treatment	Leaf Baits	Mean CFUs /ml from soil bioassay water		
		PTA	*Pythium* spp.	Bacteria
TriGene (2%)	0	0	0	0
Phytoclean (10%)	0	0	0	0
Virkon (1%)	0	0	17.5 ± 24.8	12.5 ± 17.7
Janola (5%)	8 *Pythium* spp.	0	45.0 ± 26.1	0
Citricidal	3 PTA 1 *P. cinnamomi*	5.0 ± 5.8	47.5 ± 41.0	13.3 ± 14.1
RO water control	3 PTA	10.0 ± 5.7	44.7 ± 12.0	67.0 ± 23.0

Inhibiting PTA in soil on boots

- Spray treatment of spiked soil on boots with TriGene (2%), Phytoclean (10%) and Virkon (1%) significantly decreased the number of leaf baits colonised by soil fungi and completely suppressed PTA.
- Janola did not significantly decrease the amount of soil fungi in total, but did suppress PTA after spray application.
- Post-spray treatment, PTA was only recovered from boots sprayed with Citricidal and RO water.

Summary of comparative efficacy experiments

Disinfectant	Activity against mycelium	Activity against oospores	Activity against zoospores	Ability to kill PTA in soil	Ability to kill PTA in soil on boots
TriGene (2%)	Complete mortality	Little or none	Complete kill	Complete inhibition (incl. *Pythium*)	Yes
Phytoclean (10%)	Complete mortality	Little or none	Complete kill	Complete inhibition (incl. *Pythium*)	Yes
Virkon (1%)	Inhibited radial growth to 1.5 mm/day	Some efficacy	Complete kill	Complete inhibition	Yes
Janola (5%)	Inhibited radial growth to 1.15 mm/day	Some efficacy	Complete kill	Complete inhibition	Yes
Citricidal (0.15%)	Fungistatic only - did not kill mycelium	None	None	No effect	No

Conclusions

- TriGene II Advance (2%) is a suitable disinfectant for controlling PTA, killing propagules of PTA, and reducing the infective capacity of soil containing PTA.
- Phytoclean is as effective as TriGene. Consideration could be given to registering this product, or similar quaternary ammonium products, for phytosanitary applications in New Zealand.
- Virkon and Janola effectively suppress the spread of PTA inoculum contained in soil. However these products have limited application because of reports of corrosivity to metal tools and "bleaching" of clothing.

References

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