Kauri Dieback: Kauri Hygiene – small project

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Summary

Project and Client
MAFBNZ required research to assess the efficacy of current hygiene methods to suppress (i.e. preventing it from growing or developing) and control the plant pathogen, *Phytophthora* taxon Agathis (PTA). The current hygiene product used by Auckland Regional Council (ARC) is 2% TriGene™. MAFBNZ are also keen to identify other potential candidates for consideration as alternative hygiene methods (e.g., quaternary ammonium products, sodium hypochlorite and Citricidal®, a grapefruit seed and pulp extract).

Methods
To achieve these aims, we carried out a series of *in vitro* and soil-based bioassays to obtain specific information about:

- Expt 1. The direct biocidal efficacy of the disinfectants TriGene™ (II) Advance, Phytoclean™, Virkon® S, Janola® and Citricidal®, on PTA mycelium
- Expt 2. The direct biocidal efficacy of these disinfectants on oospores and;
- Expt 2b. The direct biocidal efficacy of these disinfectants on zoospores (i.e. the inoculum of PTA)
- Expt 3. The infective capacity of PTA inoculum
- Expt 4. The direct ability of these disinfectants to kill PTA in soil and
- Expt 5. The direct ability of these disinfectants to kill PTA in soil adhering to rubber gum-boots.

Results

Expt. 1 Sensitivity of PTA mycelium to disinfectants

TriGene, and Phytoclean completely suppressed growth of PTA mycelium at all *in vitro* concentrations tested. Only TriGene and Phytoclean resulted in complete mortality of the hyphae of the pathogen contained in the mycelial plug. Virkon (at 0.2 and 0.1% a.i.) reduced growth of PTA by at least 95%. At 0.05% a.i. it reduced growth by 77%, at 0.025% a.i. it reduced growth by 54%, and at 0.0125% a.i. it reduced growth by 27% (compared with the control). Janola (at 0.2, 0.1, 0.05% a.i.) completely suppressed PTA. At 0.025% a.i. it inhibited growth by 54% and at 0.0125 % a.i., it inhibited growth by 18% (compared with the control). Citricidal was demonstrated to be fungistatic (i.e. inhibited growth but did not kill the mycelium) at all concentrations.

Expt. 2 Sensitivity of PTA oospores to disinfectants

The majority of the oospores in the unamended control were dormant (approx. 80%). About 10% of the oospores in the control were non-viable and the remaining 10% were activated. Virkon (0.2% a.i.), and Janola (0.05% a.i.) had the most significant impact on oospore viability. Virkon killed significantly more oospores than Janola – and both Virkon and Janola were more lethal than either TriGene (0.0125% a.i.), Phytoclean (0.0125% a.i.) and/or Citricidal.

Expt. 2b Sensitivity of PTA zoospores to disinfectants

The zoospores that were placed into TriGene (2%), Phytoclean (10%), Virkon (1%) and Janola (5%), did not survive the treatment. The zoospores that were placed in the Citricidal and Control (i.e. RO water) survived the treatment and produced a mean of 784 ±38 / ml, and 404 ± 70 / ml colonies of PTA after 2 days.

Expt. 3 Infective capacity of PTA inoculum

The soil "spiked" with 2000 oospores / g of PTA colonised 37% of leaf baits – confirming the infective capacity of PTA oospore-inoculum.

Expt. 4 Ability of disinfectants to kill PTA in soil

The spiked soil that was soaked in TriGene (2%) and Phytoclean (10%) completely suppressed PTA, and all soil fungi. Virkon (1%) and Janola (5%) suppressed PTA, but soil treated with Virkon (1%) and Janola (5%) did not suppress all soil fungi and bacteria.

Expt. 5 The ability of disinfectants to kill PTA in soil on boots

PTA was not recovered from soiled rubber gum-boots sprayed with TriGene (2%), Phytoclean (10%), Virkon (1%) and Janola (5%). The rinsate from spraying with RO water alone did not suppress PTA. Soil adhering to boots sprayed with TriGene (2%), Phytoclean (10%), Virkon (1%) and/or Janola (5%) significantly reduced the infective capability of PTA inoculum.

Conclusions

- Disinfecting soiled rubber gum-boots with a 2% spray treatment of TriGene II Advance effectively suppresses the inoculum of PTA. TriGene achieves this in part, by being biocidal to PTA mycelium. TriGene could also limit the spread of propagules of PTA, by its ability to kill zoospores of PTA. TriGene effectively suppresses the infective capacity of PTA in soil at its recommended label rate of 2%.
- Phytoclean demonstrated a similar efficacy to TriGene, in that it completely suppressed the growth of PTA by killing the mycelium. Phytoclean could also limit the spread of propagules of PTA, by its ability to kill zoospores of PTA. Phytoclean demonstrated efficacy at its label/recommended rate (i.e. 10%) to effectively suppress the spread of PTA inoculum contained in soil.
- Virkon only suppressed growth of PTA at higher *in vitro* concentrations and at these concentrations it was lethal to mycelium. Virkon could also limit the spread of propagules of PTA, by its ability to kill zoospores of PTA at its recommended label rate. Virkon (at 1%) also demonstrated efficacy at suppressing PTA's infective capacity in soil when applied as a spray application. Further research is required to understand the difference between spray-applied efficacy versus *in vitro* efficacy.
- Janola demonstrated a similar efficacy to Virkon, in that it completely suppressed the growth of PTA by killing the mycelium at higher *in vitro* concentrations. Janola could also limit the spread of propagules of PTA, by its ability to kill zoospores of PTA at its recommended label rate. When applied as a spray-treatment, Janola (5%) demonstrated efficacy at its recommended rate to effectively suppress the spread of PTA inoculum contained in soil, but like Virkon was not as effective *in vitro.* Further research into the "mode of action" of Virkon and Janola could assist in understanding the differences between spray-treatment efficacy and *in vitro* efficacy of these disinfectants.
- Citricidal, while demonstrating successful *in vitro* fungistatic inhibition (i.e. slowed down the growth process) of PTA, did not kill mycelium like the other disinfectants. Citricidal had no effect on zoospores of PTA, and did not reduce the infective capacity of PTA inoculum contained in soil.
- Spraying with RO water alone does not reduce the infective capacity of PTA inoculum contained in soil.

1. Introduction

Phytophthora diseases worldwide

From a global perspective, more than 66% of all fine-root diseases and more than 90% of all collar-rots of woody plants are mediated by *Phytophthora* species (Erwin & Ribeiro 1996). During the recent decades a series of devastating diseases of broad-leaved tree species in Europe and the USA has focussed research on the role of *Phytophthora* in natural ecosystems Much new information has accumulated, and several new *Phytophthora* taxa have been described in Europe (Jung et al. 2002, 2003), and the western USA (Hansen et al. 2000). In California and some localities of Oregon the airborne species *P. ramorum* is responsible for the so-called "Sudden Oak Death Syndrome": a rapidly spreading epidemic of tanoak and several oak species that is characterised by multiple cankers along the stem and in the crown (Rizzo et al. 2002). In riparian and forest stands in Western, Central, and Southern Europe various alder species are suffering from a widespread and often lethal root and collar rot caused by the alder *Phytophthora* (Brasier et al 2004).

In the agricultural and horticultural context, it has been clearly demonstrated that *Phytophthora* can be prevented from spreading by a variety of integrated chemical and physical methods. In the field or glasshouse fumigation using steam heat and/or metham sodium has been shown to suppress *Phytophthora* for at least half a year (if applied before a crop is planted). Total sanitation is very expensive, however, and in the real world, it is seldom achievable, due to the resistant nature of some of the propagules of certain *Phytophthora* species, e.g., oospores and chlamydospores (Erwin & Ribeiro 1996).

Approved *Phytophthora* disease control in natural forests has a number of risk and human-health constraints that limit the widespread application of agricultural chemical-control methods. However, successful outcomes have been reported using potassium phosphonate foliar sprays (e.g., Aberton et al. 1999) and phosphonic acid injections in stopping the growth of *Phytophthora* species (Jackson et al. 2000) in natural ecosystems. Evidence from conservation areas in Eastern Australia (Tasmania) and mining operations in Western Australia have demonstrated that spread of the inoculum of the related species *P. cinnamomi* can be limited by the removal of mud/soil at "boot wash stations", which reduces the ingress of spores (Tasmanian DPI 2004; Colquhoun & Kerp 2007).

PTA defined

Phytophthora taxon Agathis (PTA) was first recovered in 1972 from unhealthy stands of kauri (*Agathis australis*) on Great Barrier Island (Gadgil 1974), where it was associated with a distinctive collar-rot. It was initially identified as *P. heveae*, the causal agent of "black stripe" of *Hevea brasiliensis* (rubber), but subsequent molecular studies indicate that while it resembles this species it is more closely related to *P. castanea* (= *P. katsurae*) from Japan and SE Asia (Beever et al. 2009). However, it lacks the highly rugose (bullate) oospore ornamentation characteristic of this species and is probably new to science, hence it has been named PTA pending further study (Plate 1 depicts the slight raised protuberances associated with PTA, while *P. heveae* is smooth in texture). Pathogenicity tests indicate that PTA is highly pathogenic to kauri, but not to other kauri ecosystem associates (Gadgil 1974; Beever et al. 2008). PTA was recovered from the Waitakere Ranges near Auckland city in 2006 and is presently also known from Pakiri Reserve and Trounson Kauri Park, Northland. However, disease symptoms are more widespread, raising concern that PTA poses a threat to both kauri ecosystems and iconic giant trees (Beever et al. 2008, 2009).

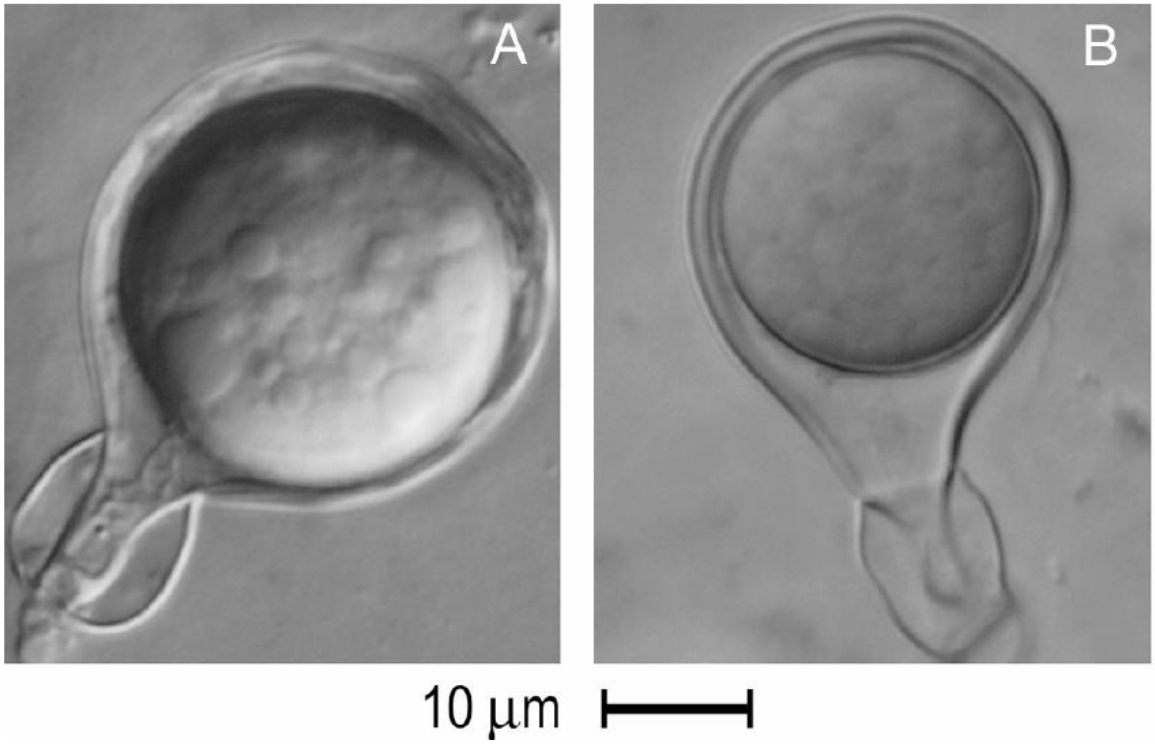

Plate 1: Oospores of PTA (A) versus *P. heveae* (B). Note oospore on left is slightly bigger and slightly rugose compared with the smaller, smoother oospore on the right hand side (from Beever et al. 2008)

Concern about this threat led the Auckland Regional Council (ARC) to initiate a management response in May 2008 (J. Craw, ARC Biosecurity, pers. comm.). In October 2008 a Joint Agency Response involving MAF Biosecurity, Department of Conservation and the 6 northern Regional Councils was initiated and PTA was declared an unwanted organism. Since that time, hygiene kits (aimed to limit the spread of soilborne PTA inoculum) have been provided at the start of walking tracks in the Waitakere Regional Park. The current recommended hygiene prescriptions for activities in kauri forest developed primarily by the ARC can be summarised as follows (http://www.arc.govt.nz/environment/biosecurity/kauri-dieback/kauri-dieback-how-you-can-help_home.cfm):

- Shoes, tyres and equipment are to be clean of dirt/soil before entering kauri forest.
- Shoes and any other equipment that comes into contact with soil need to be cleaned of adhering soil after every visit, and also if moving between bush areas.
- Keeping to defined park tracks at all times to prevent movement of soil that has the potential to spread the disease (ARC 2009).

Hygiene chemicals

TriGene™ (II) Advance (TriGene) has as its main active ingredient a group of halogenated tertiary amines. Until now, ARC has been using an earlier formulation of TriGene. We have tested the efficacy of *TriGene II Advance* on PTA on the understanding that this new formulation will likely to be adopted by ARC as the new standard. Microbial tests have demonstrated its efficacy against a range of micro-organisms including bacteria, viruses, and fungi (including the soil fungus, *Aspergillus niger*). Its recommended label rate is 2% (Table 1). It does not deteriorate if "stored correctly" (however, it is recommended that is be stored out of direct sunlight), is biodegradable (Medichem International Ltd 2008; Appendix 3), and has low mammalian toxicity.

Phytoclean™ (Phytoclean) is a disinfectant cleaner specifically designed for the control of *Phytophthora cinnamomi* in horticulture, plantation, and earth-moving industries. Phytoclean is based on the quaternary ammonium compound, benzalkonium chloride. It also contains

sodium tripolyphosphate and a surfactant. It is recommended that Phytoclean not be stored as a diluted solution. There is also a warning on the label, "do not contaminate streams, rivers or waterways with Phytoclean or used containers". For footbaths, the recommended label rate is 10% (Table 1). Noske and Shearer (1985) demonstrated that quaternary ammonium products were more effective than sodium hypochlorite at suppressing growth of *P. cinnamomi.* This work was repeated by Smith and Clements (2006), with similar suppressive results demonstrated by quaternary ammonium compounds.

Virkon® S (Virkon) is a broad spectrum disinfectant with potassium peroxymonosulphate as the main active ingredient. It is used in cleaning and disinfecting industrial, animal and agricultural facilities. It is also used for emergency disease control and is efficacious against a range of viruses, bacteria and fungi (including a range of plant pathogens, e.g., *Alternaria, Colletotrichum, Fusarium, Pythium,* and *Rhizoctonia* species). For greenhouse and horticultural applications, a 1% solution is recommended for disinfecting glasshouse structures, equipment, and tools (Table 1). There is warning on the label: "do not immerse metal objects in Virkon for long periods." The recommended maximum contact time is 10 minutes. Additionally, it is also not recommended for use on acid sensitive surfaces, e.g., copper, brass, or aluminium.

Sodium hypochlorite is available in a number of commercial formulations. Janola® (active ingredient hypochlorous acid/sodium salt solution) is a broad spectrum disinfectant at 5% concentration. Smith (1979) demonstrated that chlorine-releasing compounds (e.g., sodium hypochlorite) were fungitoxic against *P. cinnamomi.* However, sodium hypochlorite is considered hazardous in the case of skin and eye contact. It is also considered hazardous in case of inhalation in a confined space. In its diluted form, it is sensitive to light, and is extremely corrosive to brass, and moderately corrosive to bronze.

Citricidal® is synthesised from the polyphenolic compounds found in grapefruit seed and pulp. The active component of Citricidal is considered to be related to "quaternary ammonium chloride". It is recommended for the treatment of candidiasis, parasites, sinusitis, athlete's foot (in humans) and ulcers on pets and livestock. The "label rate" indicates there is 25 mg of grapefruit concentrate in each drop (i.e. approx. 0.25%).

Table 1: Percentage active ingredients of the commercial disinfectants

Disinfectant	Recommended Rates	Percentage active ingredient (% a.i.) in Label "Recommended Rates"
TriGene	2%	0.0024%
Phytoclean	10%	0.0128%
Virkon	1%	0.0020%
Janola	5%	0.0021%

NB. All MSDS information and biodegradability data (where available) is provided in Appendix 3.

In a recent comparative assessment of disinfectant products for the microbial decontamination of imported, used footwear, Cheah et al. (2009) demonstrated that sodium hypochlorite and quaternary ammonium compounds gave almost complete control of bacteria. Sodium hypochlorite and quaternary ammonium were as effective as Virkon in controlling soil fungi associated with dirty footwear.

2. Objectives

The aims of the research were to:

- Assess the efficacy of current hygiene methods (i.e. 2% TriGene) against PTA, and to
- Identify other potential candidates for consideration as alternative hygiene methods (e.g., Phytoclean (quaternary ammonium), Citricidal (grapefruit seed and pulp extract), and Janola (sodium hypochlorite)).

In order to achieve these objectives, a series of experiments were run both sequentially and concurrently. The five experiments were designed to provide specific information about:

Expt 1. The direct biocidal efficacy of the disinfectants TriGene, Phytoclean, Virkon, Janola, and Citricidal on PTA mycelium

Expt 2. The direct biocidal efficacy of these disinfectants on inoculum of PTA (i.e. oospores and zoospores)

Expt 3. The infective capacity of PTA inoculum (i.e. oospores)

Expt 4. The direct ability of these disinfectants to kill PTA in soil and;

Expt 5. The direct ability of these disinfectants to kill PTA in soil adhering to boots.

3. Methods

Experiment 1: Sensitivity of PTA mycelium to disinfectants

All PTA isolates examined to date have the same ITS sequence and are morphologically similar. Strain REB316-1 (= ICMP17021) (Beever et al. 2009), an isolate from an active tree lesion at Piha (Waitakere Ranges), was chosen for testing. Isolate REB 316-1 was grown on potato dextrose agar (PDA; Appendix 1) in petri dishes at 20ºC. From the growing edge of cultures, 6.5 mm diameter plugs of agar were placed on PDA amended with the five disinfectant treatments; TriGene, Phytoclean, Virkon, Janola and Citricidal at 0.2 (C1), 0.1 (C2), 0.05 (C3), 0.025 (C4), and 0.0125% (C5) active ingredient (a.i.) and a control containing water (5 plates per treatment).

The plates were incubated at 20ºC (under fluorescent light) and colony growth marked the same time each day at 4, 5 and 8 days after inoculation. Survival of the culture plugs was assessed by transferring them onto fresh, unamended PDA after 10 days, and growth responses assessed after a further 4 days.

Experiment 2: Sensitivity of PTA oospores and zoospores to disinfectants

PTA (isolate REB 326-1, = ICMP 18244, confirmed to be PTA by ITS obtained from the symptomatic kauri tree from Pakiri Scenic Reserve) was grown on PDA in petri dishes at 20ºC. From the growing edge of cultures, 6.5-mm-diameter plugs of agar were placed into clarified V8 juice broth (Appendix 1) and incubated at 20ºC for 56 days. PTA was harvested from the V8 juice broth and macerated in a Waring Blender for 20 seconds. Oospore numbers were estimated by haemocytometry at approx. 200 000 oospores/ml.

Four replicate oospore suspensions of 25 µl were added to plates containing 0.6% water agar amended with each of the five disinfectant treatments at their lethal concentrations as determined in Experiment 1; TriGene Advance (0.0125% a.i.), Phytoclean (0.0125% a.i.), Virkon S (0.2% a.i.), Janola (0.05% a.i.) and Citricidal (6 drops/100 ml) and a control that was the unamended water agar. The plates were incubated at 20ºC and after 10 days the viability and/or dormancy of 50 oospores from each replicate (giving a total of 200 oospores in total) were assessed by light microscopy using tetrazolium salt (BDH) as a vital stain (Jiang & Erwin 1990). Data were analysed using a χ-squared contingency table (comparing the response of the oospores in the unamended control with each of the disinfectant treatments in a pair-wise manner).

A second experiment (comprising five replicates) assessed the efficacy of the hygiene agents on zoospores of PTA. Sterile zoospore suspensions were made by incubating blocks of colonised V8 juice agar in sterilised soil extract (Appendix 1) overnight (under white and blue fluorescent light at 18ºC). The next day, the blocks were transferred to Eppendorf tubes (1.5 ml) with 50 µl of sterile soil extract and incubated in the refrigerator for 1 hour (to induce sporangial release). A 50-µl aliquot of each disinfectant at recommended label rates (i.e. TriGene 2%; Phytoclean 10%; Virkon 1%, Janola 5% and Citricidal (6 drops/100 ml)) with a sterile RO water control was added to the zoospore suspension. The tubes were vortexed and incubated for 1 minute at room temperature and the contents plated to P_5ARP Selective Medium for *Phytophthora* species (Appendix 1) selective agar and incubated in the dark for 2 days. Colony forming units (CFU's) per ml were estimated after 3 days by counting the number of fungal colonies, and representative isolates were plated to PDA and V8 juice agar to confirm their identity. This experiment was repeated 5 times.

Experiment 3: Soil spiking and quantification of PTA infection potential

Field soil from the infested Huia site was collected in sterile, 20 l plastic pails. Soil was collected around kauri (of ricker-age) exhibiting gummosis, crown decline and/or crown chlorosis and transported back to Landcare Research, Tamaki and stored at 10ºC in the dark.

Soils were passed through a 2-cm screen to remove coarse woody debris. At least 15 kg of soil was kept as the "control". The remaining soil was split into 5 lots, each having one of the five oospore suspensions added to it, before being thoroughly mixed. This resulted in five "spiked" soils with final oospore concentrations of 2000, 1000, 500, 250 or 125 oospores/g of soil.

Three 20-g sub-samples of the "control" and each of the "spiked" soils were assayed for the presence of PTA, using the extended leaf-bait soil bioassay methods (modified from Stack & Millar 1985). The soil was air-dried on the Dingley laboratory bench for two days, moist incubated for four days (see Stack & Millar 1985) and then flooded with 200 ml RO water in 400 ml beakers. The beakers were baited with 10, trimmed, Himalayan cedar (*Cedrus deodara*) needles and incubated at 20ºC for 4 days at 60%RH under blue and cool white fluorescent light (Light intensity of 180 µE). All leaf-baits were surface-sterilised for 30 seconds in 50% ethanol, rinsed three times in sterile RO water and plated onto P_5ARP selective media. Three replicate aliquots (100 µl) of the leaf-bait soil bioassay water were taken from each bioassay and were plated onto P_5ARP selective agar, and the number of CFU's/ml resembling PTA in colony morphology on the selective agar plate assessed after 3 days. Representative isolates were plated to V8 juice agar to confirm their identity. The proportion of leaf baits colonised by PTA were transformed using the angular transformation (i.e. by taking the arcsine of the square root of each proportion). Data were then compared using a t-test.

Experiment 4: Ability of disinfectants to kill PTA in soil

The ability of the disinfectants to kill/inhibit PTA in soil was assessed by soaking replicates of the spiked soil (produced for Experiment 3) in the disinfectants. Replicate spiked soil (20-g samples containing 1500 oospores/g) were placed in mesh bags and soaked in the disinfectants at their label rates (for Citricidal, the equivalent of 6 drops/100 ml was used). The control involved soaking the soil in RO water (Plates 2a, 2b). The soil was then washed three times in sterile RO water following the initial treatment and allowed to drain.

The treated soil was bioassayed using the extended bioassay methods described in Experiment 3. Three 100-µl aliquots of the soil bioassay water from each bioassay were plated directly on P_5ARP to determine the number of CFUs/ml. Representative isolates were plated on V8 juice agar to confirm their identity. The experiment was replicated three times.

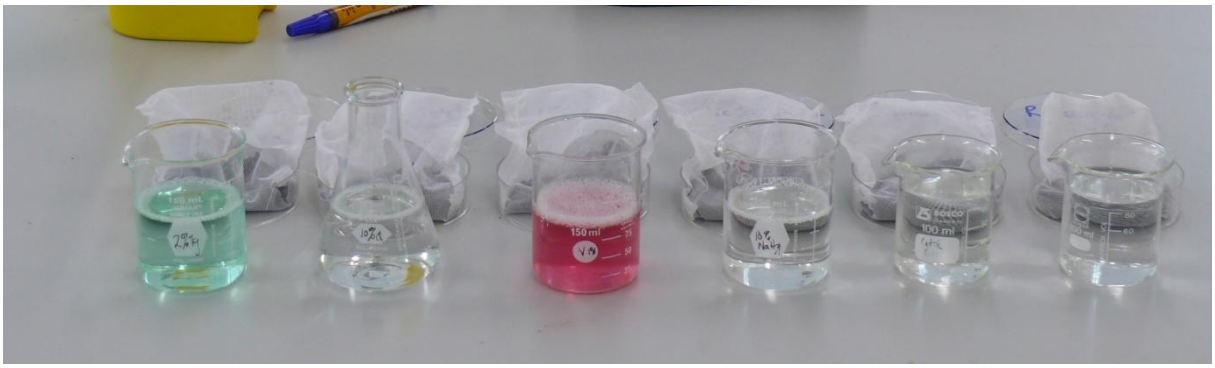

Plate 2a: Disinfectant solutions

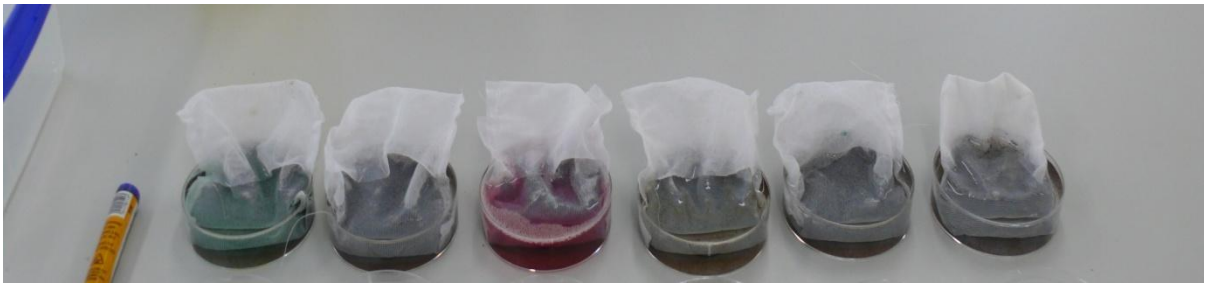

Plate 2b: Spiked soil in bags soaked in disinfectant solutions.

Experiment 5: The ability of disinfectants to kill PTA in soil on boots

The ability of the disinfectants to kill PTA in soil adhering to rubber-soled gum-boots utilised the following approach. The boot was surface sterilised by scrubbing with 95% ethanol, then rinsing three times with sterile RO water (the same boot was used throughout the experiment). A sterile cotton swab sample from the sole surface was taken before the boot was treated. The boot was then pressed into spiked soil (Plate 3a). 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 (Plate 3b). The "rinsate" from each of the chemical treatments was collected (Plate 3c) and plated (one or two plates depending upon volume of rinsate collected) to P_5ARP selective agar (15 ml per plate).

Plate 3a: Boot pressed into soil

Plate 3b: Boot sprayed

Plate 3c: Rinsate collected

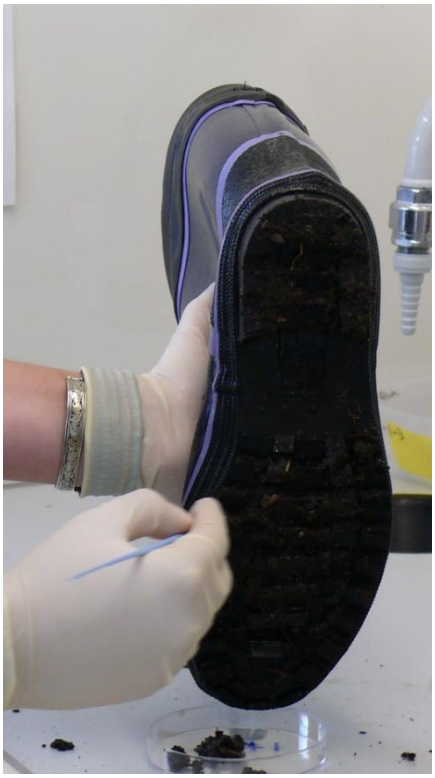

Plate 3d: Treated soil collected

The treated soil left adhering to the boot was scraped off after the spray treatment (Plate 3d) and bioassayed for PTA using the extended leaf-bait soil bioassay technique described in Experiment 3 (Plate 4).

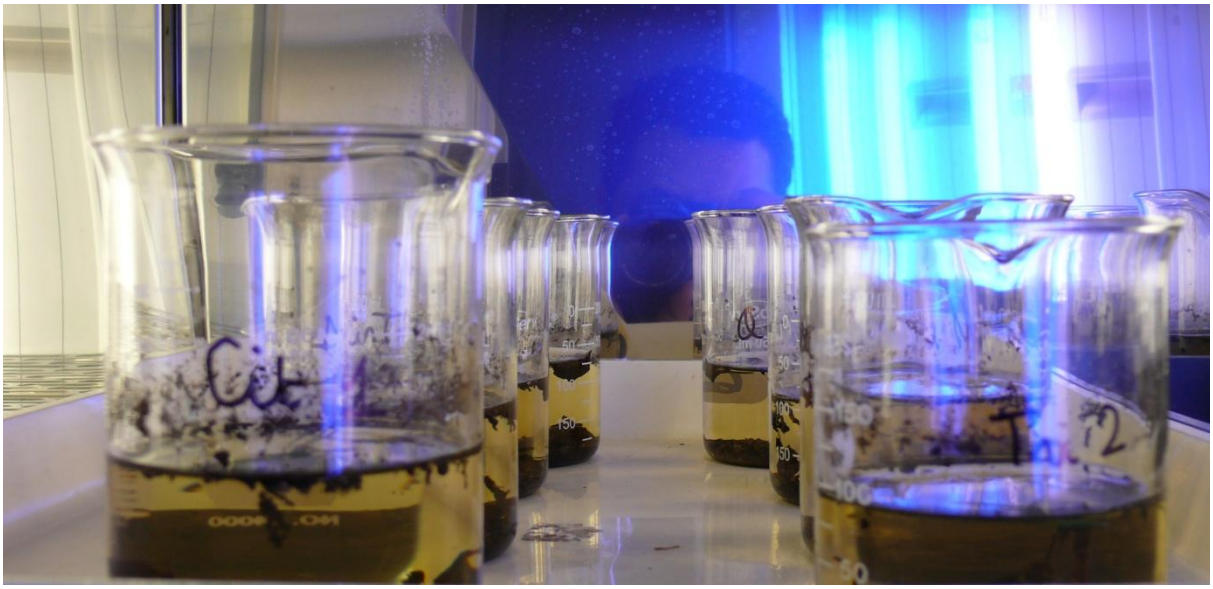

Plate 4: Leaf-bait soil bioassays after 4 days incubation in blue and cool white fluorescent light

Percentage data of leaf baits colonised by PTA before and after spray treatment with disinfectants, were transformed using the angular transformation (i.e. arcsine of the square root of each proportion). Data were then compared using a t-test. The experiment was repeated twice.

4. Results

Experiment 1: Sensitivity of PTA mycelium to disinfectants

Table 2 provides a summary of the sensitivity of PTA growth rates when grown on agar amended with the five hygiene products at five concentrations. TriGene and Phytoclean completely suppressed growth of PTA mycelium at all concentrations incorporated into agar (Table 2). Virkon (at 0.2 and 0.1% a.i.) completely suppressed growth of PTA: at 0.05% a.i. it reduced growth by 77%; at 0.025% a.i. it reduced growth by 54%; and at 0.0125% a.i. it reduced growth by 27% (compared with the control). Janola (at 0.2, 0.1, 0.05% a.i.) completely suppressed PTA: at 0.025% a.i. it inhibited growth by 54%; and at 0.0125 % a.i. it inhibited growth by 18% (compared with the control). Citricidal inhibited PTA growth at all concentrations, but did not kill the mycelium in the plug.

Table 2: Growth rates (mm/day) of PTA grown on agar amended with 5 disinfectants at 5 concentrations (mean of five replicates) after 4 days

	C1	C2	C3	C4	C5
	0.2% a.i.	**0.1% a.i.**	**0.05% a.i.**	**0.025% a.i.**	**0.0125% a.i.**
TriGene	0	0	0	0	0
Phytoclean	0	0	0	0	0
Virkon	0	0	0.6	1.45	2.10
Janola	0	0	0	1.25	2.30
Citricidal	0	0	0	0	0
Control			2.75		

Table 3 provides a summary of the ability of the PTA agar plugs exposed to the disinfectants to re-commence growth on fresh, unamended PDA agar. TriGene and Phytoclean resulted in complete mortality of the PTA in the plug at all concentrations (Table 3). Virkon was lethal at 0.2 and 0.1% a.i. Janola was lethal at a.i. concentrations of between 0.2 and 0.05%. Citricidal demonstrated *fungistasis* at all concentrations i.e. inhibited growth, but did not result in mortality at any of the concentrations assessed.

Table 3: Ability of PTA to re-grow from plugs exposed to the 5 disinfectants at 5 concentrations after 10 days. Results display re-growth after 4 days on fresh, unamended PDA (data are the number of plugs out of five that commenced growth).

	C1	C2	C3	C4	C5
	0.2% a.i.	**0.1% a.i.**	**0.05% a.i.**	**0.025% a.i.**	**0.0125% a.i.**
TriGene	0/5	0/5	0/5	0/5	0/5
Phytoclean	0/5	0/5	0/5	0/5	0/5
Virkon	0/5	0/5	5/5	5/5	5/5
Janola	0/5	0/5	0/5	5/5	5/5

	C1 0.2% a.i.	C2 0.1% a.i.	C3 0.05% a.i.	C4 0.025% a.i.	C5 0.0125% a.i.
Citricidal	5/5	5/5	5/5	5/5	5/5
Control	5/5	5/5	5/5	5/5	5/5

Experiment 2: Sensitivity of PTA oospores to disinfectants

No activated oospores (i.e. staining red in BDH) were observed in any of the hygiene treatments (Figure 1).

The majority of the oospores (approx. 80%) in the unamended control were dormant (pink bars; Fig. 1). About 10% of the oospores were activated (i.e. red oospores) and the remainder (about 10%) were non-viable (i.e. stained black).

In comparison, Virkon and Janola significantly reduced on oospore viability (Fig. 1). Virkon killed significantly more oospores than Janola – and both Virkon and Janola were more lethal than TriGene, Phytoclean or Citricidal (6 drops/100 ml). There was no difference in response between the oospores exposed to either TriGene or Citricidal, with Phytoclean having a greater negative impact than either of these two treatments (see Appendix 2 for Chi-squared results).

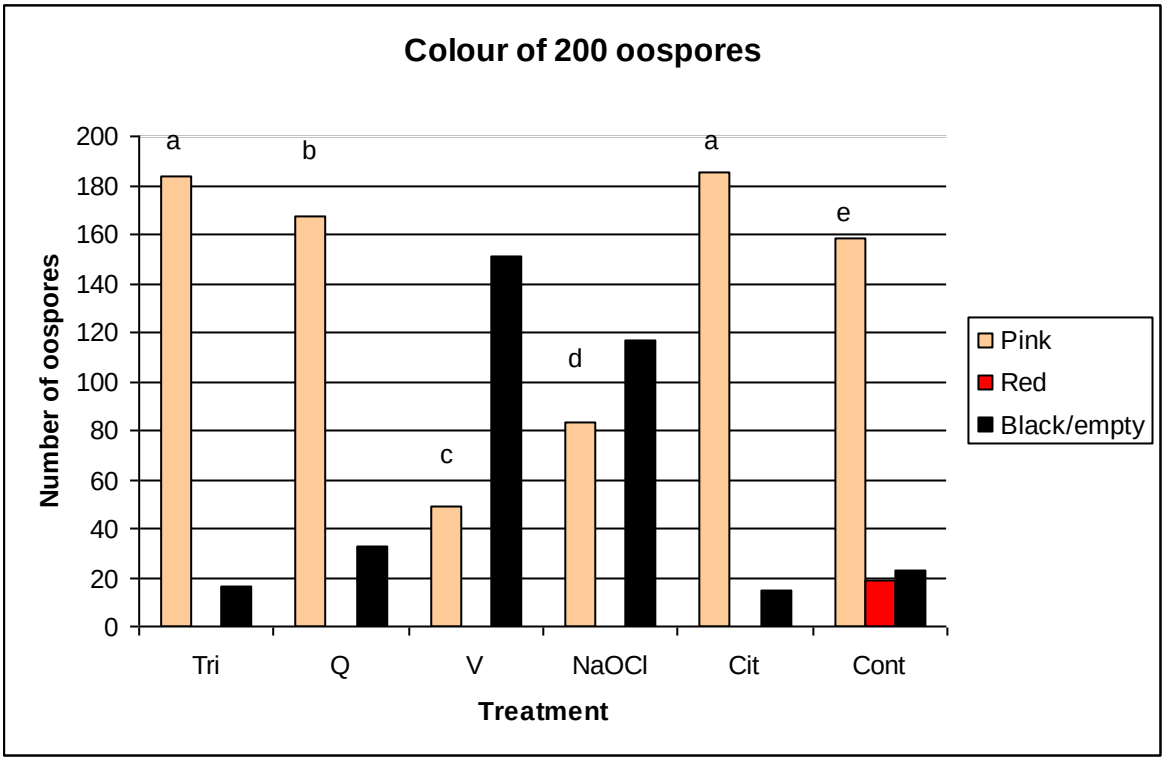

Figure 1: Oospore viability counts after 10-days being incubated in the 5 disinfectants (Tri = TriGene; Q = Phytoclean; V = Virkon; NaOCl = Janola; Cit = Citricidal; Cont = unamended control). Bars with the same letter are not significantly different (*P* = 0.05). Bar colour: Pink = dormant oospores, red = active spores, black = non-viable spores.

Experiment 2b: Sensitivity of PTA zoospores to disinfectants

Trigene (2%), Phytoclean (10%), Virkon (1%) and Janola (5%) all proved lethal to zoospores (Table 4). The zoospores that were placed in the Citricidal and Control (i.e. RO water) survived the treatment and produced a mean of 784 ±38, and 404 ± 70 colonies of PTA/ml respectively, after 3 days (see Appendix 2 for raw data).

Table 4: Survival of PTA zoospores after being treated with disinfectants at label/recommended rates. Data represent mean number of PTA colonies/ml after 3 days growth on P_5ARP (data are means ± s.e.m., n = 5).

Disinfectant treatment	Mean CFUs of PTA/ml
TriGene (2%)	0
Phytoclean (10%)	0
Virkon (1%)	0
Janola (5%)	0
Citricidal	784 ± 38
Control	404 ± 70

Experiment 3: Soil spiking and quantification of PTA infection potential

The spiked soil containing 2000 oospores/g of soil colonised approximately 37% of the leaf baits (Table 5). In comparison, the PTA recovery from the unamended Huia Composite field soil was about 3% (1 out of 30 baits from three repeats of the experiment). However, due to the high degree of variability *within* each of the oospore concentration treatments, there was no significant difference *between* treatments (*t*-value of 2.776, α=0.05; see Appendix 2 for *t*-table). From the soil bioassay water, PTA was only recovered from the soil containing 2000 oospores/g (Table 5). No CFU's of PTA were obtained from the Huia Composite soil.

Table 5: Results of spiked soil bioassay trial to enumerate PTA inoculum. Data represent number of leaf baits colonised out of 30 and mean number CFUs/ml on P_5ARP (n = 3). Leaf bait proportions with same subscripted letter are not significantly different at *P* = 0.05.

Oospore concentration	Leaf Baits	Mean CFU's/ml from soil bioassay	
		PTA	Other fungi
2000 oospores/g	$11/30_a$	23±33	233 ± 23
1000 oospores/g	$7/30_a$	0	87 ± 40
500 oospores/g	$4/30_a$	0	30 ± 19
250 oospores/g	$1/30_a$	0	0
125 oospores/g	$0/30_a$	0	150 ± 85
Huia Composite	$1/30_a$	0	76 ± 11

Experiment 4: Ability of disinfectants to kill/suppress PTA in soil

Spiked soil containing 1500 PTA oospores/g, which was soaked in TriGene (2%) and Phytoclean (10%), completely suppressed PTA and all soil fungi/bacteria (Table 6). Virkon (1%) and Janola (5%) also completely suppressed PTA (Table 6).

However, Virkon- and Janola-treatment soil did not suppress all soil fungi and bacteria (Table 6). 17.5±24.8 and 45.0±26.1 CFUs/ml of a commonly recovered zygomycete were found in the soils treated with Virkon and Janola respectively. Approximately 12.5 CFUs/ml of bacteria were also associated with the Virkon treated soil.

In comparison to the above four disinfectant treatments, Citricidal and RO water (i.e. control) did not suppress PTA. PTA was recovered from 10% of leaf baits from spiked soil soaked in Citricidal and RO water. PTA CFUs were only recovered from the soil bioassay water from the soils treated in Citricidal and RO water (Table 6).

Table 6: 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	Zygomycetes	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 (zygomycetes only)	0	45.0 ± 26.1	0
Citricidal	3 PTA 1 *Phytophthora cinnamomi* 4 zygomycetes	5.0 ± 5.8	47.5 ± 41.0	13.3 ± 14.1
RO water Control	3 PTA 1 *Pythium* sp. 8 zygomycetes	10.0 ± 5.7	44.7 ± 12.0	67.0 ± 23.0

Experiment 5: The ability of disinfectants to kill PTA in soil on boots

Rinsates collected from spray-treated boots

PTA was not recovered from the rinsate of boots sprayed with TriGene (2%), Phytoclean (10%), Virkon (1%) and Janola (5%) (Table 7). In a similar trend to that observed in Experiment 4, TriGene and Phytoclean completely suppressed all soil fungi (compare Table 7 with Table 6).

Table 7: Soil fungi and PTA recoveries from spray rinsate collected from boots being treated with hygiene treatments. Data represent mean number of CFUs/ml (n=2).

Soil treatment	Mean CFUs/ml formed on P_5ARP
TriGene (2%)	0

Soil treatment	Mean CFUs/ml formed on P_5ARP
Phytoclean (10%)	0
Virkon (1%)	15.0 ± 5.0
Janola (5%)	20.0 ± 10.0
Citricidal	115.0 ± 65.0*****
Control	260.0 ± 30.0*****

* **indicates PTA confirmed.**

Colonies of common soil zygomycetes were recovered from the rinsates produced from treatments with Virkon, Janola, Citricidal and the RO water treated control. Significantly, PTA was only recovered from the rinsates resulting from spray treatments with Citricidal and RO water (Table 7).

Before and after treatment swabs

The swabs taken from the boots before and between treatments returned negative results, i.e. no PTA (Table 8). There was, however, some carry-over between treatments, with a maximum of 20.0 CFUs/ml recovered between treatments (Table 8).

The swabs taken after the soil was removed from the spray-treated boot returned a number of soil fungi. PTA was only recovered from the boot soil treated with Citricidal and RO water (control). PTA was not recovered from boots sprayed with TriGene, Phytoclean, Virkon and Janola.

Table 8: Soil fungi and PTA recovered from cotton swabs taken before and after spray treatment of soil on boots. Data represent the mean number of CFUs/ml (n = 3).

Soil treatment	Number of colonies from swabs before spray treatment	Number of colonies from swabs after spray treatment
TriGene (2%)	2.5 ± 3.5	295.0 ± 3.0 **(no PTA)**
Phytoclean (10%)	2.5 ± 3.5	442.0 ± 3.0 **(no PTA)**
Virkon (1%)	20.0 ± 16.0	190.0 ± 160 **(no PTA)**
Janola (5%)	20.0 ± 19.8	165.0 ± 20.0 **(no PTA)**
Citricidal	0	470.0 ± 142*** (PTA)**
Control	5.0 ± 4.1	315.0 ± 4.0*** (PTA)**

* **indicates PTA confirmed.**

Soil bioassay of soil before and after spray treatment

Before spray treatment, all spiked soils produced PTA on leaf baits (Table 9), which confirmed the infective potential of the artificially spiked soil.

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 (Table 9).

Janola did not significantly decrease the amount of soil fungi in total, but did suppress PTA after spray application (Table 9).

Post-spray treatment, PTA was only recovered from boots sprayed with Citricidal and/or RO water (Table 9).

Table 9: PTA recoveries from leaf-bait soil bioassay before and after treatment with spray disinfectants. Data represents the proportion of leaf baits colonised by PTA (n = 2; total of 20 leaf baits). Data with same subscripted letter are not significantly different at *P* = 0.05.

Soil treatment	Colonised leaf baits (before spray treatment)	Colonised leaf baits (after spray treatment)
TriGene (2%)	20/20*$_a$	3/20$_c$
Phytoclean (10%)	20/20*$_a$	0/20$_c$
Virkon (1%)	17/20*$_a$	0/20$_c$
Janola (5%)	17/20*$_a$	7/20$_{a,b}$
Citricidal	16/20*$_b$	7/20*$_b$
Control	11/20*$_b$	12/20*$_b$

*** indicates PTA confirmed.**

5. Conclusions

Expt 1. Sensitivity of PTA mycelium to disinfectants

TriGene and Phytoclean completely suppressed growth (i.e. preventing it from growing or developing) of PTA mycelium at all *in vitro* concentrations tested. Only TriGene and Phytoclean resulted in complete mortality of the hyphae of the pathogen contained in the mycelial plug. Both Virkon (at 0.2 and 0.1% a.i.) and Janola (at 0.2, 0.1, 0.05% a.i.) completely suppressed PTA, but only at higher *in vitro* concentrations. Citricidal was demonstrated to be fungistatic (i.e. inhibited growth but did not kill the mycelium) at all concentrations.

Expt 2. Sensitivity of PTA oospores to disinfectants

The majority of the oospores in the unamended control were dormant (approx. 80%). The unamended control was the only treatment where activated oospores were observed (about 10%), and the remainder were non-viable (approx. 10%). Virkon (0.2% a.i.) and Janola (0.05% a.i.) had the most significant impact on oospore viability. Virkon killed significantly more oospores than Janola – and both Virkon and Janola were more lethal than TriGene (0.0125% a.i.), Phytoclean (0.0125% a.i.), or Citricidal – but again, only at higher *in vitro* concentrations than either TriGene or Phytoclean.

Expt 2b. Sensitivity of PTA zoospores to disinfectants

The zoospores that were placed into Trigene (2%), Phytoclean (10%), Virkon (1%) and Janola (5%), did not survive the treatment. The zoospores that were placed in the Citricidal and RO water Control survived the treatment and produced colonies of PTA after 2 days.

Expt 3. Infective capacity of PTA inoculum

The soil "spiked" with 2000 oospores / g of PTA colonised 37% of leaf baits – confirming the infective capacity of PTA oospore-inoculum.

Expt 4. Ability of disinfectants to kill PTA in soil

Soaking the spiked soil in TriGene (2%) and Phytoclean (10%) completely suppressed PTA and all soil fungi. Virkon (1%) and Janola (5%) suppressed PTA, but soil treated with Virkon (1%) and Janola (5%) did not suppress all soil fungi and bacteria.

Expt 5. The ability of disinfectants to kill PTA in soil on boots

PTA was not recovered from the rinsate collected from the sole of rubber gum-boots sprayed with TriGene (2%), Phytoclean (10%), Virkon (1%) and/or Janola (5%). The rinsate collected after spraying with Citricidal and RO water did not suppress PTA. Spray treatment of spiked soil on boots with TriGene, Phytoclean and Virkon 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.

6. Comparative summary of hygiene performance

- Disinfecting soiled rubber gum-boots with a 2% spray treatment of TriGene will effectively suppress the inoculum of PTA. TriGene achieves this in part, by being biocidal to PTA mycelium. TriGene could also limit the spread of propagules of PTA, by its ability to kill zoospores of PTA. TriGene effectively suppresses the infective capacity of PTA in soil at its recommended label rate of 2%.

- Phytoclean demonstrated a similar efficacy to TriGene in that it completely suppressed the growth of PTA by killing the mycelium. Phytoclean could also limit the spread of propagules of PTA, by its ability to kill zoospores of PTA at its label/recommended rate (i.e. 10%). Phytoclean demonstrated efficacy at its label/recommended rate to effectively suppress the infective capacity of PTA inoculum contained in soil.

- Virkon only suppressed growth of PTA at higher *in vitro* concentrations, and at these concentrations it was lethal to mycelium. Virkon could also limit the spread of propagules of PTA through its ability to kill zoospores of PTA at its label/recommended rate (i.e. 1%). Virkon (at 1%) also demonstrated efficacy at suppressing PTA's infective capacity in soil when applied as a spray-treatment.

- Janola demonstrated a similar efficacy to Virkon in that it completely suppressed the growth of PTA by killing the mycelium at higher *in vitro* concentrations. Janola could also limit the spread of propagules of PTA, through its ability to kill zoospores of PTA at its label/recommended rate (i.e. 5%). Janola (5%) demonstrated efficacy at its recommended rate to effectively suppress the spread of PTA inoculum contained in soil when applied as a spray-treatment, but did not suppress all soil fungi.

- Citricidal, while demonstrating good *in vitro* fungistatic inhibition of PTA, did not kill mycelium like the other disinfectants. Citricidal had no effect on zoospores of PTA, and did not reduce the infective capacity of PTA contained in soil.

- Spraying with RO water alone does not reduce the infective capacity of PTA contained in soil.

7. Recommendations

TriGene II Advance (2%) is a suitable hygiene prescription for controlling PTA, effectively killing propagules of PTA, and reducing the infective capacity of soil containing PTA.

Quaternary ammonium compounds registered for phytosanitary applications in New Zealand (e.g., Sterbac™, Trimove®, Flurosan®) should be considered as alternative hygiene options for controlling PTA should TriGene become unavailable. We consider it likely they will behave similarly to Phytoclean. An alternative could be to encourage registration of Phytoclean in NZ, as we have shown this to be efficacious against PTA.

Further research is necessary to understand the difference between *in vitro* concentrations and spray-efficacy of Virkon and Janola at recommended/label rates. The "mode of action" of these two disinfectants may explain, in part, why spray-treatments of Virkon and Janola effectively suppress the spread of PTA inoculum contained in soil.

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9. References

Aberton MJ, Wilson BA, Cahill DM 1999. The use of potassium phosphonate to control *Phytophthora cinnamomi* in native vegetation at Anglesea, Victoria. Australasian Plant Pathology 28: 225–234.

ARC 2009. Stop kauri dieback. Certified ARC signage associated with TriGene Disinfectant http://www.arc.govt.nz/environment/biosecurity/kauri-dieback/kauri-dieback-how-you-can-help_home.cfm.

Beever RE, Waipara NW, Ramsfield TD, Dick MA, Horner IJ 2009. Kauri (*Agathis australis*) under threat from *Phytophthora*? In: Goheen EM, Frankel SJ (technical coordinators) Proceedings of the fourth meeting of the International Union of Forest Research Organizations (IUFRO) Working Party S07.02.09: Phytophthoras in forests and natural ecosystems. Gen. Tech. Rep. PSW-GTR-221. Albany, CA: US Department of Agriculture, Forest Service, Pacific Southwest Research Station. Pp. 74-85.

Beever RE, Tsai S, Waipara NW, Horner IJ, Ramsfield TD 2008. *Phytophthora* taxon Agathis, a threat to kauri in northern New Zealand? 3rd International *Phytophthora, Pythium* and related genera workshop, Turin, Italy, 23–24 August 2008. [poster paper]

Brasier CM, Kirk SA, Delcan J, Cooke DEL, Jung J, Man In't Veld WA (2004). *Phytophthora alni.* sp. nov. and its variants: designation of emerging heteroploid hybrid pathogens spreading on *Alnus* trees. Mycological Research 108: 1172–1184.

Cheah L-H, Marsh AT, McNeill MR, Hedderly DI 2009. Evaluation of disinfectant products for microbial decontamination of imported footwear. New Zealand Plant Protection 62: 130–135.

Colquhoun I, Kerp N 2007. Minimizing the spread of a soil-borne plant pathogen during a large-scale mining operation. Restoration Ecology 15(4) Supplement: S85–S93.

Erwin DC, Ribeiro OK 1996. Phytophthora diseases worldwide. St Paul, MN, APS Press.

Gadgil PD 1974. *Phytophthora heveae:* a pathogen of kauri. New Zealand Journal of Forestry Science 4: 59–63.

Hansen EM, Goheen DJ, Jules ES, Ullian B (2000). Managing Port-Orford-Cedar and the introduced pathogen *Phytophthora lateralis.* Plant Disease 84(1): 4-14.

Jackson TJ, Burgess T, Colquhoun I, Hardy GE StJ 2000. Action of the fungicide phosphite on *Eucalyptus marginata* inoculated with *Phytophthora cinnamomi.* Plant Pathology 47: 147–154.

Jiang J, Erwin DC 1990. Morphology, plasmolysis and tetrazolium bromide stain as criteria for determining viability of *Phytophthora* oospores. Mycologia 87: 107–113.

Jung T, Hansen EM, Winton, L, Oβwald W, Delatour C 2002. Three new species of *Phytophthora* from European oak forests. Mycological Research 106(4): 397–411.

Jung T, Nechwatal J, Cooke DEL, Hartmann G, Blaschke M, Oβwald W, Duncan J, Delatour C 2003. *Phytophthora pseudosyringae* sp. nov. a new species causing root and collar rot of deciduous tree species in Europe. Mycological Research 107(7): 772–789.

Noske GL, Shearer BL 1985. Quaternary ammonium compounds were ore effective than a phenolic compound or sodium hypochlorite in inhibiting growth of *Phytophthora cinnamomi* (Rands). Australasian Plant Pathology 14(2): 37–40.

Rizzo DM, Garbelotto M, Davidson JM, Slaughter GW, Koike ST 2002. *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. Plant Disease 86: 205–214.

Smith PM 1979. A study of the effects of fungitoxic compounds on *Phytophthora cinnamomi* in water. Annals of Applied Biology 93: 149–157.

Smith IW, Clements PA 2006. Assessment of quaternary ammonium compounds as disinfectants for control of *Phytophthora cinnamomi* in washdowns situations. WHERE?, Victoria, Australia, Centre for Tree Technology, DNR&E.

Stack JP, Millar RL 1985. Relative survival potential of propagules of *Phytophthora megasperma* f.sp. *medicaginis*. Phytopathology 75: 1398–1404.

Tasmanian DPI 2004. Tasmanian washdown guidelines for weed and disease control: machinery, vehicles and equipment. 1st ed. Hobart, Tasmania, Tasmanian Department of Primary Industries, Water and Environment.

Appendix 1 Media Recipes

Potato Dextrose Agar (PDA)

Difco™ PDA	39 g
RO water	1 litre

Autoclave at 121ºC for 15 minutes at 15 p.s.i. (15 ml per plate)

V8 juice agar

V8 Juice	200 ml
$CaCO_3$	3.0 g
RO water	800 ml
Agar	15.0 g

Autoclave at 121ºC for 15 minutes at 15 p.s.i. (15 ml per plate)

Clarified V8 juice broth

Clarified V8 juice	100 ml
$CaCO_3$	2% (in 100 ml)
RO water	800 ml

Clarify V8 juice by centrifugation at 4000 rpm for 15 minutes. Vacuum filter three times through one layer of Whatman No. 42 filter paper and twice through two layers.

Autoclave at 121º C for 15 minutes at 15 p.s.i.

Sterile soil extract:

Garden soil	200 grams of soil (collcted from landscaped area in Tamaki carpark, 231 Morrin Road, St Johns)
RO water	1 litre

Stirred vigorously for 2 minutes, then stirred vigorously again 30 minutes later and allowed to stand overnight.

The solution was filtered through paper hand towel, bottled and autoclaved at 121º C for 15 minutes at 15 p.s.i. Stored in refrigerator.

PARP-CMA Selective Medium for *Phytophthora* species

Difco corn meal agar	17 g
RO water	1 litre
Pimaricin	5 mg/l
Sodium Ampicillin	250 mg/l
Rifamycin-SV (sodium salt)	10 mg/l
PCNB (75%)	66.7 mg/l

Autoclave at 121ºC for 15 minutes at 15 p.s.i. (15 ml / plate).

Appendix 2 Raw data and statistical analysis

EXPERIMENT 1 RAW DATA

Growth of the PTA colonies

		Growth in mm			
		2 days	4 days	5 days	8 days
TriC1R1	*REB 316-1, TriGene Advance 0.2% a.i, rep 1*				
TriC1R2	*REB 316-1, TriGene Advance 0.2% a.i, rep 2*				
TriC1R3	*REB 316-1, TriGene Advance 0.2% a.i, rep 3*				
TriC1R4	*REB 316-1, TriGene Advance 0.2% a.i, rep 4*				
TriC1R5	*REB 316-1, TriGene Advance 0.2% a.i, rep 5*				
TriC2R1	*REB 316-1, TriGene Advance 0.1% a.i, rep 1*				
TriC2R2	*REB 316-1, TriGene Advance 0.1% a.i, rep 2*				
TriC2R3	*REB 316-1, TriGene Advance 0.1% a.i, rep 3*				
TriC2R4	*REB 316-1, TriGene Advance 0.1% a.i, rep 4*				
TriC2R5	*REB 316-1, TriGene Advance 0.1% a.i, rep 5*				
TriC3R1	*REB 316-1, TriGene Advance 0.05% a.i, rep 1*				
TriC3R2	*REB 316-1, TriGene Advance 0.05% a.i, rep 2*				
TriC3R3	*REB 316-1, TriGene Advance 0.05% a.i, rep 3*				
TriC3R4	*REB 316-1, TriGene Advance 0.05% a.i, rep 4*				
TriC3R5	*REB 316-1, TriGene Advance 0.05% a.i, rep 5*				
TriC4R1	*REB 316-1, TriGene Advance 0.025% a.i, rep 1*				
TriC4R2	*REB 316-1, TriGene Advance 0.025% a.i, rep 2*				
TriC4R3	*REB 316-1, TriGene Advance 0.025% a.i, rep 3*				
TriC4R4	*REB 316-1, TriGene Advance 0.025% a.i, rep 4*				
TriC4R5	*REB 316-1, TriGene Advance 0.025% a.i, rep 5*				
TriC5R1	*REB 316-1, TriGene Advance 0.0125% a.i, rep 1*				
TriC5R2	*REB 316-1, TriGene Advance 0.0125% a.i, rep 2*				
TriC5R3	*REB 316-1, TriGene Advance 0.0125% a.i, rep 3*				
TriC5R4	*REB 316-1, TriGene Advance 0.0125% a.i, rep 4*				
TriC5R5	*REB 316-1, TriGene Advance 0.0125% a.i, rep 5*				
VirC1R1	*REB 316-1, Virkon S 0.2% a.i, rep 1*				
VirC1R2	*REB 316-1, Virkon S 0.2% a.i, rep 2*				
VirC1R3	*REB 316-1, Virkon S 0.2% a.i, rep 3*				
VirC1R4	*REB 316-1, Virkon S 0.2% a.i, rep 4*				
VirC1R5	*REB 316-1, Virkon S 0.2% a.i, rep 5*				
VirC2R1	*REB 316-1, Virkon S 0.1% a.i, rep 1*			<1	~1
VirC2R2	*REB 316-1, Virkon S 0.1% a.i, rep 2*			<1	~1
VirC2R3	*REB 316-1, Virkon S 0.1% a.i, rep 3*			<1	~1
VirC2R4	*REB 316-1, Virkon S 0.1% a.i, rep 4*			<1	~1
VirC2R5	*REB 316-1, Virkon S 0.1% a.i, rep 5*			<1	~1
VirC3R1	*REB 316-1, Virkon S 0.05% a.i, rep 1*	3	3	2	5
VirC3R2	*REB 316-1, Virkon S 0.05% a.i, rep 2*	2	2	1.5	5
VirC3R3	*REB 316-1, Virkon S 0.05% a.i, rep 3*	2	2	1.5	5
VirC3R4	*REB 316-1, Virkon S 0.05% a.i, rep 4*	2	3	1.5	5
VirC3R5	*REB 316-1, Virkon S 0.05% a.i, rep 5*	2	2	1.5	6
VirC4R1	*REB 316-1, Virkon S 0.025% a.i, rep 1*	5	5	3	9
VirC4R2	*REB 316-1, Virkon S 0.025% a.i, rep 2*	5	6	2.5	10
VirC4R3	*REB 316-1, Virkon S 0.025% a.i, rep 3*	5	6	2.5	10
VirC4R4	*REB 316-1, Virkon S 0.025% a.i, rep 4*	4	6	2.5	10
VirC4R5	*REB 316-1, Virkon S 0.025% a.i, rep 5*	4	6	2.5	9
VirC5R1	*REB 316-1, Virkon S 0.0125% a.i, rep 1*	8	8	4	12
VirC5R2	*REB 316-1, Virkon S 0.0125% a.i, rep 2*	7	8	2.5	11

VirC5R3	*REB 316-1, Virkon S 0.0125% a.i, rep 3*	7	9	3	12
VirC5R4	*REB 316-1, Virkon S 0.0125% a.i, rep 4*	7	9	3	12
VirC5R5	*REB 316-1, Virkon S 0.0125% a.i, rep 5*	8	8	3	11
NaOCl1R1	*REB 316-1, NaHypochlorite 0.2% a.i, rep 1*				
NaOCl1R2	*REB 316-1, NaHypochlorite 0.2% a.i, rep 2*				
NaOCl1R3	*REB 316-1, NaHypochlorite 0.2% a.i, rep 3*				
NaOCl1R4	*REB 316-1, NaHypochlorite 0.2% a.i, rep 4*				
NaOCl1R5	*REB 316-1, NaHypochlorite 0.2% a.i, rep 5*				
NaOCl2R1	*REB 316-1, NaHypochlorite 0.1% a.i, rep 1*				
NaOCl2R2	*REB 316-1, NaHypochlorite 0.1% a.i, rep 2*				
NaOCl2R3	*REB 316-1, NaHypochlorite 0.1% a.i, rep 3*				
NaOCl2R4	*REB 316-1, NaHypochlorite 0.1% a.i, rep 4*				
NaOCl2R5	*REB 316-1, NaHypochlorite 0.1% a.i, rep 5*				
NaOCl3R1	*REB 316-1, NaHypochlorite 0.05% a.i, rep 1*				
NaOCl3R2	*REB 316-1, NaHypochlorite 0.05% a.i, rep 2*				
NaOCl3R3	*REB 316-1, NaHypochlorite 0.05% a.i, rep 3*				
NaOCl3R4	*REB 316-1, NaHypochlorite 0.05% a.i, rep 4*				
NaOCl3R5	*REB 316-1, NaHypochlorite 0.05% a.i, rep 5*				
NaOCl4R1	*REB 316-1, NaHypochlorite 0.025% a.i, rep 1*		5	2.5	12
NaOCl4R2	*REB 316-1, NaHypochlorite 0.025% a.i, rep 2*		5	2.5	12
NaOCl4R3	*REB 316-1, NaHypochlorite 0.025% a.i, rep 3*		5	3	11
NaOCl4R4	*REB 316-1, NaHypochlorite 0.025% a.i, rep 4*		6	2.5	11
NaOCl4R5	*REB 316-1, NaHypochlorite 0.025% a.i, rep 5*		4	2.5	10
NaOCl5R1	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 1*	4	9	4	14
NaOCl5R2	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 2*	5	9	4	13
NaOCl5R3	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 3*	5	10	4	13
NaOCl5R4	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 4*	4	9	4	14
NaOCl5R5	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 5*	4	9	4	13
CitC1R1	*REB 316-1, Citricidal 20 drops rep 1*				
CitC1R2	*REB 316-1, Citricidal 20 drops rep 2*				
CitC1R3	*REB 316-1, Citricidal 20 drops rep 3*				
CitC1R4	*REB 316-1, Citricidal 20 drops rep 4*				
CitC1R5	*REB 316-1, Citricidal 20 drops rep 5*				
CitC2R1	*REB 316-1, Citricidal 12 drops rep 1*				
CitC2R2	*REB 316-1, Citricidal 12 drops rep 2*				
CitC2R3	*REB 316-1, Citricidal 12 drops rep 3*				
CitC2R4	*REB 316-1, Citricidal 12 drops rep 4*				
CitC2R5	*REB 316-1, Citricidal 12 drops rep 5*				
CitC3R1	*REB 316-1, Citricidal 6 drops rep 1*				
CitC3R2	*REB 316-1, Citricidal 6 drops rep 2*				
CitC3R3	*REB 316-1, Citricidal 6 drops rep 3*				
CitC3R4	*REB 316-1, Citricidal 6 drops rep 4*				
CitC3R5	*REB 316-1, Citricidal 6 drops rep 5*				
CitC4R1	*REB 316-1, Citricidal 3 drops rep 1*				
CitC4R2	*REB 316-1, Citricidal 3 drops rep 2*				
CitC4R3	*REB 316-1, Citricidal 3 drops rep 3*				
CitC4R4	*REB 316-1, Citricidal 3 drops rep 4*				
CitC4R5	*REB 316-1, Citricidal 3 drops rep 5*				
CitC5R1	*REB 316-1, Citricidal 1 drop rep 1*				
CitC5R2	*REB 316-1, Citricidal 1 drop rep 2*				
CitC5R3	*REB 316-1, Citricidal 1 drop rep 3*				
CitC5R4	*REB 316-1, Citricidal 1 drop rep 4*				
CitC5R5	*REB 316-1, Citricidal 1 drop rep 5*				
ConC1R1	*REB 316-1, Control ,water, rep 1*	10	11	4	13
ConC1R2	*REB 316-1, Control ,water, rep 2*	10	11	4.5	11

ConC1R3	*REB 316-1, Control ,water, rep 3*	10	11	5	11
ConC2R1	*REB 316-1, Control ,water, rep 1*	9	11	4	14
ConC2R2	*REB 316-1, Control ,water, rep 2*	9	11	5	16
ConC2R3	*REB 316-1, Control ,water, rep 3*	10	11	4.5	13
ConC3R1	*REB 316-1, Control ,water, rep 1*	10	10	4	13
ConC3R2	*REB 316-1, Control ,water, rep 2*	10	11	4	12
ConC3R3	*REB 316-1, Control ,water, rep 3*	10	12	5	12
ConC4R1	*REB 316-1, Control ,water, rep 1*	11	11	5	11
ConC4R2	*REB 316-1, Control ,water, rep 2*	10	11	4	13
ConC4R3	*REB 316-1, Control ,water, rep 3*	9	11	4.5	13
ConC5R1	*REB 316-1, Control ,water, rep 1*	10	11	4.5	14
ConC5R2	*REB 316-1, Control ,water, rep 2*	9	11	4.5	14
ConC5R3	*REB 316-1, Control ,water, rep 3*	9	11	4	15

Indicates Colony has hit edge of plate

	Observations			
	2 days	4 days	5 days	8 days
TriC1R1				
TriC1R2				
TriC1R3				
TriC1R4				
TriC1R5				
TriC2R1				
TriC2R2				
TriC2R3				
TriC2R4				
TriC2R5				
TriC3R1				
TriC3R2				
TriC3R3				
TriC3R4				
TriC3R5				
TriC4R1				
TriC4R2				
TriC4R3				
TriC4R4				
TriC4R5				
TriC5R1				
TriC5R2				
TriC5R3				
TriC5R4				
TriC5R5				
VirC1R1				
VirC1R2				
VirC1R3				Mycelia growing in plug but not radial growth
VirC1R4				
VirC1R5				
VirC2R1				
VirC2R2				
VirC2R3				Mycelia growing all over plug and just starting to grow radially
VirC2R4		Mycelia still alive on plug		
VirC2R5				
VirC3R1				
VirC3R2				
VirC3R3				
VirC3R4				
VirC3R5	Mycelia growing upwards into the air rather than outwards into the media	Mycelia still growing upwards into the air rather than outwards into the media		Mycelia also growing vigorously upwards of the plug
VirC4R1				
VirC4R2				
VirC4R3				Mycelia also growing vigorously upwards of the plug
VirC4R4				
VirC4R5				
VirC5R1				
VirC5R2				Mycelia growing on top

	Observations			
	2 days	4 days	5 days	8 days
VirC5R3				of plug but not as vigorous as VirC3 & 4
VirC5R4				
VirC5R5				
NaOCl1R1				
NaOCl1R2				
NaOCl1R3				
NaOCl1R4				
NaOCl1R5				
NaOCl2R1				
NaOCl2R2				
NaOCl2R3			A few mycelial threads	A few tiny mycelial threads growing radially into agar
NaOCl2R4				
NaOCl2R5				
NaOCl3R1				
NaOCl3R2				
NaOCl3R3				
NaOCl3R4				Surprisingly no action?
NaOCl3R5				
NaOCl4R1				
NaOCl4R2				
NaOCl4R3				
NaOCl4R4				
NaOCl4R5				
NaOCl5R1				
NaOCl5R2				
NaOCl5R3				
NaOCl5R4				
NaOCl5R5				
CitC1R1				
CitC1R2				
CitC1R3				
CitC1R4			Mycelia just emerging from top of plug	Very tiny mycelia emerging from top of plug
CitC1R5				
CitC2R1				
CitC2R2				
CitC2R3				
CitC2R4			Mycelia just emerging from top of plug	Very tiny mycelia emerging from top of plug
CitC2R5				
CitC3R1				
CitC3R2				
CitC3R3			Mycelia just emerging from top of plug	Very tiny mycelia emerging from top of plug
CitC3R4				
CitC3R5				
CitC4R1				
CitC4R2				
CitC4R3			Mycelia just emerging from top of plug	Some mycelia starting to grow well on top of the plug
CitC4R4				
CitC4R5				
CitC5R1	Mycelia	Mycelia still	All have	All replicas

	Observations			
	2 days	**4 days**	**5 days**	**8 days**
CitC5R2	growing upwards on agar plug, not into the surrounding agar	growing upwards on agar plug, not into the surrounding agar	mycelia emerging on the top of plug. No radial growth	have mycelia starting to grow well on top of plug
CitC5R3				
CitC5R4				
CitC5R5				
ConC1R1				
ConC1R2				
ConC1R3				
ConC2R1				
ConC2R2				
ConC2R3				
ConC3R1				
ConC3R2				
ConC3R3				
ConC4R1				
ConC4R2				
ConC4R3				
ConC5R1				
ConC5R2				
ConC5R3				

EXPERIMENT 1: AVERAGED DATA

Average growth of the PTA colonies

Virkon

		Growth in mm			
		2 days	4 days	5 days	8 days
VirC3R1	*REB 316-1, Virkon S 0.05% a.i, rep 1*	3	3	2	5
VirC3R2	*REB 316-1, Virkon S 0.05% a.i, rep 2*	2	2	1.5	5
VirC3R3	*REB 316-1, Virkon S 0.05% a.i, rep 3*	2	2	1.5	5
VirC3R4	*REB 316-1, Virkon S 0.05% a.i, rep 4*	2	3	1.5	5
VirC3R5	*REB 316-1, Virkon S 0.05% a.i, rep 5*	2	2	1.5	6
VirC4R1	*REB 316-1, Virkon S 0.025% a.i, rep 1*	5	5	3	9
VirC4R2	*REB 316-1, Virkon S 0.025% a.i, rep 2*	5	6	2.5	10
VirC4R3	*REB 316-1, Virkon S 0.025% a.i, rep 3*	5	6	2.5	10
VirC4R4	*REB 316-1, Virkon S 0.025% a.i, rep 4*	4	6	2.5	10
VirC4R5	*REB 316-1, Virkon S 0.025% a.i, rep 5*	4	6	2.5	9
VirC5R1	*REB 316-1, Virkon S 0.0125% a.i, rep 1*	8	8	4	12
VirC5R2	*REB 316-1, Virkon S 0.0125% a.i, rep 2*	7	8	2.5	11
VirC5R3	*REB 316-1, Virkon S 0.0125% a.i, rep 3*	7	9	3	12
VirC5R4	*REB 316-1, Virkon S 0.0125% a.i, rep 4*	7	9	3	12
VirC5R5	*REB 316-1, Virkon S 0.0125% a.i, rep 5*	8	8	3	11

Virkon				
	Average growth in mm			
Concentration	**2 days**	**4 days**	**5 days**	**8 days**
0.050%	2.2	2.4	1.6	5.2
0.025%	4.6	5.8	2.6	9.6
0.0125%	7.4	8.4	3.1	11.6

n = 5

Sodium hypochlorite

		Growth in mm			
		2 days	4 days	5 days	8 days
NaOCl4R1	*REB 316-1, NaHypochlorite 0.025% a.i, rep 1*	0	5	2.5	12
NaOCl4R2	*REB 316-1, NaHypochlorite 0.025% a.i, rep 2*	0	5	2.5	12
NaOCl4R3	*REB 316-1, NaHypochlorite 0.025% a.i, rep 3*	0	5	3	11
NaOCl4R4	*REB 316-1, NaHypochlorite 0.025% a.i, rep 4*	0	6	2.5	11
NaOCl4R5	*REB 316-1, NaHypochlorite 0.025% a.i, rep 5*	0	4	2.5	10
NaOCl5R1	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 1*	4	9	4	14
NaOCl5R2	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 2*	5	9	4	13
NaOCl5R3	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 3*	5	10	4	13
NaOCl5R4	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 4*	4	9	4	14
NaOCl5R5	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 5*	4	9	4	13

Sodium Hypochlorite (NaOCl)				
	Average growth in mm			
Concentration	**2 days**	**4 days**	**5 days**	**8 days**
0.025%	0	5	2.6	11.2
0.0125%	4.4	9.2	4	13.4

n = 5

Control

		Growth in mm			
		2 days	4 days	5 days	8 days
ConC1R1	*REB 316-1, Control ,water, rep 1*	10	11	4	13
ConC1R2	*REB 316-1, Control ,water, rep 2*	10	11	4.5	11
ConC1R3	*REB 316-1, Control ,water, rep 3*	10	11	5	11
ConC2R1	*REB 316-1, Control ,water, rep 1*	9	11	4	14
ConC2R2	*REB 316-1, Control ,water, rep 2*	9	11	5	16
ConC2R3	*REB 316-1, Control ,water, rep 3*	10	11	4.5	13
ConC3R1	*REB 316-1, Control ,water, rep 1*	10	10	4	13
ConC3R2	*REB 316-1, Control ,water, rep 2*	10	11	4	12
ConC3R3	*REB 316-1, Control ,water, rep 3*	10	12	5	12
ConC4R1	*REB 316-1, Control ,water, rep 1*	11	11	5	11
ConC4R2	*REB 316-1, Control ,water, rep 2*	10	11	4	13
ConC4R3	*REB 316-1, Control ,water, rep 3*	9	11	4.5	13
ConC5R1	*REB 316-1, Control ,water, rep 1*	10	11	4.5	14
ConC5R2	*REB 316-1, Control ,water, rep 2*	9	11	4.5	14
ConC5R3	*REB 316-1, Control ,water, rep 3*	9	11	4	15

Indicates that Colony has hit the edge of the plate

Control				
	Average growth in mm			
	2 days	**4 days**	**5 days**	**8 days**
Controls	9.733333	11	4.433333	13

n=15

EXPERIMENT 1: DATA AND RESULTS

Net growth

Net growth in mm

Virkon				
	Average net growth in mm			
Concentration	2 days	4 days	5 days	8 days
0.0500%	2.2	2.4	1.6	5.2
0.0250%	4.6	5.8	2.6	9.6
0.0125%	7.4	8.4	3.1	11.6

n = 5

Sodium Hypochlorite (NaOCl)				
	Average net growth in mm			
Concentration	2 days	4 days	5 days	8 days
0.0250%	0	5	2.6	11.2
0.0125%	4.4	9.2	4	13.4

n = 5

Control				
	Average net growth in mm			
	2 days	4 days	5 days	8 days
Controls	9.733333	11	4.433333	13

n = 15

Total growth

Total growth in mm (measured from start of plug each time)

Virkon				
	Average total growth in mm			
Concentration	2 days	4 days	5 days	8 days
0.0500%	2.2	4.6	6.2	11.4
0.0250%	4.6	10.4	13	22.6
0.0125%	7.4	15.8	18.9	30.5

n = 5

Sodium Hypochlorite (NaOCl)				
	Average total growth in mm			
Concentration	2 days	4 days	5 days	8 days
0.0250%	0	5	7.6	18.8
0.0125%	4.4	13.6	17.6	31

n = 5

Control				
	Average total growth in mm			
	2 days	4 days	5 days	8 days
Controls	9.733333	20.733333	25.16667	38.16667

n = 15

	Conc.	Rate of growth (mm/day)	R^2
Virkon	0.05%	1.33	0.98
	0.025%	2.72	0.993
	0.0125%	3.82	0.999

	0.025%	3.16	0.987
Sodium hypochlorite	0.0125%	3.66	0.978
Control	0%	5.07	0.999

Virkon – Average total growth in mm			
Days	0.05%	0.025	0.0125
0	0	0	0
2	2.2	4.6	7.4
4	4.6	10.4	15.8
5	6.2	13	18.9
8	11.4	22.6	30.5

Sodium hypochlorite – Average total growth in mm		
Days	0.03%	0.01%
0	0	0
2	0	4.4
4	5	13.6
5	7.6	17.6
8	18.8	31

Control – Average total growth in mm	
Days	
0	0
2	9.733333333
4	20.73333333
5	25.16666667
8	38.16666667

Note : Day 8 not included – Cultures grown to edge of plate

EXPERIMENT 2: DATA COMBINED (200 OOSPORES)

Colour of 200 oospores

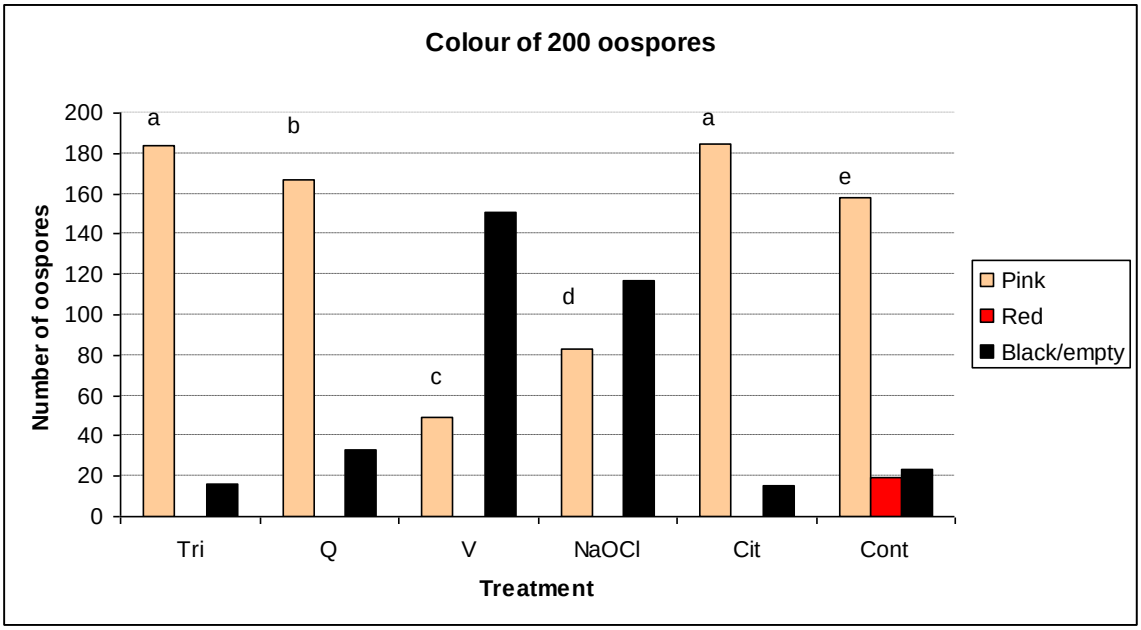
Colour of 200 oospores

	Pink	Red	Black/empty
Tri	184	0	16
Q	167	0	33
V	49	0	151
NaOCl	83	0	117
Cit	185	0	15
Cont	158	19	23

	Pink	Red	Black/empty
Tri	0.92	0	0.08
Q	0.835	0	0.165
V	0.245	0	0.755
NaOCl	0.415	0	0.585
Cit	0.925	0	0.075
Cont	0.79	0.095	0.115

Chi2 test (alpha = 0.05, critical value = 5.99)

	Distances			*Sum squares*	Chi2 (= sum squares × 200)	
	Pink	**Red**	**Black/empty**			
Tri/Cont	0.021392405	0.095	0.010652174	0.127044579	25.4089158	difference
Q/Cont	0.002563291	0.095	0.02173913	0.119302422	23.86048431	difference
V/Cont	0.375981013	0.095	3.56173913	4.032720143	806.5440286	difference
NaOCl/Cont	0.178006329	0.095	1.920869565	2.193875894	438.7751789	difference
Cit/Cont	0.02306962	0.095	0.013913043	0.131982664	26.39653275	difference
Tri/Q	0.008652695	0	0.043787879	0.052440573	10.48811468	difference
Tri/V	1.859693878	0	0.603476821	2.463170699	492.6341397	difference
Tri/NaOCl	0.614518072	0	0.435940171	1.050458243	210.0916486	difference
Tri/Cit	2.7027E-05	0	0.000333333	0.00036036	0.072072072	NO DIFFERENCE
Q/V	1.420816327	0	0.461059603	1.881875929	376.3751858	difference
Q/NaOCl	0.425060241	0	0.301538462	0.726598703	145.3197405	difference
Q/Cit	0.008756757	0	0.108	0.116756757	23.35135135	difference
V/NaOCl	0.069638554	0	0.049401709	0.119040264	23.80805272	difference
V/Cit	0.499891892	0	6.165333333	6.665225225	1333.045045	difference
NaOCl/Cit	0.281189189	0	3.468	3.749189189	749.8378378	difference



EXPERIMENT 2: Individual Replicates

Colour of 100 oospores (replicate 1)

Colour of 100 oospores

	Pink	Red	Black/empty
TriR1	100	0	0
QR3	70	0	30
VR2	28	0	72
NaOClR1	40	0	60
CitR1	94	0	6
ContR1	93	11	6

Frequency of each colour

	Pink	Red	Black/empty
TriR1	1	0	0
QR3	0.7	0	0.3
VR2	0.28	0	0.72
NaOClR1	0.4	0	0.6
CitR1	0.94	0	0.06
ContR1	0.93	0.11	0.06

Colour of 100 oospores (replicate 2)

Colour of 100 oospores

	Pink	Red	Black/empty
TriR4	84	0	16
QR4	97	0	3
VR3	21	0	79
NaOClR5	43	0	57
CitR2	91	0	9
ContR4	75	8	17

Frequency of each colour

	Pink	Red	Black/empty
TriR4	0.84	0	0.16
QR4	0.97	0	0.03
VR3	0.21	0	0.79
NaOClR5	0.43	0	0.57
CitR2	0.91	0	0.09
ContR4	0.75	0.08	0.17

Chi2 test (alpha = 0.05, critical value = 5.99)

	Distances			*Sum Squares*	*Chi2 (= sum squares × 100)*	
	Pink	**Red**	**Black/empty**			
ContR1/TriR1	0.005268817	0.11	0.06	0.175269	17.52688	difference
ContR1/QR3	0.05688172	0.11	0.96	1.126882	112.6882	difference
ContR1/VR2	0.454301075	0.11	7.26	7.824301	782.4301	difference
ContR1/NaOClR1	0.302043011	0.11	4.86	5.272043	527.2043	difference
ContR1/CitR1	0.000107527	0.11	0	0.110108	11.01075	difference
ContR4/TriR1	0.083333333	0.08	0.17	0.333333	33.33333	difference
ContR4/QR3	0.003333333	0.08	0.099411765	0.182745	18.27451	difference
ContR4/VR2	0.294533333	0.08	1.779411765	2.153945	215.3945	difference
ContR4/NaOClR1	0.163333333	0.08	1.087647059	1.33098	133.098	difference
ContR4/CitR1	0.048133333	0.08	0.071176471	0.19931	19.93098	difference
TriR1/TriR4	0.03047619	0	0.16	0.190476	19.04762	difference
QR3/QR4	0.075154639	0	2.43	2.505155	250.5155	difference
VR2/VR3	0.023333333	0	0.006202532	0.029536	2.953586	no difference
NaOClR1/NaOClR5	0.002093023	0	0.001578947	0.003672	0.367197	no difference
CitR1/CitR2	0.000989011	0.01125	0.01	0.022239	2.223901	no difference
ConTR1/ContR4	0.03483871	0.008182	0.201666667	0.244687	24.46872	difference
TriR1/QR3	0.128571429	0	0.3	0.428571	42.85714	difference
TriR1/VR2	1.851428571	0	0.72	2.571429	257.1429	difference
TriR1/NaOClR1	0.9	0	0.6	1.5	150	difference
TriR1/CitR1	0.003829787	0	0.06	0.06383	6.382979	difference

QR3/VR2	0.63	0	0.245	0.875	87.5	difference
QR3/NaOClR1	0.225	0	0.15	0.375	37.5	difference
QR3/CitR1	0.061276596	0	0.96	1.021277	102.1277	difference
VR2/NaOClR1	0.036	0	0.024	0.06	6	difference
VR2/CitR1	0.463404255	0	7.26	7.723404	772.3404	difference
NaOClR1/CitR1	0.310212766	0	4.86	5.170213	517.0213	difference

	Distances			*Sum Squares*	*Chi2 (= sum squares × 100)*	
	Pink	**Red**	**Black/empty**			
ContR1/TriR4	0.00871	0.11	0.166667	0.285376	28.53763	difference
ContR1/QR4	0.00172	0.11	0.015	0.12672	12.67204	difference
ContR1/VR3	0.557419	0.11	8.881667	9.549086	954.9086	difference
ContR1/NaOClR5	0.268817	0.11	4.335	4.713817	471.3817	difference
ContR1/CitR2	0.00043	0.11	0.015	0.12543	12.54301	difference

ContR4/TriR4	0.0108	0.08	0.000588	0.091388	9.138824	difference
ContR4/QR4	0.064533	0.08	0.115294	0.259827	25.98275	difference
ContR4/VR3	0.3888	0.08	2.261176	2.729976	272.9976	difference
ContR4/NaOClR5	0.136533	0.08	0.941176	1.15771	115.771	difference
ContR4/CitR2	0.034133	0.08	0.037647	0.15178	15.17804	difference

TriR4/QR4	0.017423	0	0.563333	0.580756	58.0756	difference
TriR4/VR3	1.89	0	0.502405	2.392405	239.2405	difference
TriR4/NaOClR5	0.39093	0	0.294912	0.685843	68.58425	difference
TriR4/CitR2	0.005385	0	0.054444	0.059829	5.982906	difference
QR4/VR3	2.750476	0	0.731139	3.481615	348.1615	difference
QR4/NaOClR5	0.67814	0	0.511579	1.189718	118.9718	difference
QR4/CitR2	0.003956	0	0.04	0.043956	4.395604	NO DIFFERENCE
VR3/NaOClR5	0.112558	0	0.084912	0.19747	19.74704	difference
VR3/CitR2	0.538462	0	5.444444	5.982906	598.2906	difference
NaOClR5/CitR2	0.253187	0	2.56	2.813187	281.3187	difference

EXPERIMENT 2b: Zoospore sensitivity data 3 Replicates 1-5

Rep 1: Zoospore CFUs/ml

	Pink
Tri	0
Q	0
V	0
NaOCl	0
Cit	840
Cont	440

Rep 2: Zoospore CFUs/ml

	Pink
Tri	0
Q	0
V	0
NaOCl	0
Cit	800
Cont	500

Rep 3: Zoospore CFUs/ml

	Pink
Tri	0
Q	0
V	0
NaOCl	0
Cit	780
Cont	320

Rep 4: Zoospore CFUs/ml

	Pink
Tri	0
Q	0
V	0
NaOCl	0
Cit	760
Cont	360

Rep 5: Zoospore CFUs/ml

	Pink
Tri	0
Q	0
V	0
NaOCl	0
Cit	740
Cont	400

	Average number of CFUs/ml	Standard deviation
Tri	0	0
Q	0	0
V	0	0
NaOCl	0	0
Cit	784	38.47076812
Cont	404	69.85699679

Experiment 3 ***Raw data and statistical analysis***

		Number of leaf baits (10 leaves/replicate)			*Total of leaf baits (out of 30)*
	Oospores / g of soil	**Replicate 1** **Leaf baits**	**Replicate 2** **Leaf baits**	**Replicate 3** **Leaf baits**	
1	2 × 103	0	8	3	11
2	1 × 103	0	5	2	7
3	500	0	4	0	4
4	250	0	0	1	1
5	125	0	0	0	0
Control	Huia Composite	0	0	0	1

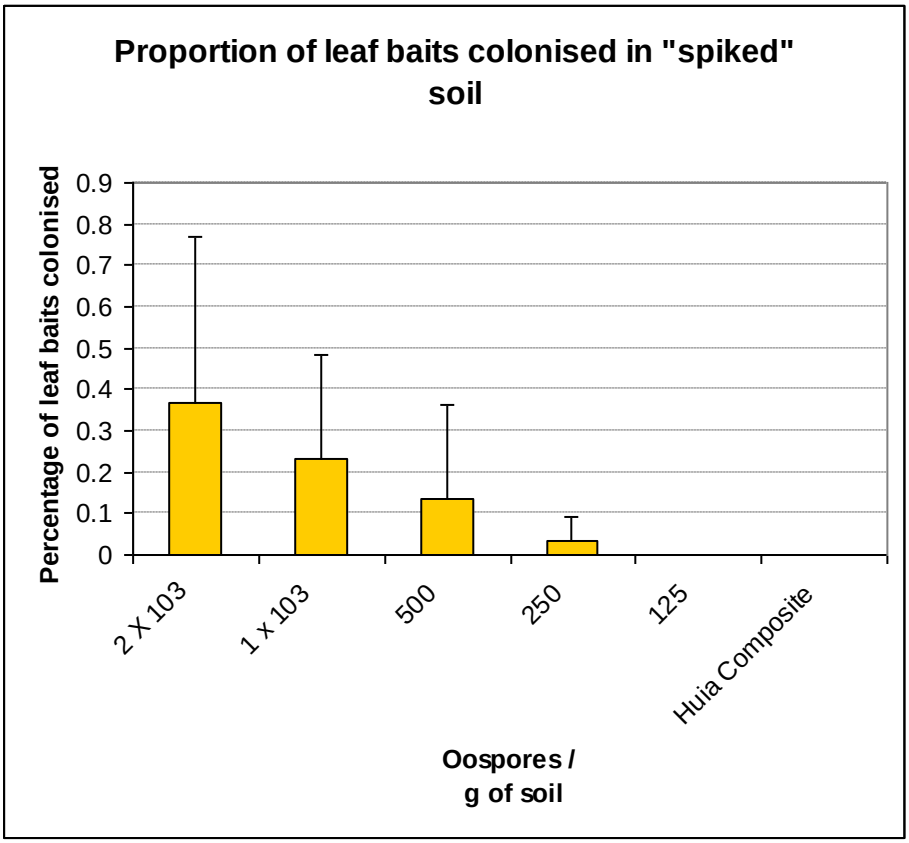
		Number of CFUs ./ ml				
Oospores/g of soil	**PTA recoveries**	**Replicate 1** **CFUs**	**Replicate 2** **CFUs**	**Replicate 3** **CFUs**	*Mean*	*Standard deviation*
2 × 103	7	220	270	210	233.3333	32.14550254
1 × 103	0	70	40	150	86.66667	56.86240703
500	0	60	10	20	30	26.45751311
250	0	0	0	0	0	0
125	0	220	220	10	150	121.2435565
Huia Composite	0	60	80	90	76.66667	15.27525232

Frequency of leaf baits

	Oospores/g of soil	**Replicate 1** **Leaf baits**	**Replicate 2** **Leaf baits**	**Replicate 3** **Leaf baits**	*Mean*	*Variance*	*SS*
1	2 × 103	0	0.8	0.3	0.366666667	0.108888889	0.326666667
2	1 × 103	0	0.5	0.2	0.233333333	0.042222222	0.126666667
3	500	0	0.4	0	0.133333333	0.035555556	0.106666667
4	250	0	0	0.1	0.033333333	0.002222222	0.006666667
5	125	0	0	0	0	0	0
Control	Huia Composite	0	0	0	0	0	0

Student test (alpha = 0.05, critical value = 2.776)

	sp2	*sX1-X2*	*t*	
1 v. Control	0.081666667	0.233333333	1.571428571	No difference
2 v. Control	0.031666667	0.145296631	1.605910137	No difference
3 v. Control	0.026666667	0.133333333	1	No difference
4 v. Control	0.001666667	0.033333333	1	No difference
5 v. Control				No difference



Angular transformation and Student test

	Oospores/g of soil	Replicate 1 Leaf baits	Replicate 2 Leaf baits	Replicate 3 Leaf baits	*Mean*	*Varpa*	*SS*
1	2 × 103	0	1.107148718	0.57963974	0.562262819	0.204447359	0.613342078
2	1 × 103	0	0.785398163	0.463647609	0.416348591	0.103926978	0.311780933
3	500	0	0.684719203	0	0.228239734	0.104186753	0.312560258
4	250	0	0	0.321750554	0.107250185	0.023005204	0.069015613
5	125	0	0	0	0	0	0
Control	Huia Composite	0	0	0	0	0	0

STUDENT TEST alpha = 0.05 limit value = 2.776

	sp2	*sX1-X2*	*t*	
1/Control	0.153335519	0.319724381	1.758585999	No difference
2/Control	0.077945233	0.227955015	1.826450674	No difference
3/Control	0.078140064	0.228239734	1	No difference
4/Control	0.017253903	0.107250185	1	No difference
5/Control				No difference

EXPERIMENT 4: After 3-days

Raw data

	Leaf baits	Soil extract	Soil extract	Soil extract
2% TriGene	0	0	0	0
2% TriGene	0	0	0	0
10% Phytoclean	0	0	0	0
10% Phytoclean	0	0	0	0
1% Virkon	0	0	0	0
1% Virkon	0	5 bact	7 zygos	0
5% NaOCl	12 zygos	0	0	0
5% NaOCl	8 zygos	3 zygos	7 zygos	0
Citricidal 6 drops/100 ml	1 pta, 1 pc, 4 zygos	7 zygos	3 zygos, 1 pta	4 zygos, 4 bact
Citricidal 6 drops/100 ml	2 pta	20 zygos	2 zygos	5 bact
Control (RO water)	1 pta, 1 py	14 zygos, 1 pta	16 bact, 16 zygos	20 bact, 10 zygos
Control (RO water)	8 zygos, 2 pta	16 zygos, 1 pta	17 bact, 10 zygos	16 bact, 5 zygos

CFUs / ml

	Mean bact	*s.d. bact*	*Mean zygo*	*s.d. zygo*	*Mean PTA*	*s.d. PTA*
2% TriGene	0	0	0	0	0	0
2% TriGene	0	0	0	0	0	0
10% Phytoclean	0	0	0	0	0	0
10% Phytoclean	0	0	0	0	0	0
1% Virkon	0	0	0	0	0	0
1% Virkon	12.5	25	17.5	35	0	0
5% NaOCl	0	0	30	60	0	0
5% NaOCl	0	0	45	36.96845502	0	0
Citricidal 6 drops/100 ml	13.33333333	20	40	17.32050808	5	5.773502692
Citricidal 6 drops/100 ml	12.5	25	55	107.0825227		
Control (RO water)	40	80	100	86.986589	10	5.773502692
Control (RO water)	40	80	97.5	46.45786622		

EXPERIMENT 5: RINSATES

CFUs / ml

	Rinsates PTA	Rinsates Zygos
2% TriGene	0	0
2% TriGene	0	0
10% Phytoclean	0	0
10% Phytoclean	0	0
1% Virkon	0	10
1% Virkon	0	20
5% NaOCl	0	10
5% NaOCl	0	30
Citricidal 6 drops/100 ml	10	180
Citricidal 6 drops/100 ml	10	50
Control (RO water)	10	290
Control (RO water)	10	230

	Mean		*Standard deviation*	
	Rinsates PTA	Rinsates Zygos	Rinsates PTA	Rinsates Zygos
2% TriGene	0	0	0	0
10% Phytoclean	0	0	0	0
1% Virkon	0	15	0	7.071068
5% NaOCl	0	20	0	14.14214
Citricidal 6 drops/100 ml	10	115	0	91.92388
Control (RO water)	10	260	0	42.42641

EXPERIMENT 5: SWABS CFUs / ml

	Swabs before Fungi	Swabs before Bacteri	Swabs after Fungi	Swabs after Bacteria
2% TriGene	0	0	230	340
2% TriGene	10	0	190	10
2% TriGene	0	0	480	630
2% TriGene	0	0	280	1000
10% Phytoclean	0	0	560	0
10% Phytoclean	1	0	440	0
10% Phytoclean	0	0	350	0
10% Phytoclean	0	0	420	0
1% Virkon	0	0	450	0
1% Virkon	0	0	290	0
1% Virkon	40	0	20	0
1% Virkon	40	0	0	0
5% NaOCl	0	0	0	0
5% NaOCl	0	1000	60	0
5% NaOCl	2	0	540	0
5% NaOCl	6	0	70	0
Citricidal 6 drops/100 ml	0	0	530	0
Citricidal 6 drops/100 ml	0	0	720	0

Citricidal 6 drops/100 ml	0	0	240	0
Citricidal 6 drops/100 ml	0	0	390	0
Control (RO water)	0	0	550	500
Control (RO water)	0	0	430	420
Control (RO water)	10	0	150	330
Control (RO water)	10	0	130	120

Mean *Standard deviation*

	Before		After		Before		After	
	Fungi	**Bacteria**	**Fungi**	**Bacteria**	**Fungi**	**Bacteria**	**Fungi**	**Bacteria**
2% TriGene	2.5	0	295	495	5	0	128.7116	421.3075
10% Phytoclean	0.25	0	442.5	0	0.5	0	87.32125	0
1% Virkon	20	0	190	0	23.09401	0	218.0214	0
5% NaOCl	2	250	167.5	0	2.828427	500	250.2499	0
Citricidal 6 drops/100 ml	0	0	470	0	0	0	204.4505	0
Control (RO water)	5	0	315	342.5	5.773503	0	208.0865	163.7834

EXPERIMENT 5: LEAF BAITS

Raw data

Number of leaf baits

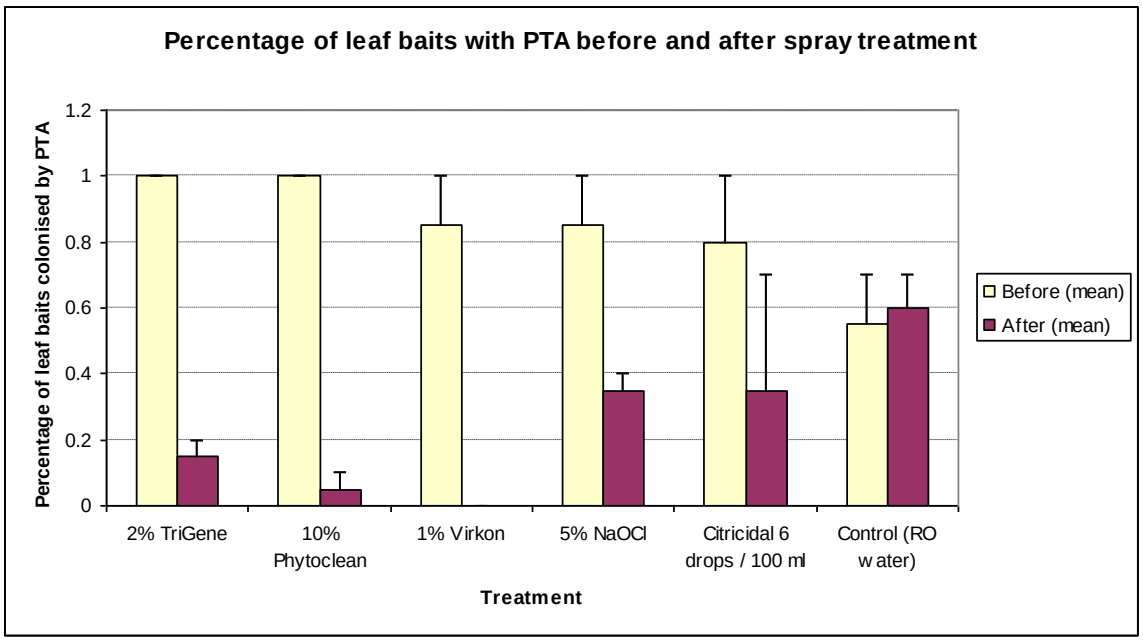
	Before	After	Total
2% TriGene	20	3	**23**
10% Phytoclean	20	1	**20**
1% Virkon	17	0	**17**
5% NaOCl	17	7	**24**
Citricidal 6 drops/100 ml	16	7	**17**
Control (RO water)	11	12	**23**
	101	**23**	**124**

Treatment	Before (Rep 1)	Before (Rep 2)	After (Rep 1)	After (Rep 2)
2% TriGene	1	1	1/5	1/10
10% Phytoclean	1	1	0	1/10
1% Virkon	1	7/10	0	0
5% NaOCl	1	7/10	2/5	3/10
Citricidal 6 drops/100 ml	3/5	1	7/10	0
Control (RO water)	7/10	2/5	7/10	1/2

	Before		After	
Treatment	*Before (mean)*	*Variance*	*After (mean)*	*Variance*
2% TriGene	1	0	0.15	0.0025
10% Phytoclean	1	0	0.05	0.0025
1% Virkon	0.85	0.0225	0	0
5% NaOCl	0.85	0.0225	0.35	0.0025
Citricidal 6 drops/100 ml	0.8	0.04	0.35	0.1225
Control (RO water)	0.55	0.0225	0.6	0.01

Student test (alpha = 0.05, critical value = 4.403)

sp2	*sX1-X*	*t*	
0.0025	0.05	17	effect of the treatment
0.0025	0.05	19	effect of the treatment
0.0225	0.15	5.666667	effect of the treatment
0.025	0.158113883	3.162278	no effect of the treatment
0.1625	0.403112887	1.116313	no effect of the treatment
0.0325	0.180277564	–0.27735	no effect of the treatment



Reduction of number of baits with lesions

Reduction of number of baits with lesions

	Rep1	**Rep2**	*Mean*	*Var*
2% TriGene	8	9	8.5	0.5
10% Phytoclean	10	9	9.5	0.5
1% Virkon	10	7	8.5	4.5
5% NaOCl	6	4	5	2
Citricidal 6 drops/100 ml	1	10	5.5	40.5
Control (RO water)	0	1	0.5	0.5

Student test (alpha=0.05, critical value=4.403)

	sp2	*sqrt(sp2)*	*t*	
Trigene/Phytoclean	0.5	0.707106781	-1.41421	no difference
Trigene/Control	0.5	0.707106781	11.3137	difference
Phytoclean/Control	0.5	0.707106781	12.7279	difference
Virkon/Control	2.5	1.58113883	5.05964	difference
Citricidal/Control	20.5	4.527692569	1.10432	no difference
NaOCl/Control	1.25	1.118033989	4.02492	difference
Trigene/Virkon	2.5	1.58113883	0	no difference
Phytoclean/Virkon	2.5	1.58113883	0.63246	no difference

Angular transformation and Student test (raw data)

Frequence of leaf baits

Before (Rep 1)	**Before (Rep 2)**	**After (Rep 1)**	**After (Rep 2)**

2% TriGene	1	1	1/5	1/10
10% Phytoclean	1	1	0	1/10
1% Virkon	1	7/10	0	0
5% NaOCl	1	7/10	2/5	3/10
Citricidal 6 drops/100 ml	3/5	1	7/10	0
Control (RO water)	7/10	2/5	7/10	1/2

Sqrt(frequence)

	Before (Rep 1)	Before (Rep 2)	After (Rep 1)	After (Rep 2)
2% TriGene	1	1	17/38	6/19
10% Phytoclean	1	1	0	6/19
1% Virkon	1	41/49	0	0
5% NaOCl	1	41/49	43/68	23/42
Citricidal 6 drops/100 ml	55/71	1	41/49	0
Control (RO water)	41/49	43/68	41/49	70/99

Arcsin(sqrt(frequence))

	Before (Rep 1)	Before (Rep 2)	After (Rep 1)	After (Rep 2)
2% TriGene	1 4/7	1 4/7	32/69	28/87
10% Phytoclean	1 4/7	1 4/7	0	28/87
1% Virkon	1 4/7	1	0	0
5% NaOCl	1 4/7	1	63/92	40/69
Citricidal 6 drops/100 ml	70/79	1 4/7	1	0
Control (RO water)	1	63/92	1	11/14

STUDENT TEST

alpha = 0.05 limit value = 4.403

	Before			After		
	Mean	*Variance*	*SS*	*Mean*	*Variance*	*SS*
2% TriGene	1 4/7	0	0	11/28	0.005033694	0.010067387
10% Phytoclean	1 4/7	0	0	14/87	0.025880855	0.05176171
1% Virkon	1 25/89	0.083995557	0.167991114	0	0	0
5% NaOCl	1 25/89	0.083995557	0.167991114	55/87	0.002760423	0.005520847
Citricidal 6 drops/100 ml	1 8/35	0.117210097	0.234420193	1/2	0.245597845	0.491195689
Control (RO water)	31/37	0.023475967	0.046951935	8/9	0.010584132	0.021168264

sp2	*sqrt(sp2)*	*t*	
0.00503	0.070948527	16.604957	effect of the treatment
0.02588	0.160875277	8.764062907	effect of the treatment
0.084	0.28981987	4.419905563	effect of the treatment
0.08676	0.294543682	2.20271907	no effect
0.36281	0.602335406	1.216694926	no effect
0.03406	0.184553785	-0.272763195	no effect

Angular transformation and Student test (reduction of number of baits)

Reduction of number of baits with lesions

	Rep1	Rep2	*Mean*	*Var*	*SS*
2% TriGene	0.4	0.45	0.425	0.000625	0.00125
10% Phytoclean	0.5	0.45	0.475	0.000625	0.00125
1% Virkon	0.5	0.35	0.425	0.005625	0.01125
5% NaOCl	0.3	0.2	0.25	0.0025	0.005
Citricidal 6 drops/100 ml	0.05	0.5	0.275	0.050625	0.10125

Control (RO water)	0	0.05	0.025	0.000625	0.00125

Reduction of number of baits with lesions (angular transformation)

	Rep1	**Rep2**	*Mean*	*Var*	*SS*
2% TriGene	0.6847192	0.735314453	0.710016828	0.00064	0.00127994
10% Phytoclean	0.78539816	0.735314453	0.760356308	0.0006271	0.001254189
1% Virkon	0.78539816	0.633051836	0.709225	0.0058024	0.011604702
5% NaOCl	0.57963974	0.463647609	0.521643675	0.0033635	0.006727087
Citricidal 6 drops/100 ml	0.22551341	0.785398163	0.505455785	0.0783677	0.156735471
Control (RO water)	0	0.225513406	0.112756703	0.0127141	0.025428148

STUDENT TEST alpha = 0.05 limit value = 4.403

	sp2	*sqrt(sp2)*	*t*	
Trigene/Phytoclean	0.00126706	0.035595847	-1.41419531	no difference
Trigene/Control	0.01335404	0.115559698	5.168411941	difference
Phytoclean/Control	0.01334117	0.115503976	5.606729959	difference
Virkon/Control	0.01851642	0.136075071	4.383376715	difference
Citricidal/Control	0.09108181	0.30179763	1.301200018	no difference
NaOCl/Control	0.01607762	0.126797546	3.224723067	no difference
Trigene/Virkon	0.00644232	0.080264068	0.009865286	no difference
Phytoclean/Virkon	0.00642945	0.080183822	0.637676114	no difference

EXPERIMENT 5: SOIL EXTRACTS

CFUs / nl

	Soil extract 1	**Soil extract 1(b)**	**Soil extract 2**	**Soil extract 2(b)**
2% TriGene (fungi)	80	0	30	90
2% TriGene (bacteria)	0	0	0	0
10% Phytoclean (fungi)	120	20	0	0
10% Phytoclean (bacteria)	0	0	0	0
1% Virkon (fungi)	50	30	0	30
1% Virkon (bacteria)	0	0	0	0
5% NaOCl (fungi)	20	70	0	30
5% NaOCl (bacteria)	60	0	0	0
Citricidal 6 drops/100 ml (fungi)	40	40	0	90
Citricidal 6 drops/100 ml (bacteria)	0	0	80	0
Control (RO water) (fungi)	50	40	60	80
Control (RO water) (bacteria)	0	0	0	0

	Mean	**Standard deviation**
2% TriGene (fungi)	50	42.42640687
2% TriGene (bacteria)	0	0
10% Phytoclean (fungi)	35	57.44562647
10% Phytoclean (bacteria)	0	0
1% Virkon (fungi)	27.5	20.61552813
1% Virkon (bacteria)	0	0
5% NaOCl (fungi)	30	29.43920289

5% NaOCl (bacteria)	15	30
Citricidal 6 drops/100 ml (fungi)	42.5	36.85557398
Citricidal 6 drops/100 ml (bacteria)	20	40
Control (RO water) (fungi)	57.5	17.07825128
Control (RO water) (bacteria)	0	0

Appendix 3 MSDS of Disinfectants Assessed in this Study

NB: MSDS Sheets Commence on Next Page