Assessment of quaternary ammonium compounds as disinfectants for control of *Phytophthora cinnamomi* in washdown situations.

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Abstract

Quaternary ammonium compounds (quats) are more effective disinfectants for sanitation requirements in nurseries than hypochlorite and phenol based products. A laboratory study was undertaken to evaluate some quats available in Victoria and appropriate rates for their use for control of *Phytophthora cinnamomi* in washdown situations.

Of the three quats tested, Phytoclean had the greatest effect on the fungus in inhibiting growth, and in killing the fungus when in contact with the chemical. It was effective at rates as low as 0.01% a.i. in preventing zoospore infection in soil and killed the fungus on agar at rates as low as 0.025%. No trace of the fungus could be isolated from soil drenched in a 1% a.i. solution for 30 seconds, and levels of infective propagules were dramatically reduced at 0.2% a.i. No phytotoxicity was observed when Phytoclean at 1% a.i. was applied as a soil drench to potted *Pinus radiata* seedlings, although some needle browning was observed when applied as a foliage spray.

ABF42 was effective at rates of 0.2% a.i. in killing the fungus on agar, but although reducing fungal growth at 0.1% a.i., was completely ineffective below these rates. Chemene was also effective at 0.2% a.i. in killing the fungus on agar and inhibited growth at all rates tested; however below 0.2% a.i. it was also ineffective in killing the fungus.

None of the disinfectants tested were able to kill the fungus within host tissue within the times tested (up to 10 minutes of immersion).

Quat products vary considerably in their chemical composition and, as shown in these experiments, also in their effectiveness to control *Phytophthora cinnamomi*.

Introduction

Phytophthora cinnamomi (Rands) is an important soil-borne root pathogen known to cause dieback and death of susceptible plants in native forests, plantations, farm and roadside shelterbelts, nurseries and home gardens. A major source of spread of the fungus is via soil adhering to vehicles, machinery and tools for which adequate hygiene and washdown procedures have not been taken.

Previously, hypochlorite and phenol based products such as Biogram have been recommended as disinfectants to enhance washdown procedures. Noske and Shearer (1985) however showed that sodium hypochlorite at concentrations below 10% active ingredient (a.i.) and Biogram below 2.5% a.i. were ineffective in suppressing growth of the pathogen within reasonable time frames. They also recorded that quaternary ammonium compounds (quats) at a.i. above 1.2% were more effective than either phenolic or hypochlorite compounds and found no significant difference between quats tested.

Noske and Shearer (1985) also showed that quat concentrations as low as 0.3% a.i. could significantly suppress fungal growth. Hygiene manuals have been written, based on this information, incorporating quats into washdown management. Recommended concentrations used however (.025% a.i., Anon 1993), do not correlate with data of Noske and Shearer (1985).

This report details seven experiments undertaken to evaluate quaternary ammonium compounds for control of *Phytophthora cinnamomi* and determine rates of quats that can be used effectively for washdown.

Methods

Experiment 1

Phytophthora cinnamomi (CFTT isolate No. 41) was grown on autoclaved potato dextrose agar (PDA) in petri dishes at 25 ^{o}C for 4 days. From the edge of the cultures, a 6.5 mm diameter plug of agar was cut and placed in the centre of 2 lines (x & y) drawn at right angles on 40 plates of PDA amended with three disinfectants at 0.07% a.i. (Table 1) and a control that contained no additives (10 plates per treatment). The plates were incubated at 25 ^{o}C and the colony diameter growth along each line was measured at the same time each day at 1, 2, 3, 4, 7, 8 and 9 days after plating as for Noske and Shearer (1985) and the average linear diameter growth of the culture recorded using:

$$\text{Average colony diameter} = \frac{\text{diameter x ? y of colony \& agar plug}}{2} - \text{diameter of plug}$$

Final colony diameter was analysed using analysis of variance. Survival of the plugs where no growth occurred was assessed by plating on PDA agar after 10 days.

Experiment 2

Phytophthora cinnamomi (CFTT isolate No. 41) was grown and transferred to petri dishes as for experiment 1 but on 160 plates of PDA amended with the three disinfectants at 0.2, 0.1, 0.05, 0.025, and 0.0125% a.i. and a control that contained no additives (10 plates per treatment). The plates were incubated for 1, 2, 3, 4 & 7 days and measured as for experiment 1. Survival of the plugs was assessed after 7 days.

Experiment 3

Phytophthora cinnamomi (CFTT isolate No. 41) was grown and transferred to petri dishes as for experiment 1 but on 49 plates of PDA amended with Chemene and Phytoclean at 0.025, 0.0125 and 0.00625% a.i. and a control that contained no additives (7 plates per treatment). The plates were incubated for 1, 4 & 11 days and measured as for experiment 1.

Experiment 4

The ability of quaternary ammonium compounds to kill *Phytophthora cinnamomi* in plant material was assessed using infected *Eucalyptus sieberi* cotyledons. Cotyledons were floated in naturally infested soil for 4 days and following infection 30 were dipped in each of the following treatments:

(i) distilled water
(ii) ABF42 at 1.0, 0.2 and 0.05% a.i. for 30 seconds, 5 minutes and 10 minutes
(iii) Chemene at 1.0, 0.2 and 0.05% a.i. for 30 seconds, 5 minutes and 10 minutes
(iv) Phytoclean at 1.0, 0.2 and 0.05% a.i. for 30 seconds, 5 minutes and 10 minutes

After dipping, the cotyledons were washed in distilled water and plated onto PDA agar and survival assessed through growth on the agar.

Experiment 5

The ability of quaternary ammonium compounds to kill *Phytophthora cinnamomi* in soil was assessed by baiting soil treated with Phytoclean. Infested soil (100 g) was placed in cloth bags and soaked in Phytoclean at 1.0, 0.2 and 0.05% a.i. for 30 seconds, 5 minutes and 10 minutes. The soil was then washed 3 times in distilled water following the initial treatment, allowed to drain and then 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19 and 0.098 g of treated soil were weighed into 100 ml plastic cups and flooded with distilled water (three replicates per series). *Eucalyptus sieberi* cotyledons were floated on the surface of the water and after 4 days were assessed for the presence of *P. cinnamomi* using sporangial characteristics.

Experiment 6

The ability of Phytoclean to inhibit *P. cinnamomi* zoospore infection of plants from soil was assessed by baiting infested soil flooded with the disinfectant. Infested soil (30 g) was placed in 50 x 100 ml plastic cups and flooded with a 1.0, 0.1, 0.01 and 0.001% solution of Phytoclean and distilled water (10 cups per treatment). *Eucalyptus sieberi* cotyledons were floated on the surface of the water and after 4 days were assessed for the presence of *P. cinnamomi* using sporangial characteristics or, in the absence of sporangia, plating on PDA agar.

Experiment 7

The phytotoxicity of a 1% solution of Phytoclean was tested on nine, 18 month old (8 months in three litre pots) *Pinus radiata* seedlings grown in a glasshouse maintained at 22 ^{o}C. Seedlings were subjected to the following treatments (three per treatment).

1. 300 ml of 1% a.i Phytoclean applied as a soil drench to each pot. The plants were allowed to sit in trays until all the disinfectant was absorbed. The plants were then rewatered.
2. Phytoclean at 1% a.i. applied as a foliage spray until drip point.
3. Control. Distilled water applied as above without the disinfectant.

Results

Experiment 1

Chemene and Phytoclean at 0.07% a.i. completely suppressed growth of *P. cinnamomi* on the agar (Figure 1). ABF42 reduced growth by 57%. Phytoclean also resulted in complete mortality of the fungus after ten days on the plate (Table 2).

Experiment 2

Phytoclean at all rates tested gave complete suppression of the pathogen. Chemene also completely suppressed growth down to 0.025% a.i. with some growth at 0.0125% a.i. (Figure 2). ABF42 reduced growth by 75% at 0.1% a.i. and at 0.2% a.i. completely suppressed growth. Phytoclean, Chemene and ABF42 at 0.2% a.i. also resulted in complete mortality of the fungus after ten days on the plate (Table 3), although Chemene and ABF42 did not reduce survival below this level. Phytoclean effectively killed the fungus down to 0.025% a.i.

Experiment 3

In this experiment, Phytoclean again completely suppressed the pathogen at all rates tested. Chemene also completely suppressed growth down to 0.0125% a.i. with some growth at 0.00625% a.i. (Figure 3) after 11 days.

Experiment 4

Reisolation of the fungus from all treatments showed that no disinfectant at any rate or time tested was able to kill the fungus within the infected cotyledon. The disinfectants in fact provided a good surface sterilant of the cotyledon in that few contaminants were found growing on the agar after plating.

Experiment 5

Phytoclean at 0.2% a.i. significantly reduced the amount of infective propagules of *P. cinnamomi* present in the soil (Figure 4). At 1.0% no fungus could be isolated. Time of immersion appeared to have little effect on isolation of the fungus.

Experiment 6

Phytoclean at rates down to 0.01% a.i. prevented zoospore infection of the cotyledon over the 4 days that the test ran (Table 4). At 0.001% a.i. however, it was ineffective with all cotyledons examined showing sporangia around the edge of the bait.

Experiment 7

Some degree of phytotoxicity was shown when a 1% a.i. solution of Phytoclean was applied to the foliage of *P. radiata* seedlings (Table 5). No phytotoxicity was observed when applied as a drench. No mortality was observed.

Discussion

Phytoclean had the greatest effect on the fungus in both inhibition of growth and in killing the fungus when in contact with the chemical. It was effective at rates as low as 0.01% a.i. in preventing zoospore infection in soil and killed the fungus on agar at rates as low as 0.025%. No trace of the fungus could be isolated from soil drenched in a 1% a.i. solution for 30 seconds and the chemical dramatically reduced levels of infective propagules at 0.2% a.i. No phytotoxicity was observed when Phytoclean at 1% a.i. was applied as a soil drench to potted *P. radiata* seedlings, although some needle browning was observed when applied as a foliage spray.

ABF42 was effective at rates of 0.2% a.i. in killing the fungus on agar but although reducing fungal growth at 0.1% a.i. was completely ineffective below these rates. Chemene was also effective at 0.2% a.i. in killing the fungus on agar and inhibited growth at all rates tested. However, below 0.2% a.i. it was ineffective in killing the fungus. None of the disinfectants were able to kill the fungus within host tissue within the times tested (up to 10 minutes of immersion).

Noske and Shearer (1985) showed that quaternary ammonium compounds (quats) are more effective than hypochlorite and phenol based products in inhibiting the growth of *Phytophthora cinnamomi*. Care has to be taken however in ensuring the correct rate is used when translating research data into management prescriptions. For example, the management plan for *Phytophthora cinnamomi* in the Tasmanian Wilderness World Heritage Area, recommends the use of ABF42 at a rate of 1:400. As the active quat within the concentrate is only at 10g/l (ie. 1%), this provides them with an effective concentration of only 0.025% which is below that recommended by Noske and Shearers (1985) of >1.2% a.i. of quat and that found to be partially effective in this study of 0.2% a.i.

The study reported here also shows that all quats are not as effective as each other in their effect on *P. cinnamomi*. Chemene and Phytoclean are quats made up of 128 g/l benzalkonium chloride (12.5% a.i) whereas ABF42 is 10g/l Poly [Oxyethylene (Dimethyliminio) Ethylene Dichloride] (1% a.i.). Benzalkonium chloride itself is a general name for a variety of different compounds of alkyldimethylbenzylammonium chlorides that differ in the attached alkyl functional group ranging from C_8H_{17} to $C_{18}H_{37}$ (Budavari *et al* 1989). The effect of these different alkyl groups on the pathogen is unknown. Phytoclean also contains additives that may have synergistic effects in the performance of the chemical.

Acknowledgements

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Table 1. Quaternary ammonium compounds tested for inhibition of growth of *Phytophthora cinnamomi*.

Product name	Active ingredient	Manufacturer
ABF42	10g/l Poly [Oxyethylene (Dimethyliminio) Ethylene Dichloride]	Chemsearch Australia
Chemene	128 g/l Benzalkonium chloride	Chemsearch Australia
Phytoclean	128 g/l Benzalkonium chloride & proprietary additives	Avis Chemicals Australia

Table 2. Survival of *Phytophthora cinnamomi* 10 days after plating on PDA agar amended with 0.07% a.i. of ABF42, Chemene and Phytoclean.

Disinfectant	Survival (%)
ABF42	100
Chemene	100
Phytoclean	0
Control	100

Table 3. Survival of *Phytophthora cinnamomi* 7 days after plating on PDA agar amended with 0.2, 0.1, 0.05, 0.025 and 0.0125 % a.i. of ABF42, Chemene and Phytoclean.

Disinfectant	Rate (% a.i.)	Survival
ABF42	0.2 0.1 0.05 0.025 0.0125	0 100 100 100 100
Chemene	0.2 0.1 0.05 0.025 0.0125	0 100 100 100 100
Phytoclean	0.2 0.1 0.05 0.025 0.0125	0 0 0 0 100
Control	0	100

Table 4. Infection of *Eucalyptus sieberi* cotyledons by zoospores of *Phytophthora cinnamomi* 4 days after baiting soil flooded with 1.0, 0.1, 0.01, 0.001% a.i. of Phytoclean.

Disinfectant	Rate (% a.i.)	Cotyledon infection (%)
Phytoclean	1.0 0.1 0.01 0.001	0 0 0 100
Control	0	100

Table 5. Phytotoxicity of a 1% a.i. solution of Phytoclean to *Pinus radiata* seedlings when applied as a soil drench and a foliage spray

Treatment	Replicate	Foliage Health
Soil Drench	1	Healthy green
	2	Healthy green
	3	Healthy green
Foliage Spray	1	Some browning
	2	Slight browning
	3	Slight browning
Control	1	Healthy green
	2	Healthy green
	3	Healthy green

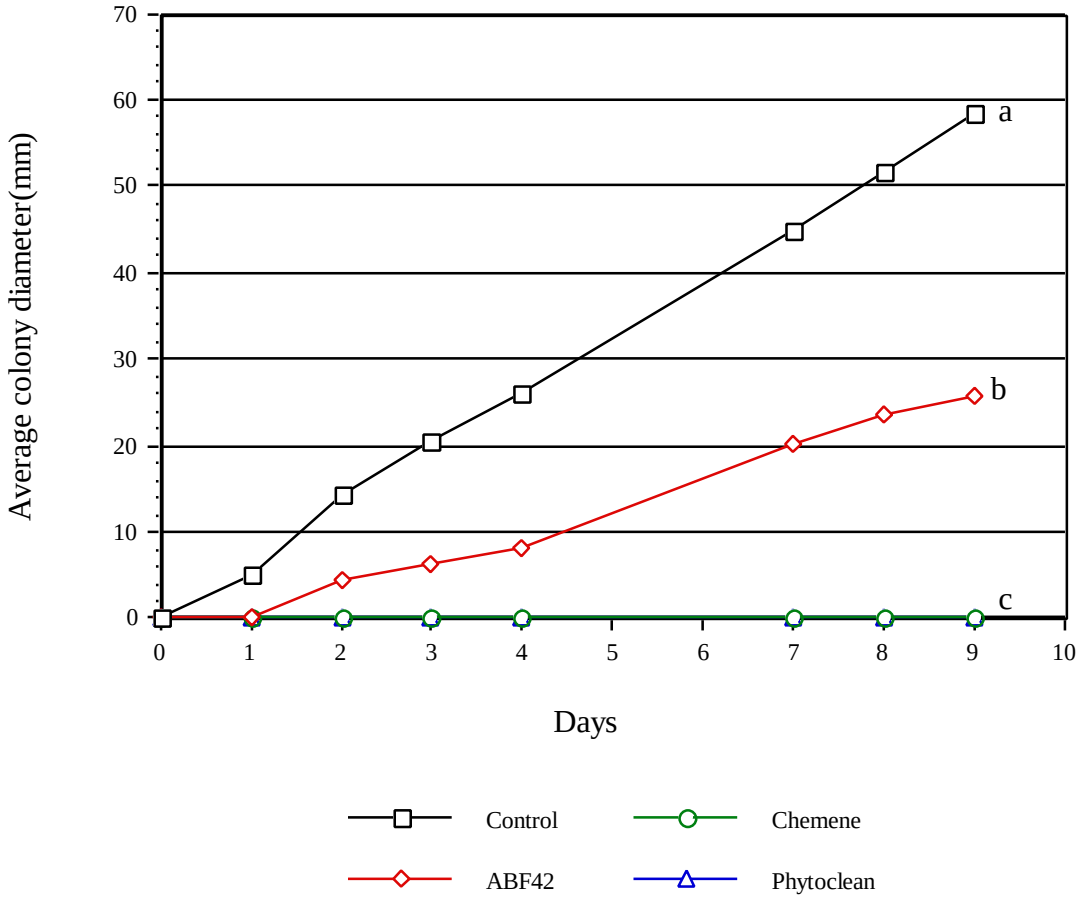

Figure 1 Growth of *Phytophthora cinnamomi* plated on PDA agar amended with 0.07% a.i. of ABF42, Chemene and Phytoclean. Treatments with different letters are significantly different at P<.05.

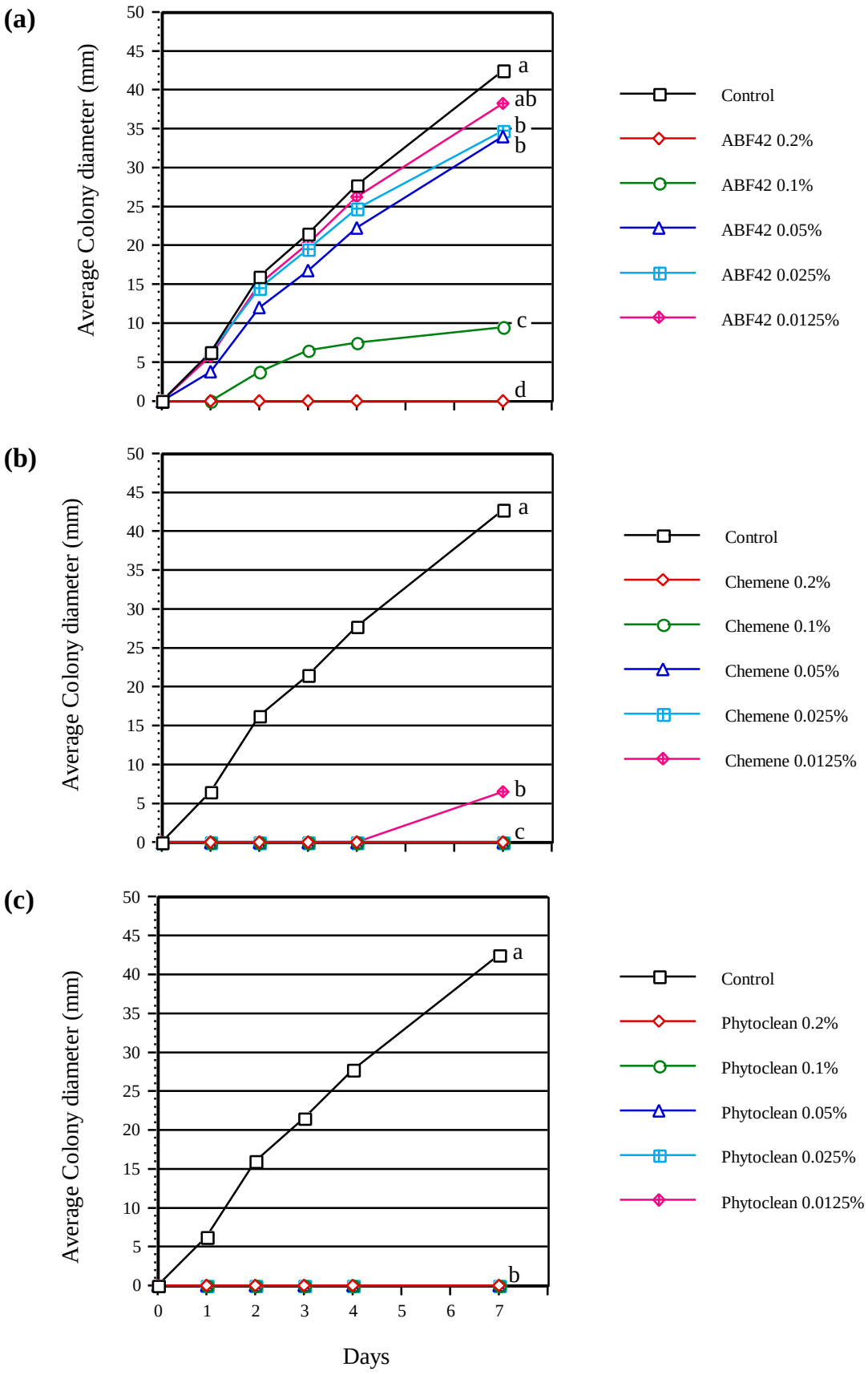

Figure 2 Growth of *Phytophthora cinnamomi* plated on PDA agar amended with 0.2, 0.1, 0.05, 0.025 and 0.0125% a.i. of ABF42, Chemene and Phytoclean. Treatments with different letters are significantly different at P<.05.

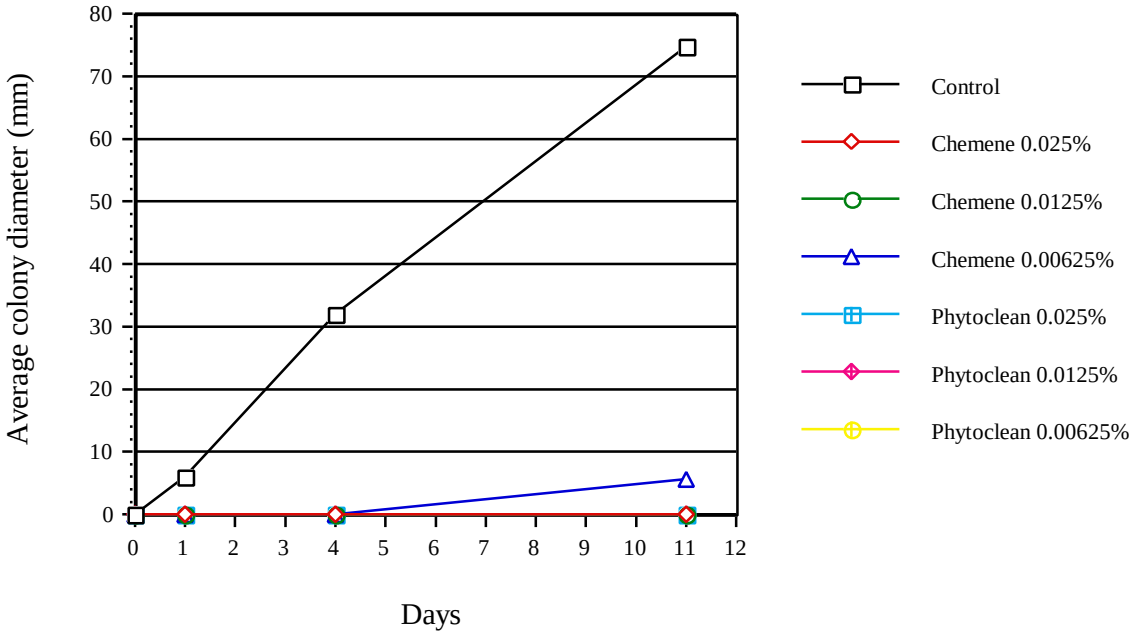

Figure 3 Growth of *Phytophthora cinnamomi* plated on PDA agar amended with 0.025, 0.0125 and 0.00625% a.i. of Chemene and Phytoclean.

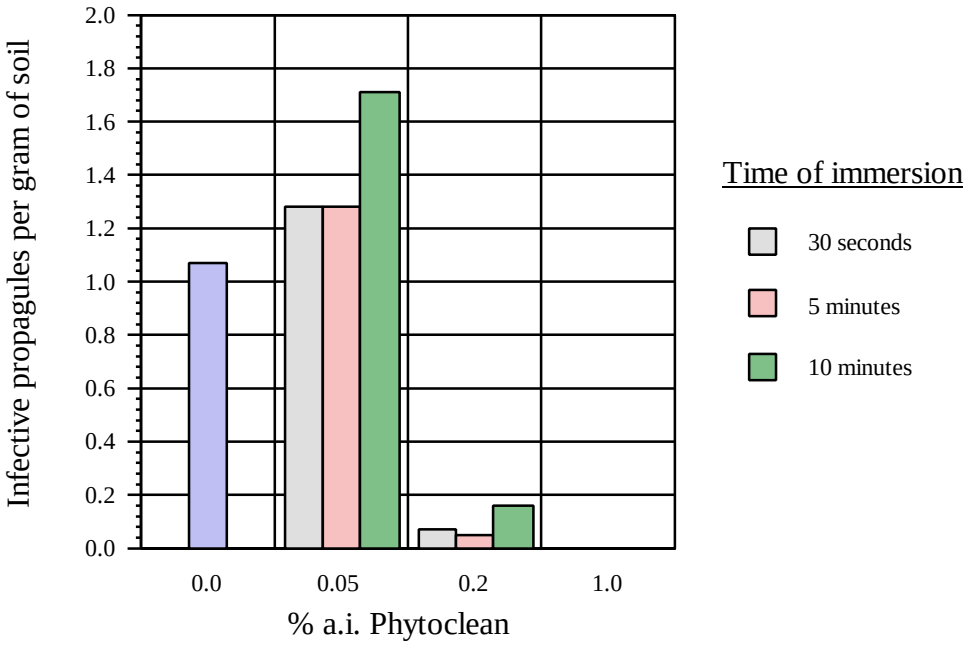

Figure 4 Number of infective propagules of *Phytophthora cinnamomi* per gram of soil treated with Phytoclean at 1.0, 0.2, 0.05% a.i. for 30 seconds, 5 minutes and 10 minutes.