Biological control of *Phytophthora cinnamomi* in *Thryptomene* spp. using manure treatments

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Summary

Results from this project clearly demonstrate that antagonists can reduce *Phytophthora cinnamomi* dieback severity in *Thryptomene calycina* in the field as it can in glasshouse pot trials. The addition of compost or compost with antagonists significantly reduces the rate of infection, reduces *Phytophthora cinnamomi* in the soil, and promotes the growth of *Thryptomene calycina.*

Phosphonate was shown to protect *Thryptomene calycina*, *Banksia grandis* and *Banksia spinulosa* against *Phytophthora cinnamomi* in the field. It significantly reduced symptom severity and plant deaths due to *Phytophthora cinnamomi* without affecting pathogen survival or plant vigour.

Previous studies had shown that compost containing chicken manure was found to be the most effective compost for eliminating *Phytophthora cinnamomi,* and although this material was suitable for *Thryptomene calycina*, the phosphorous concentration in the compost was toxic for *Banksia grandis* and *Banksia spinulosa*, negating any beneficial effects of antagonists. Further investigations are proposed to determine suitable composts for use with phosphorus-sensitive plants like *Banksia.*

Objective

To increase the survival rates of *Phytophthora cinnamomi* sensitive plants in the Royal Botanic Gardens, Cranbourne using organic soil amendments including antagonistic microorganisms.

Background

The control of *Phytophthora* in locations such as the Royal Botanic Gardens Cranbourne is essential to prevent restrictions on the range of plant taxa that can be grown. Chemical treatment with phosphonate has been successful in controlling *Phytophthora* in native forests in Western Australia (Komorek & Shearer, 1995), and in commercial plantations of crops such as avocado (Guest, Pegg & Whiley, 1995). Another approach has been investigated with success by the School of Botany at The University of Melbourne using antagonistic microorganisms to eliminate *Phytophthora cinnamomi* from infected pots. The current project has been designed to determine the effectiveness *of* antagonistic microorganisms in reducing the effect *of Phytophthora cinnamomi* on susceptible plants in field situations.

Results from stage one of the field trial did not unambiguously confirm the results of the glasshouse trials, that showed significant protection of *Thryptomene calycina* against *Phytophthora cinnamomi* dieback, following the addition of antagonistic microorganisms. Although the addition of the antagonist to the field plots did appear to decrease the death rate for *Banksia spinulosa* and *Thryptomene calycina*, it did not significantly improve the survival of *Banksia grandis.* The inconsistent results may have been a reflection of the limited time one season gives for the antagonists to have an effect on the pathogen, and the trial was extended over a second season using twice the number of new plants in each of the original plots.

Results from the extended trial showed phosphonate consistently reduced the number of deaths for all three species *Thryptomene calycina*, *Banksia grandis* and *Banksia spinulosa* over the two seasons. However, although antagonists by themselves reduced deaths for *Banksia spinulosa* and *Thryptomene calycina* in stage one of the trial, in stage two none of the antagonist or compost treatments reduced the rate of plant death. In addition, plant survival in the control plots of the *Banksia grandis* and *Banksia spinulosa* was higher than for the antagonist, compost/antagonist or compost treatments in stage two of the trial even though *Phytophthora cinnamomi* was still detected in these control plots. It is impossible to explain these results because of two confounding effects. Firstly, the unintentional bias introduced because of the unpredictable unevenness of the trial site in terms of such factors as pathogen distribution, and drainage. Secondly, the need for hand watering due to the extremely dry summer and autumn of the second season of the trial which makes comparison of the two seasons difficult.

An additional trial, taking into account what was learnt from the first trial was set up using *Thryptomene calycina* only. In this trial a different compost was used, and it was set up in a section of the original trial area where soil conditions and *Phytophthora cinnamomi* distribution were more even. Also, this trial was set up in autumn rather than spring.

The results from the additional *Thryptomene calycina* trial are presented here.

Participants

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Locations

Research Area of the Royal Botanic Gardens, Cranbourne
School of Botany, The University of Melbourne

Methods

1. Additional Thryptomene trial

Using the knowledge built up from stage one of the main trial, an additional trial using *Thryptomene calycina* only was set up in a section of the special collections area where soil conditions, including the presence of *Phytophthora cinnamomi,* were found to be more uniform. This trial was set up as for stage two of the main trial (see Methods 1) with the following modifications:
trial set up in autumn (May) rather than spring or summer;
commercial compost (chicken manure compost, Attunga Garden Products Pty Ltd, Reservoir, Victoria) was used instead *of* a composted mixture of chipped prunings and chicken manure; ten replicate plots, each with five plants were used.

2. Testing for phosphonate tolerance and effectiveness of phosphonate in protecting plant from *Phytophthora cinnamomi*

Phosphonate (PO_3) stock solution was prepared by adding 600 g H_3P0_3 to 1.0 1 deionised water and adjusting the pH to 6 using KOH. Pots infected with *Phytophthora cinnamomi,* and planted with *Thryptomene calycina, Banksia grandis* or *Banksia spinulosa* were dipped briefly into one of four concentratrions *of* PO_3 (0, 1, 2.5 and 5 g/1) made from the stock solution. The five replicates for each treatment were grown in the glasshouse, and plant deaths recorded.

3. Phosphorus toxicity on *Banksia spinulosa*

Banksia spinulosa was planted into sieved washed river sand combined with 2.5, 5, 10, and 15% (v/v) Munro chicken manure compost. Five replicates for each treatment were made. Phosphorus levels for each treatment were established using the method of Bray & Kurtz (1945), and plant deaths were recorded.

4. Measuring available phosphorus in the soil

Available phosphorous was measured in samples taken from all composted plots, and the control plots without compost of *Banksia spinulosa.* The testing was done by Pivot using the Colwell Phosphorus method.

Results

1. **Additional *Thryptomene* trial**

The results from the additional *Thryptomene calycina* trial very clearly demonstrate that antagonists are able to significantly (chi square test) reduce plant death rates in field situations for species that are susceptible to *Phytophthora.* Also, the antagonist/compost combination, and compost by itself were able to significantly reduce plant deaths (Figure 1).

Figure 1: Number of plant deaths over 10 months of *Thryptomene calycina* for additional *Thryptomene* trial

Importantly, compost either by itself or in combination with antagonists, was able to reduce pathogen survival in the soil. These combinations have a distinct advantage over phosphonate which is able to protect the plant from the pathogen, but does not reduce the level of the pathogen in the soil (Figure 2).

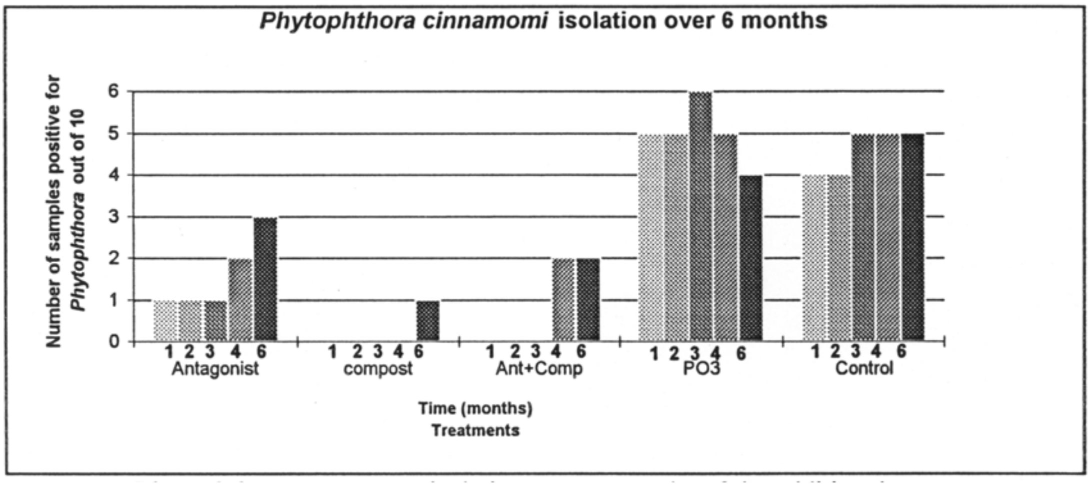

Figure 2: *Phytophthora cinnamomi* isolation over 6 months of the additional *Thryptomene* trial

It was also found that compost both with and without antagonists promoted better growth of the *Thryptomene calycina* as indicated by the number of branches (Figure 3). Branching was not promoted at all in the antagonist, phosphonate or control treatments.

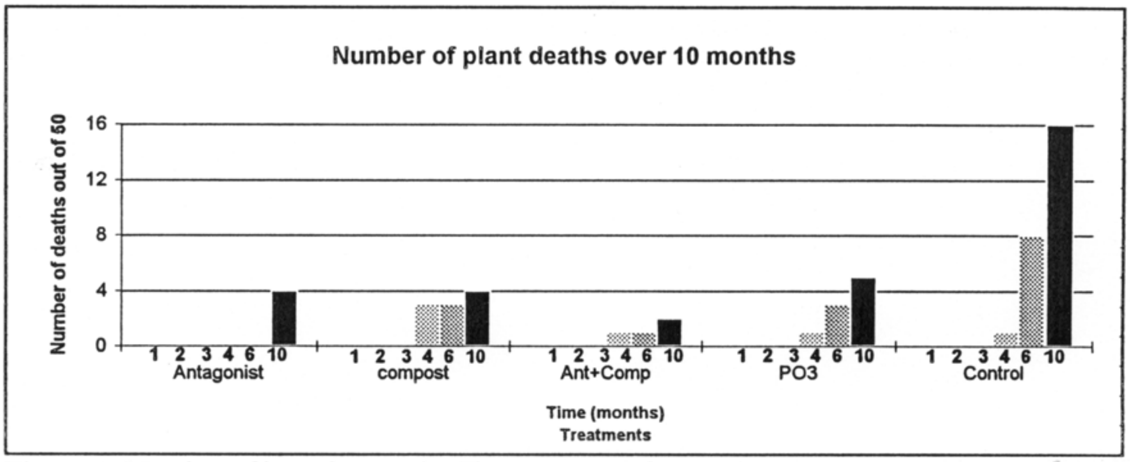

Figure 3: Number of branches on the *Thryptomene calycina* plants over 6 months of the additional *Thryptomene* trial.

The results with phosphonate in this trial replicated those of the original trial, that is, phosphonate caused a significant reduction in plant deaths (Figure 1).

Table 1 summarises the effects of the different treatments.

Table 1: Summary of the effects of the different treatments on plant survival, plant growth, and detection *of Phytophthora in* the soil for the additional *Thryptomene* trial (+ = increase; 0 = no effect; - = decrease).

Treatment	Effect		
	Plant survival increased	Plant vigour	Pathogen survival
Antagonist	+	0	0/-
Compost/antagonist	+	+	-
Compost	+	+	-
Phosphonate	+	0	0

3. Testing for phosphonate tolerance and effectiveness of phosphonate in protecting plant from *Phytophthora cinnamomi*

The graphs in Figure 4 show plant deaths in *Phytophthora cinnamomi* infected pots at four different concentrations of phosphonate (0, 1, 2.5 and 5 g/1) for *Banksia grandis, Banksia spinulosa* and *Thryptomene calycina.* As expected, *Phytophthora*-related deaths occurred in the absence of phosphonate for all species. The high number of deaths at 5g/1 of phosphonate can be attributed to the toxicity of the chemical for the three species. Plant deaths recorded for *Banksia grandis* and *Banksia spinulosa*, when grown in the absence of *Phytophthora cinnamomi,* are only recorded at the higher concentrations of phosphonate (Figure 5). However, the graphs of Figure 4 show that phosphonate protects the plant without toxic side-effects at 1 g/l. Indications of toxicity are evident at the higher concentration of 2.5g/l for both *Banksia spinulosa* and *Thryptomene calycina.*

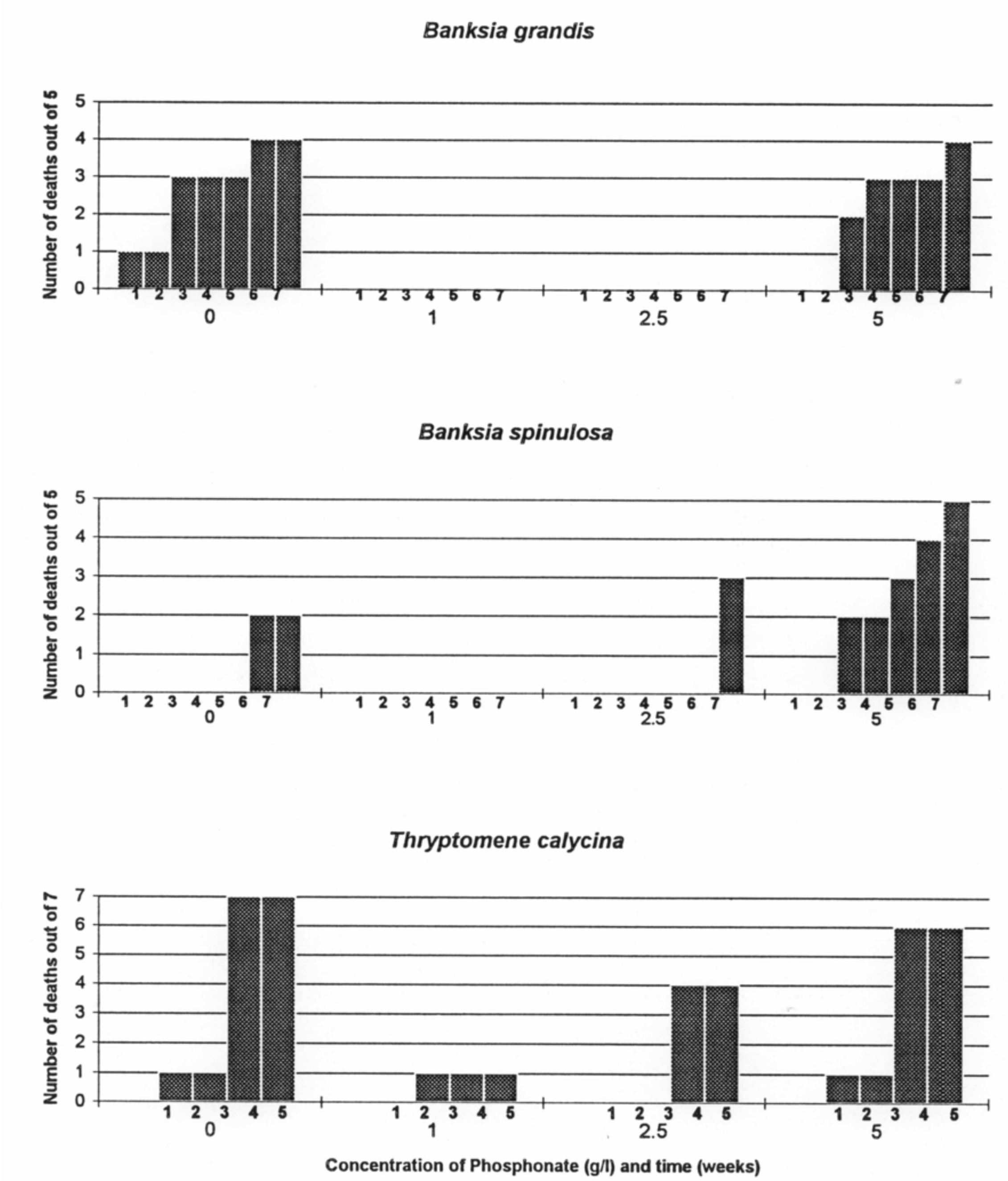

Figure 4: Plant deaths *of Banksia grandis*, *Banksia spinulosa,* and *Thryptomene calycina* when grown in the presence *of Phytophthora cinnamomi,* and at different concentrations of phosphonate (0, 1, 2.5 and 5 g/l).

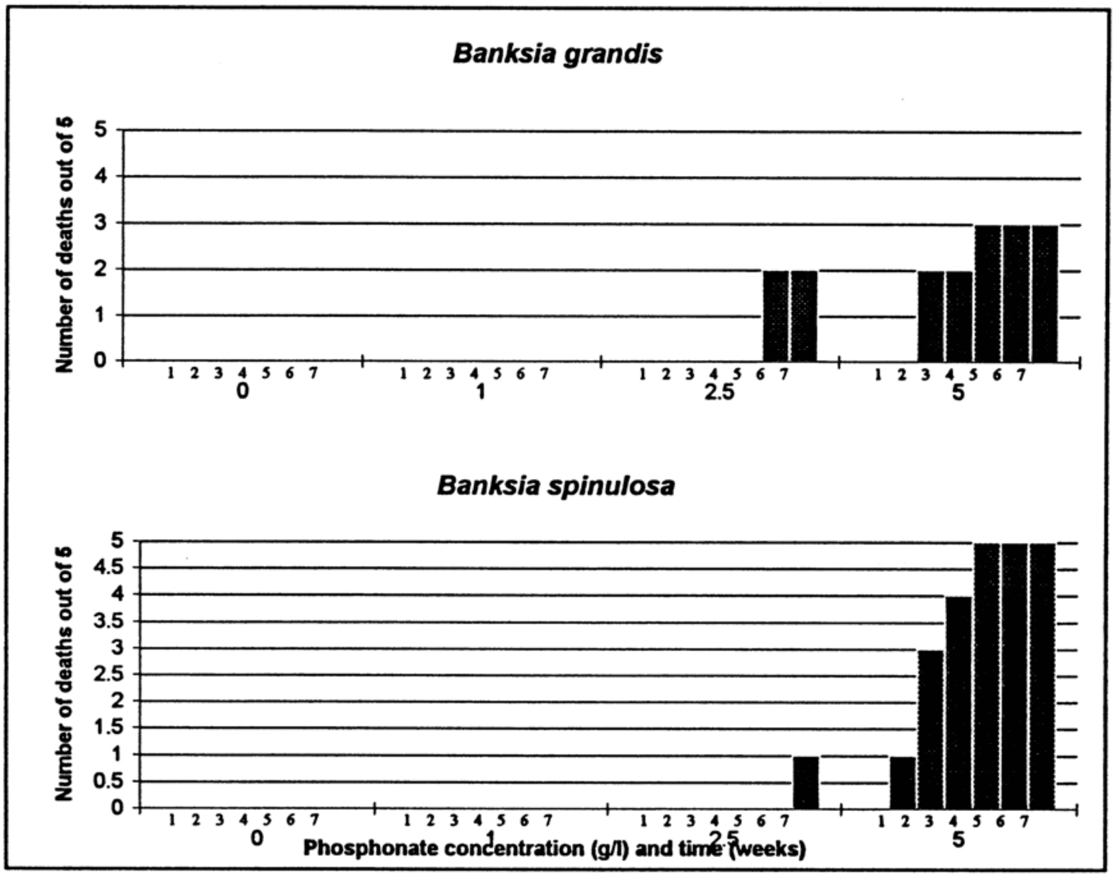

Figure 5: Plant deaths of *Banksia grandis* and *Banksia spinulosa* after dipping in different concentrations of phosphonate (0, 1, 2.5 and 5 g/1)

4. Phosphorus toxicity for *Banksia spinulosa*

Earlier experimentation at the School of Botany *of* The University of Melbourne indicated that chicken manure compost was the best to eliminate *Phytophthora cinnamomi,* but percentages lower than 15% gave partial control. This level may be appropriate for many species, but for some species of Proteaceae the phosphorus content in the manure can be toxic. The results of stage one of the main field trial indicated that phosphorus toxicity was a problem for the two *Banksia* species. Results of phosphorus testing confirmed that composted plots did have high phosphorus levels (Figure 6).

A glasshouse pot trial was undertaken to determine the concentration of phosphorus that could be tolerated by *Banksia spinulosa,* and therefore the percentages of chicken manure that could be used. Figure 7 shows that phosphorus concentrations as low as 17 ppm (5% compost), cause toxicity in *Banksia spinulosa.*

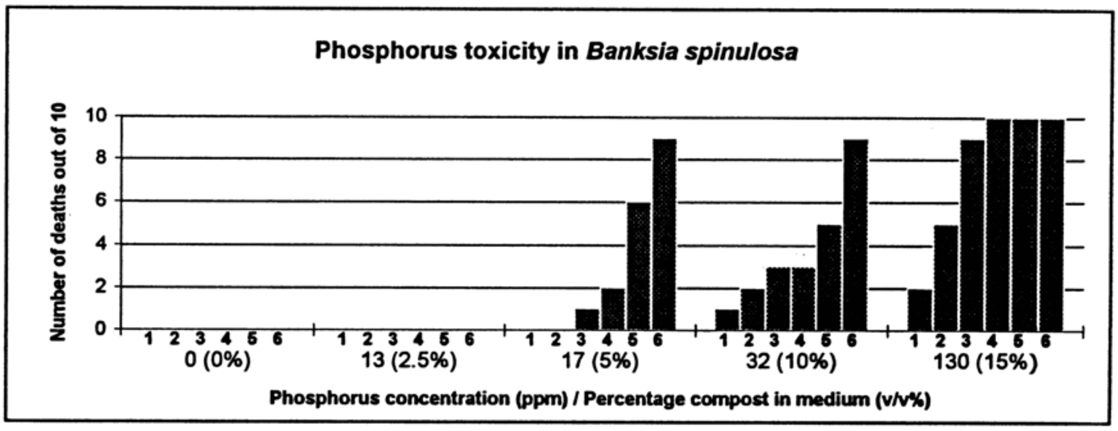

Figure 7: Plant deaths *of Banksia spinulosa* with different concentrations of phosphorus in the potting medium

5. Microbial activity, organic matter content and pH

Compost increases soil organic matter levels and stimulates microbial activity (Figure 8). Antagonist or phosphonate drenches do not significantly affect organic matter or soil microbial activity.

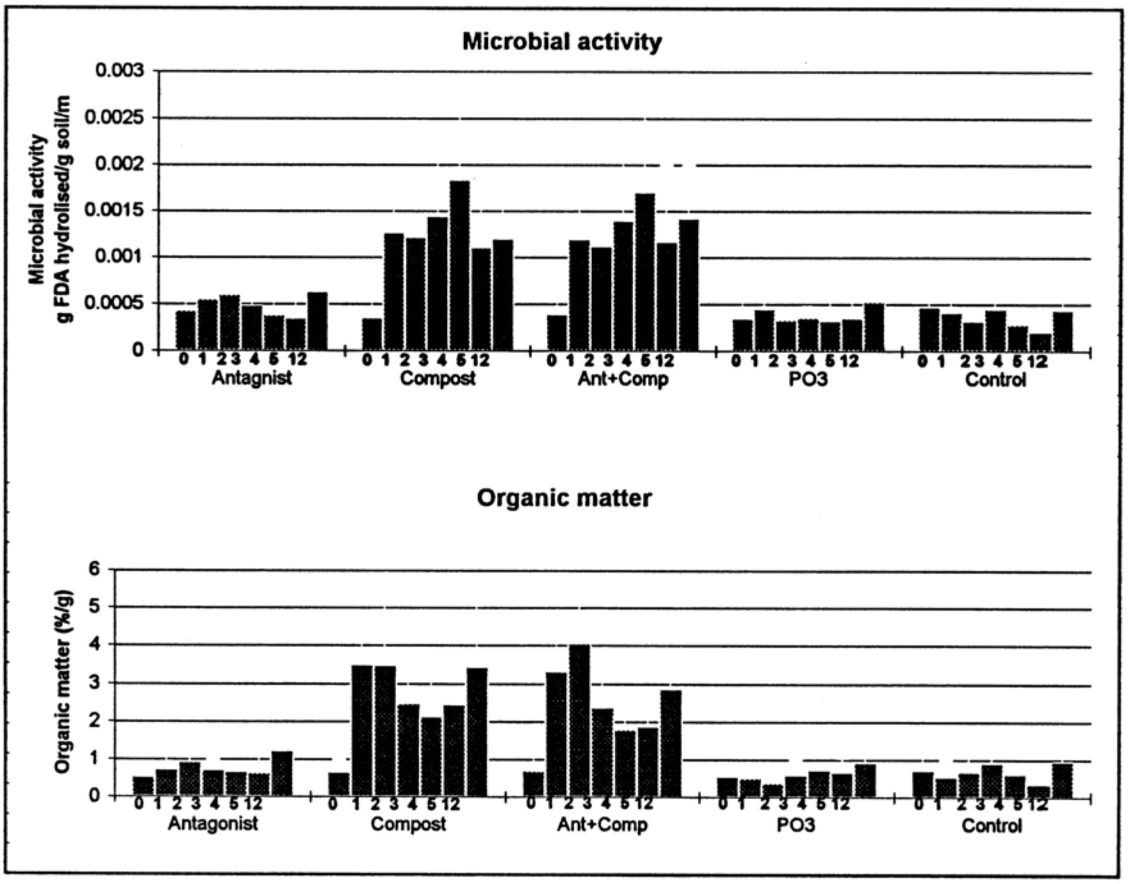

Figure 8: Microbial activity and organic matter content in the soils of the plots measured over a 12 month period from the start of stage one of the main trial.

The different treatments did not affect the pH of the soil (Figure 9)

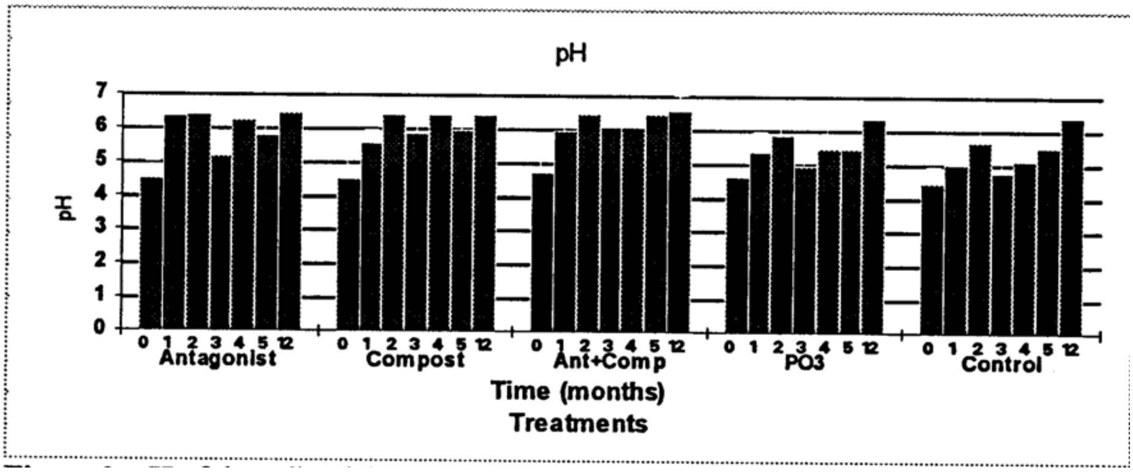

Figure 9: pH of the soils of the plots measured over a 12 month period from the start of stage one of the main trial.

Discussion

Phytophthora cinnamomi, when introduced into areas beyond its natural distribution, can cause devastation of natural flora. In horticultural situations it can be equally harmful, destroying important cultural landscapes or limiting the full potential of new ones by restricting the taxa that can be grown.

In recent years, phosphonate has been used as a chemical treatment for *Phytophthora cinnamomi* dieback, and the results from this trial support its efficacy in doing so for *Banksia grandis, Banksia spinulosa* and *Thryptomene calycina.* Phosphonate consistently protected all three susceptible species against *Phytophthora cinnamomi* over consecutive seasons in the original trial, and over one season in the *Thryptomene* trial.

Concentrations of 1 g/l of phosphonate protected *Banksia grandis, Banksia spinulosa* and *Thryptomene calycina* against *Phytophthora cinnamomi* dieback, without causing phytotoxicity damage. Toxicity was observed at concentrations of 2.5 g/1 or above in each species. These results match the effective, but safe levels determined for other species such as *Xanthorrhoea australis Xanthorrhoea minor,* and *Epacris impressa* that have been determined at the School of Botany of The University of Melbourne.

Phosphonate does not offer all the answers however. This trial has shown that phosphonate protects the plant, but leaves the *Phytophthora cinnamomi* population in the soil intact. Thus, phosphonate does not diminish the reservoir of inoculum remaining in the soil. This reservoir of inoculum forms the basis for disease outbreaks in the following spring, necessitating further fungicide treatments.

As a result of this trial the potential for using a biological control for *Phytophthora* in field situations has become a reality. Antagonists to *Phytophthora cinnamomi* have been shown to reduce mortality of *Thryptomene calycina* due to *Phytophthora cinnamomi,* when applied to infested soils. More importantly, antagonists, particularly in combination with compost, and compost by itself, offer a distinct advantage over phosphonate by reducing the survival of *Phytophthora cinnamomi* in the soil. Compost, with or without added antagonists, offers an additional advantage by promoting plant growth as indicated by the increased branching in *Thryptomene.* Increased root vigour allows quicker recovery following damage caused by pathogen attack. As a result, foliar symptoms such as wilting and dieback are less severe.

The combined effect of antagonist, compost and phosphonate has not been tested yet, but should be as it might ultimately give the maximum protection to the plant.

Compost containing chicken manure was found to be the most effective for the elimination *of Phytophthora* from infected pots in earlier glasshouse pot trials at The University *of* Melbourne, and it was therefore chosen for the field trials. Chicken manure compost worked well for the *Thryptomene calycina*, however, for the phosphorus-sensitive plants *Banksia grandis* and *Banksia* spinulosa, phosphorus toxicity disguised any advantage the compost and antagonists may have given in reducing the effects *of Phytophthora.* Assessment of a range of composts, initially for phosphorus content and ultimately for their ability to promote the action of antagonists, is required to enable biological control *of Phytophthora cinnamomi* to be extended to phosphorus-sensitive taxa.

It appears from the additional *Thryptomene* trial that planting in autumn may have benefits over planting in spring. Autumn planting allows a longer period for the plant to establish before the onset of water stress, and before the most active period for *Phytophthora* in spring when soils are moist and warm. It also allows for a longer period for the antagonists to reach an equilibrium in the soil prior to the period *of* greatest *Phytophthora* activity. It is expected that adding the antagonist to the soil prior to planting will allow a more robust and therefore effective antagonist population to establish for the protection of newly planted plants. During the colder months, *Phytophthora cinnamomi* survives as dormant propagules. The pathogen population resulting when these propagules germinate in the following spring depends on the rate that these propagules remain viable, and is vulnerable to attack by antagonists and hyperparasites.

Further work

Future trials will be conducted in the same section of the trial site as the additional *Thryptomene* trial to minimise variation in pathogen distribution, and soil moisture content.

Further work that has been planned will investigate:

- the effect of the combination of antagonist, compost, and phosphonate on *Thryptomene* survival;

- the identification of low phosphorous composts and the use of these in trialing antagonists with the phosphorus-sensitive taxon *Banksia.*

Funding will be sought from granting agencies to build upon the strong foundation that has been established in this area of *Phytophthora cinnamomi* control, made possible with the generous support of the Maud Gibson Trust.