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Phosphorous acid for controlling Phytophthora taxon agathis in Kauri

Horner IJ, Hough EG

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Horner IJ, Hough EG

Plant & Food Research, Havelock North

SPTS No. 5802

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Executive summary

Phosphorous acid for controlling *Phytophthora* taxon agathis in Kauri

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Phytophthora taxon Agathis (PTA) is a serious problem, killing kauri trees of all ages in forests in Auckland and Northland. Treatment with phosphite (phosphorous acid, PA) is one of the few potential options for treatment of infected or threatened trees. This report summarises *in vitro* tests of phosphite inhibition of PTA, and glasshouse trials testing various phosphite applications for control of PTA in inoculated kauri seedlings.

In vitro trials

Six isolates of *Phytophthora* taxon Agathis and two isolates each of *P. cactorum* and *P. cinnamomi* were grown on V8 agar amended with various concentrations of phosphorous acid (0, 5, 15, 40, 100 and 250 ug/ml). Mycelial growth rate of all three *Phytophthora* species was significantly inhibited by phosphorous acid. PTA was the most sensitive, with a 50% reduction in growth (EC_{50}) at 4.0 mg/L phosphorous acid, compared with EC_{50} values of 25.2 and 37.9 mg/L for *P. cinnamomi* and *P. cactorum* respectively. Thus, PTA seems more sensitive to phosphorous acid than other *Phytophthora* species commonly controlled by this chemical.

Glasshouse trials

Two-year-old kauri seedlings growing in potting mix were inoculated with PTA, either by introducing inoculum to the soil, or by insertion of a colonised oat grain into a wound on the trunk. Some trees were left as un-inoculated controls. Trees were treated with phosphorous acid applied as a foliar spray (three rates), trunk injection using a hypodermic needle (two rates), or soil drench (two rates). There were also a Ridomil® and untreated control treatments. The treatments were applied either 5 days before or 5 days after inoculation with PTA. Trees were grown in a glasshouse maintained below 30°C. Detailed disease assessments were made after 10 and 20 weeks. Root and foliar symptoms were recorded for all soil-inoculated trees, and foliar symptoms and lesion expansion from the inoculation point were recorded for all trunk-inoculated trees.

In trees soil-inoculated with PTA, there was a significant increase in root disease and foliar symptoms compared with those in uninoculated controls. All 14 soil-inoculated control (untreated) trees were dead within 20 weeks of inoculation. In contrast, all 15 trees treated with phosphorous acid by trunk injection were still alive and healthy. Phosphorous acid applied as a soil drench or foliar spray, or soil application of Ridomil were less effective, but still slightly better than the untreated control.

In trees that were trunk-inoculated with PTA, all untreated control trees plus many of the treated trees died, with substantial lesions rapidly expanding and girdling the trunk. Only the phosphorous acid trunk injection treatment provided significant disease control; all five trees injected before inoculation survived, as did five of the ten trees injected after inoculation. In surviving trees, lesion expansion was halted before girdling occurred, and by 20 weeks, lesions had healed and calloused around the margins.

There was not a strong rate effect noted in any of the treatments applied, but any such effects may have been overwhelmed by the aggressiveness of the pathogen in the assays used. Similarly, there were no significant differences in treatments applied before or after inoculation,

although there was a tendency for improved health scores (or delayed mortality) when trees were treated before PTA inoculation.

From these trials, there is sufficient evidence of the potential effectiveness of phosphorous acid to control PTA to justify testing on kauri trees in the forest. Field trials with kauri rickers or slightly larger trees should be the next step. Trunk injection in particular is seen as the most promising treatment, although foliar sprays and soil drenches should not be ruled out for specific situations. However, further work will be required to improve chemical absorption and translocation when the chemical is foliar or soil applied.

Minor phytotoxicity symptoms were noted in seedlings with the trunk injection treatment, particularly in the lower branches within a few centimetres of the injection point. It is difficult to predict how this result with small seedlings might extrapolate to larger trees, but caution in selecting rates is recommended. Preliminary trials should be carried out on low-risk trees, not necessarily in a PTA-infested area, to determine the rates that field-grown kauri trees can tolerate.

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1 Introduction

Phytophthora taxon Agathis (PTA) is a serious problem, killing kauri trees of all age classes in forests in Auckland and Northland (Beever et al. 2008). Very few treatment options are available for infected or threatened trees.

Treatment with phosphite (phosphorous acid, PA) is a potential control for PTA. It has been used successfully for treating a wide range of *Phytophthora* diseases of many plant species. Its predominant use is horticulture and nurseries, with some use in forest systems.

Before testing PA on large kauri trees in the field, it was considered necessary to test the efficacy of phosphorous acid against PTA *in vitro* and on kauri seedlings. This gives an early indication of whether phosphorous acid is likely to be a useful field control, helps to predict minimum rates required for control, and identifies an upper concentration threshold to avoid phytotoxicity. The current work aimed to:

- Determine the *in vitro* sensitivity of PTA to phosphorous acid, and compare this with sensitivity of other *Phytophthora* species commonly controlled by this product
- Determine phosphorous acid efficacy against PTA infection in kauri seedling roots and trunks
- Test efficacy of phosphorous acid at preventing new infections and curing established infections
- Determine phosphorous acid concentration thresholds for phytotoxicity in kauri.

This report summarises *in vitro* tests and glasshouse trials testing a range of phosphorous acid application techniques and formulations. The *in vitro* work was reported previously (Horner & Hough 2011).

2 *In vitro* response to phosphorous acid

2.1 Methods

Six isolates of *Phytophthora* taxon Agathis and two isolates each of *P. cactorum* and *P. cinnamomi* were used. All *Phytophthora* cultures were grown on V8 agar for 5 days, and then 5-mm diameter plugs from the margin of cultures were subbed to V8 agar amended with various concentrations of phosphorous acid. There were three replicate plates of each isolate for each PA concentration. PA concentrations tested were 0, 5, 15, 40, 100 and 250 ug/ml. The PA used was taken from a commercial preparation of Agrifos®600 (Key Industries), a solution of 60% phosphorous acid present as a mono- and di-potassium phosphonate. The PA was added to the agar after autoclaving, when it had cooled to about 70°C.

One day after subbing onto PA-amended media, the margins of all colonies emerging from the inoculum plug were marked using a fine marker pen on the bottom of the Petri plate. All subsequent measurements were taken from this reference point. Growth measurements were taken at three radii on each plate, 4 and 7 days after subbing, and growth rates per day were calculated. Response curves were fitted to data averaged across each species, using a third order polynomial. From this, EC_{50} values were calculated.

Microscopic examinations of the cultures were made after 7 days, to assess potentially anomalous growth or sporulation on agar amended with PA.

2.2 Results

Summaries of *Phytophthora* colony growth on PA-amend media are given in Table 1 and Figures 1 and 2.

Mycelial growth of all three *Phytophthora* species was significantly inhibited by phosphorous acid. PTA was the most sensitive to phosphorous acid, with a 50% reduction in growth (EC_{50}) at 4.0 mg/L phosphorous acid, compared with EC_{50} values of 25.2 and 37.9 mg/L for *P. cinnamomi* and *P. cactorum* respectively.

In microscopic examinations, an increasing delay in sporulation of PTA was noted with increasing phosphorous acid concentration. In un-amended media, oogonial formation occurred on hyphae that were approximately 1.5 days old. At concentrations of 5 and 15 mg/L phosphorous acid, oogonial formation occurred on hyphae 2-3 and 3-6 days old, respectively. At 40 mg/L, two of the six PTA isolates had not produced oogonia within 8 days. The remaining four isolates had produced a small number of oogonia after 4-6 days, but these oogonia did not appear to mature normally, and in many cases were plasmolysed.

Table 1. Average colony growth (mm/day) of *Phytophthora* species on V8 agar media amended with various concentrations of phosphorous acid.

		Phosphorous acid concentration (mg/L)					
Species	Culture ID	0	5	15	40	100	250
PTA	HNM09003	5.00	1.93	1.06	0.52	0.08	0.00
PTA	H270	5.78	1.98	1.28	1.20	0.54	0.08
PTA	H263	4.56	2.07	1.57	1.04	0.59	0.24
PTA	H294	4.37	2.22	1.41	1.13	0.83	0.29
PTA	H303	5.11	2.87	1.59	1.37	0.50	0.00
PTA	H315	5.80	3.28	1.48	0.93	0.04	0.00
P. cactorum	H243	3.61	2.69	2.20	1.70	1.26	0.24
P. cactorum	H244	3.39	2.65	1.96	1.80	1.04	0.16
P. cinnamomi	H275	7.39	5.39	3.02	1.70	0.98	0.54
P. cinnamomi	H289	5.39	5.37	4.78	3.17	2.33	1.39

PTA = *Phytophthora* taxon Agathis

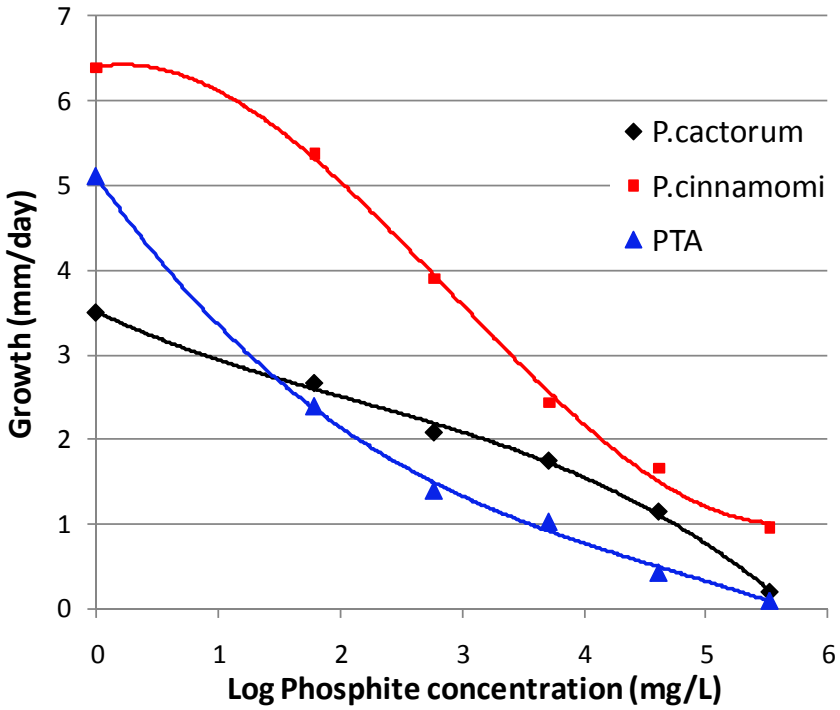
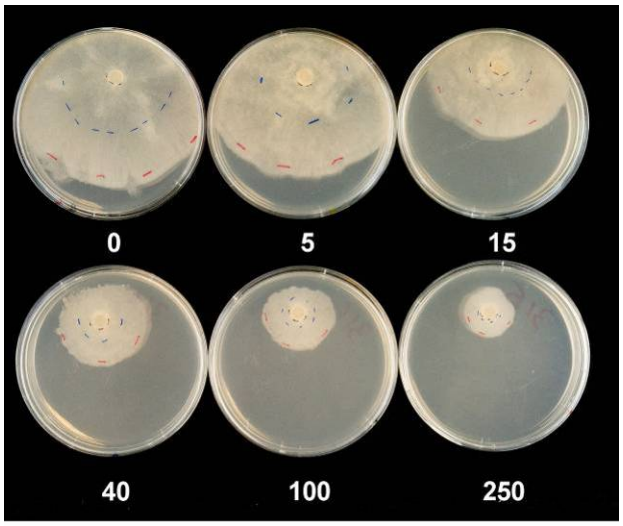
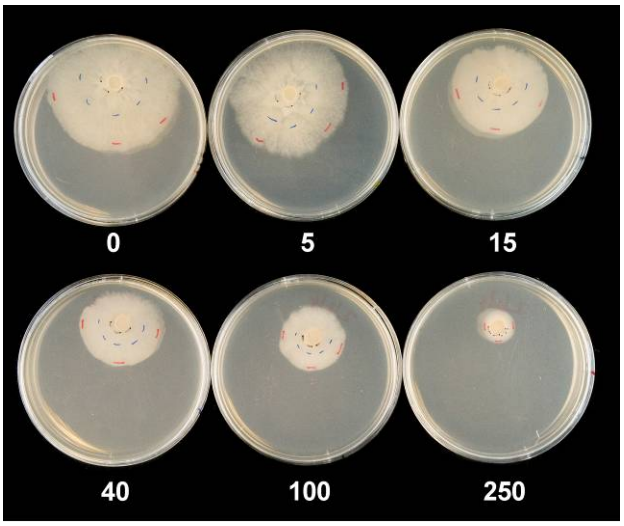


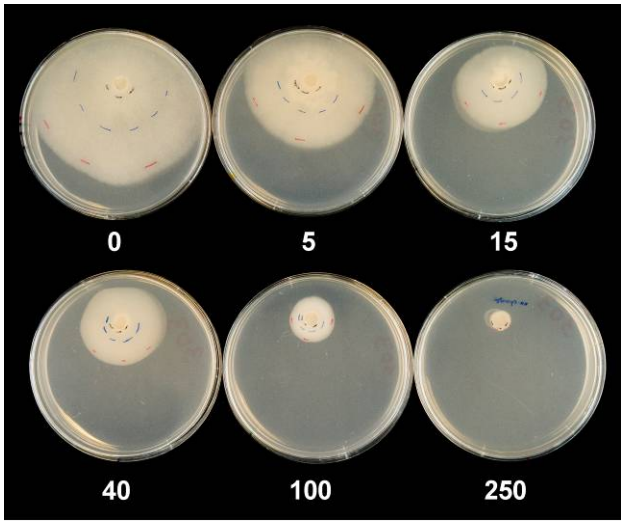
Figure 1. Growth of mycelial cultures of *Phytophthora cactorum*, *P. cinnamomi* and *Phytophthora* taxon Agathis (PTA), grown on V8-agar amended with various concentrations of phosphorous acid. Growth data are averaged for each species.



Phytophthora cinnamomi



Phytophthora cactorum



Phytophthora taxon Agathis

Figure 2. Cultures of *Phytophthora cinnamomi*, *P. cactorum* and *Phytophthora* taxon Agathis (PTA) grown for 8 days on V8-agar media amended with various concentrations of phosphorous acid. Numbers are phosphorous acid concentrations in mg/L. Black, blue and red marks in plates indicate colony margins after 1, 4 and 7 days, respectively.

3 Kauri seedling trials

The next step for testing potential chemicals for control of PTA was to carry out trials on PTA-inoculated kauri seedlings in the glasshouse. The aim was to assess PTA disease progression or control in kauri seedlings inoculated on roots or stem, and treated either before or after inoculation with phosphorous acid applied to foliage, stem or soil.

3.1 Methods

Two-year-old kauri seedlings were sourced from the Scion Nursery in Rotorua. Trees were growing in potting mix in PB3 bags, and were approximately 60 to 80 cm tall, and 8 to 10 mm diameter at the base of the trunk at the start of the experiment.

The experiment was conducted in the glasshouse at Plant & Food Research in Havelock North under strict containment protocols.

3.1.1 Inoculation methods

PTA-colonised V8-juice agar was used to seed jars of sterilised oat grains. Colonised oats were subsequently used for trunk inoculation. Cubes of colonised V8 agar were also used to seed flasks of dilute V8 juice, and incubated on an orbital shaker for 7 days. The mycelia mats produced were subsequently macerated in a blender, and used for soil inoculation.

Seedlings were inoculated with PTA in one of two ways:

1. Trunk inoculation: An oat grain colonised *in vitro* by PTA was inserted in a small incision in the bark on the trunk, approximately 10 cm above the soil. The wound and oat inoculum was then sealed with grafting tape (Figure 3). Uninoculated oat grains were used as uninoculated controls.

2. Soil inoculation: Four weeks before inoculating the seedlings, two 8-mm diameter wooden dowels were inserted approximately 6 cm into the soil (Figure 4). Immediately before soil inoculation, these dowels were removed, leaving a cavity for pouring in the PTA inoculum (macerated mycelial mat). Ten ml of the PTA suspension was poured into each of the two holes in the soil (Figure 4), then soil was gently pressed to fill in the holes. Soil was then flooded for 24 h by raising the water level to approximately half way up the soil profile in the bag (approximately 4 cm deep) (Figure 4). After 24 h, trays were drained. For the following 10 weeks, frequent misting in the glasshouse as part of the evaporative cooling system ensured that soil water was maintained at a high, but not flooded state.

All PTA inoculations were carried out on the same day using the same batch of inoculum.

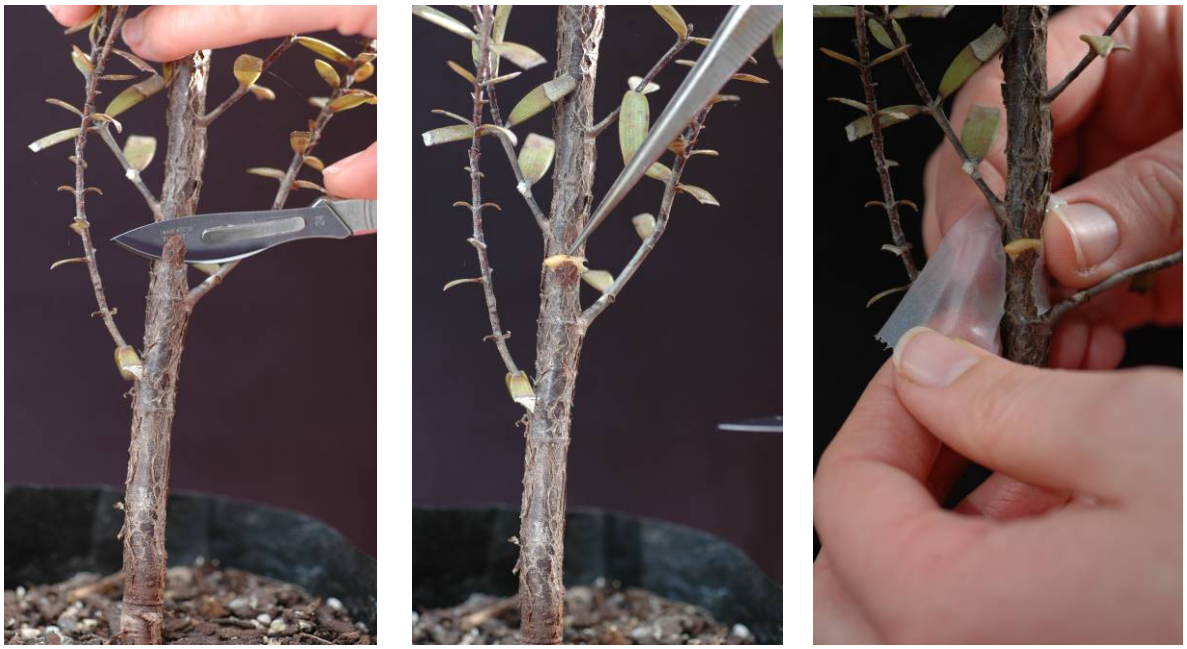


Figure 3. Trunk inoculation of kauri seedlings. Incision using scalpel (left), insertion of *Phytophthora* taxon Agathis (PTA)-colonised oat grain (centre), sealing of wound using budding tape (right)

Figure 4. Soil inoculation of kauri seedlings. Dowels inserted to create holes (top left), pouring of *Phytophthora* taxon Agathis (PTA) inoculum suspension into hole in soil (bottom left), flooding for 24 hours (right).

3.1.2 Fungicide Treatments

The following treatments were applied to trees, either 5 days before or 5 days after inoculation with PTA:

1. Untreated control

2. Phosphite spray (low rate, 2 ml/L phosphorous acid)

3. Phosphite spray (med rate, 4 ml/L phosphorous acid)

4. Phosphite spray (high rate, 8 ml/L phosphorous acid)

5. Phosphite injection (low rate, 0.16 ml of 150 ml/L phosphorous acid)

6. Phosphite injection (high rate, 0.32 ml of 150 ml/L phosphorous acid)

7. Phosphite soil drench (low rate, 20 ml of 2 ml/L phosphorous acid)

8. Phosphite soil drench (high rate, 20 ml of 8 ml/L phosphorous acid)

9. Ridomil 2.5G, 1.33 g granules (33 mg metalaxyl /plant).

The phosphite formulation used was Agrifos600®, a 60% solution of mono- and di-potassium phosphonate.

Phosphite sprays were applied to foliage using a hand held mist sprayer, with saturation of the leaves (to drip). To avoid PA application directly to the soil, thick newspaper was used to cover the soil surface during spray application and left in place until leaves were dry.

Phosphite injections were carried out by drilling a 0.45 mm diameter hole on a 45º downward angle, 2/3 of the way through the trunk, 2 – 5 cm above soil level (Figure 5). A hypodermic needle (25 gauge, 0.5 mm) containing the required volume of phosphorous acid, was immediately inserted into the hole. To aid pressurising of the syringe, approximately 0.4 ml of air was included above the PA in the syringe. The plunger was manually depressed and held in place with rubber bands, compressing the air behind the PA solution and thus pressurising the syringe (Figure 5). Syringes were monitored to determine the flow of PA into the plant. When none of the PA solution remained in the syringe, the pressure was released and the needle was removed. In most cases, the syringes emptied in 1 to 12 hours. When the volume in the syringe didn't change over a 1 – 2 hour period, the syringe was depressurised, removed, and re-inserted in a new hole drilled at least 1 cm away from the original hole. The injection point was marked with a dot of white paint, for subsequent checking of potential tissue damage (Figure 15). Before finalising the injection system, various techniques were trialled using dye to determine whether the solution was getting into and dispersing within the tree (Figure 6). Because of technical problems with the injection system, the high rate of phosphite injection was not included in the treatments *before* inoculation. The technique was subsequently refined on spare trees, and the high injection rate was included in the *after*-inoculation treatments.

Soil drenches were applied by adding the required volume of PA to 20 ml of water, and pouring it over the soil surface.

Ridomil was applied by sprinkling the required volume of Ridomil granules to the soil surface, gently working them in to the top few millimetres of soil, then drenching the soil surface with 20 ml of water per pot.

There were five replicate trees of each fungicide treatment, for each PTA inoculation system (trunk or soil), for each timing (treated pre- or post-inoculation).

Uninoculated controls were included for comparison, with five uninoculated trees of each treatment.

To test the potential for phytotoxicity from excessive chemical application rates, extra high rates of phosphorous acid were applied to spare trees, and symptoms observed. PA at 20 ml/L a.i. (i.e. 2.5 X the highest concentration in the main trial) was sprayed onto trees. Other trees were stem-injected with 0.5 ml of 150 ml/L a.i. solution.

3.1.3 Glasshouse regime

Throughout the experiment, the aim was to maintain glasshouse temperature below 28°C, as this is regarded as the upper limit of activity for PTA (Horner, unpublished). With the experiment commencing in the height of summer (late January), it was difficult at times to keep below that limit. A combination of heavy shading, misting and evaporative cooling was used. Almost continuous misting ensured that plants remained wet most of the time over the first few weeks, and additional watering was not required. A summary of ambient glasshouse temperatures is presented in Appendix 1. After inoculation, soil temperatures remained below 28°C, although this temperature was approached at time in the first few days after inoculation. Air temperatures within the canopy occasionally exceeded 30°C, though never for prolonged periods.

To prevent cross contamination between treatments and to minimise contamination of glasshouse benches, all seedlings were maintained in individual aluminium trays, after inoculation and treatment. These aluminium trays where held within large plastic or stainless steel bench-trays with water containment, as a further measure to prevent contamination of the glasshouse. All surfaces potentially exposed to PTA were thoroughly decontaminated following completion of the experiment. Any water drained from trays was collected and decontaminated using hypochlorite or Virkon®.

Figure 5. Micro-drilling kauri seedlings with 0.45-mm drill bit (left) and injection using a 25-gauge hypodermic needle, with air layer and pressurisation using rubber bands (right).

Figure 6. Injection technique testing, using blue dye (left). Dispersal of dye within the kauri trunk, 20 minutes after commencing the injection (right).

3.1.4 Assessments

The first disease assessment was made ten weeks after inoculation. Foliage health was scored by dividing each tree into three sections (Top=upper 10 cm, Base=lower 10 cm, Middle=remainder). Each section was given a disease rating on a scale from 0 to 4, where 0=healthy, 1=slight wilt symptoms, 2=severe wilt symptoms, 3=dead. The three scores for each tree were averaged to give and overall foliar health score. For trees that had been soil-inoculated, root health was assessed on a 0 to 5 scale, where 0= 0-5% diseased, 1= 5-25%, 2=25-50%, 3=50-75%, 4=75-95%, 5=95-100% of feeder roots diseased (Figure 7). A selection of diseased feeder roots was plated onto *Phytophthora*-selective media (PARPH) to determine whether PTA had colonised the roots. Live trees were re-potted into the same soil. For trees that had been stem-inoculated, lesion expansion was assessed by measuring the length above and below the oat-grain inoculation point, and by estimating the greatest percentage girdling of the trunk. Trunk tissue samples from within and outside lesion boundaries were plated on Phytophthora-selective media.

One week after the initial disease assessment, all inoculated trees still alive were retreated with the same fungicide treatment as they received initially, and retained in the glasshouse at between 15 and 25°C. These trees were re-assessed 10 weeks later, scoring overall foliar health of all trees on the same scale as above, but ignoring occasional dead or broken braches at the base of the trunk. Root health was scored for soil-inoculated trees only, and lesion activity or healing was noted for trunk-inoculated trees.

Data were analysed using regression analysis for an unbalanced design in Genstat (Version 13, 2010, VSN International Ltd, Hemel Hempstead, UK). Lesion length data were log-transformed before analysis, but no other data were transformed.

Figure 7. Various severities of root disease in kauri trees, 10 weeks after soil inoculation with *Phytophthora* taxon Agathis (PTA). Clockwise from top left, disease scores of 0, 1, 2, 3, 4 and 5.

3.2 Results

3.2.1 Soil-inoculated trees

When trees were soil inoculated with PTA, there was a significant increase in root and foliar disease scores, compared with un-inoculated controls (Appendices 2 and 3). In general, only inoculated trees showed foliar symptoms.

There were no significant differences in disease scores between the various treatments in un-inoculated trees. When trees were inoculated, the differences in root score between treatments were highly significant (P<0.001) at both 10- and 20-week assessments (Figure 8, Appendix 2). Trees injected with phosphite had, on average, significantly lower root disease scores than all other treatments. Trees treated with a soil drench of phosphite had next lowest root disease scores, significantly better than the phosphite sprayed, Ridomil or untreated trees. For the average foliar score, the order of disease severity among the treatments was similar to that with root scores, but the statistical significance was marginal (P=0.062) at 10 weeks, although high (P<0.001) at 20 weeks (Figure 9, Appendix 3).

After 20 weeks, all 15 phosphite-injected trees were still alive, with the next best survival in the phosphite drench (9/20), Ridomil (3/10) and phosphite spray (6/30) treatments. All the 14 untreated trees were dead within 20 weeks (Table 2).

Whether the treatment was applied before or after inoculation had no significant effect on root or foliar disease scores, and the dose rates within the application method did not have a major effect (Appendices 2 and 3).

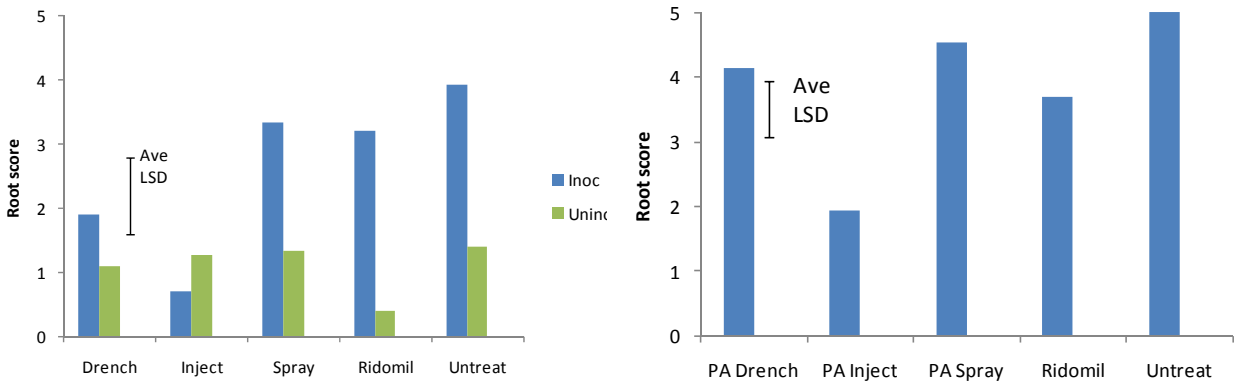


Figure 8. Average root disease score, assessed 10 weeks (left) or 20 weeks (right) after soil inoculation with *Phytophthora* taxon Agathis (PTA). Kauri trees were treated with either Ridomil or phosphite (PA) applied at various rates and three application techniques, either 5 days before or 5 days after inoculation. For simplicity, data for 'before' and 'after' treatments have been combined, as have different rates of the same treatment. Full data are presented in Appendix 2. 'Root score' is the average of root disease scored on a 0 to 5 scale, where 0 = all healthy, and 5 = all dead.

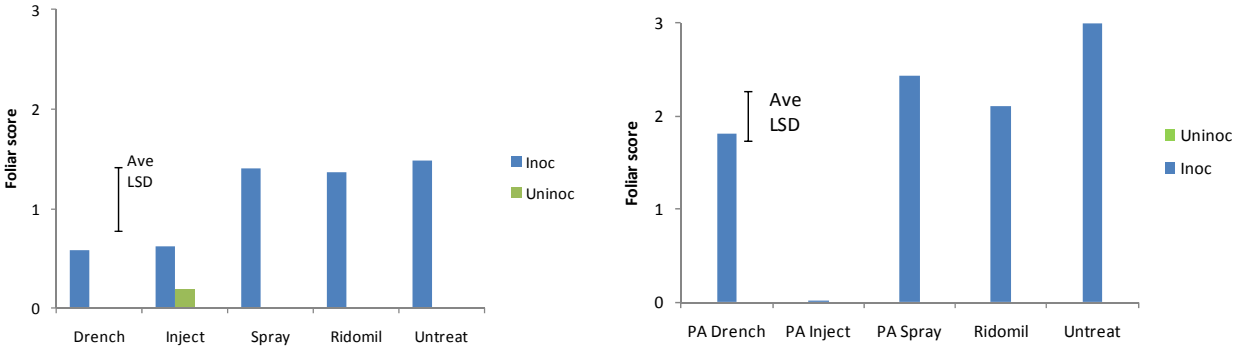


Figure 9. Average foliar disease score, assessed 10 weeks (left) or 20 weeks (right) after soil inoculation with *Phytophthora* taxon Agathis (PTA). Kauri trees were treated with either Ridomil or phosphite (PA) applied at various rates and three application techniques, either 5 days before or 5 days after inoculation. For simplicity, data for 'before' and 'after' treatments have been combined, as have different rates of the same treatment. Full data are presented in Appendix 3. 'Foliar score' is the average of foliar disease symptoms scored on a 0 to 3 scale, where 0 = healthy, and 3 = dead. Week 10 data are averages of scores for top, middle and bottom sections of the tree. Week 20 data are from a single overall tree health score.

When feeder roots from a selection of trees were plated onto Phytophthora-selective media after 10 weeks, PTA was isolated from all except trees in the trunk injection treatment and from un-inoculated trees. Isolation frequency was highest in untreated control trees. *Phytophthora cinnamomi* was also isolated from a small proportion of roots, even though this pathogen was not deliberately introduced into the trial. *P. cinnamomi* was also commonly isolated from feeder roots of un-inoculated control trees, suggesting that trees were probably infected with this pathogen when they came from the nursery. The moderate feeder root disease noted in some un-inoculated control trees (some had root **scores** of '4') was probably a result of *P. cinnamomi* infection. The presence of *P. cinnamomi* may have had some impact on the trial. However, no trees that were un-inoculated with PTA died, implying that any influence *P. cinnamomi* had was small in comparison with the impact of PTA.

3.2.2 Trunk inoculation

Where un-inoculated oat grains were inserted in the trunk, kauri seedlings remained healthy, with no lesion above the oat insertion point and, on average, lesions of less than 4 mm below the oat insertion point. When trees were trunk-inoculated with PTA-colonised oat grains, major trunk lesions formed. Lesions established and progressed rapidly in untreated trees and also in some of the trial fungicide treatments, in many instances girdling and killing the trees (Figure 10). In contrast, lesions established, but were limited in trees injected with phosphite (Figure 10). The treatment differences were highly significant for lesion length (Figure 11 and Appendix 4), percentage girdling of the trunk (Figure 12 and Appendix 5), and foliar disease symptoms (Figure 13 and Appendix 6). Most of the trees that were alive at the 10-week assessment were still alive after 20 weeks. All the five trees injected with phosphite *before* inoculation with PTA were still alive and apparently healthy when assessed after 20 weeks (Table 2, Figure 14). Five of the ten trees injected with phosphite *after* inoculation were still alive. The only other survivors at 20 weeks were two of the ten Ridomil-treated trees. All untreated controls (14 trees), phosphite-sprayed (30 trees) and phosphite-drenched (20 trees) trees were dead after 20 weeks (Table 2, Figure 14). All surviving trees have been retained in the glasshouse for further observation.

Lesion extension was not measured at the 20-week assessment, but the extent of lesion healing was noted. In all cases where trees were still alive at 20 weeks (predominantly phosphite-injected trees), the lesions appeared to have stopped expanding and had callused over (Figure 10). Pieces of lesion tissue from these apparently-healed lesions failed to yield PTA when plated onto selective agar media. In earlier isolations, ten weeks after inoculation, PTA was readily isolated from lesion tissue in all but the phosphite-injection treatments. No PTA was isolated from outside the lesion margin.

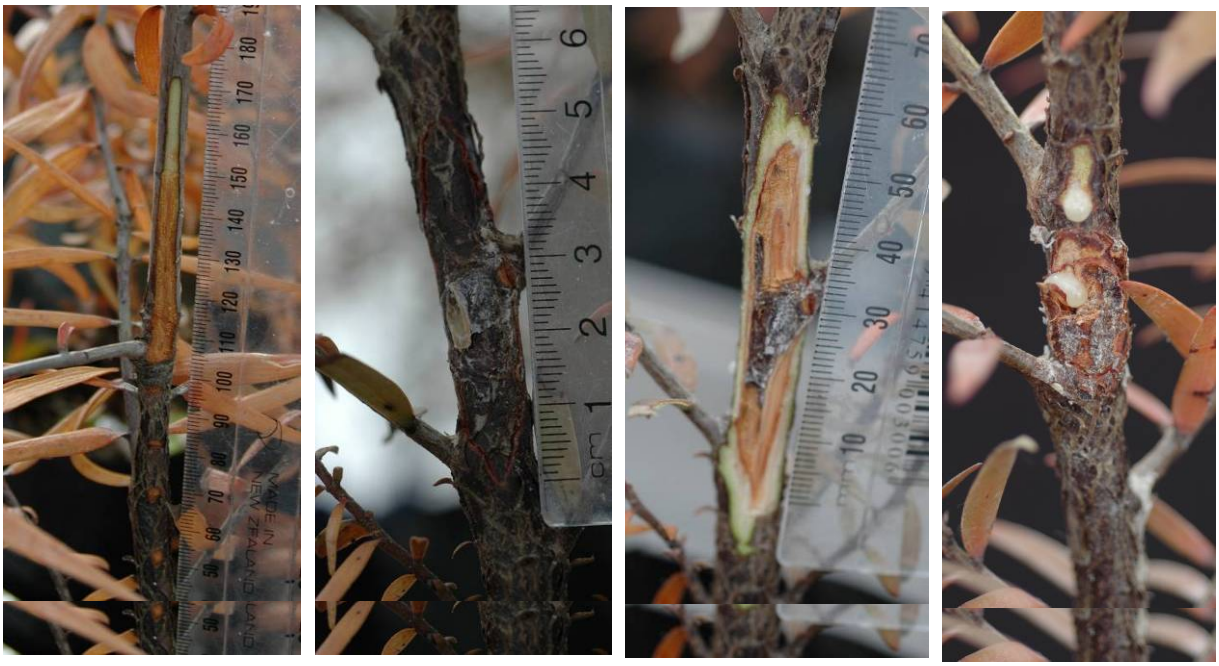


Figure 10. Trunk lesions on kauri seedlings 10 or 20 weeks after trunk inoculation with *Phytophthora* taxon Agathis (PTA). Left: actively spreading lesion on the trunk of an untreated control kauri seedling (10 weeks). Note the lesion margin at the 150 mm mark. Centre left: restricted lesion on a kauri seedling treated with a stem injection of phosphite (10 weeks). Note the cracking around the lesion margin. Centre right: the same lesion as in the centre left photograph, with the bark cut away. Right: healed lesion on phosphite-injected tree, 20 weeks after inoculation. The sap bleeding is caused by cuts to examine lesion integrity.

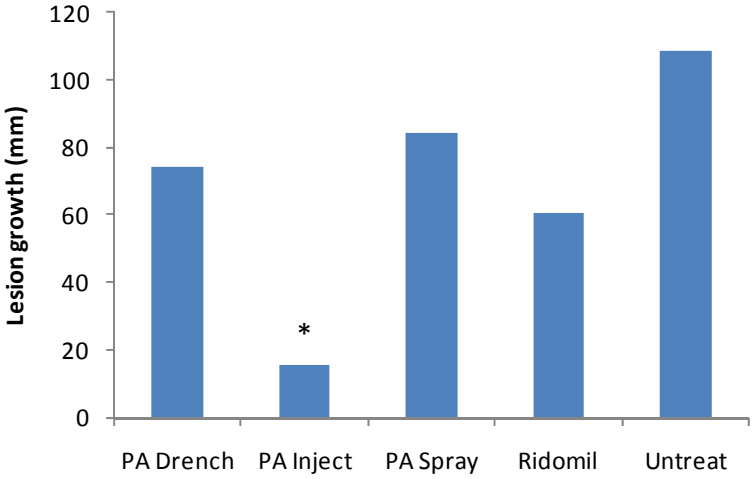


Figure 11. Average lesion extension upwards from the inoculation point, assessed 10 weeks after kauri trunk inoculation with *Phytophthora* taxon Agathis (PTA). Trees were treated with either Ridomil or phosphite (PA) applied at various rates and three application techniques, either 5 days before or 5 days after inoculation. Data are back-transformed following analyses of log-transformed lesion length data. For simplicity, data for 'before' and 'after' treatments have been combined, as have different rates of the same treatment. Full data are presented in Appendix 4. The asterisk indicates lesion growth significantly (P<0.05) less than in the untreated control.

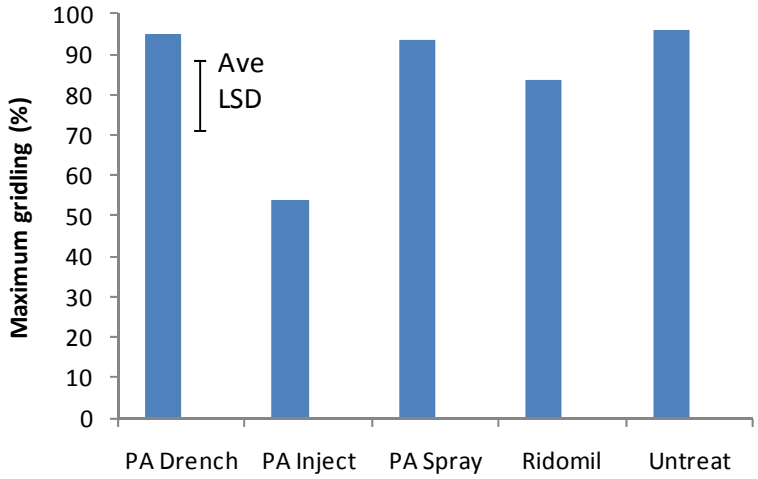


Figure 12. Percentage girdling of the trunk by lesions extending from the inoculation point, assessed 10 weeks after kauri trunk inoculation with *Phytophthora* taxon Agathis (PTA). Trees were treated with either Ridomil or phosphite (PA) applied at various rates and three application techniques, either 5 days before or 5 days after inoculation. For simplicity, data for 'before' and 'after' treatments have been combined, as have different rates of the same treatment. Full data are presented in Appendix 5.

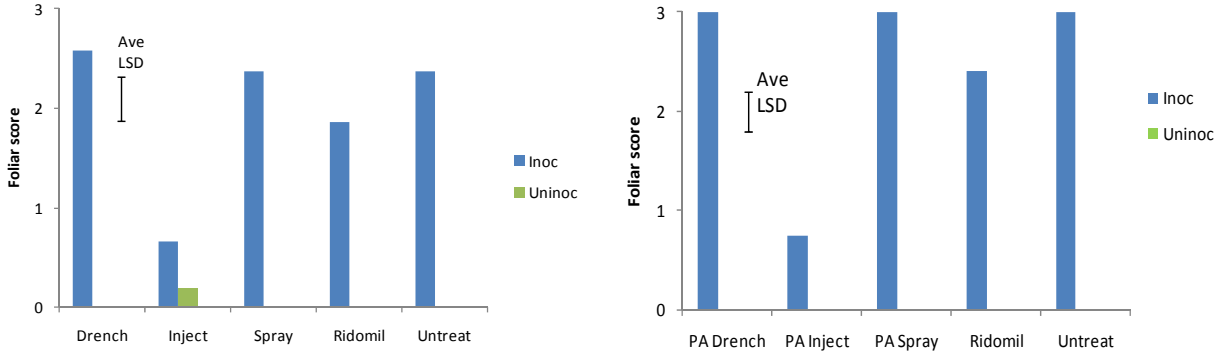


Figure 13. Average foliar disease score, assessed 10 weeks (left) and 20 weeks (right) after kauri trunk inoculation with *Phytophthora* taxon Agathis (PTA). Trees were treated with either Ridomil or phosphite (PA) applied at various rates and three application techniques, either 5 days before or 5 days after inoculation. For simplicity, data for 'before' and 'after' treatments have been combined, as have different rates of the same treatment. Full data are presented in Appendix 6. 'Foliar score' is the average of foliar disease symptoms scored on a 0 to 3 scale, where 0 = healthy, and 3 = dead. Week 10 data are averages of scores for top, middle and bottom sections of the tree. Week 20 data are from a single overall tree health score.

Table 2. Number of kauri seedlings remaining healthy 10 or 20 weeks after inoculation with *Phytophthora* taxon Agathis (PTA), either to soil or on stems. Trees were treated with either Ridomil or phosphite applied at various rates and three application techniques, either 5 days before or 5 days after inoculation. Numbers in brackets indicate the total number of trees in each treatment at the start of the experiment.

	Soil inoculation						Stem inoculation						Uninoculated		
	Treat before inoculation			Treat after inoculation			Treat before inoculation			Treat after inoculation					
	(n)	10 wk	20 wk	(n)	10 wk	20 wk	(n)	10 wk	20 wk	(n)	10 wk	20 wk	(n)	10 wk	20 wk
Untreated control				(14)	7	0				(14)	0	0	(5)	5	5
Phosphite spray (high)	(5)	2	0	(5)	3	3	(5)	0	0	(5)	1	0	(5)	5	5
Phosphite spray (med)	(5)	1	1	(5)	2	0	(5)	0	0	(5)	0	0	(5)	5	5
Phosphite spray (low)	(5)	2	1	(5)	3	1	(5)	0	0	(5)	0	0	(5)	5	5
Phosphite injection (low)	(5)	5	5	(5)	5	5	(5)	5	5	(5)	3	3	(5)	5	5
Phosphite injection (high)	-	-	-	(5)	5	5	-	-	-	(5)	3	2	(5)	5	5
Phosphite drench (high)	(5)	4	2	(5)	3	0	(5)	1	0	(5)	0	0	(5)	5	5
Phosphite drench (low)	(5)	5	4	(5)	4	3	(5)	0	0	(5)	0	0	(5)	5	5
Ridomil® granules	(5)	3	3	(5)	2	0	(5)	1	1	(5)	1	1	(5)	5	5

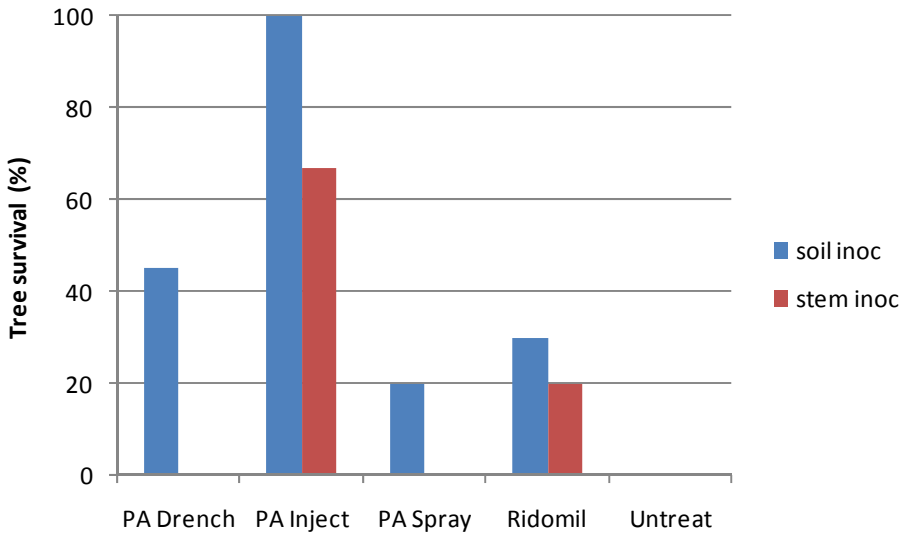


Figure 14. Percentage of kauri seedlings remaining healthy 10 or 20 weeks after inoculation with *Phytophthora* taxon Agathis (PTA), either to soil or on stems. Trees were treated with either Ridomil or phosphite (PA) applied at various rates and three application techniques, either 5 days before or 5 days after inoculation. For simplicity, data for 'before' and 'after' treatments have been combined, as have different rates of the same treatment. Full data are presented in Table 2.

3.2.3 Phytotoxicity

No phytotoxicity symptoms were noted with any of the phosphite spray or drench treatments, even on the trees sprayed with the 2.5 times the highest rate used in the main trial.

Minor phytotoxicity symptoms were noted with the injection treatment. In some of the injected seedlings, the lowest branches showed leaf-scorch symptoms, and in some cases died. These branches were in all cases within a few centimetres of the needle injection point. The positive foliar disease scores noted in the 10-week assessment of uninoculated PA-trunk-injected trees (Figure 9) were in all cases a result of symptoms in the lower branches, probably caused by a phytotoxic effect. Symptoms were greatest when extra trees outside the main experiment were injected with a very high rate of phosphite (0.5 ml of 150 ml/L a.i.) (Figure 15). With this higher rate, there was also occasionally some minor burning of the very soft new leaves (Figure 15).

Tissue damage around the injection point was minimal, with browning of tissue only about 1 mm from the needle injection point (Figure 15).

Figure 15. Left: Damage to lower branches of a kauri seedling, following injection with a high rate of phosphite (0.5 ml of 150 ml/L a.i.). Centre: minor leaf scorching on very young kauri leaves following phosphite injection with the high rate. Right: minor browning of trunk tissue, but no extensive necrosis, at the needle insertion point (between two white paint dots, bark cut away).

4 Discussion

The *in vitro* studies, in which PTA, *P. cinnamomi* and *P. cactorum* were grown on agar amended with phosphite, demonstrated that PTA is highly sensitive to this chemical. PTA's sensitivity *in vitro* appeared even greater than that of other *Phytophthora* species commonly controlled by phosphite in agricultural systems. This result justified further investigation of the potential for phosphite to PTA control in kauri.

The glasshouse trials with kauri seedlings were seen as a necessary step before treating larger trees in the forest. Although slightly artificial in that the scale of the trees was substantially different from trees likely to be treated in the forest, it was considered an effective 'low risk' way of determining potential efficacy and toxicity on kauri without jeopardising large trees.

The two different PTA-inoculation techniques tested gave some insight into infections in different portions of the trees and how they might react to various treatments. The soil inoculation was considered to provide a realistic representation of root infection. By making a hole in the soil four weeks before adding inoculum, it ensured that the root system was not wounded and that the infection was via intact roots. Flooding of soil for just 24 h after introduction of inoculum, followed by a more normal watering regime, is not too stressful on trees and probably similar to water regimes encountered in the field. The fact that all the untreated control trees died under this regime demonstrates just how virulent PTA is towards kauri, and also gives confidence that the observed survival of trees in some treatments reflects a real treatment effect on the disease. The wounding and trunk inoculation with PTA was a severe treatment for such small trees, and a very rigorous test of the experimental control treatments. Disease lesions developed in all cases. All untreated control trees, plus many treated trees, were girdled and dead within 10 weeks, again demonstrating the strong pathogenicity of PTA on kauri.

The various phosphite application methods trialled (i.e. spray, soil drench and trunk injection) represent the main ways that phosphite has historically been applied in orchard and forest settings. Another application method is trunk or canker paint, but such an application was considered unrealistic with the small seedlings in the current trial. Ridomil was included for comparison, as this chemical has been used for many years for *Phytophthora* control in horticulture, and was demonstrated to reduce kauri seedling root disease and mortality in forests infested with *P. cinnamomi* significantly (Horner 1984, Johnston et al. 2001).

By far the most effective treatment for controlling PTA was the trunk injection with phosphite. All trees in the soil-inoculated and two-thirds of the trees in the trunk-inoculated treatments were still alive and healthy after 20 weeks. Trunk lesions in these surviving trees had, in all cases, healed and callused over. The 100% survival in root-inoculated trees injected with phosphite demonstrates that the chemical was translocated through the tree and into the roots in sufficient quantity to suppress the pathogen effectively. The failure to isolate PTA from these trees also reflects this suppression. Localised phytotoxicity on lower branches next to the injection point was observed following trunk injection, but this may be an artefact of the very small trees in the trial. This is not considered likely to be a problem with larger trees, provided total chemical doses are within the correct range.

Phosphite applied as a soil drench had a positive impact on root and foliar health and tree survival when trees had been soil-inoculated with PTA. This might reflect either direct suppression of the pathogen in the soil, or absorption of phosphite by roots giving internal protection. The soil drench treatment had minimal impact on lesion extension or tree health

when applied to trunk-inoculated trees. This indicates that insufficient chemical had been absorbed by roots and translocated to shoots in time to inhibit lesion development and prevent girdling.

Spray application of phosphite was not effective in this trial. The rates applied were comparable to those used in commercial application of phosphite in horticultural tree crops, but had minimal, if any, impact on PTA in the current trial. It is possible that the anatomy of the kauri leaf is not conducive to absorption of phosphite, or that the phosphite was not translocated from leaves to trunks and roots. Addition of an agent such as Pentra-bark™, normally used to aid phosphite absorption through bark, may be beneficial. However, the manufacturers did not recommend its use in the current trial, suggesting that it would be too phytotoxic for leaf application.

The aim of treating trees with chemicals either before or after inoculation with PTA was to evaluate the potential of phosphite for both the control of existing infections and protection against new infections. Although there wasn't a highly significant difference in treatment before or after inoculation, there was a tendency towards lower disease scores and for slightly higher survival rates when trees were treated before infection. An example of this is with the PA injection treatment: the only trees to die were those that were trunk-inoculated 5 days before treatment. It is likely that lesions had established and rapidly girdled the thin trunks before the treatment had a chance to be effective. An advance of a few millimetres in 5 days could be the difference in survival or death in such small trees. This should not be a factor with larger forest trees, provided treatment is administered before a substantial portion of the tree is girdled.

5 Conclusion and next steps

These trials demonstrate that phosphite has potential for controlling PTA in kauri trees. Field trials with kauri rickers or slightly larger trees should be the next step. Site selection and experimental design will be critical to obtaining useful data from forest trials – the patchy distribution and unpredictable disease expression will complicate design and interpretation, and necessitate high replicate numbers in trials.

Trunk injection in particular looks likely to be able to reduce infection or suppress disease symptoms. A cautious approach should be taken when determining chemical rates to be used, as it will be difficult to extrapolate from the doses applied to 10-mm diameter trunks to trunks 100 times that size. Preliminary trials should be carried out on low-risk trees, not necessarily in a PTA-infested area, to determine the rates that field-grown kauri trees can tolerate. Various injection techniques should also be considered, as there are likely to be technical difficulties with injecting kauri trees (thick bark, copious resin bleeding in wounds, etc.).

Further investigation of techniques such as foliar spraying or soil drenching with phosphite should not be ruled out, as there may be specific situations where such treatments would be desirable. However, more work is needed to improve phosphite absorption and translocation. Drench application in particular could be problematic in a forest setting, given the thick litter and humic layer on the forest floor, but may be feasible in a garden or park setting. Ridomil is not considered a likely candidate for forest control for a number of reasons, including penetration through the humic layer, the rapid biodegradation of this chemical, and cost.

6 Acknowledgments

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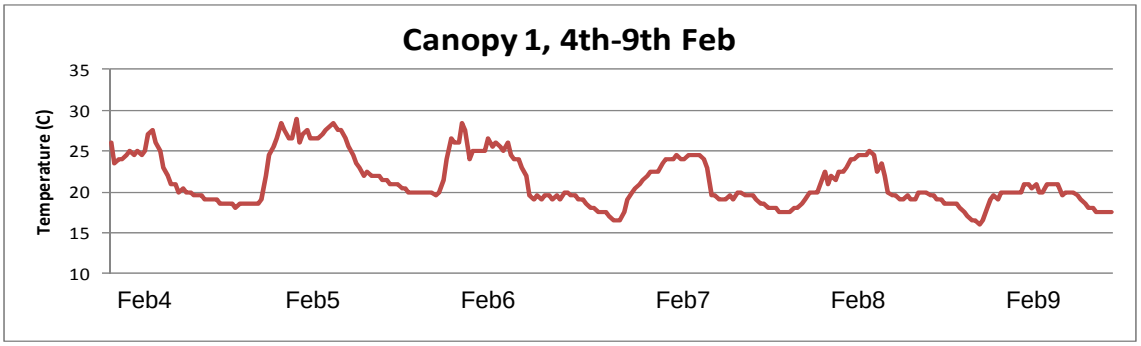
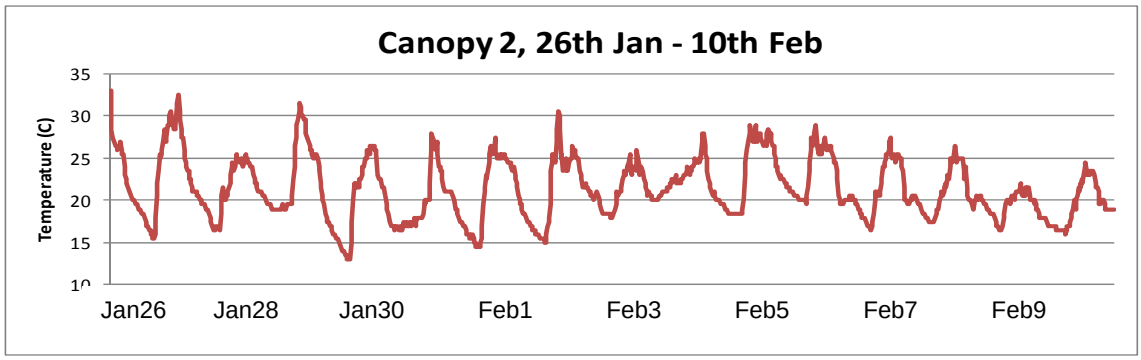
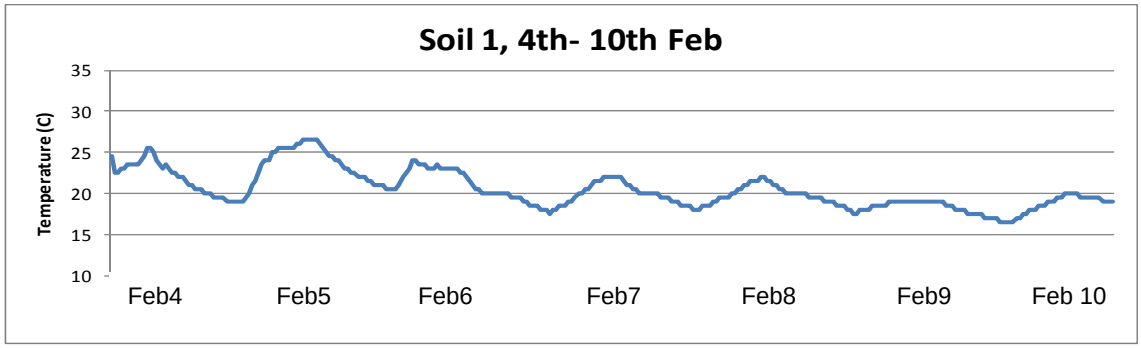
Appendices

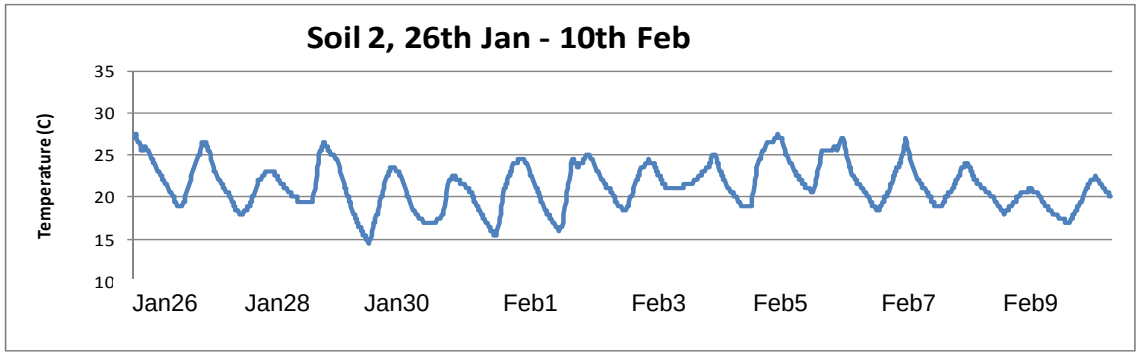
Appendix 1

Temperatures recorded by microloggers suspended in the kauri seedling canopies, or buried 5 cm deep in soil in the planter bags containing the kauri seedlings during the main seedling trial. *Phytophthora* taxon Agathis (PTA) inoculation was on Jan 26th. Beyond February 10, glasshouse temperature was maintained between 15 and 25 C at all times.









Appendix 2

Analyses of root disease scores, assessed 10 weeks and 20 weeks after soil inoculation with PTA. Kauri seedlings were treated with either Ridomil or phosphite (PA) applied at various rates and three application techniques, either 5 days before or 5 days after inoculation. 'Root score' is the average of root disease scored on a 0 to 5 scale, where 0 = all healthy, and 5 = all dead.

A: ANOVA table, 10-week assessment

	d.f.	s.s.	m.s.	v.r.	F pr.
Inoc/uninoc	1	69.051	69.051	23.5	<.001
Inoc_pre_or_post	1	0.53	0.53	0.18	0.672
Treat Type	4	65.323	16.331	5.56	<.001
Treat Dose	4	13.776	3.444	1.17	0.327
Inoc/uninoc.Treat Type	4	39.009	9.752	3.32	0.013
Inoc/uninoc.Treat Dose	4	1.533	0.383	0.13	0.971
Inoc_pre_or_post.Treat Type	4	2.683	0.671	0.23	0.922
Inoc_pre_or_post.Treat Dose	3	6.667	2.222	0.76	0.521
Residual	112	329.15	2.939		

B: Mean root disease scores, 10-week assessment. A dash (-) indicates not assessed.

Treatment	Treated after inoculation	Treated before inoculation	Uninoculated
PA Drench (low rate)	2.6	2.2	1.4
PA Drench (high rate)	1.8	1	0.8
PA Injection (low rate)	0.6	0.4	1
PA Injection (high rate)	1.4	-	1.75
PA Spray (low rate)	2.4	3.8	0.8
PA Spray (med rate)	3.4	3.2	1.8
PA Spray (high rate)	4	3.2	1.4
Ridomil	3.6	2.8	0.4
Untreat	4.2	3.667	1.4

C: ANOVA table, 20-week assessment

	d.f.	s.s.	m.s.	v.r.	F pr.
Inoc/uninoc	-	-	-	-	-
Inoc_pre_or_post	1	0.591	0.591	0.41	0.525
Treat Type	4	92.575	23.144	15.54	<.001
Treat Dose	4	10.67	2.667	1.84	0.131
Inoc/uninoc.Treat Type	-	-	-	-	-
Inoc/uninoc.Treat Dose	-	-	-	-	-
Inoc_pre_or_post.Treat Type	4	32.592	8.148	5.62	<.001
Inoc_pre_or_post.Treat Dose	3	7.317	2.439	1.68	0.178
Residual	72	104.4	1.45		

D: Mean root disease scores, 20-week assessment. A dash (-) indicates not assessed.

Treatmnt	Treated after inoculation	Treated before inoculation	Uninoculated
PA Drench (low rate)	5	4.4	-
PA Drench (high rate)	4.4	2.8	-
PA Injection (low rate)	0.8	2.6	-
PA Injection (high rate)	2.2	-	-
PA Spray (low rate)	3.2	5	-
PA Spray (med rate)	5	5	-
PA Spray (high rate)	4.6	4.4	-
Ridomil	5	2.4	-
Untreat	5	5	-

Appendix 3

Analyses of foliar disease scores, assessed 10 and 20 weeks after soil inoculation with PTA. Trees were treated with either Ridomil or phosphite (PA) applied at various rates and three application techniques, either 5 days before or 5 days after inoculation. 'Foliar score' is the average of foliar disease symptoms scored on a 0 to 3 scale, where 0 = healthy, and 3 = dead. Week 10 data are averages of scores for top, middle and bottom sections of the tree. Week 20 data are from a single overall tree health score.

A: ANOVA table, 10-week assessment

	d.f.	s.s.	m.s.	v.r.	F pr.
Inoc/uninoc	1	35.7333	35.7333	41.87	<.001
Inoc_pre_or_post	1	0.7241	0.7241	0.85	0.359
Treat Type	4	7.8797	1.9699	2.31	0.062
Treat Dose	4	1.9256	0.4814	0.56	0.689
Inoc/uninoc.Treat Type	4	5.7214	1.4304	1.68	0.16
Inoc/uninoc.Treat Dose	4	0.9577	0.2394	0.28	0.89
Inoc_pre_or_post.Treat Type	4	1.6335	0.4084	0.48	0.751
Inoc_pre_or_post.Treat Dose	3	1.2722	0.4241	0.5	0.685
Residual	112	95.5778	0.8534		

B: Mean foliar disease scores, 10-week assessment. A dash (-) indicates not assessed.

Treatmnt	Treated after inoculation	Treated before inoculation	Uninoculated
PA Drench (low rate)	0.6667	0.6667	0
PA Drench (high rate)	1	0	0
PA Injection (low rate)	0.6	0.4	0.2
PA Injection (high rate)	1	-	0.1667
PA Spray (low rate)	1.2	1.2667	0
PA Spray (med rate)	1.6667	1.8667	0
PA Spray (high rate)	1.1333	1.2667	0
Ridomil	1.5333	1.2	0
Untreat	1.7333	1.2222	0

C: ANOVA table, 20-week assessment

	d.f.	s.s.	m.s.	v.r.	F pr.
Inoc/uninoc	1	117.8982	117.8982	206.32	<.001
Inoc_pre_or_post	1	0.4101	0.4101	0.72	0.399
Treat Type	4	50.2123	12.5531	21.97	<.001
Treat Dose	4	6.6163	1.6541	2.89	0.025
Inoc/uninoc.Treat Type	4	31.4132	7.8533	13.74	<.001
Inoc/uninoc.Treat Dose	4	3.2497	0.8124	1.42	0.231
Inoc_pre_or_post.Treat Type	4	12.9954	3.2489	5.69	<.001
Inoc_pre_or_post.Treat Dose	3	7.0667	2.3556	4.12	0.008
Residual	112	64	0.5714		

D: Mean foliar disease scores, 20-week assessment. A dash (-) indicates not assessed.

Treatmnt	Treated after inoculation	Treated before inoculation	Uninoculated
PA Drench (low rate)	3	1.8	0
PA Drench (high rate)	1.6	0.8	0
PA Injection (low rate)	0	0	0
PA Injection (high rate)	0	-	0
PA Spray (low rate)	1.2	3	0
PA Spray (med rate)	3	2.6	0
PA Spray (high rate)	2.4	2.4	0
Ridomil	3	1.2	0
Untreat	3	3	0

Appendix 4

Analyses of lesion extension upwards from the inoculation point, assessed 10 weeks after trunk inoculation with PTA. Trees were treated with either Ridomil or phosphite (PA) applied at various rates and three application techniques, either 5 days before or 5 days after inoculation.

A. ANOVA table, log-transformed lesion length data

	d.f.	s.s.	m.s.	v.r.	F pr.
Inoc_pre_or_post	1	0.3785	0.3785	2.75	0.101
Treat Type	4	5.7922	1.448	10.53	<.001
Treat Dose	4	0.6567	0.1642	1.19	0.321
Inoc_pre_or_post.Treat Type	4	2.9413	0.7353	5.35	<.001
Inoc_pre_or_post.Treat Dose	3	0.5916	0.1972	1.43	0.24
Residual	72	9.914	0.1377		

B. Mean lesion lengths (mm) 10 weeks after trunk inoculation (back-transformed data following analyses on log-transformed data). A dash (-) indicates not assessed.

Treatment	Treated after inoculation	Treated before inoculation
PA Drench (low rate)	105	25
PA Drench (high rate)	116	99
PA Injection (low rate)	42	6
PA Injection (high rate)	30	-
PA Spray (low rate)	94	78
PA Spray (med rate)	141	58
PA Spray (high rate)	77	78
Ridomil	34	108
Untreat	80	148

Appendix 5

Analyses of percentage girdling of the trunk by lesions extending from the inoculation point, assessed 10 weeks after trunk inoculation with PTA. Trees were treated with either Ridomil or phosphite (PA) applied at various rates and three application techniques, either 5 days before or 5 days after inoculation.

A. ANOVA table, percentage trunk girdling

	d.f.	s.s.	m.s.	v.r.	F pr.
Inoc_pre_or_post	1	757.8	757.8	1.31	0.257
Treat Type	4	11667	2917	1.62	0.178
Treat Dose	4	1402	350	0.19	0.940
Inoc_pre_or_post.Treat Type	4	2770.7	692.7	1.2	0.320
Inoc_pre_or_post.Treat Dose	3	1666.7	555.6	0.96	0.417
Residual	72	41715.2	579.4		

B. Mean percentage girdling of trunks. A dash (-) indicates not assessed.

Treatmnt	Treated after inoculation	Treated before inoculation
PA Drench (low rate)	100	80
PA Drench (high rate)	100	100
PA Injection (low rate)	70	37
PA Injection (high rate)	54	-
PA Spray (low rate)	86	96
PA Spray (med rate)	100	100
PA Spray (high rate)	100	80
Ridomil	82.4	85
Untreat	92	100

Appendix 6

Analyses of foliar disease scores, assessed 10 and 20 weeks after trunk inoculation with PTA. Trees were treated with either Ridomil or phosphite (PA) applied at various rates and three application techniques, either 5 days before or 5 days after inoculation. 'Foliar score' is the average of foliar disease symptoms scored on a 0 to 3 scale, where 0 = healthy, and 3 = dead.

A: ANOVA table, 10-week assessment

	d.f.	s.s.	m.s.	v.r.	F pr.
Inoc/uninoc	1	131.1726	131.1726	333.39	<.001
Inoc_pre_or_post	1	0.0066	0.0066	0.02	0.897
Treat Type	4	21.6004	5.4001	13.72	<.001
Treat Dose	4	1.4398	0.36	0.91	0.458
Inoc/uninoc.Treat Type	4	17.116	4.279	10.88	<.001
Inoc/uninoc.Treat Dose	4	0.5338	0.1335	0.34	0.851
Inoc_pre_or_post.Treat Type	4	1.9025	0.4756	1.21	0.311
Inoc_pre_or_post.Treat Dose	3	0.9611	0.3204	0.81	0.489
Residual	112	44.0667	0.3935		

B: Mean foliar disease scores, 10-week assessment. Data are averages of scores for top, middle and bottom sections of the tree. A dash (-) indicates not assessed.

Treatmnt	Treated after inoculation	Treated before inoculation	Uninoculated
PA Drench (low rate)	2.733	2.2	0
PA Drench (high rate)	2.533	2.867	0
PA Injection (low rate)	1	0.2	0.2
PA Injection (high rate)	0.867	-	0.1667
PA Spray (low rate)	2.133	2.067	0
PA Spray (med rate)	2.533	2.533	0
PA Spray (high rate)	2.533	2.4	0
Ridomil	1.933	1.8	0
Untreat	2.2	2.556	0

C: ANOVA table, 20-week assessment

	d.f.	s.s.	m.s.	v.r.	F pr.
Inoc/uninoc	1	204.7442	204.7442	636.98	<.001
Inoc_pre_or_post	1	0.0591	0.0591	0.18	0.669
Treat Type	4	37.3606	9.3402	29.06	<.001
Treat Dose	4	1.579	0.3948	1.23	0.303
Inoc/uninoc.Treat Type	4	21.3659	5.3415	16.62	<.001
Inoc/uninoc.Treat Dose	4	0.3653	0.0913	0.28	0.888
Inoc_pre_or_post.Treat Type	4	3.1655	0.7914	2.46	0.049
Inoc_pre_or_post.Treat Dose	3	0.0000	0.0000	0.00	1.000
Residual	112	36	0.3214		

D: Mean foliar disease scores, 20-week assessment. Data are from a single overall tree health score. A dash (-) indicates not assessed.

Treatmnt	Treated after inoculation	Treated before inoculation	Uninoculated
PA Drench (low rate)	3	3	0
PA Drench (high rate)	3	3	0
PA Injection (low rate)	1.2	0	0
PA Injection (high rate)	1.2	-	0
PA Spray (low rate)	3	3	0
PA Spray (med rate)	3	3	0
PA Spray (high rate)	3	3	0
Ridomil	2.4	2.4	0
Untreat	3	3	0