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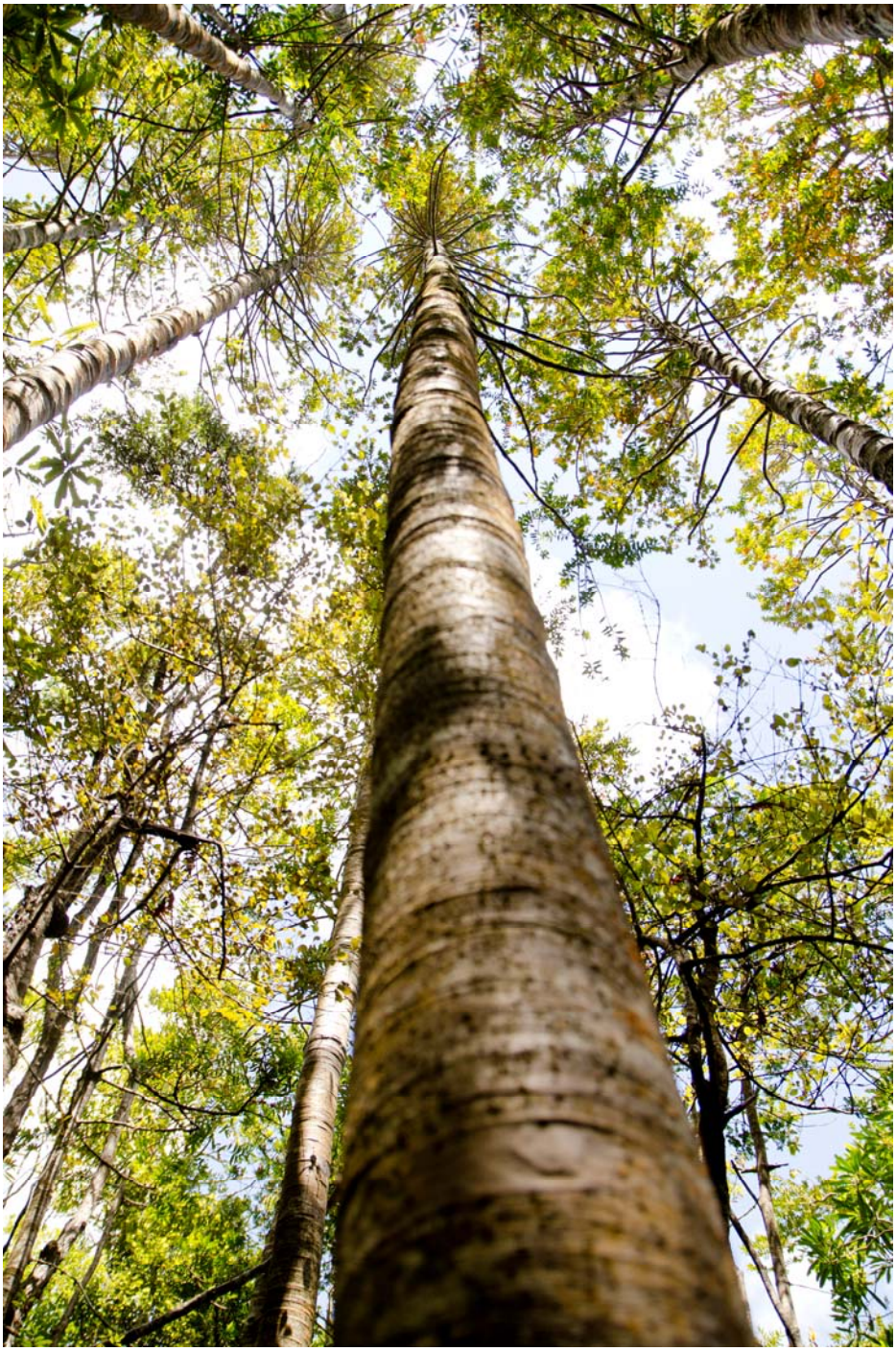
Phosphorous acid for controlling *Phytophthora* taxon Agathis in kauri: Field trials progress report for June 2013

Horner IJ, Hough EG

July 2013



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Phytophthora taxon Agathis (PTA) threatens the health and survival of kauri trees in Auckland and Northland. *In vitro* studies and glasshouse trials evaluating phosphorous acid (phosphite) for control of PTA-infected kauri seedlings gave promising results; therefore, long-term field trials were established in four forest sites between January and March 2012. Trial sites were selected at Huia and Whatipu (Waitakere ranges, Auckland) and Raetea and Omahuta Forests (Mangamuka Ranges, Northland). Trial trees are kauri rickers with girths from 40 to 120 cm, and all 160 trees showed symptoms of PTA infection at the start of the trial programme.

Small-scale preliminary trials testing various phosphite rates on trees outside the main trial established that phytotoxicity may be an issue, and symptoms were noted in a number of trees. There was leaf yellowing, some defoliation, and even premature twig abscission, particularly with the high phosphite rates (e.g. 20%). Observations on these trees in January and June 2013, 12-17 months after injection, found few signs of phytotoxicity symptoms. Although canopies were slightly less dense, they were predominantly green and apparently healthy, with signs of new growth.

At the start of the main trial, baseline assessments were made of canopy health and trunk lesions. All canopies plus the base of every trunk (at cardinal points) were photographed for future comparisons. Trunk lesions were measured (maximum height and width) and some lesion margins were marked so that future advance could be accurately measured. Trees were injected with either high (20%) or low (7.5%) concentrations of phosphite (20 ml/20 cm trunk circumference), or left untreated. In January 2013, half the previously injected trees were re-treated with phosphite, in all cases with the low concentration (7.5%), regardless of the initial concentration used. Remaining trees, including the untreated control trees, were left untreated.

Assessments of canopy health and lesion activity or spread were made in June 2013. Canopy health was either similar or slightly worse than that in the baseline photographs, regardless of whether trees were injected or left untreated. However, it is too early in the trial to expect obvious changes in the canopy; such differences are expected to become more apparent as the trial progresses.

At all four sites, a higher proportion of trunk lesions appeared active (expressing fresh ooze) in untreated trees than in the phosphite-injected trees, regardless of the concentration applied or the treatment regime used. This differential was greatest at the Raetea site, where 61% of monitored lesions in the untreated trees were classed as ‘active’, compared with no active lesions in trees treated either once or twice with phosphite. In most cases, in phosphite-injected trees there were signs of cracking, bark peeling and healing around the margins of PTA cankers.

Where lesion margins were marked at the start of the trial, then re-measured in June 2013, the lesion advance was greater in untreated trees than in phosphite-injected trees at three of the four sites. Again, this differential was greatest at the Raetea site, with an average lesion margin advance of 13 cm in untreated controls, compared with less than 1 cm in phosphite-injected trees.

In vitro PTA inoculation of sampled twigs taken from trial trees at the Omahuta site indicated that PTA growth was slower in tissue from trees that had been injected with phosphite than in untreated trees.

We intend to continue assessing lesion activity and canopy health at all four trial sites on a six-monthly basis throughout the trial programme, with the next assessment of the main trial planned for January 2014. This will allow future comparisons to be made between trees treated just once at the start of the trial and trees treated on an annual basis. Progress reports will follow each six-monthly assessment. The next phosphite injections are scheduled for January 2014. Discussion with interested parties regarding modification of trial protocols in advance of that treatment may be useful.

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1 Introduction

Phytophthora taxon Agathis (PTA) is a serious problem, killing kauri trees of all ages in forests in Auckland and Northland (Beever et al. 2008). There are no proven treatments for PTA-infected or threatened trees. Treatment with phosphorous acid (PA, phosphite) is one of the few options for treating infected or threatened trees, but it is as yet untested on forest kauri. Phosphite is widely used internationally on a range of *Phytophthora* diseases on many plant species. Its predominant use is in horticulture and nurseries, with some use in forest systems; in New Zealand it is commonly used for *Phytophthora* control in avocados, strawberries and other crops.

In vitro tests showed that PTA was very sensitive to phosphite (Horner & Hough 2011a) and glasshouse trials showed that phosphite injections could protect kauri seedlings from PTA (Horner & Hough 2011b). Field trials testing phosphite application in PTA-infected kauri stands were established in two Auckland and two Northland forests during January to March 2012 (Horner & Hough 2012, 2013). The project aims to determine whether phosphite treatment can protect kauri trees in the forest from infection by PTA, reduce the rate of symptom development in infected trees, and/or improved the health of trees in advanced stages of infection. It is anticipated that these trials could take 4 – 5 years to complete. This time frame will be necessary to achieve good discrimination of symptom development between treated and untreated trees, and to determine the longer-term robustness of any treatment effects.

The current report is a brief summary of results from tree assessments made in June 2013, 17 months after the first treatment applications.

2 Phosphite rate trial

Early results from trunk injection trials, testing a range of phosphite application rates on kauri trees, indicated that kauri were particularly sensitive to phosphite. Some doses were phytotoxic, resulting in leaf yellowing or browning, and in extreme cases, premature twig drop. The full methods and results of this trial were presented in the June 2012 report (Horner & Hough 2012).

All trees in the phosphite rate trial at the Huia forest site were checked again in June 2013. An updated summary of observations is presented in Table 1. In all cases, tree canopies looked green and healthy, regardless of the application rate (Figure 1). This suggests that although trees were initially adversely affected by the application of high rates, they recovered and did not continue to deteriorate. Only one of the trees in this trial showed signs of new shoot growth over the last summer season, although this lack of growth was common across the wider Huia trial site, in both treated and untreated trees. Monitoring of these trees will continue, to determine if normal growth resumes in the future.

In another rate trial on ricker trees destined for felling in Waipoua, trees were injected with concentrations of 15% or 60% phosphite, to determine phosphite toxicity at high rates. Within three months many leaves went yellow or brown, and by seven months there was significant and dramatic premature abscission of branches from the lower and central portion of the trunk (Horner & Hough 2012). Only branches from the upper part of the trunk remained intact, but even here there was some leaf yellowing. By June 2013, 19 months after the initial treatment, the remaining canopies on all treated trees appeared healthy and green. All trees showed signs of new growth over the 2012-2013 season, indicating that the trees were able to survive and ultimately grow out of the initial toxic shock of extreme doses of phosphite. Monitoring of these trees will also continue.

Table 1. A summary of notes on kauri tree canopy health, 6, 8, 20, 50, and 72 weeks after trunk injection with various concentrations of phosphite at the Huia trial site. Tree canopies were compared with photographs taken on the day of treatment application. The notes refer to observations on two trees in each treatment.

Trt	Phosphite (%)	volume (ml) per trunk circumf. (cm)	6 weeks	8 weeks	20 weeks	50 weeks	72 weeks
A	15	20/20	Most leaves yellow, some brown, esp. on small shoots	Many leaves yellow or brown, some leaf drop	Much thinner canopy. Remaining leaves green & healthy	Thinner canopy. Leaves green & healthy	Slightly thinner canopy. Leaves green & healthy
B	10	20/20	No change	One tree slightly yellow, other tree very yellow, some brown leaves, minor leaf drop	Much thinner canopy. Remaining leaves green & healthy	Slightly thinner canopy. Remaining leaves green & healthy on one tree, yellow on other	Slightly thinner canopy. Leaves green & healthy
C	15	20/30	Similar to photograph, but slightly more yellow	Very yellow leaves & many brown. Some leaf drop	Thinner canopy. Remaining leaves green & healthy	Slightly thinner canopy. Leaves green & healthy	One tree similar to photograph, other tree slightly thinner canopy. Leaves green and healthy
D	20	20/40	Similar to photograph, but 1 tree slightly yellow	Some yellow and brown leaves, minor leaf drop	Thinner canopy. Remaining leaves green & healthy	Slightly thinner canopy. Leaves green & healthy	One tree similar to photograph, other tree slightly thinner canopy. Leaves green and healthy
E	7.5	20/20	Similar to photograph, but 1 tree slightly yellow	One tree similar to photograph, other tree with many yellow and some brown leaves	One tree similar to photograph, other tree slightly thinner canopy, remaining leaves green and healthy	One tree similar to photograph, other tree slightly thinner canopy. Leaves green and healthy	One tree similar to photograph, other tree slightly thinner canopy. Leaves green and healthy
F	0	0	No change	No change	No change	No change	No change

Figure 1. Phytotoxicity symptoms in kauri trees following trunk injection with phosphite, Huia trial site, Auckland. Clockwise from top left: Pre-treatment (January 2012), 12 weeks post-treatment (April 2012), 20 weeks post-treatment (June 2012), 72 weeks post-treatment (June 2013). The tree at the lower/centre of each picture was injected with 20 ml of 15% phosphite per 20 cm trunk circumference. The tree on the upper left of the picture was injected with 20 ml of 10% phosphite per 20 cm trunk circumference. Note the yellowing and browning of leaves in the photograph taken 12 weeks post-treatment, and the thinner, but otherwise healthy canopies after 20 and 72 weeks.

3 Main forest trials

3.1 Methods

Trials were established in two Waitakere (Huia dam and Whatipu) and two Northland (Omahuta and Raetea) forest sites in January to March 2012. All four sites had confirmed diagnoses of the presence of PTA in soil, and only kauri trees showing symptoms consistent with PTA infection (e.g. lesions or bleeding sap at the base of the trunk, or thinning or yellowing canopies) were included in the trial. There were a total of 160 trial trees across the four sites. Most trees were at the ricker or advanced ricker stage, with girths ranging from 40 to 120 cm.

Basal trunk lesions (if present) were measured, noting position, height and width. In many cases lesion margins were marked using a chinagraph pencil for future reference. Photographs of the trunk base were taken at cardinal points, and the tree canopy was also photographed.

Trees were injected with either a high (20%, Waitakere sites only) or low (7.5%, all sites) phosphite concentration. Both concentrations were applied at a dose rate of 20 ml of phosphite solution injected per 20 cm trunk circumference. Some trees were left untreated as controls. Care was taken to ensure a balanced allocation of trees with different starting symptom severity across the various treatments and controls. Full details of trial sites and methods are provided in the June 2012 report (Horner & Hough 2012). In January 2013, 10 to 12 months after the initial treatment, half the previously treated trees at each site were re-injected with a low (7.5%) concentration of phosphite, regardless of whether they were treated with the high or low rate at the start of the trial. Remaining injected trees and untreated controls were left untreated, making a total of five different treatments in the trial as follows:

1. *'High PA/low PA':* 20% phosphite (January 2012) and 7.5% phosphite (January 2013)
2. *'High PA/nil PA':* 20% phosphite (January 2012)
3. *'Low PA/low PA':* 7.5% phosphite (March 2012) and 7.5% phosphite (January 2013)
4. *'Low PA/nil PA':* 7.5% phosphite (March 2012)
5. Untreated control.

All phosphite treatments were a single dose of 20 ml per 20 cm trunk circumference, injected into the trunk 0.4 to 0.8 m above the ground, using a Chemjet® tree injector. All treatments were applied at Huia and Whatipu. Only treatments 3, 4 and 5 were applied at Omahuta and Raetea.

In January 2013 and again in June 2013, the tree canopy health and vigour were compared with those in the photographs taken at the start of the trial. The dimensions of lesions at the base of the trunk were re-measured, and where margins on particular lesions had been marked, any advance of the lesion margin was measured. Lesion activity, as indicated by freshly oozing sap, was recorded as either not active (healed/dry), active (fresh ooze or sap), or unclear (possibly active).

3.2 Results

3.2.1 Canopy health

Comparisons of canopy health using the photographs taken at the start of the trial as references were particularly difficult to make. The growth of the understory, in particular tree ferns, blocked many of the gaps where photographs of the canopy had earlier been taken. Nevertheless, in most instances a portion of the canopy could be observed and a judgement made.

At the Huia, Whatipu and Raetea sites approximately one-third of the trees in the trial appeared to have the same or better status of canopy health and vigour as that in the baseline photographs. Most of the remaining trees showed a slight decline in canopy health. At the Omahuta site, all but one trial tree had similar or better canopy health and vigour than in their respective baseline photographs. Overall, across all trial sites, there were no consistent differences between treated and untreated trees (data not shown). The yellowing and leaf loss noted in some treated trees soon after the initial treatments last year may have confounded discrimination between treatments. However, it is still very early in the trial programme and dramatic changes in canopy health are unlikely in the short term. It is expected that canopy health assessments will become a more useful measure as the trial progresses, with relative differences in canopy health increasing with decline and death of some trees and potential improvement in others.

3.2.2 Lesion expansion

A summary of data on lesion expansion and activity is presented in Table 2 and Figures 2 and 5. Although it is early in the trial period, a trend is already appearing, with many lesions on untreated trees remaining active and continuing to advance (Figure 3), compared with cracking around the margins and healing in most trees injected with phosphite (Figure 4). Cracking and healing of lesions was also noted on some untreated trees, but this was often ‘outflanked’ by further waves of PTA advance, something rarely seen in phosphite-treated trees. On all four sites, there was a higher proportion PTA-lesions judged ‘active’ in the untreated controls than in any of the phosphite treatment regimes. Raetea, the site with the highest number of monitored lesions (95), had the greatest difference in lesion activity between treated and untreated trees, with 61% of monitored lesions on untreated trees deemed ‘active’ at 15 months, and no active lesions detected in any of the phosphite-treated trees (Figure 2). It is too early in the trial to determine any consistent differences between the various phosphite regimes. However, at the Whatipu site, a high proportion of monitored lesions in trees which received the single application of the low rate phosphite treatment were deemed ‘possibly active’ in the June 2013 assessment. The next assessment, planned for January 2014, should identify whether any of these lesions are indeed still classed as active.

At the Huia, Whatipu and Raetea sites, the distance of advance of lesions that were marked at the start of the trial was greater in the untreated control trees than in phosphite-injected trees, regardless of the phosphite concentration or injection regime (Figure 5, Table 2). The differential was greatest at the Raetea site, where there were many rapidly progressing lesions in the untreated control trees and none in the phosphite-treated trees. At the Omahuta site, the trend appears different, with the greatest lesion spread in the Low PA/nil PA treatment (Treatment 4). However, at this site the number of marked lesions is small, and the result is strongly biased by two very aggressive lesions on a single tree in the Low PA/nil PA treatment.

Table 2. Assessment of lower trunk lesion activity on *Phytophthora* taxon Agathis-infected kauri trees in June 2013, 15 – 17 months after trees were injected with phosphite (PA) at high (20%) or low (7.5%) concentrations, or left untreated. All lesions visible above ground were monitored; a subset of these had lesion margins marked at the time of treatment application, with lesion advance measured in June 2013.

		Monitored lesions						Marked and measured lesions					
Site	Treatment 2012/2013	No. of trees	No. of monitored lesions	No. active	No. possibly active	No. Not active	% active	No. of marked lesions	No. active	No. possibly active	No. Not active	% active	Average advance (cm)
HUIA	High PA/High PA	11	9	0	1	8	0	4	0	1	3	0	0.9
	High PA/nil	10	9	0	0	9	0	4	0	0	4	0	0.2
	Low PA/low PA	10	7	0	0	7	0	6	0	0	6	0	0.1
	Low PA/nil	10	10	0	0	10	0	8	0	0	8	0	0.2
	Untreated	11	10	2	2	6	20	8	1	1	6	33.3	3.5
WHATIPU	High PA/High PA	10	6	0	1	5	0	3	0	1	2	0	0.8
	High PA/nil	11	4	0	1	3	0	1	0	0	1	0	1.0
	Low PA/low PA	10	8	1	0	7	12.5	3	0	0	3	0	0.8
	Low PA/nil	10	4	0	2	2	0	5	0	1	4	0	0.2
	Untreated	11	6	3	0	3	50	3	1	0	2	33.3	3.1
OMAHUTA	Low PA/low PA	5	9	0	1	8	0	8	0	0	8	0	0.6
	Low PA/nil	5	9	2	0	7	22.2	6	2	0	4	33.3	5.1
	Untreated	5	8	5	0	3	62.5	8	2	0	6	25	1.7
RAETEA	Low PA/low PA	14	34	0	1	33	0	23	0	1	22	0	0.9
	Low PA/nil	14	25	0	1	24	0	19	0	0	19	0	0.8
	Untreated	14	36	22	6	8	61.1	17	7	3	7	41.2	13.3

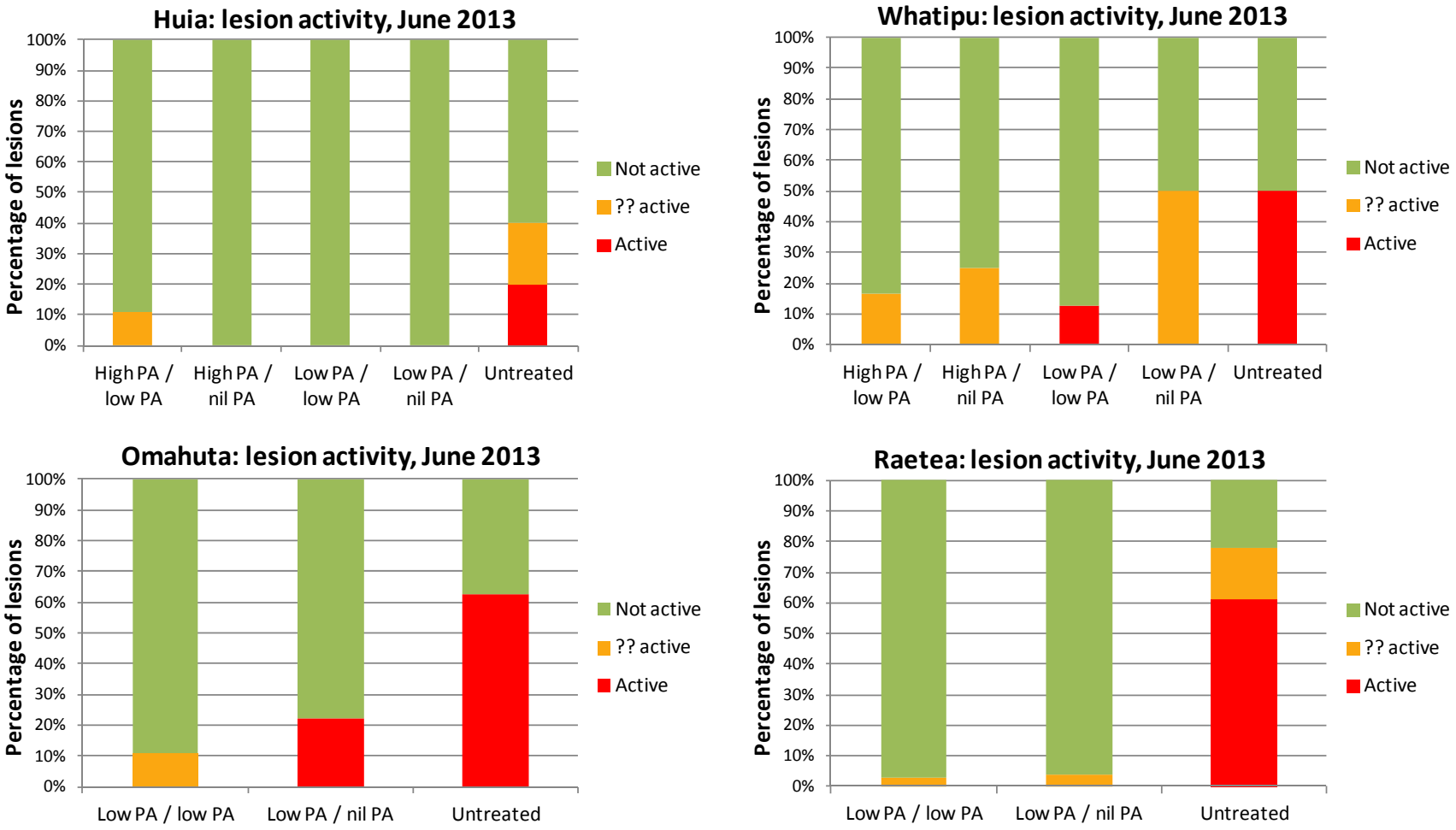

Figure 2. Activity of monitored lower-trunk lesions on *Phytophthora* taxon Agathis-infected kauri trees on four sites, where trees were treated with high (20%) or low (7.5%) concentrations of phosphite (PA), or left untreated. X-axis labels refer to treatments applied in January-March 2012 / January 2013. Assessments were made in June 2013, 15-17 months after initial treatment application.

Figure 3. *Phytophthora* taxon Agathis lesion advance in an untreated kauri at the Raetea trial site, Northland. Left: March 2012, Right: June 2013.

Figure 4. Cracking and peeling of bark around the margins of *Phytophthora* taxon Agathis lesions in kauri trees at the Omahuta trial site, Northland, 15 months after injection with 7.5% phosphite.

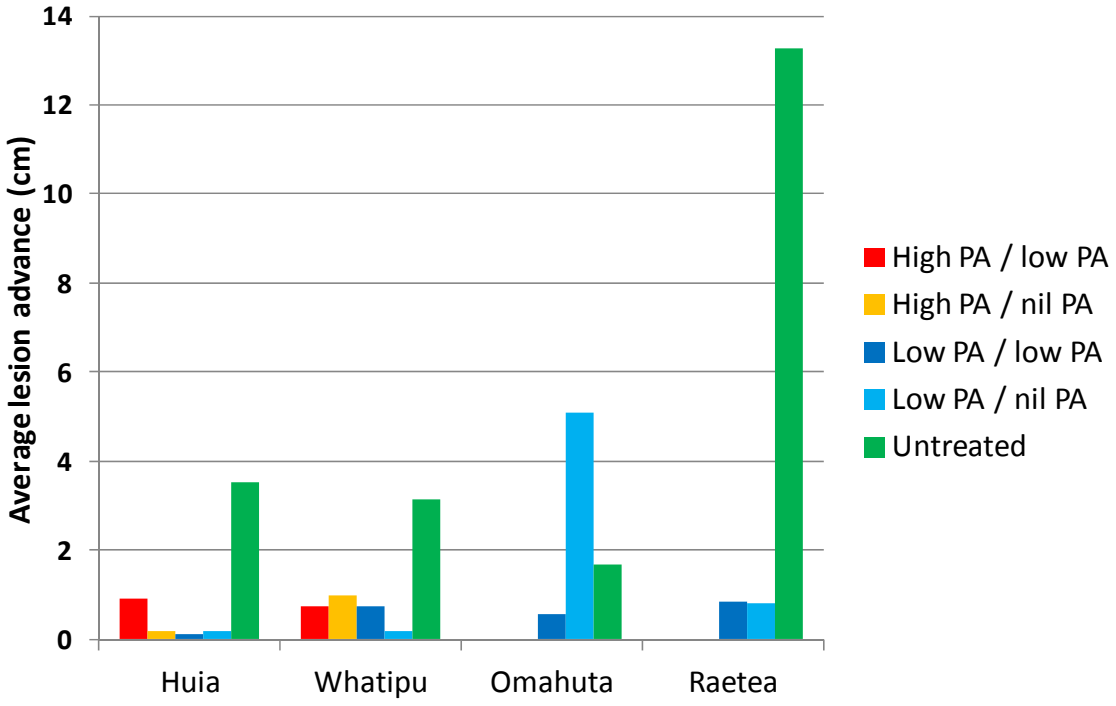

Figure 5. Mean advance of lower-trunk lesion margins marked at the time of treatment application and re-measured 15 – 17 months later. Kauri trees on four sites were treated with high (20%) or low (7.5%) concentrations of phosphite (PA), or left untreated. Legend labels refer to PA treatments applied in January-March 2012 / January 2013. The high PA treatments were not applied at the Omahuta and Raetea sites.

4 Inoculated shoots

4.1 Methods

To gain some insight into the differential effects of phosphite within treated kauri trees, twig samples were taken from treated and untreated trees at the Omahuta trial site and inoculated with PTA. All samples were collected from branches within three metres of the ground, in early May 2013. This was 14 months after the first phosphite treatment application and three months after the second. Only healthy-looking twigs were collected. Not all trees in the Omahuta trial site had suitable branches for inclusion in the twig assay. To supplement the number of trees in the 'untreated' treatment, samples were also collected from adjacent untreated trees that were not part of the trial. Numbers of trees sampled and the number of twigs used in the trial are given in Table 3.

Samples were transported to Hawke's Bay and stored in the refrigerator for one week before inoculating. A mechanical malfunction meant that many of the samples froze while in storage, and this was reflected in the browning of many leaves.

In the laboratory, the sampled twigs were divided into segments 100 to 150 mm long, and the approximate age of each segment (estimated from the growth points on the twigs) was noted. On each segment, one third of the way up from the base, a 3 x 3 mm section of bark was removed using a sharp scalpel. A 3-mm diameter plug of V8-agar colonised with PTA was then placed on the wound, and the segment was placed in a plastic bag with a moist paper towel, sealed and then incubated at 15-20 C on a laboratory bench. After 3½ weeks, twigs were removed, cut into pieces 1 – 2 cm long, split in half longitudinally, and plated onto *Phytophthora*-selective agar (PARP), with care being taken with the orientation of each segment. After 2 and 3 days, *Phytophthora*-like colonies emerging from the twig portions were marked on the bottom of the plate. To confirm their identity, these colonies were subsequently checked under the microscope for characteristic PTA oospores. The origin of each colony in the twig was estimated, and the distance of spread 'up' and 'down' from the inoculation point was determined. Data were analysed using ANOVA (Minitab 16).

4.2 Results

The age of twig portions plated ranged from 0 years (current season growth) to 10 years, with the vast majority of samples in the 0 – 4 year range. There did not appear to be any effect of twig age on the distance that PTA spread within the twigs, with an R^2 value of just 0.03 for 'Age' v. 'Distance'. Therefore, data from different aged twigs were pooled for analysis.

Data for PTA spread within the twigs are presented in Table 3. For both 'up' and 'down' assessments, growth from the inoculation point was similar in twigs from untreated trial trees and from adjacent untreated trees. In comparison, PTA growth was significantly ($P<0.05$) less on twigs from trees that had received two applications of phosphite. PTA growth on twigs from trees treated with a single phosphite application was less than that on untreated controls, but the difference was not statistically significant.

Although the trend between treatments follows the expected pattern, with greatest PTA growth on twigs from untreated trees and least growth on trees treated twice with phosphite, the magnitude of the difference was not as great as that expected. It is not known what effect tissue freezing had on the assay, but it may have partially neutralised any phosphite effect. Phosphite works by direct inhibition of *Phytophthora* in combination with enhanced host defence response; the freezing injury may have compromised the latter effect.

Table 3. Mean growth of *Phytophthora* taxon Agathis on excised kauri twigs, inoculated with PTA-colonised agar plugs. Twigs were from trees in the Omahuta trial site that had been previously treated with phosphite (PA) at the low rate (7.5%) in March 2012, January 2013, or left untreated. Additional untreated trees adjacent to the trial trees were included in the assay. Numbers followed by the same letter are not significantly different at P<0.05.

Treatment	No. trees sampled	Total no. of shoot segments inoculated	Ave. PTA spread DOWN (mm)	Ave lesion spread UP (mm)
7.5% PA May 2012 & 7.5% PA Jan. 2013	4	45	23.02 B	38.49 B
7.5% PA May 2012 & untreated Jan. 2013	4	46	26.35 AB	42.63 AB
Untreated	3	22	29.27 AB	50.82 A
Untreated (adjacent)	2	30	31.03 A	48.45 A

5 Conclusions

Evidence from forest trials on 'ricker' sized kauri trees suggests that trunk injection with phosphite is suppressing the activity of PTA within infected trees. The best evidence to date is the differential activity and spread of lesions in phosphite-treated versus untreated trees, supported by evidence from PTA growth studies on excised twigs. Whether the treatment is sufficient to save trees already infected, and ultimately restore them to good health, will become more apparent in future assessments. The longevity of treatment efficacy and the required frequency of treatment for long-term control are also yet to be determined.

6 Future Plans

Tree health measurements will continue on a six-monthly basis during mid-summer and mid-winter in each year of the trial, with the next assessment planned for January 2014. A progress report will be prepared immediately after each assessment. At each assessment, the canopy health will be re-scored, and notes taken on whether canopy health appeared better, worse, or the same as in the original photographs. Lesions will be re-measured, with notes taken on lesion activity. Where margins have been marked, any advances will be measured. Another assessment of PTA growth on excised twigs will be made early in 2014.

The next treatment application is planned for January 2014. There is room for flexibility in future treatment applications within this trial. Depending on results, treatment application in future years could be modified, for example by changing rates, application intervals or phosphite formulations. Bark applications using phosphite paints may also be considered in future years. These decisions will be made in consultation with the Kauri Dieback Planning and Intelligence team and other interested parties. This consultation should happen well before the next planned applications in January 2014.

7 Acknowledgments

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