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Phosphorous acid for controlling *Phytophthora* taxon Agathis in kauri: field trials 2½ years on, June 2014

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July 2014

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Executive summary

Phosphorous acid for controlling *Phytophthora* taxon Agathis in kauri: field trials 2½ years on, June 2014

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July 2014

Phytophthora taxon Agathis (PTA) is a serious problem, killing kauri trees in many forests in the Auckland, Northland and Coromandel regions. Phosphorous acid (PA, phosphite) is one option for treating infected or threatened trees. In January 2012, trials were established in four PTA-infected kauri stands in Huia, Whatipu, Omahuta and Raetea forests, to determine whether phosphite could be an effective treatment for PTA-diseased or infected kauri. This report summarises assessments made 2½ years after the first treatment applications.

There are 162 trees in the trial, all between 10 and 45 cm diameter and most classed as kauri 'rickers'. All trial trees showed symptoms of kauri dieback (canopy thinning and/or basal trunk lesions) at the start of the trial. Diseased trees were trunk-injected with phosphite either once (January/March 2012) or twice (January/March 2012 and January 2013) or left untreated. Phosphite injections were with a high (20% a.i.) or low (7.5% a.i.) concentration of phosphite, injected at a rate of 20 ml of solution per 20 cm trunk circumference.

Phytotoxicity symptoms, including leaf yellowing or browning and some premature twig or lower branch abscission, were noted in many phosphite treated trees, especially where the high concentration of phosphite was used. Vertical cracks in the bark above injection points were recently noted in a very small number of trees. Most trees recovered from these initial phytotoxicity symptoms, except in a few cases. A small number of injected trees have died at the Huia and Whatipu sites; in all cases these trees were showing advanced signs of infection at the start of the trial, and most had been injected with the high rate of phosphite (20%). It is possible that some trees in an advanced state of decline were unable to tolerate the phytotoxic effect caused by the high rate of phosphite, causing their rapid decline. After the first treatment (January 2012), only a low rate of phosphite (7.5%) was used in the trials.

Assessments of tree health and growth, plus PTA-lesion activity and spread, have been made at all four sites at 6-monthly intervals since the first treatment application. At all four sites, PTA-lesion activity has been substantially greater in untreated control trees than in phosphite-injected trees. Very few lesions remain active in injected trees, regardless of the treatment regime. Expansion of lesions was negligible in trees of all phosphite-injected treatments, with cracking around lesion margins and peeling off of diseased bark, with healthy bark apparent beneath peeled bark in most treated trees. In contrast, in untreated control trees (particularly at the Raetea site) there has been aggressive advancing of lesions, with a large proportion remaining active and expanding.

Results to date indicate that phosphite has good potential as a treatment for kauri dieback. Phosphite injection suppresses lesion expansion and facilitates healing. There are considerable differences between phosphite-treated and untreated controls trees with respect to lesion activity and growth, and it is anticipated that as time goes on the tree mortality will increase dramatically in untreated trees. It is not yet known whether treated trees will recover to full health or merely maintain their current health status.

There are still many issues to resolve, including optimum phosphite rates and associated phytotoxicity, longevity of treatment effects, dose rates for large trees, other phosphite formulations or application techniques, treatment timing and use of phosphite as a PTA containment tool. It is recommended that monitoring and perhaps further treatment of trees in the current trials continue, in conjunction with a series of new trials to build on results to date and address many unanswered questions.

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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). Treatment with phosphorous acid (PA, phosphite) is one of the few potential options for treating infected or threatened trees. 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).

The current project aims to determine whether phosphite treatment can protect kauri trees in the forest environment from infection by PTA, reduce the rate of symptom development in infected trees and/or improve the health of trees in advanced stages of infection. Forest trials were established in early 2012, with the expectation that they would take about 5 years to complete. That time period was considered necessary to achieve good discrimination of symptom development between treated and untreated trees, and to determine the longer-term robustness of any treatment effects. This report summarises progress 2½ years after the forest trial was initiated. Parts of this work have been summarised in previous reports (Horner & Hough 2012, 2013, 2014a) which are available on the Kauri Dieback website (http://www.kauridieback.co.nz/publications).

2 Phosphite rate trials

Before starting the main forest trials, it was necessary to determine the phosphite dose rates that were safe to use on forest kauri. There were no serious signs of phytotoxicity in glasshouse trials (Horner & Hough 2011b); there were leaf scorch symptoms in branches within a few centimetres of the injection point in kauri seedlings injected with 150 ml/L (=15%) phosphite, but otherwise leaves remained healthy. From these seedling trials, kauri was considered reasonably tolerant to phosphite treatment.

A range of rates have been used for trunk injection of phosphite into trees of various species suffering from *Phytophthora* infection. Tree canopy diameter is often used for calculation of the volume of chemical that should be injected. For example, the label instruction for injection Agrifos600® into avocado in New Zealand is 10 ml of 15% phosphite per 1 m canopy diameter. But such calculations are difficult with kauri, especially in a forest situation. For the current trials, trunk circumference was used to determine rates applied to individual trees.

2.1 Waipoua rate trial

Healthy kauri ricker trees growing beneath a power line in Waipoua forest, destined to be felled within the next few years, were selected for the rate trial in November 2011. Rates of one 20-ml injection of 15% phosphite (=250 ml/L Agrifos600®) per 20 cm trunk circumference were used as a standard treatment. To determine the effects of extreme rates of phosphite, 20 ml of undiluted Agrifos600 (60% phosphite solution)/20-cm trunk circumference was applied to other trees. In checks of these trees 2–3 weeks after treatment, no obvious symptoms were reported. On this basis, the initial treatment application in the Huia and Whatipu trial sites proceeded, using 20 ml of 20% phosphite per 20 cm trunk circumference (see below). However, subsequent checks of the Waipoua trees in late January 2012, 12 weeks after treatment, revealed a number of symptoms ranging from yellowing or browning of leaves to loss of many leaves and even some branchlets (Figure 1). With the branchlet loss, the normal abscission that occurs with kauri branches appeared to occur prematurely and rapidly in response to treatment with phosphite. Further checks of the Waipoua trees in June 2012, 7 months after treatment, showed that many of the leaves and branchlets that were brown in late January had abscised and dropped. The abscission zones appeared normal (Figure 1). The remaining canopy on these trees appeared healthy and green. Further checks of these trees were made in January and June 2013 and June 2014. All trees showed signs of new growth over the 2012/13 and 2013/14 seasons, indicating that the trees were able to survive and ultimately grow out of the initial toxic shock of extreme doses of phosphite (Figure 2). Apart from the initial response of leaf and twig loss, there is no sign of long-term adverse effect from the phosphite treatment.

2.2 Huia rate trial

Following the observation of phytotoxicity symptoms in the Waipoua trees in late January 2012, a second rate trial was carried out at the Huia site, adjacent to the main trial area (see below). Trees were treated with a range of rates, as follows:

A. 15% phosphite, 20 ml per 20 cm trunk circumference

B. 10% phosphite, 20 ml per 20 cm trunk circumference

C. 15% phosphite, 20 ml per 30 cm trunk circumference

D. 20% phosphite, 20 ml per 40 cm trunk circumference

E. 7.5% phosphite, 20 ml per 20 cm trunk circumference

F. Untreated control.

Each treatment was applied to two trees. Photographs were taken of the tree canopies at the time of treatment application, and these were used as references for determining changes in canopy health in assessments made 6, 8, 12 and 20 weeks after treatment, and 6-monthly thereafter for a further 2½ years. Leaf yellowing, browning and leaf drop were observed in many of the treatments (Table 1, Figure 3), although symptom severity was not as severe as those seen in the Waipoua trial. Even the lowest concentration of phosphite applied (7.5%), one of the two trees showed slight yellowing and minor leaf drop, although the trees remained healthy.

By June 2013, 17 months after the initial treatment, and in all subsequent assessments, tree canopies looked green and healthy, regardless of the application rate (Figure 3). This suggests that although trees were initially adversely affected by the high rates, they recovered and continued to grow normally.

2.3 Selected rates

The Waipoua and Huia rate trials demonstrated that if high rates of phosphite are injected into kauri trees, severe phytotoxicity symptoms may result. Symptoms include leaf yellowing, browning and abscission, and in some cases premature twig or lower branch abscission. But all the trees in these rate trials appeared to recover, and within 2 years canopies were green and in most cases showing signs of new growth. It must be noted that these rate trials were carried out on healthy trees. Responses to phosphite may differ on trees with severe symptoms of kauri dieback, as was observed in the main phosphite trials discussed below.

As a result of the Waipoua and Huia rate trials, the lower rate of 7.5% phosphite was selected as the standard for the Northland sites and the remaining treatments of the Huia site and Whatipu sites (see below). This low rate was selected as precaution against causing severe phytotoxicity in trees. Rates will be re-evaluated as more information and experience with injecting kauri is gained.

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. After 2 ½ years, all trees appeared green and healthy.

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. Premature leaf browning and abscission of kauri branchlets following trunk injection with a high concentration (60%) of phosphite at the Waipoua trial site, Northland. Similar symptoms also occurred with 15% and 20% solutions of phosphite.

Figure 2. Kauri trees before & after trunk injection with a high concentration of phosphite at the Waipoua trial site, Northland. In November 2011, the kauri on the right was injected with 60% phosphite solution; the tree on the left was left untreated. In the lower photograph, 7 months after the injection, note the slightly thinner, but otherwise healthy canopy in the treated tree on the right.

Figure 3. 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

After consultation with the Department of Conservation (DoC), Regional Councils, and Manawhenua iwi, four sites were selected for the main trials testing phosphite efficacy. Trial sites are:

1. Huia dam, Waitakere ranges, Auckland
2. Whatipu, Waitakere ranges, Auckland
3. Raetea Forest, Mangamuka ranges, Northland
4. Omahuta Forest, Mangamuka ranges, Northland.

Both the Auckland sites are in naturally regenerating forest, in stands of kauri rickers (Figure 4). Both Northland sites are in plantation kauri, planted by the New Zealand Forest Service in the 1950s and 1960s (Figure 4). All four sites had confirmed diagnoses of PTA in soil or trees.

The trials were established January to March 2012. 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 52, 53, 42 and 15 trees in the trial at the Huia, Whatipu, Raetea and Omahuta sites respectively, a total of 162 trees. Most trees were at the ricker or advanced ricker stage, with girths ranging from 40 to 120 cm.

Before treatments were assigned to trees, all potential trial trees were mapped, measured (girth), and canopy symptoms were scored on a 1–5 scale (where 1 is healthy and 5 is dead). Canopy photographs were taken for future reference, and the trunk base was photographed at cardinal points. 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. Lesion activity (i.e. whether they appeared fresh and active or dried and inactive) was noted. Once sufficient trial trees were identified, they were assigned into groups at each site, based on similarity of symptoms. This exercise was done away from the forest, using only the information previously collected for each tree. Trees within each group of ‘similar’ trees were then randomly assigned treatments, such that there was an even number of trees from each treatment in each disease severity group. This ensured that across each site, the ‘average’ disease severity of trees in each treatment at the start of the trial was similar.

Trees were injected with either a high (20% a.i., Waitakere sites only) or low (7.5% a.i., all sites) phosphite concentration (Agrifos600®) (Figure 5) using spring-loaded Chemjet® tree injectors (Figure 6). 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. 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 at 20 cm intervals around the trunk circumference, injected into the trunk 0.4 to 0.8 m above the ground. Following removal of the injector, the drilled hole was filled with silicon sealant. All five treatments were applied at Huia and Whatipu. Only treatments 3, 4 and 5 were applied at Omahuta and Raetea. There have been no further applications of phosphite since January 2013.

In January and June 2013, and January and June 2014, tree canopy health and vigour were compared with those in the photographs taken at the start of the trial. The scoring system, based on the original photographs and comparing canopy density was: -2 = substantially worse, -1 = slightly worse, 0 = similar, 1 = slightly better, 2 = substantially better. The subjective ratings of ‘substantially’ or ‘slightly’ different were based on whether the difference was immediately obvious (substantial) or whether the original photograph and current canopy had to be examined carefully, making comparisons down to the individual twig or leaf level (slight). Photograph comparison data were analysed using a Kruskal-Wallis non-parametric ANOVA. Binoculars were used to check for new shoot growth. At each assessment time, the dimensions of lesions at the base of the trunk were re-measured. 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).

Figure 4. Trial sites for testing phosphite for control of *Phytophthora* taxon Agathis in kauri. Top left, Huia dam site, Auckland; top right, Whatipu site, Auckland; bottom left, Raetea site, Northland; bottom right, Omahuta site, Northland.

Figure 5. Trunk injection of a kauri tree with phosphite at the Huia trial site, Auckland.

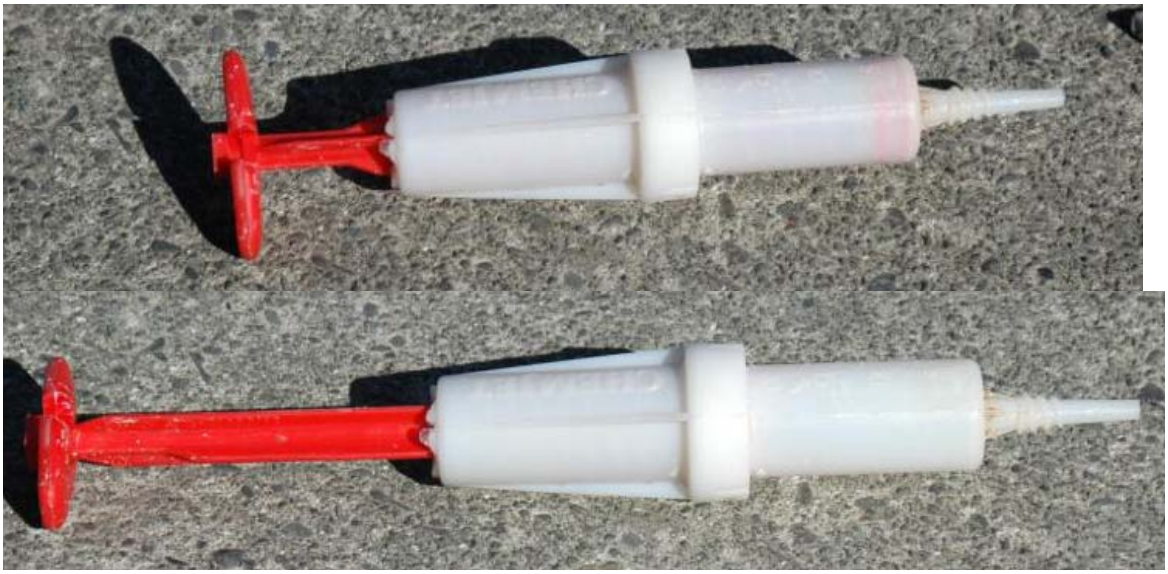

Figure 6. Chemjet® trunk injector used for injection of phosphite into kauri trees.

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 often difficult to make as the growth of the understory, in particular tree ferns, blocked many of the clearings from which earlier photographs of the canopy had been taken. Nevertheless, in most instances a portion of the canopy could be observed and a judgement made.

In June 2014, the yellowing and leaf loss that was noted in some treated trees soon after the initial treatments were applied was not as obvious as in previous assessments, although treated canopies (particularly with the high PA rate) were on average slightly thinner than at the start of the trial. Canopies that were recorded as yellowed in earlier assessments were generally green in the June 2014 assessments, with no obvious signs of the phytotoxicity noted previously.

Changes in the tree canopies were by then becoming apparent. However, trends in canopy data varied at the four sites, perhaps in response to different factors (Figure 7). At Huia, on average there was a slight decline in canopy density in all treatments, perhaps reflecting phytotoxicity in injected trees, plus some tree deaths. There was a similar trend at Whatipu, with the most substantial decline occurring in trees treated with either the high concentration of phosphite, or receiving two applications of the low concentration.

At Omahuta, canopy density had changed little in both the untreated trees or in those injected just once, but had improved in trees injected twice with the low phosphite concentration, reflected in new leaf growth in many trees. At Raetea, injected trees were, on average, of similar canopy density to that prior to treatment. However, canopy density/health of untreated control trees had declined significantly (P = 0.002).

In a small number of trees at the Huia and Whatipu site, vertical cracks in the bark were noted in some treated trees (Figure 8). These appeared to be related to the injection points, and may be a further symptom of phytotoxicity. This symptom was noticed for the first time in June 2014, and will be systematically recorded in future assessments.

3.2.2 Tree mortality

At the Huia and Whatipu sites, there have been no deaths among the 22 untreated control trees, although some have declined noticeably in health. In contrast, 10 trees out of a total of 84 previously treated with phosphite at Huia or Whatipu had died. Eight of these dead trees had been treated with the high concentration and two with the low concentration of phosphite. In all cases, the trees that died had shown advanced symptoms of kauri dieback (severe canopy loss) at the time the first treatments were applied, and all but one were below the average size (tree girth) for their respective sites. It is considered likely that these very unhealthy trees with limited canopies were unable to survive the phytotoxic effects of the phosphite treatment, and their decline was accelerated. It is also possible that the dose calculations for smaller trees are too high.

At the Omahuta site, no trial trees have died. At Raetea, none of the 28 phosphite-treated trees have died, but four of the 14 untreated control trees have died.

It is still early in the trial programme and dramatic changes in canopy health and tree survival are unlikely in the short term. It is expected that canopy health assessments and tree survival 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 improvement in others.

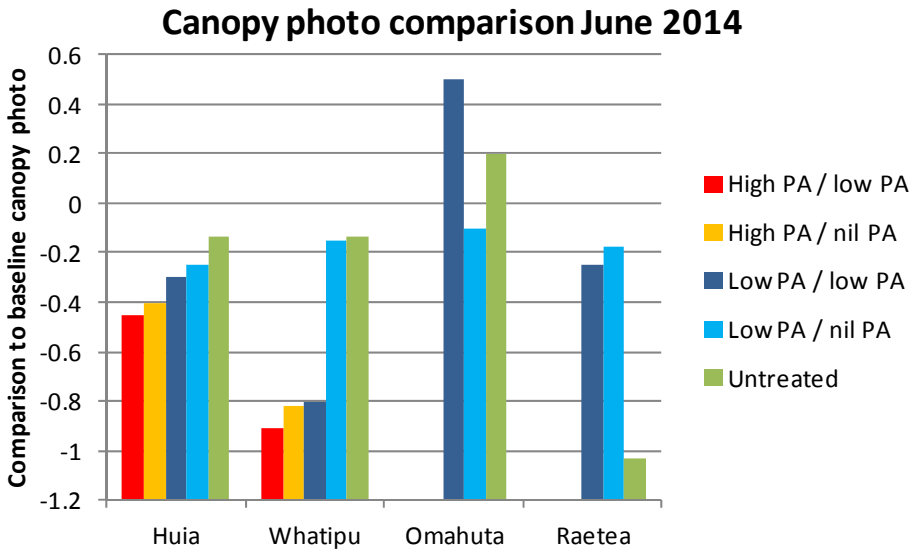

Figure 7. Comparisons of kauri tree canopy health and density in June 2014 with photographs taken before the first treatment application in February 2012. Data are means for each treatment at each of four forest sites. The scoring system, based on the original photographs and comparing canopy density was: -2 = substantially worse, -1 = slightly worse, 0 = similar, 1 = slightly better, 2 = substantially better. Trees were treated either once or twice with high (20%) or low (7.5%) concentrations of phosphite (PA), or left untreated.

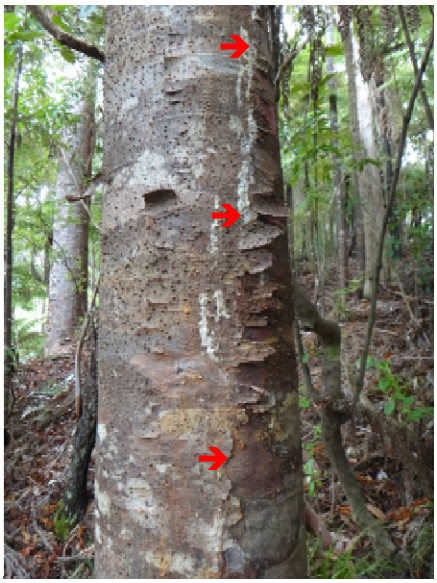

Figure 8. Vertical cracking on bark (arrows), noted on a small number of kauri trees 2 years after injection with phosphite.

3.2.3 Lesion activity

Lesion activity, as indicated by freshly oozing sap at lesion margins, is summarised in Figure 9 and Table 2. Very few lesions on phosphite-treated trees were deemed ‘active’ in June 2014, compared with many active lesions in untreated control trees. On three sites (Huia, Whatipu and Raetea) none of the monitored lesions in phosphite-treated trees showed any signs of recent activity. In contrast, on the same sites 30–50% of monitored lesions in untreated control trees were active. At Omahuta, a single lesion on one tree in each of the phosphite-injected treatments was active in June 2014. Neither of these lesions had been recorded as active in the previous (January 2014) assessment.

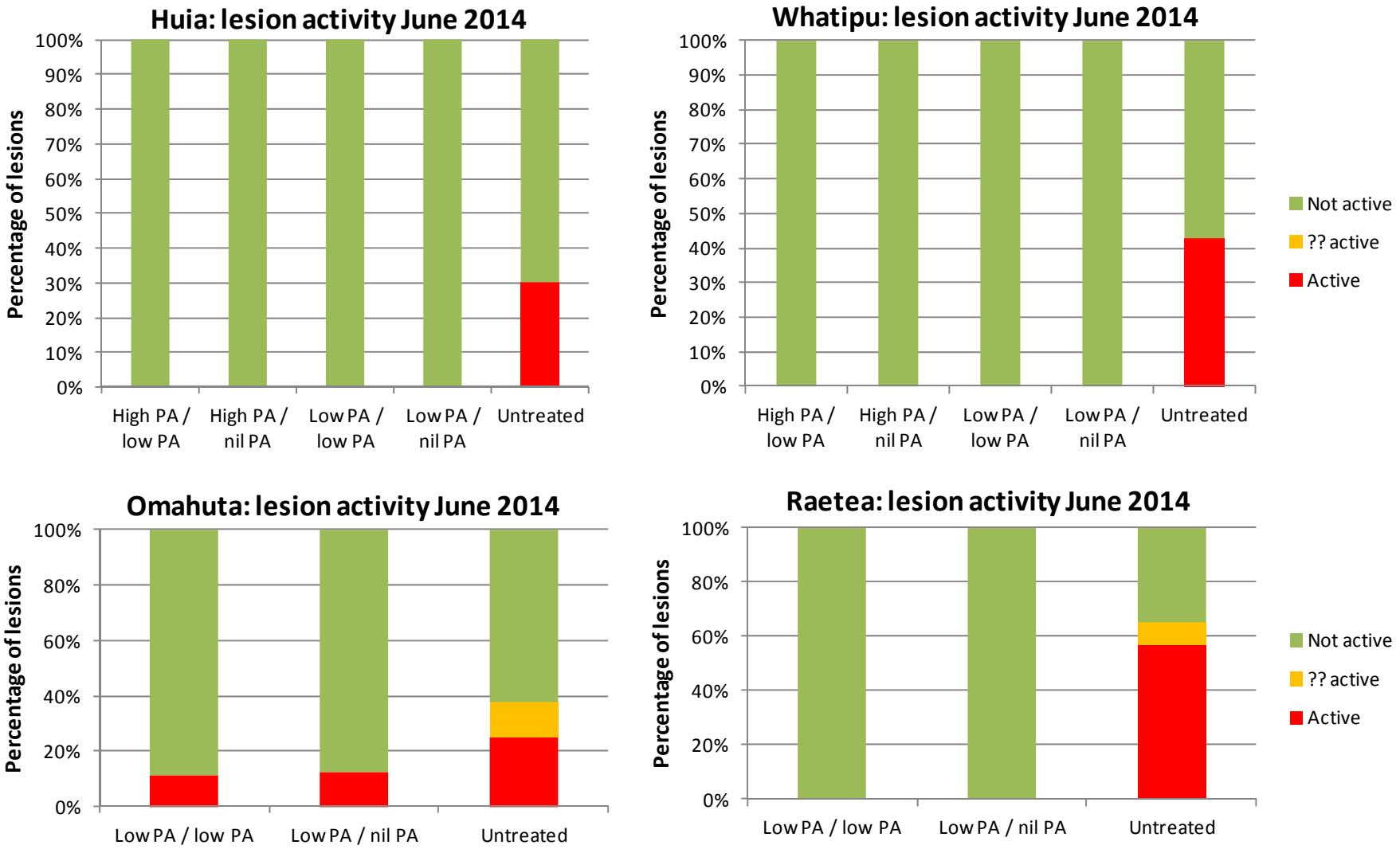

Figure 9. Activity of monitored lower-trunk lesions on *Phytophthora* taxon Agathis-infected kauri trees at four sites, where trees were treated with high (20%) or low (7.5%) concentrations of phosphite (PA), or left untreated. X-axis labels refer to treatments applied in January 2012 / January 2013. Assessments were made in June 2014, 2½ years after initial treatment application.

Table 2. Assessment of lower trunk lesion activity and advance on *Phytophthora* taxon Agathis-infected kauri trees in June 2014, following treatment with phosphite (PA) at high (20%) or low (7.5%) concentrations. Treatments were applied in January 2012 and January 2013. All lesions visible above ground were monitored; a subset of these had lesion margins marked at the time of treatment application.

			Monitored lesions					Marked and measured lesions					
Site	Treatment (Jan'12/Jan'13)	No. of trees	No. of monitored lesions	No. of lesions active	No. of lesions possibly active	No. of lesions NOT active	% active	No. of marked lesions	No. of lesions active	No. of lesions possibly active	No. of lesions NOT active	% active	Ave. Lesion advance (mm)
HUIA	Untreated	11	10	3	0	7	30%	8	2	1	5	25%	82.9
	High PA/Low PA	10	9	0	0	9	0%	4	0	0	4	0%	4.2
	HighPA/ nil	10	11	0	0	11	0%	5	0	0	5	0%	3.8
	Low PA/Low PA	11	8	0	0	8	0%	6	0	0	6	0%	2.5
	Low PA/nil	10	10	0	0	10	0%	8	0	0	8	0%	1.7
WHATIPU	Untreated	11	7	3	0	4	43%	3	0	1	2	0%	31.3
	High PA/Low PA	11	6	0	0	6	0%	3	0	1	2	0%	7.5
	HighPA/ nil	11	5	0	0	5	0%	1	0	0	1	0%	0
	Low PA/Low PA	10	6	0	0	6	0%	3	0	0	3	0%	7.5
	Low PA/nil	10	4	0	0	4	0%	5	0	0	5	0%	0
OMAHUTA	Untreated	5	8	2	1	5	25%	8	1	1	6	12%	23.0
	Low PA/Low PA	5	9	1	0	8	11%	9	0	0	9	0%	4.6
	Low PA/nil	5	8	1	0	7	12%	6	0	2	4	0%	3.3
RAETEA	Untreated	14	37	21	3	13	57%	17	4	0	13	24%	178.8
	Low PA/Low PA	14	31	0	0	31	0%	23	0	0	23	0%	11.7
	Low PA/nil	14	26	0	0	26	0%	20	0	0	20	0%	5.6

3.2.4 Lesion expansion

At all four sites, lesion expansion has been greatest in untreated control trees, where many lesions have remained active and advancing (Figures 10 and 11, Table 2). In comparison, in trees injected with phosphite very few lesions remained active, lesion expansion since the start of the trial has been negligible, and there were signs of cracking and healing around the margins of almost all lesions (Figure 12). In many cases, lesion expansion in treated trees was recorded as 'negative', as the outer diseased bark initially marked had sloughed off, with healthy bark beneath (Figure 12). In all such cases, the 'negative' lesion advances were assigned a 'zero' value for data analyses of lesion expansion. Cracking and healing of lesions was also noted occasionally on some untreated trees, but this was often overtaken by further waves of PTA advance (Figure 12), something rarely seen in phosphite-treated trees.

There are no obvious or consistent differences in lesion activity and expansion between the various phosphite regimes. All of the test treatments have to date proven to be effective.

Lesion advance in untreated trees was greater at Raetea than at the other sites, with some particularly aggressive lesions observed. While reasons for this difference are beyond the scope of this project, possible explanations include the 'softer' fast-growing trees in this plantation environment, or perhaps a particularly susceptible seed line.

Observations of lesions activity and expansion suggest that there may be reduction in activity and expansion of lesions over summer, with re-activation in winter months. This may reflect drying out of bark over summer, of perhaps stronger resistance to canker spread when trees are more physiologically active. However, more observations over a number of seasons will be required to confirm this observation.

It has also been noted that lesions appear to stop growing when the tree dies. The clear margin does not appear to advance, although no assessment of PTA spread within the dead bark tissues has been made. This could ultimately affect analyses of lesion expansion in the trial as more trees die, but to date this has had minimal impact on results.

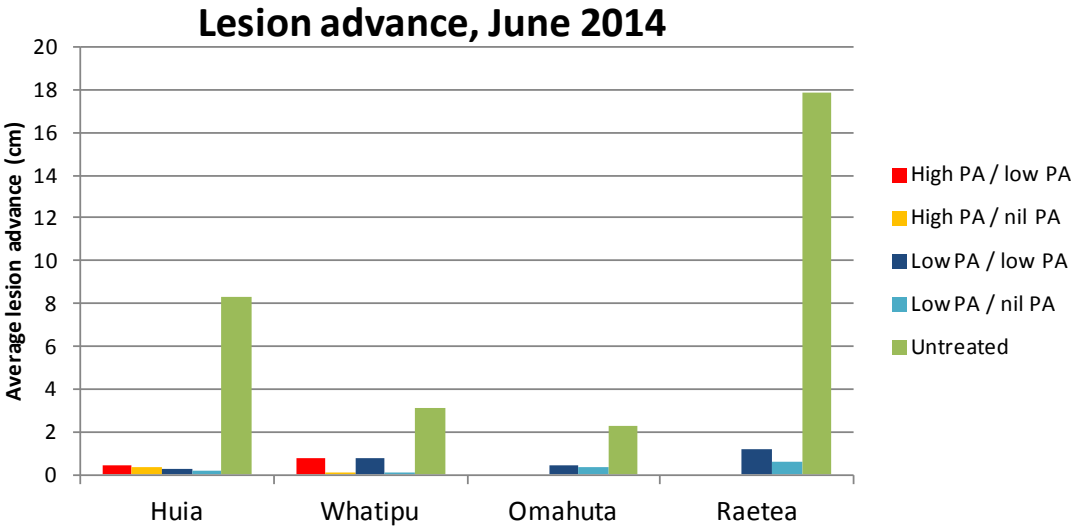

Figure 10. Mean advance of lower-trunk lesion margins marked at the time of treatment application and re-measured 2½ years later. Kauri trees on four sites were treated with high (20%) or low (7.5%) concentrations of phosphite (PA), or left untreated. Legend labels refer to phosphite (PA) treatments applied in January 2012 / January 2013. The high PA treatments were not applied at the Omahuta and Raetea sites.

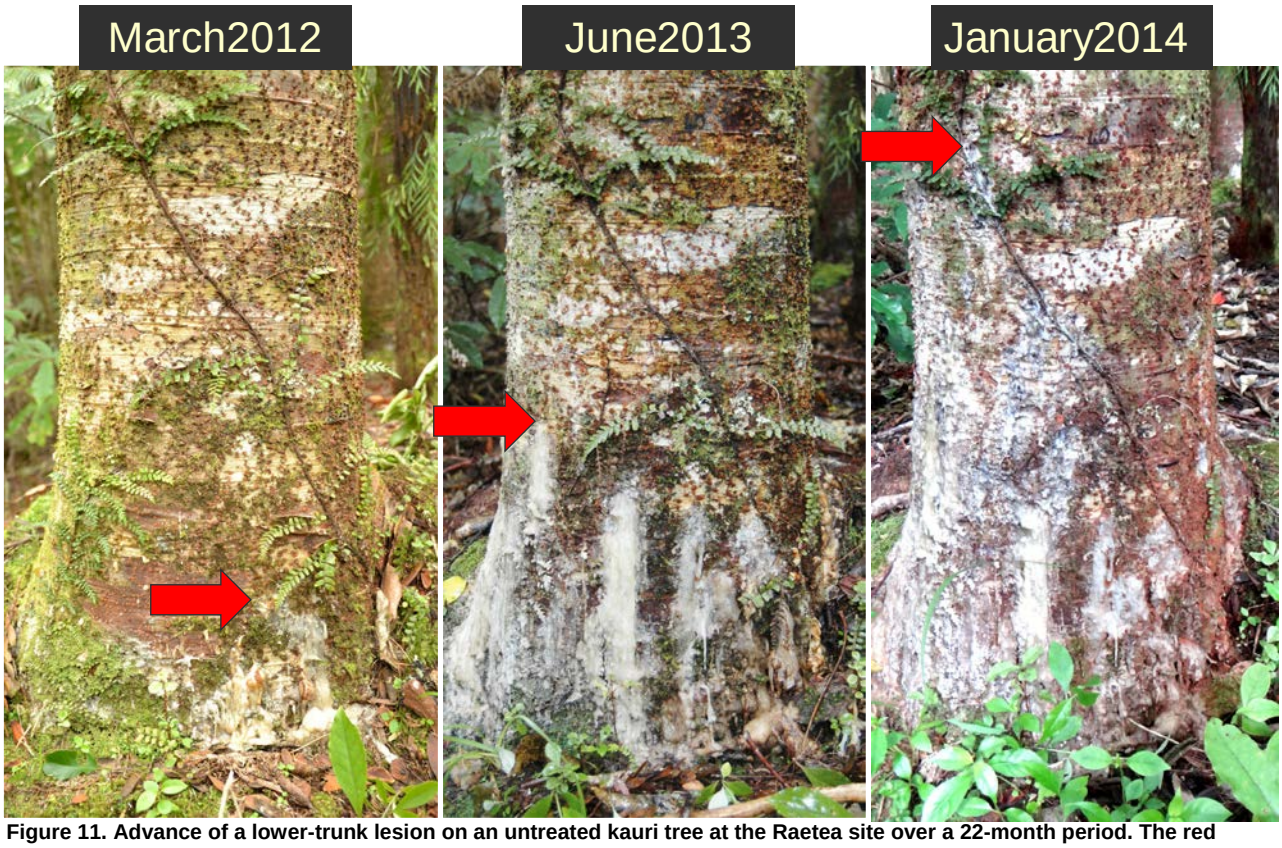

Figure 11. Advance of a lower-trunk lesion on an untreated kauri tree at the Raetea site over a 22-month period. The red arrow indicates the uppermost boundary of the lesion.

Figure 12. Cracking and peeling of bark around margins of *Phytophthora* taxon Agathis-lesions on kauri trunks. Top and bottom left: phosphite-treated trees, showing bark peeling around lesions, with healthy bark beneath. Bottom right: untreated tree, with bark peeling, but active PTA lesion on the new bark beneath.

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. The first round of samples was collected in early May 2013, 14 months after the first phosphite treatment application and 3 months after the second application. The second round of samples was collected in January 2014, approximately 2 years after the first treatment application and 1 year after the second application. All samples were collected from branches within three metres of the ground. 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.

In 2013, samples were transported to Hawke's Bay and stored in the refrigerator for 1 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 2014 samples were inoculated 24 h after collection.

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 (2013 samples only). On each segment, a 3 x 3 mm section of bark was removed using a sharp scalpel one third of the way from the base. 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 24 days (2013) or 18 days (2014), twigs were removed, cut into pieces 1–2 cm long, 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. Leaves were inoculated (2014 only) by placing an PTA-colonised agar plug the leaf surface, incubating in a humid chamber at 15–20°C for 10 days, then measuring lesion spread. Data were analysed by ANOVA.

4.2 Results

In the 2013 samples, 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 was a slight positive association between shoot age and the rate of spread of PTA on inoculated shoots. However, this effect was small in comparison with the shoot-to-shoot and tree-to-tree variation, and had a minimal effect on interpretation of treatment effects. Therefore, data from different aged twigs were pooled for analyses of phosphite treatment effects. Twig age was disregarded as a factor in the 2014 sampling.

Data for PTA spread within the twigs are presented in Table 3. In 2013, for both 'up' and 'down' assessments, spread from the inoculation point was similar in twigs from untreated trial trees and from adjacent untreated trees. In comparison, PTA growth was less on twigs from trees that

had received two applications of phosphite. PTA spread on twigs from trees treated with a single phosphite application was roughly midway between that on trees treated twice and untreated controls. However, because of the wide variation between shoots and trees, these differences were not statistically significant. Similarly, in 2014 PTA growth was, on average, less in shoots from phosphite-injected trees than on untreated controls. But once again, wide variation in the data meant that the differences were not statistically significant.

The growth rate of PTA on the field-collected kauri shoots (~2 mm/day) and leaves (~1 mm/day) in this trial was less than the 3 mm/day growth observed in a similar assay on glasshouse-grown kauri shoots and leaves (Horner & Hough 2014b). This probably reflects the 'toughness' of the field versus glasshouse-grown material.

Although the trends in lesion growth on excised shoots are what might be expected, with reduced PTA growth in twigs from phosphite-treated trees, the magnitude of the difference is small relative to background variability, and such assays may have limited usefulness. An assay on twigs on the living tree may be more discriminating, but could be problematic when working with an 'unwanted organism' such as PTA.

Table 3. Mean growth of *Phytophthora* taxon Agathis (PTA) on twigs excised from kauri trees in May 2013 and January 2014, then 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.

		May 2013			January 2014			
Treatment	No. trees sampled	Total no. of shoot segments inoculated	Ave. PTA spread DOWN (mm/day)	Ave lesion spread UP (mm/day)	Total no. of shoot segments inoculated	Ave shoot lesion spread up (mm/day)	Total no. of leaves inoculated	Ave. leaf lesion spread (mm/day)
7.5% PA Mar. 2012 & 7.5% PA Jan. 2013	4	46	0.99	1.62	30	1.15	48	1.03
7.5% PA Mar. 2012 & untreated Jan. 2013	4	46	1.12	1.79	19	1.14	37	0.92
Untreated	3	22	1.25	2.14	17	1.67	29	1.10
Untreated (adjacent)	2	30	1.19	1.95	15	1.22	28	1.00
P value			*0.278*	*0.107*		*0.456*		*0.495*

5 Forest trial overview

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. Almost all lesions on phosphite treated trees at all four trial sites have stopped expanding and appear to have healed. Whether the treatment is sufficient to save trees already infected and ultimately restore them to good health will become more apparent in assessments over the next few years. The longevity of treatment efficacy and the required frequency of treatment for long-term control are also yet to be determined.

The potential phytotoxicity of phosphite remains a concern, and this will have to be monitored carefully so that rates can be optimised. While healthy trees seem capable of recovering from the toxic effects of high doses of phosphite, there is evidence that severely diseased trees are less tolerant and that phosphite injection may accelerate their demise. There is also the symptom of bark cracking above injection points in some treated trees. This has only recently been noted in a small number of trees and will need to be carefully monitored in the future. However, phosphite treatment of diseased trees in most cases reduced the spread of PTA and has the potential to either protect trees from PTA or restore them to health. Therefore the relatively low risk of enhanced decline of severely diseased trees must be balanced against the improvement in health of the majority. The alternative is, almost inevitably, the death of most trees in PTA-infected stands if no treatment is made.

6 Future plans and unanswered questions

Trials with phosphite have, to date, given very encouraging results and demonstrated the potential of this treatment for management of PTA-infected kauri. But there are many questions that now need to be addressed, building on the results and observations to date.

The trials to date have only been on trees of the 'ricker' stage. Determination of dose rates and treatment regimes for larger trees is important, as linear extrapolation from small trees will not necessarily provide effective yet safe dose rates. There could also be a need to use differential dose rates relating to canopy density rather than just tree girth. Thus trees with significantly reduced canopy, as a result of dieback or other cause, may require lower doses than would otherwise be calculated by trunk girth alone. There is also potential for investigating lower dose rates of phosphite to see if they are effective, or to alternate treatments by applying half the dose in consecutive years, rather than a full dose in a single year. This may help minimise potential phytotoxic effects. There is also little known about optimal timing of injection, e.g. season, time of day, or even weather conditions. Is it OK to inject in the rain? Different formulations of phosphite, or perhaps trunk paints or sprays could also be investigated as alternative application methods. There is also a big question around potential use of phosphite as a PTA containment tool or protective treatment, i.e. using it to minimise spread of PTA infection foci, rather than solely as a curative treatment for already-diseased trees. Related to this could be treatment of species other than kauri, which could potentially be harbouring or propagating the pathogen without overtly showing disease symptoms.

Some of these questions will be answered by continuation of the current trials in the four ricker sites. In these sites, tree health measurements should continue on a 6-monthly basis during mid-summer and mid-winter for at least another 2 years. The next assessment would be in January 2015. At each assessment, the canopy health should be re-scored, and notes taken on whether canopy health appeared better, worse, or the same as in the original photographs. Lesions should be re-measured, with notes taken on lesion activity. Where margins have been marked, any advances should be measured. Any long-term signs of phytotoxicity should be carefully assessed. Continuation of these trials will give some insight into whether phosphite treatment has a long-term effect of improving health of trees already showing symptoms of PTA, ultimately allowing full recovery. There is also potential for determining longevity of treatment effects and requirements for repeat phosphite applications. The last treatment application was in January 2013. There is room for flexibility in future treatment applications within this trial. Any decisions regarding future treatment of the current trial trees should be made in conjunction with planning of new trials looking at various aspects of phosphite treatment.

New trials, either on additional trees in the current sites or on new sites, will be required to answer many of the questions above, and decisions regarding prioritising these should be made in conjunction with the Kauri Dieback Planning and Intelligence team and other interested parties.

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