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Phosphite toxicity and impact: an investigation of seasonal variability in injection uptake

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CONTENTS

EXECUTIVE SUMMARY

Phosphite toxicity and impact: an investigation of seasonal variability in injection uptake

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In recent years, phosphite trunk injections have been trialled to determine efficacy for treating kauri dieback. However, nothing is known about the optimal time for injecting, and whether factors such as season, time of day or weather conditions have any effect on uptake. The current trials, initially using water injections, aim to determine whether timing or weather factors affect injection uptake time.

At the Huia Dam (Auckland) trial site, 20 kauri ricker trees (10 healthy and 10 showing kauri dieback symptoms) were selected for the trial. At about midday on fine days in spring, summer, autumn and winter, each tree was injected with 20 mL of water using a Chemjet® spring-loaded injector. The time for the syringe to empty (uptake time) was recorded. The uptake time for the spring injection was significantly slower than for the other times of the year, although average uptake time was still only about 5 minutes.

In February (summer), injections were also carried out in the early morning and late afternoon, plus on a rainy day for comparison. Neither time of day nor injection in wet or dry conditions had any significant effect on uptake time.

Uptake was not significantly related to either tree health or tree girth, and which side of the tree was injected had no effect.

From this trial, there is evidence that there will be reasonable uptake from trunk injections regardless of season, time of day or weather conditions at the time of injecting.

To determine whether seasonal or other timing factors influence the efficacy of phosphite treatment, or the incidence/expression of phosphite toxicity symptoms, trials would need to be carried out with phosphite injections in four seasons. Subsequent observations of phytotoxicity symptoms and lesion healing will help to determine whether there are particularly beneficial or risky times to inject, in terms of phytotoxicity expression or treatment efficacy. Useful data relevant to this could soon be available within the Kauri Rescue™ programme.

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1 INTRODUCTION

Over the past six years phosphite trunk injections have been trialled to determine efficacy for treating kauri dieback, caused by *Phytophthora agathidicida* (formerly known as *Phytophthora* taxon Agathis or PTA), but nothing is known about the optimal time for injecting, and whether factors such as season, time of day or weather conditions have any effect on uptake. The current trials, initially using water injections, aim to determine whether timing or weather factors affect injection uptake time.

In established forest trials investigating phosphite injection for control of kauri dieback, suppression of *Phytophthora* lesions appears very good, but phytotoxicity symptoms were noted in some trees. These symptoms included leaf yellowing or browning, leaf drop, twig drop and occasionally trunk cracking or bleeding. The factors associated with phytotoxicity symptoms and reasons why they are more severe on some trees than others are yet to be determined. It is possible that seasonal or other timing factors, or prevailing weather conditions at the time of injection may influence phytotoxicity symptom development. Based on results from the current trials with water, phosphite injections could then be applied to determine whether seasonal or other factors influence phosphite efficacy or expression of phytotoxicity symptoms.

This report extends the report of July 2017 (PFR report #15015), with the inclusion of the final trunk health assessments made in October 2018.

2 METHODS

In November 2015 a trial was established at the Huia Dam site in the Waitakere Ranges, adjacent to the existing long-term phosphite forest trial. Two groups of 10 trees were selected. The first group ('good health site') was of symptomless trees (all scoring '1' on a 1–5 tree canopy health scoring scale, where 1 was healthy and 5 was dead), approximately 200 m upslope from the existing Huia trial. The second group of trees ('poor health site') was a few metres across the slope from the existing trial and trees were showing moderate symptoms of kauri dieback (seven out of 10 of them scoring a '3' on a 1–5 canopy scoring scale). All but one tree had a trunk diameter between 18 and 37 cm. The exception measured 61 cm diameter.

Canopy volume was assessed on a relatively subjective 1–4 scale, where the volume was judged on the density and size of the canopy in relation to the trunk dimension. A '4' was a large and full canopy, as expected on a healthy tree. A '1' was a very sparse and thin canopy. This was highly correlated with the canopy health score noted above. All trees on the 'good health site' had canopy density scores of 3 or 4. All trees on the 'poor health site' had canopy density scores of 1, 2 or 3.

At various times over a one-year period, the trunk of each of the 20 trial trees was injected with a single dose of 20 mL water, and uptake time (time to empty the 20-mL injector) was measured to the nearest minute. Chemjet® spring-loaded injectors were used, with injection points 50–80 cm above ground level. The orientation of each injector around the trunk was randomly determined at each injection time (one of 12 possible points, with '12.00' being upslope), but avoiding re-injection of the same point.

Injection dates were 17 November 2015, 15 February 2016, 17 February 2016, 18 April 2016 and 5 August 2016. On 15 February 2016, injections were applied in early morning (between 0700 and 0900 h), around noon and late afternoon (1800 to 1930 h). On all other dates, a single round of injections was made within 1½ hours either side of noon. The 17 February injections were applied during a period of steady rainfall. At all other times injections were in fine weather.

Soil temperature (at 10 cm depth), air temperature (1.5 m above ground), prevailing weather conditions and wind strength (on the Beaufort scale) were recorded at each injection time. Gravimetric soil water content was calculated by oven drying samples taken from the top 10 cm on the trial areas. Relative humidity in the shade at 1.5 m above ground level was recorded throughout the injection period using an EasyLog® USB version 7.4.0.0.

In early September 2016, April 2017, February 2018 and October 2018, all injection points were assessed for the amount of bleeding, and for the hardness of the exudate (i.e. whether it had stopped bleeding). The amount of bleeding was assessed on a 1–5 scale, where 1 = nil or very small dribble, and 5 = an extensive bleed. The hardness was assessed by pressing a fingernail into the bleed, and scoring as 'hard', 'soft', 'sticky' or 'very sticky'.

Analyses of injection time data were carried out using analysis of variance to test for differences in uptake time between various treatment times. Data from the 'poor' and 'good' health sites were analysed both separately and together.

3 RESULTS AND DISCUSSION

Site environmental data recorded at each injection time are summarised in Table 1.

Table 1. Summary of site environmental data recorded at each injection time. Numerical data are the average or range from repeat measurement during each injection period.

Month (2015–16)	Soil temp.	Air temp.	Humidity (% RH)	% Soil water content*	Weather	Wind (Beaufort scale)
November	12.9–13.2	13.3–13.8	83–87	150.5	mostly cloudy, sunny breaks	3-5 gentle-moderate – fresh breeze (variable)
February (morning)	19.4–19.6	19.8–20.5	81–88	-	overcast	0-1-2 calm – light breeze
February (noon)	19.5–19.7	20.5–22.0	76–79	108.8	heavy overcast	2-3. light - gentle breeze
February (afternoon)	19.5–19.7	21.2–22.1	72–76	-	heavy overcast	1-2-3-4 light air to moderate breeze (variable)
February (rain)	19.0–19.8	19.5–19.7	88–93	118.2	steady rain	3-5 gentle-moderate – fresh breeze (variable)
April	16.5–16.8	16.4–17.4	83–85	151.4	partly cloudy/ sunny	4-5 moderate – fresh breeze
August	10.3–12.8	12.0–12.2	80–84	179.2	overcast	3-4 gentle – moderate breeze
November	#	17.5-19	80-88	#	overcast/close to rain	3-5 gentle-moderate – fresh breeze (variable)

* gravimetric soil water content = weight of water/weight of oven-dried soil.

Across the trial, the maximum uptake time for any injection was 10 minutes, regardless of tree health, season, time of day or weather. This differed from previous trials on the same site, where injection times sometimes exceeded 20 minutes.

Season: There was a significant influence of time of year on injection uptake time (Figure 1). Uptake in spring (November) was significantly slower both years than in the other seasons (P<0.01). However, the difference was not large, and uptake time in November was still considered reasonable, with the average being about 5 minutes.

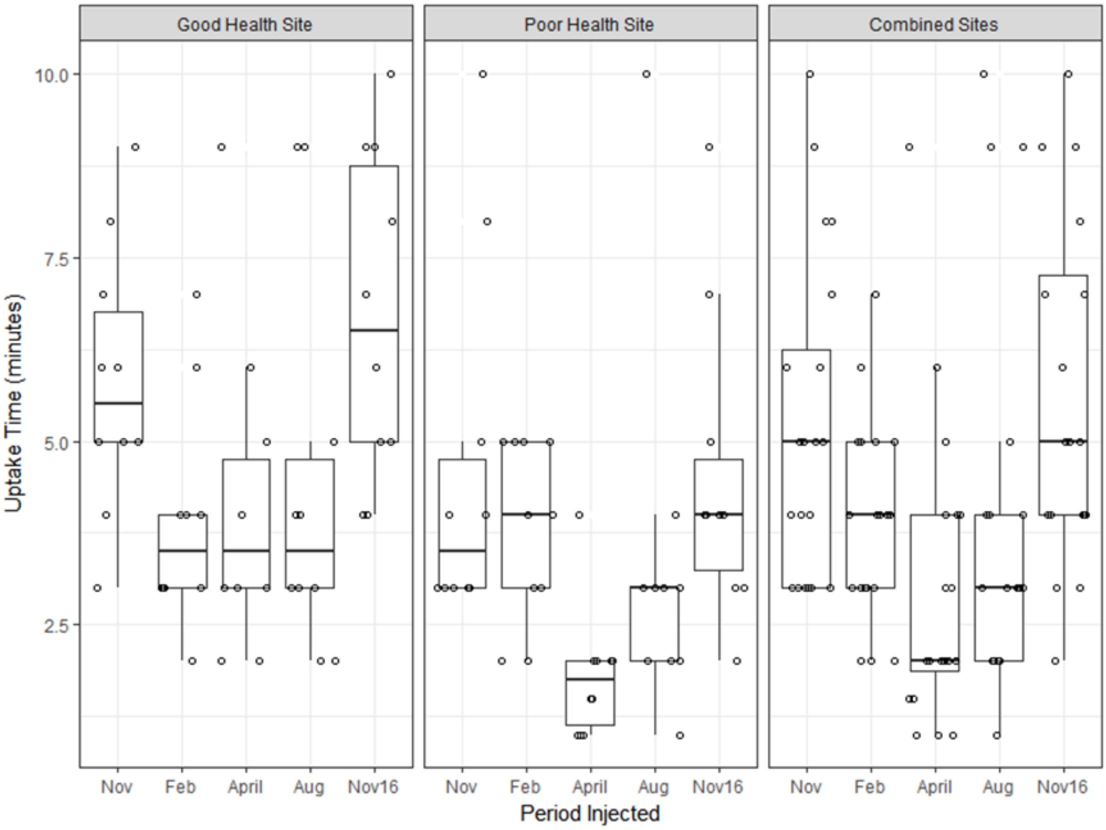

Figure 1. Effect of time of year on uptake time of 20 mL water injected into 10 kauri trees on each of two sites, injected approximately 3-monthly from November 2015 until November 2016. Bold lines within each box indicate median values, boxes indicate the 25% and 75% quartiles, and whiskers indicate points within 1.5 X quartiles. Points indicate raw data values.

Time of Day: There was no significant difference (*P*=0.517) in uptake time between early morning, noon, and late afternoon injections (Figure 2), suggesting that any time of day is suitable for injecting.

Rain versus dry: There was no significant difference (*P*=0.741) in uptake time when injecting in the rain versus dry weather in February (Figure 3), a surprising result given the assumption that uptake is related to the transpiration flow. This result indicates that injecting during wet weather should still be possible.

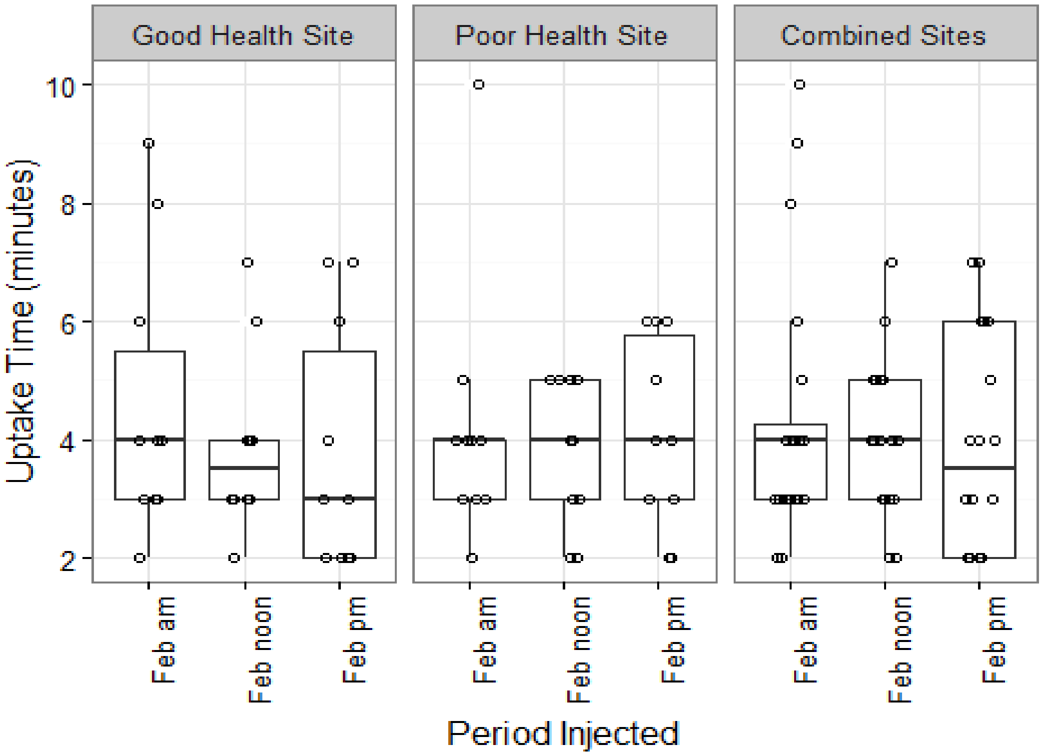

Figure 2. Effect of time of day on uptake time of 20 mL water injected into 10 kauri trees on each of two sites. Bold lines within each box indicate median values, boxes indicate the 25% and 75% quartiles, and whiskers indicate points within 1.5 X quartiles. Points indicate raw data values.

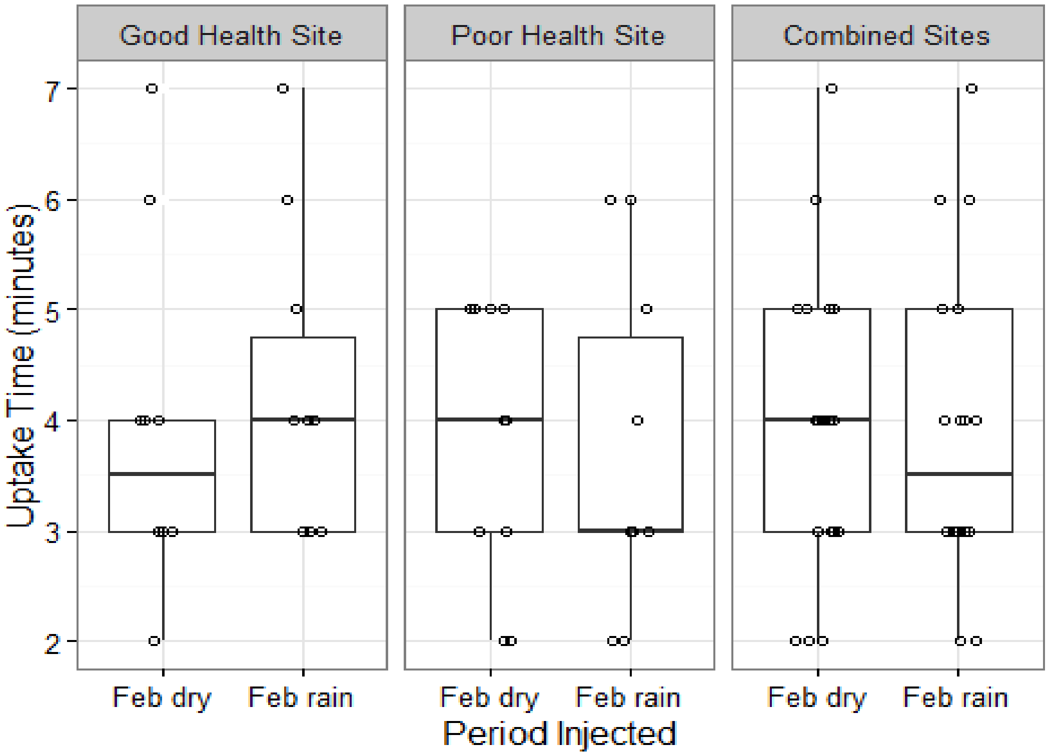

Figure 3. Effect of injecting in dry or wet weather on uptake time of 20 mL water injected into 10 kauri trees on each of two sites. Bold lines within each box indicate median values, boxes indicate the 25% and 75% quartiles, and whiskers indicate points within 1.5 X quartiles. Points indicate raw data values.

Other observations included:

- Uptake time differences between trees were not consistent across different injection times, i.e. a 'slow' tree on one date would not necessarily be a slow tree on another date.
- There was no evidence for a difference in uptake time related to the side of tree injected.
- There was no evidence that canopy health or volume influenced uptake time, although it should be noted that all but one tree in the trial scored between 1 and 3 on the canopy health scoring scale, so only one tree was severely diseased.
- There were no statistically significant differences in uptake time on the good and poor sites.
- There was no evidence that tree girth influenced the uptake time.

Injection point healing: There was no obvious difference in the amount of bleeding from injection points made at different times of the year, time of day, or weather condition (Table 2). When assessed in April 2017, bleeds on all but three out of 140 injections made previously to November 2016 had dried up and hardened. Only four of the 20 bleeds from the November 2016 injections were still soft, with remainder being hardened and dry. Minor cracking around the injection point, interpreted as part of the healing process, was noted in a majority of the injection points (Table 2, Figure 4). Observations on injection points and trunks in February and October 2018 showed that all injection points from all injection times had healed, with hardened gum plugging the hole. There were no signs of trunk cracking associated with any of the injection points, other than the minor cracking around the injection point noted above and in Figure 4).

Table 2. Bleeds from water injection points following kauri trunk injection in various seasons, times of day and weather conditions, assessed in April 2017. Bleeding was assessed on a 1–5 scale, where 1 = nil or very small dribble, and 5 = a massive bleed. The hardness was assessed by pressing a fingernail into the bleed, and scoring as 'hard', 'soft', 'sticky' or 'very sticky'. 'Crack' indicates number of cases with minor cracking around the injection point (as shown in Figure 4).

Date	Time	Weather	Ave. bleed	Bleed hardness*				Crack*
				Hard	Soft	Sticky	Very sticky	
Nov 2015	Noon	fine	2.6	20	0	0	0	19/20
Feb 2016	Morning	fine	2.2	20	0	0	0	20/20
Feb 2016	Noon	fine	2.2/2.35	19	1	0	0	20/20
Feb 2016	Afternoon	fine	2.6	20	0	0	0	20/20
Feb 2016	Noon	raining	2.7	19	0	1	0	20/20
Apr 2016	Noon	fine	2.2	20	0	0	0	20/20
Aug 2016	Noon	fine	2.4	19	1	0	0	16/20
Nov 2016	Noon	fine	2.1	16	4	0	0	9/20

* In the October 2018 assessment, bleeds were recorded as 'hard' for all injection points at all injection times, and all but one injection point showed localised cracking/healing.

Figure 4. Minor cracking around the injection point on a kauri trunk, 10 months (left) and 15–18 months (right) after trunk injection of 20 mL water

In conclusion, there was no evidence from this trial that season, time of day or weather conditions will prevent uptake of liquids injected into kauri trunks. Although the uptake time in November was significantly slower than at other times, the difference was not great and uptake was still within a time considered acceptable. Thus treatment may be possible at any time of the year or time of day.

It is yet to be determined whether seasonal or other timing factors influence the efficacy of phosphite treatment or the incidence/expression of phosphite toxicity symptoms.

4 RECOMMENDATIONS FOR ONGOING RESEARCH

In response to an MPI request for recommendations for ongoing work and an outline of trial parameters, the following was provided in July 2017:

The water trial described in this report gives little insight into potential phytotoxicity symptoms following injection at different times of the year. To determine this, phosphite injection would be required in different seasons. Before doing the water trials, there was an expectation that variables such as season, time of day and weather would substantially influence injection uptake time, and that this would influence the choice of parameters when seasonal/timing effects were investigated with phosphite applications. However, differences observed to date in the water trial have been small.

For phosphite application studies, there seems little benefit in investigating time of day and prevailing weather conditions, although such factors should at least be noted in future trial work. However, there is merit in determining phosphite efficacy and tree phytotoxicity responses at different times of the year. Even though uptake time was not substantially influenced by season, because both tree and pathogen growth are influenced by season it seems reasonable to expect that disease control and tree expression of phytotoxicity might be affected. For future broad-scale phosphite treatment of kauri forest, it is important to avoid injection times that might either be more detrimental to the tree or less effective against the pathogen.

Trial parameters should include:

- *Trees showing symptoms of kauri dieback (using symptomless trees would simplify the work and would help to determine phytotoxicity effects, but would not provide information on efficacy). Ricker stands would be preferable*
- *Injections in each of the four main seasons should be investigated*
- *Injections in each season would have to be on a separate group of trees. At least five trees (preferably up to 10) would need to be injected each season, meaning 20 to 40 trees would be required*
- *Time of day and weather conditions should be varied as little as possible, but there would be no need to look at these factors as variables*
- *A moderate rate of phosphite should be applied (perhaps 7.5–10%) where phytotoxicity symptoms have been experienced (on some sites) previously*
- *Injection number on each tree would reflect current recommendations, i.e. one 20-mL injection for every 20 cm of trunk girth*
- *Recording of canopy phytotoxicity symptoms (yellowing, leaf loss, twig loss) should be three-monthly for one year following treatment. Recording of trunk phytotoxicity symptoms (bark peeling, cracks or bleeds above injection points) should be yearly for 3 years following treatment. Recording of kauri dieback symptoms (e.g. lesion activity or spread) should be six-monthly for 3 years*
- *Timing: Could start any time, with a trial duration of 3 years. Brief progress report annually, final report after 3 years.*

While the plan as outlined above is still sound, the recommended work may not be necessary. Records from similar work could be captured from the Kauri Rescue™ programme, a citizen science project funded through the Biological Heritage National Science Challenge, which commenced in 2017. To date, approximately 50 landowners with kauri dieback have injected phosphite into kauri trees on their properties, with timing of applications spread throughout the year. Approximately 800 trees have so far been treated. All participants are collecting 6-monthly data on lesion healing, tree health, and any signs of phytotoxicity (including trunk cracking symptoms). This presents a good opportunity to observe responses over a range of sites, injection times, tree sizes and health. It could give some real insight into any seasonal or other factors related to the risk of phytotoxicity. Although it could be argued that these trials are not set up in the systematic and controlled way described in the parameters above, the sheer weight of numbers and diversity of sites could provide information not possible in more formal trials. Kauri Rescue participant data collected over the past 18 months are currently being collated and analysed, and should at least provide summary statistics of the diversity of situations and timing of application. It is hoped that the programme will continue at least for the next few years if a funding source can be found, with the expectation that a considerable amount of tree response data will be generated and regressed against the diversity of parameters and situations. There is also the opportunity for a more formal assessment, perhaps by a single expert, over selected sites treated at different times, to ensure consistency of data relevant to toxicity symptoms.

5 ACKNOWLEDGEMENTS

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