Efficacy and persistence of phosphite for control of RNC in *Pinus radiata*

Results from two trials testing different application methods

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REPORT INFORMATION SHEET

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EXECUTIVE SUMMARY

Report Title: Efficacy and persistence of phosphite for control of RNC in *Pinus radiata*
Authors: Carol Rolando, Nari Williams and Martin Bader

The problem: A chemical control strategy is required to manage severe outbreaks of red needle cast (RNC) in mature stands of *Pinus radiata*. The aim of the trials described in this report was to determine the efficacy of phosphite over time against the causal agent of RNC, *P. pluvialis*, when applied as either a stem injection treatment or as a high volume foliar spray. *Phytophthora kernoviae* was also included in all assays, as it has also been associated with *P. radiata* needle disorders.

This project: Four concentrations of phosphite were applied as a stem injection treatment (0, 0.25, 0.5 and 1.00 g a.i. cm^{-1} dbh) to each of 10 six-year-old *P. radiata* trees. Using detached needle assays with either *P. pluvialis* or *P. kernoviae*, we tested the efficacy and persistence of these treatments at 33, 126, 220 and 381 days after application. In a separate experiment, we tested the effect of timing of application (February or April) and concentration (0, 0.2% and 2%) of a high volume, foliar phosphite application on infection response, as determined by detached needle assays with either *P. pluvialis* or *P. kernoviae*. Following foliar phosphite application to four-year-old *P. radiata* trees in February or April 2013, detached needle assays were carried out in July and October 2013, and March 2014. Mixed effects models were used in both trials to determine whether the phosphite treatments reduced the length of lesions relative to an untreated control, and the period over which this effect persisted, up to one year after application.

Key Results:

- Needles sampled from trees injected with phosphite at 1 g a.i. cm^{-1} dbh had consistently smaller lesions than needles sampled from control trees *for up to one year* after injection for both species of *Phytophthora* tested. Persistence and efficacy was lower for the intermediate concentrations tested, particularly for *P. pluvialis*.
- Where a high volume foliar application was used, significant reductions in lesions formed by *P. pluvialis* were only observed where a 2% phosphite solution was applied. This effect persisted *for up to eight months* after application. In contrast, detached needle assays using *P. kernoviae* gave consistently smaller lesions on needles treated with 0.2% and 2% phosphite, in comparison to the control trees, *for up to one year* after treatment application.
- Persistence of phosphite is dose dependent.
- *P. kernoviae* appears to be more sensitive to phosphite application than *P. pluvialis*.

Implications of Results for Client: The results indicate that phosphite is an effective chemical to control *P. pluvialis* and that further work with phosphite should be carried out. Efficacy and persistence increase with dose and a chemical control treatment has to use the optimum dose for *P. radiata*. The full dose response profile for phosphite application on *P. radiata* still needs to be determined and will be the focus of trials in the next financial year.

Further Work: We recommend that we conduct controlled and field scale dose response trials with phosphite to define the effective dose range for *P. radiata* and *P. pluvialis*. These trials should also include foliar analyses of phosphite such that efficacy (using bioassays) can be related to a foliar concentration of phosphite. Epidemiological work suggests the period for highest risk of infection with red needle cast is over a very wide period (at least late summer to early spring). Further work with phosphite will aim to apply the active ingredient over the most appropriate period.

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Introduction

Red needle cast (RNC), a foliar disease of *Pinus radiata* caused by *Phytophthora pluvialis*, has the potential to cause up to 16% reduction in stem periodic annual cross sectional area increment per severe RNC event (Beets et al., 2013; Dick et al., 2014). A cost-effective, FSC compliant, chemical control strategy is needed to provide a short-term management option for control of severe outbreaks of RNC in existing *P. radiata* forests. Phosphite, a fungicide known to be effective against diseases caused by *Phytophthora*, is the key active ingredient currently being investigated for its potential to manage RNC.

There are few reports describing the use of phosphite to control *Phytophthora* foliar diseases in mature forest trees. This is likely because there are very few tree infecting aerial *Phytophthora* spp. currently known, and those which are known, such as *P. ramorum, P. pinifolia, P. kernoviae,* and *P. pluvialis*, have been relatively recent observations (Ahumada et al., 2013; Dick et al., 2014). Kanaskie et al. (2011) showed the potential for phosphite to control outbreaks of sudden oak death, caused by *P. ramorum*, in *Notholithocarpus densiflorus* (south-western Oregon tanoak). Graham (2011) suggested options for using phosphite to control *Phytophthora* associated diseases in forest trees based on research in citrus orchards. Phosphite is already used to control *Phytophthora* root rot in *Pinus radiata* nurseries (Reglinski et al., 2009). However, the nursery application cannot readily be extrapolated to airborne *Phytophthora* pathogens infecting mature *P. radiata* trees.

A cost effective management programme for control of RNC in *P. radiata* plantations will ultimately require aerial application of an effective fungicide to infected trees. Low-volume aerial applications of phosphite have been effective for the control of *Phytophthora* in other forest environments (Shearer et al., 2012; Kanaskie et al., 2011), and if demonstrated to be effective, will be used for control of RNC in *P. radiata*. Before conducting aerial application trials, however, the efficacy of phosphite for controlling RNC needs to be determined, together with factors such as dose, timing of application and persistence of protection. Since aerial application trials are expensive to conduct, and their scale of operation not conducive to testing a wide array of treatments, smaller-scale controlled trials are initially more suitable to screen active ingredients (a.i.) rather than large scale, aerial trials. Once we have a better understanding of the efficacy of phosphite, aerial trials will be conducted to determine effective management prescriptions. Small scale trials also offer the opportunity to carry out controlled inoculations which reduce the reliance on natural inoculum which is typically highly variable.

Using detached needle assays, the aim of the trials described in this report was to determine the efficacy and persistence of phosphite when applied either as a stem injection treatment or as a high volume foliar spray on the formation of foliar lesions by *P. pluvialis* and *P. kernoviae*. *Phytophthora kernoviae* was included in all assays, as it has also been associated with *P. radiata* needle disorders.

Stem injection of phosphite is not a viable control option for management of RNC in plantation forests. The size of the area, the number of trees infected, as well as the nature of the terrain would make this an extremely slow and expensive application method. This method of application was included in initial small scale trials because it provided a direct method of chemical delivery to enable differential dosages to be applied to neighbouring trees and ensure sufficient uptake of phosphite required to induce a treatment response, if any.

Materials and Methods

Experiment 1. Stem injection of phosphite (hereafter stem injection trial)

The objective of this experiment was to evaluate the effect of phosphite concentration on lesion development in *P. radiata* needles. The trial was located at the Scion experimental Long Mile forest. Forty six-year-old *P. radiata* trees were allocated to one of four treatments in which 0, 0.25, 0.50 and 1.00 g phosphite (Agrifos®600, Key Industries, Auckland; 600 g L^{-1} phosphorous acid) was injected per cm diameter at breast height (dbh). The average diameter of the trees was 12.0 ±2.9 cm.

Injection of the phosphite solution was made at the base of each tree into predrilled holes, drilled at an acute, downward angle into the cambium. The phosphite solution was passively injected using three Chemjet Injectors (Queensland Plastics, Australia) spaced equidistant around each tree. Trees, blocked into ten replications of four trees, were injected on 26 February 2013. This was late summer in Rotorua and ensured a rapid uptake of the injected solution due to high levels of transpiration. Detached needle assays with *P. pluvialis* and *P. kernoviae* were conducted at 33, 136, 220 (no *P. kernoviae*) and 381 days after the injection of phosphite. At each assessment date thirty fascicles were sampled from each tree, exposed to *P. pluvialis*, *P. kernoviae* or pond water in a detached needle assay, and assessed after 10 days for signs of lesions which were measured to the nearest millimetre.

Experiment 2. High volume foliar application of phosphite (hereafter foliar application trial)

The objective of this trial was to determine the effect of application timing (late summer (February) and autumn (April) application) and concentration (0, 0.2% and 2%) of foliar phosphite application on infection response, as determined by detached needle assays using *P. pluvialis* or *P. kernoviae*. The trial, located at the Scion experimental Long Mile forest, was designed as a 2 x 3 factorial (date x concentration) with individual trees assigned as treatment plots. Trees were 3.5 years old at the time of treatment application. There were 10 trees per treatment (timing x concentration combination), with treatments applied in a completely randomised design.

The phosphite treatments, 0, 0.2% or 2% solution of phosphite (Agrifos®600, 600 g L^{-1} phosphorous acid), were applied to run-off to crown foliage of each tree in February or April 2013 using a Solo back-pack sprayer. All solutions contained 0.2% DuWett (Etec Crop Solutions Ltd, Auckland), to optimise the uptake of the fungicide (Rolando et al., 2014). Treatments were based around Agrifos®600 label recommendations (Anon, 2013) for phosphite application to horticultural crops, most of which are to run-off with a 0.2% solution (around 300 ml/100 L water or around 3-3.5 L product per hectare, which is roughly 1.8 to 2.1 kg ha^{-1} phosphite). Thirty fascicles were harvested from each tree in July 2013, October 2013 and March 2014 and exposed to *P. pluvialis*, *P. kernoviae* or pond water, in a detached needle assay. After 10 days lesion length was assessed.

Detached needle assays

Detached needle assays were used in both trials to assess the efficacy of the phosphite treatments at regular intervals after application for up to one year. Zoospore suspensions of *P. pluvialis* and *P. kernoviae* were prepared using the RNC standard operating procedures (Williams, 2013). Suspensions were cultured and prepared in Scion's Forest Protection laboratory to produce sufficient quantities of inoculum containing zoospores at a final concentration of ~ 1 x 10^3 per ml. Autoclaved pond water was used for controls.

Statistical analysis

Linear mixed-effects models were used to analyse the average lesion length per fascicle caused by the two *Phytophthora* species in both experiments (R version 3.0.2, R Development Core Team 2013, R-package *nlme*). For both experiments separate models were run for each *Phytophthora* pathogen. Graphical model validation tools were used to check the model assumptions of variance homogeneity and normality. Variance heterogeneity was detected in all models and accounted for within the model syntax. For all models, the significance of the fixed term was assessed using a backwards selection procedure based on likelihood ratio testing until the 'optimal' model was obtained (Zuur et al. 2009). A significant interaction term was followed up with a multiple comparison procedure using Tukey contrasts (R-package *multcomp*).

Experiment 1. Stem injection trial

The models contained assessment date, phosphite concentration and their interaction as fixed effects. A 'block' corresponded to an individual tree and was included as a random effect.

Experiment 2. Foliar application trial

The models contained application date, phosphite concentration, assessment date, and their interaction as fixed effects. A 'block' corresponded to an individual tree and was included as a random effect.

Following a two-sided outlier test for the standardized residuals, three observations from the *P. pluvialis* data and two observations from the *P. kernoviae* data were removed. The outlier test identified absolute standardized residuals greater than the 1 – residual value/2 quantile of the standard normal distribution.

Results and Discussion

Experiment 1. Stem injection trial

Phytophthora pluvialis

The highest phosphite concentration showed the greatest efficacy in reducing lesion length. However, this effect emerged over time as a function of the increasing length of lesions as time after treatment application increased (significant phosphite concentration × date interaction, Fig. 1). At the first assay, one month after stem injection of phosphite, there were only minor lesions present with no significant differences between phosphite concentrations (Fig. 1). At all remaining assessment dates, the untreated control needles showed on average significantly larger lesions than needles from trees treated with the highest phosphite concentration of 1 g cm^{-1} dbh (Fig. 1). Trees injected with low and intermediate phosphite concentrations (0.25 and 0.50 g cm^{-1} dbh) also developed smaller needle lesions compared to the control at 136 and 220 days after stem injection, but due to the large variation seen in the control trees this effect was not statistically significant. More than one year following stem injections (381 days), lesion lengths in needles collected from trees receiving the two intermediate phosphite concentrations were very similar to the control while the highest phosphite concentration still provided strong protection against *P. pluvialis* suggesting that not only the efficacy but also the persistence increases dramatically with increasing phosphite concentration.

Phytophthora kernoviae

Already one month after stem injection there was a trend towards smaller lesions in needles from trees treated with the 0.5 and 1 g phosphite cm^{-1} dbh which became significant over time (significant phosphite concentration × date interaction, Fig. 2, Table

2: Appendix A). The lowest phosphite concentration (0.25 g cm^{-1} dbh) had only a minor effect on reducing lesion length which was insignificant compared to the control. However, at 136 and 381 days after stem injection, the small reduction in lesion length associated with the lowest phosphite concentration translated into a non-significant difference compared to the results produced by the intermediate and high phosphite concentrations.

The delayed response to phosphite application observed in both trials at the one month assessment possibly partly reflects the time taken for the phosphite to be translocated throughout the crown or to elicit a host response.

Experiment 2. Foliar application trial

Phytophthora pluvialis

Overall, the highest phosphite concentration conferred the strongest protection against *P. pluvialis,* with no significant response to application date (February or April) within each assessment (Fig. 3). The largest effect of concentration was seen at the first assessment (A) in July, when lesions in untreated control needles were on average 7-10 times larger than needles receiving the highest phosphite concentration (2%) where lesion formation was largely suppressed. Phosphite persistence proved to be relatively short-lived (<1 year) as evidenced by a weaker treatment response at the October assessment (B) and similar lesion lengths in control needles and those treated with phosphite at the March 2014 assessment (C), about 12 months after spraying. This decline in efficacy over time, together with the unusual outcome observed during the October assessment (B) of the trees that were sprayed in April (largest lesions in the 0.2 % phosphite treated needles, Fig. 3 see Apr B) resulted in a significant application date × phosphite concentration × assessment date interaction (L = 11.27, df = 4, P = 0.024).

Phytophthora kernoviae

In general, the highest phosphite concentration also provided the highest efficacy as evidenced by significantly smaller lesions, even about a year after phosphite application (Fig. 4). There was no significant three-way interaction (L = 2.08, df = 2, P = 0.35), but the efficacy of the 0.2 % phosphite concentration was substantially better when phosphite was applied in April compared to the February spray, resulting in a significant phosphite concentration × application date interaction (L = 45.45, df = 2, P < 0.001). The efficacy of the 2% phosphite concentration appeared to be independent of the timing of spraying because lesion lengths were similar across phosphite application and assessment dates (Fig. 4).

General discussion: Phosphite uptake and response

While phosphite is a systemic chemical with a potentially long life within the tree once applied, its effective dose after application will be affected by the growth rate of the tree, host biochemical interactions and phenology (McDonald et al., 2001; Thao and Yamakaw, 2010). The plants used in this study were 6 and 3.5 years old, respectively, a period where rapid growth can be expected. These complex biological interactions, and their impact on efficacy, will need to be evaluated in further trials with phosphite and its application to mature stands of *P. radiata* where RNC is most prevalent. It is also likely a more consistent deposition of spray in an aerial application will facilitate consistent uptake of phosphite across treated plants. The efficacy of the high volume foliar phosphite application indicates that foliar uptake of the active ingredient is sufficient to induce a host response: a good outcome for low volume aerial application of the active ingredient. However, at low volume aerial application rates, parameters for maximising uptake under field conditions will need to be optimised. This will require the thresholds between maximum phosphite activity in the host and phyto-toxicity to be determined under field conditions. The original design of the high volume foliar application trial included a 20%

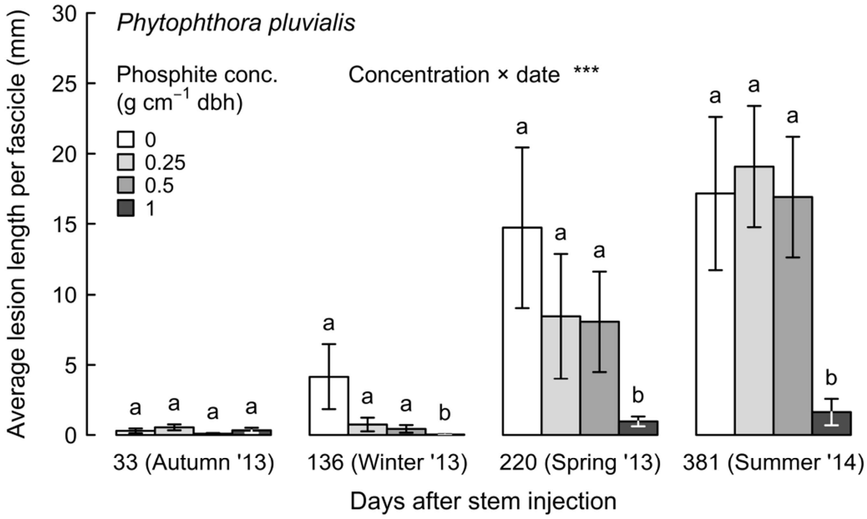

Fig. 1 Average lesion length per fascicle of *Pinus radiata* needles inoculated with *Phytophthora pluvialis.* Phosphite was applied as stem injection at four concentrations and needles were assessed for lesion formation at four subsequent dates (33, 136, 220, and 381 days after stem injection). Different lower-case letters indicate significant differences between phosphite concentrations for each date at p = 0.05 (multiple comparison test using Tukey contrasts).

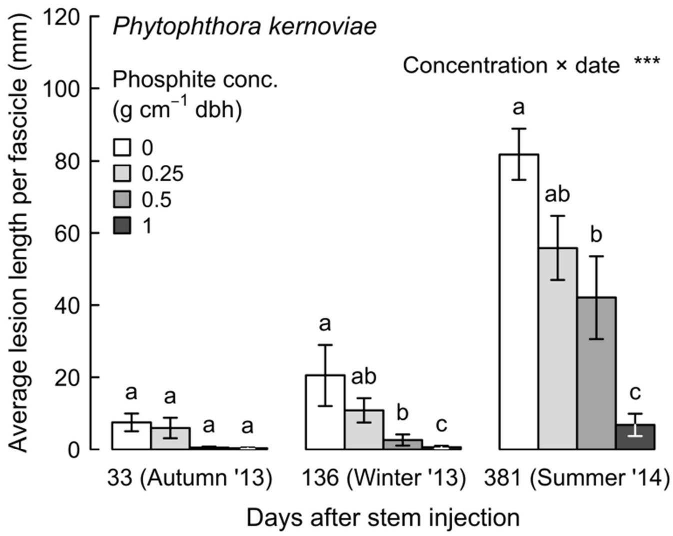

Fig. 2 Average lesion length per fascicle in *Pinus radiata* trees inoculated with *Phytophthora kernoviae.* Phosphite was applied as stem injection at four concentrations and needles were assessed for lesion formation at four subsequent dates (33, 136 and 381 days after stem injection). Different lower-case letters indicate significant differences between phosphite concentrations for each date at p = 0.05 (multiple comparison test using Tukey contrasts).

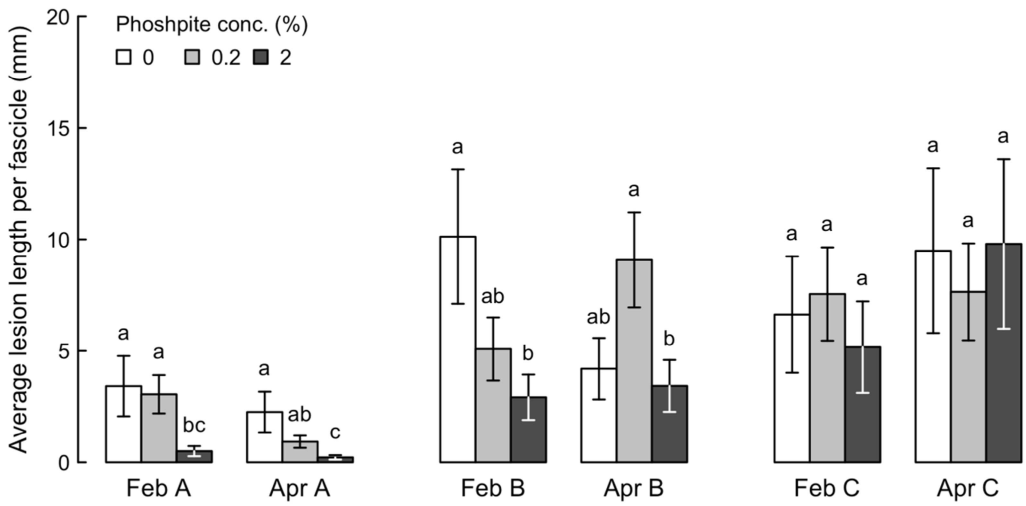

Fig. 3 Average lesion length per fascicle in *Pinus radiata* trees inoculated with *Phytophthora pluvialis.* Phosphite was applied as a spray at two dates (February, April 2013) and three concentrations (0, 0.2, 2%). Needles were assessed for lesion formation at three subsequent dates (A = July /2013, B = October 2013, C = March 2014). Different lower-case letters indicate significant differences between phosphite concentrations, which are valid across application dates within a given assessment date (multiple comparison test using Tukey contrasts, $\alpha = 0.1$). Means ± SE, n = 10 or 20 trees per phosphite concentration.

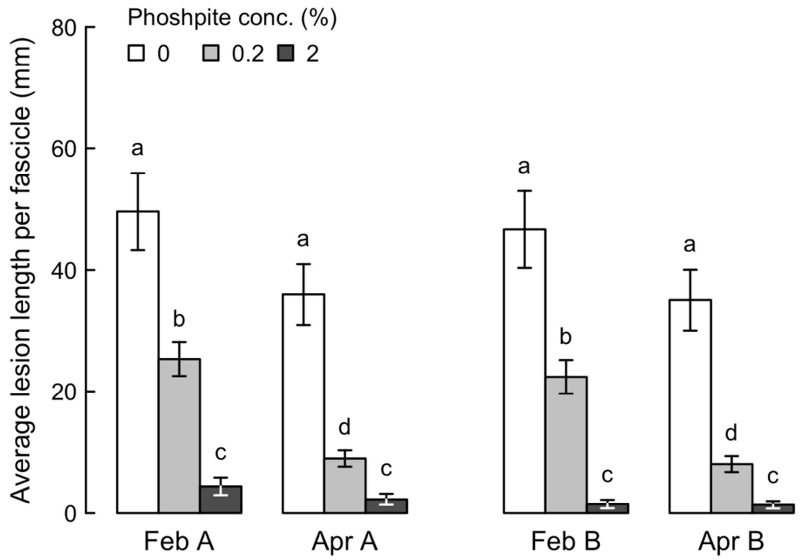

Fig. 4 Average lesion length per fascicle in *Pinus radiata* trees inoculated with *Phytophthora kernoviae.* Phosphite was applied as a spray at two dates (February, April 2013) and three concentrations (0, 0.2, 2%). Needles were assessed for lesion formation at two subsequent dates (A = July 2013, B = March 2014). Different lower-case letters indicate significant differences between phosphite concentrations, which are valid across application dates within a given assessment date (multiple comparison test using Tukey contrasts, α = 0.05). Means ± SE, n = 10 trees per phosphite concentration.

phosphite solution as a fourth treatment. However, this solution applied at a high volume killed all test trees and the treatment had to be abandoned. Parallel work has indicated that the phytotoxicity threshold of *P. radiata* is much higher for low volume spray application (P. Scott, pers comm). That there is a threshold between maximum rate and phytotoxicty has been determined and we will need to establish this for low volume foliar applications in the field.

The persistence of phosphite in both the stem injection and foliar application trials indicates that a late summer application of phosphite is likely to provide at least a full season of protection to the host. Within RNC infested stands, this period also provides the greatest needle density and high rates of transpiration which are likely to optimise the uptake of phosphite by the plant. Persistence beyond this time period may depend on host age, growth rate and site environmental conditions. As we have not yet determined the effect of phosphite when applied after infection is established, the potential for phosphite to stall/disrupt the development of a severe disease event remains unknown.

Key outcomes and recommendations

Outcomes

There are three significant outcomes from these trials:

Outcome 1: There was sufficient uptake of active ingredient with both methods of application, stem injection and foliar, to provide an assessment of dose response and persistence of phosphite at the concentrations tested. That foliar application resulted in significant reductions in lesion length is a positive outcome for this method of application indicating sufficient active ingredient can be applied to crown foliage.

Outcome 2: Results from both sets of trials indicated that not only efficacy, but also persistence of phosphite, was related the concentration applied.

- In the stem injection trial, efficacy was highest where 1 g phosphite cm^{-1} dbh was applied. Lesions formed on needles sampled from trees treated with 1 g phosphite cm^{-1} dbh were significantly smaller than lesions formed on needles sampled from the control trees for up to one year after application for both species of *Phytophthora* tested. For the intermediate concentrations efficacy and persistence were substantially reduced.
- Where a high volume foliar application was used, significant reductions in lesions formed by *P. pluvialis* were only observed where a 2% phosphite solution was applied. This effect persisted for up to eight months post phosphite application. At 12 months there was no significant difference in lesions formed on needles collected from control trees and the highest rate tested.
- Results from the *P. kernoviae* trial would indicate that this species is more sensitive to phosphite than *P. pluvialis*, since a significant reduction in lesion length was observed for both concentrations tested for up to one year after application.

Outcome 3: Timing of phosphite application within a season did not have a significant impact on efficacy

Foliar application of phosphite in either February or April did not significantly affect efficacy in detached needle assays carried out in the year following application.

Recommendations

This set of trials indicate that efficacy and persistence of the effects of phosphite are related to dose. We have yet to fully define the dose response curve for phosphite, and associated persistence, when applied to *P. radiata* for control of *P. pluvialis* across age classes and throughout the cycle of forest production. We recommend that further to these trials we conduct controlled and field scale dose response trials with phosphite to define this range for *P. radiata* and *P. pluvialis*. These trials should also include foliar analyses of phosphite such that efficacy (in bioassays) can be related to a foliar concentration of phosphite. These trials should be supported by work that determines the potential of phosphite to reduce symptoms of RNC when applied after infection has occurred. Epidemiological work suggests the period of infection for red needle cast is from late summer/to the end of spring. Further work with phosphite will aim to apply the active ingredient at the start of this period.

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Appendix 1

Table 1 Results from the final linear mixed-effects model testing the effects of assessment date (days after stem injection), phosphite concentration on lesion length development in *Pinus radiata* needles inoculated with *Phytophthora pluvialis*.

Parameter	Df_{num}	Df_{den}	*F*	*P*	
Intercept	1	141	1.33	0.251	
Phosphite concentration	3	141	2.12	0.101	
Date	1	141	12.25	0.002	**
Phosphite conc. × date	3	141	9.96	<0.001	***

Table 2 Results from the final linear mixed-effects model testing the effects of assessment date (days after stem injection), phosphite concentration on lesion length development in *Pinus radiata* needles inoculated with *Phytophthora kernoviae*.

Parameter	Df_{num}	Df_{den}	*F*	*P*	
Intercept	1	97	5.29	0.024	*
Phosphite concentration	3	97	11.40	<0.001	***
Date	1	97	5.07	0.027	*
Phosphite conc. × date	3	97	14.08	<0.001	***