Needle disease strategy Research Programme

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Pot trial to evaluate the efficacy and persistence of phosphite and cuprous oxide to control RNC

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Pot trial to evaluate efficacy and persistence of phosphite to control RNC

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Executive summary

Report Title: ***POT TRIAL TO EVALUATE THE EFFICACY AND PERSISTENCE OF PHOSPHITE AND CUPROUS OXIDE TO CONTROL RNC***
Authors: ***CAROL ROLANDO, STEFAN GOUS, MARTIN BADER AND NARI WILLIAMS***

The problem

Previous trials have indicated that phosphite is effective for control of red needle cast. However, the efficacy and persistence of phosphite as related to dose has yet to be fully defined. Initial trials with phosphite were carried out with Agrifos®, which was shown to have low penetration potential (3%) into *P. radiata* needles when applied without adjuvants. Several adjuvants were found to facilitate uptake of Agrifos®, however, the solutions were also found to be unstable thereby negating their use. More recent studies have indicated that phosphite applied as Foschek™ results in higher uptake of phosphite (up to 72%).

This project

The aim of this study was to explore in a controlled environment the efficacy of phosphite, applied as Foschek™ for control of red needle cast as assessed by length and number of lesions on detached needles 30, 180 and 360 days after treatment application. Phosphite was applied at a range of doses (6, 12 and 24 kg ha^{-1}), with and without an adjuvant (ActiWett @ 0.2%), and in combination with copper oxide (Ag Copp 75, 75% cuprous oxide, American Chemet Corporation) at 1.72 kg a.i. ha^{-1}. An additional treatment with cuprous oxide applied alone at 1.72 kg ha^{-1} was also included. Four *P. radiata* clones, of varying susceptibility to *P. pluvialis*, were used in the study.

Key results

For each of the assays, lesion length and number did not significantly differ between clones. However, averaged over the four clones, there were significant responses to treatments across all assay dates with very similar responses recorded for lesion length and number. All treatments with copper oxide significantly reduced lesion number and length up to 30 days after treatment application. This effect was no longer significant at 180 days. None of the treatments where phosphite was applied (with or without an adjuvant) were significantly different from the control for either lesion length or number at the first or subsequent assays, a surprising result not observed in any previous trials. Despite this, however, there was a non-significant trend in the response of *P. pluvialis* to the rate of phosphite applied, with lesion length reducing with increasing phosphite rate (from 6 kg ha^{-1} to 24 kg ha^{-1}) at the 30 and 180 day assays. Further, smaller and fewer lesions were generally recorded across phosphite treatments where an adjuvant had been applied. Treatments where phosphite and copper oxide had been applied together in a mix did not appear to enhance the activity of either active ingredient applied alone.

Implication for client

Both phosphite (applied as Foschek™) and copper (applied as cuprous oxide) have the potential to reduce infection of *Pinus radiata* with *P. pluvialis.* However, further work is required on both active ingredients to fully understand the host:pathogen:treatment interaction.

- As a foliar fungicide with preventative action cuprous oxide appears to be very effective against infection with *P. pluvialis* in the short-term. The full sensitivity of *P. pluvialis* to copper has yet to be determined, but applied at rates at or above that currently used for the operational treatment for dothistroma needle blight (0.86 kg a.i. ha^{-1} and above), efficacy for at least one month has been confirmed in several trials.
- The complex mode of action of phosphite renders this a more difficult active ingredient to assess. If effective, the benefit of using this active ingredient is its persistence in the plant as well as its acceptability to FSC. Based on the results of this trial, the most promising rate would appear to be 12 kg ha^{-1} phosphite, applied as Foschek™ with 0.2% adjuvant (alcohol ethoxylate). A better assay protocol able to test whole plant responses to infection with *P. pluvialis* is needed to fully understand the potential of this active ingredient. Future work with phosphite should also allocate more resources to understanding plant and soil nutrition, as well as the balance of phosphate and phosphite in host-plant tissues.

Introduction

Red needle cast (RNC) is a foliar disease of *Pinus radiata* caused by *Phytophthora pluvialis*. It can cause significant (up to 38%) annual growth loss in badly infected plantations (Beets et al., 2011). An operational, cost-effective, chemical treatment is required to control outbreaks of RNC. Phosphite is being investigated as a fungicide for management of RNC in mature *P. radiata* plantations, as is cuprous oxide, as this active ingredient is already used operationally for management of dothistroma needle blight.

Previous trials have indicated that phosphite is effective in reducing lesion development on *P. radiata* needles inoculated with *P. pluvialis* and that this effect has the potential to persist for several months, even up to a year after application (Rolando et al., 2012). However, we have yet to fully determine the dose response for phosphite, and the relation of dose to persistence. Initial trials with phosphite (Rolando et al., 2014) were carried out with Agrifos® (600 g L^{-1} phosphorous acid as mono and di potassium salts, Key Industries Ltd.), which was shown to have low penetration potential (3%) into *P. radiata* when applied without adjuvants. Several adjuvants were found to facilitate uptake of Agrifos™ into *P. radiata*, however, the solutions were also found to be unstable thereby negating their use (Horgan and Gaskin, 2015a). More recent studies indicated that phosphite applied as Foschek™ (400 g L^{-1} phosphorous acid as mono and di potassium salts, Zelam Ltd, Auckland) resulted in higher uptake of phosphite into *P. radiata* needles (up to 72%), and, further, did not require the addition of adjuvant to facilitate the higher rates of uptake (Horgan and Gaskin, 2015b).

The aim of this study was to explore in a controlled environment the efficacy of phosphite (with or without an adjuvant) and copper, applied as either Foschek™ or cuprous oxide, for control of *P. pluvialis* as assessed by lesion development (length and number). Four *P. radiata* clones, of varying susceptibility to *P. pluvialis* were used in the study.

Materials and methods

Using a belt driven tracksprayer, five potted grafts of each the four *P. radiata* clones (850_32; 850_55; 880_655; 885_001) were treated with one of nine phosphite/copper treatments in a total volume of 100 L ha^{-1} water (Table 1). Foschek™ was applied at a range of doses (6, 12 and 24 kg ha^{-1}), with and without an adjuvant (ActiWett @ 0.2%), and in combination with cuprous oxide (Ag Copp 75, 75% cuprous oxide, American Chemet Corporation) at 1.72 kg a.i. ha^{-1}. An additional treatment with copper oxide applied alone at 1.72 kg ha^{-1} was also included. The control consisted of potted trees treated with water only. Following treatment application the plants were maintained at the Scion nursery, where they were drip irrigated for one year post treatment application.

Detached needle assays with *P. pluvialis* were carried out at one (30 days), three (90 days), six (180 days) and twelve (360 days) months after treatment application, as per standard operating procedures (Williams, 2013). For each assay the number of lesions per fascicle and their total length per needle was determined. The *P. pluvialis* zoospore count was particularly low for the three month inoculation, and as a consequence very few lesions formed. The results from this assay have not been included in this report.

Data analysis

The experiment was set up as a factorial design of ten treatments (Treatment) applied to four clones (Clone). Variables included in the analysis were total lesion length (mm) per fascicle and number of lesions per fascicle.

Linear mixed effects models fitted by restricted maximum likelihood were used to analyse the lesion length and lesion count data (SAS 9.3 or R version 3.3.0, R Development Core Team 2015, R-package *nlme*, Pinheiro *et al.* 2016). Each assessment date after chemical treatment application was modelled separately. The models contained clone identity, chemical treatment and their interaction as fixed effects. The nested random term reflected the blocked design. Significant main terms were followed up applying a multiple comparison procedure using Tukey contrasts (R-packages *lsmeans* and *multcomp*, Lenth 2016, Hothorn 2008).

Table 1. Treatments applied to four *P. radiata* clones in a controlled pot trial. All treatments were applied in the equivalent of 100 L water ha^{-1}.

Treat	Phosphite (kg ha^{-1})	Actiwett (%)	Cuprous oxide (kg ha^{-1})
1	0		
2	6		
3	12		
4	24		
5	6	0.2	
6	12	0.2	
7	24	0.2	
8	0		1.72
9	6		1.72
10	12		1.72

Results

For each of the assays, lesion length and lesion number did not significantly differ between clones (Appendix A, Table 1 and Table 2). This was likely due to the very high variability observed in the infection process and also the response to the phosphite treatments, an issue which also affected interpretation of the overall responses to the application of phosphite. However, it is notable that inspection of the lesion length data for the control treatment only indicated that lesions formed on clones 885-001 and 850-32 were larger than those on 850-55 and 880-655. This was consistent across the three assays (Figure 1). This suggests a greater susceptibility to *P. pluvialis* of clones 885-001 and 850-32. The lack of significance of the factor Clone in any analyses meant that this response was not investigated further.

The main effect of Treatment was significant for both lesion length and number at 30, 180 and 360 days with very similar responses recorded for both variates across all treatments and assays (Appendix 1, Tables 1 and 2; Figures 2 and 3). For both variates at the first assay only those treatments where cuprous oxide was applied (with or without phosphite) had significantly smaller lesions from that in the control (Figure 2a and Figure 3a). This effect declined over time, with no significant effect of the cuprous oxide treatment(s) (with or without phosphite) evident on either lesion length or number at the 180 and 360 day assessments (Figure 2, Figure 3 and Figure 4).

None of the treatments where phosphite only was applied (with or without an adjuvant) were significantly different from the control for either lesion length or number at the first or subsequent assays, a surprising result not observed in any previous trials (Figures 2 and 3). However, despite this, there was a very clear response to rate of phosphite at the 30 day assessment, with lesion length reducing with increasing rate (from 6 kg ha^{-1} to 24 kg ha^{-1}) (Figure 1). Further, smaller lesions occurred across phosphite treatments where an adjuvant had been applied. This non-significant trend in response to rate was observed at subsequent assays, with the largest relative response to the control recorded at 360 days for the treatment where the equivalent of 12 kg ha^{-1} phosphite had been applied with or without an adjuvant. With time following treatment application there was a shift in the relative response to inoculation with *P. pluvialis*, with the significant effect of the copper treatments reducing over time, whilst that of the phosphite treatment seemingly persisting and increasing in efficacy at 12 kg ha^{-1}.

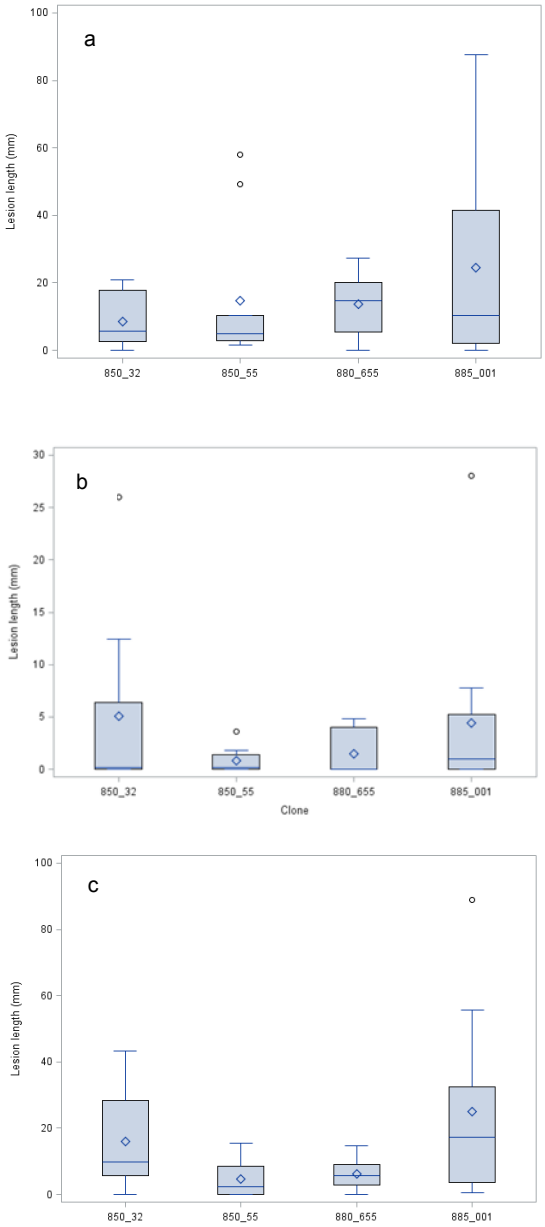

Figure 1. Lesion length (mm) in control trees for all clones assayed at a) 30 days b) 180 days and c) 360 days after trial initiation. Note that: the upper and lower whiskers represent maximum and minimum values, the upper and lower edge of boxes represent the 75th and 25th percentiles, the central line represents the 50th percentile or median and the central symbol, the mean. Extreme outliers are represented by symbols above the whiskers.

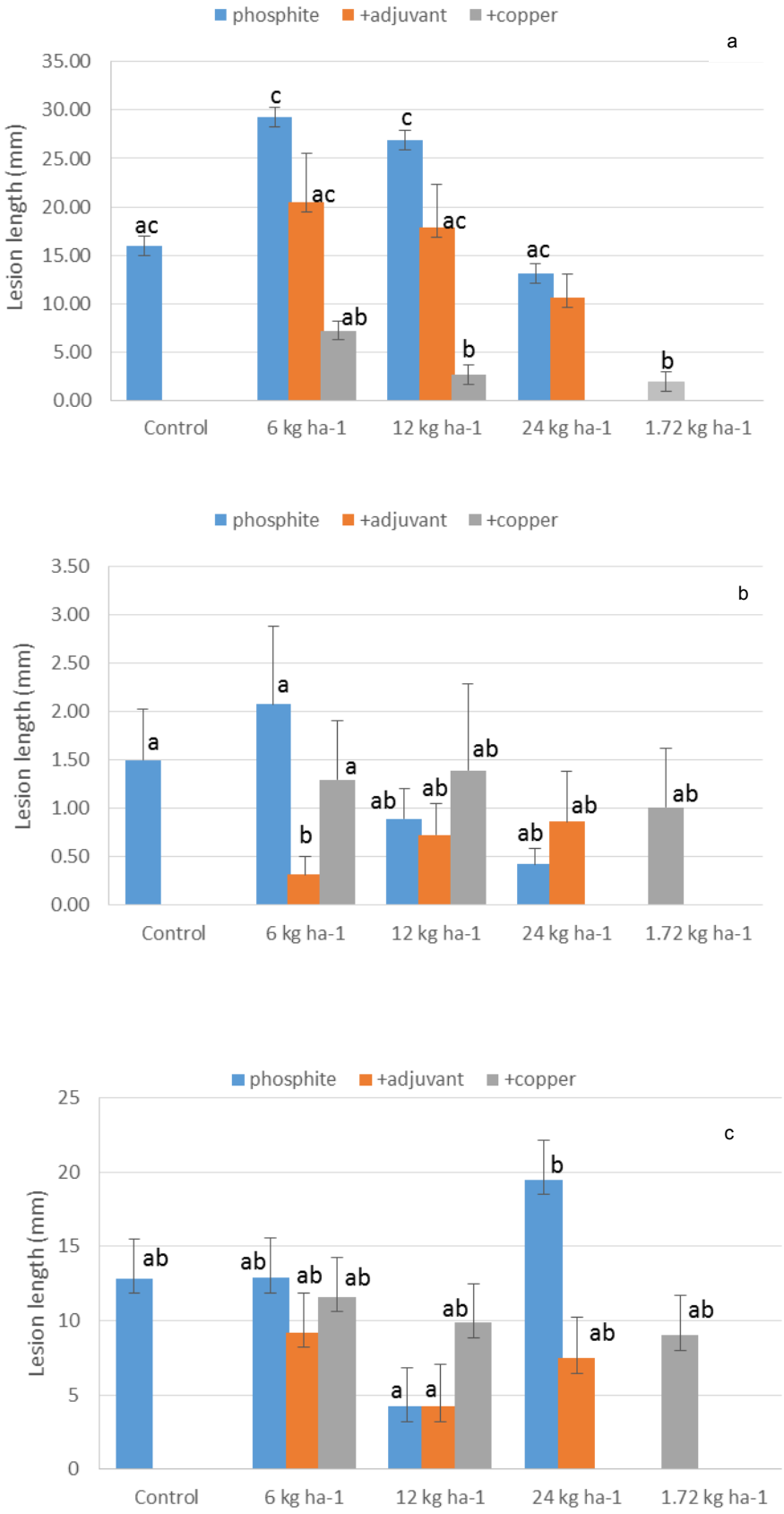

Figure 2. Average lesion length per fascicle in *Pinus radiata* needles from four clones inoculated with *Phytophthora pluvialis* in detached needle assays conducted at a) 30 days b) 180 days and c) 360 days after treatment application. Different lower case letters indicate statistically significant differences between chemicals. Bars indicate the standard error.

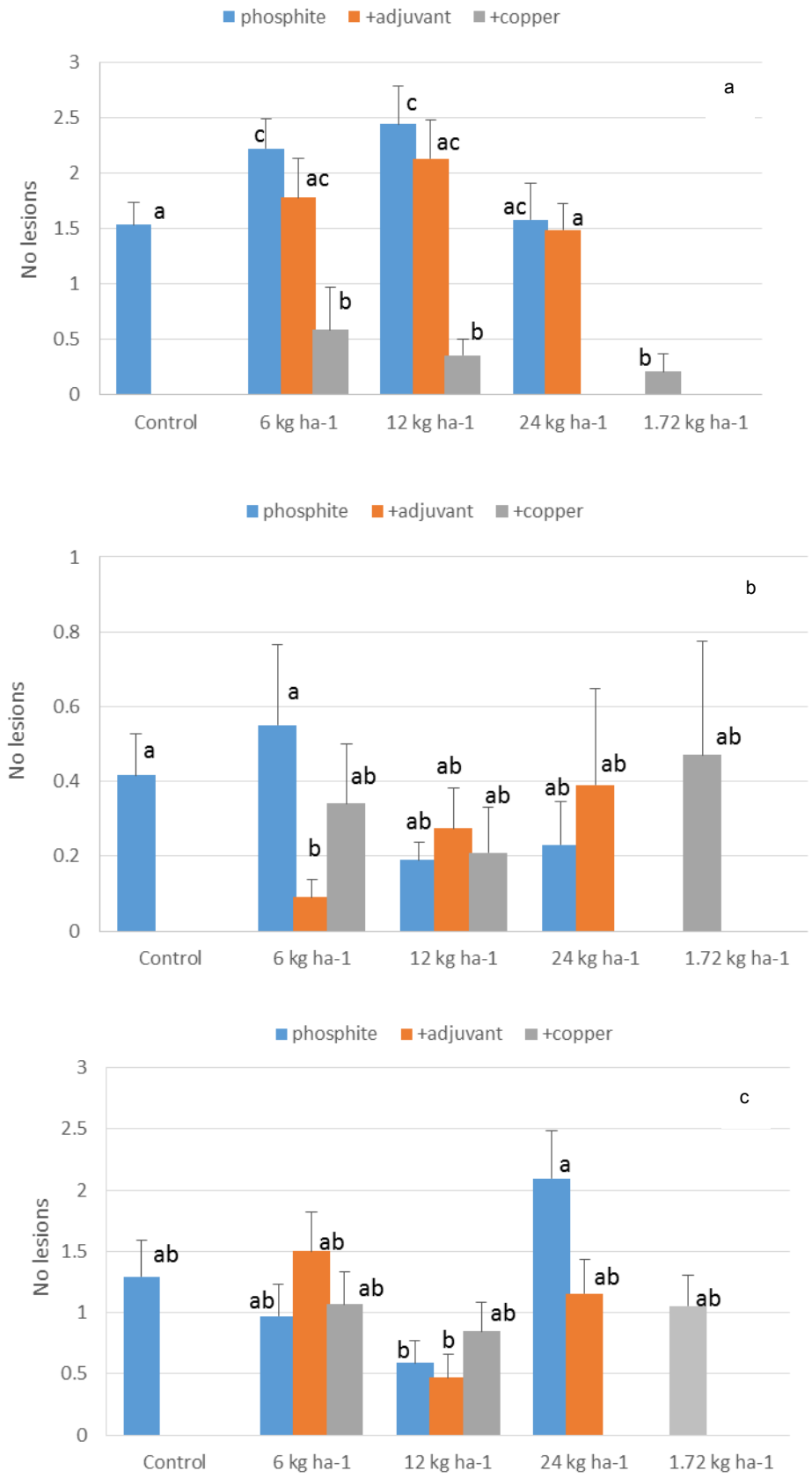

Figure 3. Average lesion number per fascicle in *Pinus radiata* needles from four clones inoculated with *Phytophthora pluvialis* in detached needle assays conducted at a) 30 days b) 180 days and c) 360 days after treatment application. Different lower case letters indicate statistically significant differences between chemicals. Bars indicate the standard error.

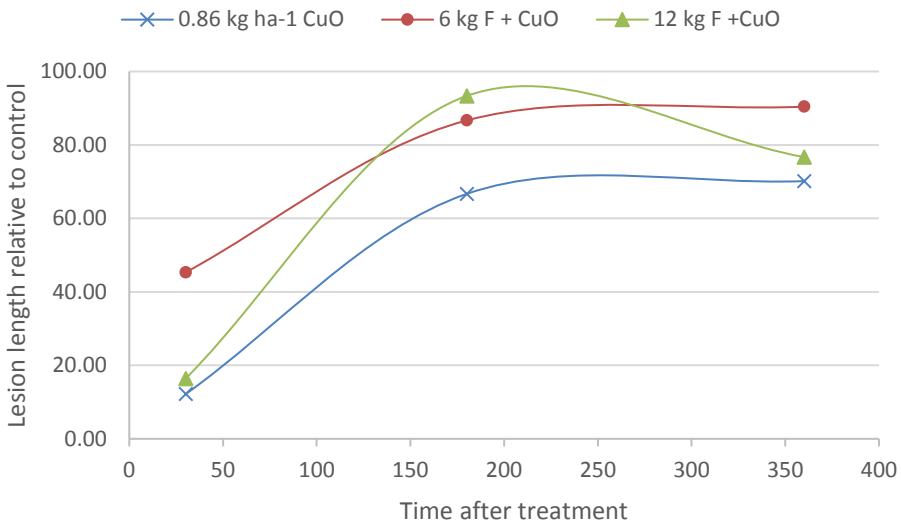

Figure 4. Lesion length in the treatments that included copper (1.72 kg ha^{-1} cuprous oxide; 6 kg ha^{-1} phosphite + 1.72 kg ha^{-1} cuprous oxide and 6 kg ha^{-1} phosphite + 1.72 kg ha^{-1}) expressed as a percentage of lesion length in the control for the duration of the trial.

Discussion

The results of this trial have both confirmed previous observations on the activity of phosphite and cuprous oxide, as well as raised some important questions. Firstly, as consistently seen in previous trials, while the detached assay method provides an efficient means to test the efficacy of treatments, it also introduces elements of variability that affect our understanding of an already highly complex host:pathogen:treatment interaction. Not only do the number and vigour of infecting zoospores vary from assay to assay, but their interaction with the needle surface may not match what happens naturally. Host physiology and therefore response to infection may also vary between assays. Further, since the needles are detached at the time of inoculation, the response (measured in terms of lesion length and number) is only that resulting from active ingredient presence/activity on or in needles, and not (whole) plant defence mobilised in response to infection. The outcome of all these factors is a highly variable response to treatment within and between assays, making interpretation of results complex and potentially masking true treatment effects. New methods to induce lesion development without compromising host response, with reduced variability are needed for further trials.

The significant effect of cuprous oxide on lesion length and number observed at 30 days was not unexpected, and supports all previous trials with copper conducted in this research programme (Gous et al., 2015; Rolando et al., 2016). In a laboratory trial, all copper based fungicides tested were found to inhibit mycelial growth of *P. pluvialis* (Rolando et al., 2016). In two separate pot trials, testing either copper oxychloride (applied at 0.75, 1.5 and 4.5 kg a.i. ha^{-1} in 100 L water) or cuprous oxide (applied at 0.86 or 1.72 kg ha^{-1} in the equivalent of 5 L oil and water), lesion length was significantly reduced relative to that of the control for up to three months post-application. Further, these *in-vitro* observations were supported by a field trial where application of an operational treatment with cuprous oxide (@ 0.86 kg ha^{-1} in 5 L oil and water) at three sites resulted in a significant reduction in lesion length (>90%) at all three sites between assays conducted before and after the copper treatment (Gous et al., 2016).

Clearly, treatment of *P. radiata* needles with cuprous oxide has the potential to reduce infection with *P. pluvialis*. However, the mode of action of this active ingredient must be incorporated into any management plan. Cuprous oxide is a foliar fungicide with preventative action and must be present on needles before infection occurs (MacBean, 2012). The copper ions are taken up by spores and accumulate until a sufficiently high concentration is achieved to kill the spore – an activity that is limited to prevention of successful spore germination and infection. Further, it is unlikely that the copper will persist on the surface of needles over the entire period spores are produced, generally between March and September. In this regard, the full sensitivity of *P. pluvialis* to copper has yet to be determined.

There was no increase in efficacy or persistence of the cuprous oxide treatment when applied in conjunction with phosphite at either 6 kg ha^{-1} or 12 kg ha^{-1}. Little information could be found on the interaction of these two active ingredients when applied as a mix.

While there was a definite response to the rate of phosphite applied at the 30 and 180 day assays, the interpretation of this response was confounded by the size and number of the lesions in relation to the control, with no significant differences observed (see Figures 1 and 2). This could be an artefact of experimental design, high variability between clonal responses or a true response to treatment. While known to be systemic, the mode of action of phosphite is complex and affected by host plant physiology, with other authors also reporting a high variability in disease control (Taylor et al., 2011). For example Taylor et al (2011) found foliar applications of phosphite to potatoes provided effective control of *Phytophthora erythroseptica*, but that these results were highly variable. Further, the authors also reported that an increasing dose of phosphite did not always result in an increase in efficacy, a factor that was attributed to the complex mode of action of this chemical. Similarly, when testing phosphite for control of sudden oak death caused by *Phytophthora ramorum* in Oregon tanoak (*Notholithicarpus densiflorus*) Kanaskie et al. (2011) found that while foliar application of phosphite reduced lesion development on experimental material, that the rate of application had no effect on the response. Although most plants readily absorb and translocate phosphite, its activity in the plant is mediated by a number of factors, most important of which is the level of phosphate in the host plant (Thao and Yamakawa, 2012; Gómez-Merino and Trejo-Tellez, 2015). Host and soil nutrition were not monitored in this study, nor foliar levels of phosphite or phosphate, but in future studies with this active ingredient it is recommended that more resources be placed on understanding plant and soil nutrition, as well as the balance of phosphate and phosphite in host-plant tissues.

An increase in lesion length and number observed for the highest dose (24 kg ha^{-1}) at the twelve month assessment relative to the control and 12 kg ha^{-1} phosphite treatment is an interesting outcome and possibly reflects a protracted biostimulant effect of phosphite on host plant foliage when applied at the highest rate tested (Thao and Yamakawa, 2012; Gómez-Merino and Trejo-Tellez, 2015). This outcome deserves some attention to understand the complex metabolism of phosphite in the plant, especially as a similar response has also been observed in a field trial at Kaingaroa. There is extensive debate about the role and use of phosphite as a 'fertilizer' in plants, as plants cannot directly use phosphite as a source of phosphorous, only phosphate (and therefore the so-called "fertilizer" affect represents a conundrum!). However, there is evidence that, applied at the certain levels, phosphite has the potential to enhance different metabolic processes to that of phosphate and thereby positively affect yield, quality (in the case of horticultural crops) and abiotic stress tolerance (Gómez-Merino and Trejo-Tellez, 2015). The underlying mechanisms driving these responses are complex and yet poorly understood, and may be related to the level of phosphate in the plant at the time of phosphite application. Microbial conversion of phosphite to phosphate can occur in the soil, a process which can take between three to four months, thereby increasing the availability of phosphate to the plant with a resulting longer term positive effect on growth (i.e. the "the fertilizer" affect). Further, foliar conversion of phosphite to phosphate after foliar applications has also been reported, although as with soil conversion, this is expected to be a slow process (Lovatt and Mikkelsen, 2006). Consolidation of the phosphite work on *P. radiata*, and more long term studies may provide further insight into this response, but it will be important to note relative increases in lesion length and or number where higher rates of phosphite have been applied.

The trend (although non-significant) of increased efficacy noted at the 30 and 180 day assay where an adjuvant was used in combination with phosphite indicates the adjuvant either increased the foliar uptake of phosphite or was toxic to *P. pluvialis*, a factor which can be tested in the laboratory. Previous studies conducted by Horgan and Gaskin (2015) indicated that application of Actiwett at 0.2% in combination with phosphite applied as Foschek™ resulted in a non-significant increase in uptake, particularly at lower rates of phosphite (6 kg ha^{-1} and 12 kg ha^{-1}). The results of this study, together with that of Horgan and Gaskin (2015) would indicate that the use of an alcohol ethoxylate in combination with Foschek™ may enhance fungicidal activity.

Finally, it is worth noting the shift in response to phosphite and copper over time (from the 30 day assay to the 360 day assay) reflects the different modes of action of these active ingredients and possibly the role they could play in a disease management programme. As a protectant copper, potentially provides an avenue to prevent infection when inoculum levels are high. However, over a single infection period this level of protection decreases. As a systemic and activator of host-plant defence mechanisms phosphite has the potential to prevent infection over a longer period, as seen in this trial. Assay protocols able to test the whole plant response to infection with *P. pluvialis* are needed to fully understand the potential of this active ingredient as a prophylactic for control of red needle cast.

Take home points

- Both phosphite (applied as Foschek™) and copper (applied as cuprous oxide) have the potential to reduce lesion development on *P. radiata* needles caused by infection with *P. pluvialis:*
    - Applied at 1.72 kg ha^{-1} cuprous oxide significantly reduced lesion development (lesion length and number) in a detached needle assay for at least one month after application.
    - Although not significantly different from the control, lesion length and number reduced with increasing phosphite dose from 6 kg ha^{-1} to 24 kg ha^{-1} up to six months after application. The fewest and smallest lesions were recorded at twelve months after application for the 12 kg ha^{-1} phosphite treatment.
    - The use of an adjuvant (Actiwett) increased the efficacy of the phosphite treatment.
    - Application of cuprous oxide and phosphite in a mix did not improve the efficacy of either active ingredient applied alone.
- Phosphite has a complex mode of action and further work is needed to fully understand the response of *P. radiata* and *P. pluvialis* to this active ingredient.
- Our findings are limited by the detached assay process and future trials should aim to use new inoculation protocols where possible.

Further work is required on both active ingredients to fully understand the host:pathogen:treatment interaction.

- As a foliar fungicide with preventative action copper oxide appears to be very effective against infection with *P. pluvialis* in the short-term. The full sensitivity of *P. pluvialis* to copper has yet to be determined, but applied at rates at or above that currently used for the operational treatment for *Dothistroma* needle blight (0.86 kg a.i. ha^{-1} and above), efficacy up to one month has been confirmed in several trials.
- The complex mode of action of phosphite renders this a more difficult active ingredient to assess. If effective, the benefit of using this active ingredient is its persistence in the plant as well as its acceptability to FSC. Based on the results of this trial, the most promising rate would appear to be 12 kg ha^{-1} phosphite. Future work with phosphite should allocate more resources to understanding plant and soil nutrition, as well as the balance of phosphate and phosphite in host-plant tissues.

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Appendix A

Table 1. Summary of th**e** ANOVA table of the lesion length models one, six and twelve months after chemical treatment

Parameter	DF	One month	Six month	Twelve month
Clone	3	0.977	0.837	0.07
Chemical treatment	9	< 0.001***	0.047*	0.007**
Clone × chemical treatment	27	0.201	0.153	0.964

Table 2. Summary of th**e** ANOVA table of the lesion count models one, six and twelve months after chemical treatment

Parameter	DF	One month	Six month	Twelve month
Clone	3	0.055	0.449	0.1290
Chemical treatment	9	<0.001	0.020*	<0.006***
Clone × chemical treatment	27	0.275	0.086	0.848