Needle disease strategy Research Programme

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Evaluation of foliar applied copper to control red needle cast disease in *P. radiata*

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Executive summary

Objective

The objective of this work was to determine the efficacy of foliar applied cuprous oxide to control red needle cast (RNC), a foliar disease of *Pinus radiata* caused by *Phytophthora pluvialis*.

Key Results

Cuprous oxide applied as a protective foliar fungicide at 0.86 kg/ha and 1.72 kg/ha metal equivalent copper significantly reduced lesion development caused by *P. pluvialis* on *P. radiata* needles in laboratory assays.

The higher 1.72 kg/ha metal equivalent copper treatment provided significantly better RNC control after 84 days than the 0.86 kg/ha metal equivalent copper treatment.

There was a significant interaction between copper rate and time after application; therefore it is possible that higher copper rates might result in longer control of RNC.

The use of copper to control dothistroma needle blight is well recognized in commercial forestry, it should therefore be easy for the forest industry to accept and use this treatment to control RNC.

Implications of Results/Conclusions

Results suggest that copper, applied as a protective foliar fungicide, has great potential to control and manage RNC in radiata pine plantations. The use of copper is well known in New Zealand radiata pine forestry and it is generally perceived as an environmentally acceptable fungicide treatment.

Further Work

The use of different spray mixtures to increase the effective protection period of cuprous oxide should be further examined. Validation of these results in additional nursery pot trials and on large trees in operational field trials is needed.

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Introduction

Red needle cast (RNC) is a foliar disease of *Pinus radiata* caused by *Phytophthora pluvialis*. It has the potential to cause up to 16% annual growth loss in severely infected plantations (Ganley et al. 2014). A cost-effective, FSC compliant, chemical control strategy is needed to provide a management option for the control of severe outbreaks of RNC in *P. radiata* forests.

Phosphite is a foliar applied, preventative, systemic fungicide known to be effective against diseases caused by *Phytophthora* species. It releases phosphonate ions that act as a catalyst to trigger the plant's natural defence mechanisms. It is the main fungicide currently being investigated for its potential to manage RNC. However, in a study to test fungicides (including phosphite) for their ability to control RNC, both the efficacy and persistence of copper, applied as a preventative treatment, showed the most promise and therefore warranted further investigation (Rolando et al. 2014).

This trial studied the efficacy and persistence of two copper rates to control RNC.

Methods

Description of treatments

The experiment was set up as a 3 $\times$ 3 fully replicated factorial design, using three copper concentrations (control, 0.86 kg ha^{-1} cuprous oxide, or 1.72 kg ha^{-1} cuprous oxide) and three *P. radiata* clones (887-841/ 875-242/ 268-262) and 15 single tree replications. The three clones were all known to be susceptible to *P. pluvialis*. Needles were sampled and exposed to *P. pluvialis* at seven and 84 days after copper application. All potted trees were kept outside under drip irrigation for the duration of the experiment.

Treatment application

The dothistroma standard (DS) treatment was applied as cuprous oxide, at 0.86 kg ha^{-1} metal equivalent, to the same specifications as for the standard commercial treatment for dothistroma needle blight (Bulman et al. 2013), using the PPCNZ conveyor belt track sprayer on 16 October 2014. A very fine droplet spectrum (ASABE 2009) was produced by using an ULVA + controlled droplet applicator (Micron Sprayers Ltd 2014), calibrated to deliver 5 L ha^{-1} and droplets with volume median diameter (VMD) of approximately 60-70 µm. The double dothistroma standard (DDS) treatment (1.72 kg ha^{-1} metal equivalent) was achieved by doing two passes through the track sprayer, rather than decreasing belt speed or altering flow rate, as this would certainly affect droplet size.

Plant assessments

Height and root collar diameters of all plants were measured at trial initiation and termination. Foliage heath assessments, in 10% increments, were recorded at the trial initiation and prior to each of the needle collections to ensure the plants were symptom free prior to exposure to *P. pluvialis* in the detached needle assays. Prior to spraying, the point where fully expanded needles began was marked on each branch. This was so that later in the trial, it was possible to differentiate between needles that were sprayed from new needles and needles that expanded since the treatments were applied.

Disease assessments

Detached needle assays were carried out as per the standard operating procedure to determine the efficacy of treatments at seven and 84 days post treatment. Twenty healthy fascicles were collected from each plant with five fascicles assigned at random to two independent blocks (A and B) per treatment (H_2O and *P. pluvialis*-inoculated). Each tube was inoculated with either 4.5 ml of *P. pluvialis* zoospore suspension (5 x 10^3 zoospores/ml) or sterile pond water overnight (18 hours). Fascicles were placed on trays moistened with wet paper towels and incubated in a controlled environment (17 ^{o}C, 65-70 % relative humidity, 14 h photoperiod) for 10 days. The needles within each fascicle were separated and lesions measured.

Data analysis

A linear mixed effects model (LMM) fitted by restricted maximum likelihood was used to analyse the lesion length data (Pinheiro et al. 2015; R Core Team 2015). The model contained copper treatment, clone identity, assessment date, and their interaction as fixed effects. The nested random term comprised assessment date nested in ramet to reflect the repeated measures nature of the data.

The significance of the fixed terms was assessed using a backwards selection procedure based on likelihood ratio tests (Zuur AF et al. 2009). Graphical model validation tools were used to test the underlying assumptions of variance homogeneity and normality (plots of standardised residuals vs. fitted values and against all explanatory variables to evaluate variance patterns, quantile-quantlie plots to assess the normality criterion). The data showed strong heteroscedasticity, which was modelled using a combination of a constant plus power variance function structure (varConstPower,) using the fitted values as variance covariate and copper treatment as grouping variable to allow for stratified variance modelling.

The significant copper treatment × clone × assessment date interaction was followed up applying a multiple comparison procedure using Tukey contrasts (Hothorn et al. 2008; R Core Team 2015). Specifically, copper treatments were compared within each clone and assessment date. Furthermore, assessment dates were compared within each treatment within clone.

Results

Plant health assessments

The first foliage heath assessments carried out at seven days post treatment indicated that, with the exception of four plants, all plants scored 0% needle browning. The aforementioned four plants all scored less than 10% browning. The second foliage heath assessments done at 84 days post treatment indicated that with the exception of six plants, all plants scored a 0% needle browning. Five of these plants scored less than 10% browning and one plant scored less than 20% browning. The above observed damage was totally random and not confounded to any treatment.

Lesion development

The control developed pronounced lesions, on average between 12 and 23 mm per fascicle (Fig. 1). Copper treatment largely suppressed lesion development and this effect varied with clone and time. There was a significant interaction between copper treatment, clone and assessment date (Table 2, Fig. 1). In two of the three radiata clones (268-262, 875-242), the standard copper solution used for dothistroma needle blight control applied twice proved to be a significant improvement over the standard application 84 days after inoculation (Fig. 1).

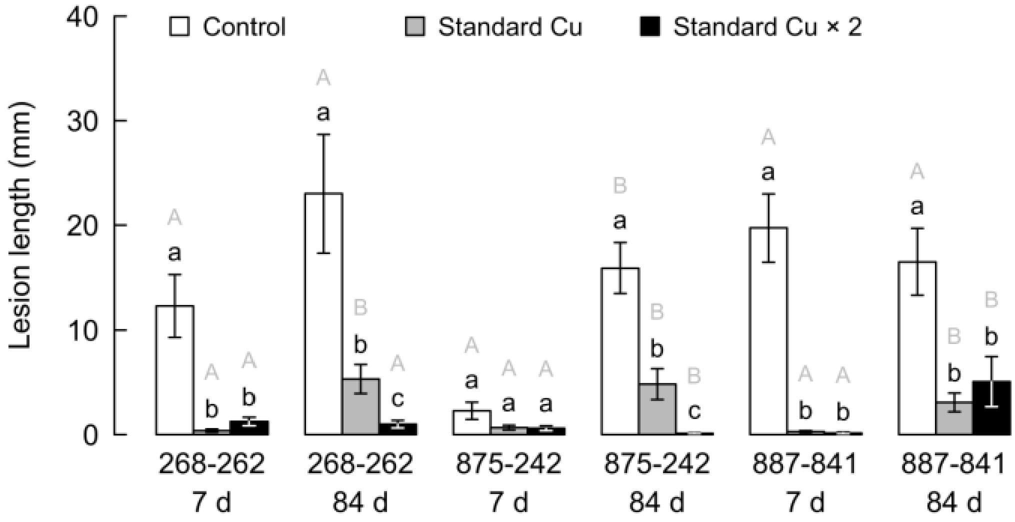

Fig. 1 - Average lesion length per fascicle in *Pinus radiata* needles inoculated with *Phytophthora pluvialis*. X-axis labels: six-digit numbers indicate clone identities; 7 d = assessed seven days after inoculation, 84 d = assessed 84 days after inoculation. Control = *Phytophthora*-inoculated samples not treated with copper, Standard Cu = standard copper solution used for *Dothistroma* control, Standard Cu $\times$ 2 = standard copper solution applied twice. Different lower-case letters indicate statistically significant differences between copper treatments within clone and assessment date. Different upper-case letters (grey) indicate statistically significant differences between assessment dates within copper treatment and clone (multiple comparison procedures using Tukey contrasts at $\alpha = 0.05$).

Table 2 - ANOVA table of the final model (DFnum = numerator degrees of freedom, DFden = denumerator degrees of freedom, F = F-value, P = P-value).

Parameter	DFnum	DFden	F	P	
Intercept	1	138	43.35	< 0.001	***
Treat	2	138	30.01	< 0.001	***
Clone	2	138	1.89	0.155	
Date	1	9	0.01	0.919	
Treat × clone	4	138	15.26	< 0.001	***
Treat × date	2	138	22.16	< 0.001	***
Clone × date	2	138	0.26	0.769	
Treat × clone × date	4	138	3.67	0.007	**

Discussion & Conclusions

This trial highlighted that copper applied as cuprous oxide at 0.86 kg ha^{-1} (DS) and 1.72 kg ha^{-1} (DDS) metal equivalent copper, significantly reduced lesion development caused by *P. pluvialis* on *P. radiata* needles in laboratory assays. There is strong evidence that the DDS treatment provided significantly better RNC control after 84 days than the DS treatment. Given the strong and significant interaction between copper treatment and time after application, it is likely that higher copper doses will result in longer control of RNC. As well as being an effective treatment, the use and application of copper is a well-known and documented treatment to control dothistroma needle blight in commercial forestry. Therefore it should be readily accepted by forest growers and easy to employ to control RNC. These results need to be validated in field trials next summer before any field recommendations are made.

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