Research

Can Copper Be Used to Treat Foliar *Phytophthora* Infections in *Pinus radiata*?Carol Rolando,^{1,†} Chanatda Somchit,¹ Martin K-F. Bader,² Stuart Fraser,¹ and Nari Williams¹¹ Scion, New Zealand Forest Research Ltd., Rotorua 3010, New Zealand² School of Science, Auckland University of Technology, Auckland 1010, New Zealand

Abstract

Red needle cast is a significant foliar disease of commercial stands of *Pinus radiata* caused by *Phytophthora pluvialis* in New Zealand. The effect of copper, applied as a foliar spray of cuprous oxide at a range of doses between 0 and 1.72 kg ha⁻¹, was investigated in two controlled trials with potted plants and in an operational trial with mature *P. radiata*. In all trials, lesions formed on needles after artificial exposure to the infecting propagules (zoospores) of *P. pluvialis* were used to determine treatment efficacy, with the number and/or length of lesions as the dependent variable. Results across all trials indicated that cuprous oxide was highly effective at reducing infection of *P. radiata* with *P. pluvialis*. Application rates equivalent to ≥ 0.65 kg ha⁻¹ significantly reduced infection levels relative to a control treatment, with foliar surface copper levels as low

as 13 to 26 mg kg⁻¹ of needle tissue preventing infection. Greater copper content was associated with a reduction in the proportion of needles with *P. pluvialis* lesions, with the probability of lesions developing decreasing approximately 1% for every 1 unit (in milligrams per kilogram) increase in copper content. Over a 90-day period, surface copper content declined to 30% of that originally applied, indicating an approximate period of treatment efficacy of 3 months. Our findings highlight the potential of cuprous oxide for the control of red needle cast in *P. radiata* stands. Further information about the optimal field dose, timing, and the frequency of foliar cuprous oxide application is key to prevent infection and also reduce the build up of inoculum during severe outbreaks of this pathogen.

Copper has been applied as a fungicide in one form or another for controlling plant disease since at least the 18th century (Morton and Staub 2008). A variety of different forms of copper have been used for disease control, including copper hydroxide [Cu(OH)₂], copper oxychloride [3Cu(OH)₂·CuCl₂], copper sulfate [CuSO₄·5H₂O], and cuprous oxide [Cu₂O] (MacBean 2016). Copper fungicides are foliar active, broad-spectrum preventative fungicides that have been effective in controlling a wide range of diseases, including those caused by fungi, oomycetes, and bacteria. On application of copper fungicides to foliage, solubilization of the initially insoluble copper compound occurs, which allows contact of copper ions with germinating spores and bacteria-disrupting enzyme systems and prevents further cellular and mycelial development (Franich and Wells 1975; Giannousi et al. 2013). Copper ions must be present on the crop before fungal spores germinate (MacBean 2016). After application, cycles of resolubilization and drying locally redistribute copper ions on individual leaves, improving ion-spore contact and disease control (Hislop and Cox 1970). Copper compounds generally adhere strongly to plant surfaces after application, even after rainfall events (Bulman and Carlson 2011; Franich and Wells 1975).

Phytophthora species are among the most serious pathogens in both native and production plant systems, with outbreaks often having devastating ecological and economic effects (Davis et al. 2014; Guenther et al. 2001). *Phytophthora* pathogens are not true fungi, yet the sensitivity of *Phytophthora* species to copper fungicides is widely reported (Babadoost et al. 2015; Coelho et al. 2005; Lucero

et al. 2014). This active ingredient has been used to manage many *Phytophthora* species-related diseases, with primary success in controlling foliar infecting *Phytophthora* species (Coelho et al. 2005; Giannousi et al. 2013; Lawrence et al. 2017; Rolando et al. 2017a). Copper has also been used to control the foliar pathogen *Phytophthora infestans* (Mont.) de Bary, which causes late blight in plants belonging to the Solanaceae family (tomato, potato, eggplant, and peppers) (Giannousi et al. 2013). Although copper has been successfully applied to control ink disease of sweet chestnut (*Castanea sativa* Mill.) caused by the soilborne pathogens *Phytophthora cambivora* (Buisson Petri) and *Phytophthora cinnamomi* Rands (Coelho et al. 2005; Vetrano 2010), its use for the management of soilborne infections is limited and needs very careful consideration within natural ecosystems.

Phytophthora pluvialis Reeser, W.L. Sutton and E.M. Hansen is the causal agent of red needle cast in stands of *Pinus radiata* D. Don in New Zealand (Dick et al. 2014; Reeser et al. 2013). *P. radiata* accounts for 90% of plantation forest in New Zealand and as such, this disease has the potential to incur severe economic effects domestically (NZFOA 2016). Red needle cast was first observed in *P. radiata* stands on the east coast of New Zealand in late April 2008 (Dick et al. 2014). Since then, the disease has been reported from sites across both the North and South Islands, but with most damage observed in the center and the northeast coast of the North Island. Disease occurrence is often sporadic, with severe outbreaks occurring approximately every 3 years. Symptoms of red needle cast typically first appear in autumn (March to May) and are characterized by the development of olive-colored lesions on affected needles, which are frequently accompanied by resinous black bands. Affected needles quickly turn orange to brown, lending a reddish hue to the affected crown before being cast prior to the emergence of new needles in late spring. Studies have shown that severe defoliation events associated with red needle cast can reduce tree growth by up to 38% in the year after defoliation, with a further 10% loss in the next year provided that no further infection occurs.

Following the discovery of red needle cast in New Zealand, research was initiated to identify chemical options for management (Rolando et al. 2014, 2017a, 2017b). Early in vitro studies and in planta experiments identified copper as one of three active

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ingredients, along with metalaxyl-M and phosphite, that showed promise as a control agent for red needle cast (Rolando et al. 2017a). This was an important outcome from the initial screening studies because copper is already widely used in New Zealand for the control of *Dothistroma* needle blight, caused by the true fungus *Dothistroma septosporum* (Dorog.) M. Morelet, on *P. radiata*. Current industry practice is to apply cuprous oxide aerially in November (spring) to stands where an average of 10 to 20% of the total crown is affected by the disease. The fungicide is applied with a helicopter at an equivalent rate of 0.855 kg ha⁻¹ active ingredient, in 2 liters of oil per hectare made up to 5 liters per hectare with water (Bulman et al. 2013, 2016).

Following the initial *in vitro* and *in planta* studies, which were limited in the dose applied and also formulation, it was necessary to carry out further work to explore the efficacy of copper (applied as cuprous oxide) as a prophylactic treatment or management tool for red needle cast in *P. radiata*. This article details a combination of a pot trial and an operational field trial carried out to determine the response of *P. pluvialis* to cuprous oxide applied to *P. radiata* foliage as per the current formulation used by the New Zealand forest industry for control of *Dothistroma* needle blight. A further pot trial was carried out to evaluate the full dose response of *P. pluvialis* to cuprous oxide applied in oil to *P. radiata* foliage.

Materials and Methods

Experiment one: Copper applied at the industry standard and two times the industry standard. A 3 × 3 fully replicated factorial design was established using three copper (AG Copp 75; American Chemet Corporation) concentrations (0 kg ha⁻¹ control-oil only; 0.86 kg ha⁻¹ cuprous oxide, industry standard; or 1.72 kg ha⁻¹ cuprous oxide, two times the standard) and three potted (4.6-liter pots) *P. radiata* clones (A, B, and C). Each treatment comprised 15 single tree replications where the average height and diameter of the seedlings was 80 and 0.14 cm, respectively. The three clones were all known to be susceptible to *P. pluvialis*. The cuprous oxide treatments all contained 2 liters of mineral oil (BP Dothi oil) made up to 5 liters of total volume with water. All treatments were applied using a conveyor belt track sprayer. A very fine droplet spectrum (American Society of Agricultural and Biological Engineers 2009) was produced by using an ULVA+ controlled droplet applicator (Micron Sprayers Ltd. 2014), calibrated to deliver 5 liters per hectare and droplets with a volume median diameter of approximately 60 to 70 μm. The 1.72 kg ha⁻¹ cuprous oxide treatment was achieved by doing two passes through the track sprayer, rather than decreasing the belt speed or altering the flow rate, as this would affect the droplet size. After treatment with cuprous oxide in October 2014, plants were placed in the Scion nursery in Rotorua, New Zealand, where they were exposed to ambient conditions. Plants were also drip irrigated for 3 months to prevent excessive drying out of the pots. Needles were sampled and exposed to *P. pluvialis* in detached needle assays at 7 and 84 days.

The protocol for conducting detached needle assays with *P. pluvialis* was described in Rolando et al. (2014, 2017a). Briefly, five fascicles were collected from each potted ramet and exposed to zoospores of *P. pluvialis* overnight. Following exposure, fascicles were placed on trays moistened with wet paper towels and incubated in a controlled environment (17°C, 65 to 70% relative humidity, 14-h photoperiod) for 10 days. The needles within each fascicle were separated and the lesion number and length were assessed.

A subsample of the needles collected for the detached needle assays was submitted for determination of surface copper content. For each assessment, needles were bulked from replications 1 to 5, 6 to 10, and 11 to 15, respectively. Approximately 2 g dry weight of each sample was used for the copper analyses. The needles were digested in 1:1 10% HCl/methanol solution and analyzed by atomic absorption spectroscopy. Each bulk sample was digested in triplicate.

Experiment two: Field trial using the operational copper treatment. Current industry standard copper treatments were applied in mid-November 2015 to three different *P. radiata* stands in the central North Island region of New Zealand, aged 4 years (Kinleith), 8 years (Te Manawa O Tuhoe [TMOT]), and 15 years (Waiotapu). Treatments consisted of cuprous oxide (AG Copp 75) applied in very

fine droplets (using Micronair nozzles) by helicopter (Iroquois Bell) at 0.86 kg ha⁻¹ metal equivalents in 2 liters of mineral oil made up to 5 liters per hectare total volume with water (Baillie et al. 2017). Prior to treatment application, 20 random trees were selected and labeled at each site. Three branchlet subsamples were taken from each tree before (1 week) and after (1 month) treatment application to be used in detached needle assays with *P. pluvialis*. Each assay was carried out immediately after needle collection. For each assay, 10 fascicles were selected from each branchlet and dipped into sterilized pond water or a solution containing *P. pluvialis* zoospores overnight, after which they were placed in a controlled temperature room for 10 days as per standard detached needle assay protocols (Rolando et al. 2014, 2017a). After incubation for 10 days, the lesion number and length were assessed.

On the day of spraying, needle samples for determination of surface copper content were collected after spraying. Samples were collected from the 20 trees selected for the detached needle assay assessments and analyzed as described above.

Experiment three: Copper dose-response trial. Using the conveyor belt track sprayer (described above), copper (AG Copp 75) treatments (0, 0.22, 0.43, 0.65, 0.86, and 1.72 kg ha⁻¹ cuprous oxide) representing application rates up to two times the recommended application rate were applied in oil (2 liters per hectare), made up to 5 liters per hectare total volume with water, to three commercial genotypes of *P. radiata* obtained from a local nursery. As a result of plant availability, these were not the same genotypes as the commercial clones used in experiment 1. One-year-old ramets were lifted in November 2016 and potted in 4.6-liter pots; they were 29.6 cm tall on average, with a root collar diameter of 0.06 cm at the time of trial initiation in late March 2017. There were two control treatments, one that included spraying with water and oil only and another that did not receive any treatment (water or oil). During the application of each treatment, six steel plates (115 cm²) were placed on the conveyor belt to estimate the actual amount of copper applied (dose applied in kilograms per hectare). These were submitted to the laboratory where copper content was determined using atomic absorption spectroscopy.

Following treatment with copper, the potted ramets were placed into one of two fog chambers (inoculated or control) for 12 weeks,

Fig. 1. A and B. Average lesion length per fascicle in *Pinus radiata* needles inoculated with *Phytophthora pluvialis*. 7 d = assessed 7 days after inoculation, 84 d = assessed 84 days after inoculation. **A.** Different lowercase letters indicate statistically significant differences between copper treatments within clone and assessment date. **B.** Different uppercase letters indicate statistically significant differences between assessment dates within copper treatment and clone (multiple comparison procedures using Tukey contrasts at $\alpha = 0.05$).

each with an identical experimental layout. There were six replications of each treatment in each chamber, with each replication consisting of three ramets (one of each clone). Plants were placed into the respective fog chambers 3 days prior to the first inoculation on 20 April 2016 (and exactly 1 week after spraying with copper). To initiate infection, plants were sprayed to run off with a suspension of *P. pluvialis* zoospores at 1×10^4 zoospores ml^{-1} . To increase the likelihood of infection, *P. pluvialis* mycelial mats produced in a broth were evenly spread over fly mesh stretched 30 cm above the potted plants and left for 24 h with ongoing fogging to induce further zoospore release and opportunities for infection. Production of zoospores was previously described (Rolando et al. 2014, 2017a). Fog chamber environmental conditions were set at a 14-h/10-h day/night schedule, with fog applied for 1 min every 20 min. The temperature was set at 17°C. There were two inoculation events in the trial; the first

was carried out a week after spraying (and 1 week before the first assessment), and the second, 7 weeks after the initial inoculation.

Plants were assessed for signs of infection at 1, 5, 8, and 12 weeks after the initial infection. At each assessment, three fascicles, each containing three needles, were randomly selected from each ramet and scored for the number of needles with at least one lesion caused by *P. pluvialis* (i.e., maximum score of 3 per fascicle). Foliage samples for the determination of the surface copper content were also submitted to the laboratory immediately after spraying with copper and at the 5-week and 12-week disease assessments (6 weeks and 13 weeks after spraying). For the first analysis, surface copper content was analyzed at three different scales: using the above-ground biomass, 2 g of needle tissue, or for a single fascicle only. All further samples used 2 g of needle tissue (which was standard laboratory practice).

Statistical analysis. For experiment one (copper applied at the industry standard and two times the industry standard), a linear mixed-effects model fitted by restricted maximum likelihood was used to analyze the lesion length data (Pinheiro et al. 2015; R Core Team 2016). The model contained copper treatment (control, standard copper, and copper applied at two times the industry standard), clone identity (A, B, and C), assessment date (7 or 84 days), and their interaction as fixed effects. The nested random term comprised assessment date nested in the ramet to reflect the repeated-measures nature of the data. Graphical model validation tools were used to test the underlying assumptions of variance homogeneity and normality (plots of standardized residuals versus fitted values and against all explanatory variables to evaluate variance patterns, quantile-quantile plots to assess the normality criterion). The data showed strong heteroscedasticity, which was modeled using a combination of a constant plus power variance function structure (varConstPower), using the fitted values as the variance covariate and copper treatment as the grouping variable to allow for stratified variance modeling. The significant copper treatment $\times$ clone $\times$ assessment date interaction was followed up applying a multiple-comparisons procedure using Tukey contrasts (Hothorn et al. 2008; R Core Team 2016).

For experiment two (field trial testing operational copper treatment), a generalized least squares model fitted by restricted maximum likelihood was used to analyze the lesion lengths resulting from *P. pluvialis* inoculation (R version 3.3.0, R Core Team 2016; R-package nlme, Pinheiro et al. 2015). The model comprised site (Kinleith, TMOT, and Waitapu), spray (pre- and postcopper aerial spray), and inoculation treatment (control = sterilized pond water, pathogen = *P. pluvialis*) and all interactions as explanatory variables. Graphical model validation tools were used to assess the underlying assumptions of variance homogeneity and normality

Table 1. Analysis of variance table of the final model used for experiment 1 showing the impact of the industry standard treatment and the two times the industry standard treatment on the size of *Phytophthora pluvialis* lesions on *Pinus radiata* needles

Parameter	Numerator df	Denominator df	F value	P value ^z
Intercept	1	138	43.35	<0.001*
Treatment	2	138	30.01	<0.001*
Clone	2	138	1.89	0.155
Date	1	9	0.01	0.919
Treatment $\times$ Clone	4	138	15.26	<0.001*
Treatment $\times$ Date	2	138	22.16	<0.001*
Clone $\times$ Date	2	138	0.26	0.769
Treatment $\times$ Clone $\times$ Date	4	138	3.67	0.007**

^zSignificant values are denoted as follows: * $P < 0.001$ and ** $P < 0.01$. df = degrees of freedom.

Table 2. Needle surface copper content at 7 and 84 days after treatment in experiment 1^z

Treatment (kg ha^{-1})	7 days	84 days
Control	0.0	0.0
0.86	50.0 ± 10.0	20.0 ± 0.0
1.72	150 ± 50	60 ± 0.04

^zData are presented as means $\pm$ standard deviation (in milligrams per kilogram).

Fig. 2. Average lesion length in *Pinus radiata* fascicles collected either before (control) or 1 month after aerial copper spray application (Cu) and inoculated with *Phytophthora pluvialis*. Spray applied at three sites, with 20 trees assessed per site. Different lowercase letters indicate statistically significant ($P < 0.05$) differences within sites (based on a multiple comparison procedure using Tukey contrasts following the significant site $\times$ spray $\times$ inoculation interaction). TMOT = Te Manawa O Tuhoe.

(plots of the standardized residuals versus fitted values and against all predictors, quantile-quantile plots). Variance heteroscedasticity was detected and accounted for by incorporating a constant variance structure using spray and inoculation treatment as variance covariates, allowing for stratified variance modeling. A significant three-way interaction term was sliced using a multiple comparison procedure with Tukey contrasts (R-package multcomp, Hothorn et al. 2008). The slicing was performed in two ways, allowing the comparisons between (i) the pathogen treatment levels within copper treatment levels at each site (i.e., H₂O versus *P. pluvialis* within “control” and within “Cu₂O” at each site) and (ii) between the copper treatment levels within the pathogen treatment levels at each site (i.e., “control” versus “Cu₂O” within the H₂O control plants and within the *P. pluvialis*-infected plants at each site). The resulting *P* values were corrected for multiple testing using the Benjamini and Hochberg (1995) method.

For experiment three (copper dose-response trial), generalized linear mixed models (GLMMs) were used to investigate the efficacy of cuprous oxide dose (in kilograms per hectare) on the proportion of *P. pluvialis* lesions (out of nine needles) present on the selected needles (assuming a binomial error and logit link). The fixed effect variables were “inoculation treatment,” “cuprous oxide dose,” and the interaction between these two explanatory variables. Ramets nested in “replications” in “inoculation treatment” were included as a random effect. Graphical tools such as the Pearson residual plots were used to test for model validation. Overdispersion occurred and was modeled using a beta binomial GLMM (Zuur et al. 2009). The model parameters were estimated using Laplace approximation. Model selection for the fixed effect terms was based on backward selection using the Akaike information criterion and likelihood ratio tests (Zuur et al. 2009). The model was fitted using the R *glmmADMB* package (Skaug et al. 2015) and analyzed separately for each time period (week 1, 5, 8, and 12 assessments). Because no significant difference in lesion number was found between the two application control treatments, these were combined for all further analyses.

There was a significant response at week 5 to applied cuprous oxide dose (in kilograms per hectare) as assessed in the proportion of sampled needles with lesions of *P. pluvialis*. We used the surface copper content of foliage assessed immediately after spraying of copper, and at the time of first inoculation, to determine the relationship between foliar copper content at the time

of inoculation and presence of *P. pluvialis* at week 5. A quasi-binomial GLMM was performed using R-Mass (Ripley et al. 2015) for this analysis, with model checks and validation carried out as described above.

Results

Experiment one: Copper treatments applied at the industry standard and two times the industry standard. In the detached needle assays, the control developed pronounced lesions, on average between 12 and 23 mm long per fascicle (Fig. 1). Both copper treatments largely suppressed lesion development but this effect varied with clone and time. There was a significant interaction between copper treatment, clone, and assessment date (Fig. 1; Table 1). This interaction was caused by clone B, for which there was no significant effect of the copper treatment on lesion length at 7 days, a result largely dependent on the small lesions that developed on the control needles. Copper dose (0.844 kg ha⁻¹ or 1.76 kg ha⁻¹) had no effect on lesion development at 7 days. In two of the three *P. radiata* pine clones (A and B), double the standard copper treatment proved to be a significant improvement over the standard application 84 days after inoculation (Fig. 1). It is likely that surface copper levels remained above the threshold levels for infection to occur in this treatment.

Seven days post-application, the copper recovery from the needle surface indicated copper levels in the two times the industry standard treatment (1.76 kg ha⁻¹ cuprous oxide) to be approximately three times more than that in the standard treatment (0.86 kg ha⁻¹ cuprous oxide) (Table 2). After 84 days of exposure to overhead irrigation and natural elements, very little of the original applied copper was still

Table 3. Results from model comparison procedures using the beta binomial generalized linear mixed models for the proportion of *Phytophthora pluvialis* lesions in response to copper dose (backward selection using the Akaike information criterion and likelihood ratio tests)^a

Dropped term	AIC	df	LR	<i>P</i> value
None	276.84			
Room × cuprous oxide dose	273.89	5	6.87	0.230
Room	270.76	1	0.17	0.676
Cuprous oxide dose	268.94	5	28.30	<0.001*

^a AIC = Akaike information criterion, df = degrees of freedom, and LR, likelihood ratio test. Significant values are denoted as follows: *P < 0.001.

Fig. 3. Predicted incidence of *Phytophthora pluvialis* lesions at the week 1, 5, 8, and 12 assessments in response to cuprous oxide dose (in kilograms per hectare) for the duration of the trial. Bars indicate the standard error and arrows indicate inoculation days.

present on the needle surface, yet this was sufficient to suppress lesion development in the detached needle assay (for both treatments).

Experiment two: Field trial using operational copper treatment. Prior to the application of the operational copper treatment (0.86 kg ha⁻¹ cuprous oxide in oil), *P. radiata* needles treated with sterilized pond water showed minimal lesion development across sites, while exposure of needles to *P. pluvialis* in the detached assay resulted in substantial lesion development (Fig. 2). This initial sampling established the baseline susceptibility of the trees to infection with *P. pluvialis*. The length of the lesions, however, differed by site. This could be attributed to a difference in susceptibility in the genotypes planted at each site or stand age or needle physiology.

Needles collected 1 month after aerial application of copper oxide showed suppressed lesion development relative to the prespray samples at all three sites (Kinleith, TMOT, and Waiotapu) (Fig. 2). Suppression was 100, 96, and 90% at Kinleith, TMOT, and Waiotapu, respectively. Together these findings produced a significant site × spray × inoculation interaction (likelihood ratio statistic [L] = 14.78, 2 degrees of freedom, *P* < 0.001). Surface copper content of needles just after spraying was 26.5, 22.0, and 13.5 mg kg⁻¹, respectively.

Table 4. Results from the beta binomial generalized linear mixed models for the proportion of *Phytophthora pluvialis* in the copper dose-response trial^z

Parameter	Estimate	SE of the estimate	z statistic	P value
Fixed effects				
Intercept	-2.50	0.426	-5.88	<0.001*
Cuprous oxide dose (kg ha ⁻¹)				
0.22	0.07	0.364	0.20	0.845
0.43	-1.56	0.586	-2.67	<0.01**
0.65	-1.20	0.525	-2.29	<0.05***
0.86	-0.87	0.458	-1.91	0.055
1.72	-1.65	0.644	-2.57	<0.05***
Random effects				
Random intercept	Estimated variance of 1.32			

^z Models included ramets, nested in “replications” in “inoculation treatment,” as a random effect. Significant values are denoted as follows: *P < 0.001, **P < 0.01, and ***P < 0.05.

Experiment three: Dose-response trial. Following inoculation, the greatest numbers of *P. pluvialis* lesions were observed at the week 5 assessment, followed by the week 12 assessment (Fig. 3). Both of these assessments were conducted 5 weeks after an inoculation. Lower numbers of lesions were scored for the assessments carried out in weeks 1 and 8, each 1 week after an inoculation. This result may reflect that each inoculation event triggered one cycle of disease that took around 3 to 5 weeks to fully express but which did not progress (i.e., become polycyclic) or that infected needles were cast and not scored.

There was no statistical evidence of an effect of cuprous oxide on symptom expression at weeks 1, 8, and 12. A statistically significant effect of dose (applied in kilograms per hectare) on infection level was observed in week 5 (Tables 3 and 4). The threshold for a significant reduction in infection at the week 5 assessment fell between the 0.22 and 0.43 kg ha⁻¹ copper treatments, equating to between ~30 to ~60 mg kg⁻¹ of surface copper content (either whole plant, 2-g sample, or one fascicle only) at the time of infection (Fig. 4; Table 5). Despite this reduction in the amount of surface copper, the infection level at week 5 was reduced by about 90% in contrast to both the control and 0.22 kg ha⁻¹ dose. There was no significant dose response at the week 12 assessment (Fig. 3). Surface foliar copper content determined at the week 5 assessment was between 26 and 60 mg kg⁻¹ of copper on all treatments except the highest dose, where content was still >100 mg kg⁻¹ (Fig. 4). The second inoculation was carried out 7 weeks after spraying when copper would have been at or just below these levels, and possibly still high enough to prevent infection. Levels of surface copper had declined to ≤60 mg kg⁻¹ for all treatments by the final assessment at week 12 (Fig. 4).

Analysis of the proportion of lesions assessed at week 5 in relation to surface copper content at the time of spraying (Table 5) indicated a highly significant effect of foliar surface copper content on the proportion of *P. pluvialis* lesions (*P* < 0.01). Greater surface copper levels were associated with a reduction in the proportion of needles with *P. pluvialis* lesions, with the probability of lesions developing decreasing approximately 1% for every 1-unit increase in copper content (in milligrams per kilogram) (Fig. 5).

Discussion

All of the trials described provide strong evidence that cuprous oxide is effective at reducing symptoms of red needle cast on *P. radiata*. The amount of copper present on the surface of mature *P. radiata* foliage after operational application of cuprous oxide

Fig. 4. Needle surface copper content determined by atomic absorption spectroscopy in experiment 3. Arrows indicate inoculation events.

in November for control of Dothistroma needle blight is around 20 to 30 mg kg⁻¹ (Bulman and Carlson 2011; Gilmour and Noorderhaven 1973), as shown for the foliage samples collected shortly after spraying in the operational trial (experiment 2). This surface copper content is substantially lower than the surface levels of copper detected in the dose-response trial (experiment 3), where equivalent rates of cuprous oxide were applied to the foliage (0.86 kg ha⁻¹), and is also below the threshold of sensitivity identified in the same trial for prevention of *P. pluvialis* infection, estimated at between 30 and 50 mg kg⁻¹. The detached needle assay carried out 1 month after spraying in the operational spray trial indicated high levels of efficacy against infection with *P. pluvialis* despite the lower levels of surface copper present on these needles shortly after spraying (13 to 26 mg kg⁻¹ of copper). Lower levels of active ingredient on the surface of mature tree foliage than potted clones for the same application rate would be expected as a result of the differences in tree size and leaf area; however, the differential response does raise questions as to the minimum foliar surface copper content required to prevent infection of *P. radiata* with *P. pluvialis* in situ. Does *P. pluvialis* have a similar sensitivity to copper as *D. septosporum*? Relative sensitivities of the different pathogens would need to be confirmed, as well as whether the application rate of 0.86 kg ha⁻¹ cuprous oxide will be effective in the long term for management of red needle cast.

Research into Dothistroma needle blight indicated that the copper acted against the pathogen in two ways (Franich 1988). First, copper deposits on conifer needles dissolved slowly in water distributing copper ions across the needle surface, thereby killing spores released during rainfall events and also protecting foliage from new infections. The life cycle of *P. pluvialis*, an oomycete, is quite different from that of *D. septosporum*, an ascomycete. Fruit bodies are not

produced by *P. pluvialis* (or other *Phytophthora* species). Under wet conditions, transient hyphae will grow out onto the needle surface through stomata and produce sporangia, which give rise to infective zoospores that disperse in water on the needle surface (N. Williams, *personal communication*) and are presumed to spread in water splash based on demonstrated dispersal in similar systems. Despite this difference, copper is likely to have similar effects on both infection and sporulation by *P. pluvialis* to those described for *D. septosporum*. Copper on the needle surfaces will kill zoospores of *P. pluvialis* released during wet conditions, reducing infection. Copper on needle surfaces will likely also reduce sporulation by inhibiting the growth of hyphae out of the needles and the production of sporangia. A previous study demonstrated the inhibitory effect of copper on *P. pluvialis* mycelial growth in vitro (Rolando et al. 2017a), and copper has been shown to be effective in inhibiting *Phytophthora* growth in many systems and for many years (Halsall 1977).

Franich (1988) also showed that copper prevented fruiting bodies from producing and releasing spores. Data on the epidemiology of red needle cast indicate that inoculum is present from around autumn to early summer, with detection more frequent after periods of rainfall (Hood et al. 2017). High incidences of red needle cast result from polycyclic epidemics, where more than one cycle of infection occurs in any one season with the intensity of the epidemic dependent on the rate of infection in each cycle and the amount of initial inoculum (Hood et al. 2017). As with the management of Dothistroma needle blight, spraying with a copper fungicide at critical times will control the disease by stopping spore production, particularly during the production of primary inoculum at the start of an outbreak. Several studies have shown that copper inhibits mycelial growth of *Phytophthora* in vitro (Coelho et al. 2005; Lucero et al. 2014; Rolando et al. 2017a). Copper ions were also found to be inhibitory to sporangial production of *Phytophthora* and zoospores at a concentration of 10⁻⁷ M (Coelho et al. 2005). Epidemiological results obtained thus far suggest that with *P. pluvialis*, a fungicidal spray treatment may be effective if applied during autumn (March) as temperatures begin to cool, with possibly a second spray later in the season (Hood et al. 2017). The efficacy of such a regime in the field is yet to be determined.

Almost all data collected on the persistence of copper indicate that 3 months is common (Bulman and Carlson 2011; Gilmour and Noorderhaven 1973), with surface residue levels decreasing in the order of 80% of the initial application concentrations in this time frame. Such observations were confirmed in this study, where both pot trials showed copper levels reducing to ~30% of the original amount applied over a period of roughly 90 days. This would support the notion

Table 5. Amount of surface copper per plant, 2-g sample, and fascicle at first sampling after spraying, as well as the amount of copper detected on calibration plates collected during spray operation

Treatment (kg ha ⁻¹)	Plates (kg ha ⁻¹)	Fascicle (mg kg ⁻¹)	2-g sample (mg kg ⁻¹)	Whole plant (mg kg ⁻¹)
0	0	5.3 ± 1.6	3.4 ± 0.9	2.1 ± 0.4
0.22	0.30	30.9 ± 5.7	30.9 ± 12.4	20.6 ± 6.3
0.45	0.39	79.0 ± 38.9	59.7 ± 22.8	54.8 ± 3.2
0.65	0.68	89.9 ± 58.8	102.5 ± 25.8	68.7 ± 14.4
0.86	0.65	83.2 ± 69.2	90.3 ± 42.6	75.2 ± 18.4
1.72	1.47	322.2 ± 187.4	245.8 ± 90.7	197.8 ± 42.7
0	0	6.1 ± 1.6	3.5 ± 0.6	1.2 ± 0.2

Fig. 5. Fitted proportions of *Phytophthora pluvialis* needles with lesions in experiment 3 as a function of surface copper content at the time of infection with predicted 95% confidence intervals based on the fitted model.

that any second application of copper should be applied 2 to 3 months after the original spray.

These results have shown that copper is effective at reducing the symptoms of red needle cast on *P. radiata*. Our study provides the foundation for the development of a cost-effective management tool for red needle cast and motivates future research into the optimization of dose, timing, and frequency of application. Field-based observations show inoculum production and disease build up from late autumn to late spring (May to October), with inoculum build up early in the season likely to be a significant factor in disease impact under conditions favoring disease. Based on the results of this study, treatment may be optimized to enable timely inoculum knockdown and further refinement of application regimes. Infection criteria or thresholds of infection to inform spraying, however, will need to be determined from field studies and detailed epidemiological models. Using existing technologies and under current environmental conditions, the aerial application of copper for control of Dothistroma needle blight in New Zealand has been shown to pose a low risk to the receiving aquatic environment (Baillie et al. 2017). However, the impact of additional sprays for protection of trees from red needle cast on beneficial fungi and bacteria would need to be determined in future trials. Optimized spray timing is critical for the development of cost-effective and responsible implementation of integrated pest management for red needle cast.

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