Chemical Control of Red Needle Cast

Milestone 3.1 Alternative chemical testing

Test five chemicals for preventative control of RNC using detached needle assays

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REPORT INFORMATION SHEET

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EXECUTIVE SUMMARY

Report Title: Chemical Control of Red Needle Cast. Milestone 3.1. Alternative chemical testing. Test five chemicals for preventative control of RNC using detached needle assays.
Authors: Carol Rolando, Nari Williams and Martin Bader

The problem: A chemical control strategy is required to manage severe outbreaks of red needle cast in mature stands of *Pinus radiata*. The objective of this study was to test the ability of five active ingredients to reduce the symptoms of *Phytophthora* infection in *P. radiata* needles when exposed to *Phytophthora pluvialis* and *Phytophthora kernoviae*.

This project: Three agrichemical products Agrifos® 600, AGPRO Copper Oxychloride 800 WP® and Ridomil® Gold SL were applied at four concentrations to two clones of *P. radiata*. The clones differed in their susceptibility to red needle cast, with Clone A described as moderately susceptible and Clone B, highly susceptible. Two disinfectants, Omniwett® and 30 Seconds®, were also tested and applied to ramets of the same two clones. The disinfectants were applied at commercially recommended rates and with or without a surfactant. Needles were collected from the treated ramets at 1 week and 3 months after treatment application and exposed to either *P. pluvialis* or *P. kernoviae* in a detached needle assay. Mixed effects models were used to determine whether the active ingredients reduced development of lesions.

Key Results

1. The efficacy of the active ingredients was dependent on host susceptibility to the pathogens, with higher rates of chemicals required to reduce lesion development in the more susceptible clone (Clone B).
2. Three months after application AGPRO Copper Oxychloride 800 WP®, Ridomil® Gold SL and Agrifos® 600 showed efficacy against *Phytophthora pluvialis* and *Phytophthora kernoviae.*
3. One week after treatment application the disinfectants 30 Seconds® and Omniwett® showed variable efficacy against the two Phytophthora species. Neither of these products were effective three months after treatment application.
4. The pathogen response to the concentration range applied for Agrifos® 600 and AGPRO Copper Oxychloride 800 WP® provides an effective dose range on which to base future trials with these active ingredients.

Implications of Results for Client: Any chemical management programme for red needle cast will need to incorporate strategies that encompass host variation in susceptibility to this disease. Further testing is required to confirm the potential of the three most promising products, especially that of copper as it is already widely used (as cuprous oxide) by the forest industry, although not commonly in regions where RNC is most problematic. Known cases of agricultural *Phytophthora* diseases developing resistance to the active ingredient in Ridomil Gold® SL render this product a less attractive long-term option. Copper based products and also salts of phosphorous acid, like Agrifos 600®, are low cost fungicides presenting a cost effective option. The lower efficacy of the disinfectants 30 Seconds® and Omniwett® relative to the three fungicides tested indicates that further work with these active ingredients should not be continued.

Further Work: In light of the results presented in this report it is recommended that further work with Agrifos 600® continues, as well as repeated studies to confirm the efficacy of the copper based products. The ability of phosphite to reduce disease development needs to be determined in trials where the chemical is applied after infection has occurred.

Milestone 3.1 Alternative chemical testing

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Introduction

Red needle cast, 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 red needle cast in existing *P. radiata* forests. Phosphite, a fungicide known to be effective against diseases caused by *Phytophthora* species, is the key active ingredient currently being investigated for its potential to manage red needle cast in *P. radiata* plantations. However, it is important a range of chemicals are investigated for their efficacy against the causal agent of red needle cast. This is to ensure that there are known alternatives to phosphite should: 1) the disease show signs of resistance to this chemical, 2) the use of phosphite become restricted due to high cost, environmental or social concerns and/or 3) the alternatives prove to provide better and more prolonged protection of the host from the causal agents of red needle cast.

Research to evaluate the efficacy of a number of fungicides for control of *P. pluvialis* was initiated in 2012. A study conducted in the laboratory to identify fungicides with *in-vitro* activity against *P. pluvialis* indicated that phosphorous acid (Agrifos®), metalaxyl-M (Ridomil® Gold SL), copper (AGPRO Copper Oxychloride 800 WP®), dimethomorph (Sphinx®) and metiram (Polyram® DF) showed efficacy against the target pathogen (Rolando et al., 2012). The recommendation from that study was to test the most promising chemicals in small scale pot trials using detached needle bioassays, or on-plant inoculations, to give a better indication of their potential *in-planta*.

The aim of this study was to test the efficacy and persistence of five active ingredients (Table 1) for preventing infection of *P. radiata* needles with the pathogens *P. pluvialis* and *P. kernoviae*. *P. kernoviae* was included in the study as it has been recovered from foliage of trees suffering red needle cast and other foliar disorders. Two susceptible clones were used to determine the interaction between active ingredient, rate of application and host susceptibility to infection.

This report summarises results for Milestone 3.1 of the FOA Needle Disease Strategy:

3.1 Alternative chemical testing. Using detached needle assays test five chemicals for preventative control in a pot trial on two susceptible clones.

The report consists of two parts:

1. A dose response trial with three fungicides applied at four rates.
2. A trial testing two active ingredients applied at commercially recommended rates and with or without a surfactant. The standard commercial treatment for management of dothistroma needle blight was included in this trial as a point of comparison.

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Table 1. Products and active ingredients included in a screening trial to determine their efficacy for preventing infection of *P. radiata* needles with the pathogens *Phytophthora pluvialis* and *P. kernoviae*.

Product	Active Ingredient and rate	Chemical group	Mode of action	Cost per unit ($ kg^{-1} L^{-1})	Manufacturer	Research Provider
Agrifos®600	600 g L^{-1} phosphorous acid	phosphonate	systemic fungicide	9.00	Key Industries Ltd, Auckland, NZ	Scion
AGPRO Copper Oxychloride 800 WP	500 g L^{-1} copper oxychloride	inorganic copper	protectant fungicide	10.20	AGPRO NZ Ltd, Auckland NZ	Scion
Ridomil® Gold SL	480 g L^{-1} metalaxyl-M	phenylamide	systemic fungicide	45.00	Syngenta Crop Protection Ltd, Auckland, NZ.	Scion
Omniwett®*	400 g L^{-1} didecyl dimethyl ammonium chloride + other ingredients	quaternary ammonium compound	Adjuvant		Omnia Nutriology, Auckland NZ	TreeLab
30 Seconds®*	50 g L^{-1} sodium hypochlorite + other ingredients	bleach	Algaecide	5.00	30 Seconds, Auckland	TreeLab

*Applied with and without Hasten (BASF, New Zealand), a non-ionic surfactant aimed at improving penetration of agrichemicals

Materials and Methods

Plant material

Two grafted clones of *P. radiata* were used in this study. Previous testing indicated Clone A was "moderately susceptible" and Clone B was "highly susceptible" to needle infection by *P. pluvialis* and *P. kernoviae* (NM Williams pers comm).

Description of trial

Part 1. Dose response trial with three fungicides

This trial was set up as a full factorial design with three fungicides applied at four concentrations to each of two clones (Table 2). Treatments were applied to five ramets per clone. Detached needle assays with *P. pluvialis* and *P. kernoviae* were carried out at 6 days (November 2013) and 90 days (February 2014) after spray application. Lesion length was used to assess the efficacy of the treatment.

Table 2. Product and amount applied for the three fungicides tested in a dose response trial.

Product	Concentration range of each product (kg/L ha^{-1})				Volume (L ha^{-1})	Surfactant
Agrifos® 600*	0	5.00	20.00	40.00	100	0.2% Du Wett
AGPRO Copper Oxychloride 800 WP*	0	1.50	3.00	6.00	100	0.025% AgPro Wetter Penetrant
Ridomil® Gold SL	0	0.75	1.50	3.00	100	

Part 2. Disinfectants applied with or without surfactant

This trial consisted of two disinfectants applied with or without a surfactant, applied to each of two clones (Table 3). Treatments were applied to five ramets of each clone. In addition, the current commercial treatment for dothistroma needle blight was included as a point of comparison (Table 3). Detached needle assays with *P. pluvialis* and *P. kernoviae* were carried out at 6 days (November) and 90 days (February) after spray application and lesion length was used to assess the efficacy of the treatments.

Table 3. Treatments applied in part 2 of the study.

Treatment	Description	Volume (L ha^{-1})
Omni	0.2% Omniwett®	100
Omni +H	0.2% Omniwett® with 0.5% Hasten®	100
30 S	50% 30 Seconds ®	100
30 S +H	5% 30 Seconds with 0.5% Hasten®	100
H	0.5% Hasten®	100
Dothi Standard	1.1 kg ha^{-1} cuprous oxide (Ag Copp® 75) in 2 L oil made up to 5 L	5
Control	Not treated	

Application of chemicals

All treatments, except the commercial treatment for dothistroma needle blight, were applied in the equivalent of 100 L ha^{-1} using a calibrated, moving head tracksprayer (PPC_{NZ}, Rotorua) fitted with a 1 m boom and two twinjet nozzles (TJ60-8002 EVS). The standard, commercial treatment for dothistroma needle blight was applied using an ULVA + controlled droplet applicator calibrated to deliver 5 L ha^{-1} and droplets with volume mean diameter (VMD) of approximately 60-70 µm.

Once the trees had been sprayed they were placed in a polyhouse in the nursery where they were drip irrigated . Six weeks after spraying the plants were moved out of the polyhouse to be exposed to ambient weather conditions.

Assessments

Detached needle assays

Detached needle assays were undertaken at 6 days and 90 days after spraying. Zoospore suspensions of *P. pluvialis* and *P. kernoviae* were prepared using the red needle cast SOP (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 10^4 ml^{-1}. Autoclaved pond water was used for controls.

Thirty healthy fascicles were collected from each potted ramet, including the controls. Ten fascicles from each potted ramet were exposed to either a suspension in pond water of 1 x 10^4 *P. pluvialis* or *P. kernoviae* zoospores per ml for 24 hours. Controls were exposed to autoclaved pond water only. Following exposure, needles were placed on trays moistened with wet paper towels and incubated in a controlled environment (17 ^oC, 65-70% relative humidity, 14 h photoperiod) for 10 days. After this time, the needles within each fascicle were separated and the total number of lesions and lesion length was measured.

Statistical analysis

For both parts of this study the *P. pluvialis* and *P. kernoviae* assays were analysed separately as each species varies in its epidemiology. The results have been presented in separate figures.

Part 1.

Using a mixed effects model the data were analysed as a full factorial (clone x product x dose x time) with all interactions apart from the four way interactions considered. 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 to adjust *P*-values for multiple testing (R-package *multcomp*).

Part 2.

Using a mixed effects model the data were analysed as a two way factorial (clone x treat). A significant interaction term was followed up with a multiple comparison procedure using Tukey contrasts to adjust *P*-values for multiple testing (SAS Version 9.3).

Results and Discussion

Part 1. Dose response trial with three fungicides

No signs of phytotoxicity to radiata pine were observed for any of the treatments applied in this study.

Phytophthora pluvialis

Overall, lesions assessed in November (6 days after treatment application) were significantly smaller than those assessed in February (90 days after treatment application), when larger lesions occurred (Figure 1). This response is independent of the application of chemicals and instead reflects that needle and pathogen physiology play an important role in lesion development within each assay. The factors affecting lesion length are poorly understood at this stage but could include needle phenology, moisture status and interaction with plant secondary metabolites.

With few exceptions, the untreated control needles showed on average significantly larger lesions than all fungicide-treated needles across both sampling dates (Fig. 1; Appendix A). In addition, fewer (data not shown), smaller lesions were noted on Clone A than Clone B, highlighting the inherent difference in susceptibility to the pathogen between the two clones (Fig. 1). This generally resulted in a higher response to concentration in Clone A rather than Clone B. These effects varied across assessment dates and gave rise to two significant three-way interactions: a date × clone × fungicide and a clone × fungicide × concentration interaction (Fig. 1, Appendix B: Table A).

The response to AGPRO Copper Oxychloride 800 WP® produced similar effects in both clones and across dates, with a reduction in lesion length noted with increasing concentration (Fig. 1). This was a surprising outcome given that copper-oxychloride is a protectant fungicide and would likely wash-off from needles or degrade on exposure to the environment. It is possible that the period of time in the polyhouse, before being deployed to the open environment, extended the efficacy of this treatment beyond that which would occur in the field. However, it is interesting to note, that in other field trials with copper, applied in oil, persistence up to four months has been found (Bulman and Carlson, 2011). The response to Agrifos® 600 also persisted over time, and whilst not significant, a trend of decreasing lesion length with increasing concentration was evident (Fig. 1). Persistence was more pronounced in Clone A, than Clone B, where fungicide application reduced lesion length by more than 50% compared to the untreated control needles at the second assessment in February (Fig. 1; Appendix A). A similar outcome to Agrifos® 600 was recorded for Ridomil® Gold SL. Interestingly, in almost all cases fungicide efficiency did not significantly increase with increasing concentration.

Phytophthora kernoviae

In general, a similar trend of smaller lesions in the November assay (6 days after treatment application) than the February assay (90 days after treatment application) was also observed for *P. kernoviae* as described for *P. pluvialis* above. Larger lesions were observed for Clone B, the more susceptible clone to red needle cast, than Clone A (Fig. 2, Appendix C: Table B). This, together with responses in lesion length to concentration effects across fungicides meant that there were three significant three-way interactions for these data (date × clone × concentration; date × fungicide × concentration and clone × fungicide × concentration: Appendix C: Table 2) indicating a complex of significant outcomes.

All chemicals and rates significantly reduced lesion length in the assay carried out in November (6 days after treatment application), particularly for Clone A (Fig. 2; Appendix D) indicating a high efficacy of all products and concentrations. As with the *P. pluvialis*

assays, the efficacy of the AGPRO Copper Oxychloride 800 WP® persisted through 90 days to the second assessment in February for both clones, indicated by the significantly reduced size of lesions relative to the control in February (Fig. 2, Appendix D). Ninety days after treatment both clones treated with Ridomil® Gold SL or Agrifos® 600 developed lesions of similar length to the control, particularly where Agrifos®600 had been used at lower rates (Figure 1). As with the *P. pluvialis* assay, where a fungicide was shown to be effective the concentration seemed to be of relatively minor importance.

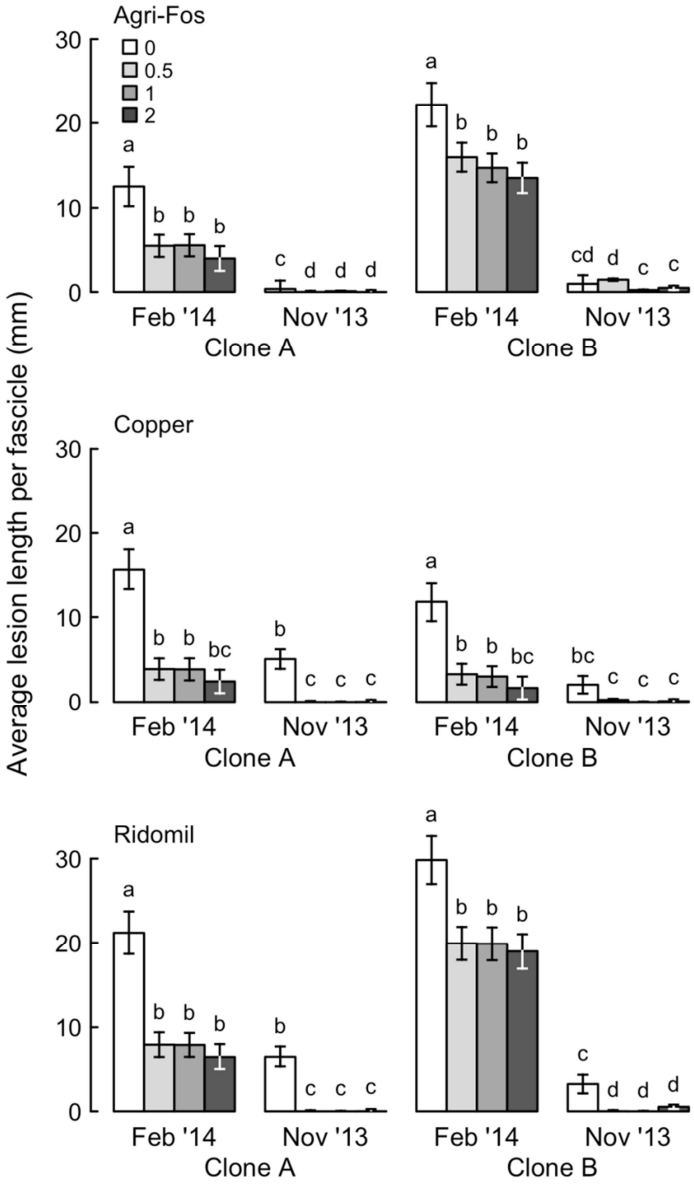

Figure 1. Average lesion length per fascicle in ramets from two *Pinus radiata* clones (A, B) inoculated with *Phytophthora pluvialis.* The fungicides AgriFos®, Rimodil® Gold SL as well as AGPRO Copper Oxychloride 800 WP were applied at four concentration and needles were assessed for lesion formation at two subsequent dates. Different lower-case letters indicate significant differences between plant materials at α = 0.05 (multiple comparison test using Tukey contrasts). Means ± SE, n = 5 ramets per clone.

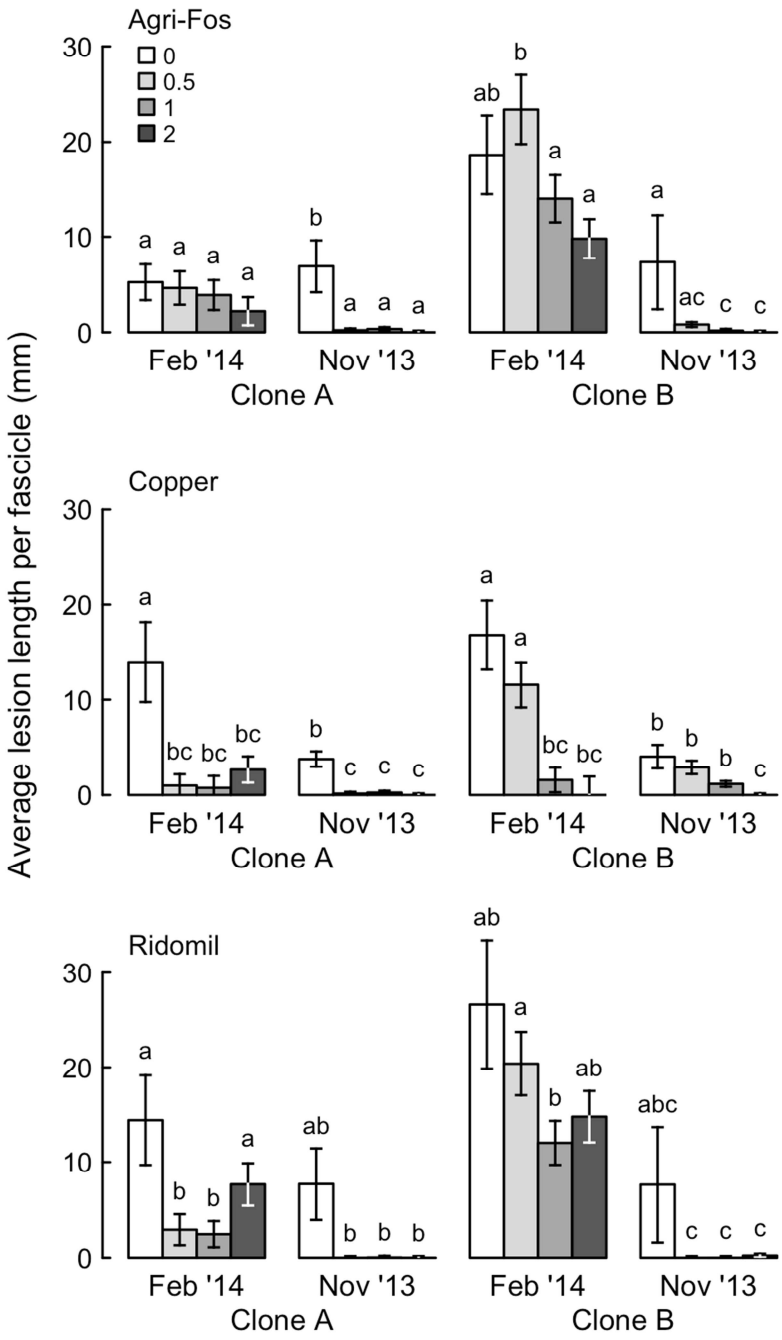

Figure 2. Average lesion length per fascicle in ramets from two *Pinus radiata* clones (A, B) inoculated with *Phytophthora kernoviae.* The fungicides Agrifos® 600, Rimodil® Gold SL as well as AGPRO Copper-oxychloride 800 WP® were applied at four concentration and needles were assessed for lesion formation at two subsequent dates. Different lower-case letters indicate significant differences between plant materials at α = 0.05 (multiple comparison test using Tukey contrasts). Means ± SE, n = 5 ramets per clone.

Part 2: Disinfectants applied with or without surfactant

No signs of phytotoxicity to radiata pine were observed for any of the treatments applied in this study.

Phytophthora pluvialis

There was a significant interaction between treatment and clone at the first assay made 6 days after treatment application in November (Appendix E). However, this effect did not persist to the second assay, made in February 90 days after treatment application. At that time, no chemical treatment effect was observed on lesion length, and lesion length differed significantly only between clones (Figure 3, Appendix E). At the November assay lesion length in all treatments differed significantly from the control treatment, for Clone A, however, there were no significant differences between the control and treated ramets for Clone B (Figure 3). The lower efficacy of chemicals on the more susceptible Clone B was a trend also observed in part 1 of this study.

Phytophthora kernoviae

There was a significant main effect of treatment at the first assay made 6 days after treatment application in November (Appendix E). However, this effect did not persist to the February assay, where chemical treatment had no effect on lesion length, and lesion length differed significantly only between clones (Figure 3, Appendix E). At the November assessment, lesions were significantly smaller than the control for Clone B in treatments: Omni, 30S and the Dothi standard. For the same assessment for Clone A lesion length was significantly reduced relative to the control for treatments: 30S, 30S + H and the Dothi standard.

Since the mode of action of all treatments in this study including the copper treatment currently used for dothistroma control (Dothi Standard) was contact-based with little systemic uptake by the plant, it is not surprising that efficacy of most treatments did not persist. The significantly longer lesions observed across the study for the 90 days after-treatment assessment made in February is evidence of the variation in susceptibility of different clones of radiata pine to needle infection by *Phytophthora* species. The difference in persistence observed between the two copper applications (a water based treatment versus oil based treatment) highlights the need to further investigate the potential of this active ingredient in studies where plants are exposed to ambient conditions from the time of spraying.

The high variability noted throughout this trial reflects a general trend in detached needle assays carried out in this project, and others, in the Needle Disease Programme. This has been identified as partly attributable to within tray and growth chamber effects which can now be corrected for through experimental design. Some of this experimentally induced variability may account for the variation observed in these trials. An improved experimental design is now being incorporated into subsequent trials by applying a completely randomised alpha design accounting for within tray, growth chamber and clonal effects.

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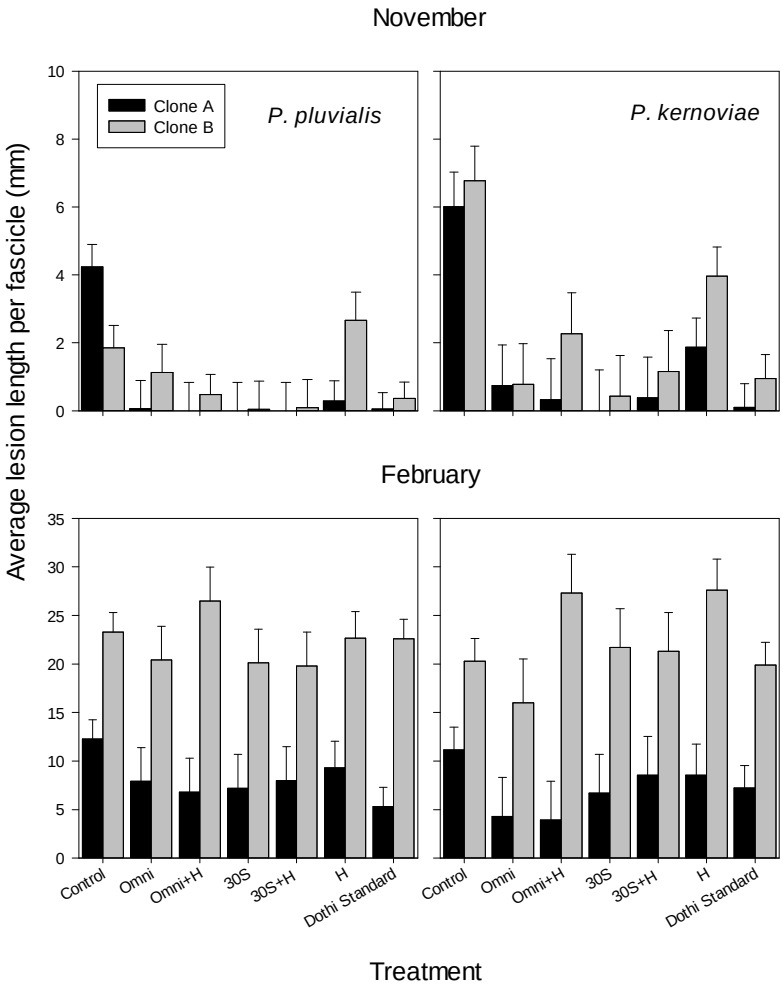

Figure 3. Average lesion length for two *P. radiata* clones inoculated with either *P. kernoviae* or *P. pluvialis* at one week (November 2013) and three months (February 2014) after chemical application. The disinfectants Omniwett® and 30 Seconds® were applied with or without a surfactant. The commercial treatment for dothistroma needle blight was included in the study. Bars indicate standard error.

Key outcomes and implications of this study

Outcome 1: Clonal variation of radiata pine in susceptibility to needle infection by *Phytophthora* species affects the efficacy of chemicals.

Implication: *Potentially higher rates of chemicals may be required to reduce infection of highly susceptible clones.*

Outcome 2: Over a period of three months after treatment application AGPRO Copper-oxychloride 800 WP® and Ridomil® Gold SL showed similar efficacy to Agrifos® 600 against needle infection by *Phytophthora* species.

Implication: *Further studies are needed to confirm this result, particularly that for copper which is already widely used by the forest industry. It is possible that the effects of copper observed here would be compromised on full exposure to the environment (sunlight and rainfall) following application. Known cases of agricultural* Phytophthora *diseases developing resistance to the active ingredient in Ridomil Gold® SL render this active ingredient the least attractive long-term alternative to Agrifos® 600.*

Outcome 3: The disinfectants 30 Seconds® and Omniwett® showed variable efficacy against needle infection by *P. kernoviae* and *P. pluvialis* 6 days after treatment application. None of these treatments reduced lesion length three months after treatment application most likely because they are contact rather than systemic fungicides.

Implication: *The lower efficacy of 30 Seconds® and Omniwett® relative to the three fungicides tested indicates that further work with these active ingredients should not be continued.*

Outcome 4: The pathogen response to the concentration range applied for Agrifos® 600 and AGPRO Copper-oxychloride 800 WP® provides an effective dose range on which to base future trials with these active ingredients.

Implication*: We are a step closer to defining optimum rates of active ingredients to test in field for field control of red needle cast.*

Further work should aim to re-assess the potential of copper products to control red needle cast as well as examine the curative action of copper and phosphite fungicides.

Acknowledgements

The authors would like to acknowledge Catherine Banham, Caro Gous, Nalini Navaranjan, Nadine Rea and Madeline Myers for technical assistance. Stefan Gous must also be acknowledged for providing the technical leadership required to spray these trials in the Plant Protection NZ, tracksprayer facility. Jenny Aitken, The TreeLab, provided two chemicals for this trial. The contributions of the TreeLab treatments to this trial work is acknowledged. This work was funded by the Forest Owners Association, New Zealand under the Needle Disease Strategy.

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Appendix A

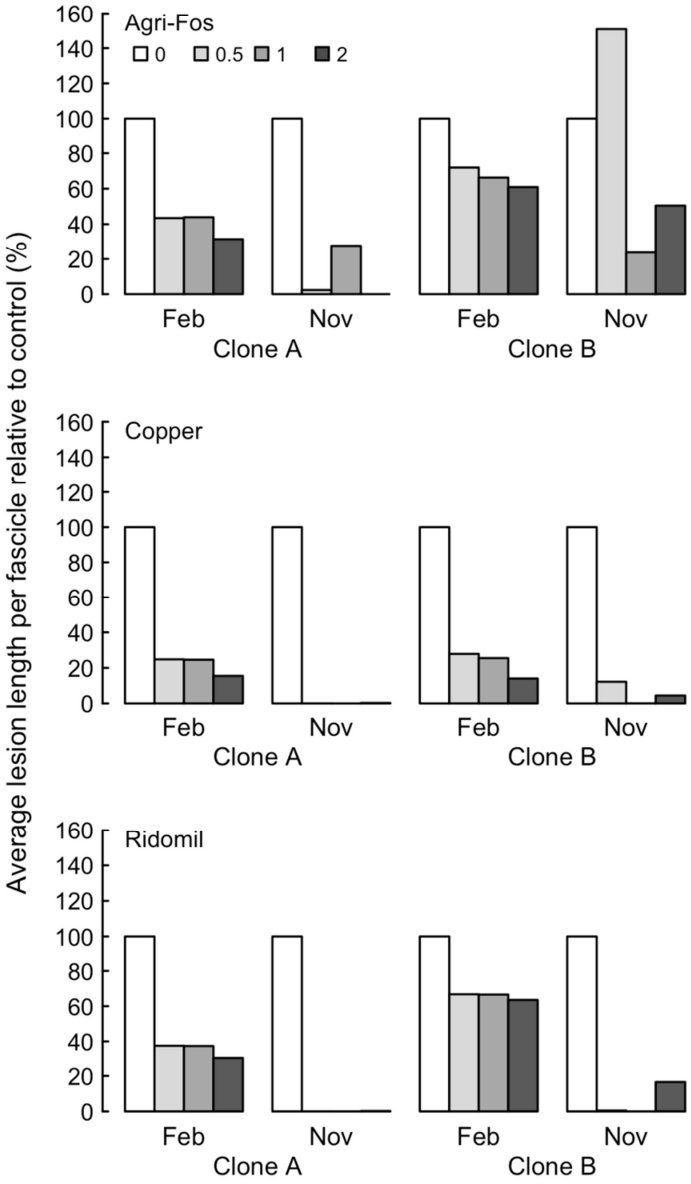

Figure A. Lesion length per fascicle in ramets from two *Pinus radiata* clones (A, B) inoculated with *P. pluvialis* expressed as a percentage relative to the control (0 concentration). The fungicides Agrifos®, Ridomil® Gold SL and AGPRO Copper Oxychloride were applied at four concentrations and needles were assessed for lesion formation at two subsequent dates.

Appendix B

Table A Summary of the final linear mixed-effects model testing the effects of assessment date, host clone, fungicide and fungicide concentration on lesion length development in *Pinus radiata* needles inoculated with *Phytophthora pluvialis*.

Source of variation	Df_{num}	Df_{den}	*F*	*P*	
Intercept	1	108	27.81	<0.001	***
date	1	108	206.51	<0.001	***
clone	1	96	10.47	0.002	**
fungicide	2	96	12.20	<0.001	***
concentration	3	96	19.39	<0.001	***
date × clone	1	108	10.50	0.002	**
date × fungicide	2	108	29.73	<0.001	***
clone × fungicide	2	96	6.43	0.002	**
date × concentration	3	108	4.47	0.005	**
clone × concentration	3	96	7.45	<0.001	***
fungicide × concentration	6	96	5.79	<0.001	***
date × clone × fungicide	2	108	15.67	<0.001	***
clone × fungicide × concentration	6	96	4.47	<0.001	***

Appendix C

Table B Results from the final linear mixed-effects model testing the effects of assessment date, host clone, fungicide and fungicide concentration on lesion length development in *Pinus radiata* needles inoculated with *Phytophthora kernoviae*.

	Df_{num}	Df_{den}	*F*	*P*	
Intercept	1	96	21.49	<0.001	***
date	1	93	93.87	<0.001	***
clone	1	96	1.96	0.165	
fungicide	2	96	2.03	0.137	
concentration	3	96	20.02	<0.001	***
date × clone	1	93	25.74	<0.001	***
date × fungicide	2	93	11.06	<0.001	***
clone × fungicide	2	96	0.49	0.620	
date × concentration	3	93	1.99	0.120	
clone × concentration	3	96	1.09	0.356	
fungicide × concentration	6	96	2.36	0.036	*
date × clone × fungicide	2	93	9.15	<0.001	***
date × clone × concentration	3	93	5.47	0.002	**
date × fungicide × concentration	6	93	2.22	0.047	*
clone × fungicide × concentration	6	96	2.61	0.022	*

Appendix D

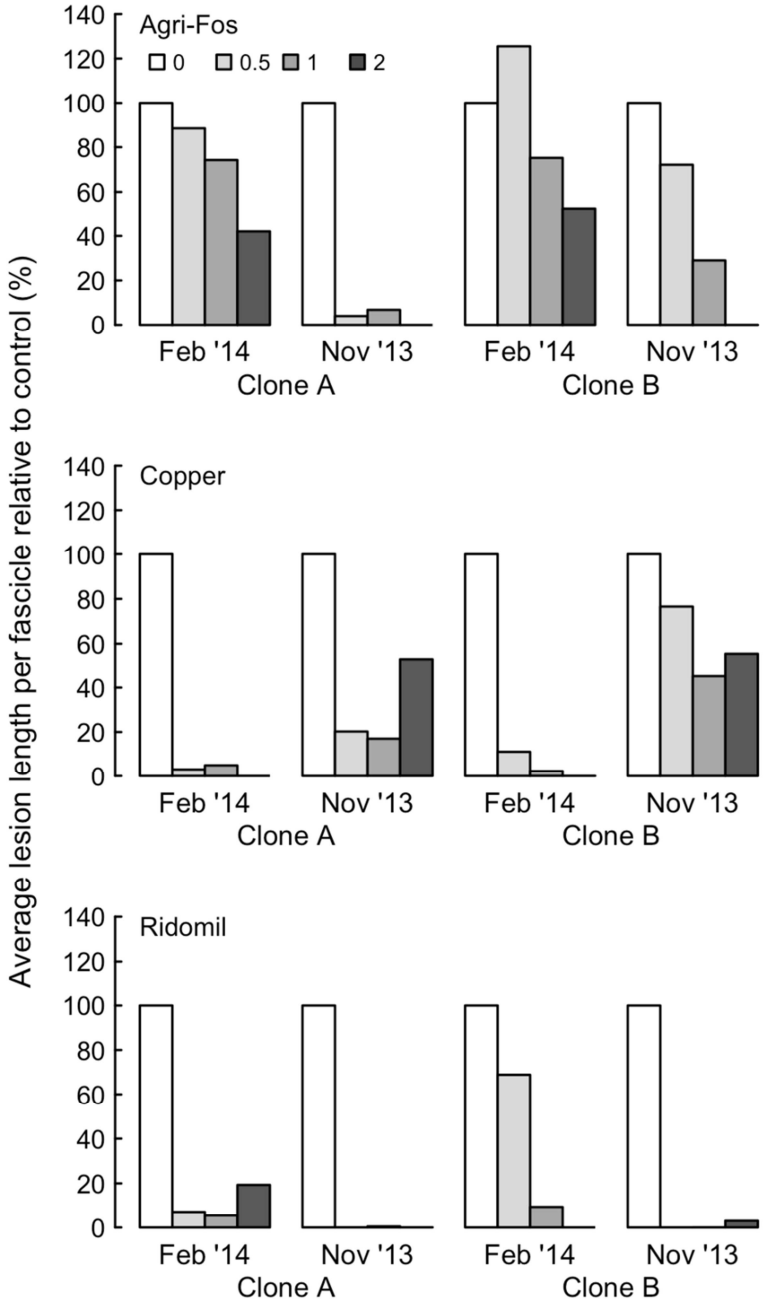

Figure B. Lesion length per fascicle in ramets from two *Pinus radiata* clones (A, B) inoculated with *P. kernoviae* expressed as a percentage relative to the control (0 concentration). The fungicides Agrifos®, Ridomil® Gold SL and AGPRO Copper Oxychloride were applied at four concentrations and needles were assessed for lesion formation at two subsequent dates.

Appendix E

Table C. Summary of the final linear mixed effects model testing the effects of clone and treatment on lesion length development in *Pinus radiata* needles inoculated with *Phytophthora pluvialis* and *Phytophthora kernoviae*.

	P. pluvlialis					
	November			**February**		
Source of variation	***Df* (num/den)**	***F***	***P***	***Df* *(num/den)***	***F***	***P***
clone	1/102	0.54	0.463	1/97	73.91	<0.001
treat	6/47	4.10	0.002	6/97	0.89	0.504
clone*treat	6/102	3.64	0.003	6/97	0.68	0.667
	P. kernoviae					
clone	1/101	3.12	0.080	1/97	66.78	<0.001
treat	6/46.1	6.79	<0.00	6/97	1.02	0.418
clone*treat	6/101	0.27	0.948	6/97	1.15	0.341