Control of *Neonectria ditissima* with copper based products in New Zealand

M. Walter¹, O.D. Stevenson¹, N.T. Amponsah¹, R.W.A. Scheper², D.G. Rainham³, C.G. Hornblow³, U. Kerer³, G.H. Dryden⁴, I. Latter⁵ and R.C. Butler⁶

¹The New Zealand Institute for Plant & Food Research Ltd (Plant & Food Research), Old Mill Road, RD3, Motueka 7198, New Zealand

²Plant & Food Research, Private Bag 1401, Havelock North 4130, New Zealand

³AgFirst Consultants Ltd., PO Box 33, Motueka 7143, New Zealand

⁴Fruition Horticulture (SI) Ltd, PO Box 3194, Richmond 7050, Tasman, New Zealand

⁵GroChem, AgriNova NZ Ltd, PO Box 50-080, Porirua, New Zealand

⁶Plant & Food Research, PO Box 51, Lincoln, New Zealand

Corresponding author: monika.walter@plantandfood.co.nz

Abstract New Zealand pipfruit crops require postharvest fungicides for control of European canker, caused by *Neonectria ditissima*. Fungicide efficacy trials to protect leaf scars from *N. ditissima* infections were conducted during autumn 2013 and 2014. Disease control of artificially inoculated leaf scars was achieved by single applications of copper oxychloride and copper oxide, but not copper hydroxide, applied at 4.3, 1.1 and 0.6 kg elemental copper/ha respectively. Control of leaf scar infections by copper oxide (0.65 and 0.95 kg elemental copper/ha) was similar to control by captan. Leaf scars (0-10 days old) remained susceptible to infections up to 10 days after leaf fall; disease control was achieved by re-distribution of copper and captan fungicides onto new leaf scars. Disease control using copper oxide was consistent and similar to control using captan. The copper product, and concentration of elemental copper, are important for successful control of leaf scar infections by *N. ditissima*.

Keywords European canker, *Neonectria galligena*, *Nectria galligena*, copper oxychloride, copper hydroxide, copper oxide, fungicide.

INTRODUCTION

Copper-based fungicides were introduced by Millardet around 1845 with the use of Bordeaux mixture containing copper(II)sulphate (CuSO_4) and calcium hydroxide (lime, Ca(OH)_2) for the control of grapevine downy mildew (causal agent: *Plasmopara viticola*) in France. The lime in the Bordeaux mixture was required to neutralise the copper sulphate, rendering it less phytotoxic. Bordeaux mixture is still a commonly used copper fungicide in the cropping and horticultural industries and by home gardeners, but other copper-containing products have been developed for control of bacterial leaf spots, blights, downy

mildews, anthracnoses and cankers. In these alternative copper products, the copper is 'fixed' or less soluble than in Bordeaux mixture, thereby reducing their phytotoxicity. The fixed copper products contain either basic copper sulphate, copper chlorides, cupric hydroxide, copper oxides, or mixtures thereof (Agrios 1997). While the water-soluble copper products such as copper sulphate are short lived, the less soluble copper products release active copper ions over a period of time when the plant surface is wet, and are thus relatively residual in nature. It is this slow release of the insoluble copper products that also reduces the phytotoxicity

to plants (Anonymous 2013). In contrast, the water-soluble copper products tend to be more effective at lower rates of application (CEFS 2010).

The mode of action of copper ions (Cu^{2+}) is based upon the inhibition of spore germination by fungal pathogens. Active growth of germ tubes is generally accompanied by acidification of the area surrounding the spore. This small and localised acidification process dissolves sufficient quantities of the water-insoluble copper deposits to allow uptake into the fungal cell (Ruberson 1999). Following cellular absorption, the copper ions bond to chemical groups on proteins such as imidazoles, phosphates, sulphhydryls, and hydroxyls, thereby disrupting normal protein function. The mode of action of copper fungicides therefore is nonspecific denaturation and disruption of cellular proteins (CEFS 2010).

In New Zealand, pipfruit growers may make two to six fungicide applications during the leaf fall period, using a range of products including captan and copper-based products to protect leaf scars from infection by *Neonectria ditissima* (syn. *Nectria galligena*, *Neonectria galligena*, *Cylindrocarpon heteronema* (anamorph)), the causal agent of European canker. Autumn or 'leaf fall' has been considered the most important infection period, because of an abundance of entry wounds via leaf scars, coinciding with inoculum from sporulating cankers and sufficient rain, which is required for spore dissemination. Copper-based products applied during leaf fall have provided adequate control against *N. ditissima* leaf scar infections in many apple-growing areas. However, product efficacy has been dependent on the type of copper product used (Swinburne 1975; Weber 2014a, b). Copper fungicides for control of *N. ditissima* infections have not been evaluated formally under New Zealand conditions. The aim of this study was to compare the efficacy of copper-based products that are commonly used by New Zealand pipfruit growers for the control of *N. ditissima*.

MATERIALS AND METHODS

Fungicides and trial sites

The fungicides evaluated were copper oxychloride, copper oxide, copper hydroxide and captan,

compared with an untreated control. Fungicide treatments, product descriptions and timing of applications are described in Table 1. There were three independent experiments carried out in the Riwaka-Motueka region of Nelson. Experiment 1, was conducted at the Plant & Food Research (PFR) orchard block L2, consisting of seven rows of 'Braeburn' and approximately 150 trees. Trees were grafted onto 'MM106' rootstock, planted in 1995 at a spacing of 2.5×4 m. Experiments 2 and 3 were conducted on a commercial 'SciFresh'/JazzTM block on 'M9' rootstocks in Riwaka. The experimental block consisted of two rows with approximately 200 and 150 trees for Experiment 2 and 3, respectively. These trees were planted at a spacing of 1.2×3.3 m (Experiment 2) and 1.3×3.2 m (Experiment 3).

In Experiment 1, there were five treatments (Table 1) replicated four times in a Latinized block design over the seven rows with three treatment plots per row and seven to eight trees per treatment plot. Fungicide treatments were applied once, at 5% leaf fall on 28/05/2013. In Experiment 2, there were four treatments (Table 1) arranged in a randomised block design with four replicates over two rows, with eight trees/plot. Fungicide treatments were applied four times, at 25% leaf fall on 31/05/2013, 50% leaf fall on 12/06/2013, 75% leaf fall on 18/06/2013 and 95% leaf fall on 25/06/2013. In Experiment 3, there were four treatments (Table 1) arranged in a randomised block design with five replicates arranged over two rows, with six trees per plot. Fungicide treatments were applied once, at 5% leaf fall on 27/05/2014.

Fungicides were applied using a STIHL® SR450 air-assisted motorised knapsack at water rates of 1700 and 1000 litres/ha for the 'Braeburn' and 'SciFresh'/JazzTM trees, delivering approximately 500 ml and 320 ml/tree, respectively. The 'Braeburn' trees on 'MM106' rootstock were larger than the 'SciFresh'/JazzTM trees on 'M9' rootstock. In each row, the outside bays (five to nine trees/bay) were used as guard trees. All trees within a plot were sprayed, and the two adjacent trees on the edge of adjoining plots were also used as guard trees. In the commercial block, there were two unsprayed guard rows on either side of the experimental rows to minimise accidental overspray. The

research orchard block was surrounded by a grass strip and shelter trees.

Inoculum production and inoculation methods

A spore suspension of *N. ditissima* was prepared by collecting sporulating cankers from the field. Field cankers were collected 3–14 days before use and kept at 18–20°C in a clear plastic bag by the window to encourage further sporodochia production. Spore suspensions were produced by cutting the lesion area from the branches and suspending the lesions in 4°C pre-cooled sterile distilled water for 10–15 min. This allowed the spores to dehisce naturally. The spore suspensions were checked for purity under the microscope and the concentration adjusted using a haemocytometer. Spore suspensions were always prepared fresh on the day of use and stored at 4°C or on ice until required. Spore germination was determined in 20 µl droplets on glass slides then incubated on the laboratory bench for 24 h at 18–20°C in humidity chambers.

For inoculation, 1-year-old, non-fruit bearing shoots were labelled with flagging tape the day before the fungicide applications. Generally 50 (Experiments 2 and 3) or 100 (Experiment 1) suitable shoots were labelled in each treatment plot, with approximately half on each side of the plot. Three (Experiments 1 and 2) or two (Experiment 3) new leaf scars were created on each shoot by gently flicking off leaves close to natural senescence. The leaf scar positions were marked with a paint pen and then the fungicide treatments were applied later in the afternoon. Once the treatments had dried, the labelled leaf scars were inoculated with approximately 15–25 µl of a *N. ditissima* spore suspension (adjusted to 1×10^5 conidia/ml). Droplets were brushed onto leaf scars with a camel hair brush fixed to the tip of a plastic 5-ml Pasteur pipette. This inoculation technique allowed targeted application of inoculum onto a leaf scar with very little run-off. During Experiment 2, additional spray treatments were carried out for control of European canker from orchard inoculum within the experimental sites, but artificial inoculations were only made after the first fungicide treatment. There were no additional spray treatments in Experiments 1 and 3.

Effect of leaf scar age on infection

In Experiments 1 and 3, the effect of leaf scar age was also studied. Leaf scars were created before the treatments as described above, but were artificially inoculated on different days, ranging from 0- to 10-day-old leaf scars. In Experiment 1, leaf scars were 0, 3, 7 and 10 days old at the time of inoculation. In Experiment 3, leaf scars were 0, 1, 2 and 6 days old. In Experiment 1, at each inoculation time, fresh (0-day-old) leaf scars were also created and inoculated within 2 h to determine the potential maximum infection on each particular date. These leaf scars had no direct exposure to the corresponding fungicide treatments. For the 0-day old leaf scar, one leaf scar was made before the spray application and one after the spray application. For convenience these have been termed 'fresh scar'. In Experiment 3, a fresh scar was included at only the last inoculation time (6-day-old leaf scars).

Assessments

Before the experiments started, all cankers within reach from the ground were pruned and removed from all experimental trees in all trial areas. After inoculation, typical symptoms of European canker were assessed at intervals of 4–6 weeks until December by visually monitoring the appearance of symptoms from natural infections and artificial inoculations on all trees, including guard trees and pollinators. The number of leaf scars on inoculated and non-inoculated shoots, including non-marked shoots and branches, was counted at each assessment time. Early symptoms with lesions <5 mm were recorded, and when advanced lesions developed or lesions increased in size to >1 cm, the affected shoot was cut off and removed from the experimental site.

Statistical analyses

Data for natural infections were analysed separately from those for infections of inoculated shoots. For inoculated shoots, the percentage lesions formed and percentage shoots removed were analysed using a hierarchical generalised linear modelling approach (Lee et al. 2006). This allowed inclusion of random effects such as row, replicate, plot and tree as well as fixed effects such as fungicide treatment and leaf scar age (McCullagh & Nelder 1989). Both

fixed and random effects were fitted with a logit link. For natural infections, the number of lesions per shoot and numbers of removed shoots were analysed in the same way as for the inoculated shoots, but, as the total shoot number and scars were not known, a Poisson distribution was used for the fixed effects, while a gamma distribution was used for random effects, with both effect types fitted with a logarithmic link.

For all three experiments, the importance of random effects was assessed using a Chi-Square test of the change in deviance on dropping the term, as implemented in GenStat's HGRTEST procedure (GenStat Committee 2014a). Only

significant random effects were retained in the final analyses. For Experiment 1, these were plots and trees within plots; for Experiments 2 and 3, they were plots (no record was taken of the tree from which shoots were assessed). Fixed effects, including specific contrasts between the treatments, were assessed similarly to random effects, using GenStat's HGFTEST procedure. All analyses were carried out with GenStat (GenStat Committee 2014b). Results have been presented as means with associated 95% confidence limits. These were obtained on the link (logit scale for percentages, logarithmic scale for counts) and back-transformed for presentation.

Table 1 Treatment descriptions, number of fungicide applications and apple cultivars used in three field experiments for control of leaf scar infections by *Neonectria ditissima* in 2014 and 2015.

Trt No.	Product	Active ingredient (ai)	Product rate/100 litres	Water rate (litres/ha)	Elemental copper (kg/ha)	No. spray applications
Experiment 1: 'Braeburn' 2013¹						
1.1	Kocide® (Nufarm)	Copper hydroxide	110 ml	1700	0.6	1
1.2	Nordox 75WG (GroChem)	Copper oxide	90 g	1700	1.1	1
1.3	GroChem Oxi-Cup™ (GroChem)	Copper oxychloride	500 g	1700	4.3	1
1.4	Captan 900WG (Nufarm)	Captan	110 g	1700	0	1
1.5	Control	Unsprayed	-	-	-	0
Experiment 2: 'SciFresh'/Jazz™ 2013²						
2.1	Nordox 45 WG (GroChem)	Copper oxide	145 g	1000	0.653	4
2.2	Nordox 75 WG (GroChem)	Copper oxide	130 g	1000	0.975	4
2.3	Captan 80WG (Fruitfed)	Captan	125 g	1000	0.64	4
2.4	Control	Unsprayed	-	-	-	0
Experiment 3: 'SciFresh'/Jazz™ 2014³						
3.1	Nordox 45 WG (GroChem)	Copper oxide	145 g	1000	0.653	1
3.2	Nordox 75 WG (GroChem)	Copper oxide	130 g	1000	0.975	1
3.3	Captan 80 WG (Fruitfed)	Captan	125 g	1000		1
3.4	Control	Unsprayed	-	-	-	0

¹In Experiment 1, fungicide treatments were applied once, at 5% leaf fall on 28/05/2013.

²In Experiment 2, fungicide treatments were applied four times, at 25% leaf fall on 31/05/2013, 50% leaf fall on 12/06/2013, 75% leaf fall on 18/06/2013 and 95% leaf fall on 25/06/2013.

³In Experiment 3, fungicide treatments were applied once, at 5% leaf fall on 27/05/2014.

RESULTS

In all three experiments, the percentage of lesions formed matched the percentage of shoots removed, with a very high correlation between the two sets of means (correlation co-efficient $r > 0.99$). Therefore, only results for percentage shoots removed are presented.

Experiment 1

All fungicides, except copper hydroxide ($P = 0.250$; Figure 1a), significantly ($P < 0.002$) reduced the number of shoots removed compared with that in the unsprayed control treatment. The number of uninoculated shoots removed (natural infections from background inoculum) did not vary significantly ($P = 0.266$) between the different treatments.

For the “age of leaf scar” part of the experiment, at each inoculation time a fresh batch of inoculum was produced. For shoots where leaf scars were created before the fungicide application, there generally was no decrease in susceptibility to *N. ditissima* with increasing leaf scar age in the treatments (data not shown). Overall, infection rates were similar ($P > 0.20$) between the different

leaf scar ages for copper oxychloride, copper hydroxide and captan treatments.

The percentages of shoots removed in the fresh scars (no direct fungicide exposure) overall were 27, 14, 20 and 14% for the four inoculation times and all fungicide treatments, but these differences were not statistically significant ($P = 0.162$). Although the fresh leaf scars were created after the fungicide application and just before inoculation, differences between the different fungicide treatments were still observed ($P = 0.079$; Figure 1b). These differences were found irrespective of the timing of the fresh scar inoculations (0, 3, 7 or 10 days) after fungicide application ($P > 0.30$, data not shown). Overall, the development of European canker in the fresh scars on shoots that had not been treated with fungicide (18.2% shoots removed) was higher ($P = 0.019$) than on shoots where the fungicide was sprayed (11.9% shoots removed).

Experiment 2

The percentage of shoots removed after artificial inoculation was higher in the unsprayed control treatment ($P < 0.001$) than in any of the fungicide treatments. There were no significant differences

Figure 1 Shoot infection by *Neovectria ditissima* (% inoculated shoots removed) on (a) leaf scars created before fungicide application (fungicide-treated scars) or (b) fresh leaf scars (made after the fungicide application) on artificially inoculated ‘Braeburn’ apple shoots in 2013 in Experiment 1. Apple trees were sprayed with one of four fungicides or left unsprayed. Three leaf scars per shoot were inoculated. Values are the mean for four inoculation application times. Error bars are 95% confidence limits.

($P>0.05$) between Nordox 45, Nordox 75 and captan in terms of the percentage of shoots that were removed (Figure 2). The numbers of lesions and shoots infected from background orchard inoculum were very low and statistically similar ($P=0.685$) for all treatments. For example, only 10 lesions in the unsprayed control treatment were found in the four replicate plots.

Experiment 3

The percentage shoots removed from the artificially inoculated shoots (fungicide treated leaf scars) did not vary substantially between the unsprayed control and any of the fungicide treatments ($P>0.1$; Figure 3a) 6 days after the fungicide applications. Shoots with fresh scars (and no direct fungicide exposure) expressed more disease ($P<0.001$) than shoots with leaf scars that had been formed 6 days before and received a fungicide spray (Figure 3b). While there were no fungicide treatment differences between the percentage shoots removed for

Figure 2 Shoot infection by *Neonectria ditissima* (% inoculated shoots removed) on leaf scars created before fungicide application with copper oxide (Nordox 45 and Nordox 75) and captan on artificially inoculated 'Scifresh'/Jazz™ shoots compared with that in the untreated control in 2013 in Experiment 2. Three leaf scars per shoot were artificially inoculated 0 days after treatment application. Values are the mean of one inoculation. Error bars are 95% confidence limits.

the fungicide treated leaf scars for both 0- and 6-day-old leaf scars ($P=0.557$; Figure 3a and b, respectively), there were significant treatment differences ($P<0.05$) for Nordox 75 and captan compared with the unsprayed control for the fresh scar inoculations 6 days after the fungicide application (Figure 3b).

DISCUSSION

The fungicides evaluated in all three experiments effectively controlled artificial leaf scar infections when used as protectants. While both copper oxide concentrations (0.653 and 0.975 kg elemental copper/ha) provided equal control in Experiments 2 and 3, copper hydroxide provided less control of European canker at 0.6 kg elemental copper/ha (Experiment 1). However, copper hydroxide using elemental copper concentrations >1 kg/ha has provided consistent control of *N. ditissima* leaf scar infections in Germany (Graf 1985; Palm 2009; Palm & Kruse 2012). Copper oxychloride with an elemental copper concentration of approximately 4.3 kg/ha also significantly reduced European canker infection from naturally occurring inoculum (Experiment 1). This is likely to be because of the higher copper deposits, its residual nature, and the gradual release of Cu ions during wet weather.

The present study also shows that copper-based fungicides were controlling *N. ditissima* leaf scar infections to a degree similar to that obtained with captan. This finding demonstrates that copper-based fungicides represent another option for orchard managers seeking effective postharvest control of *N. ditissima* leaf scar infections. The German horticultural extension services in the Hamburg area recommend a captan spray at the beginning of leaf fall followed by the application of copper hydroxide during mid and late leaf fall stages, with an additional midwinter copper application. Importantly, this strategy allows orchard managers to adhere to the allowable maximum of three copper-based applications per year, as required by the German regulatory authorities (Weber 2014b). In New Zealand, there is no restriction on the amount of copper conventional growers can apply as

Figure 3 Shoot infection by *Neovectria ditissima* (% inoculated shoots removed) on (a) leaf scars created before fungicide application (fungicide-treated scars) or (b) 0-day-old leaf scars (fresh leaf scars, no direct fungicide application, made 6 days after the fungicide sprays) and 6-day-old leaf scars (leaf scars created before fungicide application) on artificially inoculated ‘Scifresh’/Jazz™ shoots in 2014. Fungicide treatments were copper oxide (Nordox 45 and Nordox 75), captan and an untreated control. Two leaf scars per shoot were artificially inoculated. Values are the mean for (a) four or (b) one inoculation application times. Error bars are 95% confidence limits.

fungicides, but many orchard managers have chosen to adhere to the limit set by organic regulatory authorities of 3 kg elemental copper/ha/year (Tim Herman, Pipfruit New Zealand Inc., personal communication). However, in practice many orchard managers in New Zealand may not be able to access their blocks and spray during leaf fall, as high autumn rainfall causes wet and boggy conditions. Orchard managers also need to consider the choice of product and rate/ha. If environmental copper contamination becomes a concern, then soluble copper products and concentrations should be further evaluated for disease control efficacy. If access to the orchard is limited because of rain and wet ground conditions, copper deposits, based on product and rate of application, also need to be evaluated.

Generally, artificial inoculations in late May/early June result in peak lesion expression during November/December. Multiple assessments were made only to remove those lesions that expressed earlier. Observations have shown that 50% of young leaf scar lesions of approximately 1 cm

length are able to produce and release conidia (M. Walter, unpublished data). Therefore, lesions were removed regularly from artificial inoculations to prevent potential secondary infections. The early shoot removal also means that the number of lesions formed from artificial inoculations is likely to be higher than the results presented here. All shoots were removed at the end of the trial to remove potential *N. ditissima* infections, which may remain latent for several years (McCracken et al. 2003).

In glasshouse studies using field-collected conidia (R.W.A. Scheper, unpublished data), and pathogenicity trials using single spore isolates (Scheper et al. 2010; Amponsah et al. 2014), variation in number of lesions formed between inocula and isolates was observed. Equally, inoculum dose, duration of wet period temperature and wound age may affect the success rate of artificial inoculation with *N. ditissima* in field experiments (Xu et al. 1998; Latorre et al. 2002). Therefore, it is recommended that for sequential inoculation over time, fresh scars be included at each inoculation

time. There was no significant difference in lesion development between inoculation times in Experiment 1. However, in Experiment 3, the effect of inoculum and/or potential inoculation time was quite pronounced with 11% of untreated, fresh leaf scars expressing lesions from the first inoculation (0 days after the fungicide application, Figure 3a) and 68% of untreated, fresh leaf scars expressing from the second inoculation (6 days after the fungicide application, Figure 3b).

Interestingly for the fresh scar inoculations, treatment effects from the fungicide sprays were still apparent even in the absence of direct contact with the fungicides in Experiments 1 and 3. For example, leaf scars were created in Experiment 1 approximately 4–6 h (0 days), 3, 7 and 10 days after the spray applications. The observed reduction in European canker development shown in Figure 1 therefore was probably the result of fungicide redistribution onto the leaf scars either during the inoculation process and/or through wetness events and rain. This re-distribution of chemicals has been described by Weber (2014b) as the ‘depot effect’ of copper fungicides that accounts for their long-term efficacy and ability to redistribute. In the present study, this effect was observed for the copper products and the captan fungicide.

Wound susceptibility to infection generally decreases with age, as a result of wound healing. Wound healing depends on temperature and plant physiological activity and therefore would be expected to be higher during the vegetative growth stages in summer than in winter. Resistance to pathogen infection associated with wound age has been attributed to the formation of boundary zone tissues and wound periderm (Zeller 1926; Krähmer 1980; Xu et al. 1998). Wound susceptibility may also be affected by the *N. ditissima* inoculum dose, as reviewed by Weber (2014a). It has also been reported that leaf scar susceptibility to *N. ditissima* infections drops significantly within 48 h (Weber 2014b). In Experiment 3, wound susceptibility decreased with leaf scar age with approximately 67.4% and 8.1% shoots removed in the unsprayed treatment for 0 and 6 day old leaf scars, respectively

(Figure 3b). However, in Experiment 1 of this investigation, no significant reduction in leaf scar susceptibility to *N. ditissima* was observed for up to 10 days. Also, in earlier research, Walter et al. (2013) used a similar inoculum dose for the leaf scar inoculations in late May 2012 and reported a significant effect of leaf scar age in the same research block that was used in 2013. Xu et al. (1998) found similar conflicting results when studying pruning wound infections by *N. ditissima* (formerly *N. galligena*). Clearly, the driving parameters of tissue susceptibility include temperature, inoculum dose, wetness duration and plant physiological activity (Crowdy 1949, 1952; Dubin & English 1974; Xu et al. 1998), but their interactions and relationships and other contributing factors are not yet fully understood, and warrant further investigation.

Based on the fungicide results presented here and experience overseas, an autumn spray programme for European canker control that alternates captan with a copper-based product is recommended. The copper oxide-based product Nordox 75WG currently has a label claim for European canker control in New Zealand, and at this time should be the copper-based fungicide alternated with captan. The label rates of most other copper-based products relate to control of black spot caused by *Venturia inaequalis*, and there is limited New Zealand-based research on control of *N. ditissima* with copper-based products other than Nordox 75. Studies to progress label claims for other copper-based fungicides are recommended.

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