Effect of a protectant copper application on Psa infection of kiwifruit trap plants

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Abstract *Pseudomonas syringae* pv. *actinidiae* (Psa) causes bacterial canker of kiwifruit, which is an ongoing threat to New Zealand kiwifruit production. Disease control depends on orchard practices such as removal of visibly diseased material, pruning during low-risk periods, and the application of foliar bactericides. Although the use of copper compounds on *Actinidia* species (kiwifruit) can cause phytotoxicity, copper-based formulations remain a key component of Psa control in New Zealand. The effect of single copper applications on Psa infection of 'Hort16A' trap plants was studied over the Spring of 2014 (Sept–Nov). Psa leaf spots were observed at the beginning of October, appearing first on the untreated plants. Although the copper sprays did not achieve complete protection, particularly as the inoculum built up during November, the copper-sprayed plants always had less disease than the untreated plants.

Keywords *Pseudomonas syringae* pv. *actinidiae*, *Actinidia chinensis* var. *chinensis* 'Hort16A'.

INTRODUCTION

Pseudomonas syringae pv. *actinidiae* (Psa) causes bacterial canker of kiwifruit, which is currently the most important disease of cultivated *Actinidia* species worldwide, and is an ongoing threat to New Zealand kiwifruit production (Tyson et al. 2016). Disease control depends on orchard practices such as removal of obviously diseased material, pruning during dry periods to minimise the potential inoculum load on susceptible wounds, and the strategic application of foliar bactericides (copper formulations, antibiotics and biologicals) to minimise infection. Although use of copper compounds on kiwifruit can cause phytotoxicity problems (Donati et al. 2014), copper-based formulations remain a key component of Psa control in New Zealand.

Recent studies of Psa inoculum production and infection over extended periods (Beresford & Tyson 2014; Tyson et al. 2014) have shown that rainfall is essential for disease development on trap plants. There were also indications of a strong seasonal component to Psa epidemiology. Over the spring of 2013 (Sept, Oct, Nov), Psa leaf spots developed on the trap plants after every rain event. From late summer to late winter, disease development was more erratic (Tyson et al. unpub. data).

During spring, kiwifruit vines break dormancy, with leaves emerging first, then blossoms. Young leaves are particularly susceptible to Psa (Tyson

et al. 2015); the flower buds can also be affected by blossom blight, making this a key time for Psa control.

This project aimed to study the effect of a copper application (Nordox) on Psa infection of trap plants over the spring of 2014 (Sept-Oct-Nov).

MATERIALS AND METHODS

From 8 September to 28 November 2014, trap plants were exposed for 4-day periods in two Psa-infected blocks (Blocks 40 and 50) of mature kiwifruit on the Te Puke Research Orchard (TPRO). Block 50 contained *Actinidia chinensis* var. *deliciosa* 'Hayward' and block 40 contained a mixture of new and non-commercial varieties. Both blocks were known to have substantial amounts of Psa leaf spotting over the previous two years.

Trap plants

Tissue-cultured plantlets of *A. chinensis* var. *chinensis* 'Hort16A' were grown in a glasshouse in a Psa-free area. At approximately 3 months post-tissue culture, the plants were 30–40 cm high and had 10–15 leaves. To obtain plants that were as uniform as possible, plants were tipped at a height of 40 cm.

Within each experimental block, four sets of five plants were exposed for 96 h periods (09:00 Monday – 09:00 Friday). Hanging containers were used to suspend the plants just below the canopy. Within each block, two sets of plants were untreated controls and two sets were sprayed with copper oxide (Nordox at 37.5 g/100 L), targeting both leaf surfaces, to run-off before being exposed to potential infection in the orchard.

After 96-h exposure in the field, the plants were incubated in a laboratory under growth lights at c. 20°C for 21 days and then assessed for symptom expression (leaf spots). Any leaves that developed Psa-type leaf spots were sent to the Plant & Food Research Mt Albert Research Centre (MARC) for diagnosis.

Assessment of phytotoxicity

After 96-h exposure in the orchard and a further 21 days incubation, the trap plants were visually assessed for signs of phytotoxicity on the leaves.

Psa isolation and identification

Areas of leaf with disease symptoms were excised, macerated in 200 µL bacteriological saline (0.85% NaCl in sterile distilled water) and left for 5 min, after which 100 µL of the resulting suspension was spread across KBC, an agar medium semi-selective for *Pseudomonas* species (Mohan & Schaad 1987). The isolation plates were incubated at 20°C for 72 h and then assessed for bacterial growth.

DNA extraction, and qPCR conditions and analysis, were as described by Tyson et al. (2012), using the primers PsaF3 and PsaR4 developed by Rees-George et al. (2010). In addition, bacterial 23S primers (Anthony et al. 2000) were used in qPCR as an internal control to check that the DNA was PCR-competent. In this study, a Cp (crossing point or threshold value) value below 30 was interpreted as a Psa positive result, 30–35 as a weak positive, and a Cp value above 35 as a negative result.

Weather data

Hourly temperature and rainfall data were collected from the weather station at TPRO over the period of trapping. The total amount of rainfall collected during each 96-h exposure period was then calculated. The weather data were downloaded from the National Institute of Water and Atmospheric Research Ltd (NIWA) National Climate Database (CliFlo) for the period of the trial (Te Puke Ews, station no. 12428, latitude 37.822, longitude 176.324).

RESULTS

No phytotoxicity was observed on any of the copper-treated trap plants during the trial.

No Psa leaf spots developed on any copper-treated or untreated plants exposed during September 2014. The first Psa leaf spots were

observed at the beginning of October, appearing first on the untreated control plants (Table 1). Leaf spots were not observed on copper-treated plants until the end of October. The number of copper-treated plants with Psa leaf spots was always lower than for untreated plants so the copper treatment appeared to have a protective effect. Low levels of leaf spots observed at many of the sampling dates meant that statistical analysis of variation among the replicate sets of blocks was not meaningful. The amount of rainfall collected over each 96-h exposure period is shown in Figure 1 along with the percentage of plant with Psa leaf spots observed for either untreated or copper-treated plants.

DISCUSSION

In this trial, copper-treated and untreated control trap plants of the highly susceptible kiwifruit cultivar 'Hort16A' were exposed to natural Psa inoculum in an infected orchard over 12 weeks in September, October and November 2014, which is the New Zealand spring. Infection of kiwifruit plants by Psa is known to be affected by leaf age (Tyson et al. 2015), with young leaves being more susceptible to the bacterium, making spring a crucial period for disease control.

Throughout the spring of 2014, the copper-treated trap plants always had less disease than the untreated plants. However, the copper treatment did not completely protect the plants from Psa, particularly as Psa inoculum built up in the orchard blocks during November.

Psa leaf spots developed after 7 of the 12 weeks of the trial, always after periods of rain. No spots developed after the single exposure period during which there was no rainfall. This result is consistent with the results of previous

Table 1 The number of *Actinidia chinensis* var. *chinensis* 'Hort16A' trap plants with *Pseudomonas syringae* pv. *actinidiae* (Psa) leaf spots from 12 sets of 40 plants, either treated with copper oxide or left untreated, 21 d after exposure in an infected orchard for 96-h periods during spring 2014.

Date exposed	Cumulative rain (mm over the 96-h exposure period)	Number of plants with Psa leaf spots		Reduction in Psa after application of copper oxide (%)	Crop growth stage
		Copper treated (n=20)	Untreated (n=20)		
8-Sep-14	16.7	0	0	-	
15-Sep-14	23.4	0	0	-	budburst
22-Sep-14	4.1	0	0	-	
29-Sep-14	3.4	0	0	-	budburst
6-Oct-14	1.8	0	1	100	
13-Oct-14	0.0	0	0	-	budburst
20-Oct-14	0.3	0	1	100	
28-Oct-14	9.9	0	7	100	leaves
3-Nov-14	10.3	9	11	18	
10-Nov-14	4.7	1	7	86	leaves, flowers
17-Nov-14	17.2	5	16	69	
24-Nov-14	0.9	0	3	100	leaves, flowers

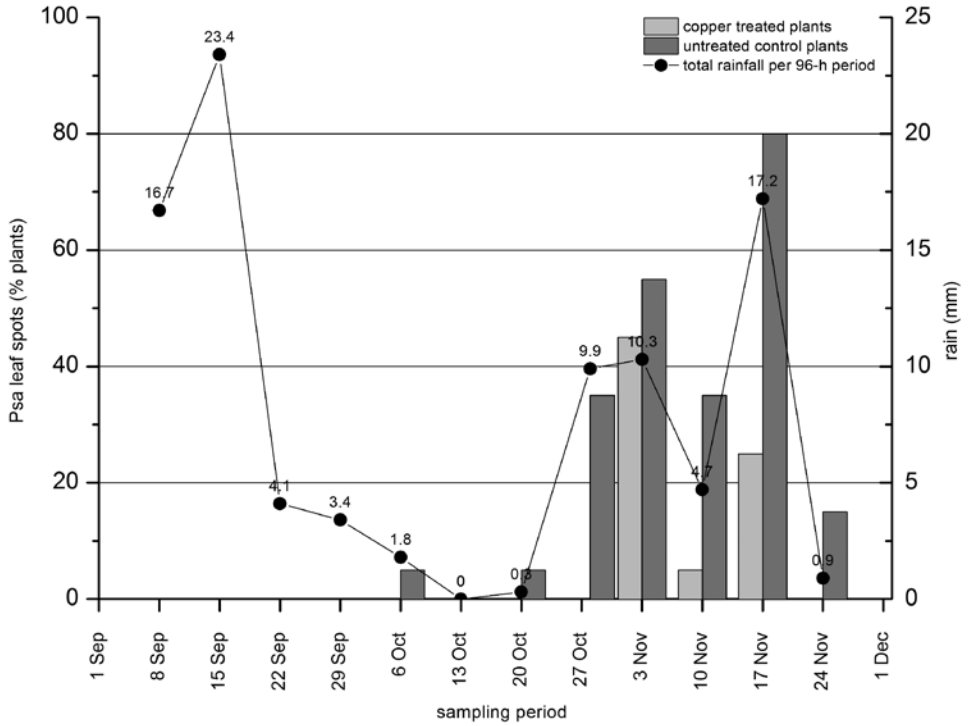

Figure 1 *Actinidia chinensis* var. *chinensis* 'Hort16A' trap plants, either treated with copper oxide or left untreated, with *Pseudomonas syringae* pv. *actinidiae* (Psa) leaf spots after exposure in an infected orchard for 4-day periods during spring 2014.

studies that have shown that leaf infection is dependent on rainfall (Tyson et al. 2014; Beresford et al. 2017).

The two major instances where the copper-treated plants were not fully protected by copper applications were marked by periods of high inoculum availability, as indicated by the increased incidence of disease on the untreated control plants and increasing amounts of leaf spotting in the mature kiwifruit plants.

The fact that some leaf spots were found on copper-treated plants is an important issue of concern to the kiwifruit industry. Strains of Psa with copper-resistance were detected as early as 1987 in Japan (Nakajima et al. 2002) and in New Zealand isolates of Psa in 2014 (Colombi et al. 2017), 4 years after the first detection of kiwifruit canker in 2010 (Everett et al. 2011). The strain of Psa causing leaf spotting on the copper-treated plants was not tested for resistance to copper, however, and there was no indication that there was resistance to copper in the orchard being studied.

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