2013/14 Potted Plant Field Trial Report

Copper products on Bruno plants

December 2013– February 2014

March 2014

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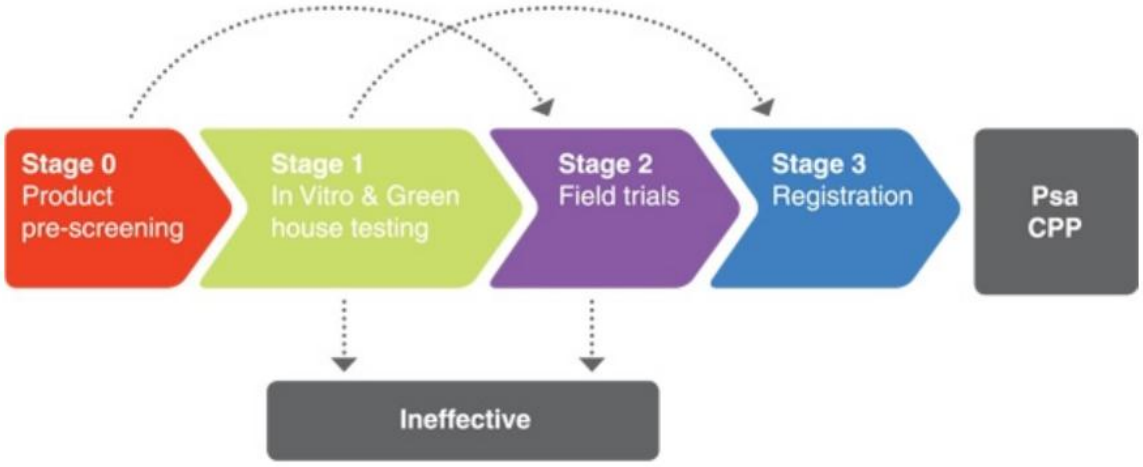
Introduction

Zespri, with support from KVH, is coordinating the screening of the effectiveness of a wide range of products to control *Pseudomonas syringae pv. actinidiae* (Psa-V). The screening programme has been developed to identify options for managing Psa-V. To understand the steps in the product testing programme the process is outlined in the diagram below.

An important stage in the testing programme is field testing which is the subject of this report. The efficacy of products for the control of Psa-V is being evaluated using potted plants in an infected orchard in Te Puke. The plants have been propagated Psa-V free and typically are treated with products prior to being shifted to the trial site where they are actively inoculated with Psa-V. Symptoms are subsequently monitored in the field. Products are applied using protocols agreed with the suppliers.

For the second year running, Zespri has contracted HortEvaluation Ltd to undertake these field trials. The results are reported directly to Zespri so that publications of this nature can be produced.

This report documents the findings from a trial conducted from December 2013 to February 2014 on Bruno potted plants in which various copper products were tested.



Objective(s)

This trial was established to determine the efficacy of three, previously untested copper products, (copper hydroxide, copper oxide and chelated copper), in reducing Psa infection in Bruno kiwifruit plants. Two products were tested at different concentrations to determine the most effective rate and one product, the chelated copper, was applied four times. Nordox was used as the positive control.

Methodology

All spraying, inoculating, transportation and disposal of plants was performed under the relevant MPI / ACVM and KVH approvals. All products were tested with the permission and guidance of the suppliers.

Plants

This trial utilised Bruno kiwifruit potted plants, sourced from a kiwifruit nursery in Kerikeri. The plants were believed to be Psa-V free at the start of the trial as there were no observed symptoms of Psa-V disease. The plants were transported from Kerikeri to HortEvaluation in Hamilton, where the plants were randomly assorted into treatment groups and labelled, prior to the start of the trial.

Treatments

There were 10 treatment groups, with 15 plant replications per group. Table 1 lists the treatment groups, rates, number and timing of applications relative to Psa inoculation (-1 = 1 day prior to Psa inoculation; + days = post Psa inoculation) and final concentration of product per plant.

Table 1.

Treatment	Active Ingredient	Rate (per 100L water)	Application timing (days)	Final active concentration / plant
Grosafe Copper	copper hydroxide	50g	-1	0.05g
Grosafe Copper	copper hydroxide	70g	-1	0.07g
Grosafe Copper	copper hydroxide	90g	-1	0.09g
Copper Red	copper oxide	50g	-1	0.08g
Coptyzin +Fructol	chelated copper	80ml + 100g	-1, +10, +20, +30,	0.025g (per application)
Coptyzin +Fructol	chelated copper	100ml + 100g	-1, +10, +20, +30,	0.03g (per application)
Coptyzin +Fructol	chelated copper	125ml+ 100g	-1, +10, +20, +30,	0.04g (per application)
Nordox	copper oxide	37.5g	-1	0.09g
Water (negative)	N/A	N/A	N/A	N/A
Psa (positive)	N/A	N/A	N/A	N/A

Treatment application

Spraying of treatments at -1 day was performed at HortEvaluation, Hamilton. All post inoculation spraying was performed at the trial site, 866 No. 2 Road, Te Puke. A gas assisted backpack sprayer was used to produce fine droplets. The entire canopy of each plant was thoroughly sprayed. Spraying was performed between December 2013 and January 2014.

Plants were inoculated on 9th December 2013. On the day of inoculation, the plants were transported to Plant and Food Research, Te Puke. The plants were placed inside a gazebo, which itself was housed inside a shed, to ensure double containment of inoculum at time of application.

Inoculum was cultured by Plant and Food Research, Te Puke to a concentration of 10^8 cfu/ml bacterium. A sample of the inoculum was taken at the beginning, middle and end of plant inoculation to monitor the concentration of bacteria. The inoculum concentration remained at 10^8 cfu/ml throughout the procedure.

Plants were inoculated in groups, with plants being randomly chosen from each treatment group to be inoculated at any one time, to account for any variation in inoculation that may have occurred throughout the day.

The inoculum was sprayed onto the undersides of the leaves until wet, with 5L hand-held pressure sprayers with fine nozzles. The water treatment group was sprayed in an identical manner with tap water.

Initial wetting of plants

Once inoculated the plants were transported from Plant and Food Research, Te Puke, to the trial site. The plants were placed under overhead water misters for 48 hours with continuous water flow, to ensure the wet climatic conditions required for disease incidence. After 48 hours of misting, the plants were relocated to their final trial site positions. The plants were watered twice a day, for 2 hours, via drippers that were placed over their pots.

Assessments

The level of leaf spotting, as a percentage of leaves covered in spots, and secondary symptoms were visually estimated and recorded at days 10, 22, 27 and 38 post inoculation. The same assessors were used to score the plant disease symptoms, to ensure continuity in the scoring. Assessments were performed during December 2013 and January 2014. Table 2 lists the secondary symptoms that were measured and the ranking used to score secondary disease symptoms.

Each time, the amount of total leaf area covered in spots was estimated. The parts of the plants that were mature at the time of inoculation were assessed separately from the parts that were expanding.

Table 2.

Secondary symptom(s)	Score given
None	0
Leaf curl	1
Leaf curl + 1 die back shoot	2
Leaf curl + > 1 die back shoot	3
Leaf curl + > 1 die back shoot, ooze	4
Plant dying / death	5

While visual assessments are subjective, the same assessor performed each assessment to ensure consistency of scoring. Throughout treatment application, inoculation and assessment, the focus was on ensuring consistency across treatments.

Weather

During the inoculation stage of the trial, there was 0.2 mm of rainfall. This was followed by a 10 day dry spell, followed by a 12 day wet spell when 106.6mm of rainfall was recorded. An additional dry spell ensued until the last two days of the trial when 3mm of rainfall was recorded. Thus in total the plants received 109.8mm of rain. The average maximum air temperature throughout the trial period was 23.7^{o}C (range 15.9 – 27.6).

Statistical Analysis

Analysis of the leaf spotting data was performed in Microsoft Excel (Microsoft Office 2010). An ANOVA was performed comparing all of the treatment groups at the different assessment times. Further analysis was performed using the student T-Test to determine the differences between each treatment versus Psa alone at each assessment.

Results and interpretation

There was a good level of disease incidence throughout the trial, with 13% leaf spotting in the Psa only group at day 10 post inoculation, rising to 47% by day 38 (17th January 2014). In contrast, the water treatment group had negligible leaf spotting until day 27, with a final leaf spotting assessment of 13% at day 38. The level of leaf spotting in the water group remained significantly lower than the Psa group throughout the trial (T-test $p < 0.001$).

Figure 1 shows the average percentage leaf spotting per treatment group at the four assessment time points. Figures 2a – e are photos showing typical examples of the state of the plants for each treatment at the highest concentration given.

At days 10 and 22, all treatment groups had significantly less leaf spotting compared with the Psa only group; ANOVA $p < 0.001$, F = 7.52, 9 d.f. and $p < 0.001$, F = 15.2, 9 d.f. respectively.

At day 27, Coptyzin at concentrations of 8ml and 10ml / 10L water no longer had significantly less leaf spotting compared with the Psa only group (T-test $p > 0.05$). All other treatment groups had significantly less leaf spotting compared with the Psa group (T-test $p < 0.05$).

At day 38 leaf spotting in the all of the Coptyzin groups, Copper Red treatment group and Nordox control group were not significantly reduced compared with the Psa group (T-test $p > 0.05$). In the HortCare Grosafe copper treatments (all concentrations) leaf spotting remained significantly less than the Psa group (T-test $p < 0.05$).

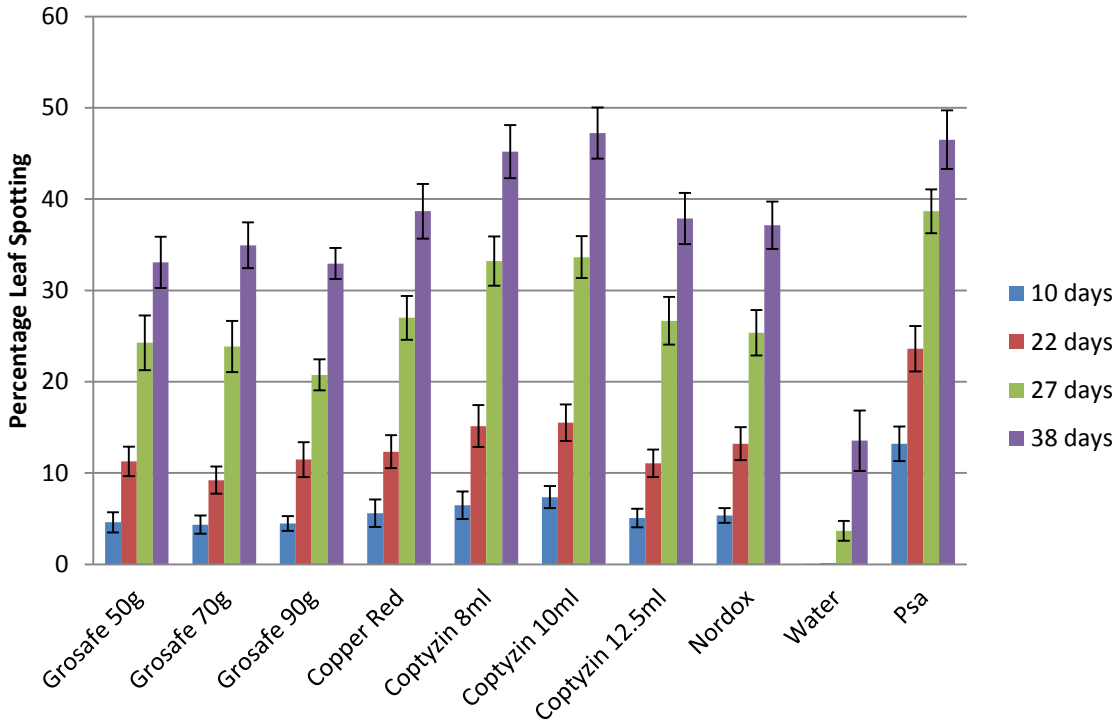


Figure 1. The error bars are the +/- SEM. No indication of significance is given on the graph, as this would require a large number of symbols, making it difficult to interpret. Please refer to the text regarding significant differences in leaf spotting.

Secondary symptoms were measured at days 27 and 38 using a disease score index (refer to Table 2). Figure 3 shows the secondary symptom index score for each treatment group. At day 38, 81% of plants across all groups had a disease score of '1', 13% had a disease score of '2' and one plant in the Nordox group had a score of '3', which was the highest disease score recorded. The water control group had scores ranging from '0' to '1', compared with the Psa group which ranged from '1' to '2'. This highlights that, except for leaf curl, secondary symptoms of disease were not a major problem in this experiment.

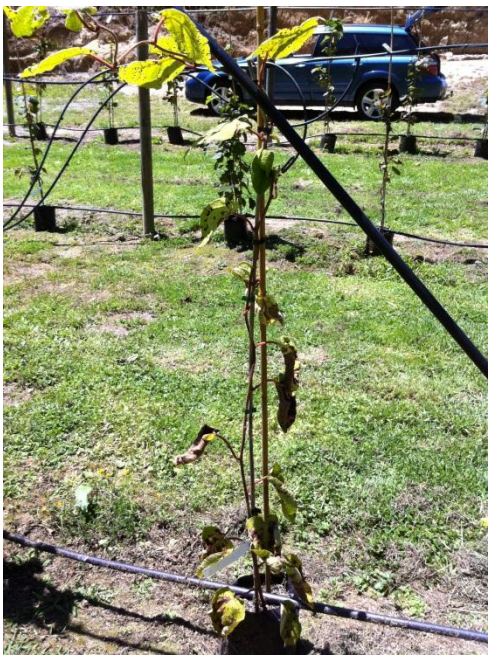


Figure 2a. A typical Psa treatment group plant at day 38.

Figure 2b. A typical water treatment group plant at day 38.

Figure 2c. A typical Grosafe 90g group plant at day 38.

Figure 2d. A typical copper oxide group plant at day 38.

Figure 2e. A typical Coptyzin (12.5ml / 10L) group plant at day 38.

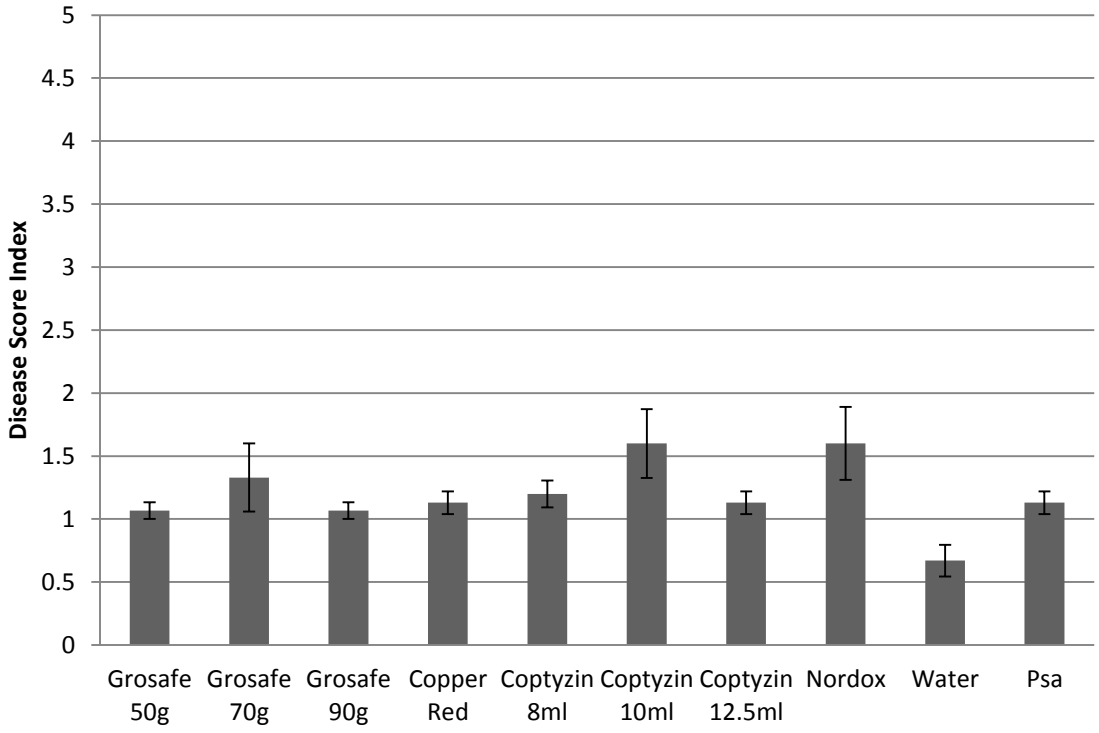


Figure 3. Secondary symptom score at day 38. A '0' score records no symptoms, a score of '5' records plant dying or death of plant.

Summary

Spray inoculation of Bruno plants with 10^8 cfu / ml of Psa-V resulted in a good level of infection, as determined by leaf spot analysis. There was a 12 day wet spell during the middle part of the trial, with 109.8mm of rainfall, which may have contributed to the level of infection observed. Up to 22 days post inoculation, all the copper products were able to significantly reduce leaf spotting by an average of 52% compared with the Psa only group. At 27 days, despite re-application, the two lower concentrations of Coptyzin (8 and 10 ml/ 10L water) were no longer able to significantly decrease leaf spotting compared with the Psa only group. At day 27 Coptyzin had been applied three times, thus the amount of active chelated copper applied to each plant by this time, was *now* in the range that was received by plants sprayed with Grosafe Hortcare and Copper oxide products. At day 38, only the Grosafe HortCare copper hydroxide treated plants (all concentrations) had significantly lower leaf spotting compared with the Psa only plants. However, in all groups leaf spotting increased over time, with all treatments except water only, having over 30% of leaf spotting at day 38.

All treatment groups suffered from a degree of leaf curl, but other secondary symptoms were low in incidence and so not a major problem in this trial.

There was no evidence of phytotoxicity in this trial.

83% of the plants across all treatment groups had new growth at the end of the trial (data not shown).

A number of suggestions can be made from this data:

1. The copper products tested in this trial can significantly reduce the incidence of leaf spotting up to 3 weeks (22 days) after Psa exposure.
2. Some differences in effect were observed, which may be due to copper formulation, rather than rate applied, as Grosafe Hortcare copper hydroxide still showed a significant decrease in leaf spotting at the end of the trial for all concentrations applied.
3. Once Psa has established itself on and within the plant, repeated applications of copper may no longer be able to reduce leaf spotting. Please note only one copper product was repeatedly applied in this trial and there was less active copper applied per application than other products tested.
4. The copper products tested here exhibited a similar efficacy to the Nordox copper control group.