2013/14 Potted Plant Field Trial Report

Kasumin® and other products on Hort 16A

January 2014– March 2014

May 2014

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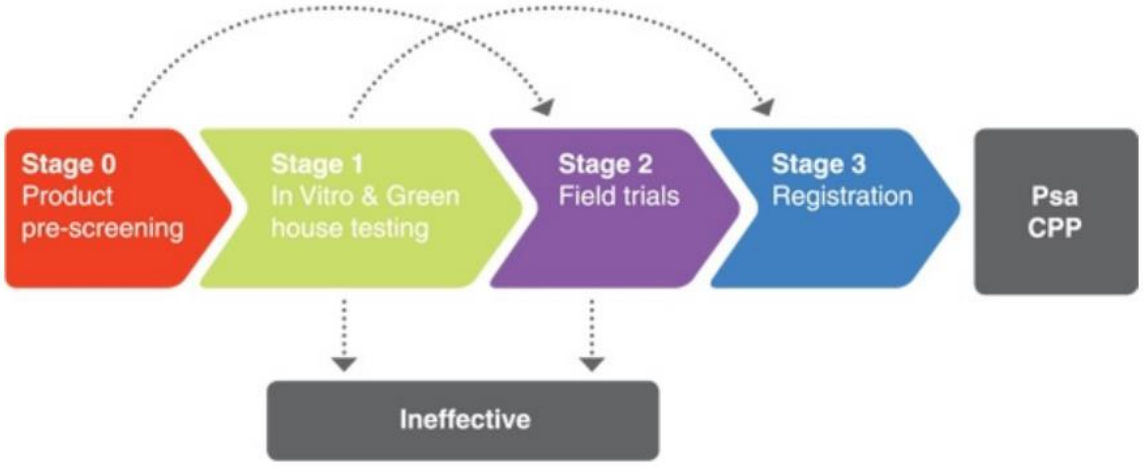
Introduction

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 Te Puke region 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 third 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 January to March 2014 on 16A potted plants in which Kasumin, copper products with or without rotation with seaweed extracts and Nanospada 500 were tested.



Objective(s)

This trial was established to determine the most effective time to apply Kasumin in response to an infection event. In addition a number of other products were tested including a copper product Ag Copp 75 and Nanospada 500. In addition two seaweed extracts were tested to determine if alternating spraying with copper had any effect on efficacy and could reduce phytotoxicity symptoms.

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 Hort 16A kiwifruit potted plants, sourced from kiwifruit nurseries in the Northland and Nelson regions. 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 the nurseries 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 15 treatment groups, with 15 plant replications per group. Table 1 lists the treatment groups, active ingredient, rate of product application, and the number and timing of applications relative to Psa inoculation (-1 = 1 day prior to Psa inoculation; + days = post Psa inoculation; T0 time of Psa inoculation).

Table 1.

Treatment	Active Ingredient	Rate (per 100L water)	Application timing (days)
Kasumin	Kasugamycin	500ml	-14 days
Kasumin	Kasugamycin	500ml	-7 days
Kasumin	Kasugamycin	500ml	-1 day
Kasumin	Kasugamycin	500ml	+3 days
Kasumin	Kasugamycin	500ml	+7 days
Kasumin + Nordox	Kasugamycin + Copper oxide	500ml + 37.5g	-1 day
Nanospada 500	QAC mixture	25L	-1 and +14 days
Nordox 75WG + Acadian (Ascophyllum spp)	Copper oxide + Seaweed extract	37.5g + 5g	-8, -9, -10 Acadian, -3, -4 Acadian, -1 Nordox, +10 Acadian, +20 Nordox, +30 Acadian
Nordox 75WG + Alga 600 (Ascophyllum, Laminaria & Sorgossum)	Copper oxide + Seaweed extract	37.5g + 5g	-1 Nordox, +10 Alga 600, +20 Nordox, + 30 Alga 600
KeyStrepto	Streptomycin	60g	-1 day
Nordox 75 WG	Copper oxide	37.5g	-1 day
Nordox 75 WG	Copper oxide	37.5g	-1 day, +20 days
Ag Copp 75	Copper	37.5g	-1 day
Water	N/A	N/A	T0
Psa	N/A	N/A	T0

Treatment application

Spraying of treatments up to -1 day were 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 January 2014 and March 2014.

Plants were inoculated on 4th February 2014. On the day of inoculation, the plants were transported to Plant and Food Research, No. 1 Road, 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 at a rate of 2L per hour, via drippers that were placed over their pots.

Assessments

The level of leaf spotting, as a percentage of total leaf area covered in spots, and secondary symptoms were visually estimated and recorded at days 10, 17, 28 and 37 post inoculation. The same assessors were used to score the plant disease symptoms, to ensure continuity in the scoring. Assessments were performed during February 2014 and March 2014. Table 2 lists the secondary symptoms that were measured and the scoring used to rank secondary disease symptoms.

Table 2.

Secondary symptom(s)	Score given
None	0
Browning of shoot or stem	1
Tip die back	2
Shoot die back	3
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

Pre-Psa inoculation treatment applications were made at intervals ranging from 14 (21 January) to 1 day (3rd February) before inoculation. During this time 16.4mm of rain fell. Plants were then moved from Waikato to Te Puke for inoculation.

From inoculation on 4 February 2014 to final assessment on 13 March 2014 only 4.6mm of rain was recorded in the 37 day period. The air temperature range through the trial period was 3.4^{o}C

on 4 March 2014 to 26.4°C on 5 February 2014. The average daily air temperature was 16.4°C and the average minimum and maximum were 11.1°C and 23.2°C respectively.

Statistical Analysis

Analysis of the leaf spotting data and secondary symptoms was performed in JMP 10 Statistical Package (SAS Institute). An ANOVA was performed comparing all of the treatment groups at the different assessment times. If a significant difference was indicated, further analysis was performed using a 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 10% leaf spotting in the Psa only group at day 10 post inoculation, rising to 20% by day 37 (13thMarch 2014). In contrast, the water treatment group had 1% leaf spotting at day 10, with a final leaf spotting assessment of 3% at day 37. The level of leaf spotting in the water group remained significantly lower than the Psa group from Day 17 ($p < 0.001$). Disease Severity Scores (secondary symptoms) remained significantly decreased compared with the Psa group throughout the trial ($p < 0.001$).

Figure 1 shows the average percentage leaf spotting per treatment group at the four assessment time points (Day 10, 17, 28, 37). The slight decrease in leaf spotting observed at Day 28 for the Water + Psa group is an artefact due to the presence of 5 dead plants at this assessment, resulting in leaf spot assessments being made for 10 plants, rather than 15, in the group.

At ***Day 17*** significant decreases in leaf spotting were observed for:
Kasumin applied at -14 days and – 1 day
Nanospada 500
Nordox with Acadian.

At ***Day 37*** significant decreases in leaf spotting were also observed for:
Kasumin +7 days
Kasumin with Nordox
KeyStrepto.

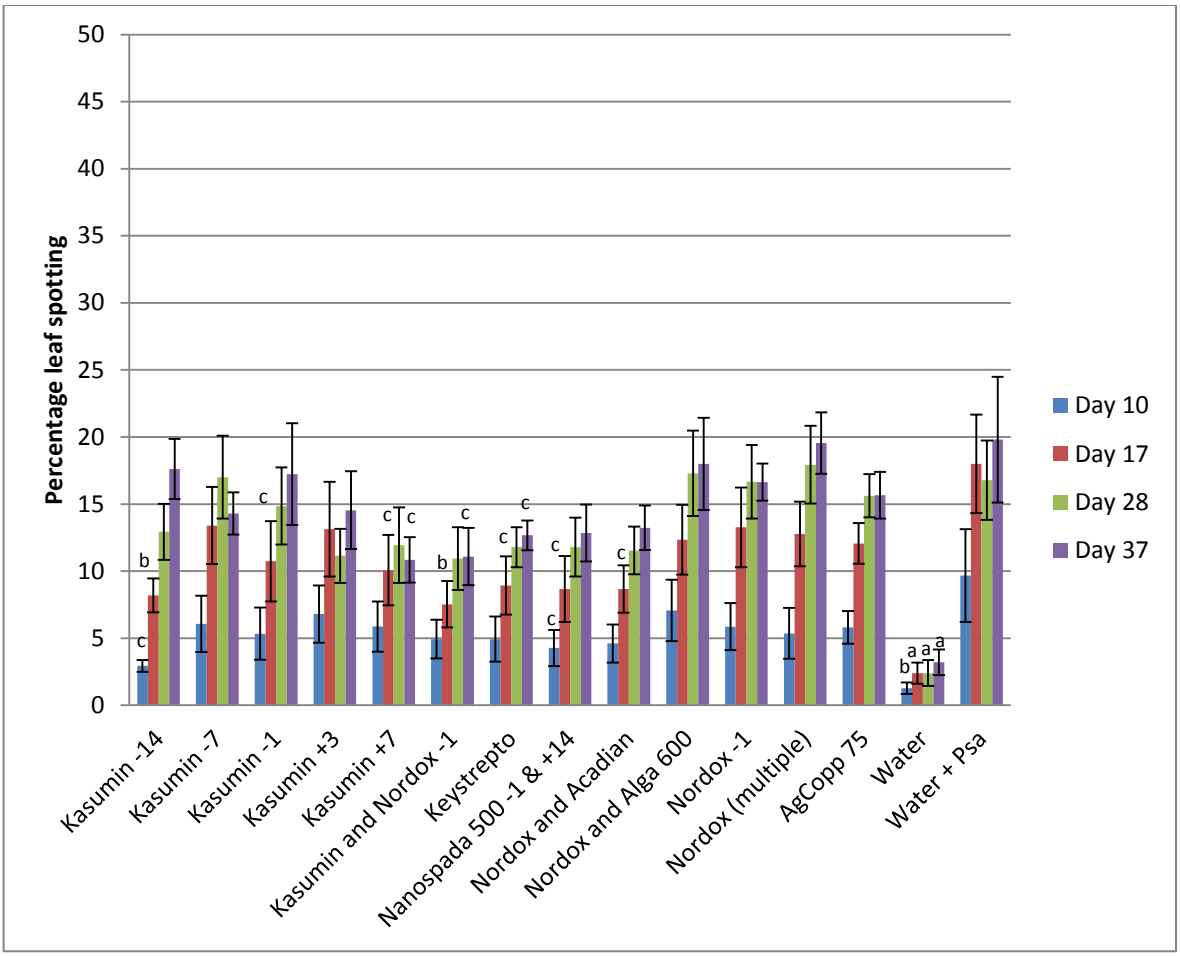


Figure1. Percentage leaf spotting in Hort 16A potted plants inoculated with 10^8 cfu/ml Psa. Significance levels: a = $p < 0.001$; b = $p < 0.01$; c = $p < 0.05$. Error bars are +/- SEM.

Secondary symptom scores are shown in figure 2. This shows the level of disease severity at Day 28 post Psa inoculation. Kasumin applied at -1 day, Nanospada 500 and two applications of Nordox significantly reduced the symptoms compared with Psa at this time. By Day 37, the disease had progressed in all treatment groups (except water), resulting in no significant differences in symptoms between the treatment groups and Psa group, with all groups averaging a Disease Severity Score of 3 – 4.

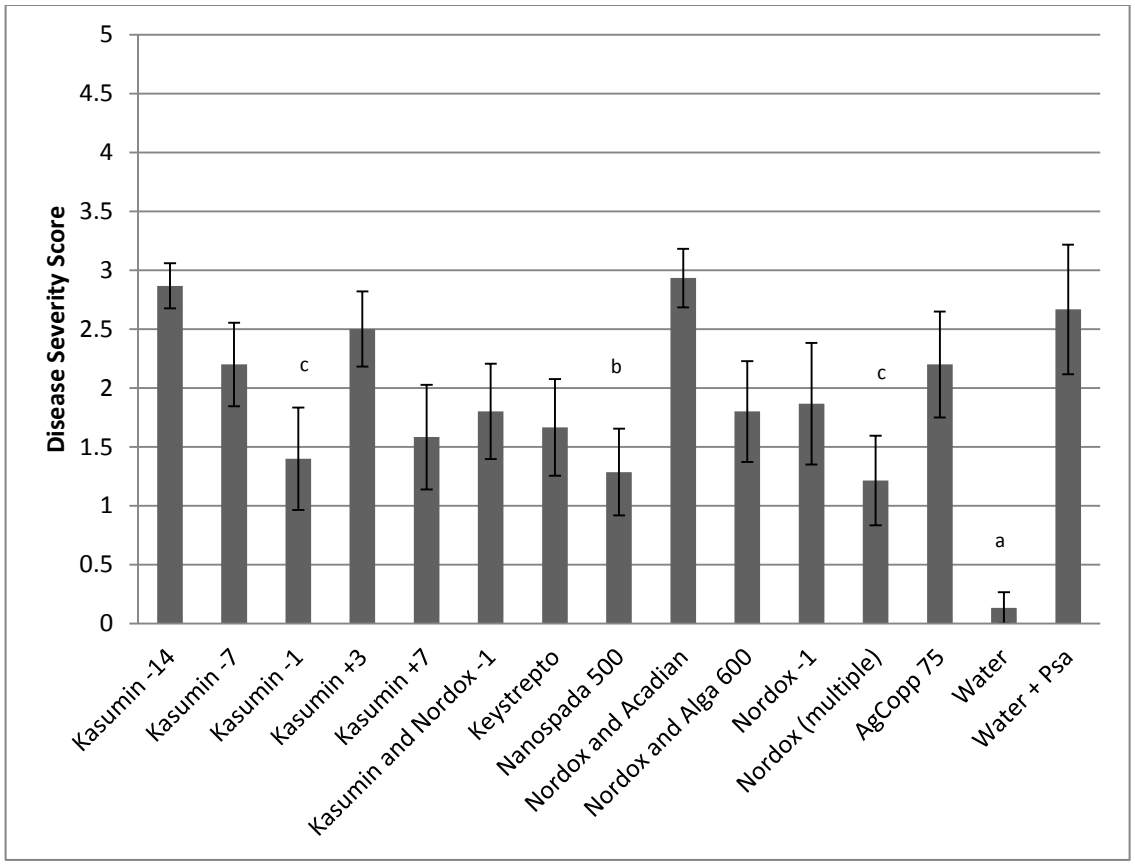


Figure 2. Secondary disease severity scores on Hort 16A plants spray inoculated with 10^8 cfu/ml Psa 28 days post inoculation. A '0' score records no symptoms; a score of '5' records plant dying or death of plant. Significance level: a = $p < 0.001$; b = $p < 0.01$, c = $p < 0.05$. Error bars are +/- SEM.

Appendix 1, figures 3 - 17 show photos of the condition of the plants for each treatment at the end of the trial (37 days post Psa inoculation).

Summary

Spray inoculation of 16A plants with 10^8 cfu/ml of Psa-V resulted in a good level of infection, as determined by leaf spot analysis and Disease Severity Score (assessment of secondary symptoms).

Different application times of kasumin, ranging from 14 days prior to and 7 days post Psa inoculation, were assessed using the label rate of 100ppm. Even though significant decreases in leaf spotting were observed with -14 days, -1 day and +7 days treatment groups, at Day 28 only the – 1 day group had a significant decrease in secondary disease symptoms. Kasumin applied with Nordox at -1 day again produced significant decreases in leaf spotting up to Day 37, but at Day 28 there was no significant difference in secondary symptoms compared with Psa group. KeyStrepto was used as the positive control and significantly decreased leaf spotting up to 37 days post Psa inoculation. Both bactericides appear to have similar efficacy in controlling Psa.

Another potential Psa control product was identified, Nanospada 500, which significantly reduced leaf spotting for up to 17 days and secondary symptoms for up to 4 weeks post Psa inoculation, when applied at -1 and +14 days.

The effect of the seaweed extracts on copper phytotoxicity was not determined as phytotoxicity was not observed in the trial. The seaweed extracts did not have any effect on Psa symptoms as there were no significant decreases in leaf spotting and secondary symptoms compared with Psa treatment group throughout the trial.

A number of observations and suggestions can be made from the data:

1. Kasumin at the label rate of 100ppm applied 1 day prior to an infection event produces the greatest protection against Psa disease in Hort 16A kiwifruit potted plants for up to 4 weeks, compared with all of the other application times of Kasumin tested in this trial. This data supports the current method of use of Kasumin.
2. Nanospada 500, a QAC product previously found to show efficacy against Psa in a glasshouse trial, again showed a level of Psa disease control similar to that observed with bactericides Kasumin and KeyStrepto. This product is currently not registered for use as an agrichemical, but will be tested further.
3. Two applications of Nordox significantly decreased secondary symptoms at 4 weeks post Psa inoculation, whereas a single application did not. Multiple applications of Nordox provided greater protection than a single application, supporting the current method of use of copper products.
4. A single application of Ag Copp 75 had a similar effect on leaf spotting and secondary symptoms as a single application of Nordox.
5. Alternating applications of Nordox with seaweed extracts did not significantly decrease secondary symptoms, in fact Nordox alternated with Acadian had Disease Severity Score similar to the Psa treatment group. However, Nordox with Acadian did significantly decrease leaf spot at Day 17 compared with the Psa group. The effect of the seaweed extracts in reducing phytotoxicity caused by copper was unable to be assessed as phytotoxicity was not observed during the trial. This could suggest that seaweed extracts used to mitigate copper phytotoxicity may affect Psa control, however more research would be required to confirm this.

Appendix 1

The plants are arranged in the photos to show the most severe symptoms, i.e. dead plant, on the left hand side of the photo, going through to plants with least severe symptoms, i.e. none or stem discolouration on the right hand side.

Figure 3. Kasumin -14 days

Figure 4. Kasumin -7 days

Figure 5. Kasumin -1 day

Figure 6. Kasumin +3 days

Figure 7. Kasumin +7 days

Figure 8. Kasumin + Nordox – 1 day

Figure 9. Nanospada 500 -1 and + 14 days

Figure 10. Nordox and Acadian. Acadian -8, -9 or -10 days; Acadian -3 or -4 days, Nordox – 1 day; Acadian +10 days; Nordox + 20 days; Acadian + 30 days.

Figure 11. Nordox and Alga 600. Nordox – 1 day; Alga 600 + 10 days; Nordox + 20 days; Alga 600 + 30 days.

Figure 12. KeyStrepto – 1 day

Figure 13. Nordox single application – 1 day

Figure 14. Nordox two applications – 1 day and + 20 days

Figure 15. Ag Copp 75 – 1 day

Figure 16. Water

Figure 17. Psa