2014/15 Potted Plant Field Trial Report

Psa Control Products on Bruno Potted Plants

December 2014 – January 2015

July 2015

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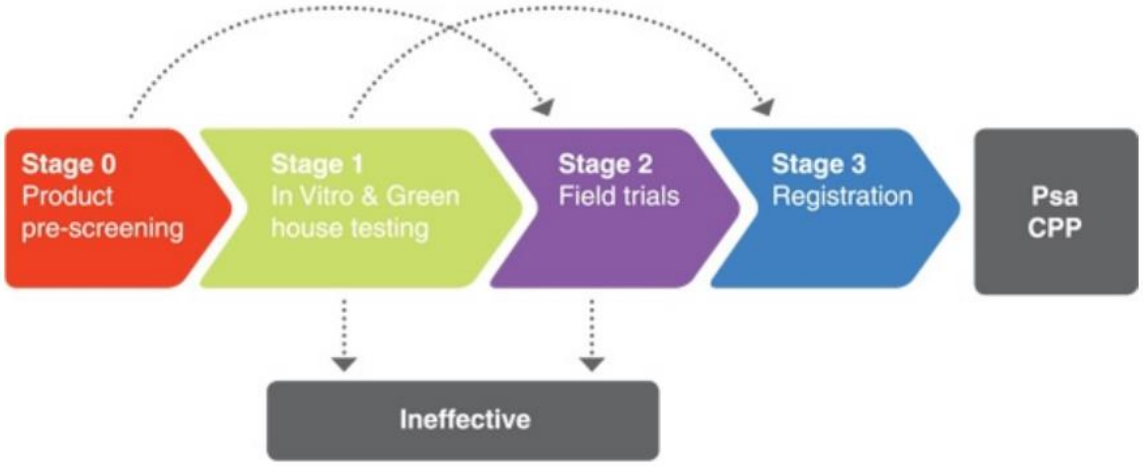
Introduction

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 fourth 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 2014 to January 2015 on Bruno potted plants in which a range of protectants were tested, with streptomycin as the positive control.



Objective(s)

This trial was established to determine the efficacy of a range of Psa control products in reducing Psa symptoms, using Bruno potted plants.

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 kiwifruit a nursery in the Nelson region. 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 13 treatment groups, with 15 plant replications per group. Table 1 lists the treatment groups, active ingredient, amount of active ingredient, rate of product application, and the timing of applications relative to Psa inoculation (-1 = 1 days prior to Psa inoculation; +25 = 25 days post Psa inoculation).

Treatment application

Spraying of products prior to Psa inoculation was performed at HortEvaluation, Hamilton. Spraying post Psa inoculation was performed at the trial site. A gas assisted backpack sprayer was used to produce fine droplets. The entire canopy of each plant was thoroughly sprayed. Spraying was performed on 15th December 2014 for all products. The Nanospada treatments had additional applications on the 9th January 2015, 24 days after inoculation.

Plants were inoculated on 16th December 2014. On the day of inoculation, the plants were transported to the trial site in Pukehina. The plants were placed inside a gazebo, to ensure 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 and end of plant inoculation to monitor the concentration of bacteria. The inoculum concentration reduced to 10^7 cfu/ml by the end of the applications, but this is still a high enough concentration to induce disease expression.

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.

Table 1.

Treatment	Active Ingredient	Rate	Amount of active ingredient	Application timing (days)
Nanospada	Quaternary Ammonium Chloride	1L product/3L water	1.25%	-1 and +24
Nanospada	Quaternary Ammonium Chloride	1:20 of treatment 1	0.0625%	-1 and +24
Nanospada	Quaternary Ammonium Chloride	1:200 of treatment 1	0.006%	-1 and +24
KOF Creme Clean plus KOF Sulfur	wax and fertiliser	1ml /L + 1ml/L	42% substrate and 50% sulphur	-1
KOF Creme Clean plus KeyStrepto	wax and streptomycin	2ml/L (KOF) & 6g/10L Keystrepto	42% substrate 1g streptomycin	-1
KOF Creme Clean & KOF Sulfur & KeyStrepto	wax and fertiliser and streptomycin	1ml/L + 1ml/L & 6g/10L Keystrepto	42% substrate, 50% sulphur 1g streptomycin	-1
TNL 3067	Component A (buffer) Component B (active ingredient)	105g/10L water 15g/10L water	Confidential	-1
TNL 3137	Not disclosed - chemical	25ml/10L	Confidential	-1
TNL 3141	Not disclosed - chemical	50ml/10L	Confidential	-1
TNL 3214	Not disclosed - chemical	5ml/10L	Confidential	-1
KeyStrepto	streptomycin	6g/10L	1g	-1
Water	N/A	N/A	N/A	N/A
Psa	N/A	N/A	N/A	N/A

Initial wetting of plants

Once inoculated 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 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 21, 28, 35 and 44 post inoculation.

Assessments were performed during January 2015.Table 2 lists the secondary symptoms that were measured and the score 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 and continuity of scoring. Throughout treatment application, inoculation and assessment, the focus was on ensuring consistency across treatments.

Weather

Conditions were warm and dry on 15th December 2014, when treatments were applied. A very wet and windy day occurred on 17th December 2014, the day after inoculation, with maximum wind speed of 34km/h and 74.8 mm rain. These conditions were not likely to have affected the trial plants adversely, as the plants were already being wetted by overhead misters within a well sheltered nursery area.

Rain fell on 17 out of 44 days, from inoculation date to the end of the assessments. Weather was generally warm with temperature averaging 19.5^0C with average relative humidity of 83%, favouring satisfactory plant growth.

Statistical Analysis

Analysis of the leaf spotting data and secondary symptoms was performed in JMP 11 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 leaf spotting in this trial, with the Psa treatment group displaying an average leaf spot of 11% of the total plant at the end of the trial. In contrast, the water treatment

group had approximately 2% leaf spotting at the end of the trial. Figure 1 shows the leaf spotting data throughout the trial.

The highest concentration of Nanospada (manufacturer's recommendation) significantly decreased leaf spotting ($p < 0.05$) 3 weeks post Psa inoculation, but not at later time points. The diluted concentrations of Nanospada did not significantly reduce leaf spotting.

The KOF Creme Clean plus Sulphur treatment did not significantly reduce leaf spotting. However the KOF treatments when combined with streptomycin did significantly reduce leaf spotting up to 3 weeks post Psa inoculation for Creme Clean plus streptomycin ($p < 0.05$), and for up to 4 weeks post Psa inoculation for Creme Clean plus Sulphur plus streptomycin ($p < 0.05$).

TNL 3067, 3137 and 3141 did not significantly decrease leaf spotting throughout the trial.

TNL3214 did significantly decrease leaf spotting compared with Psa for up to 6 weeks post Psa inoculation ($p < 0.05$).

Streptomycin, the positive control, significantly decreased leaf spotting for up to 3 weeks post Psa inoculation ($p < 0.05$).

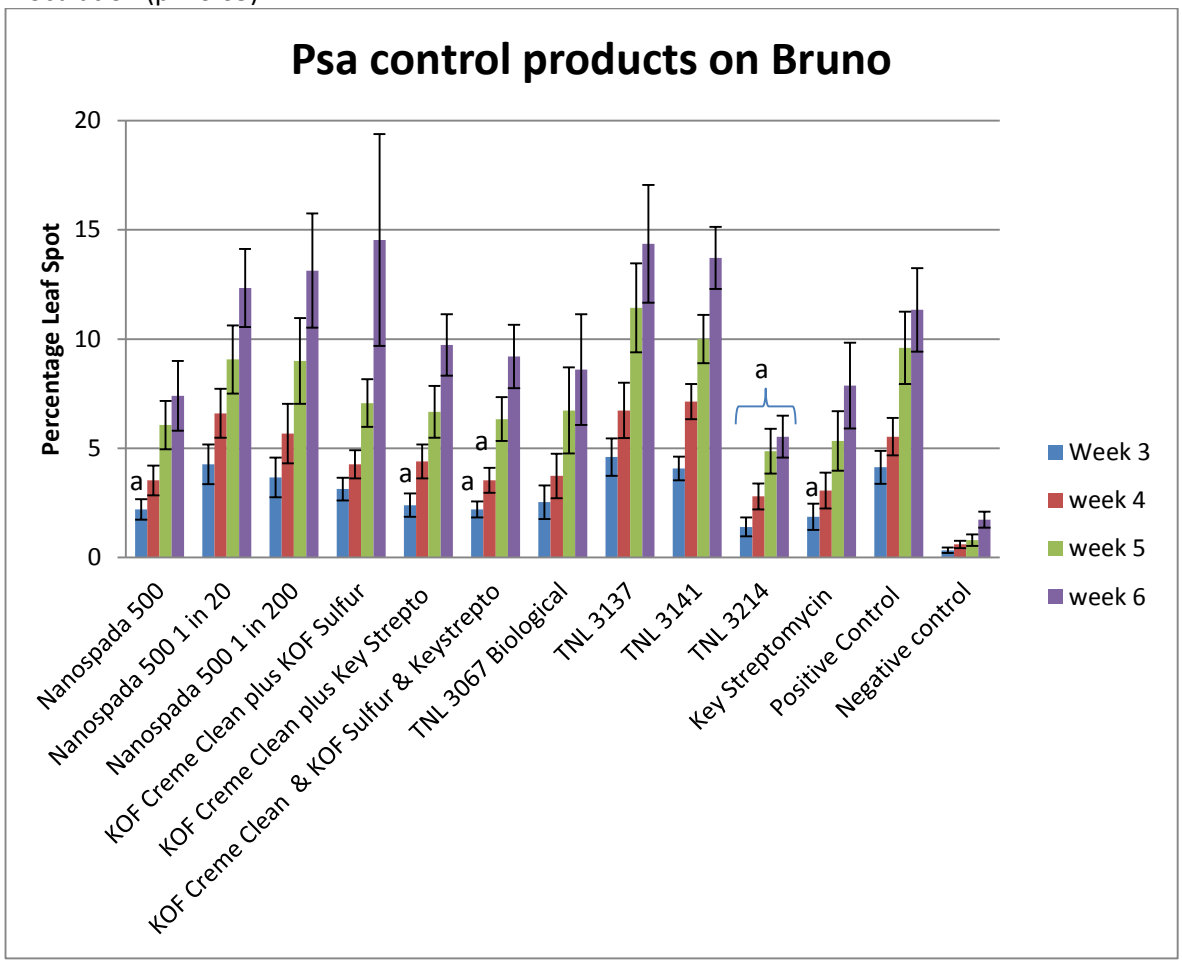


Figure1. Percentage leaf spotting in Bruno potted plants inoculated with 10^7 or^8 cfu/ml Psa. Error bars are +/- SEM. Significance is indicated on the graph: a = $p < 0.05$.

The development of secondary symptoms was low, with only 22 plants exhibiting any sign (stem discoloration, tip or shoot die back) of secondary symptoms 6 weeks after Psa inoculation. Hence, no analysis of this data has been performed.

Figures 2 and 3 are representative images of symptoms assessed throughout the trial; leaf spotting and secondary symptoms. Figures 4 and 5 show the comparison of a Psa inoculated plant and water treated plant at the end of the trial.

Figure 2. Image of a plant showing leaf spotting and halos.

Figure 3. Image of a plant showing shoot die back, secondary symptom score of '3'.

Figure 4. Image showing a plant inoculated with Psa.

Figure 5. Image of a plant treated with water only.

Summary

Spray inoculation of Bruno plants with $10^{7 \text{ or } 8}$ cfu/ml of Psa-V resulted in a good level of infection, as determined by leaf spot analysis. A low level of secondary symptoms was observed as determined by Disease Severity Scores.

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

1. Nanospada has previously shown efficacy against Psa when tested on Hort16A potted plants and in glasshouse trials. At the recommended concentration, Nanospada significantly decreased leaf spotting for up to 3 weeks post Psa inoculation. When diluted, Nanospada has no significant effect, supporting the use of the recommended rate. This data also supports earlier data indicating that Nanospada could be used to control Psa. However, further efficacy and residue tests need to be performed, but this will only happen once the formulation of Nanospada has been modified to suit the spray technology used in New Zealand.

2. The KOF Creme Clean and sulphur products only showed efficacy when combined with streptomycin. This indicates that it is streptomycin which is providing Psa control in these treatments. Hence, the KOF products will not be tested further.

3. TNL3214 significantly reduced leaf spotting throughout the trial. Discussions are underway with the product supplier to further test the efficacy and analyse residues on orchard.