2015/16 Potted Plant Field Trial Report

Actigard and Estim rates on Bruno and Gold3 Potted Plants

March – April 2016

January 2017

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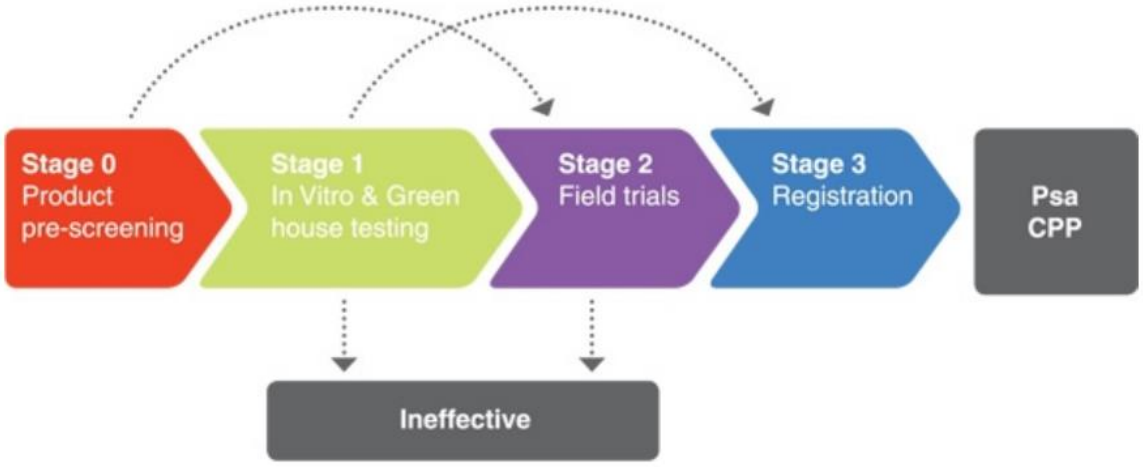
Introduction

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 Gold3 potted plants in which a range of elicitors were tested, with Actigard as the positive control.



Objective(s)

This trial was established to determine the efficacy of different rates of elicitors, Actigard and Estim, in inducing a plant immune response to Psa, using Bruno and Gold3 potted plants. Estim (from a Spanish based company, Cintrave) is a novel elicitor, formulated from plant antimicrobial peptides, which indicated efficacy in the control of Psa in a Hayward potted plant glasshouse trial.

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 and Gold3 on Bruno rootstock kiwifruit potted plants, sourced from a kiwifruit 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 nursery 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 seven treatment groups for Bruno potted plants, and six treatment groups for Gold3 potted plants, with 15 plant and 10 replications per group respectively. 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. Actigard treatments were applied seven days before Psa inoculation. Estim treatments were applied four days before Psa inoculation, then at 6 days and 17 days after Psa inoculation.

Table 1.

Treatment	Active Ingredient	Rate (per 100L water)	Amount of active ingredient	Application timing (days)
Actigard	Acibenzolar-S-methyl	20g	10g	-7
Actigard	Acibenzolar-S-methyl	13.3g	6.65g	-7
Actigard	Acibenzolar-S-methyl	10g	5g	-7
Estim	Plant antimicrobial peptides	40ml*	U	-4, +6, +17
Estim	Plant antimicrobial peptides	15ml	U	-4, +6, +17
Water	N/A	N/A	N/A	N/A
Psa	N/A	N/A	N/A	N/A

U = Unknown. * Estim was only used at the 40ml rate for Gold3. -7 = 7 days before Psa inoculation, +6 days after Psa inoculation

Treatment application

Spraying of elicitors 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.

Plants were inoculated on 1st March 2016. On the day of inoculation, the plants were transported to the trial site at 232 Billing Road, 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, 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 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.

The potted plants were then randomly placed into their final sites for the assessment period. Each pot had a water dripper placed over, to ensure each plant was watered twice daily.

Assessments

The level of leaf spotting, as a percentage of total leaf area covered in spots were visually estimated and recorded at days 15, 21, 31 and 35 post inoculation. The same assessors were used to score the plant disease symptoms, to ensure continuity in the scoring. Assessments were performed during March 2016 and April 2016. Only a low percentage of secondary symptoms were observed during this trial, hence no analysis of secondary data has been undertaken and no results are reported.

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

Elicitors were applied either 23 or 26 February 2016. Conditions were warm in the week following from 23 February with daytime temperature maxima typically 25-30 ^{0}C. Rain fell on each of 27, 28 and 29 February, accumulating to 32mm in that time.

Conditions were fine and warm on the Psa inoculation date and continued similarly for the next couple of days, whilst the plants were receiving the overhead misting.

Warm humid conditions occurred through the symptom development and assessment phase, with average daily temperature of 18.03^{0}C and relative humidity of 86%. Rainfall was frequent, occurring on 16 of the 33 days of the trial. Total rainfall was 85.8mm, averaging 2.52mm per day or 5.36mm per rain day.

Estim treatments were reapplied at 10 day intervals after initial application in suitable spray weather conditions.

Statistical Analysis

Analysis of the leaf spotting data and secondary symptoms was performed in JMP 13 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 Tukey-Kramer 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 60% of the total Bruno plant at the end of the trial, and at 13% of the total Gold3 plant. In contrast, the water treatment group had approximately 34 and 3% leaf spotting at the end of the trial for Bruno and Gold3 respectively. Bruno plants are more susceptible to Psa leaf spotting, compared with Gold3, and this may explain the higher incidence of leaf spotting in the water only control. No leaf spotting was observed at the beginning of the trial. Figure 1 shows the leaf spotting data for the Bruno potted plants, and Figure 2 for the Gold3 potted plants, throughout the trial.

In Bruno potted plants, all rates of Actigard significantly decreased leaf spotting (ANOVA $F < 0.0001$; Tukey – Kramer $p < 0.0001$) at the first assessment. At the last assessment the 100g and 200g / ha rates significantly reduced leaf spotting (Tukey – Kramer $p < 0.01$ and $p < 0.05$ respectively). Even though the 133g / ha rate did not significantly decrease leaf spotting at the last assessment, a rate effect can be discounted as the other two rates resulted in a significant effect.

At the first assessment there was a significant decrease in leaf spot at the higher rate of 40ml of Estim, (ANOVA $F < 0.0001$; Tukey – Kramer $p < 0.05$), but not at the lower rate of 15ml. At the last assessment, both rates significantly reduced leaf spot ($p < 0.05$). This would indicate that at the earlier stage of Psa infection, with fewer Estim applications, there is a rate response. However this was no longer observed as time progressed.

In Gold 3 potted plants there was no significant decrease in leaf spot for all three Actigard rates throughout the assessments, even though there seems to be a rate effect, with the 200 g / ha rate resulting with a lower average of leaf spot. This lack of significant effect may be due to the low level of leaf spot in the Gold3 plants. Likewise, Estim at the one rate tested (40ml) also did not significantly reduce leaf spot compared with Psa.

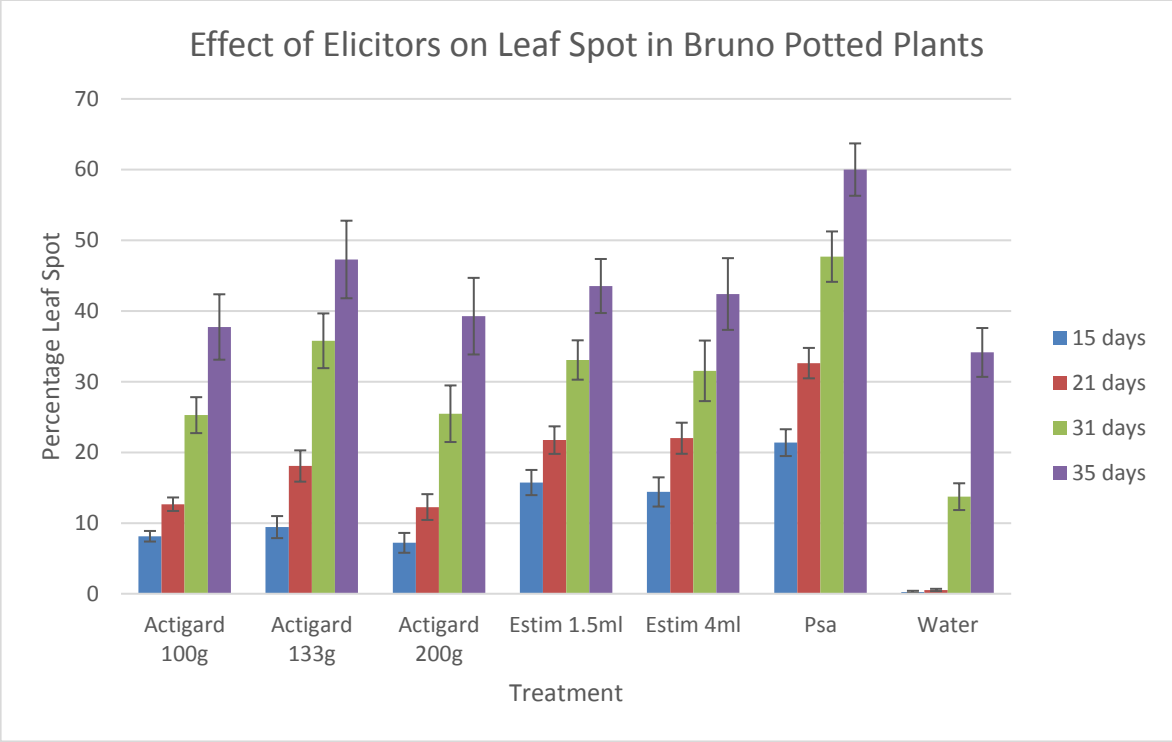


Figure1.

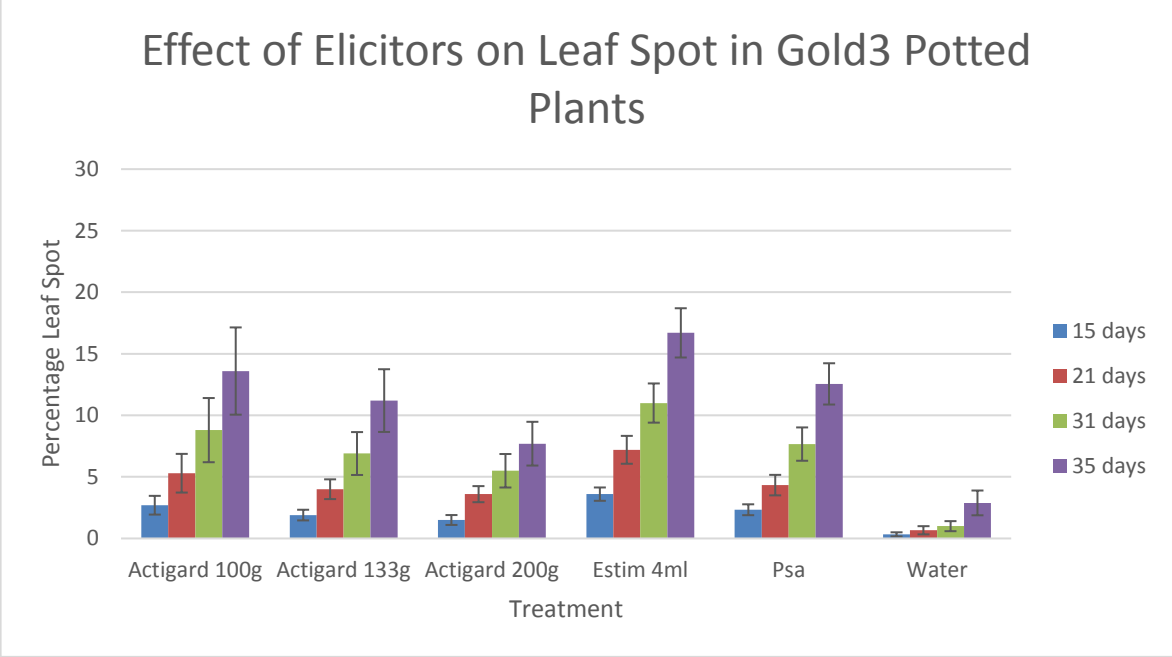


Figure 2.

Summary

Spray inoculation of Gold3 plants with 10^8 cfu/ml of Psa-V resulted in a good level of infection, as determined by leaf spot analysis. Typically, mature Gold3 on orchards does not show leaf spot, so

the degree of leaf spotting observed in this trial could be due to the age of the plants (young potted vines) and / or the heavy inoculum load. However, there was a lower level of Psa incidence / leaf spot observed in the Gold3 potted plants in this trial, compared with the Bruno potted plants.

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

1. The different rates of Actigard had no impact on efficacy against Psa in Bruno potted plants, as all rates significantly reduced leaf spotting. This trial was run in parallel to an on orchard trial investigating the impacts of the rates of Actigard on canopy development in Hayward and Gold3 potted plants where no negative impact was observed in the trial for any of the rates (Snelgar et al., 2016).

2. Even though the different rates of Actigard did not have a significant effect on leaf spot in Gold3, previous trial work has indicated that Actigard is efficacious on Gold3. The lack of a significant effect in this trial may be due to the lower level of Psa incidence / leaf spot observed in the Gold3 potted plants, compared with the Bruno potted plants.

3. Estim, a trial elicitor formulated from plant antimicrobial peptides, continues to show promise as a Psa control product, having previously shown efficacy in Hayward potted plants in a glasshouse trial. The Bruno results in this trial support its efficacy in green kiwifruit varieties. However, efficacy on Gold varieties cannot be discounted at this stage and an on-orchard trial with both Hayward and Gold3 is currently underway.

Reference

Snelgar et al., (2016), Impact of spring application of Actigard® on Gold and Green Kiwifruit; PFR Repor