



2014/15 Potted Plant Field Trial Report

Elicitors on Gold3 Potted Plants

January 2015 – March 2015



July 2015

Disclaimer

This report has been prepared based on information available at the time of publication which is inherently preliminary in nature and subject to change. No party, including without limitation, Kiwifruit Vine Health Incorporated, Plant & Food Research and Zespri Group Limited, makes any warranty, representation or guarantee as to the accuracy and/or completeness of the information regarding Psa, potential treatments and/or best treatment practice, and none of those parties shall be liable to any person for any loss arising from that person's reliance on the information and/or for any damages arising out of or connected with the use of the enclosed information. No obligation is accepted or undertaken to update this or any other information or publicly release revisions to this document to reflect additional information, circumstances or changes in expectations which occur after the date of this document.

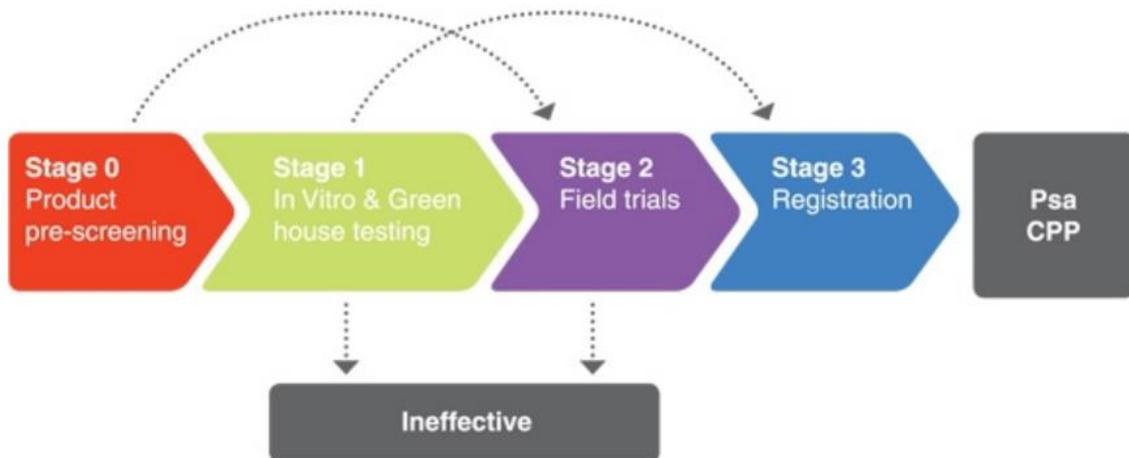
Introduction

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 January to March 2015 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 a range of elicitors in inducing a plant immune response to Psa, using Gold3 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 Gold3 kiwifruit potted plants, sourced from kiwifruit nurseries 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 11 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 (-7 = 7 days prior to Psa inoculation; +14 = 14 days post Psa inoculation).

Table 1.

Treatment	Active Ingredient	Rate (per 100L water)	Amount of active ingredient	Application timing (days)
TNL3454	TNL3454	100ml	30g	-7
TNL3454	TNL3454	200ml	60g	-7
TNL3454	TNL3454	400ml	120g	-7
Bayer AB48414	AB48414	200ml	40g	-7
Luna Care	Fluopyram and Fosetyl-aluminium	150g	Fluopyram 50g/kg Fosetyl – Al 666g/kg	-7
Citrox BioAlexin	Phytoalexin	350ml	unknown	-7, +7, +17 +28 and +35
ProAct + silver	Harpin Protein + silver	20g Harpin, 10g Silver	0.02g Harpin 100ppm silver	-9 for Harpin and -7 for silver
Silver	silver	10g	100ppm	-7
Actigard	Acibenzolar-S-methyl	20g	10g	-7
Water	N/A	N/A	N/A	N/A
Psa	N/A	N/A	N/A	N/A

Treatment application

Spraying of elicitors 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 20th January 2015 for all elicitors, except Harpin which were sprayed on 18th January 2014. Citrox BioAlexin had additional applications on the 3rd, 13th, 23rd February and 3rd March 2015.

Plants were inoculated on 27th January 2015. 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 trial, 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.

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 14, 29, 37 and 44 post inoculation. Assessments were performed during February 2015 and March 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 assessors 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 during treatment application, inoculation and initial wetting were fine, warm and dry. With no rainfall during this period at either HortEvaluation, Hamilton or the trial site at Pukehina, Bay of Plenty, no natural infection risk periods occurred.

No rain fell until 30 January 2015. From that date, rain was recorded daily until 5 February 2015, with a total of 41.2mm rain accumulated in that time. Over 26 and 27 February, 33.4mm rain fell. A further wet period occurred 5-7 March with 9mm rain recorded.

Average daily temperature during the trial was 18.8 °C and average relative humidity was 82%.

Conditions were favourable for plant growth without unusual climatic stress.

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 16% 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 elicitors TNL3454 (at 20 and 40 ml concentrations), AB48414 and Actigard (positive control) produced significant decreases ($p < 0.01$; TNL3454 $p < 0.05$ at week 6) in leaf spotting for up to 6 weeks post Psa inoculation. Citrox BioAlexin showed a significant decrease at week 4 only ($p < 0.05$).

Silver alone also produced significant decreases in leaf spotting for up to 6 weeks post Psa inoculation ($p < 0.01$ week 5; $p < 0.05$ week 6).

TNL3454 at 10ml concentration, ProAct and silver combination and Luna Care did not produce a significant decrease in leaf spotting during the trial.

Phytotoxicity was not observed during the trial.

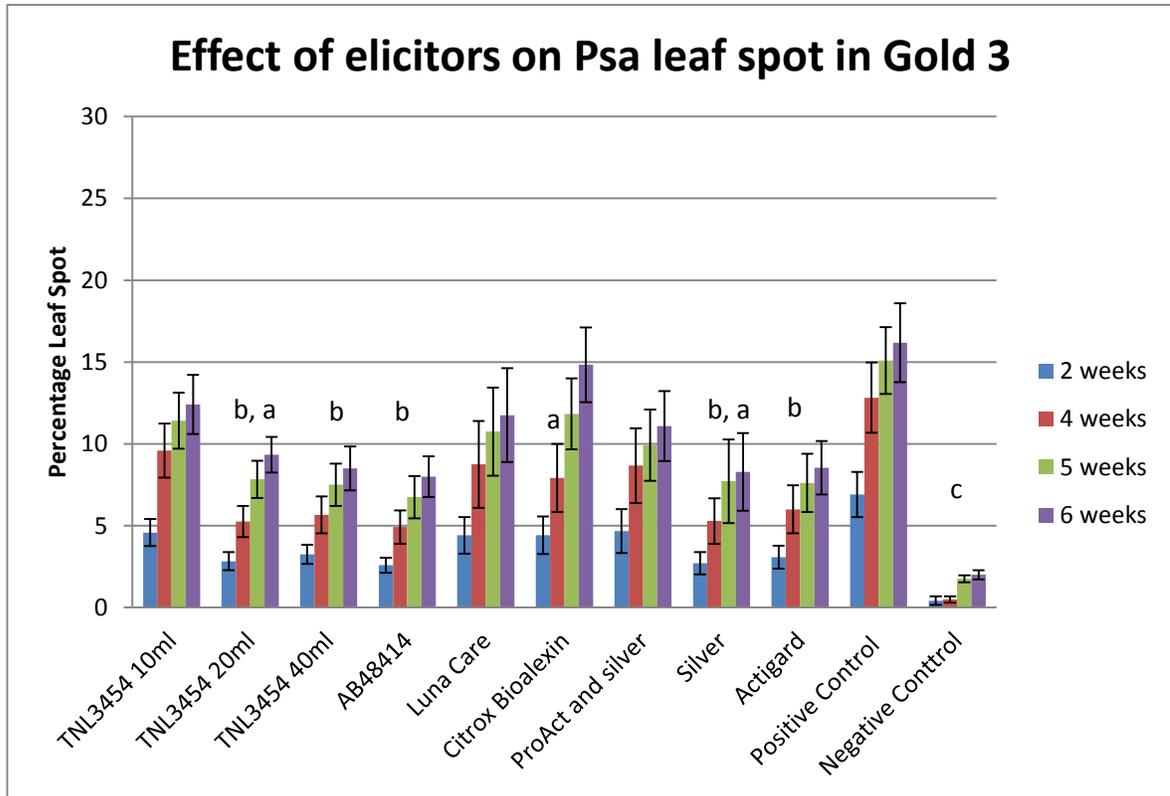


Figure1. Percentage leaf spotting in Gold3 potted plants inoculated with 10^8 cfu/ml Psa. Error bars are +/- SEM. Significance is indicated on the graph: a = $p < 0.05$; b = $p < 0.01$; c = $p < 0.001$. TNL3454 at 20ml and Silver had significance at $p < 0.01$ level up to 5 weeks post Psa inoculation, then $p < 0.05$ at week 6.

The development of secondary symptoms was low, with only 5 plants exhibiting any sign (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 Gold3 plants with 10^7 or 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. 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. The elicitors TNL3454 (at higher concentrations) and AB48414 showed a comparable level of Psa control as Actigard, for up to 6 weeks post Psa inoculation. This data suggest that these two new elicitors could also be used to control Psa, once further efficacy and residue testing has been performed.
2. CitroX BioAlexin showed some significant Psa control 4 weeks post Psa inoculation, but the efficacy decreased over time, even after multiple applications of the product. CitroX BioAlexin could offer organic growers an elicitor option, however further trials to prove consistent efficacy would be advisable.
3. ProAct with silver did not significantly decrease leaf spotting, however silver alone did for up to 6 weeks post Psa inoculation. This suggests that ProAct (harpin protein) is not having any effect on Psa, but silver is. ProAct, with and without silver, has shown some efficacy in

other trials. However, the inconsistency in effect may mean it will not provide a suitable addition to elicitor control for Psa in kiwifruit. Silver is not an elicitor but is another heavy metal with antimicrobial properties, similar to copper. There are no current plans to include silver into the Zespri Crop Protection Standard (CPS).

