



# 2013/14 Potted Plant Field Trial Report

## Elicitors on Gold3 Potted Plants

January 2014 – March 2014



*June 2014*

**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.*

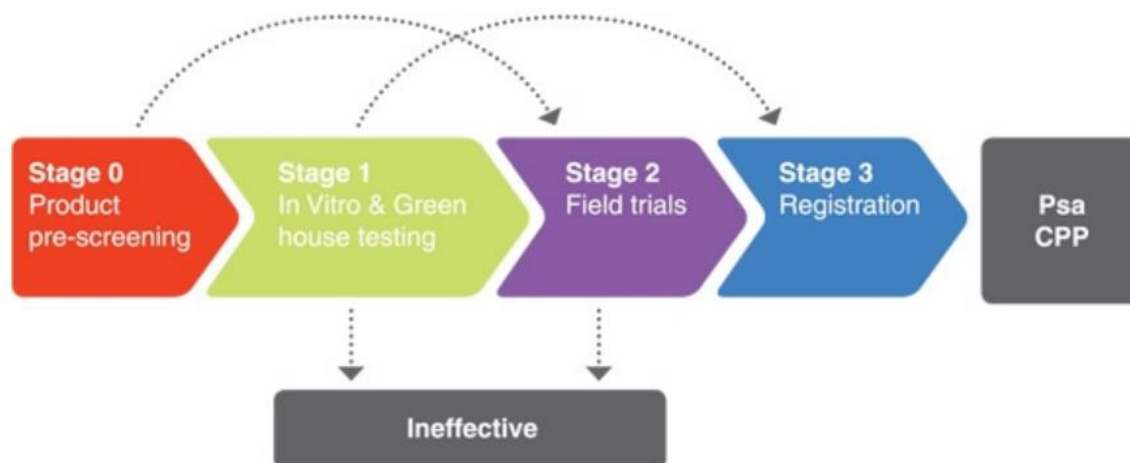
## Introduction

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 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 a range of elicitors and forchlorfenuron products 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 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 14 treatment groups, with 15 plant replications per group. Table 1 lists the treatment groups, active ingredient, amount of active ingredient (Forchlorfenuron in red, elicitors in blue) rate of product application, and the timing of applications relative to Psa inoculation (-8 = 8 days prior to Psa inoculation).

Table 1.

Treatment	Active Ingredient	Rate (per 100L water)	Amount of active ingredient	Application timing (days)
Ambitious	Forchlorfenuron	25ml	0.25g	-8
Ambitious	Forchlorfenuron	50ml	0.5g	-8
Ambitious	Forchlorfenuron	70ml	0.7g	-8
Sitofex	Forchlorfenuron	75g	0.75g	-8
Caplit	Forchlorfenuron	50g	0.5g	-8
GroSafe Elicitor	Forchlorfenuron	50ml	0.5g	-8
CropStar CPU	Forchlorfenuron	25ml	0.25g	-8
Tiadinil	1,2,3-thiadiazole	100ml	unknown	-8
Bayer AB48414	AB48414	200ml	40g	-8
Alexin*	Salicylate derivatives	500ml	15.625g	-2 and +13
ProAct	Harpin Protein	20g	0.02g	-2 and +13
Actigard	Acibenzolar-S-methyl	20g	10g	-8
Water	N/A	N/A	N/A	N/A
Psa	N/A	N/A	N/A	N/A

\*Alexin was applied 2 days prior to Psa inoculation, rather than the 7 days prior as recommended by product supplier.

## **Treatment application**

Spraying of elicitors at -8 and -2 days 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 2014 for all elicitors, except Alexin and Harpin which were sprayed on 26<sup>th</sup> January 2014, with additional applications on 10<sup>th</sup> February.

Plants were inoculated on 28<sup>th</sup> January 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, 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 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 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 of scoring. Throughout treatment application, inoculation and assessment, the focus was on ensuring consistency across treatments.

## Weather

Elicitors were applied at HortEvaluation, Hamilton on 20 January 2014. During the following week there was 16.4mm of rain recorded. Plants were moved to Te Puke on 28 January 2014, for Psa inoculation. From inoculation to final assessment on 13 March 2014, 4.6mm of rain fell in the 37 day period. The air temperature range through the trial period was 3.4°C (4 March 2014) to 26.4°C (5 February 2014). The average daily air temperature was 16.4°C and the average minimum and maximum were 11.0°C and 23.1°C respectively.

## Statistical Analysis

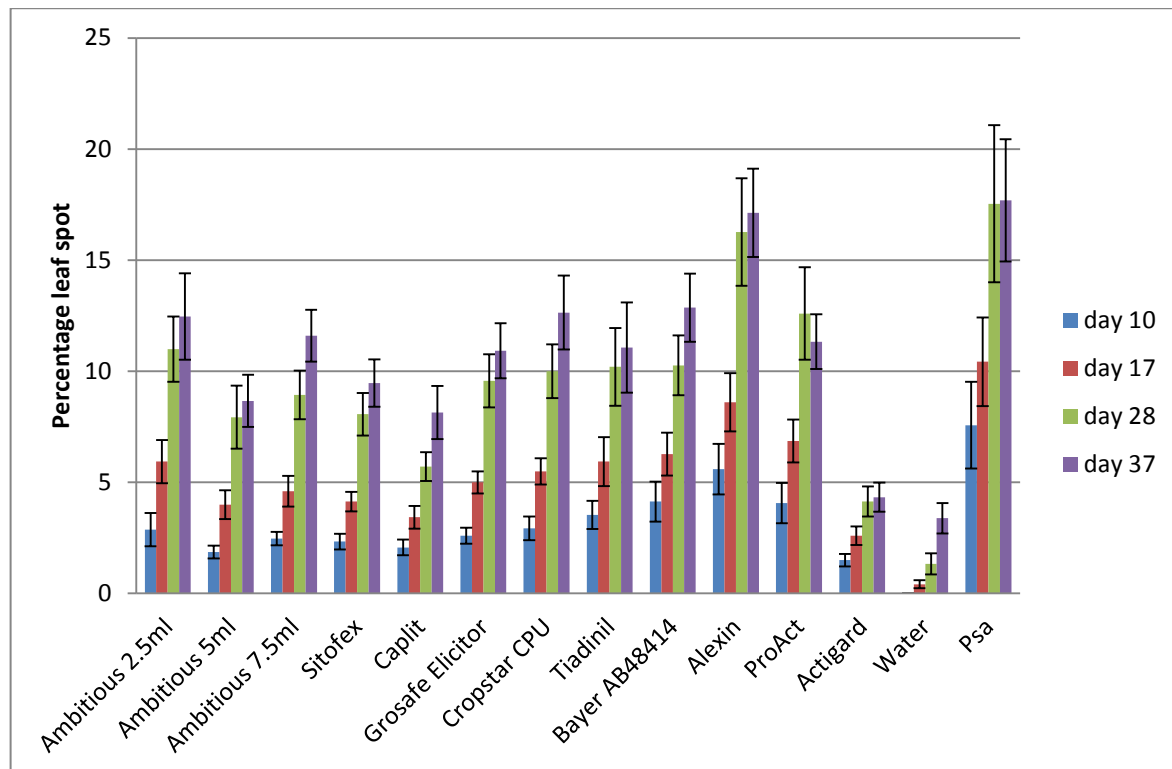
Analysis of the leaf spotting data and secondary symptoms was performed in JMP 10 Statistical Package (SAS Institute). An ANOVA was performed comparing all of the treatment groups at the different assessment times. If a significant difference was indicated, further analysis was performed using a t-test to determine the differences between each treatment versus Psa alone at each assessment. T-tests were performed to determine the difference in efficacy of Actigard in comparison with the other elicitors, and the CPPU / forchlorfenuron elicitors.

## 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 17% of the total plant at the end of the trial. In contrast, the water treatment group had approximately 3% leaf spotting at the end of the trial. Figure 1 shows the leaf spotting data throughout the trial. All elicitors, except Alexin which was applied 2 days prior to Psa infection rather than at the recommended 7 days prior\*, significantly decreased leaf spotting compared with the Psa treatment group up to 37 days post Psa inoculation ( $p < 0.05$ ). Actigard had the greatest effect.

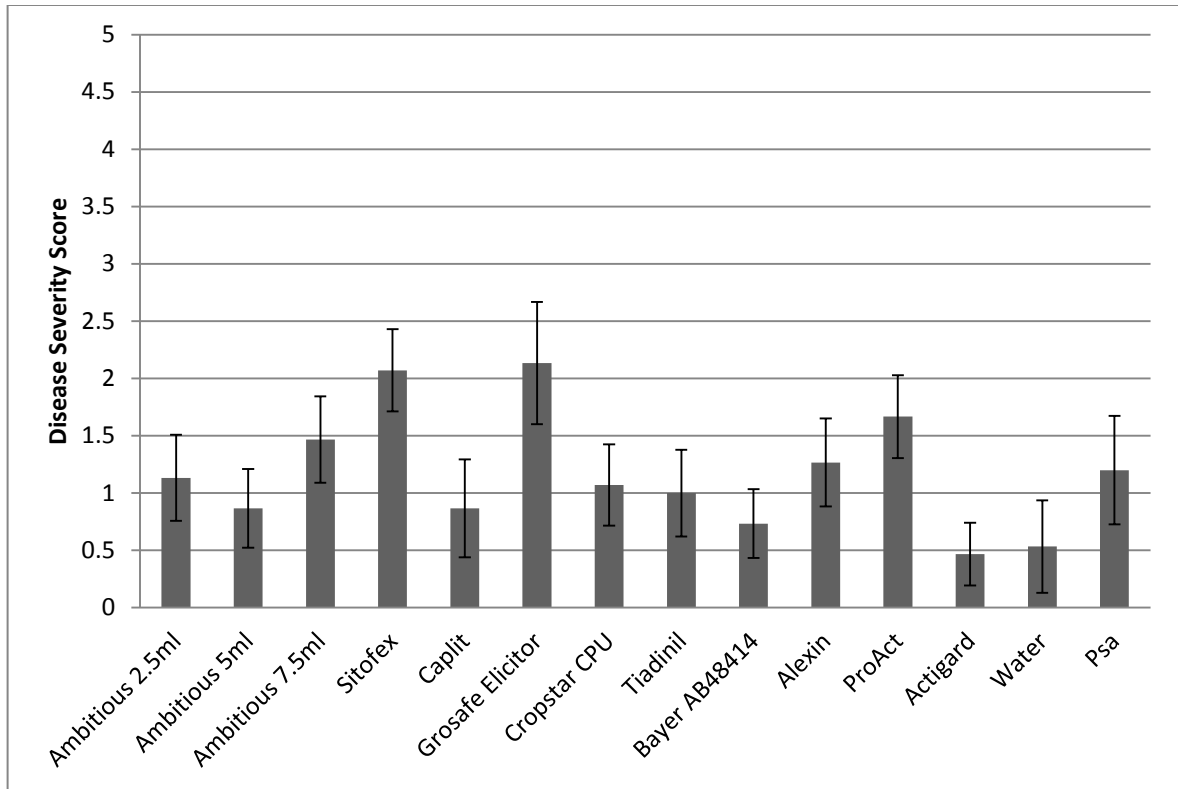
Actigard had significantly less leaf spotting compared with all of the other products ( $p < 0.05$ ), with the exception of Caplit.

Caplit had significantly less leaf spotting compared with the lower rate of Ambitious (25ml / 100L water) and CropStar CPU ( $p < 0.05$ ).



**Figure1.** Percentage leaf spotting in Gold3 potted plants inoculated with  $10^8$  cfu/ml Psa. Error bars are +/- SEM. All elicitors, except Alexin (see text above)\*, significantly decreased leaf spotting compared with Psa group ( $p < 0.05$ ). Significance is not indicated on the graph, as all treatments, were significantly decreased compared with Psa.

Figure 2 shows the average secondary symptoms disease severity score at Day 37 for each treatment group. There were no significant decreases in secondary symptoms compared with Psa treatment group, although the level of secondary symptoms was low (range 0 to 3). Actigard had the greatest effect in reducing secondary symptoms.



**Figure 2.** Secondary symptom disease severity scores on Gold3 plants spray inoculated with  $10^8$  cfu/ml Psa 37 days post inoculation. A '0' score records no symptoms; a score of '5' records plant dying or death of plant. Error bars are +/- SEM.

Figures 3 and 4 are representative images of symptoms assessed throughout the trial; leaf spotting and secondary symptoms. Figures 5 and 6 show the comparison of a Psa inoculated plant and water treated plant at the end of the trial.





**Figure 3.** Image of a plant showing leaf spotting and halos.



Shoot die back

**Figure 4.** Image of a plant treated with a CPPU product, showing shoot die back, secondary symptom score of '3'.



Shoot die back

**Figure 5.** Image showing a plant inoculated with Psa. Note the shoot die back.



**Figure 6.** Image of a plant treated with water only.



## 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. A low level of secondary symptoms was observed as determined by Disease Severity Scores.

To date, it has not been determined if CPPU (Forchlorfenuron) products act as elicitors. However they are plant growth regulators that will be inducing a response in the plant that may also be beneficial in controlling Psa in a similar manner to elicitors.

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

1. All of the elicitors, except Alexin\*, significantly reduced leaf spot in Gold3. None of the elicitors significantly reduced secondary symptoms. Testing the products in this trial on other kiwifruit varieties may result in a different response to ones observed in this trial, as an elicitor response may be kiwifruit variety-specific.
2. \*Alexin was applied 2 days prior to Psa inoculation, not at the manufacturer's recommendation of 7 days prior, and not at the same time as Actigard. Alexin was applied two weeks post Psa inoculation (recommended by supplier). However, the fact that Alexin was applied only 2 days before Psa inoculation, Alexin would not have had the time to induce a response in the plant prior to Psa inoculation, as with Actigard. Since elicitors work by priming the plant immune system *prior* to an infection event, the additional application of Alexin would not have been able to induce a plant response in time to exert any effect on Psa infection. Hence the data for Alexin is negated in this trial. However, in previous glass house trials Alexin significantly reduced Psa infection in Bruno and Hayward potted plants.
3. Actigard, which was used as a positive control, had the greatest effect in reducing leaf spotting and secondary symptoms compared with the Psa group. In addition, Actigard had significantly less leaf spotting compared with all of the other elicitors, with the exception of Caplit, supporting its current use for the control of Psa.
4. Sitofex and the GroSafe elicitor treatments showed increased secondary symptoms compared with Psa, however the increases were not significant. It is interesting to note that Sitofex had the highest concentration of forchlorfenuron active ingredient (0.75g).
5. Ambitious was applied at three different rates. The middle rate (50ml / 100L water) had less leaf spotting than the other two rates, but this decrease was not significant. The different doses applied did not result in a dose response of the plant to Ambitious in the control of Psa.
6. Of the CPPU / forchlorfenuron applications (Ambitious, Sitofex, Caplit, GroSafe and CropStar CPU), Caplit had the greatest effect in reducing leaf spotting. Caplit (0.5g active ingredient) significantly reduced leaf spotting compared with Ambitious and CropStar CPU (both 0.25g active ingredient).

