



2012/13 Potted Plant Field Trial Report

Trials 14 to 16

Biological Control Agents on Green 14

Elicitors and Protectants on Gold 9

Elicitors on Chieftain Male

March – May 2013



28 June 2013

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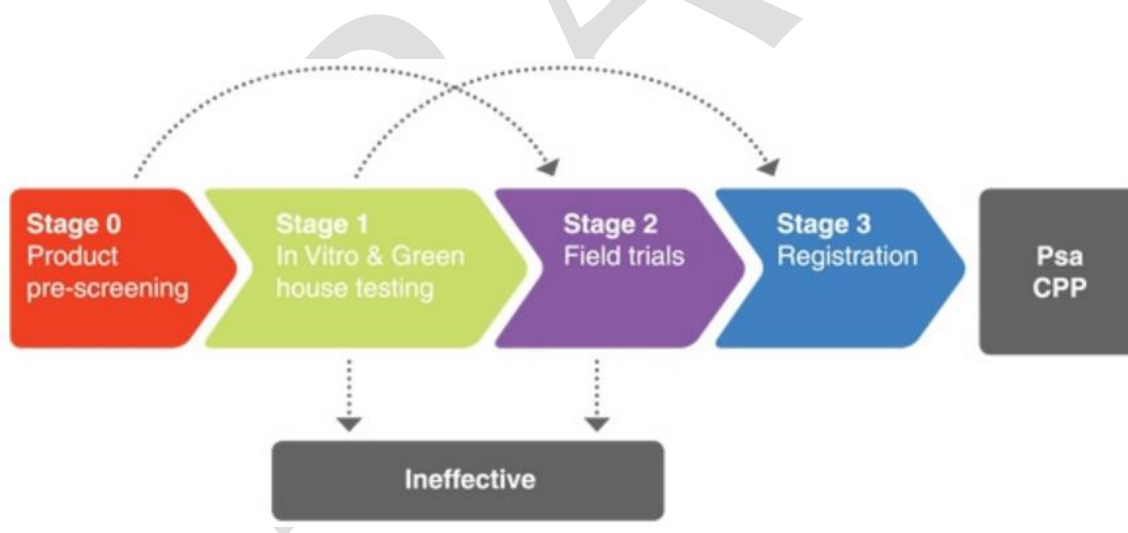
Introduction

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 trial site 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 second 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 three trials of biological control agents (BCAs), protectant and elicitor treatments conducted from March to May 2013 on potted plants.



Objective(s)

To test the efficacy of foliar applied treatments at protecting kiwifruit plants from Psa-V. Specifically, the following were studied:

- Biological Control Agents (BCAs) on G14
- Elicitors and Protectants on G9
- Elicitors on Chieftain

Methodology

Plants

These trials utilised G14, G9 and Chieftain potted plants. These were grafted onto 2 year old Bruno rootstocks in spring 2012, in Kerikeri. The plants were believed to be Psa-free at the start of the trials as no symptoms were observed previously. The plants were approximately 1.5m in height with approximately half a dozen leaves (Figure 1).

Figure 1. Example of the potted plants used in the KVH/Zespri trials. Also shown is the overhead misting system used to keep plants continuously wet for 48 hours following inoculation.



Treatments and replication

The treatments applied in each trial are listed in Table 1 to Table 3. Each treatment was applied to 10 plants (replicates).

Table 1. G14 treatments. Applied once on March 13 prior to inoculation. No repeat applications.

Product	Active(s)	Rate	Application timing (relative to inoculation)	No. of plants
Actigard	1, 2, 3 – benzothiadiazole 7 thiocarboxylic acid-S-methyl-ester	20g/100L	7 days before	10
BOTRY-Zen	<i>Ulocladium oudemansii</i>	800mL/100L	2 days before	10
PP9 + Foliacin	<i>Pseudomonas putida</i>	10mL/100L 500mL/100L	2 days before	10
BacStar + Duwett	<i>Bacillus subtilis</i> – <i>Amyloliqfacien D747</i> Organosilicone surfactant	175g/100L 35mL/100L	12 hours before	10
Serenade Max + Duwett	<i>Bacillus subtilis</i> Organosilicone surfactant	350g /100L 35mL/100L	12 hours before	10
Plant Shield + Biosea Omega Oil	A range beneficial bacteria Blend of Omega 3 and Omega 6 oils	350g/100L 200mL/100L	12 hours before	10
Psa	-	-		10
Water	-	-		10

Table 2. G9 treatments. Applied once on March 13 prior to inoculation then reapplied once 14 days later on March 27 (after inoculation).

Product	Active(s)	Rate	Application timing (relative to inoculation)	Repeat application timing (relative to inoculation)	No. of plants
Actigard	1, 2, 3 – benzothiadiazole 7 thiocarboxylic acid-S-methyl-ester	20g/100L	7 days before	14 days later	10
Citrox BioAlexin	Citrus extracts, palm oil	300mL/100L	7 days before	14 days later	10
Sitofex	CPPU	75g/100L	7 days before	14 days later	10
Bayer Product	Confidential	200mL/100L	7 days before	14 days later	10
Coptyzin + Fructol	Chelated copper	100mL/100L 100mL/100L	1 day before	14 days later	10
Nordox 75WG	Copper oxide	37.5g/100L	1 day before	14 days later	10
Psa	-	-	-		10
Water	-	-	-		10

Table 3. Chieftain treatments. Applied once 7 days prior to inoculation (on March 13) then reapplied once 14 days later on March 27 (after inoculation).

Product	Active(s)	Rate	Application timing (relative to inoculation)	Repeat application timing (relative to inoculation)	No. of plants
Actigard	1, 2, 3 – benzothiadiazole 7 thiocarboxylic acid-S-methyl-ester	20g/100L	7 days before	14 days later	10
Citrox BioAlexin	Citrus extracts, palm oil	300mL/100L	7 days before	14 days later	10
Sitofex	CPPU	75g/100L	7 days before	14 days later	10
Spotless	Benzalkonium ammonium chloride, Salicylic acid, Urea, Propiconazole	700mL/100L	7 days before	14 days later	10
Alexin	Salicylate derivatives	500mL/100L	7 days before	14 days later	10
Psa	-	-	-	-	12
Water	-	-	-	-	12

Treatment application

Treatments were applied to the Psa-free potted plants in a region free of Psa-V (Waikato - Hamilton) prior to moving the plants to the field trial site in Te Puke for inoculation. A gas-assisted backpack sprayer was used to produce fine droplets. The entire canopy of each plant was sprayed thoroughly with application rates adjusted to compensate for the smaller volumes of canopy being treated. A water rate equivalent to 1000L/ha was used. The treatments were applied between 13 and 27 March 2013.

Inoculation

Application of the Psa-V, for which MPI permission was obtained, was undertaken at the Zespri/KH trial site in Te Puke on 20 March 2013. This occurred inside a temporary spray booth to contain the spread of inoculum. One or two pallets of plants were inoculated in the spray booth at a time. On each pallet, one plant from each treatment was included to account for any variation in inoculation that may have occurred during the day.

Plant and Food Research staff from Te Puke provided fresh inoculum on the day. The target concentration for the G9 and Chieftain trials was 10^8 cfu/mL; subsequently the concentration used was measured to be between 10^7 cfu/mL and 10^8 cfu/mL. A lower concentration was targeted for the BCA trial on G14 so as not to overwhelm the treatments. This target was 10^7 cfu/mL; subsequently the concentration used was measured to be between 10^7 cfu/mL.

The inoculum was sprayed onto plants using 5L multi-purpose hand-held pressure sprayers with fine nozzles. The undersides of leaves were sprayed to wet. This lower leaf environment, where the stomata are, is more conducive to Psa infection. Inoculation occurred between 11am and 2pm.

Initial wetting of plants

Following inoculation, plants were kept continuously wet from above for approximately 48 hours by an overhead misting system (see Figure 1) i.e. from about 2pm on 20 March to 2pm on 22 March 2013. During this time, it is estimated that the equivalent of 34mm of water was applied in the trial area (of approximately 1200m²).

During the inoculation and initial wetting no rain fell. On the day of inoculation, the temperature reached approx. 19°C, 20°C the following day and 21°C the day after that. Average relative humidity during this period was approximately 80%.

Assessments

The levels of leaf spotting and secondary symptoms were visually estimated and recorded approximately 2, 3, 4 and 6 weeks after inoculation. Each time, the amount of total leaf area covered in spots was estimated. The mature and expanding parts of plants were assessed separately.

The last assessment of leaf spotting was conducted on 1 May 2013 as based on previous experience there was unlikely to be any significant progression in leaf spot beyond that time. Secondary symptoms were monitored regularly with the last assessment conducted on 24 May 2013.

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

Weather conditions during field trials need consideration when interpreting results hence a summary is presented here.

- i) *Weather during application of the treatments. Hamilton. 13 March – 20 March 2013. Appendix 1.*

Maximum daily temperatures were between 23 and 30°C and minimum daily temperatures were between 8 and 18°C. Approximately 10mm of rain fell on each of the 17 and 18th of March, 3 days after the elicitors were applied and the day before other treatments were applied.

- ii) *Weather following inoculation (based on the installed Harvest.com weather station). 20 March – 24 May 2013. Appendix 2.*

During the initial 2-day wetting period, less than 2mm of rain fell while the average daily temperature and relative humidity was approximately 13.5 °C and 80% respectively.

Subsequently, little rain fell until the April 15 when 475mm fell over a 7 day period to 21 April. Another 40mm fell at the beginning of May with another 100mm falling from mid to late May. Generally, average daily temperature ranged between 10 and 15°C, while average daily relative humidity ranged between 80 and 95%.

Results

Leaf spotting

G14 (Figure 2)

The level of leaf spotting overall was low i.e. average of 2% or lower for the untreated and inoculated Psa control. Generally, 4 to 5% leaf spotting is regarded as a minimum level that confident conclusions can be based on. Therefore the results of this trial are considered inconclusive.

The levels of leaf spotting were low on the expanding leaves and even lower on the mature leaves.

G9 (Figure 3)

The level of leaf spotting was sufficient in the mature leaves to have good confidence the results i.e. up to 5% for the untreated and inoculated Psa control. Here, there was a trend for Sitofex, the Bayer Product, Coptyzin, Nordox and Actigard to have less leaf spotting than the Psa control although the reductions were not statistically significant (due to variability in the data). Citrox BioAlexin did not reduce leaf spotting. Nordox and Actigard resulted in the least amount of leaf spotting.

The level of leaf spotting in the expanding leaves was low and therefore it is difficult to conclude much here. That said, Sitofex and the Bayer product reduced leaf spotting with the differences being statistically significant initially (at the 10% level).

Chieftain (Figure 4)

The overall level of leaf spotting was high in this trial i.e. up to 11% for the untreated and inoculated Psa control. For the mature leaves, only Actigard significantly reduced leaf spotting.

In the expanding leaves, overall the treatments did not result in reductions in leaf spotting that were statistically significant; high variability probably contributed to this. Nevertheless, Actigard and Spotless did reduce leaf spotting with the difference being statistically significant at the third assessment.

Secondary symptoms

Little or no secondary symptoms were observed in the trial. If possible, the plants will be kept until next spring to evaluate the impacts of the treatments on new growth. If this occurs, the results will be reported later.

Summary

The trials presented in this report had varying outcomes.

The G14 trial of biological control agents was inconclusive due to lack of symptom expression. One possible contributing factor to this may have been the lower inoculum concentration that was used so as not to overwhelm the treatments.

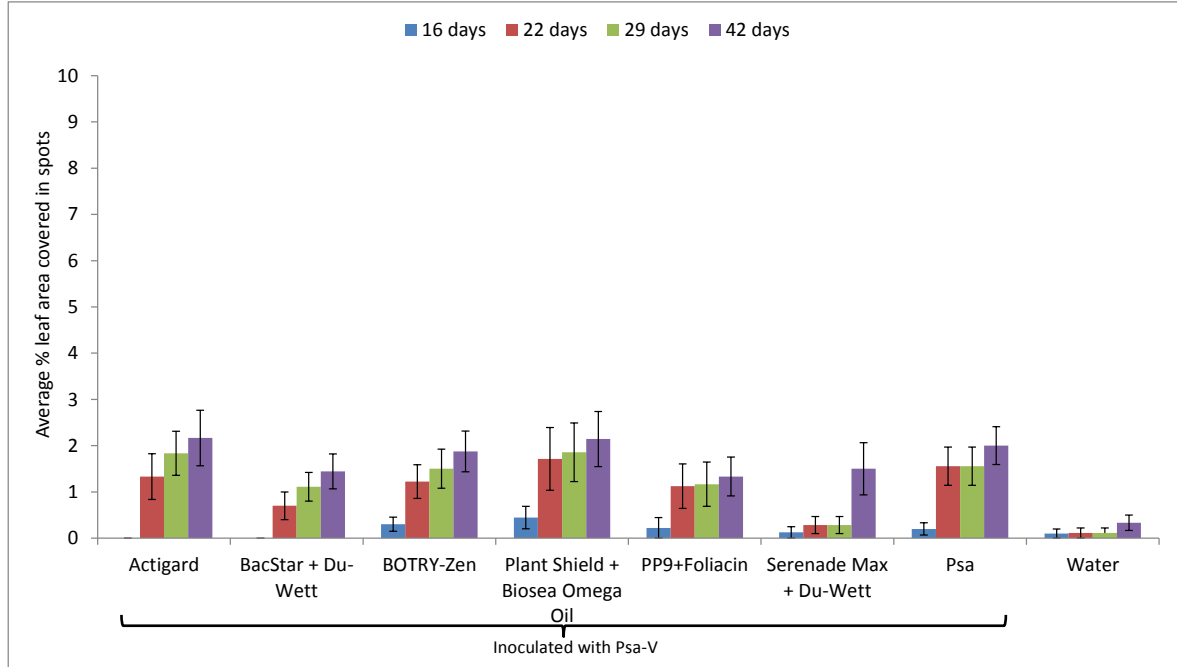
For the G9 trial, the level of leaf spotting was sufficient in the mature leaves. All treatments except Citrox BioAlexin reduced leaf spotting although the reductions were not statistically significant. The level of leaf spotting in the expanding leaves was low and therefore it is difficult to conclude much here. That said, Sitofex and Bayer product reduced leaf spotting.

A high level of leaf spotting was observed in the Chieftain trial. For the mature leaves, only Actigard significantly reduced leaf spotting. For the expanding leaves, there was some evidence that Actigard and Spotless reduced leaf spotting significantly.

Little or no secondary symptoms were observed in these trials. If possible, the plants will be kept until next spring to evaluate the impacts of the treatments on new growth. If this occurs, the results will be reported later.

Figure 2. 2012/13 Zespri/KVH trial of foliar-applied biological control agent (BCA) treatments on G14. Average percentages of leaf area covered in leaf spots at different times after inoculation. Standard error bars are shown (n = 10).

Expanding leaves



Mature leaves

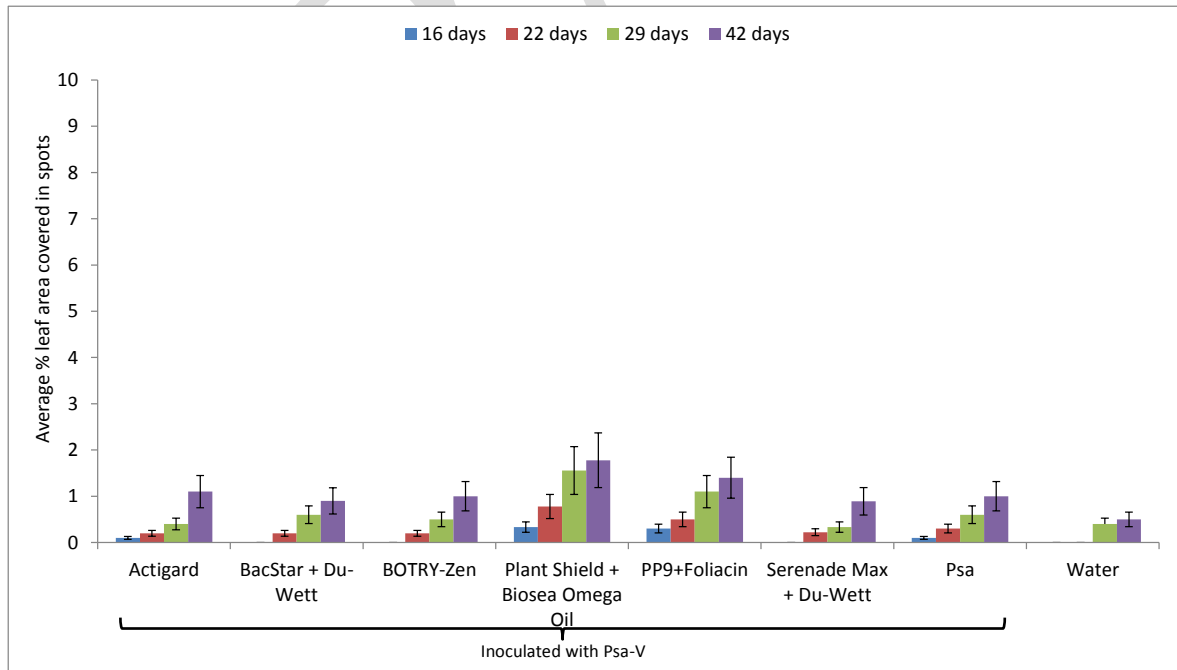
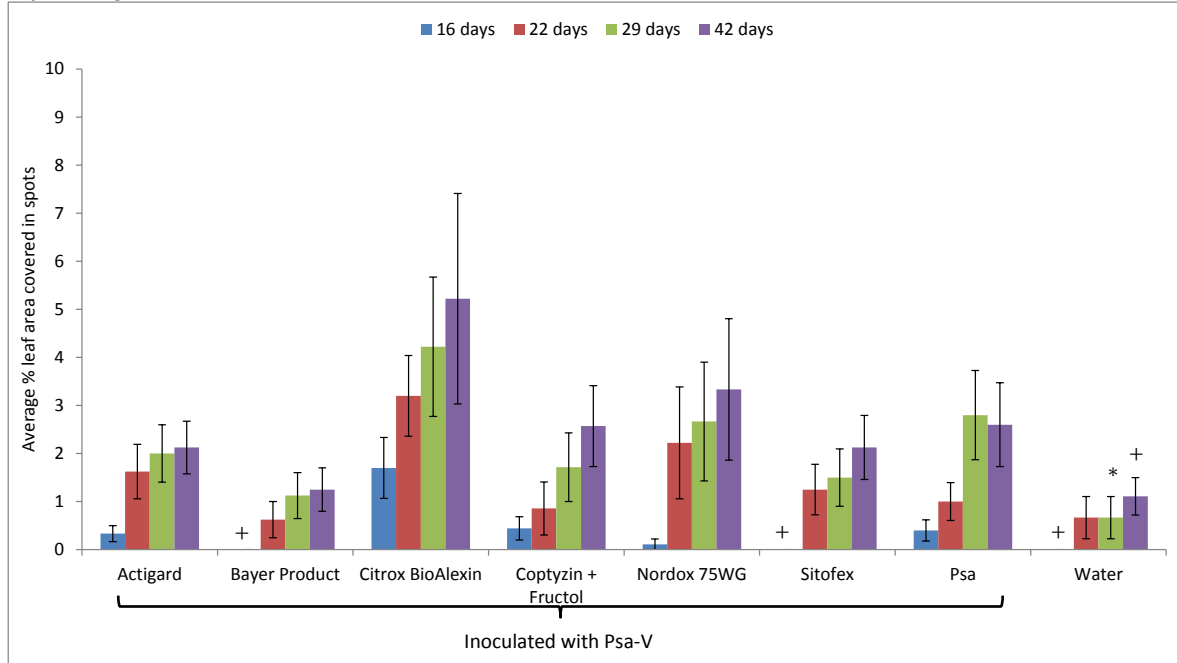
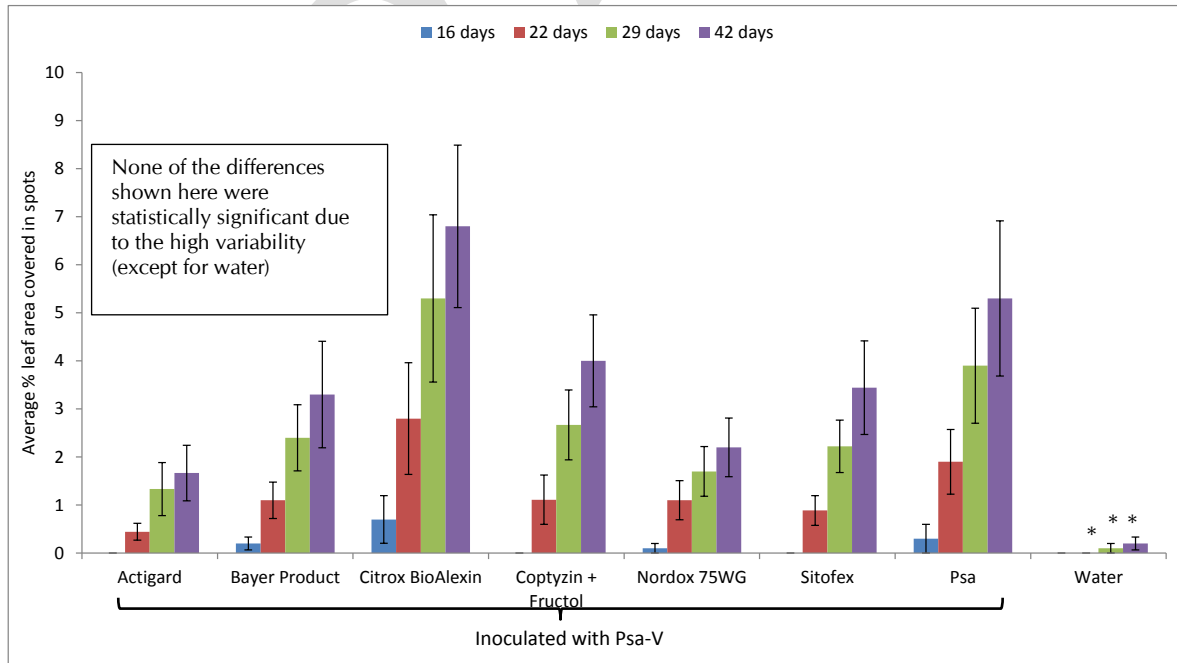


Figure 3. 2012/13 Zespri/KVH trial of foliar-applied protectant and elicitor treatments on G9 potted plants. Average percentages of leaf area covered in leaf spots at different times after inoculation. Standard error bars are shown (n = 10).

Expanding leaves



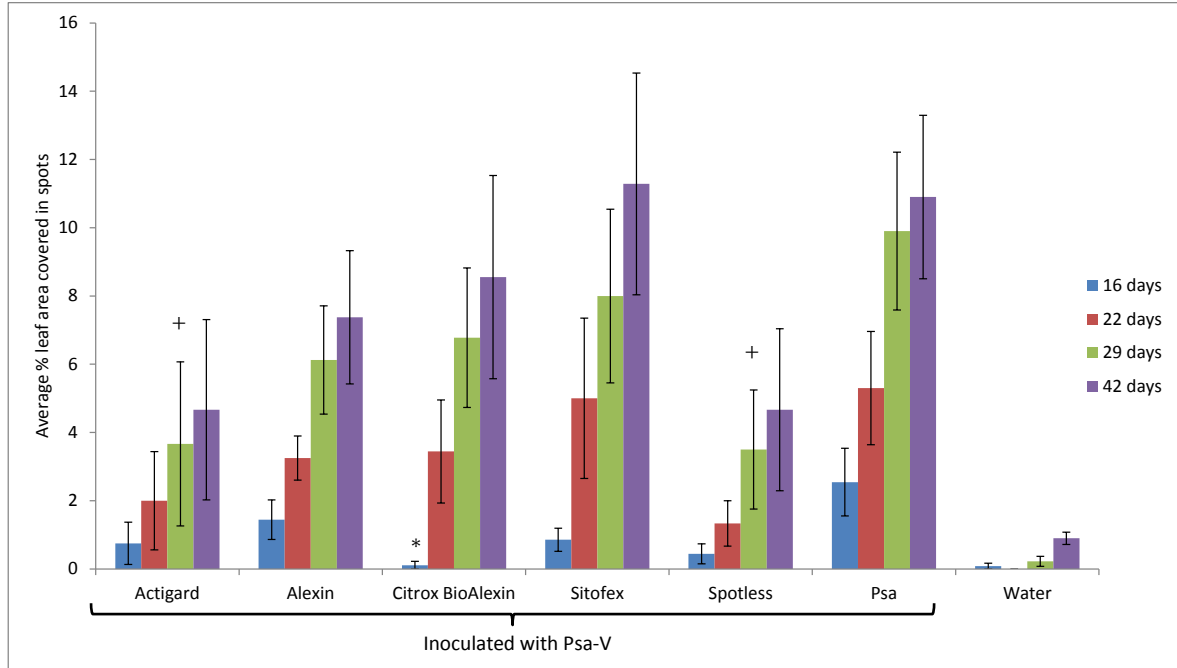
Mature leaves



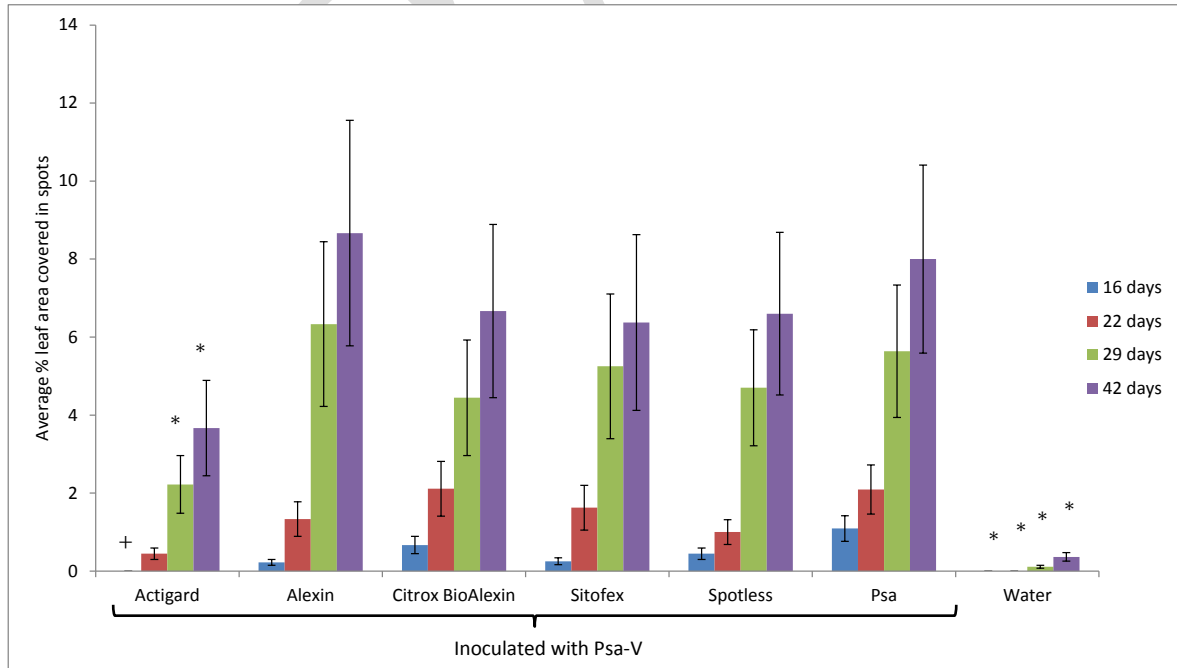
*,+ Statistically significant from Psa treatment according to a non-parametric (Wilcoxon) test, at the 5% and 10% significance levels respectively (n = 10).

Figure 4. 2012/13 Zespri/KVH trial of foliar-applied elicitor treatments on Chieftain potted plants. Average percentages of leaf area covered in leaf spots at different times after inoculation. Standard error bars are shown (n = 10).

Expanding leaves

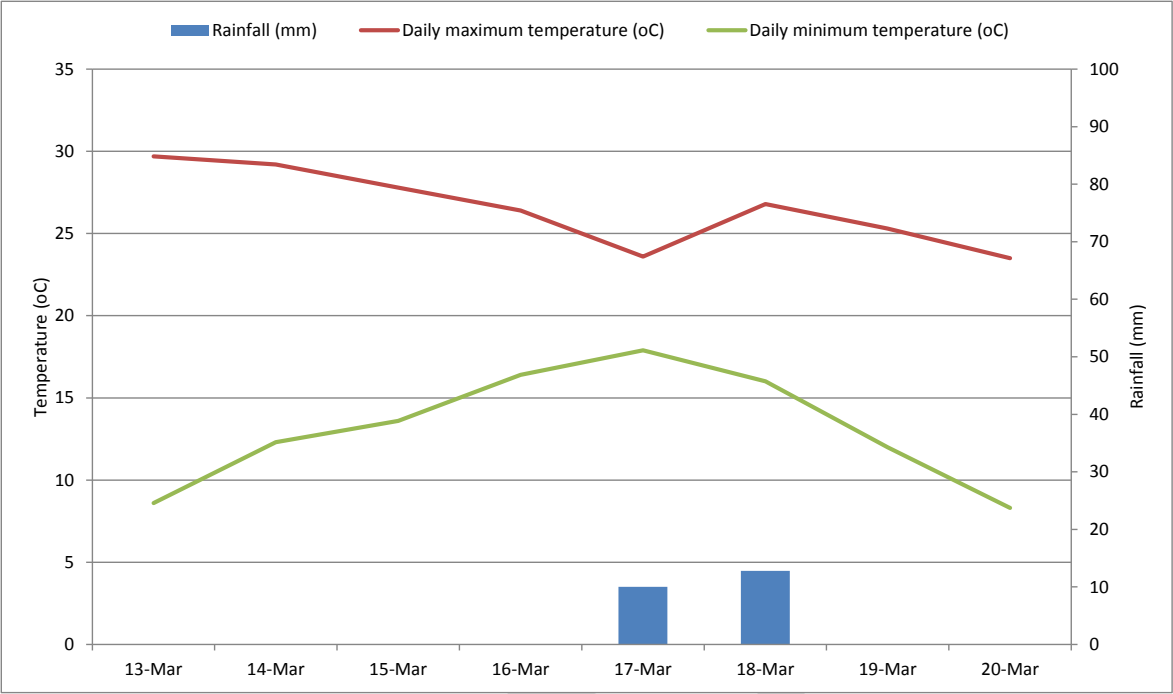


Mature leaves



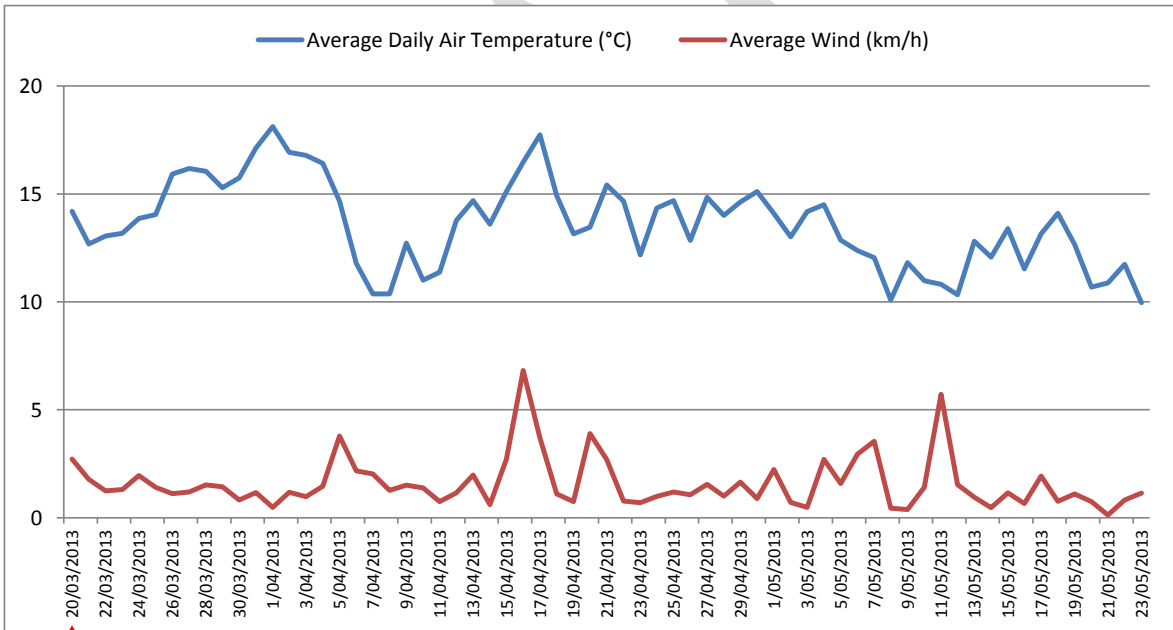
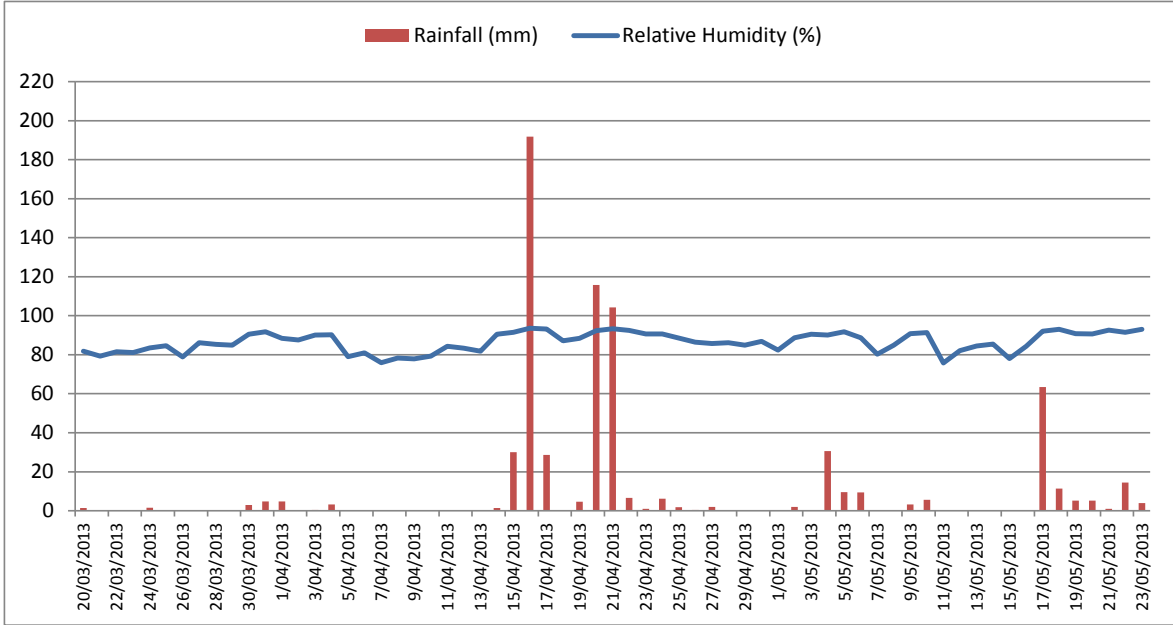
*,+ Statistically significant from Psa treatment according to a non-parametric (Wilcoxon) test, at the 5% and 10% significance levels respectively (n = 10).

Appendix 1. Weather in the Waikato during the period that treatments were applied. Source: NIWA Weather Stations i.e. Hamilton, Ruakura 2 Ews (#26117).



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Appendix 2. Weather at the Zespri/KVH field site during the trial of BCAs on G14, protectants and elicitors on G9, and elicitors on Chieftain. Source: Harvest.com (weather station on site).



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**Inoculation
 (Mar 20)**

