



2012/13 Potted Plant Field Trial Report

Trial 6

Actigard Integrated with PFR Yeast Mixes on Hayward

December 2012 – March 2013



June 2013

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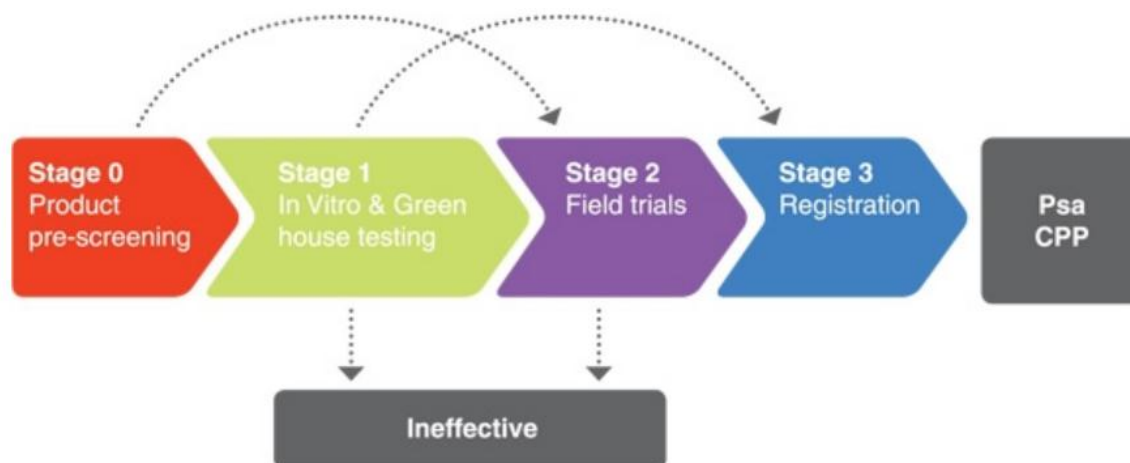
Introduction

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 a trial conducted from December 2012 on Hayward potted plants in which combinations of Actigard and yeast mixes (provided by Plant & Food) were tested.



Objective

To test the efficacy of Yeast Mixes provided by Plant & Food Research (PFR) when combined with Actigard in comparison to Actigard only.

Methodology

Plants

In this trial, Hayward plants were used. 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 trial as no symptoms were observed previously. The plants were approximately 1.2 m in height with approximately half a dozen leaves (Figure 1).

Figure 1. Example of the Hayward plants (on Bruno rootstocks) used in the KVH/Zespri trial of Actigard integrated with PFR Yeast Mixes. Also shown is the overhead misting system used to keep plants continuously wet for 48 h following Psa-V inoculation.



Treatments

These are listed in Table 1. Four different yeast mixes were applied, all in combination with Actigard. An Actigard only treatment was included. Due to a lack of plants, it was not possible to test the yeast mixes alone. All treatments were applied twice before inoculation i.e. 10 and 2 days before.

The Actigard was applied at a rate of 20 g/100 L in each treatment and was applied by PFR staff from Te Puke and Ruakura using best practice for commercial elicitors (10 days and 4 days prior to inoculation with Psa-V). The application details for the PFR yeast mixes are confidential to Plant and Food Research. The yeast mixes were applied as a tank mix with the Actigard.

Table 1. List of treatments in the KVH/Zespri Actigard+Yeast Mixes (coded “YBCA”) trial, December 2012.

Treatment No.	4/12/2012 (10 days before Psa-V inoculation)	12/12/2012 (2 days before Psa-V inoculation)
1	Nil	Nil (water control)
2	Nil	Nil (Psa-V control)
3	PFR-YBCA1 (fermented) +Actigard	PFR-YBCA1 (fermented) +Actigard
4	PFR-YBCA2 (fermented) +Actigard	PFR-YBCA2 (fermented) +Actigard
5	PFR-YBCA2 (NOT fermented) +Actigard	PFR-YBCA2 (NOT fermented) +Actigard
6	PFR-YBCA3 (fermented) +Actigard	PFR-YBCA3 (fermented) +Actigard
7	Actigard	Actigard

YBCA1-is a mixture of two PFR yeasts

YBCA-2-is a mixture of three PFR yeasts

YBCA-3-is a single yeast sp. that is in a pre-commercial stage for PFR

At the time of this trial, it was not certain whether fermentation of the yeasts was required for effective efficacy against Psa, therefore best practice yeast production was used at that time

Treatments 2–7 were inoculated with Psa-V on 14 December 2012.

Treatment application

The plants were originally held at PFR Ruakura, after delivery from Kerikeri, and were transferred to PFR Te Puke on 4 December 2012 where they were immediately treated with Actigard and the PFR yeast treatments by PFR staff from Te Puke and Ruakura Research Centre. The plants were left uncovered and light rain (less than 2 mm) fell overnight which may have reduced yeast colony forming units (cfu) on leaf surfaces. The following day, on 5 December 2012, the plants were moved to an area of the nursery under a plastic shelter. The second application of yeast treatments was on 12 December 2012. Although light rain occurred overnight, the treated plants remained relatively dry and treatment dilution and run-off was avoided. All treatments were applied to both the upper and lower leaf surfaces of each individual leaf per plant using a hand-held 500 mL mist sprayer. Actigard treatments were applied to lightly wet the leaf surfaces, whereas all yeast treatments were applied to just before run-off.

Inoculation

Application of the Psa-V, for which MPI permission was obtained, was undertaken at the Zespri/KVH trial site in Te Puke on 14 December 2012. This occurred inside a temporary spray booth to contain the spread of inoculum. Plant and Food Research staff from Te Puke provided fresh inoculum on the day. The concentration was ca. 1×10^8 cfu/mL.

The inoculum was sprayed onto plants using 5 L multi-purpose hand-held pressure sprayers with fine nozzles. The undersides of leaves were sprayed to wet. The lower leaf surface contains the majority of stomata, and is believed to be a more conducive site for Psa-V infection. Inoculation occurred between 10 and 11am.

Initial wetting of plants

Following inoculation, plants were kept continuously wet from above for approximately 48 h by an overhead misting system (see Figure 1) i.e. from about 11 am on 14 December to 11 am on 16 December. During this time, it is estimated that the equivalent of 34 mm of water was applied in the trial area (of approximately 1200 m²).

During the inoculation and initial wetting period about 0.5 mm of rainfall was recorded. On the day of inoculation, the maximum temperature reached 21°C, 26°C the following day and 23°C the day after that.

Assessments

The extent of leaf spotting and secondary symptoms were visually estimated and first recorded 17 days after inoculation, then at approximately weekly intervals. Each time, the amount of total leaf area covered in necrotic spots for whole plants was estimated. The last assessment was conducted on 8 March 2013, 84 days after inoculation. Following that, the plants were removed and destroyed in accordance with our Research Approval in order to make way for another trial.

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 (Source: NIWA Weather Station “Te Puke Ews” – located across the road from site of treatment application). 4– 14 December. Appendix 1.*

Approximately 10 mm of rain fell in the 4 days (5–8 December 2013) following the initial application of treatments. About 5 mm also fell on the night of 12 December, following the second application of treatments. However, the plants were covered during virtually all of these periods so wash-off was likely to have been minimal. Average daily relative humidity ranged between 77 and 100% while average daily temperature ranged between 14 and 18°C.

- ii) *Weather following inoculation (based on the installed Harvest.com weather station). 14 December – 8 March.* Appendix 2.

Approximately 10 mm fell in the four days following the initial wetting period. Another 140 mm fell in the second and third weeks after inoculation. Average daily relative humidity was generally above 80% while average daily temperature ranged between 15 and 20°C in the 2 to 3 week period after inoculation.

Results and interpretation

The results are presented in Figure 2 and Figure 3 and summarised as follow:

- High levels of leaf spotting were observed on the Psa-V control plants in this trial i.e. on average; between 50 and 70% of the total leaf area of plants were covered in necrotic leaf spots. These high levels indicate good infection conditions had occurred, combined with a cultivar that is highly susceptible to the leaf spotting phase of Psa-V.
- The low level of leaf spotting observed across the water control plants (i.e. less than 2% on average) confirms that there was very little natural infection.
- Considerably less leaf spotting was observed in all the other treatments throughout the trial i.e. on average, less than 10% of the total leaf area of plants was covered in necrotic leaf spots. This was largely the result of the Actigard included in each treatment (Figure 2).
- At the last leaf spot assessment (41 days after inoculation) the average amount of leaf spotting for the Actigard treatment was approximately 7%. At the same time, leaf spotting for the Actigard+Yeast Mix treatments was on average between 3 and 4% (Figure 3).
- The differences between the four Actigard+Yeast Mix treatments and the Actigard alone treatment were statistically significant ($P < 0.05$).
- There were no statistically significant differences between the four Actigard+Yeast Mix treatments at both the 5 and 10% level of probability.

No secondary symptoms were observed in this trial including at the last assessment which was conducted on 8 March 2013, 84 days after inoculation. The very dry summer may have contributed to this.

Summary

This trial on highly susceptible grafted Hayward potted plants demonstrated that Actigard alone reduced leaf spotting from 62 to 7% when applied 10 and 2 days prior to Psa-V inoculation at the recommended rate of 20 g/100 L. This trial also demonstrated that when Actigard treatments are integrated with selected PFR Yeast Mixes (e.g. YBCA2+Actigard), there is a further statistically significant reduction in leaf spotting i.e. Psa-V leaf spotting was reduced from 62 to 3.5%. This indicates that combined use of a commercial plant defence elicitor with protectant BCAs has the potential to provide better control of Psa compared to treatments applied alone.

Further trials of this nature are planned. First to confirm the efficacy of PFR Yeast Mixes when applied alone and secondly to confirm their efficacy with and without Actigard on cultivars other than Hayward.

Figure 2. 2012/13 Zespri/KVH Potted Plant Trial of Actigard+Yeast Mixes, on Hayward kiwifruit. Average amounts of total leaf area covered in Psa-V leaf spots (n = 10 replicates), for all treatments. Bars are plus and minus the standard error of the mean. All treatments were significantly different to the Psa-V treatment according to a non-parametric (Wilcoxon) test at the 5% level.

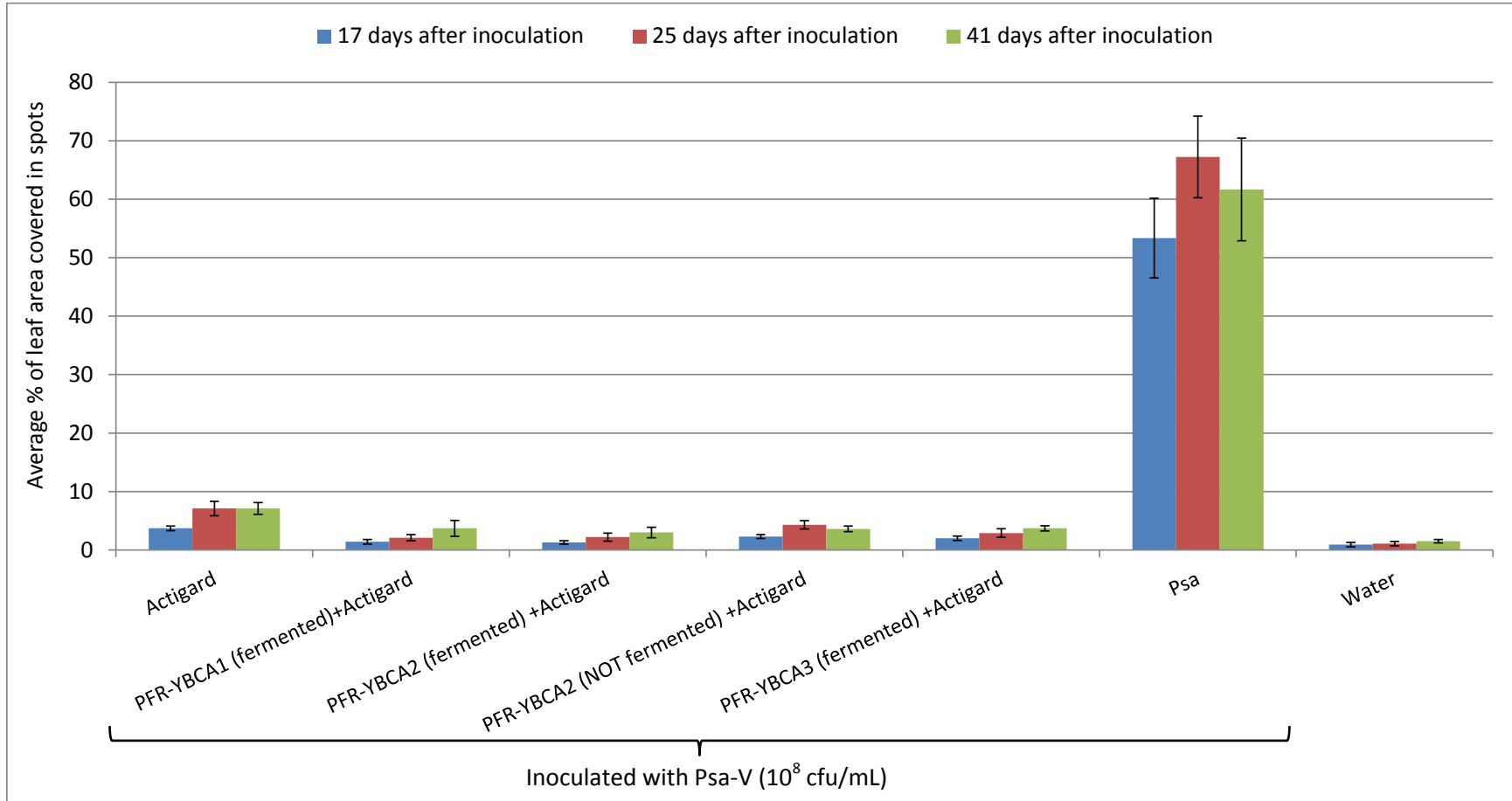
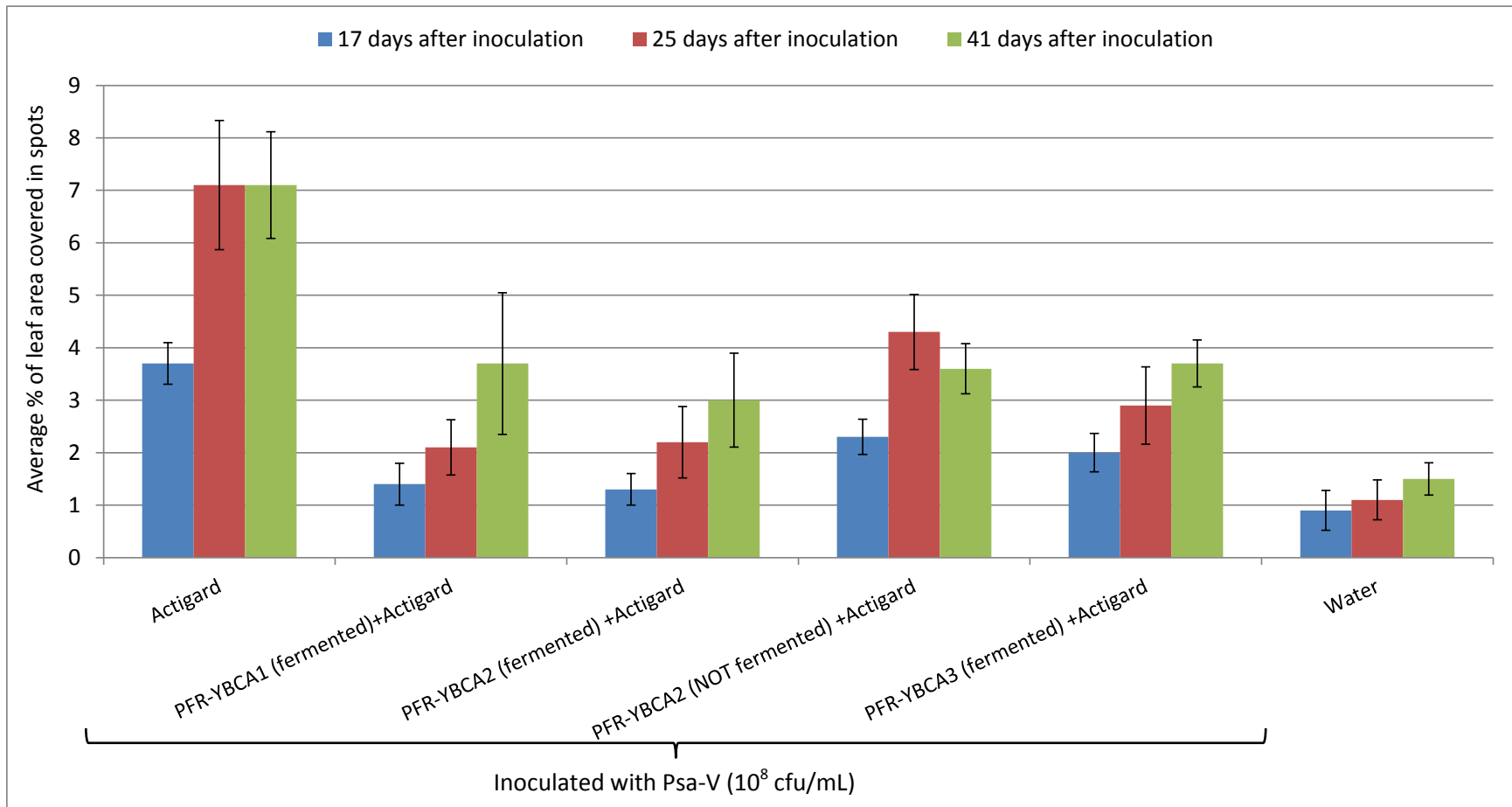
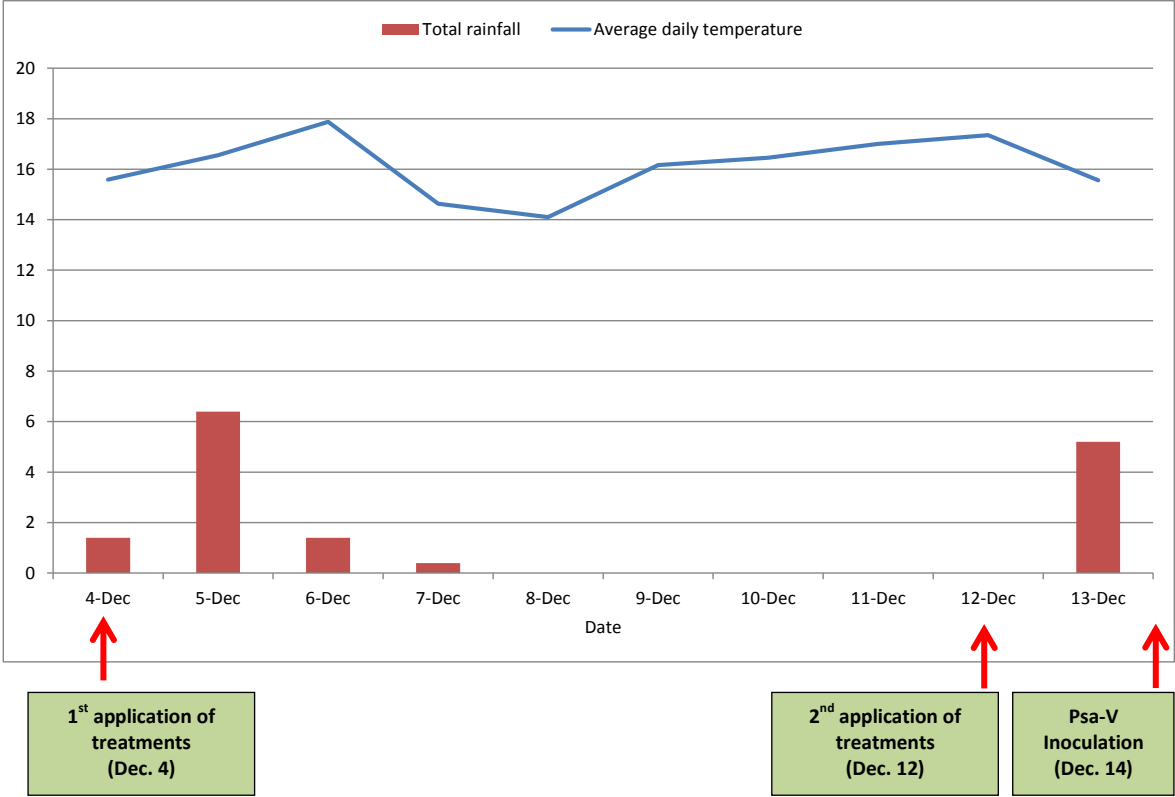


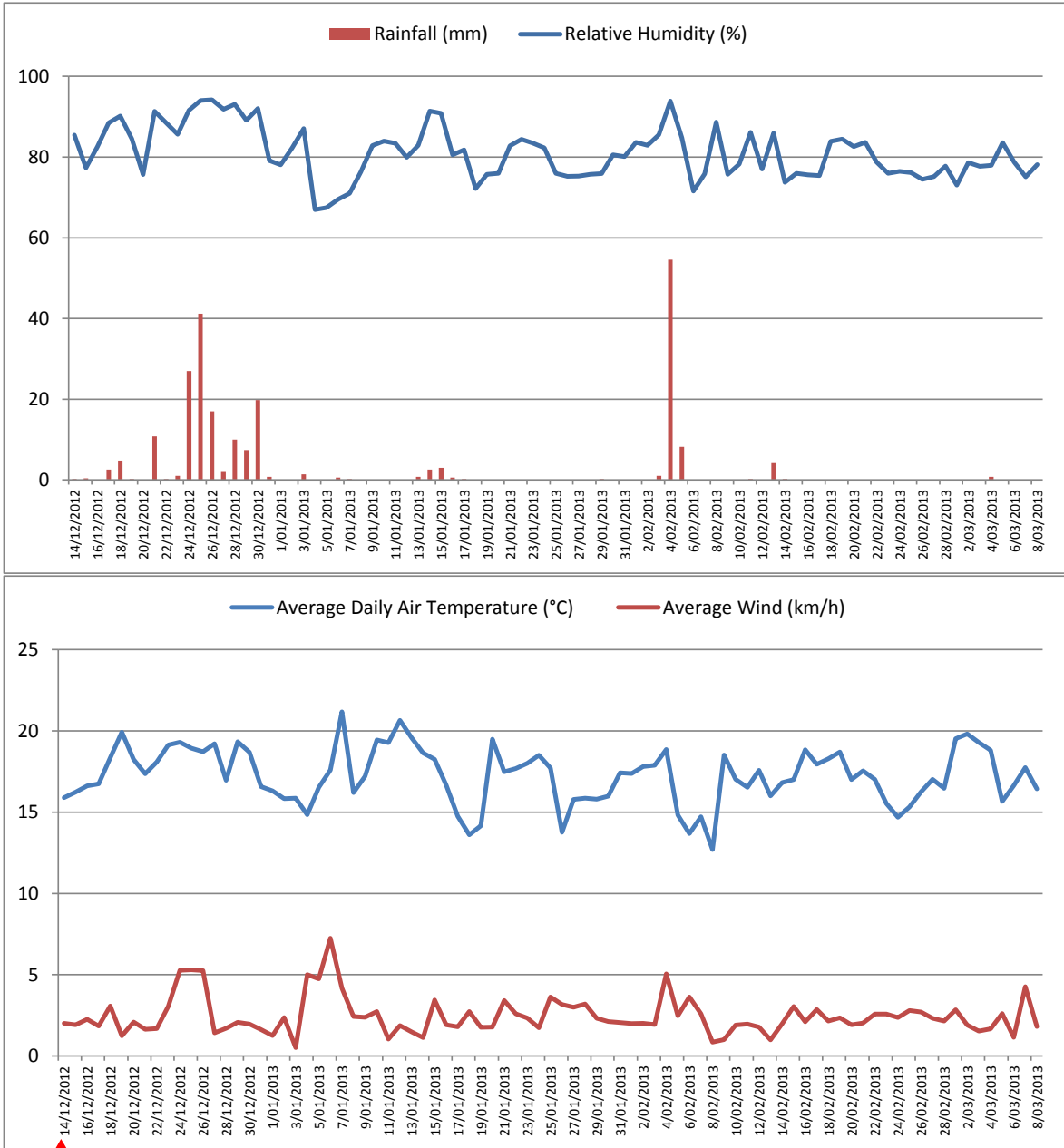
Figure 3. 2012/13 Zespri/KVH Potted Plant Trial of Actigard+Yeast Mixes, on Hayward kiwifruit. Average amounts of total leaf area covered in Psa-V leaf spots (n = 10 replicates). The nil treated Psa-V control treatment has been excluded here to better visualise treatment differences. Bars are plus and minus the standard error of the mean. All treatments were significantly different to the Actigard treatment according to a non-parametric (Wilcoxon) test at the 5% level.



Appendix 1. Weather at the Zespri/KVH field site over the period that treatments were being applied in the Actigard+Yeast Mixes trial. Treatments were applied at the nearby Plant and Food Research Station and weather would have been similar to that shown here. Source: Weather Station on Site.



Appendix 2. Weather at the Zespri/KVH field site during the trial of Actigard+Yeast Mixes which started in December 2012. Source: Harvest.com (weather station on site).



**Psa-V Inoculation
(Dec 14)**

