2012/13 Potted Plant Field Trial Report

Trial 5

Biological control agents (BCAs) on Hayward

December 2012 – January 2013

28 March 2013

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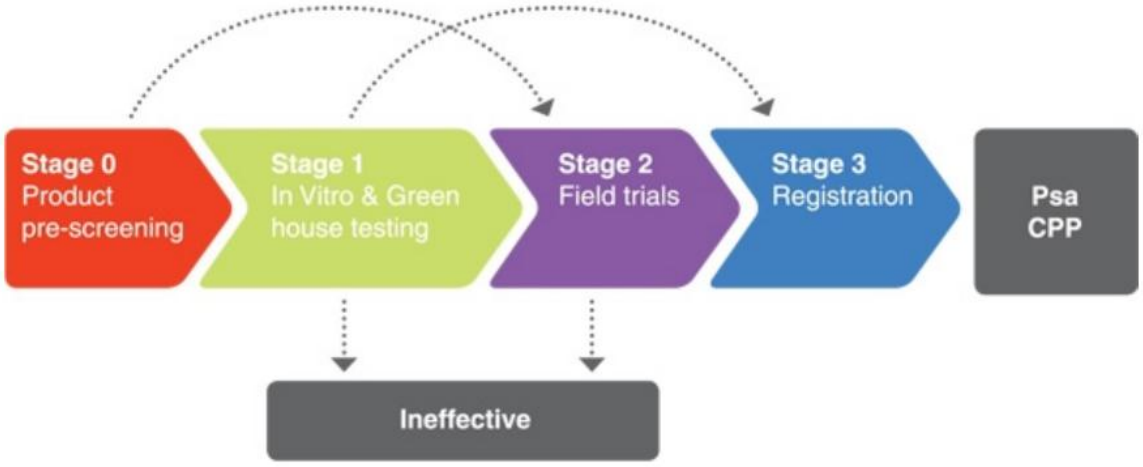
Introduction

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 the efficacy of different biological control agents (BCAs) at controlling Psa-V was studied.



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 1.5m in height with a significant number of leaves (Figure 1).

Figure 1. Example of Hayward plants (on Bruno rootstocks) used in KVH/Zespri BCA trial in December 2012/13.

Treatments

These are listed in Table 1. Nordox 75WG was included as a positive control. Each treatment was applied to 10 plants (single plant replicates). Treatments were applied from 5 days to 12 hours before inoculation following the suppliers instructions. The rates were provided by the suppliers.

Treatment application

Treatments were applied to 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 December 1 and 5.

Table 1. List of treatments applied in the KVH/Zespri biological control agent (BCA) trial in December 2012. Recommended by suppliers of products.

Treatment no.	Product	Actives	Rate per 100L	Application timing relative to inoculation
1	TripleX + Filmstar	*Bacillus amyloliquefaciens BS 1b* Terpens and fatty alcohols	200mL 60mL	5 days before
2	Clarity	*Bacillus subtilis*	53g	2 days before
3	Blossom Bless	*Pantoea agglomerans (strain p10c)*	30g	2 days before
4	BOTRY-Zen	*Ulocladium oudemansii*	800 g	2 days before
5	PP9 + Filmstar	*Pseudomonas putida* Terpens and fatty alcohols	10mL 60mL	2 days before
6	Superzyme	*Mix of species*	100g	2 days before
7	Blightban + Sequestrene	*Pseudomonas fluorescens A506* Iron chelate	100g 120g	2 days before
8	Nordox 75 WG	*Copper oxide*	37.5g	1 day before
9	Plant Shield + Biosea Omega Oil	A range of beneficial bacteria Blend of Omega 3 and Omega 6 oils	350g 200mL	12 hours before
10	BacStar* + DuWett	*Bacillus amyloliqufaciens* D747 Organosilicone surfactant	175g 35mL	12 hours before
11	Serenade Max + DuWett	*Bacillus subtilis* Trisiloxane ethoxylate	350g 35mL	12 hours before
12	Water:Water			
13	Water:Psa			

* The active in this is often referred to as Amylo X and has been tested offshore by GM Balestra.

Inoculation

Inoculation, for which MPI permission was obtained, was undertaken at the Zespri/KH trial site in Te Puke on 6 December 2012, the day after the final treatments were applied. This occurred inside a temporary spray booth to contain the spread of inoculum. A Psa-V concentration of 10^6cfu/mL[1] was requested for the inoculation. Previous work indicated anything lower than this would not produce sufficient infection and symptoms.

Plant and Food Research staff from Ruakura provided fresh inoculum on the day. 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.

[1] Subsequently, the Psa-V concentration applied was measured and estimated to be between 10^6 and 10^7 cfu/mL

Infection period

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 12pm on December 6 to 12pm on December 8. During this time, it is estimated that the equivalent of 34mm of water was applied in the trial area (of approximately 1200m^2).

Relative humidity was high and sustained on the day of inoculation and cloud cover was 100%. In addition to the artificial wetting, it also rained significantly during the night of December 6 (largely between 3am and 5.30am). Approximately 30mm fell. Approximately 4mm also fell on Dec 7 with another 10mm falling on the Dec 8. This is shown in the weather graphs in Appendix 1.

Assessments

Two formal leaf spot assessments were carried out on December 21 (+15 days) and January 8, (+33 days). The percentage of total leaf area per plant covered in leaf spotting was visually estimated each time. The presence of any secondary symptoms was also recorded.

Each time, plants were divided into two sections for leaf spot assessment:

i) Mature – leaves that were mature (fully expanded) at the time of inoculation i.e. the bottom parts of plants. These parts would have been fully covered by each treatment and by Psa-V.

ii) Expanding – leaves that were still expanding (or not present yet) at the time of inoculation i.e. the top parts of the plants. These parts would not have been fully protected by each biological later in the trial if the leaves expanded but the biologicals did not.

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.

Plants were removed shortly after the second assessment to make way for another trial.

Weather

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

i) *Weather in Hamilton between application of treatments and transfer of plants to trial site for inoculation (based on Metservice website info).* **1 to 5 December.**

During the period of treatment application, 10 – 15mm of rain fell on December 2, again on December 4 and again between December 5 and 6. Plants were kept under cover during this time to prevent wash off of treatments. Maximum daily temperatures were

between 20 and 24°C while minimum daily temperatures were between 4 and 15°C (Source: NIWA Weather Station, Raukura 2 Ews).

ii) *Weather between inoculation and the final assessment at field trial site in Te Puke (based on installed Harvest.com weather station).* **6 December to 8 January.** Appendix 1.

As discussed above significant rain fell just after inoculation. About 20mm of rain also fell on December 13. Significant amounts of rain fell from December 21 to 31 totalling about 140mm. Average humidity hovered around 80% while average daily temperature hovered was between 15 and 20°C.

Results and interpretation

Within a week, significant leaf necrosis was observed across plants from all treatments except the water control plants. This had not been observed before in Zespri/KVH trials of this nature. In the previous year, at a similar time of year (December/January) much lower symptom expression was observed[2]. Examples of the leaf necrosis observed are shown Figure 2.

After much consultation with Zespri and Plant & Food scientists, it was decided that what was observed were Psa-V symptoms, rather than some kind of phytotoxicity. This is because the symptoms were at high levels across all treatments except the water control.

The reasons for the rapid symptom expression are not known but the following factors may have contributed:

- During the inoculation process and period very good infection conditions prevailed. On the day of inoculation, it was overcast, warm and humid.
- In addition to artificial watering, significant amounts of rain fell initially which may have exacerbated infection.
- The overhead misting system used was different to that used in the previous season and designed to deliver smaller droplets and volumes of water. Previously, a sprinkler system was used that delivered significantly more water onto plants.
- The condition of the plants in this trial may have been such that they were more susceptible to infection.

The results of the formal leaf assessments are presented in Figure 3 and Figure 4. In summary:

- Visually high levels of leaf spotting were recorded for all treatments except the water control which had little or no leaf spotting.
- Average leaf spotting across all plants (excluding the water control) was about 15% and 20%, 15 and 33 days after inoculation respectively. As a guide, average leaf spotting percentages above about 5% are visually noticeable i.e. visually there is a marked difference between a plant with no leaf spotting and one with 5 or more percent.
- A high level of leaf spotting was also observed in the positive control (Nordox 75WG). This was unusual as in previous trials this treatment had shown good efficacy.
- The only statistical reductions in Psa-V found were 15 days after inoculation and only in the expanding parts of the plants. Specifically:

[2] www.kvh.org.nz/vdb/document/91134

- Botryzen, Plant Shield, Superzyme had Nordox 75WG had significantly lower leaf spotting than the Psa control[3].
- Although statistically lower, the levels of leaf spotting in all these treatments was on average between 10 and 15% which is visually high.
- The infection pressure may have been lower in the expanding parts early on which allowed some treatments to show some efficacy.

- No statistical reductions were observed 33 days after inoculation. This may have been due to treatments losing their effect and/or the plants being overwhelmed with Psa-V and infection.
- At the second assessment, 33 days after inoculation, four out of the 130 plants had shoot dieback - one from each of the following treatments: Botryzen, PP9, Serenade Max, TripleX.

Figure 2. Hayward plants in BCA trial showing significant Psa-V symptoms

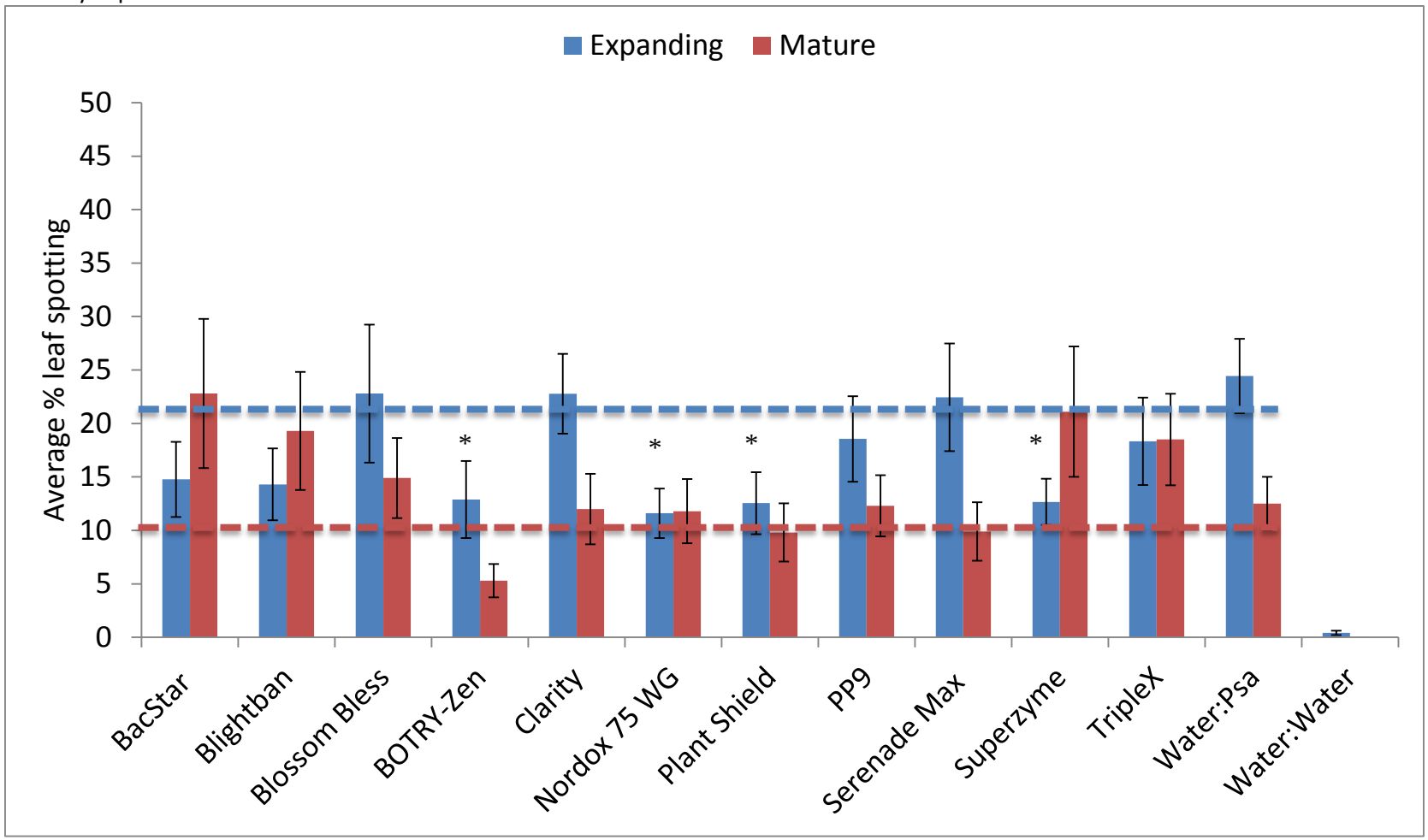
Summary

Unexpected high levels of leaf spotting were observed quickly across all treatments in this trial (except the water control). This means very good infection occurred and/or that the plants were particularly susceptible. The only statistical reductions in leaf spotting were observed 15 days after inoculation and only on the expanding sections of plants. That said the level of leaf spotting in these treatments was still high (just not as high in the Psa control).

It is likely that the biological treatments in this trial were overwhelmed by the Psa-V that was applied. Alternatives to testing biologicals will be considered including lowering the inoculum although this would increase the likelihood of getting insufficient symptom expression.

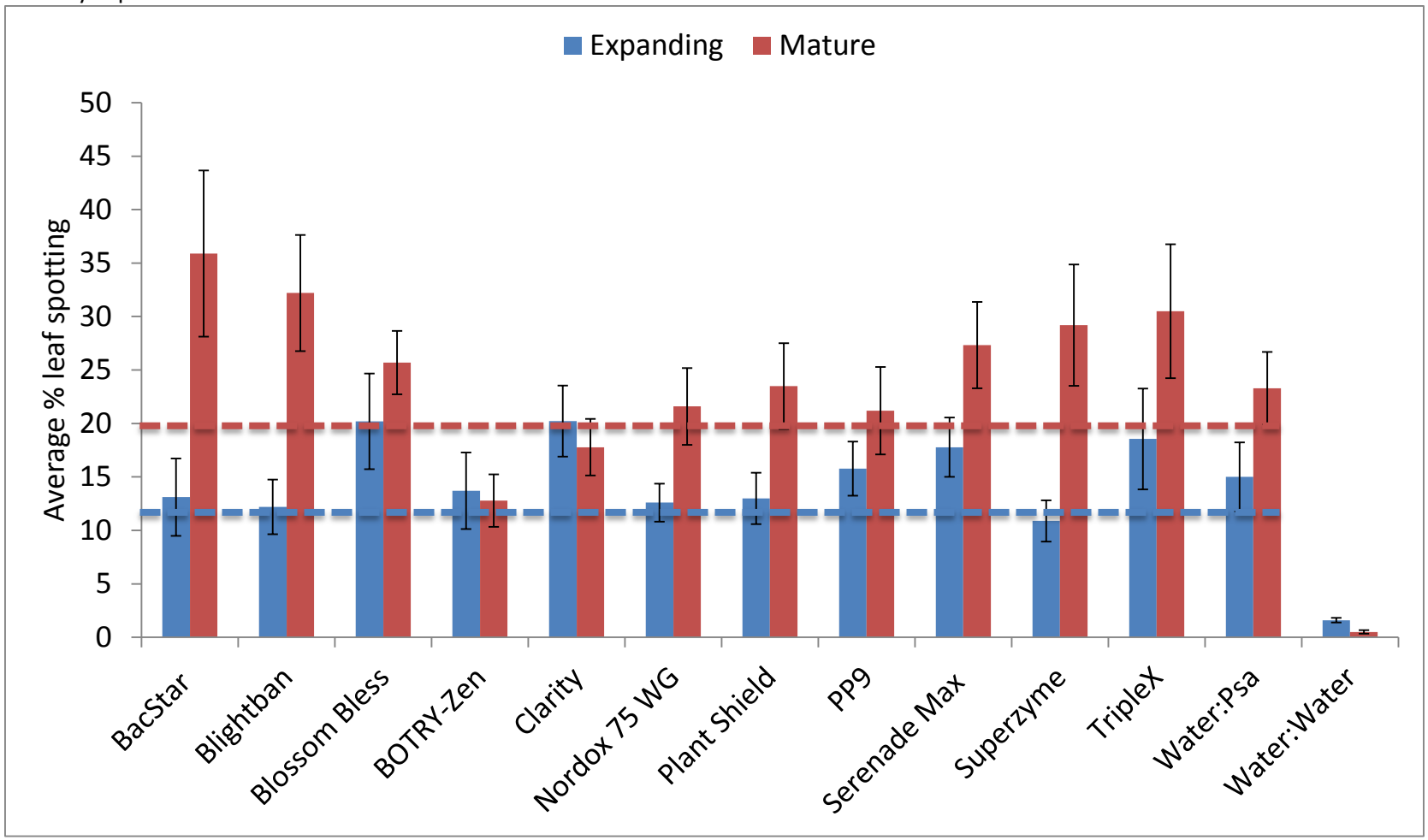
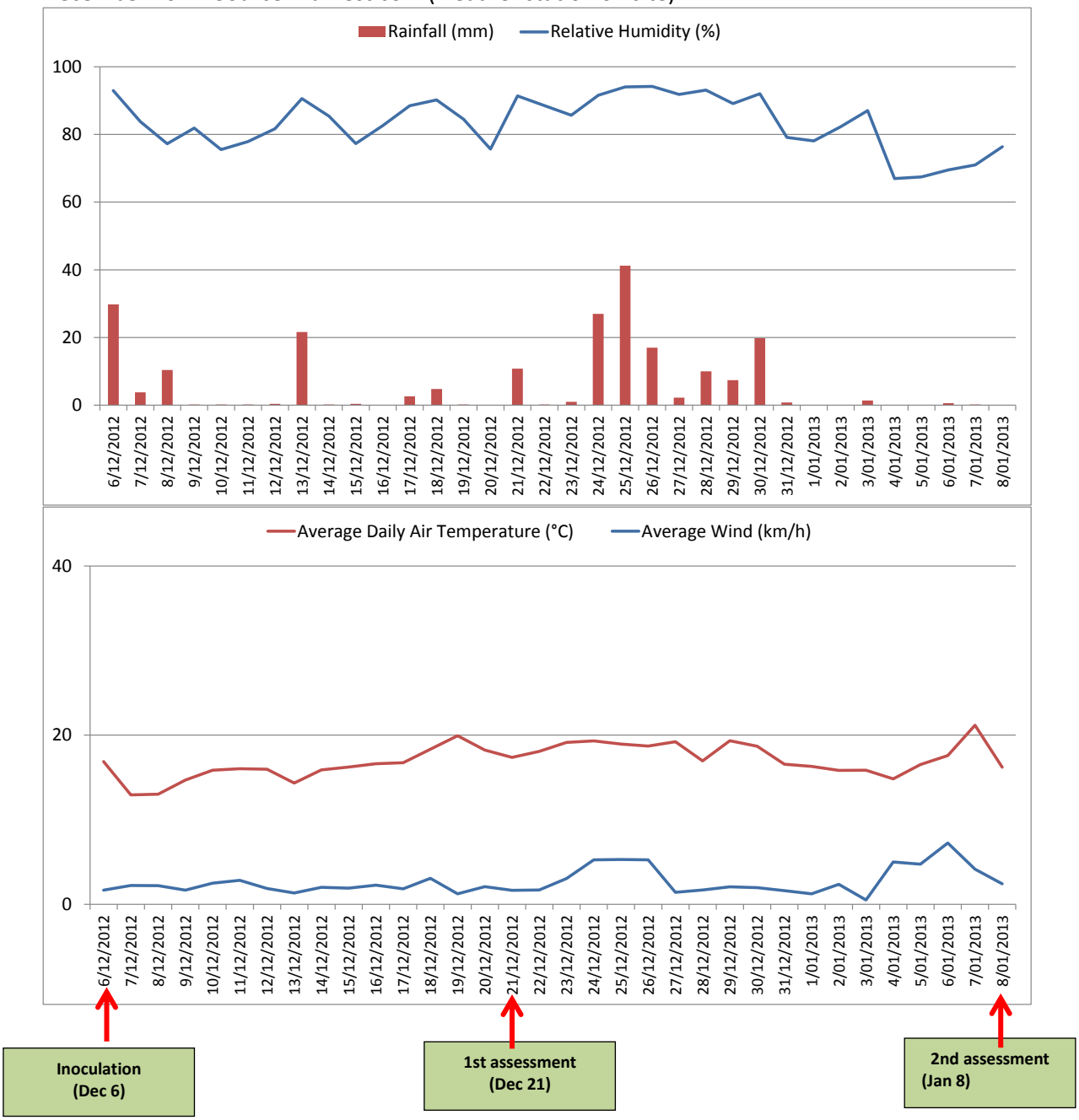
[3] According to a non-parametric (Wilcoxon) test. This was used as the data was not normally distributed.

Figure 3. Average amounts of total leaf area in Hayward potted plants covered in Psa-V leaf spots (n = 10), **15 days after inoculation**. Plants were divided into two parts for assessments: i) Mature = leaves were all mature (fully expanded) at the time of inoculation ii) Expanding = leaves were not fully expanded at the time of inoculation.



* Statistically significant from Water:Psa treatment according to a non-parametric (Wilcoxon) test.

Figure 4. Average amounts of total leaf area in Hayward potted plants covered in Psa-V leaf spots (n = 10), **33 days after inoculation**. Plants were divided into two parts for assessments: i) Mature = leaves were all mature (fully expanded) at the time of inoculation ii) Expanding = leaves were not fully expanded at the time of inoculation.



Appendix 1. Weather at the Zespri/KVH field site during the BCA trial on Hayward which started in December 2012. Source: Harvest.com (weather station on site).