Psa-V Product Testing – Field Trial Report

Trial 10

Biological control agents - Hort16A

April 2012

28 May 2012

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.

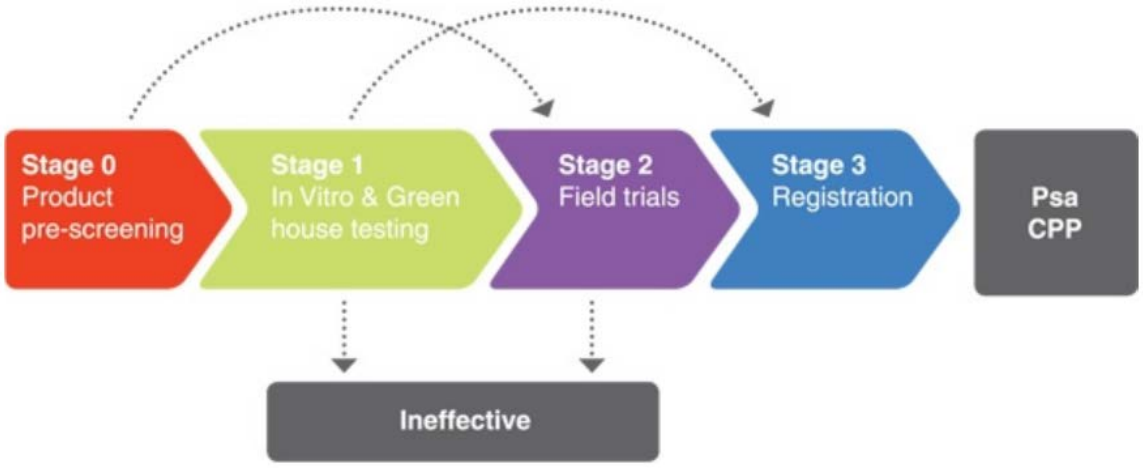
Introduction

ZESPRI, with support from KVH, is coordinating the screening of the effectiveness of a wide range of products to control the virulent type of bacterial disease caused by *Pseudomonas syringae pv. Actinidiae* (Psa-V). The screening programme has been developed to identify, rigorously test and then obtain permission to use suitable products as part of the crop protection programme (CPP) to help manage Psa-V. To understand the steps in the product testing programme the process is outlined in the diagram below.

The final 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 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.

ZESPRI has contracted HortEvaluation Ltd, led by Lynda Hawes, to undertake the 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 on Hort16A potted plants in which the efficacy of different biological control agents at controlling Psa-V was studied.



Methodology

Plants

In this trial, female Hort16A plants were used. These were grafted onto 1 year old Bruno rootstocks in spring 2011, 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 Hort16A seedlings (on Bruno rootstocks) used in KVH/ZESPRI BCA trial in April/May 2012.

Treatments

These are listed in Table 1. With the exception of Nordox (the positive control) and ARMOUR-Zen (an elicitor), all the products tested were biological control agents (BCAs). Nordox was included as a positive control and applied with and without overhead irrigation to test the impact of this on the level of phytotoxicity. Each treatment was applied to 15 plants (single plant replicates) 12 hours to 2 days before inoculation following the suppliers instructions. The rates were provided by the suppliers and applied using a water rate of 1000L/ha.

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 on 9 & 10 April 2012.

Table 1. List of treatments applied in the KVH/ZESPRI biological control agent trial in April/May 2012.

Treatment No.	Product(s)	Active ingredients	Rate per 100L*. Water rate of 1000L/ha used.	Timing of application relative to inoculation*
1	Plant Shield	*A range beneficial bacteria*	350 g	2 days before
	+ Biosea Omega Oil	Blend of Omega 3 and Omega 6 oils	200 mL	
2	BOTRY-Zen	*Ulocladium oudemansii*	800 mL	2 days before
3	ARMOUR-Zen	Chitosan	1 L	2 days before
4	Clarity	*Bacillus subtilis*	53 mL	2 days before
5	Blightban (A506)	*Pseudomonas fluorescens*	100 g	2 days before
	+ Sequestrene	Iron chelate	120 g	
6	Blossom Bless	*Pantoea agglomerans* (strain p10c)	30 g	2 days before
7	BacStar	*Bacillus subtilis – Amyloliqufacien* D747	150 g	1 day before
	+ Bond Xtra	Organosilicone surfactant	100 mL	
8	Nordox 75 WG, OH irrigated	Copper oxide	37.5 g	1 day before
9	Nordox 75 WG, not OH irrigated	Copper oxide	37.5 g	1 day before
10	Serenade Max	*Bacillus subtilis*	350 g	12 hours before
	+ DuWett	Trisiloxane ethoxylate	35 mL	
11	Water:Water			
12	Water:Psa (10^6 cfu/mL)			

"OH" = overhead irrigated

* Advised by the supplier of each product

Inoculation

Inoculation, for which MAF permission was obtained, was undertaken at the trial site on 11 April 2012, the day after the final treatments were applied. This occurred inside a temporary spray booth to contain the spread of inoculum. The Psa-V inoculum level used was 10^6 cfu/mL as it was considered that anything lower than this would not produce sufficient infection and symptoms to differentiate between treatments. This is exactly what occurred in a previous trial on Hayward.

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 is more conducive to Psa infection.

Overhead watering

Following inoculation, plants were watered from above for approximately 14 hours by overhead sprinklers to create an infection event i.e. from 3pm on April 11 to 5am on April 12. During this time, it is estimated that the equivalent of 40mm of rainfall fell in the trial area (of approximately 1200m^2). In addition to this, it also rained i.e. from 5pm on April 11 about 65mm of rain fell in the ensuing 9 hours. So effectively the plants were exposed to about 100mm of rain during a 14 hour period.

Treatment 9 (Nordox) was deliberately not overhead irrigated to evaluate the impact of watering on the level of phytotoxicity. However, the rain nullified this.

Assessments

Four leaf spot assessments were carried out i.e. April 18 (+7 days), April 26 (+15 days), May 5 (+24 days) and May 10 (+29 days). The percentage of total leaf area per plant covered in Psa-V leaf spotting was visually estimated at each assessment time. At the end of the trial on May 18, the number of plants with shoot die-back (a type of secondary symptom) was also determined.

At the first and final assessments, leaf phytotoxicity was formally assessed for the two Nordox treatments using a scale from 1 (none) to 10 (severe). A simplified version of this scale and the associated symptoms is presented in Appendix 1.

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 in Hamilton between application of treatments and transfer of plants to trial site for inoculation (based on Metservice website info). 9 - 11 April.*

The weather was fine with no precipitation.

ii) *Weather between inoculation and the final assessment at field trial site in Te Puke (based on installed Harvest.com weather station). 11 April – 10 May.* Appendix 2.

As discussed previously, during the night following inoculation significant amounts of rain fell (approx. 65mm in a 9 hour period). No further rain fell until April 26 and 27 when 10 and 20mm fell respectively. Further rain fell on May 8 (10mm) followed by a significant rainfall on May 9 (80mm), the day before the final assessment.

Average daily temperature generally decreased during the trial from around 15 degrees at the beginning to less than 10 towards the end. At the end, the rain was accompanied by an increase in temperature (up to 15 degrees).

Results and interpretation

Leaf spotting

The amount of leaf spotting (percentage of total leaf area covered) observed throughout the trial is shown in Figure 2 (all assessments) and Appendix 3 (separate graphs for each assessment). The main findings were:

- Leaf spotting increased over time throughout the trial across all treatments.
- Levels of leaf spotting at the first assessment a week after inoculation were very low across all treatments i.e. less than 1% on average for the worst affected treatment (Water:Psa i.e. unprotected plants sprayed with Psa-V). Statistically, leaf spotting for all treatments was lower than the Water:Psa control. However given the very low level of spotting, these results are tenuous.
- At the second assessment, 15 days after inoculation, the level of leaf spotting had increased with the worst affected treatment, Water:Psa, having an average of 1.7% leaf spotting. This could be considered to represent a low level of infection, perhaps equivalent to low disease pressure in the field. At this time the following treatments had statistically less leaf spotting than the Water:Psa control: Plant Shield, Clarity, Blightban (+Sequestrene), Blossom Bless, the two Nordox treatments, and Water:Water. The BOTRY-Zen, ARMOUR-Zen and BacStar treatments did not have statistically less leaf spotting at this time.
- At the third assessment, 24 days after inoculation, only the two Nordox and Water:Water treatments had significantly less leaf spotting compared to the Water:Psa control. The Serenade Max treatment was on the border of having statistically less leaf spotting.
- At the fourth and final assessment, 29 days after inoculation, only the two Nordox and Water:Water treatments had significantly less leaf spotting compared to the Water:Psa control.
- Only the Nordox treatments had significantly less leaf spotting at all assessment times compared to the Water:Psa control.
- The low level of leaf spotting observed in the Water:Water treatment indicates that the natural disease pressure in this trial was low. The significantly higher level of leaf spotting in the Water:Psa control provides evidence that actively spraying Psa-V onto the plants successfully provided greater disease pressure and symptom development. This is important for ensuring each treatment receives a similar challenge and for ensuring sufficient symptom development to differentiate between treatments.
- The conditions experienced in this trial could be regarded as very challenging for BCAs and under more favourable conditions they may be more efficacious.

Secondary symptoms

Of the 178 vines (2 died previously) that were assessed at the end of the trial, only 9 had any shoot die back (5%). 8 of these had just one shoot affected with the other having 2 shoots affected. There was no difference between treatments.

Phytotoxicity

The level of leaf phytotoxicity associated with the two Nordox treatments was below what is regarded as slight (Figure 3). There was no difference between the two treatments which was expected given that the rainfall following inoculation would have nullified any treatment difference. While not formally assessed, phytotoxicity was not evident in the other treatments.

Summary

This trial provided evidence that biological control agents (BCAs) can reduce Psa-V leaf spotting but under what could be regarded as low disease pressure; Nordox provided significantly more control. Serenade Max showed efficacy in an earlier KVH/ZESPRI field trial on Hort16A (Trial 3) and the results here further indicate that it can reduce leaf spotting in Hort16A.

The challenging conditions experienced in this trial particularly the initial significant watering and declining temperatures may have reduced the efficacy of the BCAs. Further field testing is recommended to clarify the efficacy of BCAs under different field conditions.

Figure 2. Average amounts of total leaf area in Hort16A potted plants covered in Psa-V leaf spots (n = 15). Appendix 3 displays the same results but with each assessment on a separate graph and with treatments that significantly reduced leaf spotting noted. The horizontal dashed lines align with the lower standard error bars for the Water:Psa control at each assessment time. "OH" = Overhead.

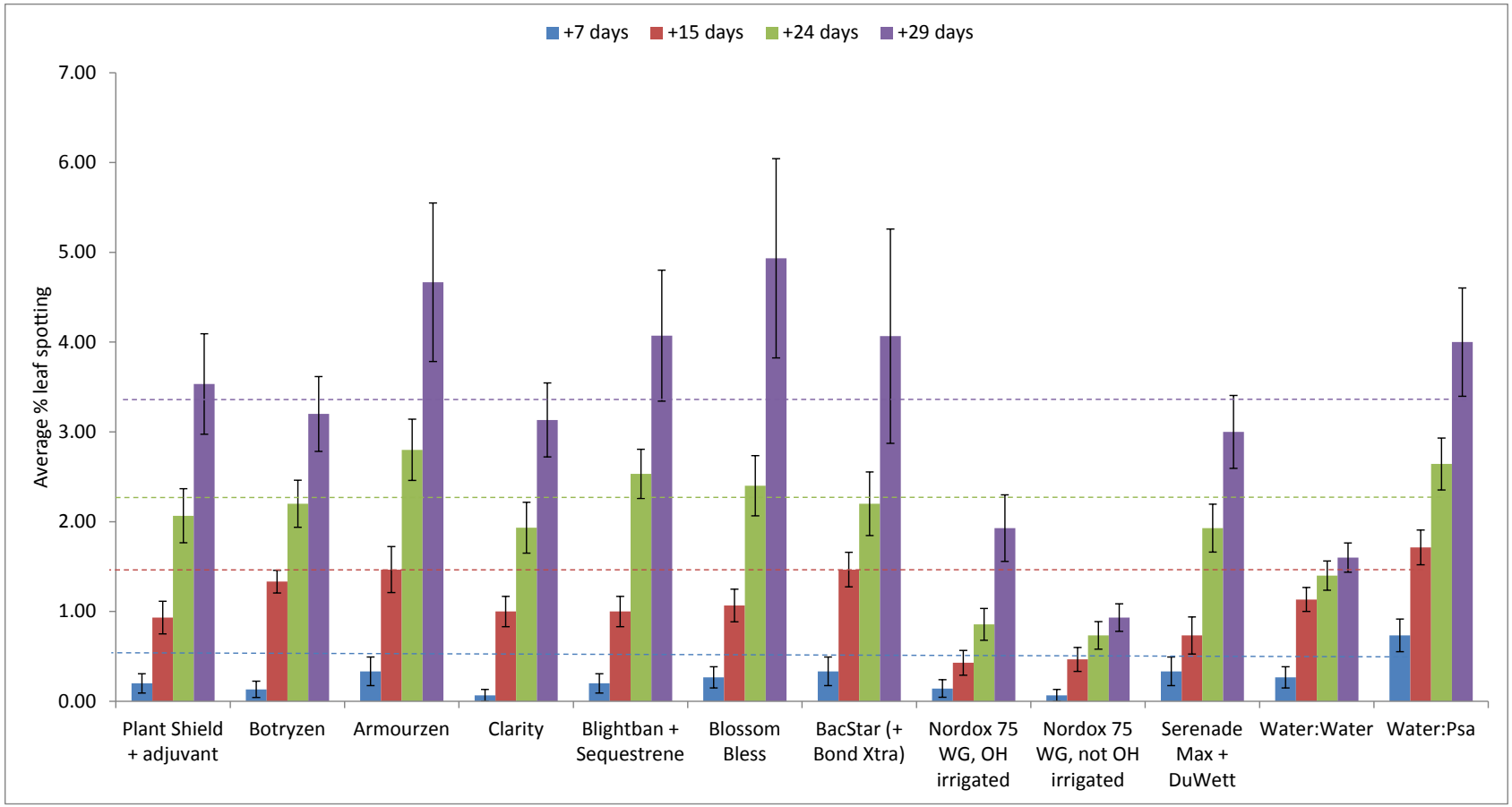
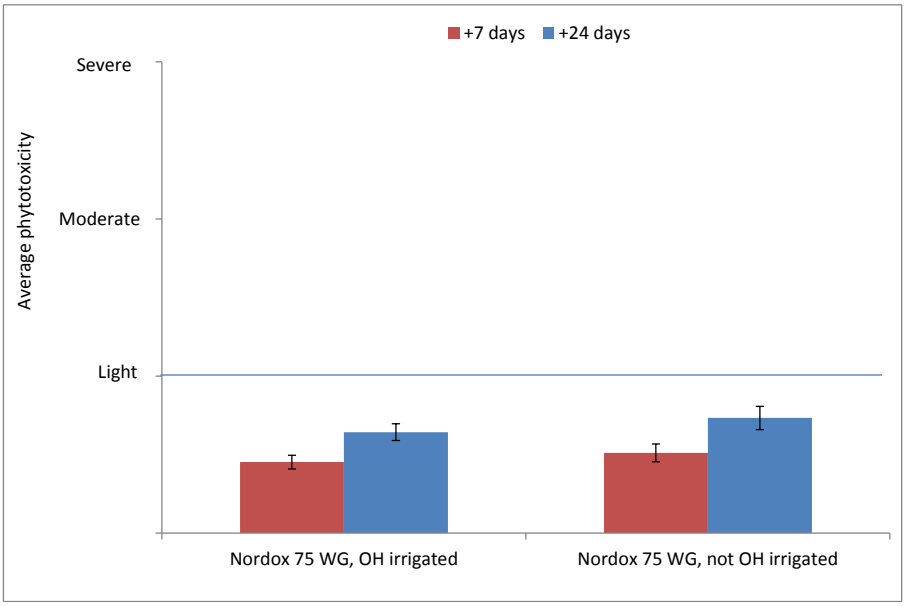


Figure 3. Average amounts of leaf phytotoxicity observed with the two Nordox treatments in BCA trial conducted on Hort16A potted plants in Te Puke in Apr/May 2012 (n = 15).



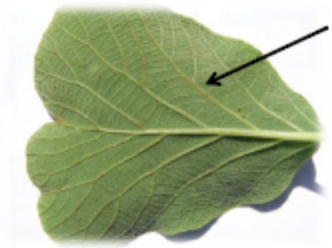
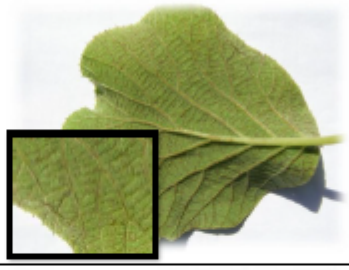
Appendix 1. KVH phytotoxicity scoring system (Source: KVH Website)

0 = No symptoms

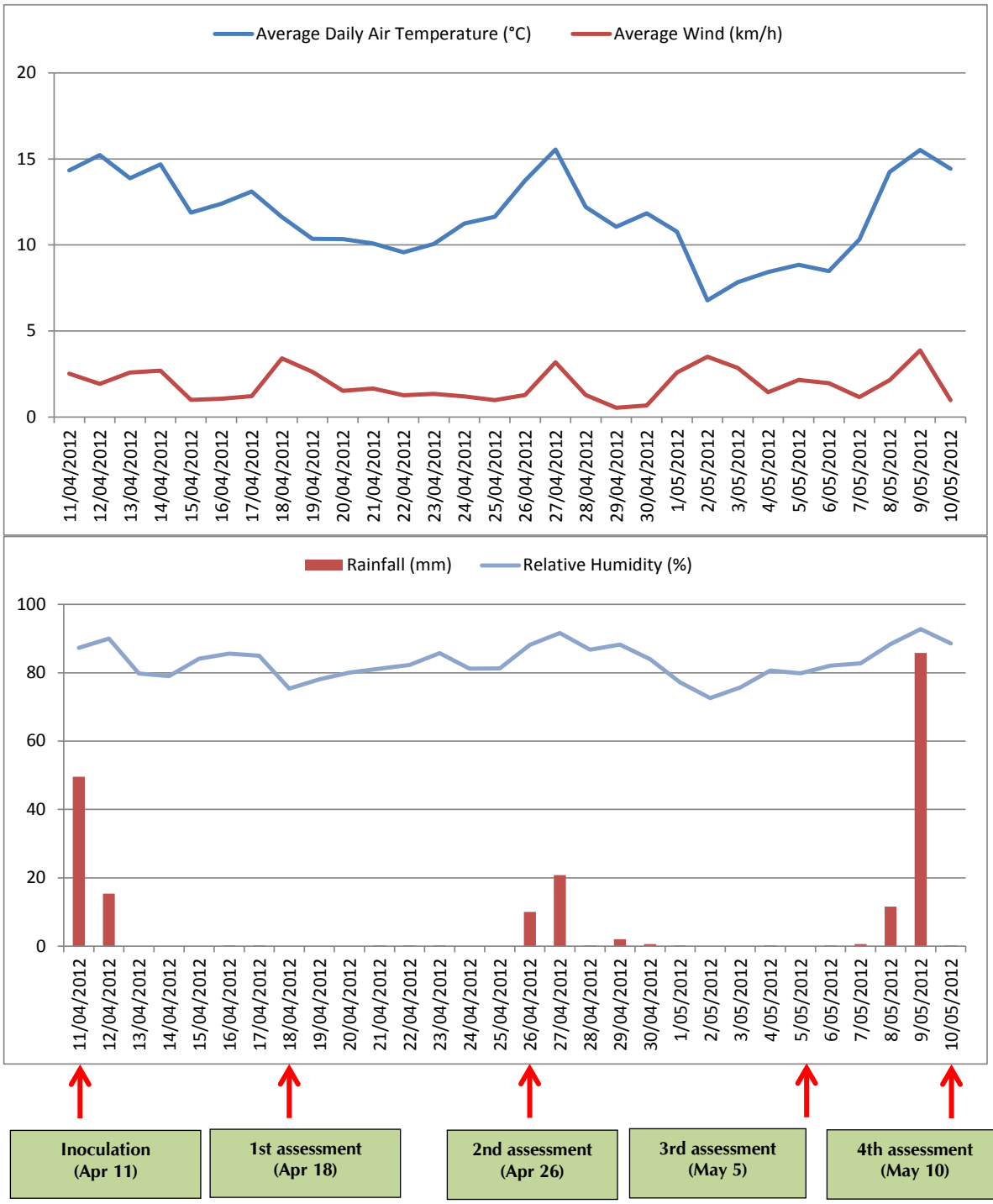
1 = Light symptoms (vein staining, bronzing)

2 = Moderate symptoms (vein staining, 'cross hatching', mild yellowing)

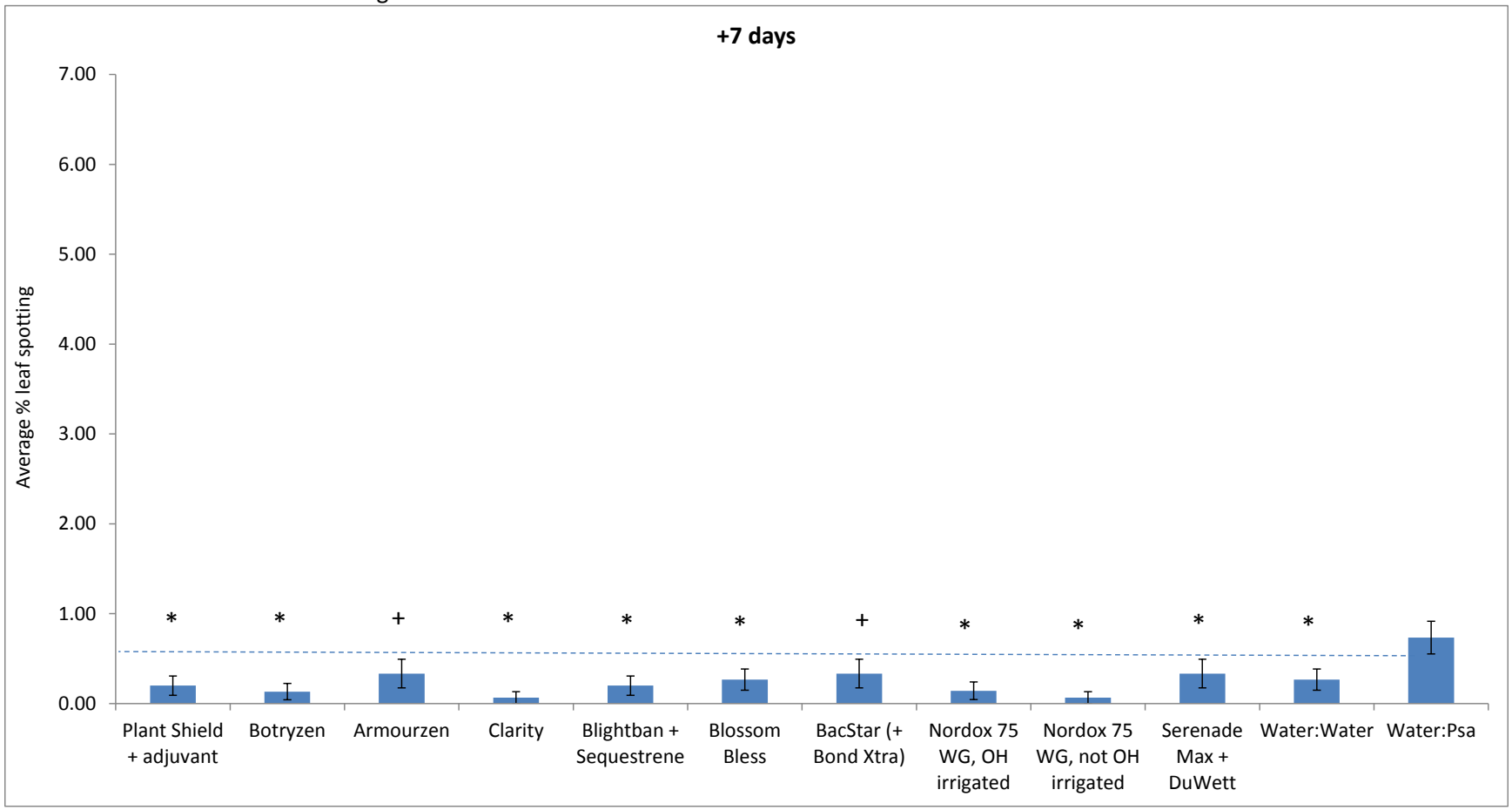
3 = Severe symptoms (vein staining, 'cross hatching', heavy yellowing and leaf breakdown)

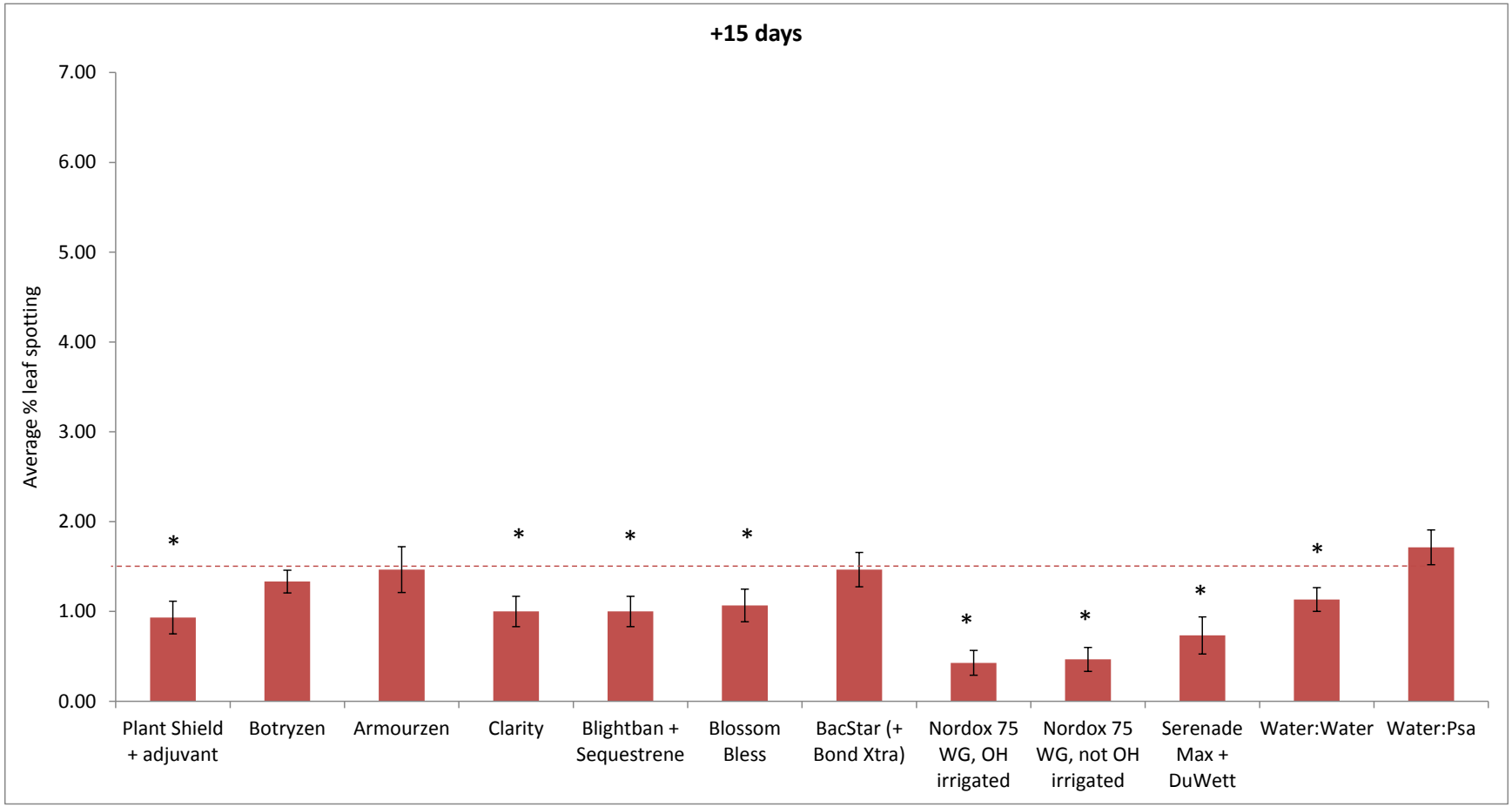
Level	Upperside of leaf	Underside of leaf	Description/details
0			No effects
1			The early symptom of light vein staining can be seen on the underside of the leaf. Vein staining may darken over time.
2			Cross hatched vein staining/darkening. Early signs of yellowing may appear on the topside of the leaf.
3			Severe yellowing and leaf beginning to breakdown. Leaf may deform as a result.

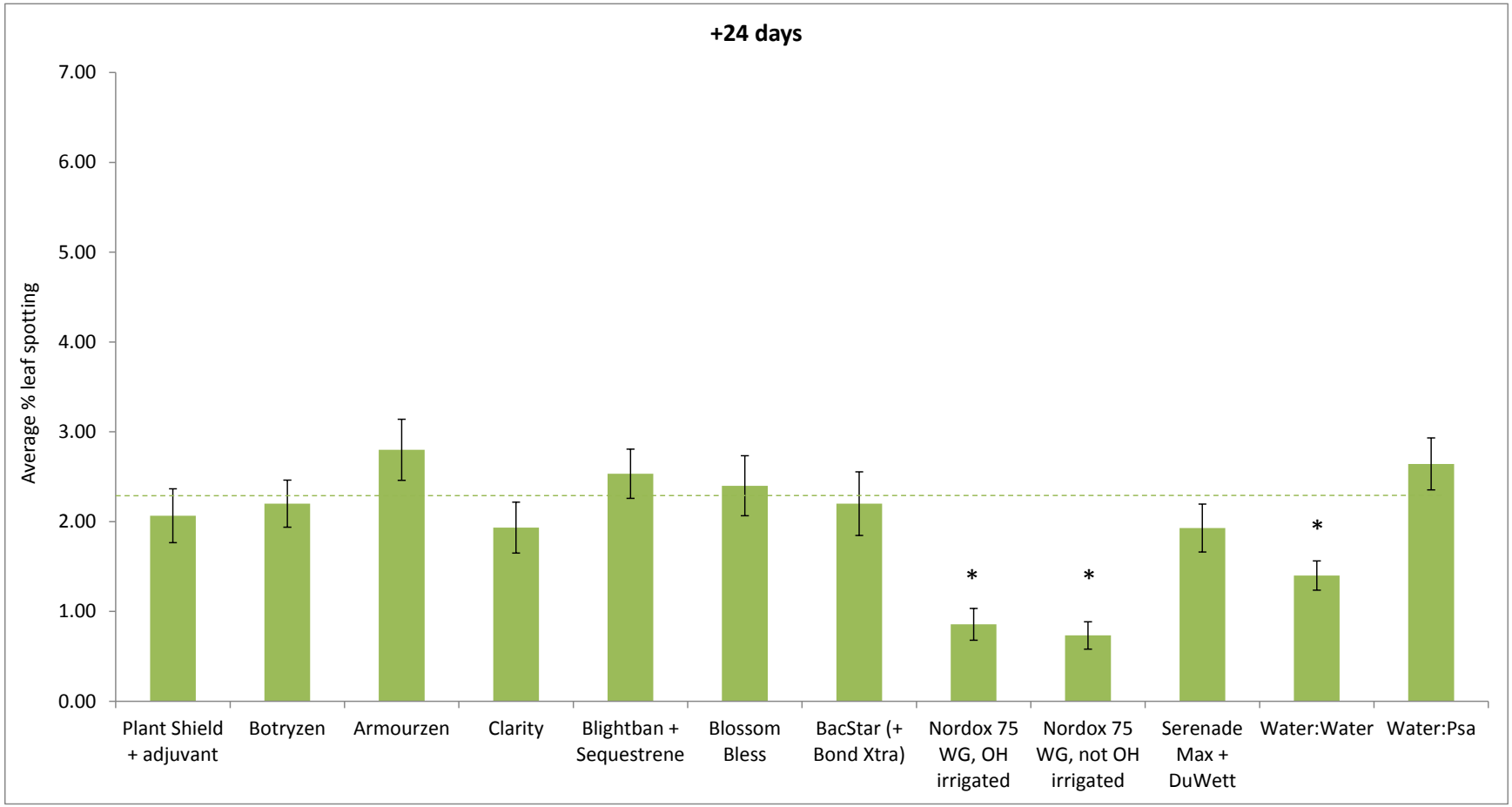
Appendix 2. Weather in the field during KVH/ZESPRI BCA field trial in April/May 2012. Source: Harvest.com (weather station on site).

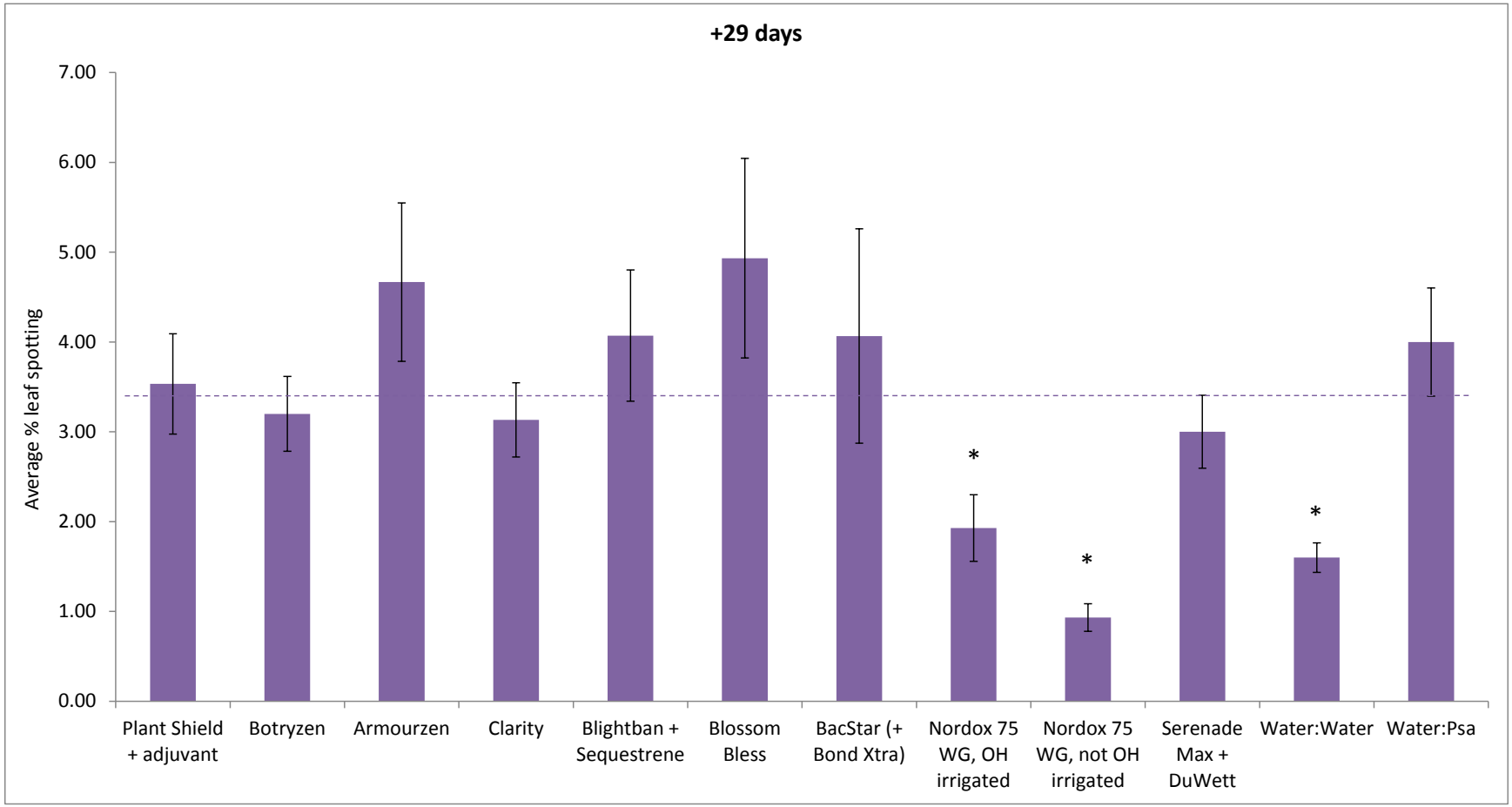


Appendix 3. Average amounts of total leaf area in Hort16A potted plants covered in Psa-V leaf spots per treatment. Separate graphs for each assessment time. Asterisks (*) and plus signs (+) denote values were significantly lower than the Water:Psa control values at the 5% and 10% levels respectively (according to a Wilcoxon test). ns = not significantly different. Error bars are standard error bars (n = 15). "OH" = Overhead. The horizontal dashed lines align with the lower standard error bars for the Water:Psa control at each assessment time.




+15 days
Average % leaf spotting
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Plant Shield + adjuvant
Botryzen
Armourzen
Clarity
Blightban + Sequestrene
Blossom Bless
BacStar (+ Bond Xtra)
Nordox 75 WG, OH irrigated
Nordox 75 WG, not OH irrigated
Serenade Max + DuWett
Water:Water
Water:Psa


+24 days
Average % leaf spotting
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
*
*
*
Plant Shield + adjuvant
Botryzen
Armourzen
Clarity
Blightban + Sequestrene
Blossom Bless
BacStar (+ Bond Xtra)
Nordox 75 WG, OH irrigated
Nordox 75 WG, not OH irrigated
Serenade Max + DuWett
Water:Water
Water:Psa


+29 days
Average % leaf spotting
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
*
*
*
Plant Shield + adjuvant
Botryzen
Armourzen
Clarity
Blightban + Sequestrene
Blossom Bless
BacStar (+ Bond Xtra)
Nordox 75 WG, OH irrigated
Nordox 75 WG, not OH irrigated
Serenade Max + DuWett
Water:Water
Water:Psa