Psa-V Product Testing – Field Trial Report

Trial 9

Copper efficacy - Hort16A

March/April 2012

26 April 2012

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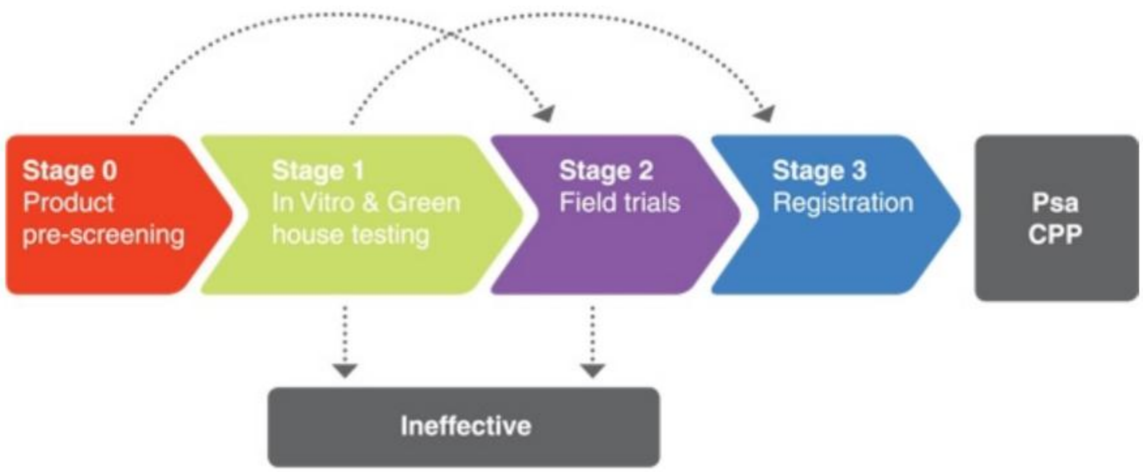
Introduction

ZESPRI, with support from KVH, is coordinating the screening of the effectiveness of a wide range of products to control 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 which compared the efficacy of different copper products and copper rates at controlling Psa-V.



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. At the time of laying out the trial, 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 copper efficacy trial.

Treatments

The four main commercial copper products (Kocide Opti, Nordox 75 WG, Champ DP and Liquicop) were each tested at three different rates i.e. the amount of copper was standardised to 30, 20 and 10 g Cu / 100L. Table 1 lists these treatments in detail. The highest rate of 30 g Cu / 100L was close to the highest summer rate for Kocide Opti, Nordox 75 WG and Champ DP (Table 2). The highest summer rate for Liquicop is around 20 g Cu / 100L.

Each treatment was applied to 14 plants (single plant replicates) the day before inoculation on March 7. Plants were then actively inoculated in the field using 10^6 cfu/mL of Psa-V then laid out randomly in the trial block.

Following inoculation, plants were watered from above for approximately 30 hours by overhead sprinklers to maximise infection.

Table 1. List of treatments applied in the KVH/ZESPRI copper efficacy trial.

Treatment	Product	Active	Copper rate (g Cu / 100L)	Product rate (g or mL / 100L)
1	Kocide Opti	Copper hydroxide	30	100
2			20	67
3			10	33
4	Nordox 75 WG	Copper oxide	30	40
5			20	27
6			10	13
7	Champ DP	Copper hydroxide	30	80
8			20	53
9			10	27
10	Liquicop	Copper ammonium acetate	30	333
11			20	222
12			10	110
13	Water: Water		0	
14	Water: Psa (10^6 cfu/mL)		0	

Table 2. Recommended summer rates for above copper products.

	Amount of active copper	Percentage active copper	Recommended rate (per 100L)	Copper rate (g /100L)
Kocide Opti	300 g/kg	30%	70 - 90 g	21 - 27
Nordox 75 WG	750 g/kg	75%	25 - 37.5 g	19 - 28
Champ DP	375 g/kg	37.5%	50 - 75 g	19 - 28
Liquicop	92.8 g/L	9%	200 mL	19

Treatment application and inoculation

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.

The treatments were applied on 6 March 2012. Inoculation, for which MAF permission was obtained, was undertaken at the trial site the following day inside a temporary spray booth to contain the spread of inoculum. 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.

Assessments

Four assessments were carried out i.e. March 14 (+7 days), March 23 (+16 days) and March 29 (+23 days) and April 5 (+30 days). The percentage of total leaf area per plant covered in Psa-V leaf spotting was visually estimated at each assessment time.

At the third assessment, leaf phytotoxicity was also assessed 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). 6 March.

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). 7 March – 5 April. Appendix 2.

On the day of inoculation and the following three days no significant rain fell. For this reason, plants were watered by overhead sprinklers for approximately 30 hours to create an infection period. The amount of water delivered by the irrigation system to plants is difficult to gauge. Our best estimate is that during this time the plants were exposed to the equivalent of 35 – 50mm rainfall. Following this, on March 11 and 12, significant amounts of rain fell i.e. a total of 60mm. The plants therefore received significant amounts of water directly on the leafy canopy in the first week of the trial.

Significant rain also fell in the second half of the second week of the trial i.e. a total of 100mm. In the third and fourth weeks of the trial, relatively little rain fell i.e. a total of approximately 20mm.

Average daily temperature ranged between 12 and 20 degrees.

Results and interpretation

Leaf spotting

The amount of leaf spotting observed throughout the trial is shown in Figure 2 and Appendix 3. The main findings were:

- Very little leaf spotting was observed at the first assessment, 1 week after inoculation. The average amount of leaf area covered per plant was 0.13% with the highest treatment average being 0.5% for the water:Psa control. This first assessment result likely reflects that there had not been enough time since inoculation for symptom expression rather than any treatment effect per se.
- From the second to fourth assessments, 16, 23 and 30 days after inoculation respectively, there were inverse and linear relationships between the amounts of copper applied and the amounts of leaf spotting for Nordox, Champ and Liquicop i.e. as copper rate increased, leaf spotting decreased, and vice versa. Figure 3 shows that on average across the four products the rate of leaf spot development slowed as the rate of copper applied increased. Leaf spot development was fastest in the water:Psa treatment.
- By the end of the trial, with the exception of Champ DP and Liquicop at 10 g Cu / 100L, all the copper treatments had reduced leaf spotting significantly.
- Unlike the other products there was no clear relationship between the level of leaf spotting and copper rate for Kocide Opti. Bacterial control from copper sprays is achieved from the release of cupric ions. These results indicate that under the conditions of this trial the release of cupric ions did not differ between copper rates as much as it did for other products.
- A low level of leaf spotting was observed in the water:water treatment i.e. approximately 1% by the end of the trial. This suggests that the much higher leaf spotting observed in the other treatments was a consequence of the Psa that was actively applied.
- No treatment completely prevented the development of Psa symptoms; on average, across assessments 2 – 4, leaf spotting across the treatments was 55% of the level observed in the unprotected plants sprayed with Psa-V (i.e. the water:Psa controls). It is not clear why the copper treatments did not reduce leaf spotting more than they did. Some hypotheses which have been suggested to explain this are as follows:
    - When each treatment was applied, 100% coverage of plants surfaces was not achieved with each product. Therefore 100% coverage with cupric ions was not achieved. 100% coverage of all plant surfaces with cupric ions is unlikely in an orchard situation.
    - The copper treatments were applied the day before inoculation. More time may be required for optimal cupric ion release.
    - Hort16A is a highly susceptible variety and while good amounts of protection may have been provided, this was not sufficient to suppress symptoms more. This was further demonstrated by a number of the plants exhibiting shoot dieback.
    - The inoculum load of 10^6 cfu/mL was too high for optimum protection in which case the coppers would be expected to provide greater protection at lower levels of inoculum. This level of inoculum was used to ensure leaf spotting developed.

- To create an infection period, plants were overhead watered for approximately 30 hours and it is estimated that during this period the plants were exposed to the equivalent of 35 – 50mm rainfall. Also, significant amounts of rain fell following this in the first week of the trial i.e. a total of 60mm. This overhead water may have affected product efficacy.

Secondary symptoms

Secondary symptoms were observed in this trial with about 5% of the vines having at least one shoot with dieback (Figure 4). No relationship was apparent between the products used and/or rates and the expression of secondary symptoms. There were no statistical differences between treatments. It is possible that plants suffered some damage during transportation and handling after treatment and before or during inoculation. This would have occurred randomly and allowed unprotected wounds to become infected at inoculation.

Phytotoxicity

Across all four copper products, a positive relationship was observed between copper rate and the level of phytotoxicity i.e. as the copper rate increased so did the level of phytotoxicity (Figure 5). With the exception of Liquicop at the two highest rates of 20 and 30 g Cu/100L, the level of phytotoxicity was on average below what is regarded as light phytotoxicity i.e. brown veins on the under-surfaces of leaves. This level is not expected to significantly impact on production.

In a recent similar trial conducted on Hort16A potted plants in Rotorua using the same copper products and application rates but no Psa inoculation, much less leaf phytotoxicity was observed (Figure 6). The reason for the difference in phytotoxicity is not known. A key difference between the trials was that the plants in Rotorua were not overhead irrigated following product application whereas the plants in the field trial were. One hypothesis is that the overhead irrigation and rain in the first week of the trial released more cupric ions from the products onto the plants thereby causing greater phytotoxicity.

The greater phytotoxicity associated with Liquicop at higher rates highlights potential formulation differences. Some thoughts on why Liquicop caused more phytotoxicity are provided below directly from the supplier (Glynn Douglas, Hortigro):

- Liquicop produces much smaller particle sizes of copper hydroxide on the plant surface once it dries, compared to other copper formulations, with generally an average size of 0.3 micrometers. Therefore the smaller particle size increases the surface area for cupric ions to dissolve off, so comparatively there are more cupric ions released than for the other formulations.
- For an equivalent amount of copper applied per 100L, the Liquicop is releasing a larger amount of cupric ions and so there is potential for a greater uptake by the plant.

The potentially higher phytotoxicity associated with Liquicop is the reason it is recommended to be applied at rates which deposit a lower amount of actual copper compared to most other copper products.

Summary

This trial has again demonstrated a reduction in Psa-V leaf spotting through the use of copper products. Generally, the higher the copper rate the lower the amount of leaf spotting (with the exception of Kocide Opti).

While leaf spotting was reduced particularly at higher rates it is not clear why leaf spotting was not reduced more than it was. The inability to achieve 100% coverage of plant surfaces with active cupric ions, a high inoculum load, significant amounts of overhead water in the first week, physical damage to plants during handling, and the inherent high susceptibility of Hort16A may have all contributed to this.

Light phytotoxicity (brown veins underneath leaves) was observed and increased as the copper rate increased. Liquicop at the two highest rates caused more phytotoxicity than other products at the same rates demonstrating the potential differences that can arise between formulations.

These results highlight the challenges of utilising coppers to control Psa-V. Increasing rates will provide a higher level of control but increases the risk of phytotoxicity. These results relate to a single application. It is likely that repeat applications, as required in a commercial situation to provide cover of emerging growth, will see a build-up of leaf copper levels of older leaves providing improved control but also increasing the risk of leaf or fruit damage.

Future trials are required to better understand the impact of repeat applications of copper products and to clarify the impact of overhead water on the levels of infection, efficacy and phytotoxicity.

Figure 2. Average amounts of total leaf area in Hort16A potted plants covered in Psa-V leaf spots. Appendix 3 displays the same results but with each assessment on a separate graph.

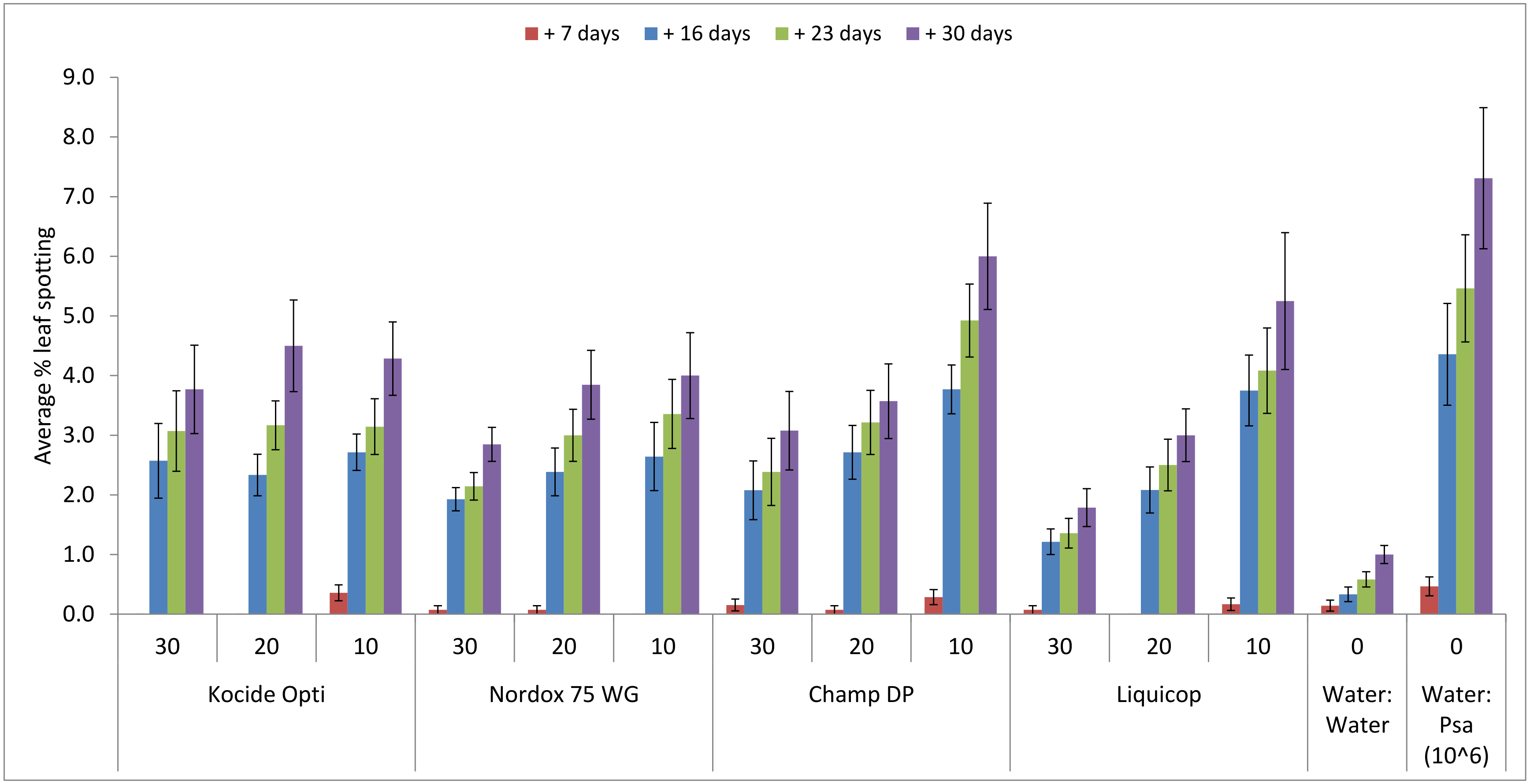


Figure 3. Average amounts of total leaf area in Hort16A potted plants covered in Psa-V leaf spots for each level of copper.

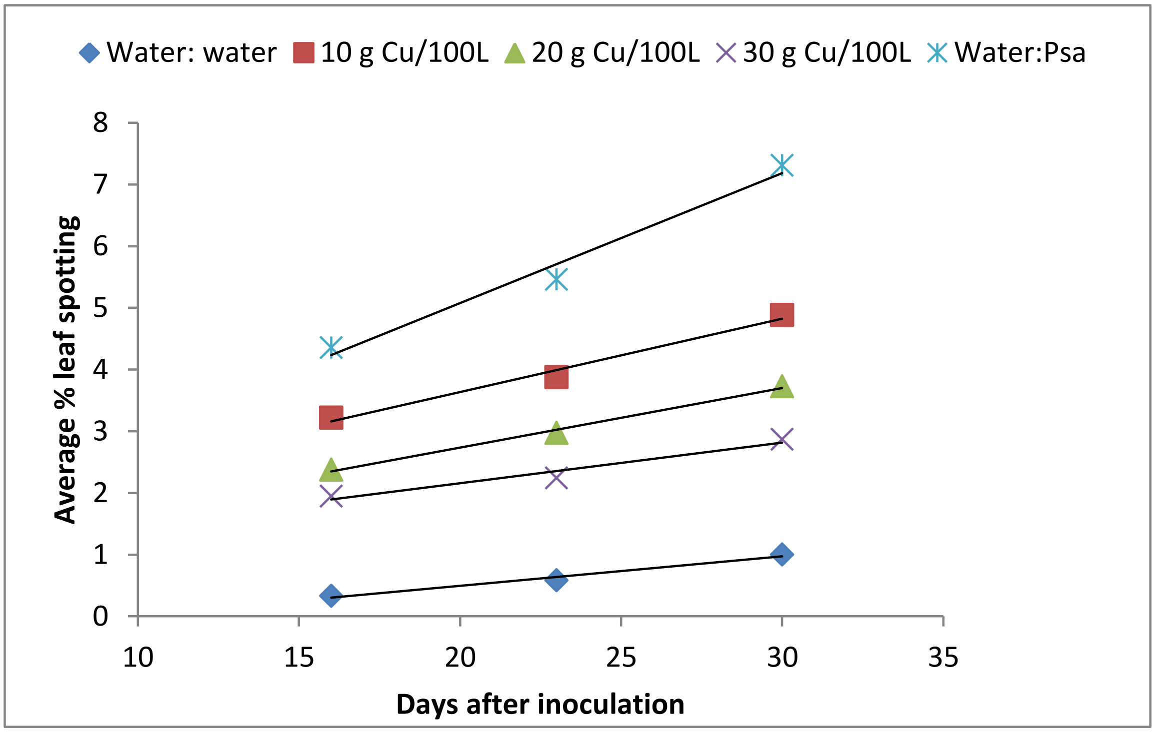


Figure 4. Proportion of Hort16A potted plants which had at least one shoot with dieback. According to a Wilcoxon test, none of the values were significantly different from each other.

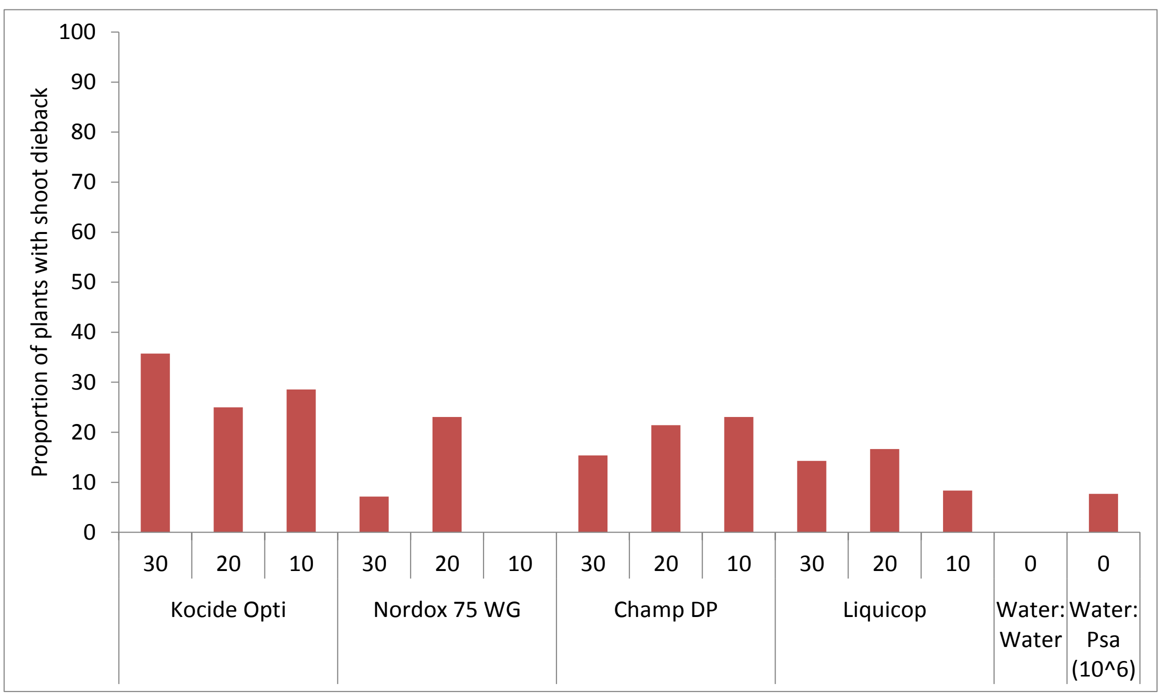
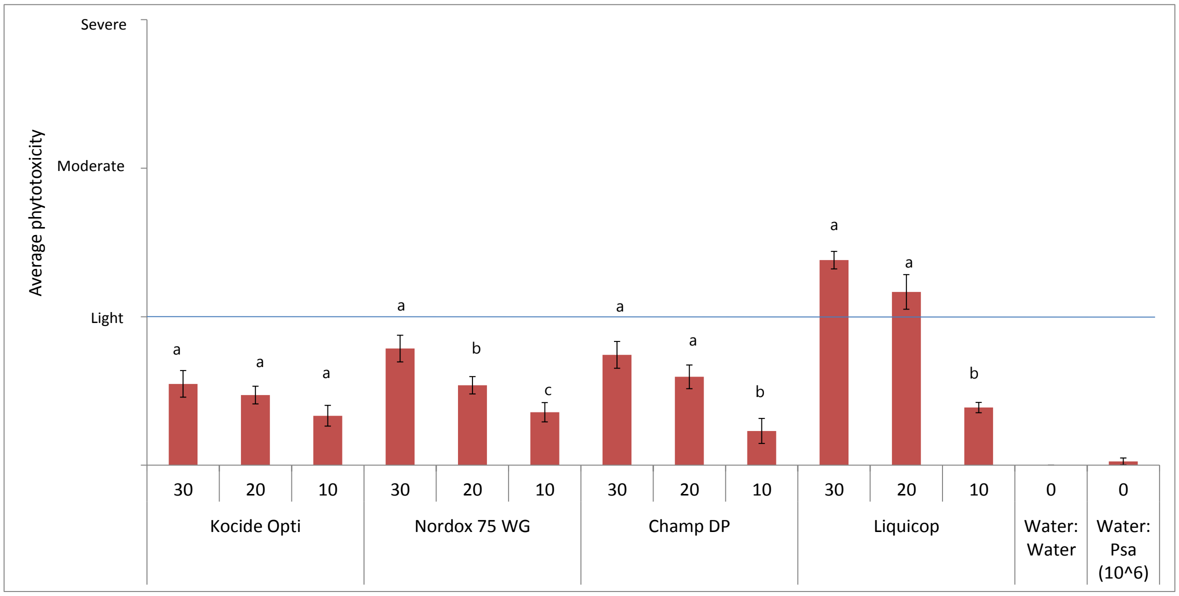
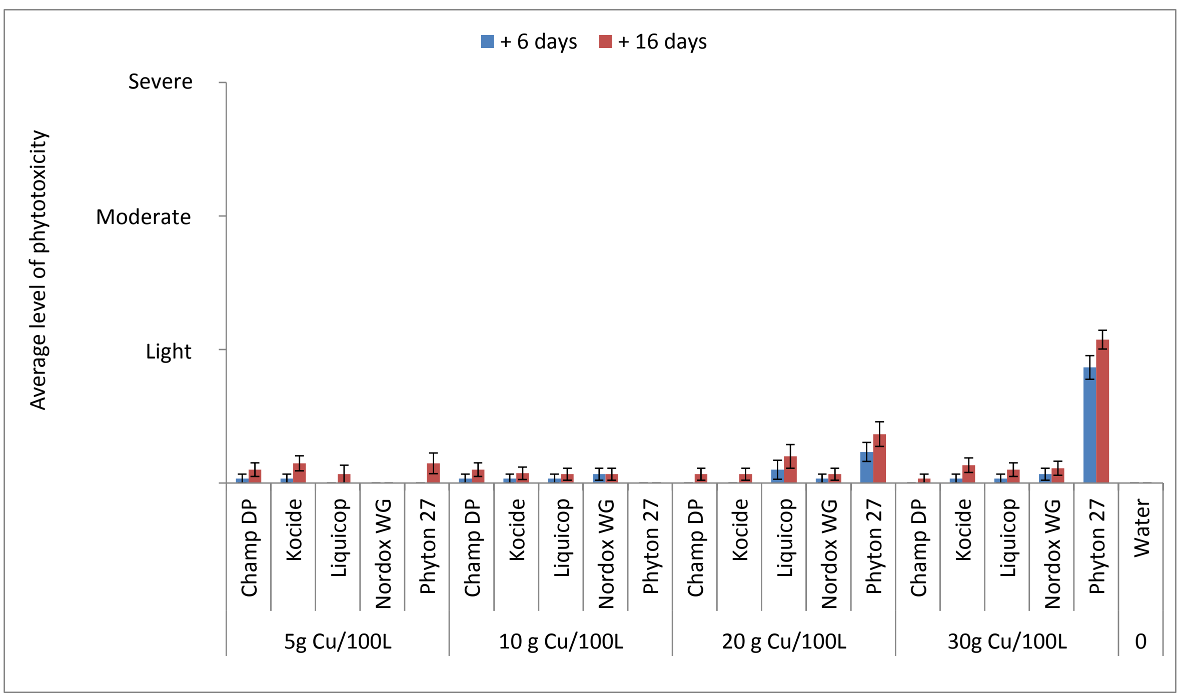


Figure 5. Average amounts of leaf phytotoxicity observed in copper efficacy trial conducted on Hort16A potted plants in Te Puke in Mar/Apr 2012 (n = 14).



For each product, values with the same letter are not significantly different from each other at the 5% level according to a Wilcoxon test. Error bars are standard error bars (n = 14).

Figure 6. Average amounts of leaf phytotoxicity observed in copper phytotoxicity trial conducted on Hort16A potted plants in Rotorua in Feb/Mar 2012 (n = 10).



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

0 = No symptoms

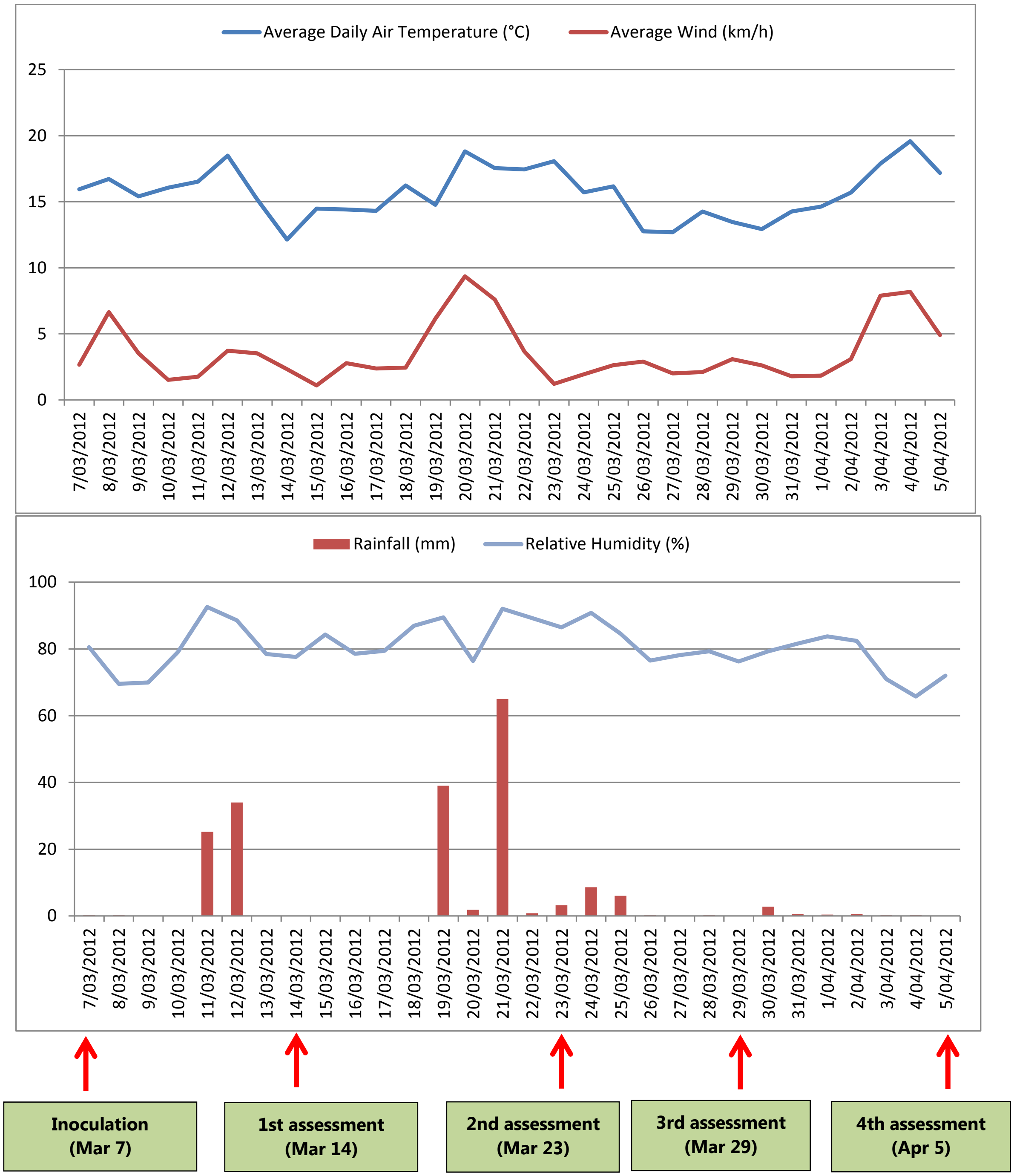
1 = Light symptoms (vein staining, bronzing)

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

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

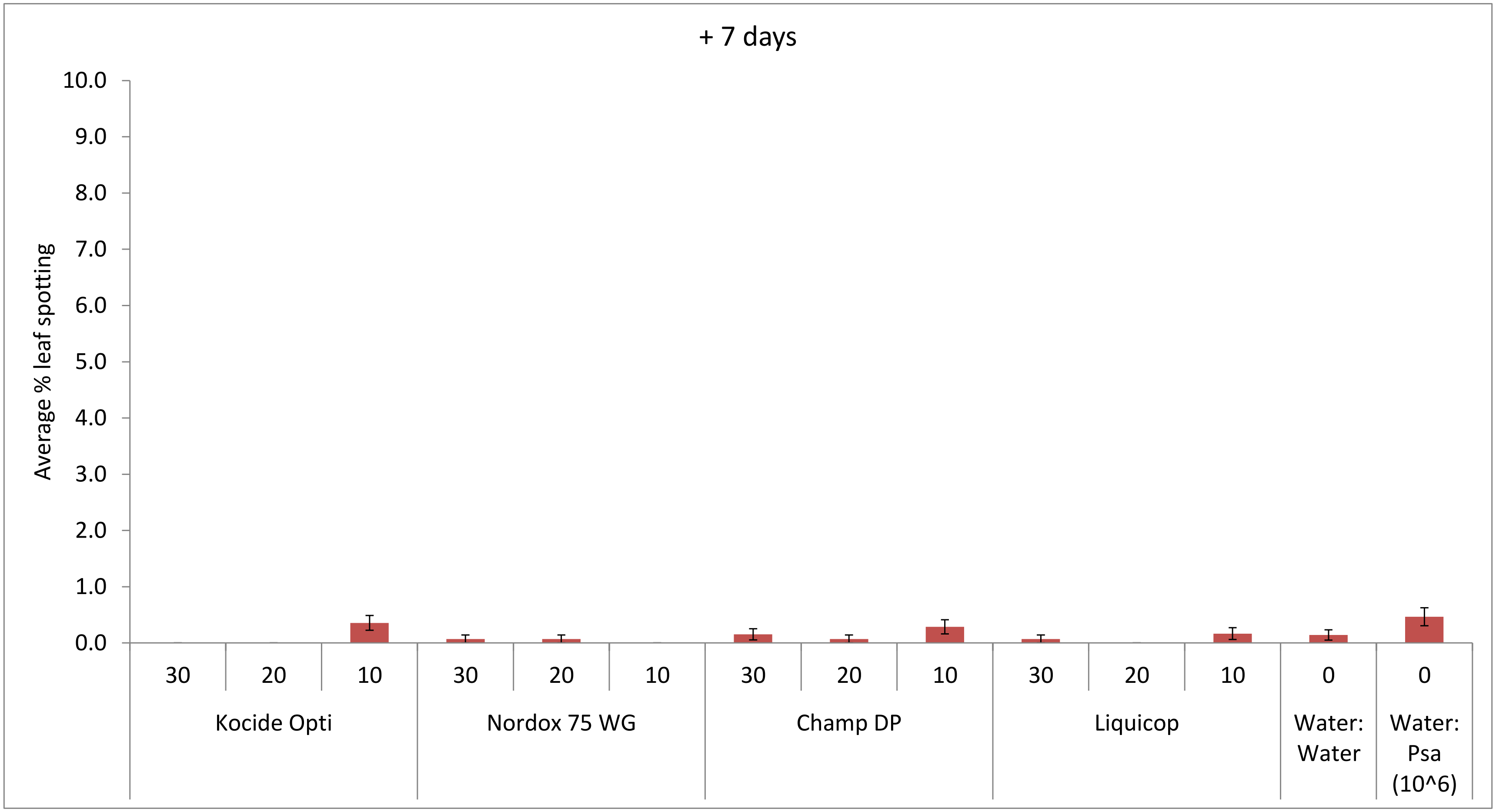
Level	Upperside of leaf	Underside of leaf	Description/details
0			No effects
1			The early symptom of light vein staining can be seen on the underside of the leaf. Vein staining may darken over time.
2			Cross hatched vein staining/darkening. Early signs of yellowing may appear on the topside of the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

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

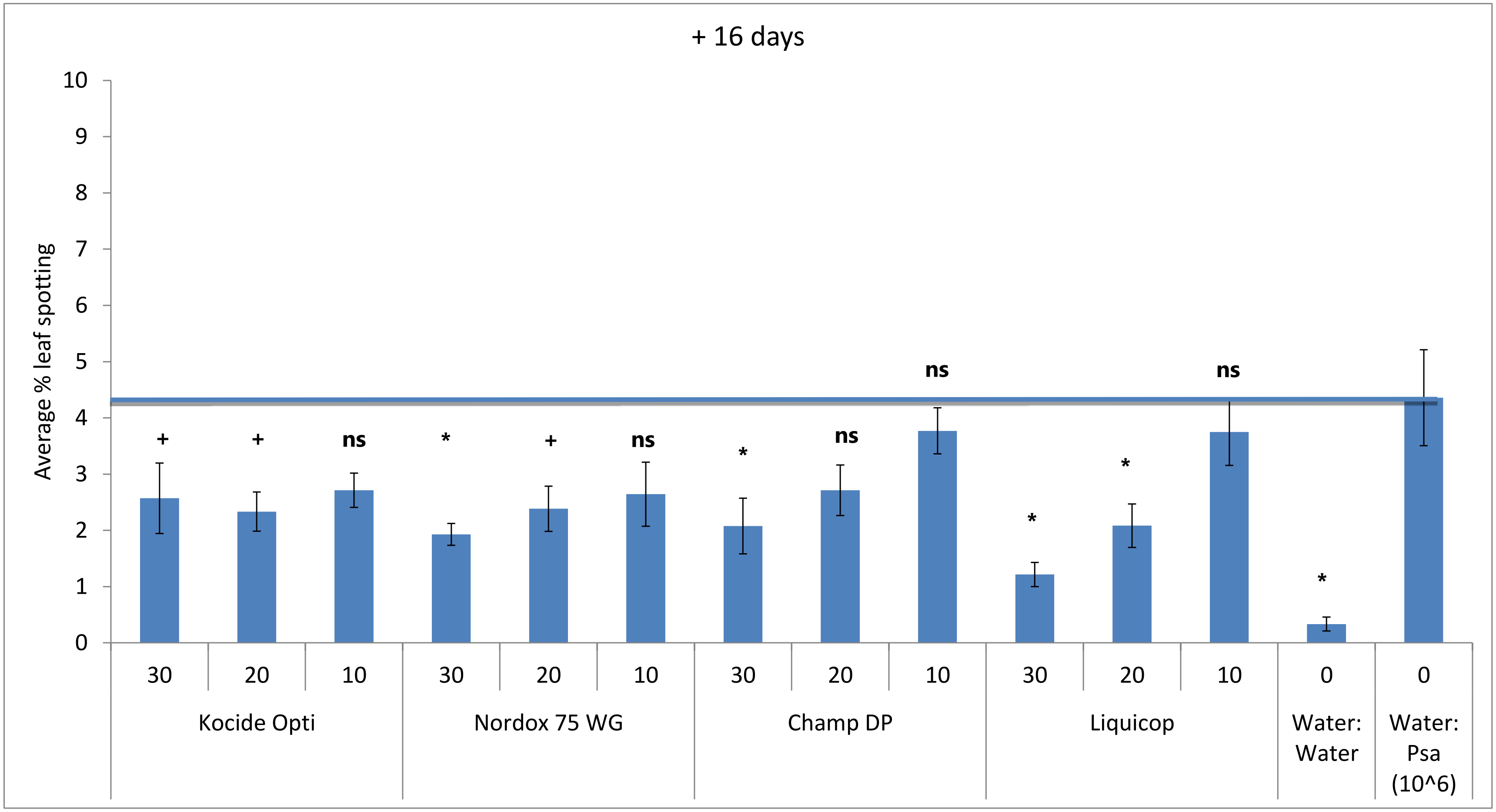


Appendix 3. Average amounts of total leaf area in Hort16A potted plants covered in Psa-V leaf spots per treatment. Separate graphs for each assessment.

7 days after inoculation. No statistics provided given the very low level of leaf spotting.

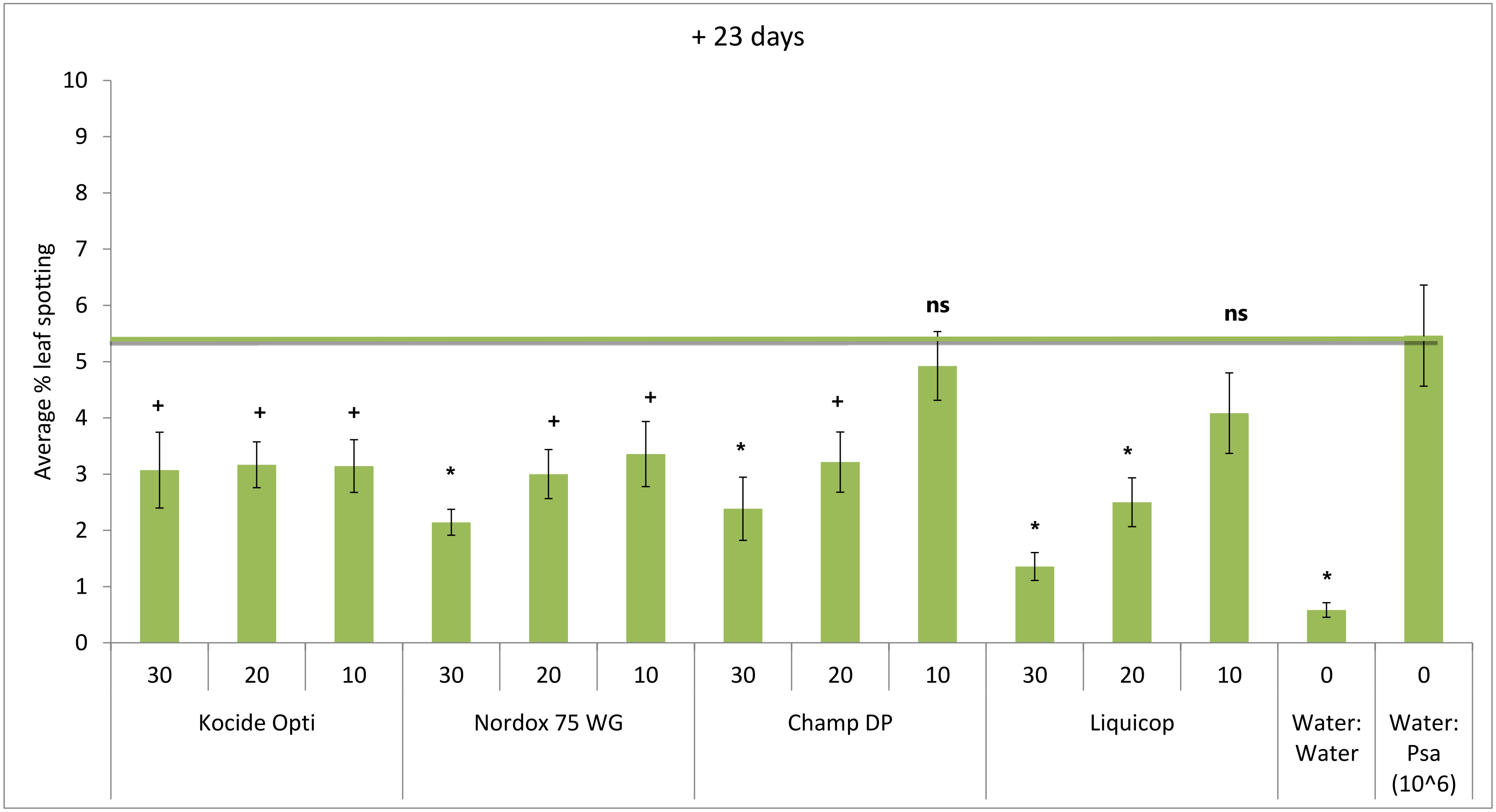


16 days after inoculation.



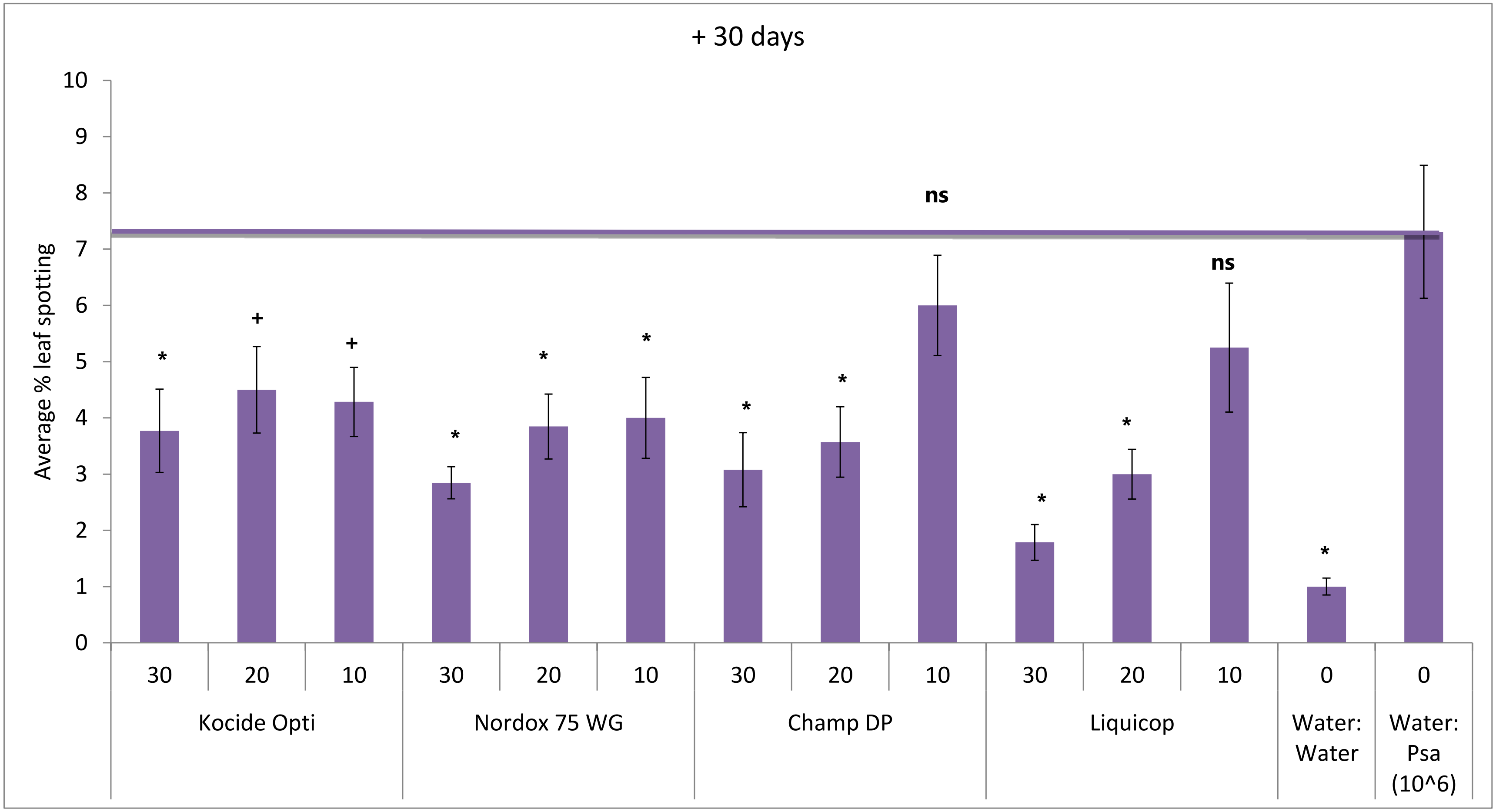
At each assessment time, asterisks (*) and plus signs (+) denote values were significantly lower than the Water:Psa control values at the 5% and 10% levels respectively (according to a Wilcoxon test). ns = not significantly different. Error bars are standard error bars (n = 14).

23 days after inoculation.



At 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 = 14).

30 days after inoculation.



At 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 = 14).