Psa-V Product Testing – Field Trial Report

Trials 1 & 2

Preliminary inoculation trials on Hort16A and Hayward

November/December 2011

26 April 2012

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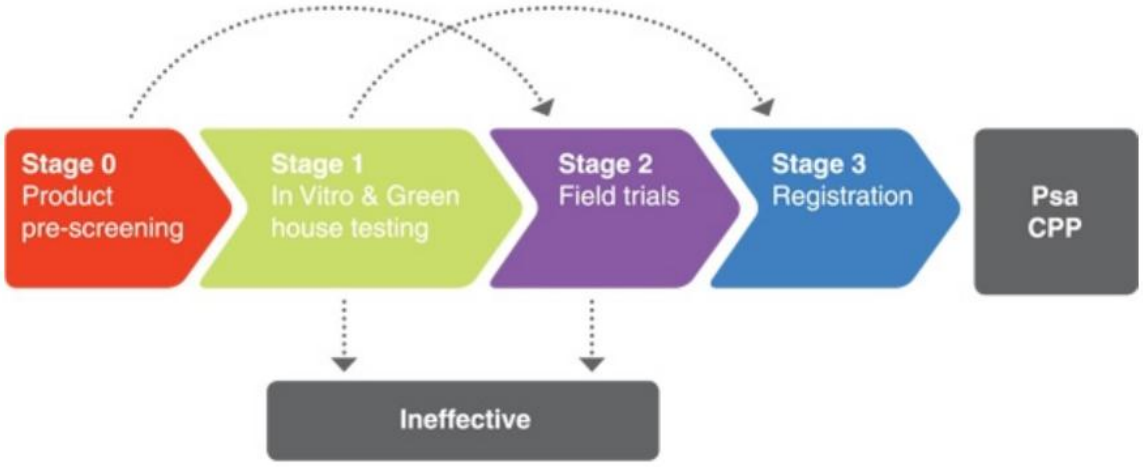
Introduction

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 two preliminary trials which were conducted on Hort16A and Hayward to identify an appropriate level of Psa-V to inoculate plants with in subsequent product testing trials.



Methodology

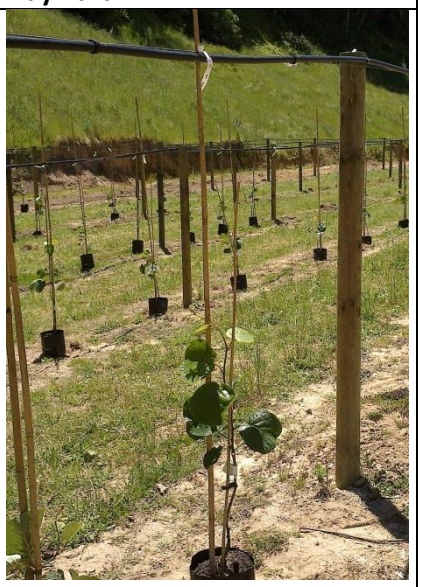
Plants

In this trial, female Hort16A and Hayward plants were used. The Hort16A were 2 year old scion material on 1 year old Bruno rootstocks, sourced from Pyes Pa (Tauranga) in September 2011. These were kept at a greenhouse facility in Rotorua to advance growth until needed. The plants were believed to be Psa-free at the start of the trial as no symptoms were observed previously. Although not a guarantee that the plants were clean, Psa-V was not detected in leaf samples taken in October 2011. At the time of laying out the trial, the plants were approximately 2m in height with a significant number of leaves

The Hayward plants used were much smaller as they were grafted onto 1 year old Bruno rootstocks in spring 2011, in Kerikeri. Like the Hort16A, these 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 1m in height with a small number of leaves.

Examples of the plants used are shown in Figure 1.

Figure 1. Example of a Hort16A and Hayward on Bruno rootstocks used in KVH/ZESPRI preliminary inoculation trials.

Hort16A	Hayward
	

Treatments and inoculation

Three different levels of Psa-V (10^5, 10^7 and 10^9 cfu/mL) were each applied to 20 plants per variety. Water was also applied to 20 plants per variety as a control. This was carried out at the KVH/ZESPRI trial site in Te Puke inside a temporary spray booth to contain the spread of inoculum. Plant and Food Research staff from Ruakura provided fresh inoculum on the day and sprayed the inoculum 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. Samples of the Psa-V inoculum used were collected throughout the day to check the correct levels were applied. Subsequent lab analyses confirmed this occurred.

The Hort16A were treated on November 15 and the Hayward on November 25 (2011).

MAF permission was obtained for inoculating with Psa-V at the KVH/ZESPRI trial site.

Overhead watering

At the time of conducting these initial trials, the irrigation system was still in the process of being set up. For this reason, only overhead drippers had been installed. These were used twice daily, morning and late afternoon, for 2 hours each time. The leaves of each plant were therefore wetted for four hours each day throughout the course of the trial. 4.5 – 5 L of water per hour was delivered each time to each plant.

Assessments

In the Hort16A trials, four assessments were made on November 25, December 1, December 8 and December 14 i.e. 10, 16, 23 and 29 days after inoculation respectively. In the Hayward, two assessments were made on December 6 and 14 i.e. 11 and 19 days after inoculation respectively. The percentage of total leaf area per plant covered in Psa-V leaf spotting was visually estimated each time.

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, particularly rainfall, during field trials need consideration when interpreting results hence a summary is presented here.

Appendix 1 shows that in the first two weeks of the Hort16A trial, little or no rain fell. In the third and fourth weeks, which correspond with the Hayward trial, significant amounts of rain fell. Specifically:

- Third week of Hort16A trial and first week of Hayward trial: approximately 80mm fell over a 5 day period between December 2 and 6 with approximately 40mm falling on December 4.

- Fourth week of Hort16A trial and second week of Hayward trial: approximately 55mm fell over a 3 day period between December 12 and 14 with approximately 30mm falling on December 13 (just prior to the last assessments).

Throughout the trial, average daily temperature increased very slightly while relative humidity noticeably increased averaging 70% in the first week of the Hort16A trial and close to 90% in the last week.

Note, in addition to the natural rainfall, the plants were watered twice a day with overhead drippers.

Results and interpretation

As expected, in both varieties, the level of leaf spotting increased over time across all treatments (Figure 2). As the inoculum level increased so did the amount of leaf spotting with the differences overall being significantly different.

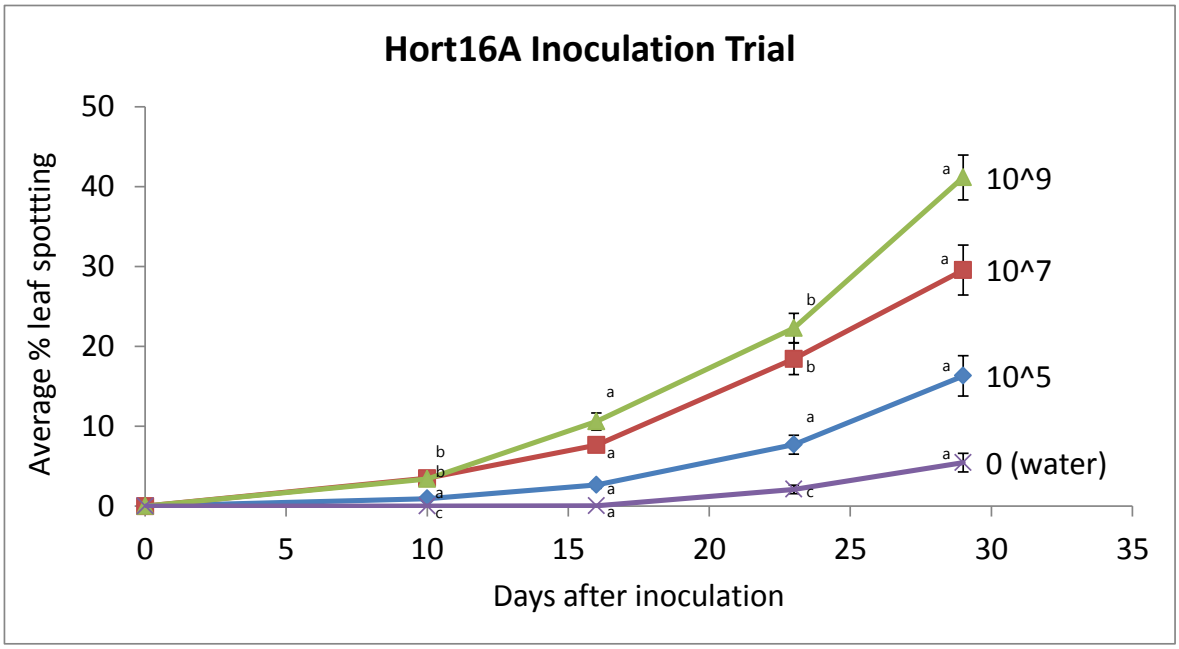
The leaf spotting observed across the water control plants showed that the active inoculation with Psa-V was successful in causing greater leaf spotting and that the natural inoculum level was much lower than 10^5 cfu/mL.

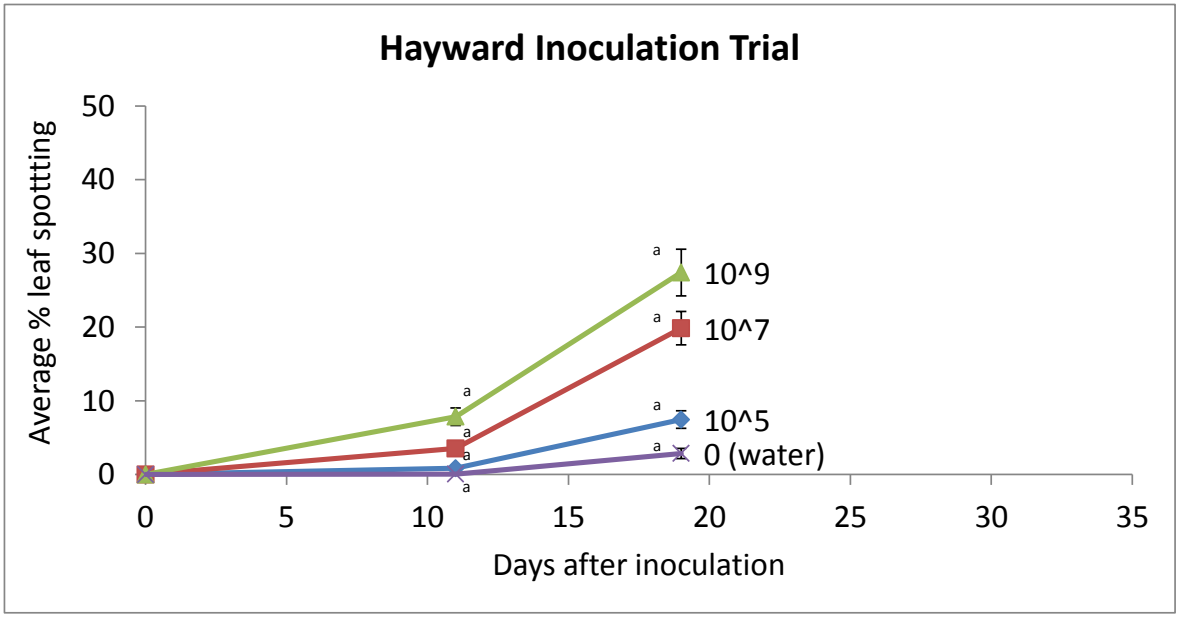
Although caution is advised when comparing different varieties and trials, the rate of leaf spot development was slightly faster in the Hayward. Significantly amounts of rain fell during the first two weeks of this trial compared to the first two weeks of the Hort16A trial which may have contributed to this.

Conclusion

Based on the findings of these trials, it is recommended that the level of inoculum used in future product testing field trials should be no higher than 10^7 cfu/mL as this should provide sufficient amounts of leaf spotting to identify any treatment differences. It is important to keep the inoculum level as low as possible so as not to present products with a challenge which is too high for them to be effective. This particularly applies to biological products. This level should be reviewed over time as the level of infection may change as plants grow and environmental conditions change.

Figure 2. Average percentage of total leaf area covered in leaf spots in KVH/ZESPRI preliminary inoculation trials.





At each assessment time, 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 = 20).

Appendix 1. Weather in the field during KVH/ZESPRI preliminary inoculation field trials in November/December 2011. Source: Harvest.com (weather station on site).