



2012/13 Potted Plant Field Trial Report

Trials 10 & 11

Yeast and *Trichoderma* Mixes on Hayward and Gold3

January – May 2013



November 2013

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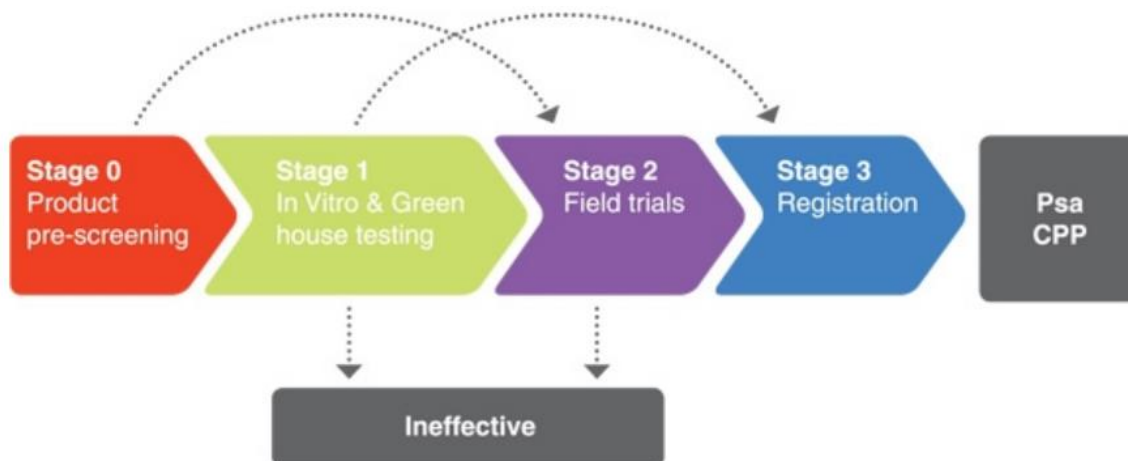
Introduction

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 trials conducted from January to May 2013 on Hayward and Gold3 potted plants in which various yeast mix (provided by Plant & Food Research) and *Trichoderma* (provided by the Bioprotection Research Centre, Lincoln University) treatments were tested.



Objective(s)

To test the efficacy of various yeast mix (provided by Plant & Food Research) and *Trichoderma* (provided by the Bioprotection Research Centre) treatments.

Methodology

Plants

In this trial, Gold3 and 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 1.5 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 Yeast Mix and *Trichoderma* treatments. Also shown is the overhead misting system used to keep plants continuously wet for 48 hours following inoculation.



Treatments

These are listed in Table 1 and Table 2. Various yeast mix (YM2), *Trichoderma* mix (TriMix1) and Actigard® treatments were applied. The number of Hayward plants available for this trial was limited to 50 and so fewer treatments were applied relative to the Gold3.

The Actigard was applied at a rate of 20 g/100 L in each treatment. The application details for the *Trichoderma* and yeast mixes are confidential to Plant and Food Research and the Bioprotection Research Centre, Lincoln University.

Table 1. Hayward treatments.

		19-Dec	18 & 24 Jan	18-Jan	25-Jan
TRT No.	No. of reps	<i>Trichoderma</i> root drench (KeriKeri)	Foliar applications of yeast mix (TPRO)	Foliar applications of elicitor (TRPO)	Inoculation with Psa-V at Zespri/KVH trial site (Te Puke)
1	10	Nil	Nil	Nil	Psa
2	10	Nil	Nil	Nil	Psa
3	10	Nil	FC	Nil	Psa
4	10	TriMix1	YM2-granules	Actigard	Psa
5	10	Nil	YM2-granules	Nil	No Psa

TriMix1 = *Trichoderma* mix; YM = Yeast Mix; FC = biological control agent (BCA) additive
 TPRO = Te Puke Research Orchard.

Table 2. Gold3 treatments.

		19-Dec	18 & 24 Jan	18-Jan	25-Jan
TRT No.	No. of reps	<i>Trichoderma</i> root drench (Keri-Keri)	Foliar applications of yeast mix (TPRO)	Foliar applications of elicitor (TRPO)	Inoculation with Psa-V at Zespri/KVH trial site (Te Puke)
1	9	Nil	Nil	Nil	Psa
2	9	Nil	Nil	Nil	Psa
3	9	Nil	FC	Nil	Psa
4	9	TriMix1	Nil	Nil	Psa
5	9	TriMix1	Nil	Actigard	Psa
6	9	TriMix1	YM2-granules	Nil	Psa
7	9	TriMix1	YM2-granules	Actigard	Psa
8	9	Nil	YM2-granules	Nil	Psa
9	9	Nil	YM2 (fermented)	Nil	Psa
10	9	Nil	YM2-granules	Actigard-foliar	Psa
11	9	Nil	Nil	Actigard-foliar	Psa
12	9	Nil	Nil	Actigard-root	Psa
13	10	TriMix1	YM2-granules	Actigard-root	No Psa

TriMix1 = *Trichoderma* mix; YM = Yeast Mix; FC = Biological control agent (BCA) additive
 TPRO = Te Puke Research Orchard.

Treatment application

The TriMix1 treatments were applied in KeriKeri where the plants were sourced from. TriMix1 was sent to staff at the nursery who applied the treatments by drenching the soil in each pot. The plants were then moved to Plant & Food Research in Te Puke for the subsequent foliar treatment applications. These 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/KH trial site in Te Puke on 25 January 2012. This occurred inside a temporary spray booth to contain the spread of inoculum. One or two pallets of plants were inoculated in the spray booth at a time. On each pallet, one plant from each treatment was included to account for any variation in inoculation that may have occurred during the day.

Plant and Food Research staff from Te Puke provided fresh inoculum on the day. The target concentration was 10^8 cfu/mL; subsequently the concentration used was measured to be 10^7 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. This lower leaf environment, where the stomata are, is more conducive to Psa infection. Inoculation occurred between 10 am and 1 pm.

Inoculation Error

On the day of inoculation there was an accidental mix-up in the inoculation of some treatments. Specifically:

Hayward trial:

- treatment 5 was not inoculated with Psa when it should have been
- treatment 1 was inoculated with Psa, but should not have been; effectively this meant there were two untreated Psa controls and no water control.

Gold3 trial:

- treatment 13 was not inoculated with Psa when it should have been
- treatment 1 was inoculated with Psa, but should not have been; effectively this meant there were two untreated Psa controls and no water control.

Initial wetting of plants

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 12 pm on January 25 to 12 pm on January 27. 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 no rain fell. On the day of inoculation, the average daily temperature was 18°C, 14°C the following day and 16°C the day after that. Average relative humidity was approximately 75% was during this period.

Assessments

The levels of leaf spotting and secondary symptoms were visually estimated and recorded from 16 days after inoculation then at approximately weekly intervals until 42 days after inoculation. A final assessment was conducted 70 days after inoculation on April 5 2013.

Each time, the amount of total leaf area covered in spots was estimated. The parts of the plants that were mature at the time of inoculation were assessed separately from the parts that were expanding.

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). 18 – 25 January.* Appendix 1.

No rain fell during the period that treatments were applied. Maximum daily temperatures ranged between 21 and 30°C which minimum daily temperatures ranged between 6 and 17°C.

- ii) *Weather following inoculation (based on the installed Harvest.com weather station). 25 January – 5 April.*

iv) **Appendix 2.**

As discussed above no rain fell during the initial wetting period. Rain did not fall until 11 days after inoculation on Feb 4 & 5 when approximately 60 mm fell. The next significant weather event occurred on March 17 when approximately 100 mm of rain fell.

Average daily relative humidity ranged between 75% and 95% while average daily temperature ranged between 12 and 20°C.

Results and interpretation

Hayward

Overall levels of leaf spotting were regarded as good i.e. 3 weeks after inoculation the average levels for the untreated but inoculated Psa controls were about 5% and 20% for the mature and expanding leaves respectively (Figure 2 and 3).

There was an indication that the combination treatment reduced leaf spotting. Unfortunately, the YM only treatment was not inoculated which explains the little or no leaf spotting associated with the treatment. There was no strong evidence that the FC alone treatment reduced leaf spotting.

Gold3

Overall leaf spotting was regarded as low in this trial. 28 days after inoculation the average levels for the untreated but inoculated Psa controls were 2.5% and 3.5% for the mature and expanding leaves respectively. Generally, 4 to 5% leaf spotting is regarded as a minimum level that confident conclusions can be based on.

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

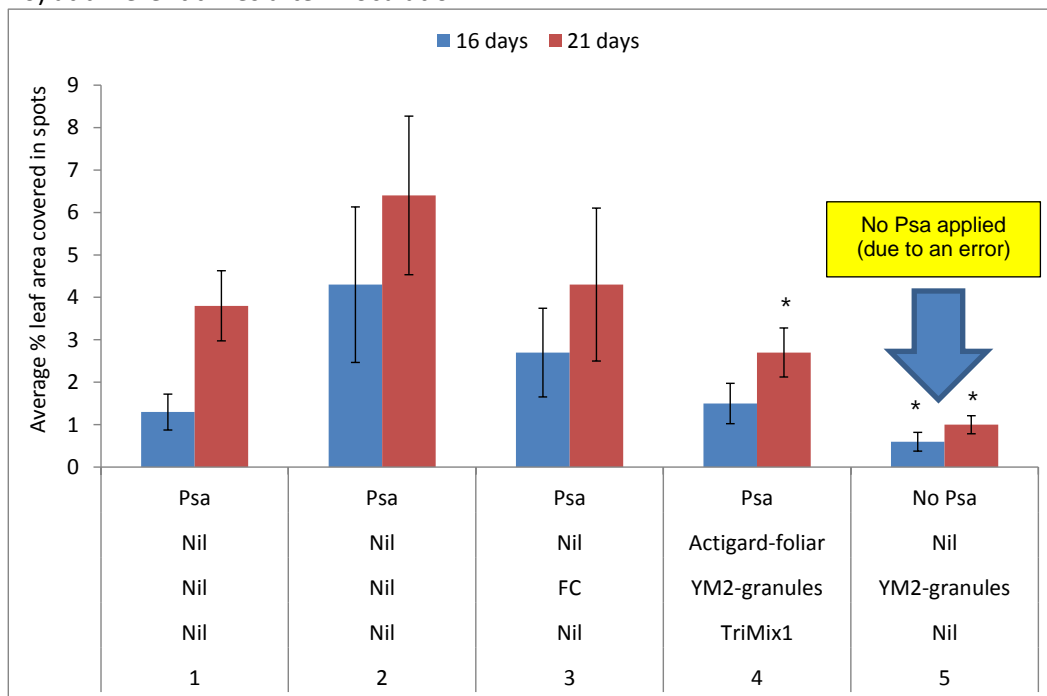
TRT No.	19-Dec	18 & 24 Jan	18-Jan	25-Jan	Percentage of leaf spotting relative to the untreated Psa controls (%)	
	<i>Trichoderma</i> root drench (Keri-Keri)	Foliar applications of yeast mix (TPRO)	Foliar applications of elicitor (TRPO)	Inoculation with Psa-V at Zespri/KVH trial site (Te Puke)	Mature leaves	Expanding leaves
1	Nil	Nil	Nil	Psa	-	-
2	Nil	Nil	Nil	Psa	-	-
3	Nil	FC	Nil	Psa	36	59
4	TriMix1	Nil	Nil	Psa	92	200
5	TriMix1	Nil	Actigard	Psa	48	59
6	TriMix1	YM2-granules	Nil	Psa	39	35
7	TriMix1	YM2-granules	Actigard	Psa	45	35
8	Nil	YM2-granules	Nil	Psa	41	82
9	Nil	YM2 (fermented)	Nil	Psa	52	82

10	Nil	YM2-granules	Actigard-foliar	Psa	35	47
11	Nil	Nil	Actigard-foliar	Psa	66	59
12	Nil	Nil	Actigard-root	Psa	72	82
13	TriMix1	YM2-granules	Actigard-root	No Psa	33	35

Summary

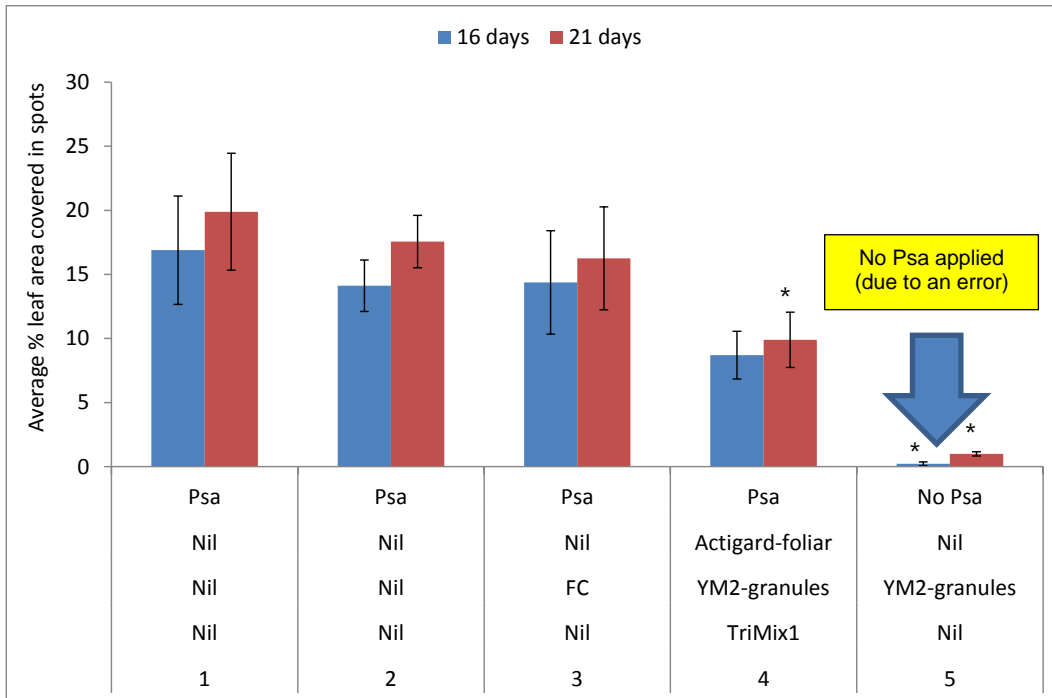
Despite a problem with some of the treatments being applied incorrectly, these trials still provide some evidence that combinations of Actigard, yeast and *Trichoderma* may be useful in the management of Psa (in terms of suppressing leaf spotting).

Figure 2. 2012/13 Zespri/KVH Potted Plant Trial of Yeast & *Trichoderma* Mixes on Hayward. Average amounts of total leaf area for the mature parts of plants covered in Psa-V leaf spots (n = 10) at different times after inoculation.



* Statistically significant at the 5% level from the Psa-only treatments (average) according to a non-parametric (Wilcoxon) test.

Figure 3. 2012/13 Zespri/KVH Potted Plant Trial of Yeast & *Trichoderma* Mixes on Hayward. Average amounts of total leaf area for the expanding parts of plants covered in Psa-V leaf spots (n = 10) at different times after inoculation.

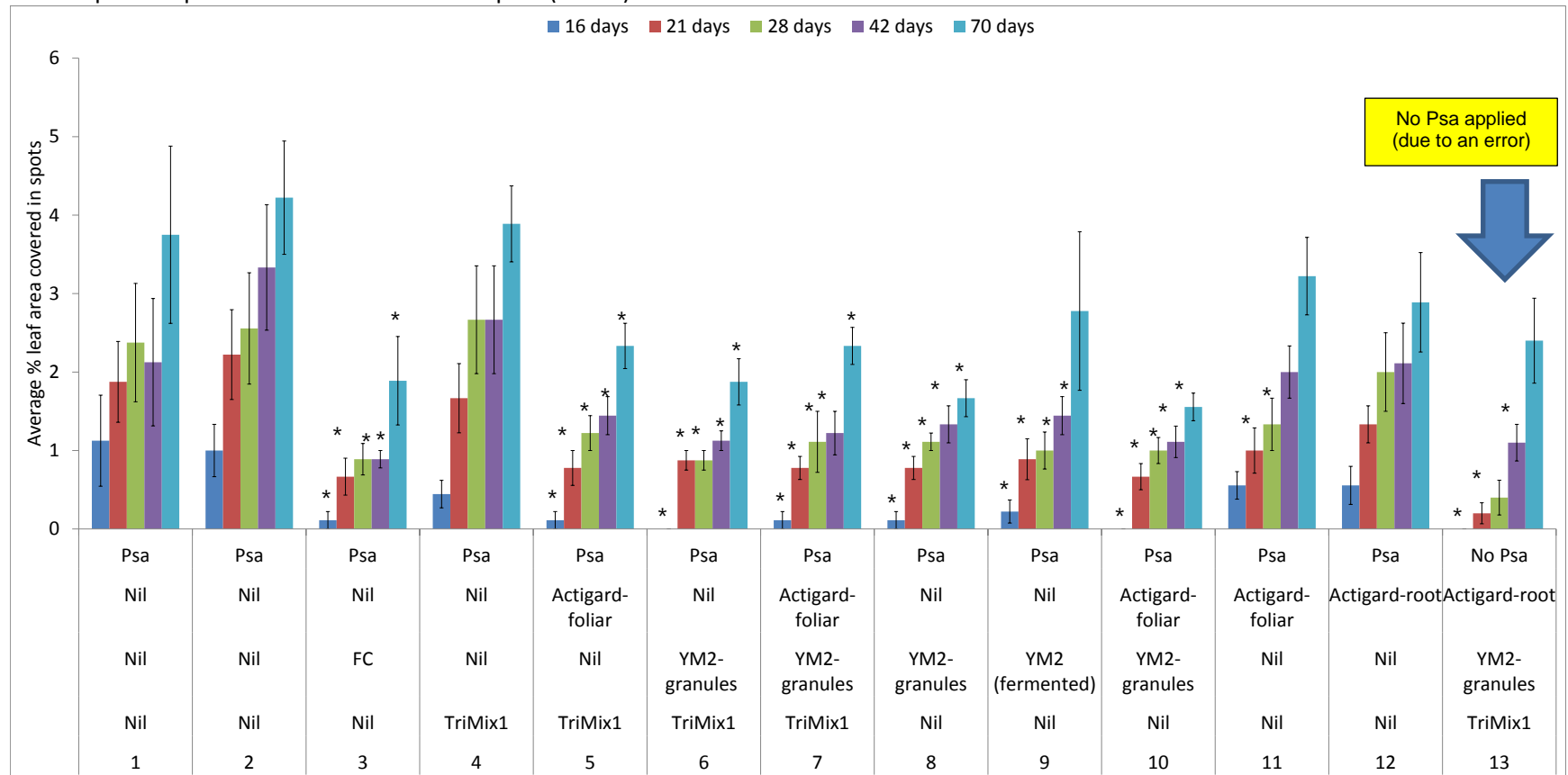


* Statistically significant at the 5% level from the Psa-only treatments (average) according to a non-parametric (Wilcoxon) test.



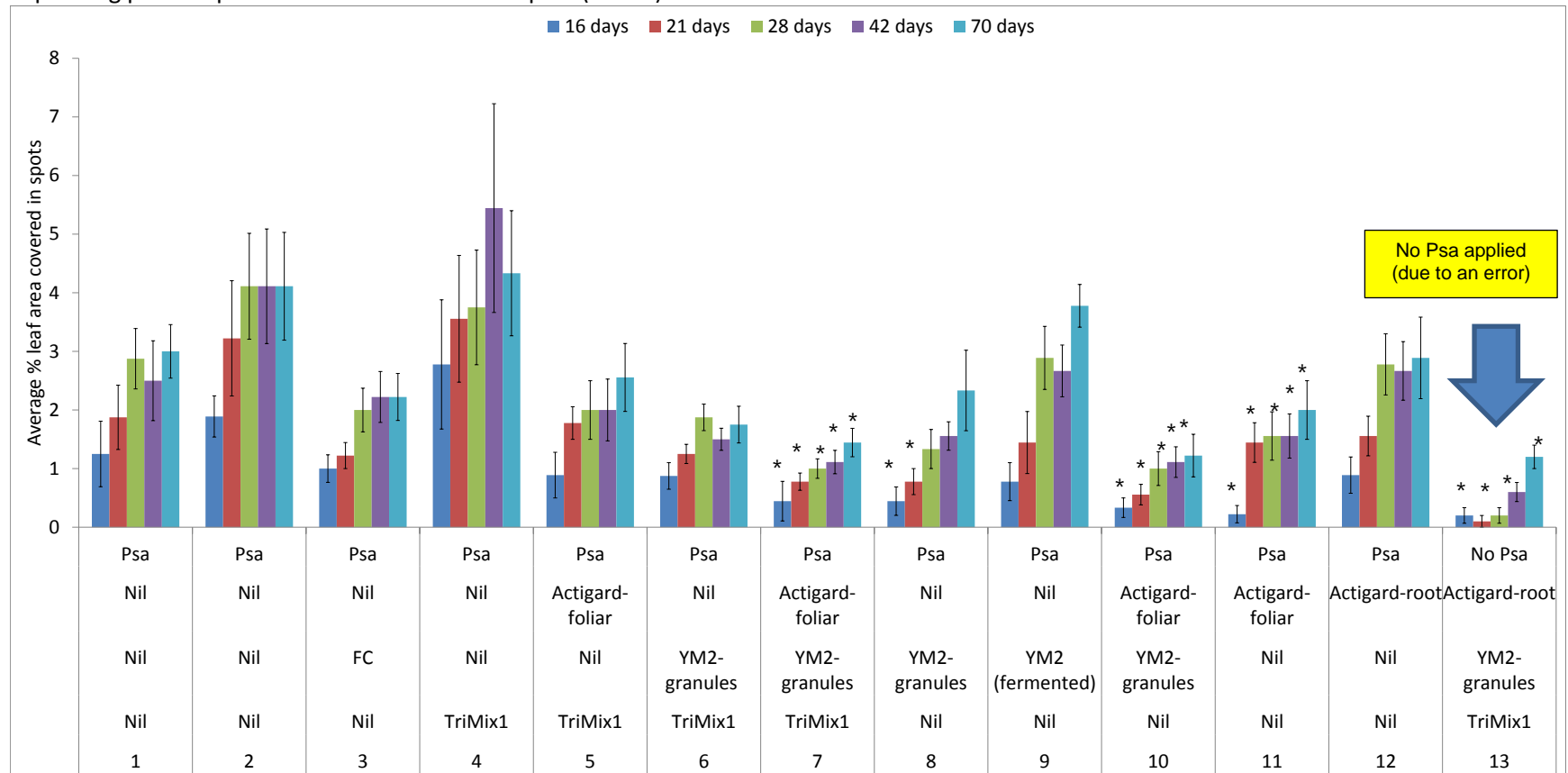
Inoculated with Psa-V (10^8 cfu/mL)

Figure 4. 2012/13 Zespri/KVH Potted Plant Trial of Yeast, *Trichoderma* and Actigard Mixes on Gold3. Average amounts of total leaf area for the mature parts of plants covered in Psa-V leaf spots (n = 10) at different times after inoculation.



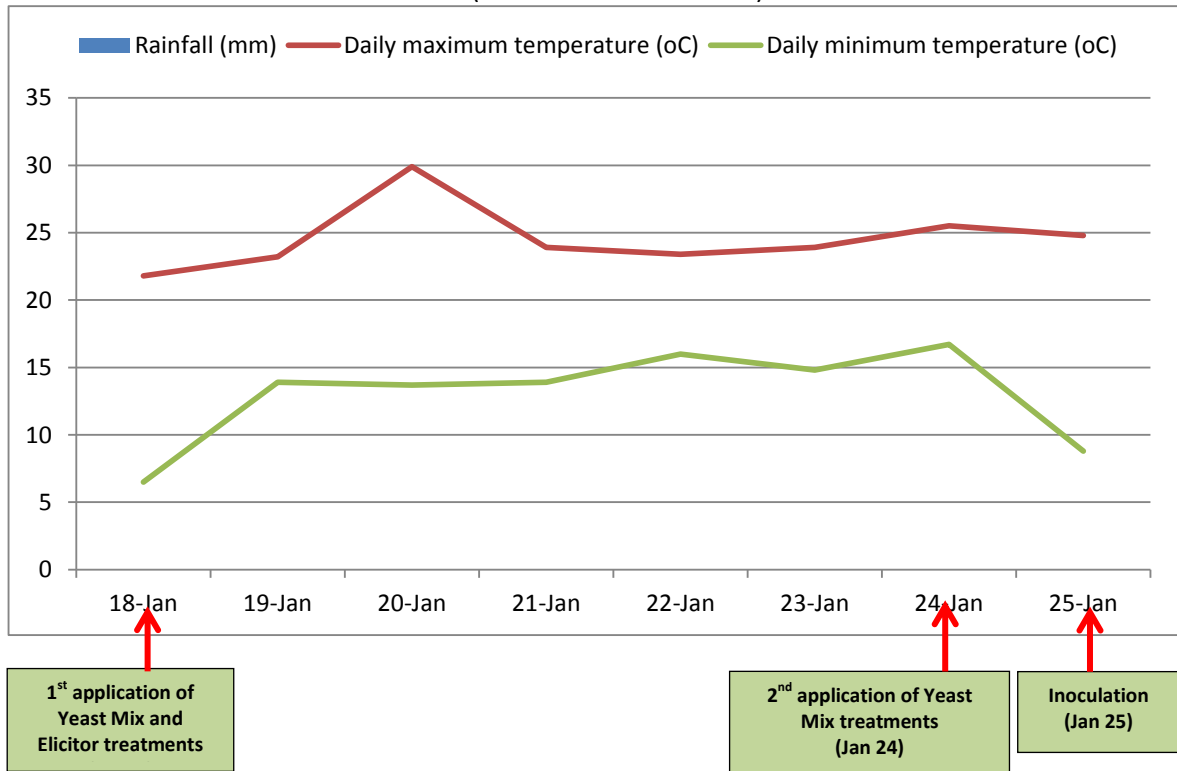
* Statistically significant at the 5% level from the Psa-only treatments (average) according to a non-parametric (Wilcoxon) test.

Figure 5. 2012/13 Zespri/KVH Potted Plant Trial of Yeast, *Trichoderma* and Actigard Mixes on Gold3. Average amounts of total leaf area for the expanding parts of plants covered in Psa-V leaf spots (n = 10) at different times after inoculation.

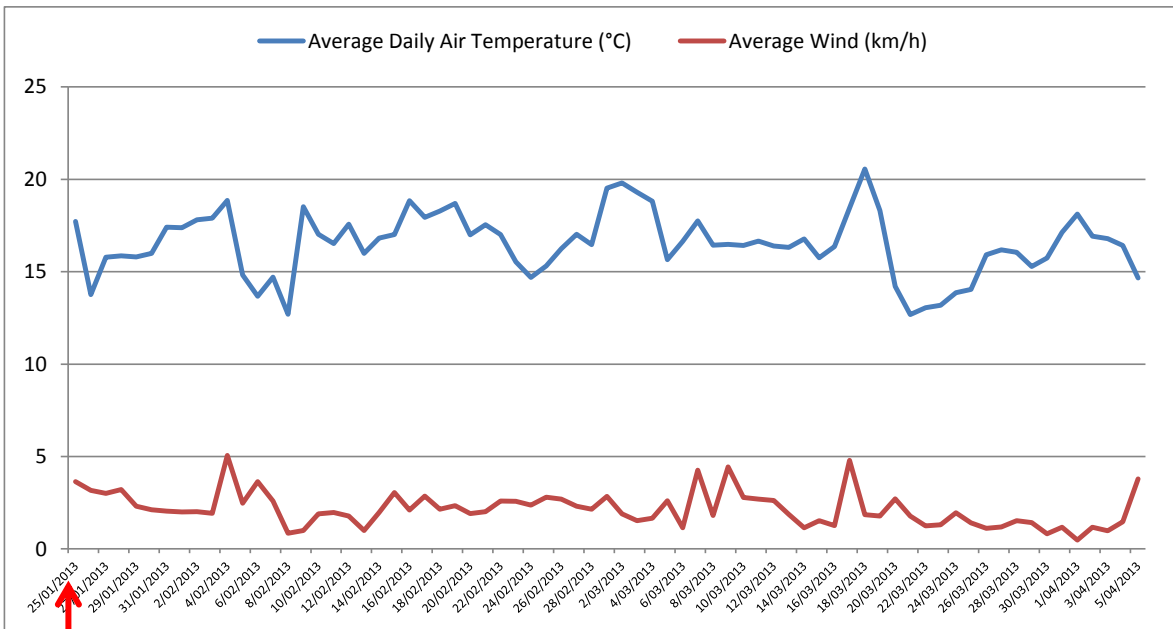
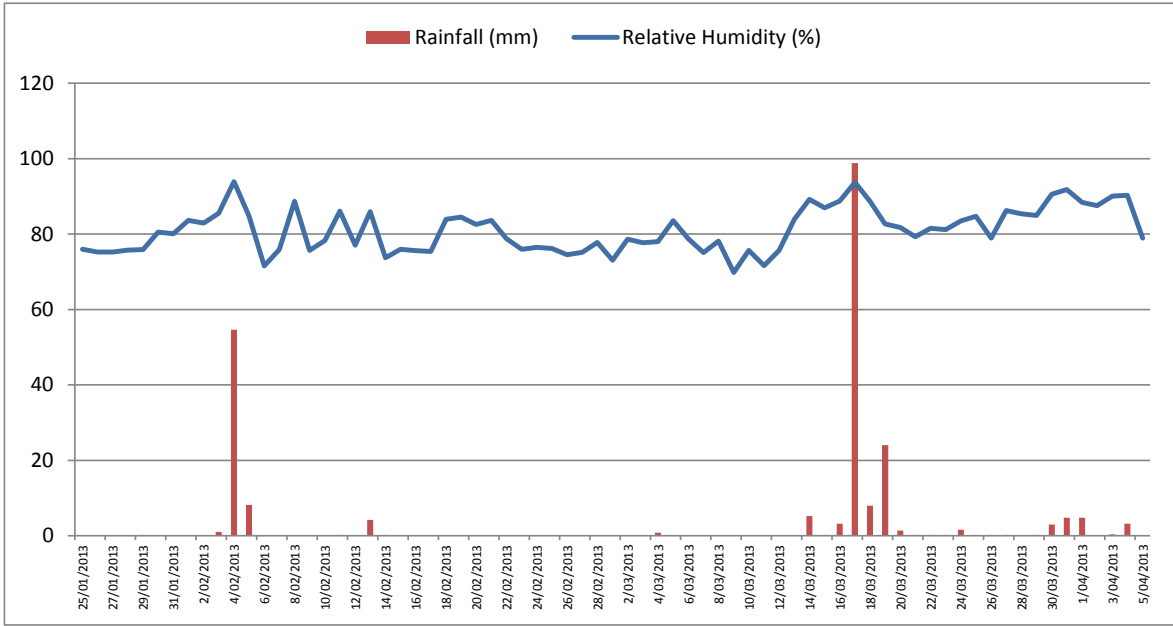


* Statistically significant at the 5% level from the Psa-only treatments (average) according to a non-parametric (Wilcoxon) test.

Appendix 1. Weather during the period that treatments were being applied in the trial of Yeast and *Trichoderma* Mixes on Hayward Gold3 which commenced in January 2013. Treatments were applied at the nearby Plant and Food Research Station and weather would have been similar to that shown here. Source: Harvest.com (weather station on site).



Appendix 2. Weather at the Zespri/KVH field site during the trial of Yeast and *Trichoderma* Mixes on Hayward and Gold3 which started in January 2013. Source: Harvest.com (weather station on site).



**Inoculation
(Jan 25)**

