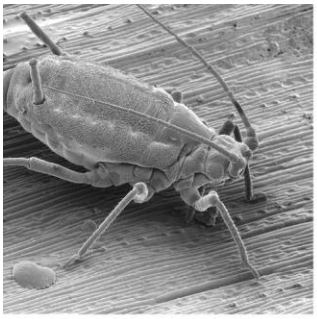
PFR SPTS No 10244

Rapid commercialization of an integrated, biologically based management package for Psa-V (V11384)

Elmer PAG, Hoyte SM, Parry FJ, Stark C, Hill RA, Taylor J, Ah Chee A, Spiers M, Mauchline N & Hall C.

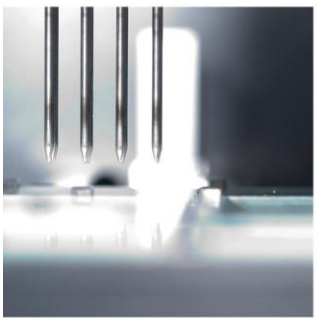
June 2014





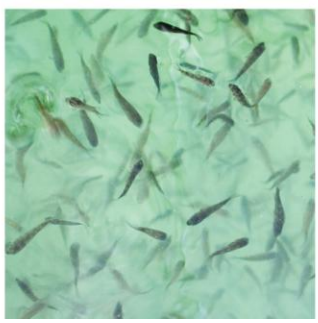
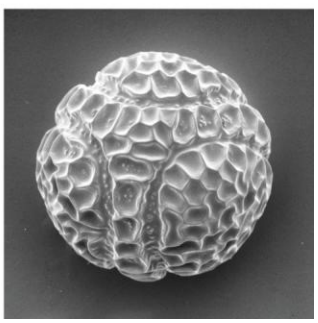










Confidential Report for:
Zespri Group Limited, V11384

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Executive summary

Rapid commercialization of an integrated, biologically based management package for Psa-V (V11384)

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June 2014

Background

Zespri Group Limited/KVH are seeking alternative products to control *Pseudomonas syringae* pv. *actinidiae* (Psa) in kiwifruit that can complement or substitute for the kiwifruit sector's current reliance upon copper-based products, antibiotics and Actigard® for the control of Psa.

Research completed as part of Zespri project VI1227 (Hoyte et al. 2013) identified that foliar applied yeast based biological control agents (BCAs) significantly reduced Psa leaf severity. In the same project, the BioProtection Centre (Lincoln University) identified that *Trichoderma* strains & mixtures applied to roots significantly reduced Psa lesion development on artificially wounded and inoculated stems of potted kiwifruit plants. In one large potted vine field trial in 2013, the most effective treatment was a combination of *Trichoderma* mixes (TMix1 and TMix2) applied to roots with yeast mixes (Yeast Mix 1 (YM1) and Yeast Mix 2 (YM2)) and the plant defence elicitor Actigard applied to foliage. The New Zealand Institute for Plant & Food Research Limited (PFR), in conjunction with the BioProtection Centre, was contracted in 2013 to carry out two follow-up projects:

Project A – aimed at identifying the most effective isolates and combinations of mixtures from the *Trichoderma* and yeast mixes already shown to have efficacy against Psa. This approach would ensure that the industry would have access to the 'MARK II' version if either TMix1 or YM2 prototypes did not perform to expectations in the grower based field trials.

Project B – aimed at validating the field efficacy (in potted plant trials and orchard based trials) of TMix1 and YM2 (the two biologically-based pre commercial products that were closest to market) and determining the compatibility of the two yeast strains (YCom1 and YCom2) in YM2 with a copper-based product. Another key aim was to identify a suitable manufacturer of TMix1 and YM2 and a distribution partner in NZ in order to facilitate a 'fast tracking' process that would move both prototype BCAs towards commercialisation as quickly as possible, on the condition that significant disease reductions were achieved in field studies.

This report summarises the progress made with regard to Project B.

Methods and Materials

YM2 copper compatibility

The compatibility of each of the two components of YM2 (YCom1 and YCom2) with a selected copper-based product (Kocide® Opti™) was evaluated using a multi-well plate assay. The compatibility of both components of YM2 with Kocide Opti were then tested on potted kiwifruit plants by sampling leaf discs and determining the number of viable colony forming units (CFUs) at five time intervals after application.

YM2 composition and adjuvants

The efficacy of YM2 and its three individual components (YCom1, YCom2 and YCom3) was evaluated on potted *Actinidia deliciosa* 'Bruno' and 'Hayward' kiwifruit plants (Assays 17 and 18). Disease control efficacy was calculated using back-transformed treatment means where: Efficacy = (% necrosis in the nil treatment - % necrosis in the treated) / % necrosis in the treated x 100).

Delivery and recovery of TMix1 in potted kiwifruit plants

Different methods of applying the root endophyte treatment, TMix1, to the roots of potted 'Bruno' kiwifruit plants were investigated. The plants were stab inoculated into the stem to evaluate the efficacy of the root treatments against systemic Psa infection.

The persistence of *Trichoderma* in the roots of kiwifruit plants used in the second Zespri/KVH trial was determined by isolating root endophytic *Trichoderma* from the roots at the completion of the above ground assessments. Five vines were selected from each of four treatments for both 'Bruno' and *A. chinensis* x *A. deliciosa* 'Zesh004' (commonly known as Green14) plants: T1 = water control, T2 = Psa control, T3 = combination treatment of TMix1+(YM2)+Actigard and T6 = TMix1 only. Using dilution plating techniques the number of root sub-samples with *Trichoderma* colonies was counted.

YM2 and TMix1 field efficacy trials

Potted plant trials: (Zespri/KVH)

Two large potted plant trials were carried out by PFR and Zespri/KVH under the Zespri/KVH field evaluation programme co-ordinated by Jayson Benge in 2013. These trials were previously reported to Zespri/KVH and are included in this report (Appendix 1 and 2) as they were part of the original project description for this project.

The first potted plant trial used both 'Hayward' (5 treatments) and *A. chinensis* 'Zesy002' (commonly known as Gold3) (13 treatments) plants and a range of YM2, TMix1 and Actigard® treatments were applied before inoculation of the leaves with a suspension of Psa on 25 January 2014. In the second potted vine trial, 'Bruno' seedlings and grafted Green14 plants were used and a range of YM2, TMix1 and Actigard® treatments (six in total) were applied before inoculation with Psa on 14 March 2013. Leaf spotting (proportion of leaf area) and secondary symptoms were visually estimated by Zespri/KVH staff and recorded from 16 days after inoculation and then at approximately weekly intervals until 42 days after inoculation. The leaves that were mature and treated at the time of inoculation were assessed separately from the newly expanding leaves.

Whole vine trials: (PFR/Lincoln University)

Three trials sites (*A. chinensis* 'Hort16A', 'Hayward' and Gold3) in the Bay of Plenty were established in the autumn of 2013 to validate the efficacy of TMix1 and YM2 in grower's orchards. There were three treatments, each with 15 replicate plots at each trial site. Three spray programmes were compared for the management of Psa: A grower standard programme, based upon copper and Actigard treatments; a TMix1- and YM2-based programme which consisted of two root zone drench applications of TMix1 (autumn and spring); and regular (2–3 weekly) foliar applications of YM2. The third treatment was a programme based upon YM2 treatment only. Visual disease assessments were carried out by PFR staff and data were expressed as the average Psa leaf spotting score, average shoot die-back and average cane die-back per vine.

Commercialisation

Manufacture and scale-up

The individual components of YM2 are manufactured by a commercial supplier. PFR put in place a non-disclosure agreement (NDA) with the commercial supplier in February 2013 which has enabled open discussions about the prototype product, manufacture, formulation and pricing.

Registration and distribution

Given that YM2 can be manufactured and packaged to specification, an agricultural distribution partner was identified and confirmed with Zespri/KVH.

Patenting

PFR has engaged an Intellectual Property Company, to complete a review of yeasts and their application for bacterial disease control.

Key results

YM2 copper compatibility (PFR)

- Lab-based studies: YCom1 was very sensitive to the copper based-product Kocide Opti (60% reduction in growth when exposed to 1/8 field rate of Kocide Opti). In contrast, YCom2 showed some tolerance to Kocide Opti (20% reduction in growth at the recommended Kocide Opti field rate).
- Plant-based studies: In this study, it was confirmed that YCom1 does not tolerate tank mixing with Kocide Opti, or when applying YCom1 to copper treated leaves. The multi well assay indicated that YCom2 may have some tolerance to Kocide Opti. However, the plant based assay did not support this and YCom2 viability was significantly reduced when either applied to copper treated leaves or when tank mixed with copper.

YM2 composition and adjuvants

- Assay 17: In the absence of any treatment, average Psa leaf severity was 29% and all treatments significantly ($P < 0.05$) reduced Psa severity by 41% (Foodcoat (FC) only control) to 78% (YCom1 + YCom2 (FC)). Importantly, the removal of YCom3 (the most expensive yeast component) from the YM2 prototype did not result in any reduction of efficacy. In this assay, the combination of YCom1 and YCom2 demonstrated significantly better efficacy against Psa, (78%) than YCom1 (61%) and YCom2 (57%) used alone.

- Assay 18: The efficacy of YCom1 and YCom2 combined was confirmed (87%) when the BCA additive, FC was replaced with a more cost effective wetter/spreader, Latron B.

Delivery and recovery of TMix1 in potted kiwifruit plants

- Assay 19: In this assay, none of the TMix1 root delivery treatments, significantly reduced Psa development after treated plants were inoculated with Psa using the stem stab method, compared with untreated plants.
- Persistence of *Trichoderma* on roots: The recovery of *Trichoderma* spp. from roots of selected vines used in the Zespri/KVH Trial 2 (below) indicated that where no TMix1 was applied (water control), the proportion of roots with endophytic *Trichoderma* spp. was 9% and 8% for 'Bruno' and Green14, respectively. In those treatments that received TMix1 (T3 and T6), endophytic root colonisation by *Trichoderma* spp. ranged from 32 to 47%, indicating that the TMix1 root drench application method had resulted in successful root colonisation.

YM2 and TMix1 field efficacy on potted vines

Potted plant trials: (Zespri/KVH)

- Trial 1 – 'Hayward': Relatively dry conditions over the duration of this trial (21 days) suppressed Psa development and the untreated, inoculated plants had 6.5% Psa leaf severity. The combination treatment of TMix1-YM2-Actigard, significantly reduced Psa leaf severity by 59% compared with the untreated Psa control. Low plants numbers meant that the individual treatment components could not be evaluated this part of the trial programme.
- Trial 1 – Gold3: Similarly, dry conditions over the duration of this trial (up to 70 days) suppressed Psa development and in the untreated, inoculated plants average Psa leaf spot severity was only 4.2%.
    - Only one individual treatment significantly reduced Psa leaf spot severity, namely the YM2-granule treatment (Psa efficacy = 60%) and this compared well with the Actigard treatment (Psa efficacy = 24%).
    - There was no significant reduction in Psa leaf severity following root treatment with TMix1.
    - On untreated and uninoculated expanding leaves, three treatments (each containing Actigard) significantly reduced Psa leaf spot severity, namely TMix1-YM2-Actigard, YM2-Actigard and Actigard.
- Trial 2 – 'Bruno': Psa leaf spot severity was 45% in the nil treated control 28 days after Psa inoculation. Three treatments – YM2, Actigard and TMix1-YM2+FC-Actigard – significantly ($P < 0.05$) reduced Psa leaf spot severity by 56%, 40%, and 62% respectively.
    - TMix1 did not significantly ($P < 0.10$) reduce Psa leaf spotting at each assessment date.
- Trial 2 – Green14: Psa leaf severity was 14% in the nil treated control 41 days after Psa inoculation. Three treatments – YM2+FC, Actigard and Tmix1-YM2+FC-Actigard, significantly ($P < 0.05$) reduced Psa leaf spot severity by 71%, 79% and 93%, respectively. The TMix1 treatment reduced Psa leaf spot severity by *ca*. 50% but this was not statistically significant even when tested at the 10% level of probability.

Whole vine trials: (PFR/LU)

- 'Hort16A': The incidence of leaf spotting and shoot dieback was not significantly different between the grower standard spray programme and the YM2 and YM2+TMix1 spray treatments. This trial block was subsequently abandoned because of severe secondary symptom development and removal of those vines by the grower.
- 'Hayward': The incidence of leaf spotting was not significantly different between the grower standard spray programme and the YM2+TMix1 spray treatment. In the YM2 only treatment, the mean Psa leaf spotting score was 2.5 (scale 0–5) and this was significantly ($P < 0.05$) higher than on plants receiving the grower standard spray programme (1.5). Shoot dieback and cane die-back was only present on the male vines and was not significantly different between the treatments.
- Gold3: Due to a dry 2013–14 season and low propensity for Gold3 vines to develop leaf spotting, there were insufficient Psa related symptoms over the entire trial block area and therefore insufficient data for ANOVA.

Commercialisation

YM2 manufacture and scale-up

- . A 3–4 month lead in time for manufacture of large quantities is required and they can package and label as per customer specifications.

YM2 registration and distribution

- Under a non-disclosure agreement (NDA), PFR has shared extensive information about YM2 with our commercialization partner. This company applied their commercialization knowledge and recommended a series of developmental milestones and a plan to address these recommendations with associated timelines was discussed with Zespri/KVH.
- Commercial R&D questions are currently being addressed through a PSAF (Pre Seed Accelerator Fund) programme. More applied questions are being addressed in Zespri/KVH – PFR potted vine field trials and the more fundamental questions are being addressed in the MBIE – 'Next Generation Biopesticides' programme.

YM2 patenting

- The review completed by an Intellectual Property Company did not identify any Freedom to Operate (FTO) issues with YM2 in New Zealand. However, some prior art (both patent and publications) was identified (e.g. the use of yeasts for control of mammalian diseases caused by bacterial pathogens).
- The search results and PFR data have also been reviewed by AJ Park patent attorneys. Their initial conclusion is that there may be a position to patent the use of YM2 against Psa and other closely related plant diseases. They are now conducting a more detailed review of the data and a decision whether to patent will be made by end of June 2014/early July, depending upon results of the Zespri/KVH and PFR potted vine trials.

TMix1 manufacture and scale up

- The BioProtection Centre, Lincoln University, is proceeding with scale-up production of the *Trichoderma* isolates in TMix1 with the aim to supply enough product to treat 2000 ha. The

TMix1 is being sold to growers who show interest in purchasing TMix1 and has been underwritten by Zespri in a separate project.

Conclusions

Our data demonstrated the potential of YM2 to reduce Psa leaf severity in kiwifruit in glasshouse trials. The removal of YCom3 from YM2 and the replacement of FC with Latron B did not significantly reduce Psa efficacy. However, FC does offer advantages for yeast BCAs and we are evaluating other alternative additives as potential replacements for Latron B since the commercial life of this adjuvant is limited. Laboratory- and plant-based studies demonstrated that both components of YM2 are sensitive to field rates of copper applied in the form of Kocide Opti. While this may be regarded as a setback, the commercial partner is comfortable with YM2 being applied the period from flowering to fruit set when there are very limited options for protecting flowers and rapidly growing shoots against Psa infection.. Controlled environment room studies at Lincoln University with TMixes suggested that Psa stem infections on young plants could be reduced (Hoyte et al. 2013: Report to Zespri Group Limited V11227-30-J). Unfortunately, data from a limited number of glasshouse studies on potted plants and data from joint trials with Zespri/KVH on larger potted vines indicate that the TMix1 root endophyte treatment did not significantly reduce Psa severity symptoms when challenge inoculated with Psa.

Recommendations

Several commercially focused research streams are recommended in order to advance the commercialisation and grower availability of TMix1 and YM2 and these have been placed under suggested funding source categories:

Zespri-funded:

- Carry out further validation of TMix1 and YM2 singly and in combination with each other and with Actigard, on potted vines under the Zespri/KVH potted plant field evaluation programme. Also, if feasible, carry out a similar trial in an Italian-based potted plant trial in order to gain extra data from a different growing region and to gain advantage from an extra spring trial evaluation in the Northern Hemisphere.
- Carry out field evaluations of YM2 in commercial orchards to confirm field efficacy of commercially acceptable rates and any changes to the composition of YM2, arising from potted plant trails being carried out under other work streams (see below).

PSAF-funded:

- Investigate more cost-effective ratios of YCom1 and YCom2 other than the 1:1 ratio used to date, and determine the minimum inhibitory concentration of YM2. Glasshouse studies are underway to determine if these changes affect Psa efficacy.
- Determine the persistence of YM2 on leaves and relate this to its efficacy against Psa in order to establish appropriate spray intervals.
- Determine the effects of YM2 on bee survival and health, with and without several adjuvants.
- Determine the compatibility of YM2 with fungicides, insecticides and nutrient products that are likely to be applied during and after flowering.
- Confirm suitable mixing procedures for YM2, including the need for spray tank cleansing, and recommendations with respect to copper tolerance.

Commercial partner-funded:

- Seek dispensation for fruit disposal requirements for YM2 so that fruit compensation will not be required for next season's field trials.
- Carry out larger scale, grower-based field trials using air-blast sprayer application to confirm efficacy of the preferred final YM2 composition and rate.

PFR-funded:

- Seek IP protection for the use of yeast strains for control of bacterial plant diseases. A decision on patenting will be made following results from the latest potted plant trials (June 2014).
- Investigate the mode of action of YM2 on kiwifruit (MBIE – "Next Generation Biopesticides").

Lincoln University/Zespri-funded:

- Commercialisation of TMix1. This is proceeding under a separate project, and provides for the production of sufficient TMix1 to treat 2000 ha during the autumn/spring of 2014.
- It will be important to have some split block comparisons established across several sites so that Psa development can be monitored in treated and untreated areas.

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1 Introduction

Zespri Group Limited/KVH are seeking alternative products to control *Pseudomonas syringae* pv. *actinidiae* (Psa) in kiwifruit that can complement or substitute for the kiwifruit sectors current reliance upon copper-based products, antibiotics and Actigard® for control of Psa.

There are several issues associated with each of these three main product groups currently used throughout the industry for Psa control. These are detailed in Table 1.

Table 1. Issues associated with three product groups most commonly used for *Pseudomonas syringae* pv. *actinidiae* control in New Zealand orchards.

Product group	Issue 1	Issue 2	Issue 3	Supporting literature
Copper-based	Up to 20 applications per season are being applied, meaning that long-term use of coppers will lead to unwanted build-up of copper in orchard soils and environments	Phytotoxicity on leaves and fruit can occur	Risk of copper resistance development in Psa bacterial populations is moderate to high	http://www.kvh.org.nz/vdb/document/91314 Copper resistance in *Pseudomonas syringae* in NZ (Vanneste et al., 2008, http://www.nzpps.org/journal/61/nzpp_610800.pdf)
Antibiotics	Restricted use. Cannot be used in the week before flowering and are not permitted after flowering	The number of applications of Kasumin™ or KeyStrepto™ per season (post-harvest to post-harvest) must not exceed four The total combined number of antibiotic applications must not exceed six	Risk of resistance development in Psa bacterial populations is high	http://www.kvh.org.nz/antibiotics Naturally occurring streptomycin resistance in *Pseudomonas syringae* in NZ (Vanneste et al. 2008 http://www.nzpps.org/journal/61/nzpp_610800.pdf)
Actigard®	Restricted use. Must not be applied as a foliar spray within producing blocks after the commencement of male flowering through to harvest	Market access requires residues below MRL detection to meet 'All MarkStatus'	Risk of resistance development is unknown	http://www.kvh.org.nz/vdb/document/91452

Research completed as part of Zespri project VI1227 (Hoyte et al. 2013), identified several biological control agents (BCAs), composed of different foliar applied yeasts (PFR, Ruakura Research Centre) and root-applied *Trichoderma* strains & mixtures from the BioProtection Centre, Lincoln University. Independently, both of these approaches demonstrated significant efficacy against Psa in the laboratory, glasshouse and in one large potted vine field trial in 2013. The most effective treatments were shown to be a combination of root-applied *Trichoderma* mixes (TMix1 and TMix2) with foliar applied yeast mixes, Yeast Mix 1 (YM1) and Yeast Mix 2 (YM2) and the plant defence elicitor, Actigard.

PFR was contracted in 2013 to carry out two follow-up projects:

Project A – aimed at identifying the most effective isolates and combinations of mixtures from the *Trichoderma* and yeast mixes already shown to have efficacy against Psa. This approach would ensure that the industry would have access to the 'MARK II' version if either TMix1 or YM2 prototypes did not perform to our expectations in the grower-based field trials.

Project B – aimed at validating the field efficacy (in potted plant trials and orchard based trials) of TMix1 and YM2 (the two biologically based pre commercial products that were closest to market) and determining the compatibility of the two yeast strains in YM2 with a copper-based product. While these work streams were being carried out, another key aim was to identify a suitable manufacturer of TMix1 and YM2 and then a distribution partner in NZ in order to facilitate a 'fast tracking' process that would move both prototype BCAs towards commercialisation as quickly as possible, on the condition that significant disease reductions were achieved in field studies. A registration package was anticipated to be prepared in conjunction with commercial partners with the intended aim of submitting this to ACVM by 20 December 2013.

This report summarises the progress made with regard to evaluations of TMix1 and YM2 (Project B) against Psa, copper compatibility of YM2 and progress on the path towards commercialisation for each of these prototype BCAs.

2 YM2 Copper Compatibility

2.1 Methods and materials

The compatibility of the two components of YM2 with copper has been carried out in laboratory-based *in vitro* assays, as well as on small potted kiwifruit plants.

2.1.1 Laboratory-based studies

The compatibility of each of the two components of YM2 with a selected commonly used copper-based product (Kocide® Opti™) was evaluated using a multi-well plate assay. This consisted of mixing a known concentration of yeast cells in Potato Dextrose Broth (PDB) with a range of concentrations of copper and monitoring the growth by measuring the optical density (increasing optical density equates to increasing growth of the yeast) using a spectrophotometer (BioTek™). A preliminary study indicated that a wavelength of 600 nm was suitable for detecting growth of the yeasts.

The two yeast components, YCom1 and YCom2, were made up in PDB and mixed directly with different concentrations of each copper product to provide final copper concentrations that were 2x, 1x, 0.5x, 0.25x and 0.1x the recommended field rate for each respective product. A control was included that had no copper product (yeast and PDB only). There were eight replicate wells for each treatment. The starting concentration of yeast was 2×10^7 cells/mL and the micro-well plates were incubated at 25°C. Optical density readings were taken with the spectrophotometer on five occasions following the initial mixing of yeast and copper and data are presented as the percentage reduction in absorbance relative to the control treatment (no copper).

2.1.2 Plant-based studies

The compatibility (or tolerance) of both components of YM2 were tested on kiwifruit plants (tissue culture propagated PFR clonal selection Red15) that had been grown in Rockwool cubes and then potted into 1.5 L pots prior to using in the assay. There were 10 plants chosen for the assay and they were approximately 30–50 cm high, each with 4–5 usable leaves per plant. The treatments are shown in Table 2.

The YCom1 and YCom2 treatments were carried out on separate plants to avoid cross-contamination of the yeast types. Four leaves were labelled on each plant and assigned to one of four treatments (including a control, which received no yeast and no copper). The leaf position for each treatment was changed for each replicate plant. On 6 August 2013, Kocide Opti (46 g/100 L) was prepared in the wetter, Latron B® (30 mL/100 L) and applied to run-off on the leaves labelled for Kocide Opti then YCom1 and Kocide Opti then YCom2 treatments. The following day YCom1 and YCom2 (both at 5×10^7 cells/mL) was applied to leaves previously treated with Kocide Opti and to the leaves labelled for YCom1 only and YCom2 only. A mixture of Kocide Opti and YCom1 and Kocide Opti and YCom2 were applied, using the same rates as described above, to the leaves labelled for the two tank mixed treatments, respectively. Latron B (30 mL/100 L) was applied to the control leaves.

Table 2. Treatments of Yeast Mix 2 components applied to kiwifruit leaves with and without Kocide® Opti™.

Treatment	Yeast applied	Kocide Opti [1] applied 24 h before yeast	Kocide Opti applied as a tank mix with yeast
Control	No	No	No
YCom1 [2] only	YCom1	No	No
Kocide Opti then YCom1	YCom1	Yes	-
Kocide Opti + YCom1 tank mix	YCom1	-	Yes
YCom2 [3] only	YCom2	No	No
Kocide Opti then YCom2	YCom2	Yes	-
Kocide Opti + YCom2 tank mix	YCom2	-	Yes

[1] Kocide Opti applied at 46 g/100 L, prepared in Latron B (30 mL/100 L)

[2] YCom1 = Yeast component 1, a component of Yeast Mix 2 applied at ~5 x 10^7 cells/mL prepared in Latron B (30 mL/100 L)

[3] YCom2 = Yeast component 2, a component of Yeast Mix 2 applied at ~5 x 10^7 cells/mL prepared in Latron B (30 mL/100 L).

At each of five sampling times (2 h, 1 day, 4 days, 7 days and 14 days) following application of the yeasts; five 10 mm diameter leaf discs were cut from each leaf. The sets of five leaf discs were then washed to recover the viable yeast cells by placing them into sterile 100 mL conical flasks containing 10 mL of Phosphate buffer and Tween80 (0.05%). The flasks were placed onto an orbital shaker for 10 min (150 rpm) and then transferred to a sonicating water bath (Bandelin Sonorex) operating at 90% capacity.

After sonication the liquid was transferred to a 15 mL centrifuge tube and were centrifuged at 900 *g* for 10 min. The supernatant was removed and the volume made back up to 10 mL with Phosphate buffer. This was vortexed to mix the yeast cells and then serially diluted twice to give stock, 10-fold and 100-fold dilutions. A 100 µL aliquot was taken from each dilution and spread onto a Petri dish containing Semi-Selective Yeast Agar (SSYA). Plates were incubated at 25°C for 4 days before counting the number of colony forming units (CFU) of yeast on each Petri dish. Calculations were carried out to generate the number of CFU/leaf disc by taking into account dilutions and appropriate volumes used.

2.2 Results and discussion

2.2.1 Laboratory based studies

The effects of directly mixing YCom1 and YCom2 (components of YM2) with Kocide Opti, followed by overnight incubation on growth is shown in Figure 1. For YCom1, the percentage growth reduction ranged from 61 to 63% for all five rates of Kocide Opti, indicating that this copper product significantly ($P < 0.001$) reduced the growth of YCom1, regardless of copper concentration. Copper also significantly ($P < 0.001$) reduced growth of YCom2 but the reduction in growth at each copper concentration was significantly less than that found withYCom1. Further, there was evidence that YCom2 demonstrated some tolerance to this

copper-based product (20% reduction in growth, Figure 1) when the full field rate of Kocide Opti was used.

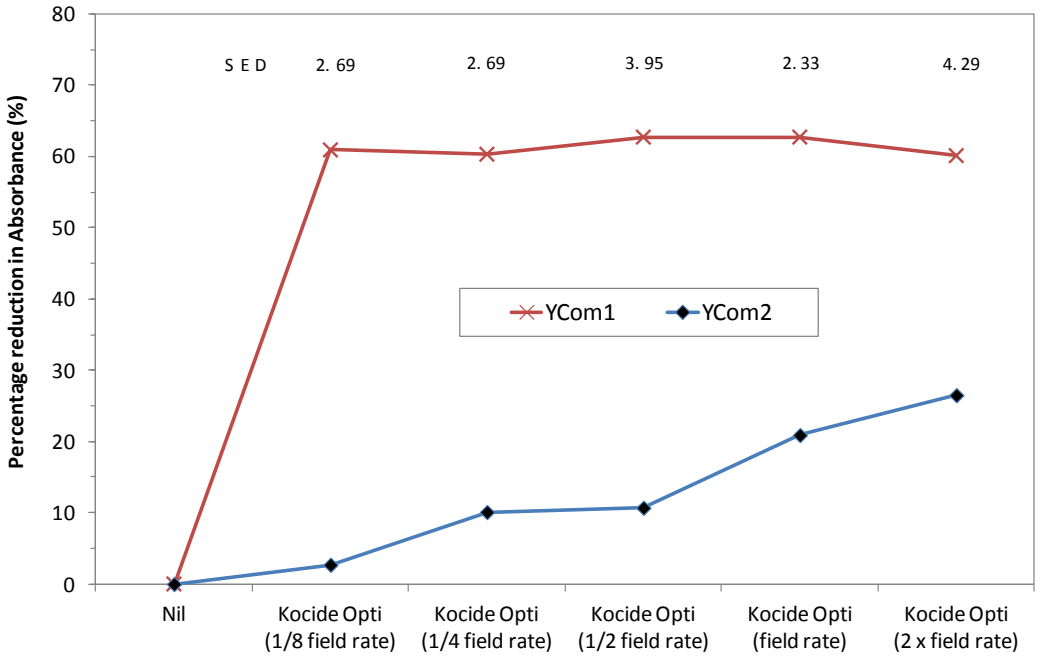

Figure 1. Percentage reduction in absorbance (600 nm) of Yeast Mix 2 components (YCom1 and YCom2) growing in potato dextrose broth in multi-well plates, when mixed with different rates of Kocide Opti. Values along the top of the chart are the standard error of the deviation of the mean (SED) for each rate of copper.

2.2.2 Plant-based study

The effects of copper, either applied to leaves prior to YCom1 and YCom2 or when tank mixed with each yeast component are shown in Figure 3. In the absence of Kocide Opti, YCom1 populations were relatively stable over the 14-day timeframe of this assay and only showed a gradual decline in numbers of CFUs recovered. When YCom1 was applied to Kocide Opti treated leaves there was a significant ($P < 0.05$) decline in survival (log 2.6 to log 1.6, Figure 2) 1 day after application. YCom1 populations then steadily declined over time and by day 14 the number of viable cells on Kocide Opti treated leaves was log 0.4 (2.5 CFU/disc).

When copper was tank-mixed with YCom1 and applied to leaves, there was also a significant ($P < 0.05$) decline in survival (log 2.6 to log 1.2, Figure 2) 1 day after application. From day 4 to the termination of the assay (day 14), very few (≤ log 0.1) viable YCom1 cells were recovered, indicating that copper appeared more toxic to YCom1 when used in a tank mix than when applied to leaves followed by the yeast mix.

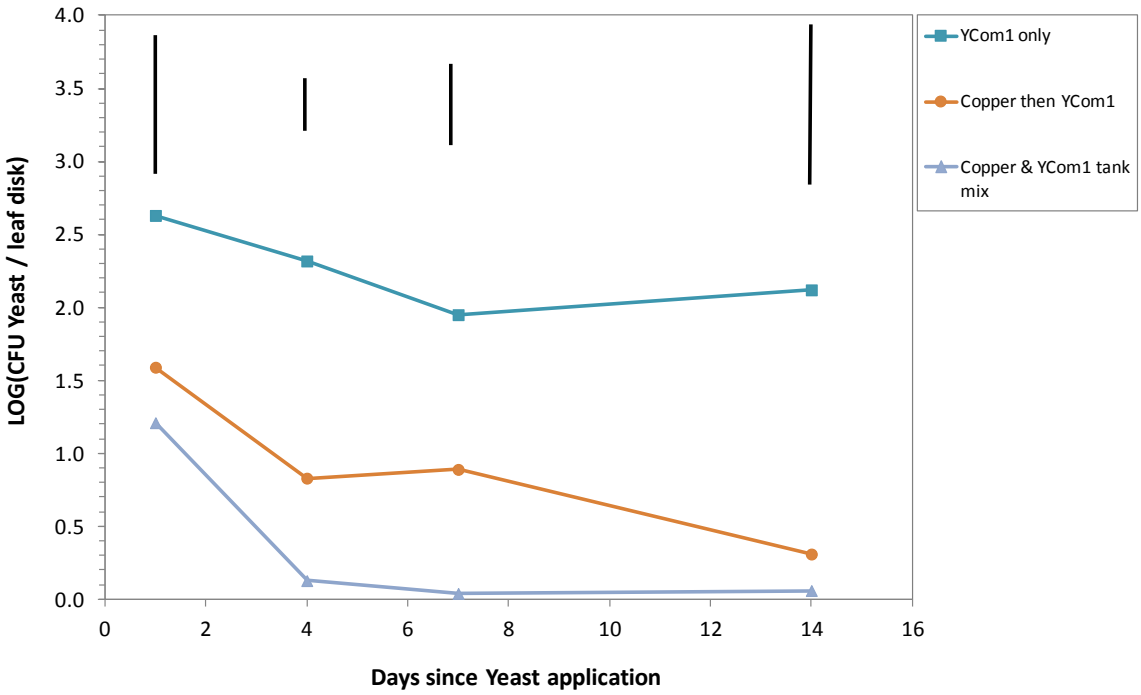

Figure 2. Log mean number of colony forming units of YCom1 per leaf disc sampled from plants treated with YCom1 only, pre-treated with Kocide Opti or YCom1 tank mixed with Kocide Opti. Black bars are Least Significant Differences ($P < 0.05$) for each time point.

In the absence of Kocide Opti, YCom2 populations steadily declined on kiwifruit leaves from log 4.2 (day 1 sample) to log 1.4 (day 14 sample). When YCom2 was applied to Kocide Opti-treated leaves there was a significant ($P < 0.05$) decline in survival (log 4.2 to log 2.6, Figure 3) 1 day after application. YCom2 populations then steadily declined over time and by day 14 the number of viable cells on Kocide Opti-treated leaves was log 0.4 (2.5 CFU/disc).

When copper was tank-mixed with YCom2 and applied to leaves, there was also a significant ($P < 0.05$) decline in survival 1 day after application (log 4.2 to log 1.5, Figure 3). From day 4 to the termination of the assay (day 14), very few (≤ log 0.3) viable YCom2 cells were recovered, indicating that the tank mixing treatment was more toxic to YCom2 than applying YCom2 to leaves that had received Kocide Opti treatment.

Overall the survival of YCom2 was similar to that of YCom1 in the presence of copper. However, in the absence of copper it appeared that YCom1 had better survival characteristics on the kiwifruit leaves than YCom2.

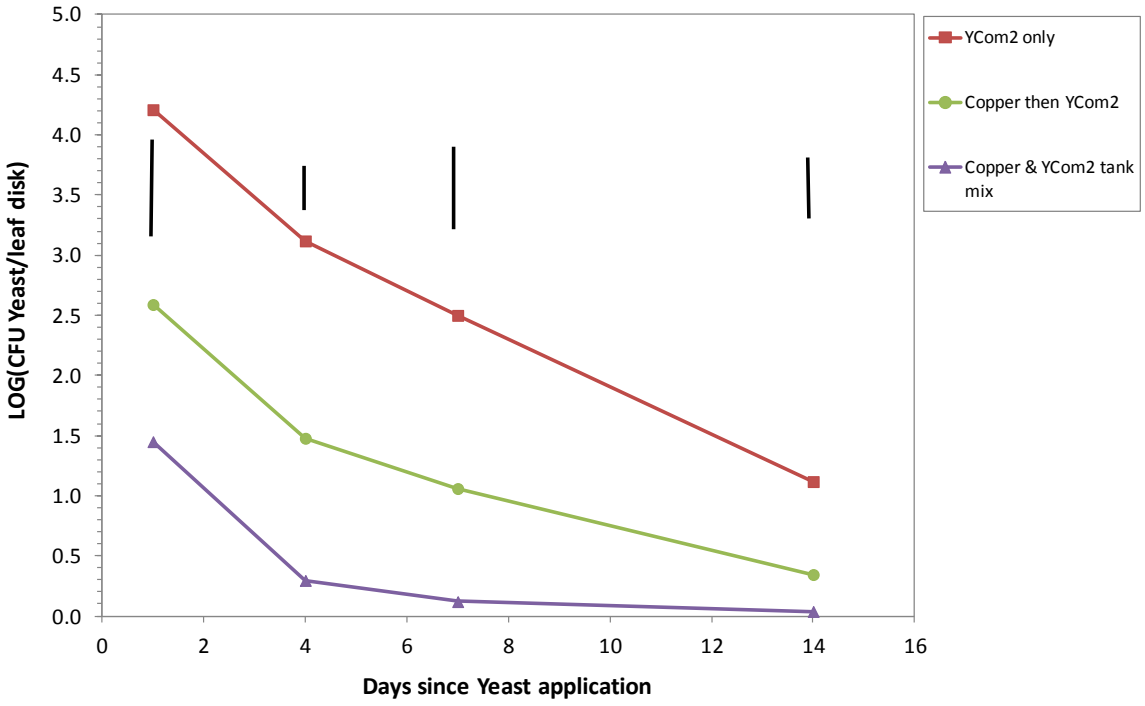

Figure 3. Log mean number of colony forming units of YCom2 per leaf disc sampled from plants treated with YCom2 only, pre-treated with Kocide Opti or YCom2 tank-mixed with Kocide Opti. Black bars are Least Significant Differences ($P < 0.05$) for each time point.

3 YM2 composition and adjuvants

3.1 Method and materials

3.1.1 Psa inoculum preparation

A Psa culture (isolate code 10627), which had been isolated from an infected *Actinidia chinensis* 'Hort16A' kiwifruit vine located in the Te Puke region during 2010, was used for all stab and spray inoculation assays included in this report. Psa inoculum was prepared by growing this strain of Psa for 2–3 days on King B (KB) medium and harvesting the bacteria by washing the plate with SDW to make a stock suspension of inoculum that was visually determined to be >1 x 10^9 CFU/mL. A subsample of this Psa stock was serially diluted and 10 µL droplets placed onto fresh KB medium so that the number of CFU/mL could be counted after 2 days' incubation. To facilitate spray inoculation in the glasshouse, Psa inoculum was transferred to a 500 mL plastic hand trigger sprayer and the underside of each leaf was sprayed to give an even coverage of droplets, without reaching run-off. Plants were always placed into a high-humidity tent after inoculation.

Note: the numbering for assays in this report, where Psa has been inoculated onto plants, has been continued from the previously funded project "Control of Psa using beneficial microbes and elicitors" (Hoyte et al. 2013).

3.1.2 PFR assay 17 – YM2 and its components in FC

Assay 17 was carried out in the PC1 glasshouse at Ruakura using seedling *A. deliciosa* 'Bruno' plants growing in 1.5 L pots to investigate the efficacy of YM2 components and to measure the effects of a commercial adjuvant (Latron B) in comparison to the previously used (too expensive) adjuvant FC. The yeast and adjuvant treatments were applied to the plants 1 day before inoculation (dbi) with Psa (1 x 10^8 CFU/mL) applied on 16 April 2013 and are described in Table 3. All yeasts treatments were applied at a final concentration of 5 x 10^7 CFU/mL. Yeast CG163 was used in this assay to provide an extra comparison to the two adjuvants, as this yeast is one of the top performing yeasts from Project A.

Plants were visually scored for the percentage area of leaf necrosis after 13 and 28 days incubation. In order to ensure consistency, only two staff members carried out Psa leaf severity assessments with regular cross-checking of the severity scores. There was no significant change in leaf necrosis between the assessment dates so data is presented for the first assessment only.

Table 3. Assay 17 treatments of YM2 components and two adjuvants, applied to *Actinidia deliciosa* 'Bruno' seedlings in pots and inoculated with *Pseudomonas syringae* pv. *actinidiae* on 16 April 2013.

Treatment	Yeast[1] used	Adjuvant used
Nil (control)	Nil	Nil
FC[2] only	-	FC (2.5%)
CG163[3] (FC)	CG163	FC (2.5%)
YCom1 (FC)	YCom1	FC (2.5%)
YCom2 (FC)	YCom2	FC (2.5%)
YM2 x2 (FC)	YCom1 and YCom2	FC (2.5%)
YM2 x3 (FC)	YCom1, YCom2 and YCom3[4]	FC (2.5%)

[1] Yeasts were all applied at a final concentration of 5 x 10^7 CFU/mL

[2] FC is a commercial adjuvant that improves yeast survival

[3] CG163 is a PFR yeast component in YM1

[4] YCom1, YCom2 and YCom3 are commercial yeasts; the three original components of YM2.

3.1.3 PFR Assay 18 – YM2 and its components in Latron B

Assay 18 was carried out in the PC1 glasshouse at Ruakura using tissue culture produced *A. deliciosa* 'Hayward' plants growing in Rockwool cubes to investigate the efficacy of YM2 components and adjuvants, including an experimental barley extract supplied by PFR, Lincoln. The yeast and adjuvant treatments were applied to the plants 1 dbi with Psa (1 x 10^8 CFU/mL) on 31 May 2013 and are described in Table 4. Plants were scored for the percentage area of leaf necrosis after 20 days of incubation.

Table 4. Assay 18 treatments of YM2 components and two adjuvants, applied to tissue cultured *Actinidia deliciosa* 'Hayward' plants growing in pots and inoculated with *Pseudomonas syringae* pv. *actinidiae* on 31 May 2013.

Treatment	Yeast[1] used	Adjuvant used
Nil (control)	Nil	Nil
Latron B only	-	Latron B (0.03%)
FC[2] only	-	FC (2.5%)
YCom1[3] (LB)	YCom1	Latron B (0.03%)
YCom2[3] (LB)	YCom2	Latron B (0.03%)
YM2[4] x2 (LB)	YCom1 and YCom2	Latron B (0.03%)
YCom1 (FC)	YCom1	FC (2.5%)
YCom1 (Barley)	YCom1	Barley extract (20%)
Barley (control)	-	Barley extract (20%)

[1] Yeasts were all applied at a final concentration of 5 x 10^7 CFU/mL

[2] FC is a commercial adjuvant that improves yeast survival

[3] YCom1 and YCom2 are commercial yeasts and are components of YM2

[4] YM2 is Yeast Mix 2, a mixture of two commercial yeasts.

3.2 Results and discussion

3.2.1 PFR assay 17 – YM2 and its components in FC

The aim of this assay was to investigate the efficacy of the YM2 components. In the absence of any treatment, the average Psa leaf severity was 29% (Figure 4). All treatments significantly ($P < 0.05$) reduced Psa severity by 41% (FC only control) to 78% (YCom1 + YCom2 FC). This assay demonstrated that absence of the relatively more expensive YCom3 did not compromise YM2 efficacy against Psa. Further, the combination of YCom1 and YCom2 was significantly ($P < 0.05$) better at controlling Psa, than when either YCom1 or YCom2 when used alone.

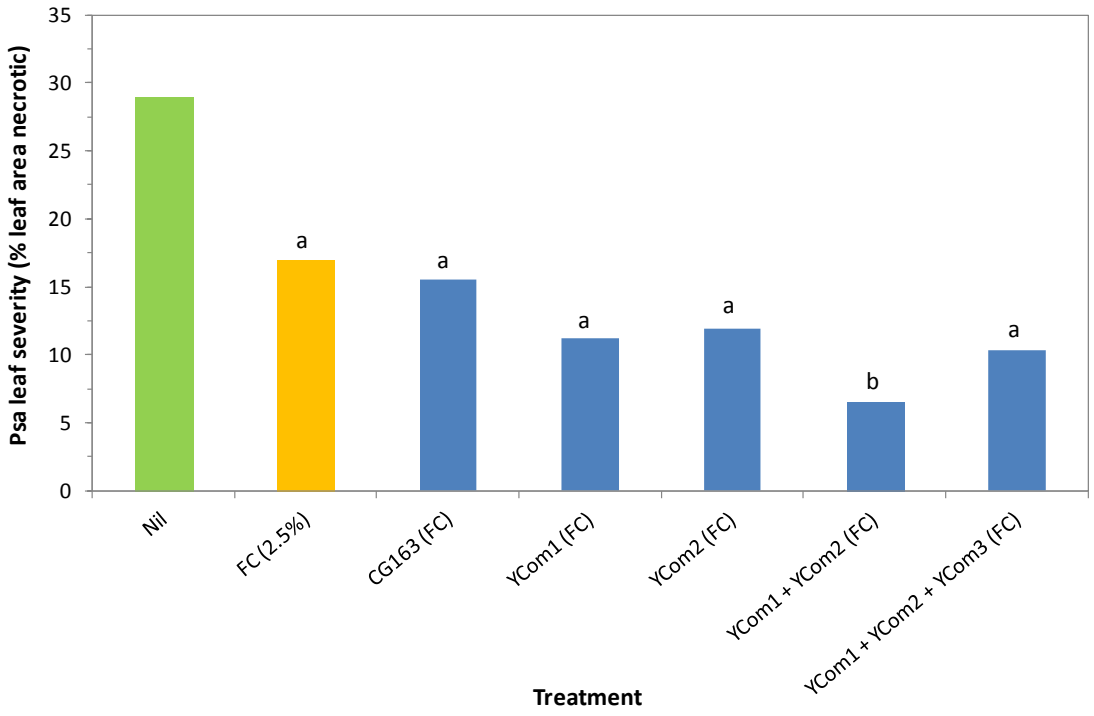

Figure 4. The effects of YM2 components alone and combined on *Pseudomonas syringae* pv. *actinidiae* leaf severity after application to *Actinidia deliciosa* 'Bruno' seedlings 1 day before inoculation (dbi) with Psa (1 x 10^8 CFU/mL) on 16 April 2013. Data are raw means (SED = 3.21, LSD(5%) = 6.38, Fprob =< 0.001). Bars with different letters are significantly different to each other ($P < 0.05$), based on log-transformed data and comparison against the FC(2.5%) control treatment (orange bar).

Where:

All yeast treatments were applied at a final concentration of 5 x 10^7 CFU/mL

CG163 is a yeast under development by PFR and is a component of YM1

FC = Foodcoat. A commercial adjuvant that has been reported to improve yeast survival in field trials overseas

YCom1, YCom2 and YCom3 are commercial yeasts; the three original components of YM2.

In this assay, FC applied at 2.5% also had efficacy against Psa, but its cost is too high to be economically viable for use in kiwifruit. Zespri/KVH requested that a further assay be carried out to identify a suitable, cost effective replacement for FC. A range of adjuvants was screened for compatibility with YM2 in multiwall studies and the most cost-effective adjuvant (Latron B) was selected for further evaluation with YM2 on potted plants in glasshouse studies.

3.2.2 PFR Assay 18 – YM2 and its components in Latron B

The primary aim of this assay was to investigate the efficacy of the two YM2 components when applied with the adjuvant, Latron B. Secondary aims were to confirm if YCom1 and YCom2 combined had greater efficacy than when they were used alone. A plant derived PFR compound was also evaluated for efficacy against Psa alone and in combination with YCom1. In the absence of any treatment, the average Psa leaf severity on 'Hayward' seedlings was 24% (green bar Figure 5) and all treatments significantly reduced Psa severity by 33% (LB) to 87% (YCom1 + YCom2 LB).

In other related assays carried out by the research team, the effect of LB on Psa leaf severity has been inconsistent, ranging from no significant effect to significant reductions in Psa. The reason for this is unclear but one hypothesis is that wetting agents are potentially interfering with Psa during the infection process. This assay demonstrated that the combination of YCom1 and YCom2 achieved significantly ($P < 0.05$) greater control of Psa (87% efficacy than YCom1 alone (66% efficacy). This assay also demonstrated that further assays are warranted to establish if PFR-BE has any efficacy at lower concentrations.

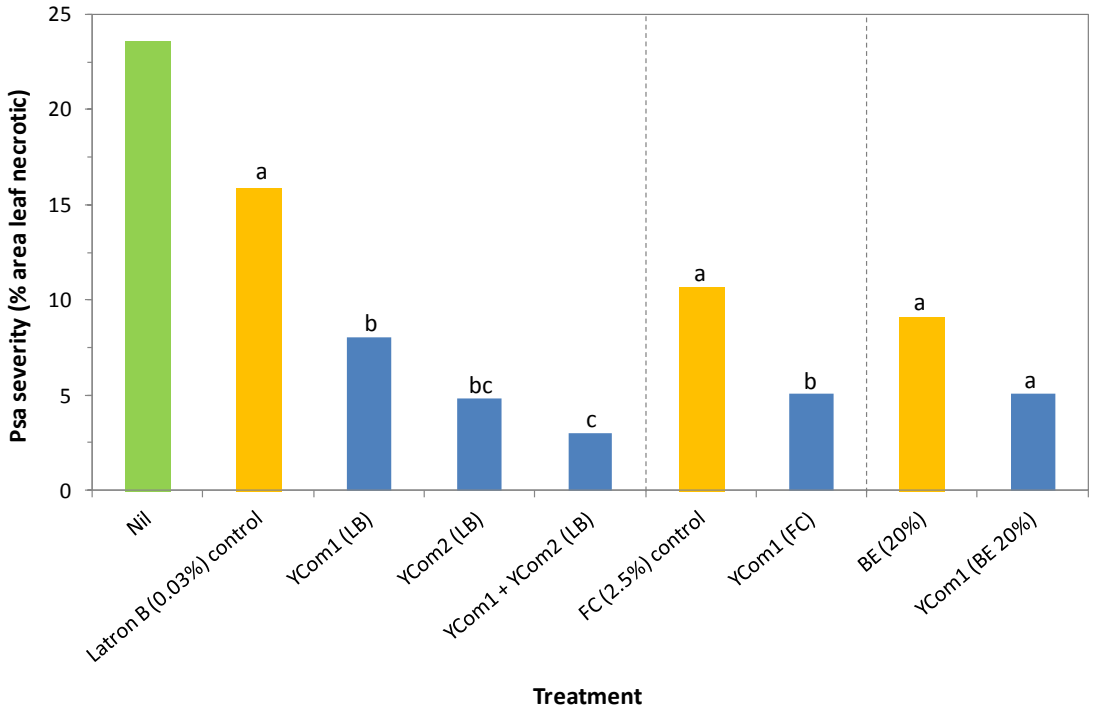

Figure 5. The effects of YM2 yeast components alone and combined on *Pseudomonas syringae* pv. *actinidiae* leaf severity after application to *Actinidia deliciosa* 'Hayward' seedlings 1 day before inoculation (dbi) with Psa (1 x 10^8 CFU/mL), on 31 May 2013. Data are raw means (SED = 2.31, LSD(5%) = 4.64, Fprob =< 0.001). Bars with different letters are significantly different to each other (*P* < 0.05), based on log-transformed data and comparison against the respective control treatments (orange bars).

Where:

All yeast treatments were applied at a final concentration of 5 x 10^7 CFU/mL

BE is a plant derived compound under development by PFR and LB = Latron B

FC = Foodcoat. A commercial adjuvant that has been reported to improve yeast survival in field trials overseas

YCom1 and YCom2 are commercial yeasts used in YM2.

4 Delivery and recovery of TMix1 in potted kiwifruit plants

4.1 Methods and materials

4.1.1 Application methods for TMix1 in potted plants

PFR Assay 19 was established to investigate different methods for applying TMix1 to potted kiwifruit and the effects of root pruning and fresh potting mix on susceptibility to Psa infection. The trial had nine treatments that were all stab inoculated with Psa using a toothpick dipped in freshly prepared Psa inoculums. There were 10 replicate plants per treatment. The treatment details are described in Table 5. TMix1 was supplied by Lincoln University and the initial treatment application, root pruning and repotting took place on 15 February 2013. Plants were grown in a glasshouse for 36 days prior to transferring to the containment glasshouse for Psa inoculation on 26 March 2013.

Following Psa inoculation, plants were held in high humidity tents to favour infection. Psa symptoms were scored after 4 weeks on 22 April 2013. The stem lesion at the point of inoculation was recorded, as well as leaf necrosis, wilting and tip death. A mean Psa severity score was derived from this data which combined these different symptoms, which had a scale ranging from 0 to 12, where 12 was complete plant collapse and decay.

Table 5. Assay 19 treatments applied to *Actinidia deliciosa* 'Bruno' seedlings consisting of combinations of TMix1 application, root pruning, repotting and YM2 application.

TRT No.	Treatment description	Potting mix	TMix1[1] applied		YM2[2] applied	Psa inoculation[3]
			15 Feb.	8 Mar.	25 Mar.	26 Mar.
1	Nil (-T)	Existing + 100 ml water	-	-	-	Stab
2	Drench[4] x1 (TMix1)	Existing mix	TMix1 (wet)	-	-	Stab
3	Drench x2 (TMix1)	Existing mix	TMix1 (wet)	TMix1 (wet)	-	Stab
4	Root prune[5] in pot (-T)	Existing mix + 100 ml water	-	-	-	Stab
5	Root prune in pot + Drench (TMix1)	Existing mix	TMix1 (wet)	-	-	Stab
6	Root prune and Repot fresh mix (-T)	Fresh + 100 ml water	-	-	-	Stab
7	Root prune + Dip[6] (TMix1) and Repot fresh mix + Dry[7] (TMix1)	Fresh mix add water to base of tray	TMix1 (wet + dry)	-	-	Stab
8	Root prune and Repot fresh mix + Dry (TMix1)	Fresh mix add water to base of tray	TMix1 (dry)	-	-	Stab
9	Repot fresh mix + Dry (TMix1)	Fresh mix add water to base of tray	TMix1 (dry)	-	-	Stab

[1] TMix1 is *Trichoderma* Mix 1, a mixture of 3 isolates of *Trichoderma* spp. Supplied by BioProtection Centre, Lincoln University

[2] YM2 is Yeast Mix 2, applied at 5 x 10^7 CFU/mL in the additive FC (2.5%)

[3] Psa stab inoculation (1 x 10^8 CFU/mL) to plant stem using a toothpick; Psa spray inoculation (1 x 10^8 CFU/mL) to underside of leaves

[4] Drench = 100 mL of TMix1 suspension (5 x 10^6 spores/mL) added to each pot

[5] Root prune = root system reduced by approximately 50% using a sharp knife

[6] Dip = Root system dipped briefly into a 1.5 L container of TMix1 suspension (5 x 10^6 spores/mL)

[7] Dry = 0.5 g of TMix1 solid substrate (equivalent to 5 x 10^6 spores) was added to the root zone at repotting.

4.1.2 Recovery of endophytic *Trichoderma* from root samples

The persistence of *Trichoderma* in the roots of older, more established kiwifruit plants was determined by isolating root endophytic *Trichoderma* from the roots of selected potted kiwifruit vines as part of the Zespri/KVH potted field trial 2 (see Section 5.1, below).
Forty representative vines were chosen as follows: Two cultivars ['Bruno' and *A. chinensis* x *A. deliciosa* 'Zesh004' (commonly known as Green14)], four treatments (T1 = water control; T2 = Psa control; T3 = combination treatment of TMix1 + (YM2 + FC) + Actigard (TMix1+); T6 = TMix1 only) and five replicates per treatment (Replicates 1, 3, 5, 7 and 9) were sampled at the beginning of July 2013 and after completion of the 'Bruno' and Green14 potted field

trials. Root sampling was carried out by cutting the entire root ball from the base of each plant, then washed thoroughly on site to remove excess soil. Each root ball was double bagged, in accordance with relevant MPI transfer protocols and sent to the Bio-Protection Research Centre (Lincoln University) for processing.

For each sample, 3 or 4 subsamples (clumps of roots) were taken from each root ball by cutting off root mass from roughly the same location of each sample (middle, outside). The subsamples were thoroughly washed and cut into ca. 1–2 cm long pieces, which were placed into a Petri dish and soaked in Virkon® (1% w:v) for 10 min for surface sterilisation. After rinsing the root pieces in sterile distilled water, five healthy, representative root pieces per sample were transferred onto one of each of five replicate plates of *Trichoderma* selective MRB Agar (Malt extract 10 g, Yeast extract 1 g, Terrachlor (quintozene) 0.2 g, Rose Bengal 0.15 g, Agar 15.0 g, make up to 1 L).

Plates were incubated at 25°C in the dark for 1 week and then under ambient light and temperature conditions (bench top) for another 1–2 weeks. Following incubation, plates were visually assessed and the total number of *Trichoderma* colonies was counted. *Trichoderma* colonies were sub-cultured onto Malt Yeast Agar (Malt extract 10 g, Yeast extract 1 g, Agar 15 g, made up to 1 L) and were classified based on colony appearance.

4.2 Results and discussion

4.2.1 Application methods for TMix1 in potted plants

The aim of this assay was to determine whether different root treatments and TMix1 application methods affected Psa development following stab inoculation of the growing stem. Lesion size only ranged from 1 to 10 mm, indicating that stem infections did not spread extensively up and down the stem in ‘Bruno’ seedlings. Leaf necrosis (> or = to 2% area) had developed in 23% of the leaves, across all treatments and there were no significant treatment effects (P = 0.199).

The mean Psa severity score for the three treatments that did not receive TMix1 application ranged from 1.9 to 3.6 (Figure 6). The chart below with all the treatments evaluated has been divided into three sections for ease of describing the key findings. In the left-hand section of this graph, there was no significant reduction in Psa severity when TMix1 was drenched onto the plants either once or twice (no root pruning and no repotting). In the middle section of the graph there were two treatments where the roots were pruned *in situ* but the plants were not repotted. In this case there was no significant difference between the TMix1 treated plants and those not receiving TMix1. In the right hand section there were four treatments that involved repotting with fresh potting mix and plus and minus TMix1 were compared. The results indicated no significant difference between the three treatments that received *Trichoderma* and the treatment that did not.

In summary, irrespective of the application method, TMix1 did not reduce the overall Psa severity score following stab inoculation of ‘Bruno’ seedlings.

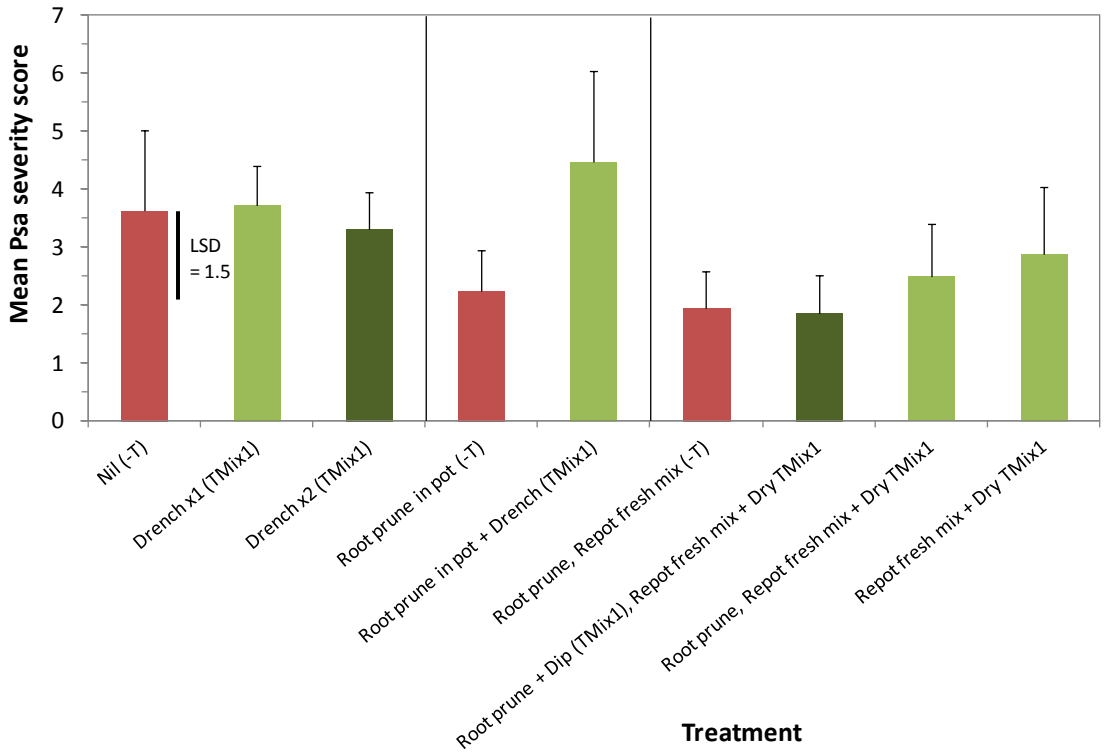

Figure 6. Mean *Pseudomonas syringae* pv. *actinidiae* severity score for *Actinidia deliciosa* 'Bruno' seedlings treated with various combinations of TMix1 application, root pruning and repotting, then stab inoculated with Psa. Treatments without *Trichoderma* application are shown in red (-T), treatments receiving two applications of *Trichoderma* are shown in dark green. Whiskers are standard errors of the mean and black bar is the Least Significant Difference (*P* < 0.05).

4.2.2 Recovery of endophytic *Trichoderma* from root samples

After 3 weeks of incubation, a range of different *Trichoderma* types were identified by their morphological appearance (e.g. white fluffy, white, white green, white yellow, yellow, green and dark green) (Table 6). The visual categorisation indicated that some colony types were more common than others.

Table 6. Percentage distribution of *Trichoderma* morphological types according to colony type identified by visual assessment presented by kiwifruit cultivar.

Cultivar	white fluffy	white	white green	white yellow	yellow	green	dark green
'Bruno'	39%	34%	3%	6%	3%	11%	3%
Green14	46%	27%	1%	9%	12%	3%	3%

Significantly greater numbers of *Trichoderma* were isolated from the roots of the treated vines (TMix1 and TMix1+) than from the untreated controls with most isolates found in the combination treatment (T3 – TMix1+) (Table 7 and 8, Figure 8). Cultivar-specific differences were also observed as greater numbers of *Trichoderma* were recovered from roots of kiwifruit vines of the cultivar 'Bruno'.

Future studies are needed to identify the correlations and interactions that lead to these differences. Developing molecular tools to positively identify the *Trichoderma* strains in present root samples to compare them to the applied strains and to be able to discriminate inoculated from naturally occurring *Trichoderma* more easily will be a first step to achieving this task.

Table 7. ANOVA results (Mean *Trichoderma* colonies, *P* values, LSD and standard errors of difference) for *Trichoderma* colonies by cultivar, treatment and cultivar treatment interaction.

					F pr.	LSD	s.e.d.
Cultivar effect	'Bruno'	Green14					
	2.4	1.8			0.005	0.419	0.213
Treatment effect	H_2O	Psa	TMix1+	TMix1			
	0.76	1.2	3.7	2.9	<0.001	0.593	0.301
Cultivar-treatment interaction	H_2O	Psa	TMix1+	TMix1			
'Bruno'	0.92	1.4	4.0	3.4	0.611	0.839	0.425
Green14	0.60	0.92	3.5	2.4			

For both cultivars, most *Trichoderma* colonies were found in the combination treatment (T3 – TMix1+) followed by the *Trichoderma* only treatment T6 (TMix1) (Table 8). Overall, more *Trichoderma* colonies were isolated from 'Bruno' roots (57% of all *Trichoderma*) than from Green14 roots (43% of all *Trichoderma*) and most *Trichoderma* were isolated from T3 (TMix1+), followed by T6 (TMix1) > T2 (Psa only) > T1 (H_2O only).

Table 8. Proportion of total number of *Trichoderma* colonies recovered from 25 root pieces/treatment for two kiwifruit cultivars (‘Bruno’ and Green14).

Treatment	‘Bruno’	‘Green14’
T1 – No Psa	9%	8%
T2 – Psa	14%	13%
T3 – TMix1+	41%	47%
T6 – TMix1	36%	32%

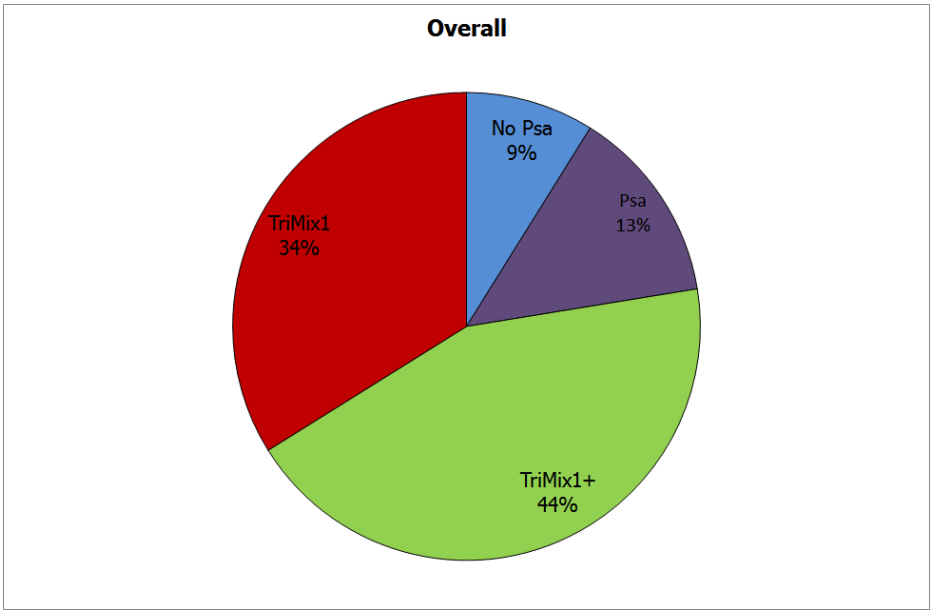

Figure 8. Average of *Trichoderma* counts for cultivars *Actinidia deliciosa* ‘Bruno’ and *A. chinensis* x *A. deliciosa* ‘Zesh004’ (commonly known as Green14) following re-isolation of *Trichoderma* from roots of potted kiwifruit vines. Treatments: No Psa (T1) – H_2O control; Psa (T2) – Psa control; TMix1+ (T3) – combination treatment of yeast, *Trichoderma* and Actigard®; TMix1 (T6) – *Trichoderma* only.

A molecular-based tool to identify the *Trichoderma* spp. isolates in treated kiwifruit root samples is being developed. This will allow us to determine the frequency with which the applied strains are recovered from roots (in this case TMix1). Results show that root inoculation with *Trichoderma* resulted in the establishment of an endophytic *Trichoderma* community in the roots.

The greater frequency with which *Trichoderma* in the roots of plants that had received the combination treatment is most likely explained by a positive feedback effect between the roots of the healthier plants and the naturally occurring soil microbial community. The health/ disease status of the plants will strongly influence the rhizosphere environment through root exudate quality and quantity. The greater number of *Trichoderma* will in turn further promote plant health, nutrient uptake and disease resistance.

5 YM2 and TMix1 field efficacy trials

5.1 Potted plant trials (Zespri/KVH)

Four potted plant trials, each with a different cultivar ('Hayward', *A. chinensis* 'Zesy002' (commonly known as Gold3), 'Bruno' and Green14) were carried out by Zespri/KVH on two separate occasions and have been previously reported to Zespri. Full copies of these reports are included in this report (Appendix 1 and 2) as these trials were part of the project description for this project.

5.2 Whole vine trials (PFR/LU)

5.2.1 Methods and materials

Three trials sites were established, with each orchard having a different cultivar ('Hort16A', 'Hayward' and Gold3), during the autumn of 2013 to field validate the efficacy of TMix1 and YM2 in growers' orchards. The spray trials commenced immediately after harvest. There were three treatments, each with 15 replicates at each trial site:

1. Grower standard. This consisted of PFR staff applying a similar Psa spray programme to that of each respective grower. Timing of these applications generally took place within a week of the growers making their applications. The same products were used, except on some occasions in the 'Hayward' block, a different copper product was used.
2. TMix1 and YM2. This treatment consisted of two applications of TMix1 (autumn and spring) and regular (2–3 weekly) applications of YM2 from fruit harvest to leaf drop and again from bud-burst to flowering. During the intervening period (winter) the growers applied their standard Psa spray programme across the whole trial site, since the YM2 is targeted at control of Psa on leaves.
3. YM2. This treatment consisted of YM2 applied to vines at the same time as in treatment 2 above.

The standard application rate for YM2 was initially 344 g/100 L until October 2013 when a new higher viability component was used which reduced the amount of YM2 applied to 183 g/100 L these (amounts are approximately equivalent to 2 x 10^7 CFU/mL). TMix1 was applied as a 20 L root drench/vine and because the soil conditions were fairly dry, at two sites the ground was pre-wetted with 20 L water and another 20 L of water was applied after application of the TMix1, at the third site irrigation was used to wet the soil. There were buffer plots around the perimeter of the trial area and these were treated the same as the grower standard treatment.

5.2.2 'Hort16A'

This trial was carried out at an orchard near Katikati. Vines in the block of 'Hort16A' were pergola trained and were growing on their own root system (i.e. no rootstock) and were double planted (two vines per bay) with a row spacing of 3.6 m and length of bay of 5 m. Psa symptoms were present within several of the vines within the block during that growing

season; however, the vines generally looked healthy and were still carrying a crop prior to commencing the trial. The treatments were applied according to the schedule shown in Table 9.

Table 9. Schedule of treatments applied to *Actinidia chinensis* 'Hort16A' vines during autumn and spring 2013.

Date	Treatments applied	Product (adjuvant)	Rates
12 April 2013	*Trichoderma* root drench[1]	TMix1	5 g made up in 20 L per vine
1 May 2013	Spray treatments	Kocide Opti (Du Wett) YM2 (Latron B)	40 g/100 L (40 mL/100 L) 344 g/100 L (30 mL/100 L)
13 May 2013	Spray treatments	Kocide Opti (Du Wett) YM2 (Latron B)	40 g/100 L (40 mL/100 L) 344 g/100 L (30 mL/100 L)
6 June 2013	Leaf drop spray	Copper sulphate (Latron B)	900 g/100 L (25 ml/100 L)
11 June	Spray treatments[2]	Kocide Opti (Du Wett) YM2 (Latron B)	40 g/100 L (40 mL/100 L) 344 g/100 L (30 mL/100 L)
3 Sept. 2013	Spray treatments[3]	Kocide Opti (Du Wett) YM2 (Latron B)	40 g/100 L (40 mL/100 L) 344 g/100 L (30 mL/100 L)
18 Sept. 2013	Spray treatments	Kocide Opti (Du Wett) YM2 (Latron B)	40 g/100 L (40 mL/100 L) 344 g/100 L (30 mL/100 L)
2 Oct. 2013	Spray treatments	Kocide Opti (Du Wett) YM2 (Latron B)	40 g/100 L (40 mL/100 L) 183 g/100 L (30 mL/100 L)[4]

[1] Irrigation sprinklers were used to moisten soil prior to TMix1 application and again afterwards to help assist soil penetration

[2] Leaf fall was not complete, estimated 20% leaf remaining on vines

[3] Bud-burst ~90% complete

[4] Newly sourced granules of YM2 used.

Between the leaf drop application on 6 June 2013 and the re-commencement of treatment applications in September 2013, the grower applied his winter spray programme across the whole trial site. This consisted of Nordox (1.1 kg/1000 L/ha) on 19 June and 31 July, and Hi-Cane® (6% at 700 L/ha) on 1 August.

Disease assessments were carried out on three occasions. Firstly, on 23 April 2013, Psa symptoms were assessed on approximately half of the trial vines, as a measure of background infections that were already established within the vines at the start of the trial. A second Psa disease assessment was carried out during dormancy on 25 June 2013, and a final assessment was carried out on 16 October 2013.

It had been agreed with the grower that any vines showing significant sign of secondary Psa symptoms (bleeding/ooze from either the trunk or main leaders) were to be cut out by the grower to reduce the risk of further Psa inoculum being produced. Although all vines appeared reasonably healthy at the beginning of the trial, many vines had been removed by the grower when the third assessment was done, effectively reducing the trial to 5–7 replicates per treatment.

5.2.3 'Hayward'

This trial was carried out at a grower's property near Katikati. This block of 'Hayward' was pergola trained and growing on 'Bruno' rootstock. The bays were 3 m wide and female vines were spaced down the row at 5 m. Every second row was strip male vines that were spaced at 15 m, with a narrow canopy running along the row. As with the 'Hort16A' trial, fruit were harvested prior to commencing the trial. The treatments were applied according to the schedule shown in Table 10. Psa disease assessments were carried out on 2 October and 18 December 2013.

Table 10. Schedule of treatments applied to *Actinidia deliciosa* 'Hayward' vines during autumn and spring 2013.

Date	Treatments applied	Product	Rates
16 May 2013	*Trichoderma* root drench[1]	TMix1	5 g made up in 20 L per vine
20 May 2013	copper/YM2	Kocide Opti (Du Wett) YM2 (Latron B)	40 g/100 L (40 mL/100 L) 344 g/100 L (30 mL/100 L)
12 June 2013	copper/YM2	Kocide Opti (Du Wett) YM2 (Latron B)	40 g/100 L (40 mL/100 L) 344 g/100 L (30 mL/100 L)
9 July 2013	copper/YM2	Kocide Opti (Du Wett) YM2 (Latron B)	40 g/100 L (40 mL/100 L) 344 g/100 L (30 mL/100 L)
1 Oct 2013	*Trichoderma* root drench[2]	TMix1	20 g made up in 20 L per vine[3]
2 Oct. 2013	copper/YM2	Kocide Opti (Du Wett) YM2 (Latron B)	40 g/100L (40 mL/100 L) 183 g/100 L (30 mL/100 L)[4]
Mid Oct. 2013	copper/YM2	Kocide Opti (Du Wett) YM2 (Latron B)	40 g/100 L (40 mL/100 L) 183 g/100 L (30 mL/100 L)
1 Nov. 2013	copper/YM2	Nordox 75WG Ambitious 10SL YM2 (Latron B)	37.5 g/100 L 75 mL/100 L 183 g/100 L (30 mL/100 L)
12 Nov. 2013	copper/YM2	Ambitious 10SL YM2 (Latron B)	75 mL/100 L 183 g/100 L (30 mL/100 L)
30 Nov. 2013	copper/YM2	Nordox 75WG (Du Wett) YM2 (Latron B)	37.5 g/100 L (20 mL/100 L) 183 g/100 L (30 mL/100L)

[1] 20 L of water were applied to the ground to moisten the soil prior to application of 20 L of TMix1, and again afterwards to help assist soil penetration

[2] 20 L of TMix1 applied, no pre or post wetting as soil was already moist

[3] Rate of TMix1 applications increased to 20 g/vine on advice from Lincoln University

[4] Newly sourced granules of YM2 used.

5.2.4 Gold3

This trial was carried out at an orchard near Te Puke. This block of Gold3 was pergola trained and growing on 'Bruno' rootstock (re-grafted over from 'Hort16A'). The bays were 5 m wide and female vines were spaced down the row at 5 m. Every second row had strip male vines that were trained to run across the rows. The treatments were applied according to the schedule shown in Table 11. Psa disease assessments in the Gold3 block were carried out on 16 June and 30 October 2013.

Table 11. Schedule of treatments applied to *Actinidia chinensis 'Zesy002' (commonly known as* Gold3) vines during autumn and spring 2013.

Date	Treatments applied	Product	Rates
17 April 2013	*Trichoderma* root drench[1]	TMix1	5 g made up in 20 L per vine
30 April 2013	copper/YM2	Kocide Opti Actigard YM2 (Latron B)	50 g/100 L 20 g/100L 344 g/100 L
14 May 2013	copper/YM2	Kocide Opti (DuWett) Actigard YM2 (Latron B)	50 g/100 L (20 ml/100L) 20 g/100 L 344 g/100 L (30 ml/100 L)
25 June 2013	Grower applied (leaf drop spray)	CuSO4	
2 July 2013	copper/YM2	Kocide Opti (Du Wett) YM2 (Latron B)	40 g/100 L (40 mL/100 L) 344 g/100 L (30 mL/100 L)
22 September 2013	*Trichoderma* root drench[2]	TMix1	20 g made up in 20 L per vine[3]
2 Oct. 2013	copper/YM2	Kocide Opti (Du Wett) YM2 (Latron B)	50 g/100 L (40 mL/100 L) 183 g/100 L (30 mL/100 L)[4]
10 Oct. 2013	copper/YM2	Kocide Opti (Du Wett) YM2 (Latron B)	40 g/100 L (40 mL/100 L) 183 g/100 L (30 mL/100 L)

[1] 20 L of water were applied to the ground to moisten the soil prior to application of 20 L of TMix1, and again afterwards to help assist soil penetration

[2] 20 L of TMix1 applied, no pre or post wetting as soil was already moist

[3] Rate of TMix1 applications increased to 20 g/vine on advice from Lincoln University

[4] Newly sourced YM2 granules used.

5.2.5 Results and discussion

Potted plant trials (Zespri/KVH)

A brief summary of these potted plant trials is provided here; the full reports are in Appendices 1 and 2.

'Hayward'

Three weeks after inoculation the average leaf spot severities in the untreated controls were 6% and 18% for the mature and expanding leaves respectively (Appendix 1, Figures 2 & 3). The FC treatment did not significantly ($P > 0.05$) reduce Psa leaf severity on mature (treated) or expanding (untreated) leaves. On the treated mature leaves and the expanding leaves (which did not receive treatment applications), the combined treatment of TMix1+YM2+Actigard significantly ($P < 0.05$) reduced Psa leaf spot severity, compared with the untreated control after 21 days. Unfortunately, the YM2 only treatment was not inoculated with Psa (due to operator error) resulting in a low leaf spot severity in that treatment and therefore no conclusions can be drawn regarding the efficacy of this treatment.

Gold3

In this trial the severity of leaf spotting on the mature leaves in the untreated control vines was relatively low (3.3%) 42 days after Psa inoculation (Appendix 1, Figure 4). This indicates that some caution is required when interpreting these results since Gold3, in our experience, is not as susceptible to leaf infection as the other cultivars used in these Zespri/KVH trials. However, statistically significant reductions in Psa infection were achieved as a result of several treatments. Data analysis showed that six treatments significantly ($P < 0.05$) reduced Psa leaf spot severity on mature leaves at each of the five assessment dates (FC, TMix1+Actigard, TMix1+YM2, TMix1+YM2+Actigard, YM2 and YM2+Actigard), compared with untreated vines. The YM2-fermented treatment significantly ($P < 0.05$) reduced Psa leaf spot severity on mature leaves at the first four assessments, but not on the final assessment 42 days after Psa inoculation. The Actigard-foliar treatment significantly ($P < 0.05$) reduced Psa leaf spot severity on mature leaves only on the 21 and 28 day assessments. There was no statistical difference in leaf spot severity between the untreated control and the TMix1 and Actigard-root treatments.

Despite a problem with some of the treatments being applied incorrectly, these trials provided some evidence that YM2 suppressed leaf spotting in Psa inoculated plants in the field. However, in this trial, there was no evidence that the TMix1 treatments contributed to a reduction in Psa leaf spot severity following leaf inoculation.

'Bruno'

The severity of leaf spotting in the untreated control was 23% 13 days after Psa inoculation and this was significantly ($P < 0.05$) reduced in the Actigard, YM2 and the TriMix1+YM2+Actigard treatments (Appendix 2, Figure 2). These same treatments also significantly reduced ($P < 0.05$) leaf spotting on the mature parts of the plants 22 and 28 days after inoculation (Appendix 2, Figure 4).

No secondary symptoms had developed when the trial ended (2 months after Psa inoculation); therefore the impact of treatments on this phase of Psa development could not be determined.

Green14

The severity of leaf spotting was 3% in the untreated control 13 days after Psa inoculation and this increased steadily over the following weeks, reaching 10% after 28 days (Appendix 2, Figures 5 and 6). The Actigard and TMix1+YM2+Actigard treatments significantly reduced ($P < 0.05$) the severity of leaf spotting at each of the assessments dates. The YM2 treatment significantly reduced ($P < 0.05$) the severity of leaf spotting at the final assessment, 41 days after Psa inoculation compared with the untreated control. At the earlier assessments the YM2 treatment had also reduced leaf spotting compared with the untreated control, but this was only significant at a lower level of significance ($P < 0.10$).

Although the TMix1 treatment had approximately 50% less leaf spotting than the untreated control, this was not a statistically significant reduction ($P > 0.05$).

Whole vine trials (PFR/LU)

'Hort16A'

The severity of Psa leaf spotting (0–5 scale) in this trial, located in Katikati, was similar across all three treatments in the 'Hort16A' plants (e.g. Standard grower Psa programme = 1.1 compared with the combined TMix1 and YM2 treatment = 1.5) (Table 12). The leaf spot severities of the combined TMix1 and YM2 treatment and YM2 alone treatment were not significantly different ($P < 0.05$) from the copper-based grower standard programme. Average shoot die-back per plot was also similar across all three treatments. A binominal analysis of the number of vines removed from this experimental site also indicated that there were no significant ($P > 0.05$) treatment effects on vine removal.

Table 12. *Pseudomonas syringae* pv. *actinidiae* leaf severity, shoot die-back and the proportion (%) of vines removed in the experimental *Actinidia chinensis* 'Hort 16A' site during spring of 2013.

Treatment	Average Psa leaf spotting score[1]	Average shoot die-back/vine	Proportion of vines removed (%)[7]
Grower standard[2]	1.1	0.8	47
TMix1 & YM2[3]	1.5	0.1	67
YM2[4]	1.4	0.4	53
Treatment SED[5]	0.36	0.59	21.5
Treatment Fprob[6]	0.50 ns	0.46 ns	0.65 ns

[1] The number of leaves on selected shoots were recorded and then the average severity of Psa spotting on leaves determined visually using a 0-5 scale; where 0 = no Psa spotting visible, 1= 1 to 5 spots on single leaves, 2 = greater than 5 spots on several leaves, 3 = Psa spotting on many leaves, 4 = moderate to severe spotting on most leaves and 5 = severe spotting on all leaves

[2] The grower standard programme consisted of seven copper based applications (four prior to leaf fall and three from bud burst to early spring (2 October 2013)

[3] The TMix1 and YM2 treatment consisted of one Trichoderma root drench treatment on 12 April 2013 and six foliar applications of YM2 (three in the autumn and three in the spring) that were timed to coincide with the grower standard (copper based) treatments, except for the copper sulphate spray used to drop leaves on 6 June 2013

[4] The YM2 treatment consisted of six foliar applications of YM2 (three in the autumn and three in the spring) that were timed to coincide with the grower standard (copper-based) treatments, except for the copper sulphate spray used to drop leaves on 6 June 2013

[5] Standard error of the deviation of the mean

[6] Data were analysed using the restricted maximum likelihood (REML) function in GENSTAT

[7] A binomial analysis was used to analysis the proportion of vines removed from each treatment within the experimental trial area.

ns = not statistically significant at $P < 0.05$.

'Hayward'

Plants that received the grower's standard Psa spray programme had an average leaf spotting severity of 1.5 (Table 13). The combined treatment of TMix1 and YM2 with an average leaf spotting severity of1.9) was not significantly ($P > 0.05$) from that of the grower standard programme. YM2 applied alone had significantly ($P < 0.05$) more leaf spotting (2.5) than on the grower standard treatment and the combined treatment. Average shoot die-back and average cane die-back in the male vines were similar across all three treatments (Table 13).

Table 13. *Pseudomonas syringae* pv. *actinidiae* leaf severity, shoot and cane die-back in the experimental *Actinidia deliciosa* 'Hayward' site during the spring of 2013.

Treatment	Average Psa leaf spotting score[1]	Average shoot die-back (male vines only)	Average cane die-back (male vines only)
Grower standard[2]	1.5	20.0	1.8
TMix1 & YM2[3]	1.9	14.8	2.5
YM2[4]	2.5	17.8	1.0
Treatment SED[5]	0.29	7.65	1.37
Treatment Fprob[6]	0.007***	0.794 ns	0.569 ns

[1] The number of leaves on selected shoots were recorded and then the average severity of Psa spotting on leaves determined visually using a 0-5 scale; where 0 = no Psa spotting visible, 1= 1 to 5 spots on single leaves, 2 = greater than 5 spots on several leaves, 3 = Psa spotting on many leaves, 4 = moderate to severe spotting on most leaves and 5 = severe spotting on all leaves

[2] The grower standard programme consisted of seven copper-based applications (four prior to leaf fall and three from bud burst to early spring (2 October 2013)

[3] The TMix1 and YM2 treatment consisted of one *Trichoderma* root drench treatment on 12 April 2013 and six foliar applications of YM2 (three in the autumn and three in the spring) that were timed to coincide with the grower standard (copper-based) treatments, except for the copper sulphate spray used to drop leaves on 6 June 2013

[4] The YM2 treatment consisted of six foliar applications of YM2 (three in the autumn and three in the spring) that were timed to coincide with the grower standard (copper based) treatments, except for the copper sulphate spray used to drop leaves on 6 June 2013

[5] Standard error of the deviation of the mean

[6] Data were analysed using the restricted maximum likelihood (REML) function in GENSTAT

ns = not statistically significant at $P < 0.05$.

Gold3

This trial was visually assessed on 16 July 2013 and only 10 shoots with die-back symptoms were observed across the entire trial area indicating that there was insufficient data for ANOVA. On 30 October 2013, there were no Psa leaf spotting symptoms across the trial area and insufficient incidence of shoot die-back for analysis to be carried out.

6 Recommendations

Several commercially focused research streams are recommended in order to advance the commercialisation and grower availability of TMix1 and YM2 and these have been placed under suggested funding source categories:

Zespri-funded:

- Carry out further validation of TMix1 and YM2 singly and in combination with each other and with Actigard, on potted vines under the Zespri/KVH potted plant field evaluation programme. Also, if feasible, carry out a similar trial in an Italian-based potted plant trial in order to gain extra data from a different growing region and to gain advantage from an extra spring trial evaluation in the Northern Hemisphere.
- Carry out field evaluations of YM2 in commercial orchards to confirm field efficacy of commercially acceptable rates and any changes to the composition of YM2, arising from potted plant trails being carried out under other work streams, see below.

PSAF-funded:

- Investigate more cost-effective ratios of YCom1 and YCom2 other than the 1:1 ratio used to date, and determine the minimum inhibitory concentration of YM2. Glasshouse studies are underway to determine if these changes affect Psa efficacy.
- Determine the persistence of YM2 on leaves and relate this to its efficacy against Psa in order to establish appropriate spray intervals.
- Determine the effects of YM2 on bee survival and health, with and without several adjuvants.
- Determine the compatibility of YM2 with fungicides, insecticides and nutrient products that are likely to be applied during and post flowering.
- Confirm suitable mixing procedures for YM2, including the need for spray tank cleansing, and recommendations with respect to copper tolerance.

Commercial partner-funded:

- Seek dispensation for fruit disposal requirements for YM2 so that fruit compensation will not be required for next season's field trials.
- Carry out larger scale, grower-based field trials using air-blast sprayer application to confirm efficacy of the preferred final YM2 composition and rate.

PFR-funded:

- Seek IP protection for the use of yeast strains for control of bacterial plant diseases. A decision on patenting will be made following results from the latest potted plant trials (June 2014).
- Investigate the mode of action of YM2 on kiwifruit (MBIE – "Next Generation Biopesticides").

Lincoln University/Zespri-funded:

- Commercialisation of TMix1. This is proceeding under a separate project, and provides for the production of sufficient TMix1 to treat 2000 ha during the autumn/spring of 2014.
- It will be important to have some split block comparisons established across several sites so that Psa development can be monitored in treated and untreated areas.

7 Acknowledgements

We would like to thank Pete Saunders for assisting with the 'Hayward' orchard spray trial and Catherine Cameron for her very valuable assistance with statistical analysis. Many thanks to Deidre Cornish, Janet Yu and Crystal Felman for providing Psa inoculum for experiments. We also thank Kate Stannard for valuable technical support with potted plant experiments at TPRO. Thanks to Bob Fullerton, Catherine Langford and Sue Muggleston for editorial assistance.

8 References

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Appendix 1: 2012-13 Potted Plant Field Trial Report to Zespri/KVH: Trials 10 & 11- Yeast and *Trichoderma* Mixes on 'Hayward' and Gold3

2012/13 Potted Plant Field Trial Report

Trials 10 & 11

Yeast and *Trichoderma* Mixes on Hayward and Gold3

January – May 2013

November 2013

Disclaimer

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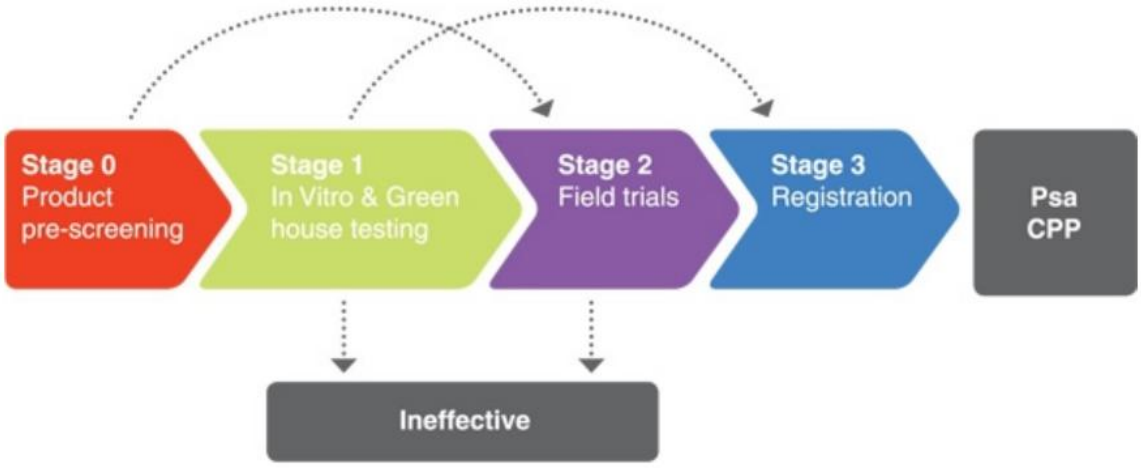
Introduction

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**	***Trichoderma* 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**	***Trichoderma* 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 m^2).

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.** 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

<u>Hayward</u>
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.

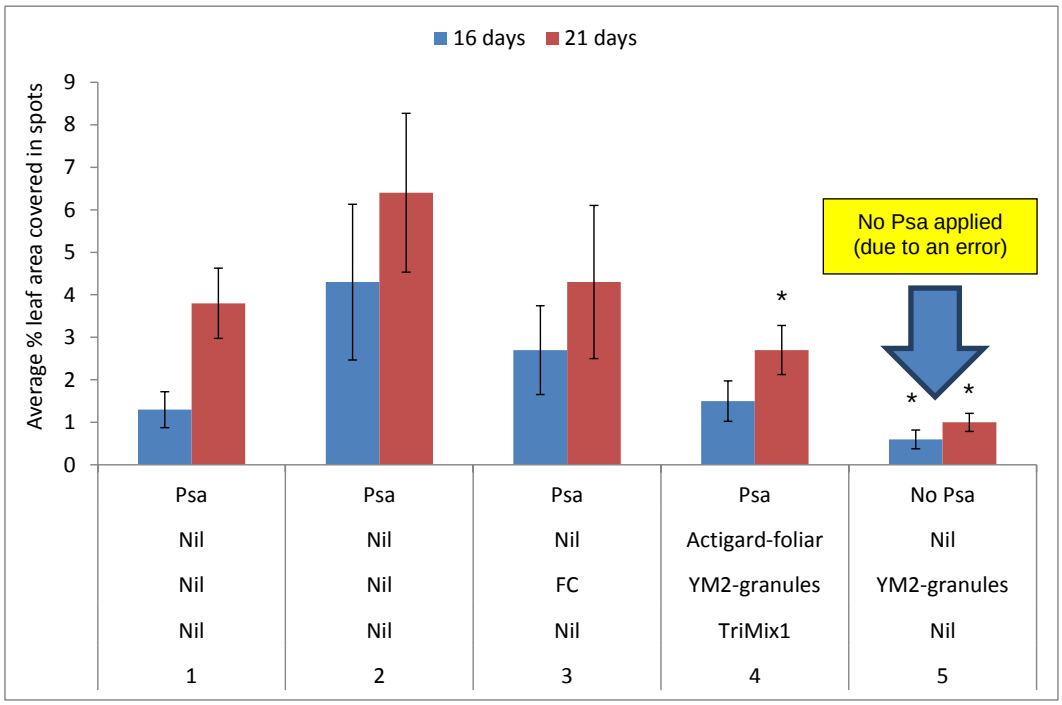
The results for Gold3 are presented in Figure 4 and Figure 5 and summarised as follows:

TRT No.	19-Dec	18 & 24 Jan	18-Jan	25-Jan	Percentage of leaf spotting relative to the untreated Psa controls (%)	
	Trichoderma 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

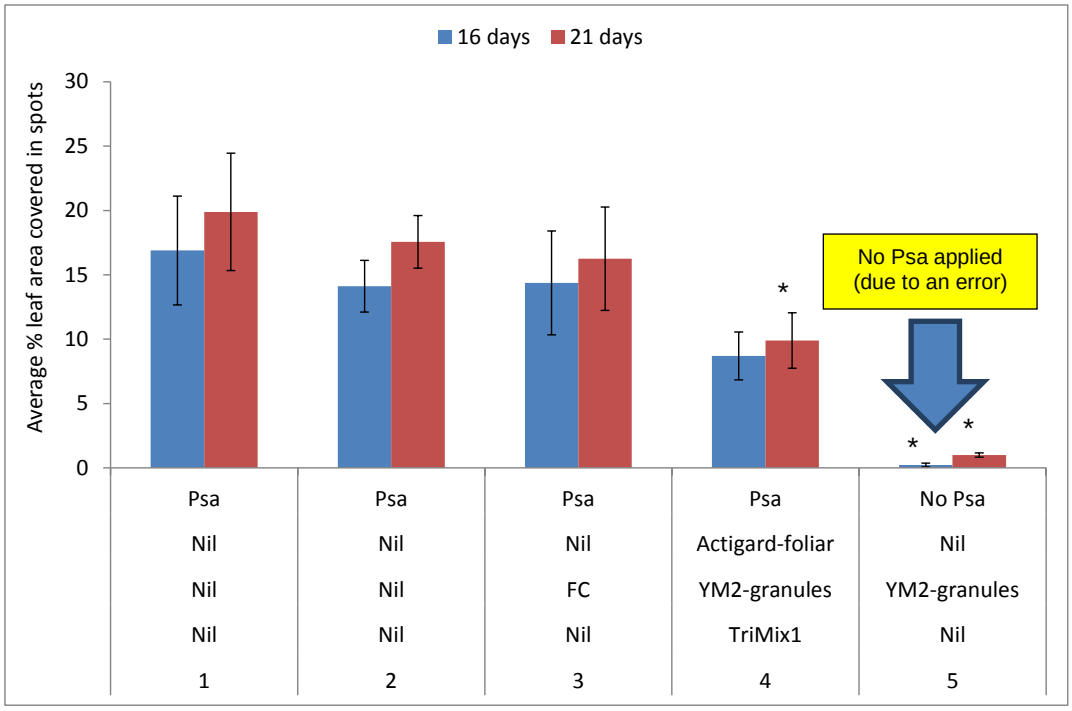
Despite a problem with some of the treatments being applied incorrectly, these trials still provide some evidence that combinations of Actigard, yeast and *Trichoderma* may be useful in the management of Psa (in terms of suppressing leaf spotting).

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.



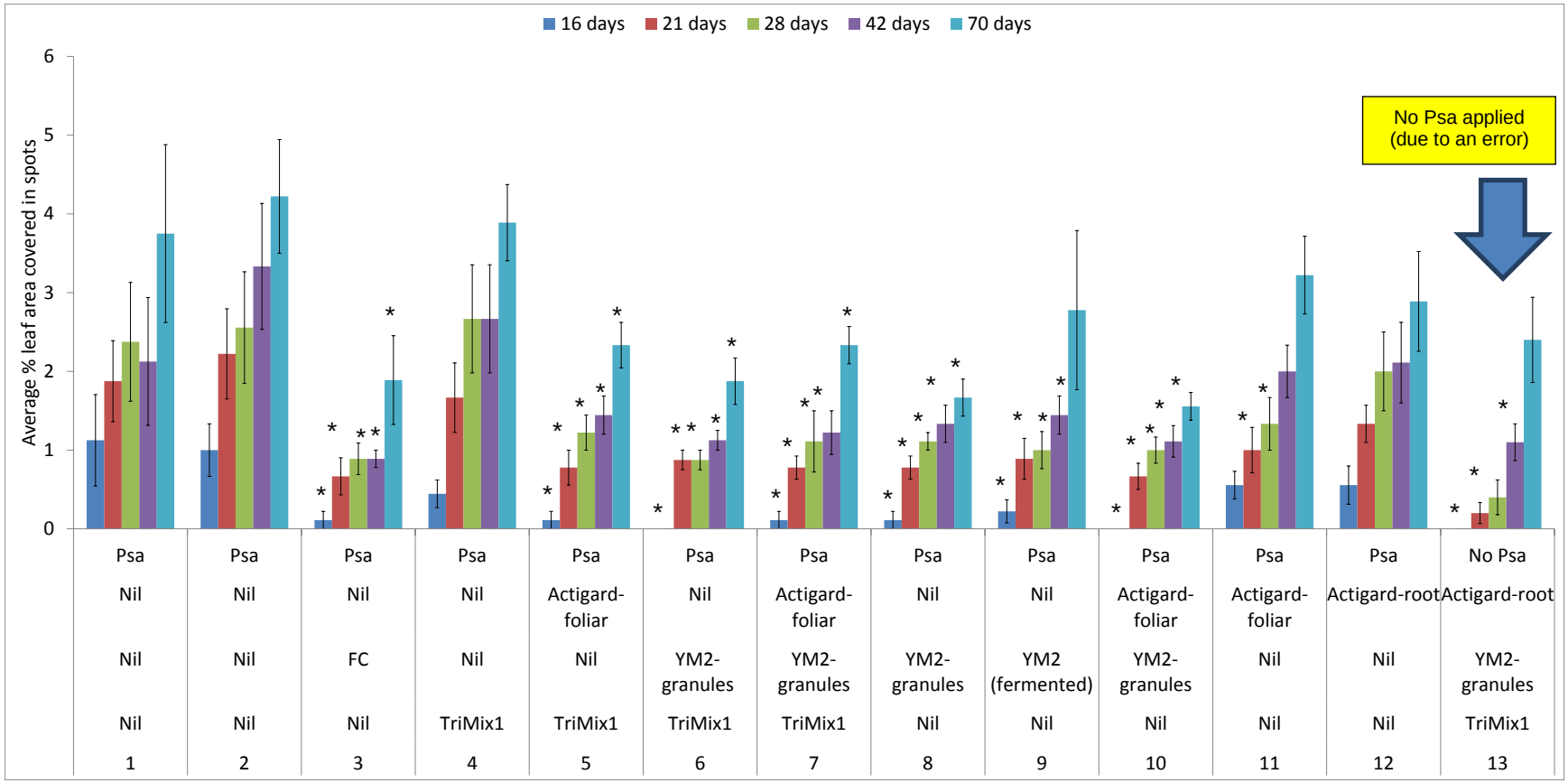
* Statistically significant at the 5% level from the Psa-only treatments (average) according to a non-parametric (Wilcoxon) test.

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.



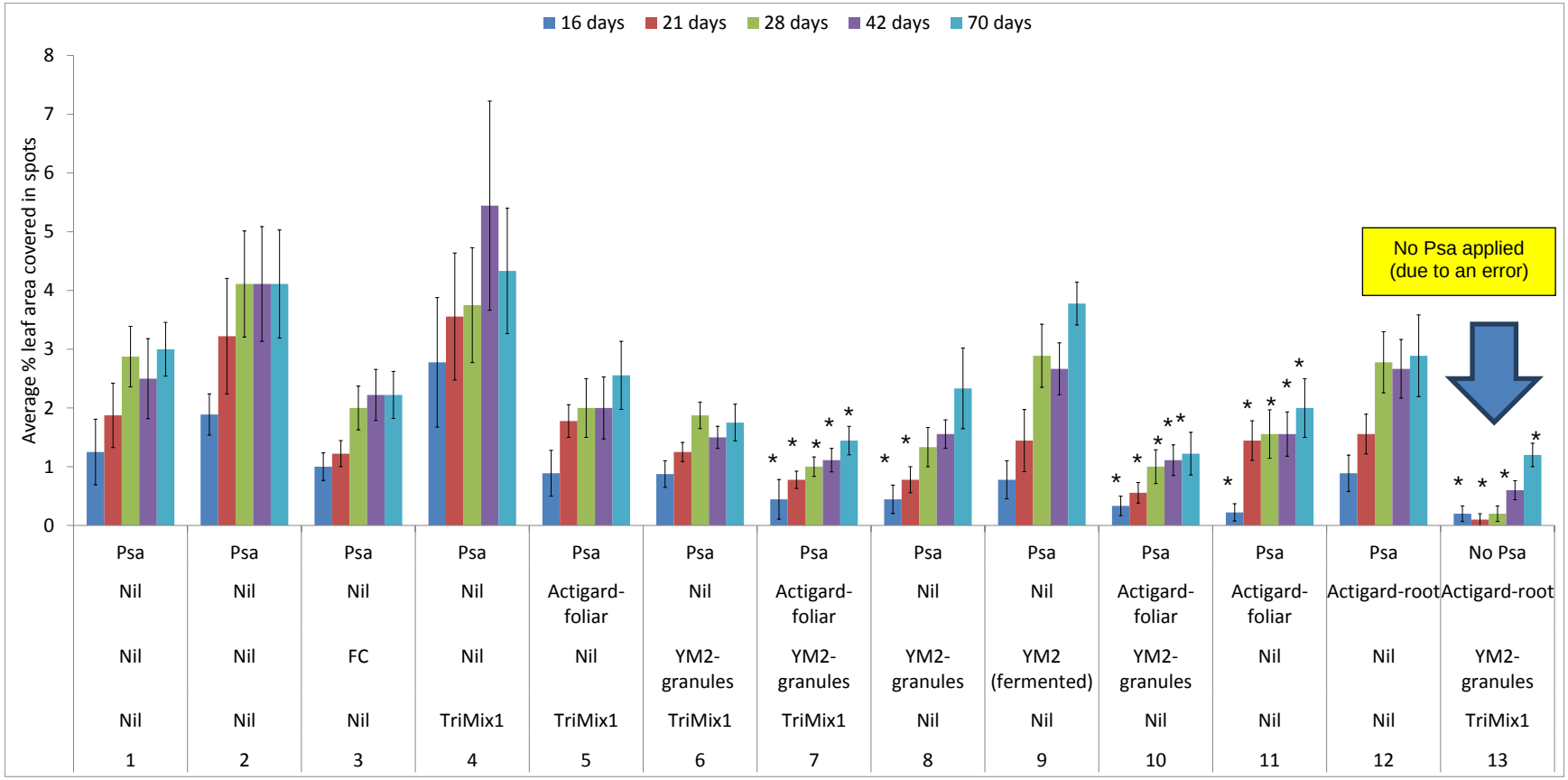
* Statistically significant at the 5% level from the Psa-only treatments (average) according to a non-parametric (Wilcoxon) test.

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.



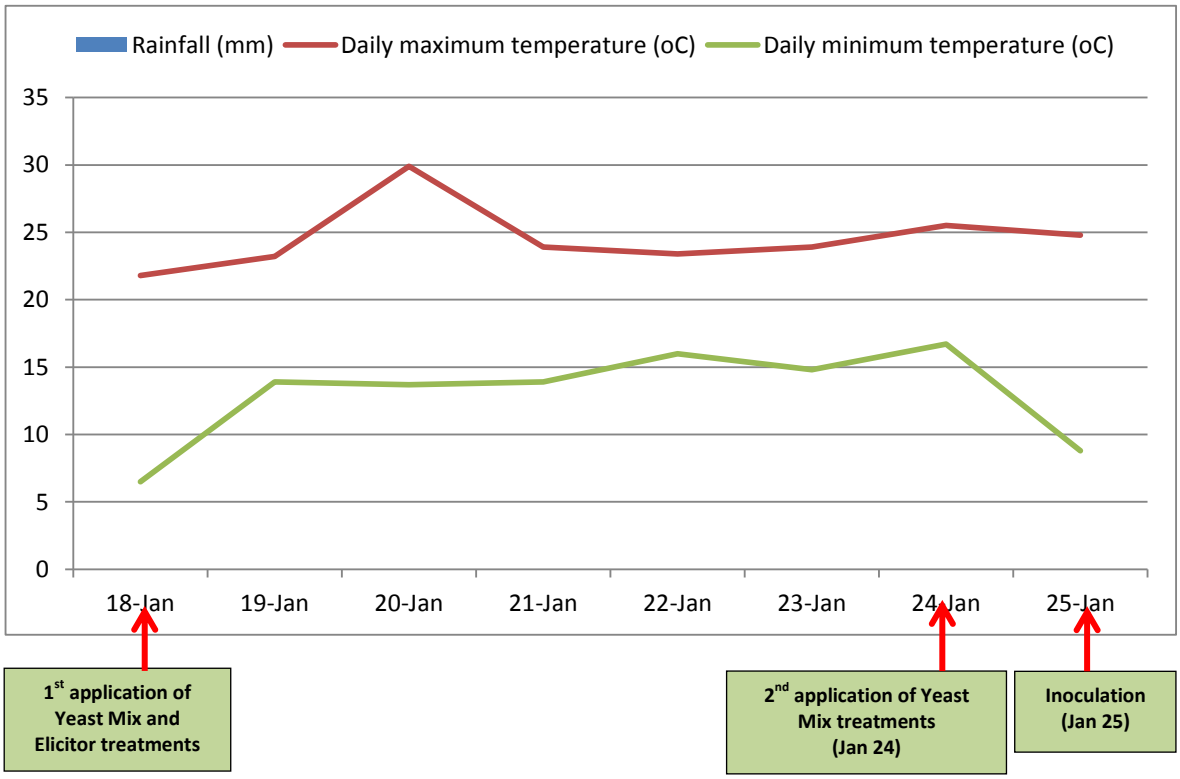
* Statistically significant at the 5% level from the Psa-only treatments (average) according to a non-parametric (Wilcoxon) test.

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.

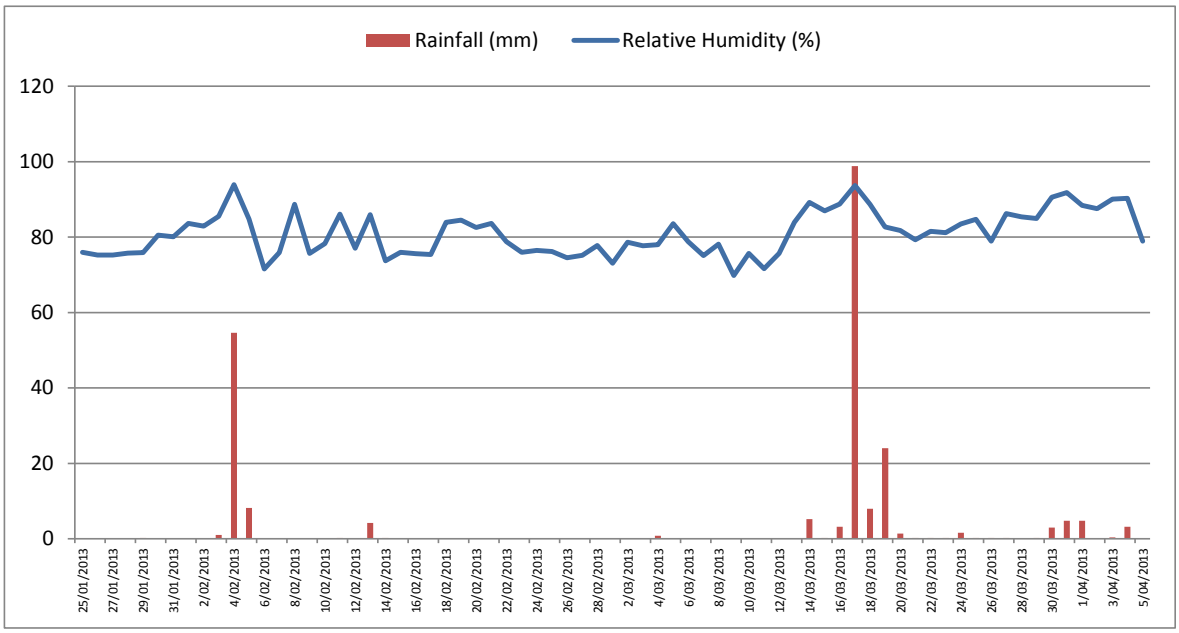


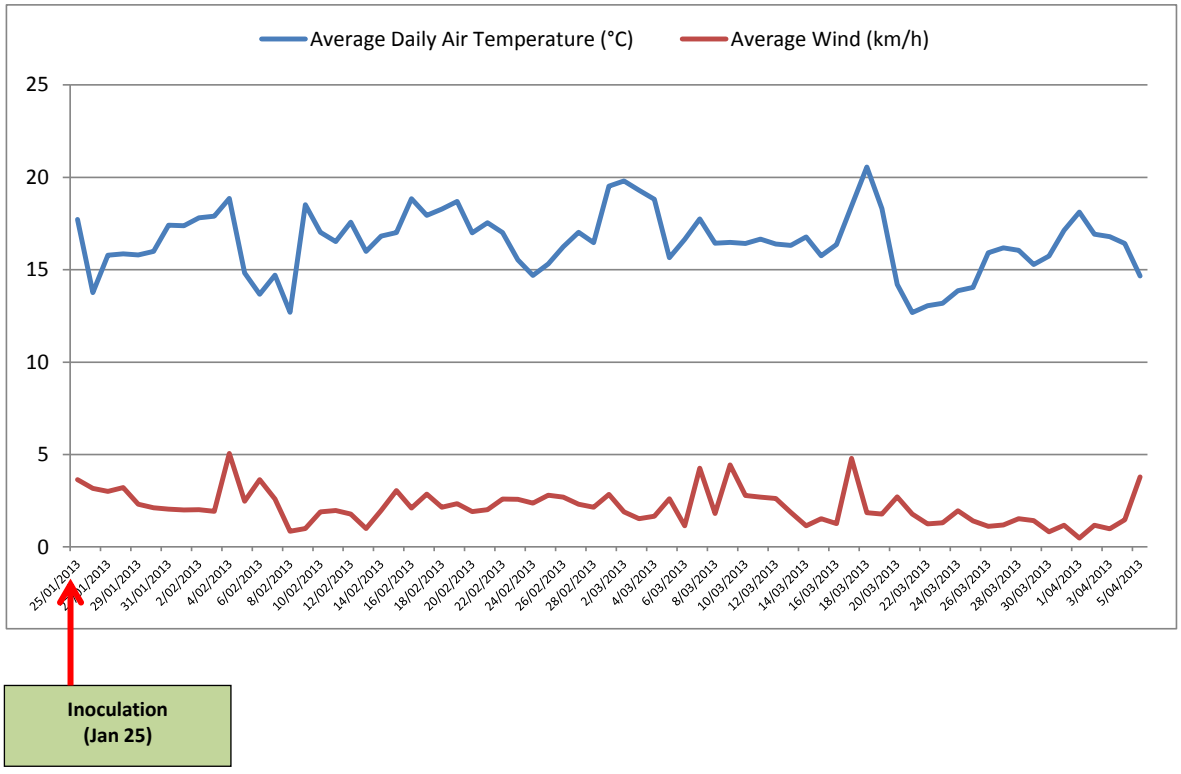
* Statistically significant at the 5% level from the Psa-only treatments (average) according to a non-parametric (Wilcoxon) test.

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 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).





Appendix 2: 2012-13 Potted Plant Field Trial Report to Zespri/KVH: Trials 12 & 13 - PFR Yeast Mix 2 and LU *Trichoderma* Mix 1 evaluation on 'Bruno' and G14

2012/13 Potted Plant Field Trial Report

Trials 12 & 13

PFR Yeast Mix 2 and LU *Trichoderma* Mix 1 evaluation on Bruno and G14

March – May 2013

November 2013

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.

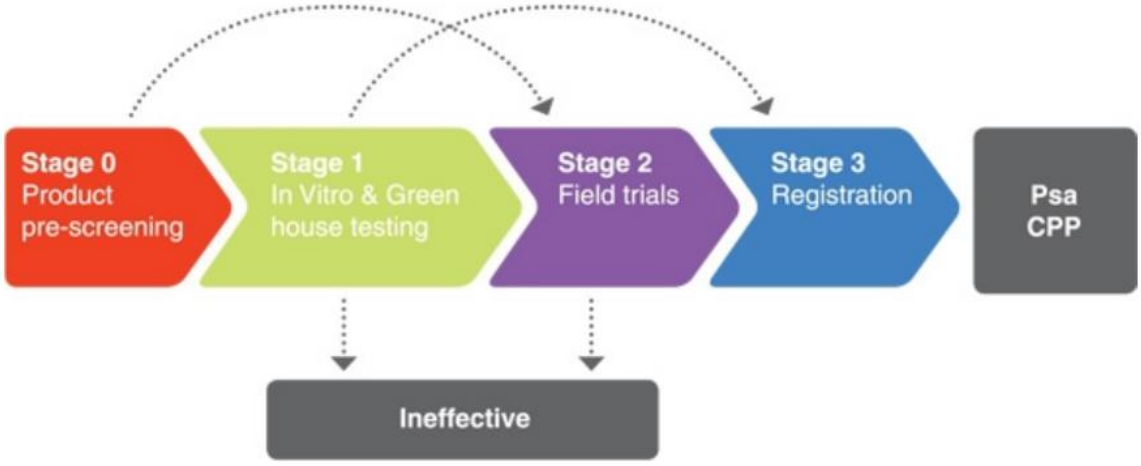
Introduction

Zespri, with support from KVH, is coordinating the screening of the effectiveness of a wide range of products to control *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 two trials conducted from March to May 2013 on Bruno seedlings and grafted G14 potted plants in which yeast mix (provided by Plant & Food Research), hereafter referred to as YM2, and a mix of *Trichoderma* spp. isolates (provided by the BioProtection Research Centre, Lincoln University), hereafter referred to as TriMix1, were tested for efficacy against Psa.



Objective(s)

To test the efficacy of a yeast mix (code YM2, supplied by Plant & Food Research) and a *Trichoderma* mix (code TriMix1 supplied by Lincoln University BioProtection Research Centre) alone and as combined treatments against Psa-V on potted plants.

Methodology

Plants

In these trials, G14 and Bruno plants were used. The G14 were grafted onto 2 year old Bruno rootstocks in spring 2012, in Kerikeri. The Bruno plants were seedlings sourced from PFR Ruakura Research Centre. The plants were believed to be Psa-free at the start of the trial as no symptoms were observed prior to treatment application. The plants were approximately 1.5 m in height with approximately half a dozen fully expanded leaves. Figure 1 shows an example of the potted plants used.

Figure 1. Example of the potted plants 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. A range of YM2, TriMix1 and Actigard® treatments were applied. The number of plants available was limited to 60 per trial due to plant availability issues.

Actigard was applied at a rate of 20 g/100 L for this treatment. The application details for the TriMix1 and YM2 are confidential to Plant and Food Research and BioProtection Research Centre, Lincoln University.

Table 1. Bruno seedling treatments.

	Treatments and dates						
TRT No.	**Root Drench (TPRO) (16dbi)**	**Root Drench (TPRO) (7dbi)**	**Foliar YM2 (TPRO) (7dbi)**	**Foliar Elicitor (TPRO) (7dbi)**	**Foliar YM2 (TPRO) (1dbi)**	**Psa inoc. at Zespri site (TPRO)**	**Questions to address**
	27 Feb	**6 Mar**	**6 March**	**6 March**	**13 March**	**14 March**	
1	Nil	Nil	Nil	Nil	Nil	No Psa	Background
2	Nil	Nil	Nil	Nil	Nil	Spray 1 x 10^8	Untreated comparison
3	TriMix1	TriMix1	YM2 (NF)+ FC	Actigard	YM2 (NF)+ FC	Spray 1 x 10^8	Is a triple mix best?
4	–	–	YM2 (NF)+ FC	–	YM2 (NF)+ FC	Spray 1 x 10^8	Does YM2 have field efficacy?
5	–	–	–	Actigard	–	Spray 1 x 10^8	Is Actigard driving the efficacy of the triple mix?
6	TriMix1	TriMix1	–	–	–	Spray 1 x 10^8	Does TriMix1 reduce Psa?

dbi = days before inoculation
TriMix1 = *Trichoderma* mix1; YM2 = Yeast Mix2; FC = biological control agent additive
TPRO = Te Puke Research Orchard, NF=not fermented

Table 2. Grafted G14 treatments.

	Treatments and dates						
TRT No.	**Root Drench (TPRO) (16dbi)**	**Root Drench (TPRO) (7dbi)**	**Foliar YM2 (TPRO) (7dbi)**	**Foliar Elicitor (TPRO) (7dbi)**	**Foliar YM2 (TPRO) (1dbi)**	**Psa inoc. at Zespri site (TPRO)**	**Questions to address**
	27 Feb	**6 Mar**	**6 March**	**6 March**	**13 March**	**14 March**	
1	Nil	Nil	Nil	Nil	Nil	No Psa	Background
2	Nil	Nil	Nil	Nil	Nil	Spray 1 x 10^8	Untreated comparison
3	TriMix1	TriMix1	YM2 (NF) + FC	Actigard	YM2 (NF)+ FC	Spray 1 x 10^8	Is a triple mix best?
4	–	–	YM2 (NF)+ FC	–	YM2-granules + FC YM2 (NF)+ FC	Spray 1 x 10^8	Does YM2 have field efficacy?
5	–	–	–	Actigard	–	Spray 1 x 10^8	Is Actigard driving the efficacy of the triple mix?
6	TriMix1	TriMix1	–	–	–	Spray 1 x 10^8	Does TriMix reduce Psa?

dbi = days before inoculation
TriMix1 = *Trichoderma* mix1; YM2 = Yeast Mix2; FC = biological control agent additive
TPRO = Te Puke Research Orchard, NF=not fermented

Treatment application

All treatments were applied by staff at Plant & Food Research's Te Puke Research Orchard (TPRO). The TriMix1 treatments were applied by drenching the soil mix in each pot. All other treatments were foliar applied. 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 14 March 2013. 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. In other words, each pallet contained a single plant from each of the treatments.

Plant and Food Research staff from Te Puke provided fresh Psa-V inoculum on the day. The target concentration was 1 x 10^8 cfu/mL; subsequently measurements indicated the concentration actually applied was between 5 x 10^7 and 1 x 10^8 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 11 am and midday.

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 midday on 14 March to midday on 16 March 2013. During this time, it is estimated that the equivalent of 34 mm of rainfall was applied in the trial area (of approximately 1200 m^2).

During the inoculation and initial wetting less than 1 mm of rain fell. On the day of inoculation, the maximum temperature reached 22^{o}C, 23^{0}C the following day and 20^{0}C the day after that.

Assessments

The levels of leaf spotting and secondary symptoms were visually estimated and recorded approximately 2, 3 and 4 weeks after inoculation. Each time, the amount of total leaf area covered in spots was estimated. At the first assessment, whole plant assessments were conducted. Subsequently, mature and expanding parts of plants were assessed separately. The last assessment of leaf spotting was conducted on 11 April 2013, as based on previous experience there was unlikely to be any significant progression in leaf spot beyond that.

Secondary symptoms were monitored regularly with the last assessment conducted on 25 May 2013.

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.

Trial duration

Final assessments were conducted on 25 May 2013. Subsequently, in June, samples of plant roots were collected from selected treatments and sent to the BioProtection Research Centre at Lincoln University for determination of *Trichoderma* colonisation.

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, Station #12428" – located across the road from site of treatment application).* 28 February – 14 March. Appendix 1.

Less than 1 mm fell at Plant & Food Research's Te Puke Research Orchard (TPRO) during the two week period that treatments were applied. Maximum daily temperatures ranged between 20 and 30^{o}C while minimum daily temperatures ranged between 10 and 18^{o}C.

ii) *Weather following inoculation (based on the installed Harvest.com weather station).* 14 March – 23 May 2013. Appendix 2.
Approximately 8 mm of rain fell during the initial 2-day wetting period following inoculation i.e. from 14-16 March. This was followed by 100 mm of rain on 17 March with a further 35 mm falling over the next 3 days. No further significant rain fell until the middle of April, from about the 15th.

Average daily temperatures generally declined throughout the trial from between 15 and 20^{o}C down to between 10 and 15^{o}C. During the initial 2-day wetting period, the average daily temperature was approximately 16^{o}C. Average daily relative humidity during the period was above 85%.

Results and interpretation

Leaf spotting

Overall

The severity of Psa leaf spotting in this trial was higher in the Bruno plants (e.g. Nil treatment = approx. 23%), compared with the G14 plants (Nil treatment = approx. 3%), 13 days after Psa inoculation.

Bruno

- At the first assessment, 13 days after inoculation, the following treatments significantly ($P<0.05$) reduced leaf spotting: Actigard, YM2&FC and the TriMix1+(YM2&FC)+Actigard treatment (Figure 2). The same treatments also significantly reduced ($P<0.05$) leaf spotting on the mature parts of the plants 22 and 28 days after inoculation (Figure 3).
- Leaf spotting on the expanding parts of the plants was much lower than that observed in the mature parts of the plants with much more variability between individual plants (as represented by the large standard error bars, Figure 4). This is not uncommon since these new tissues would not have received the treatment and would not have received the same inoculum dose that the fully expanded leaves had at the time of inoculation. Comparison of the water only and Psa controls indicate that the active inoculation was not a major factor in the leaf spotting observed in the expanding parts. It is probable that this leaf spotting was the result of natural inoculation and that the expanding parts were not fully covered by treatments i.e. leaves were not present when applied or expanded.
- The TriMix1 treatment did not significantly ($P<0.05$ or $P<0.10$) reduce Psa leaf spotting at any assessment.

G14

- At the first assessment, 13 days after inoculation, the following treatments significantly ($P<0.05$) reduced leaf spotting: Actigard, YM2&FC and the TriMix1+(YM2&FC)+Actigard (Figure 5). The same treatments also significantly reduced leaf spotting on the mature leaves of the plants 22 and 28 days after inoculation (Figure 6).
- The percentage reductions in Psa for these treatments were greater in G14 compared with Bruno. One hypothesis for this is that the protectant treatments when combined with some level of tolerance may work together more effectively compared with very susceptible cultivars.
- Only approximately 10 of the 60 G14 plants produced any new expanding growth and virtually no leaf spotting was seen on these parts. Therefore no data is presented here for the newly expanding leaves.
- For the mature parts of the plants, the TriMix1 treatment reduced leaf spotting by approximately 50%. However, this was not a statistically significant reduction even when analysed at the 10% level of probability and this was due to the variability from rep to rep.

Secondary symptoms

No secondary symptoms were observed throughout the trial including at the last assessment on 24 May 2013.

Summary

In this trial the YM2+FC treatments significantly reduced Psa leaf spotting in both Bruno and G14, particularly on the mature parts of plants. This was even after 48 h of continuous watering from above after Psa was applied indicating that this protectant treatment also has some persistence. The effect of this treatment was greatest in G14 i.e. overall it reduced leaf spotting by about two-thirds in G14 and by about a half in Bruno. One hypothesis for this is that protectant treatments when combined with some level of tolerance may work together more effectively compared with very susceptible cultivars.

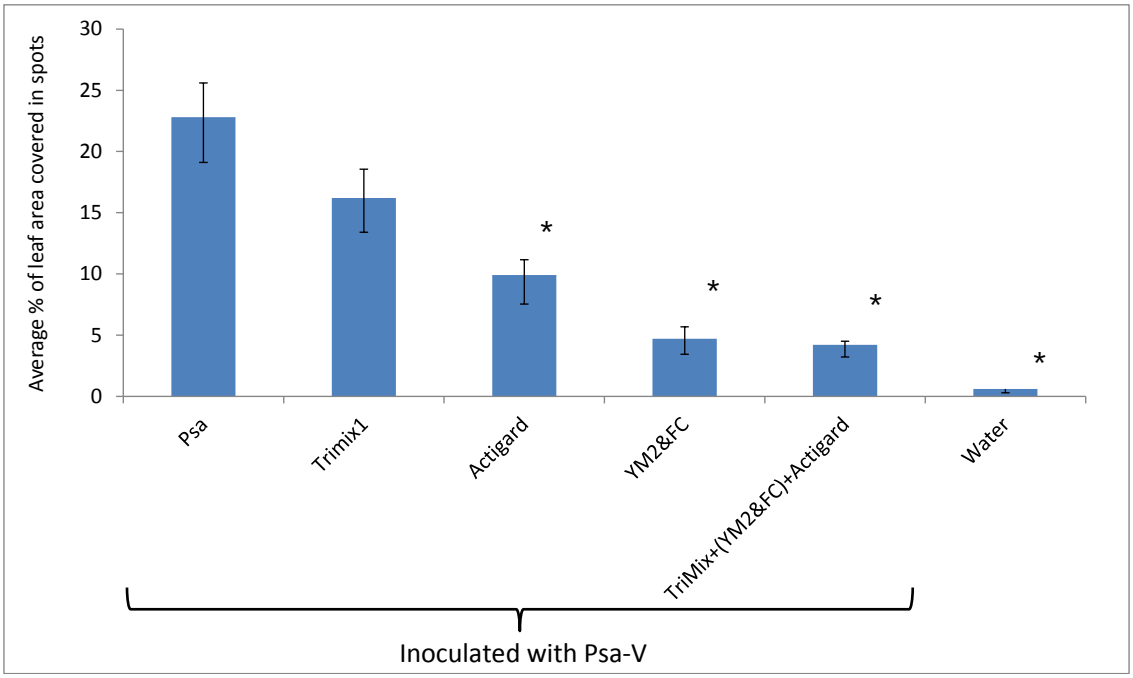
In Bruno, the YM2+FC treatment showed similar if not higher efficacy to Actigard. While this treatment reduced leaf spotting significantly in G14, Actigard reduced leaf spotting more.

The combination treatment of YM2+FC, Actigard and TriMix1 resulted in the highest reduction of Psa leaf spotting in both varieties i.e. overall, leaf spotting was reduced by about two-thirds in the Bruno and 95% in the G14 (mature parts).

There was a trend for the TriMix1 alone treatment to reduce leaf spotting particularly in the G14, however the differences were not statistically significant (due to significant variability).

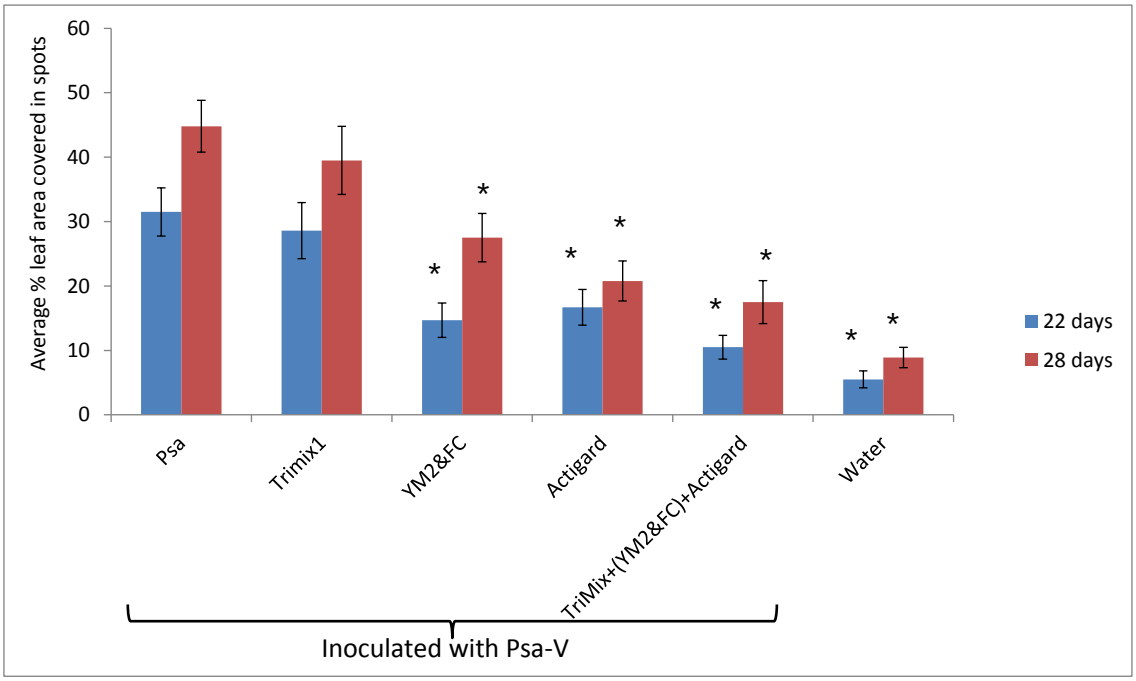
No secondary symptoms had developed when the trial ended (2 months after treatment) and the impact of treatments on this phase of Psa development could not be determined.

Figure 2. 2012/13 Zespri/KVH Potted Plant Trial of *Trichoderma* Mix1 (TriMix1) and Yeast Mix 2 (YM2) on Bruno seedlings. Data are the average total leaf area covered in necrotic Psa-V leaf spots (n = 10), 13 days after inoculation in 2013. Standard error bars are shown.



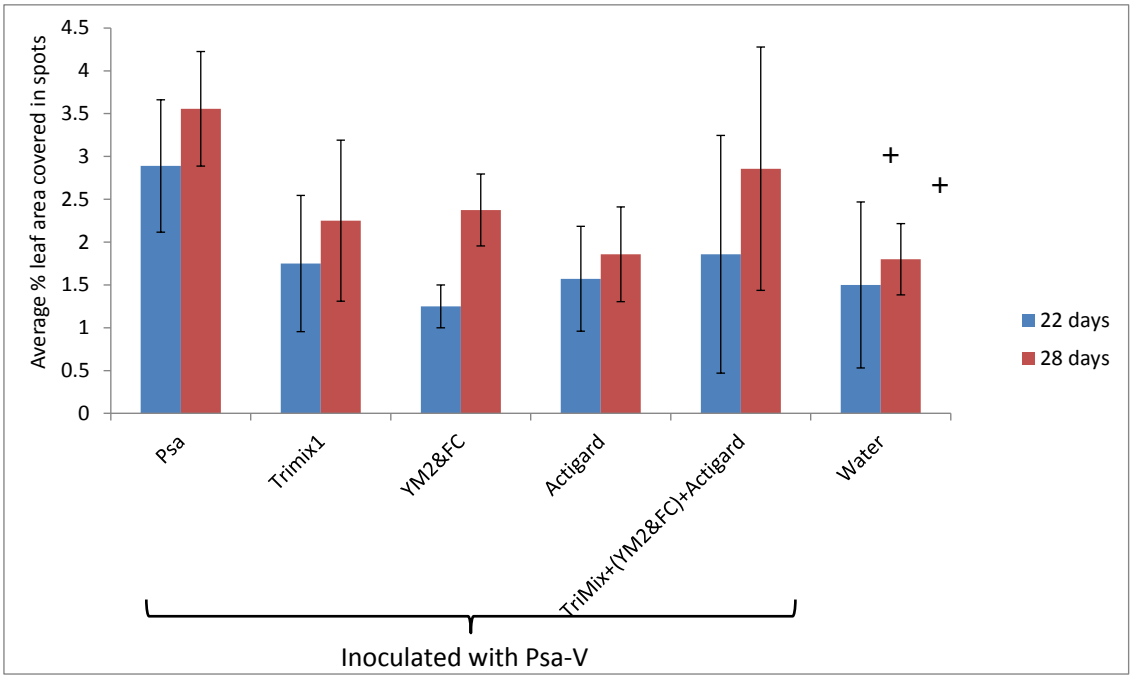
* Statistically significant from the Psa-only treatment according to a non-parametric (Wilcoxon) test, at the 5% significance level Bars are plus and minus the standard error of the mean.

Figure 3. 2012/13 Zespri/KVH Potted Plant Trial of *Trichoderma* Mix1 (TriMix1) and Yeast Mix 2 (YM2) on Bruno seedlings. Data are the average total leaf area covered in necrotic Psa-V leaf spots, 22 and 28 days after inoculation, for the mature parts of plants (n = 10). Standard error bars are shown.



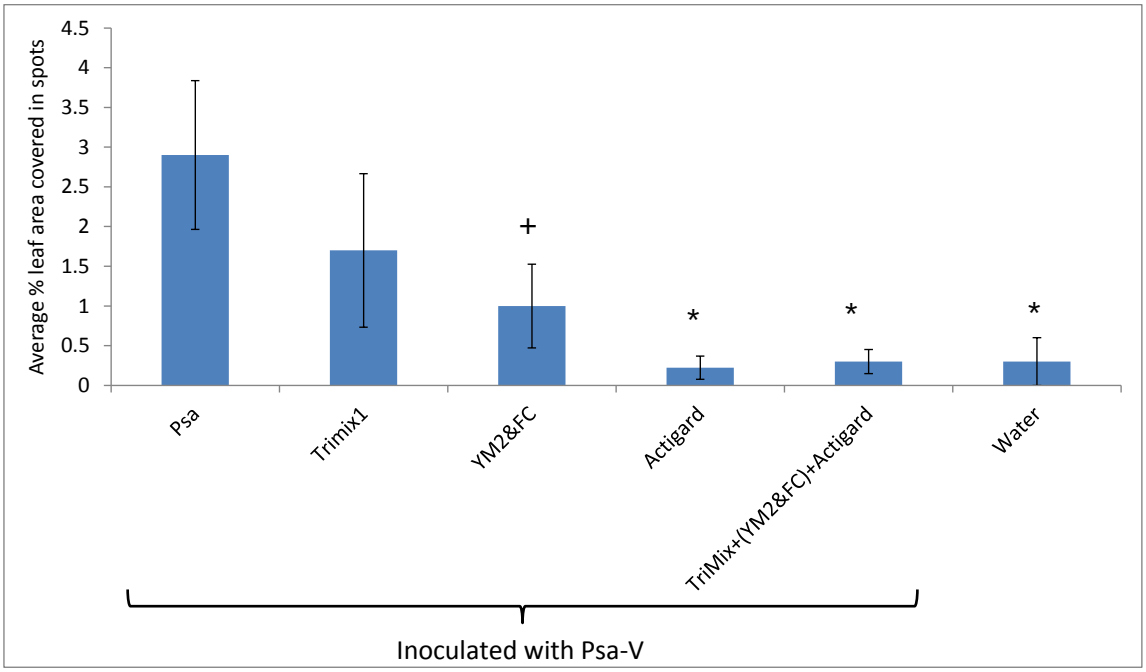
* Statistically significant from the Psa-only treatment according to a non-parametric (Wilcoxon) test, at the 5% significance level.

Figure 4. 2012/13 Zespri/KVH Potted Plant Trial of *Trichoderma* Mix1 (TriMix1) and Yeast Mix 2 (YM2) on Bruno seedlings. Data are the average total leaf area covered in necrotic Psa-V leaf spots, 22 and 28 days after inoculation, for the expanding parts of plants (n = 10). Standard error bars are shown.



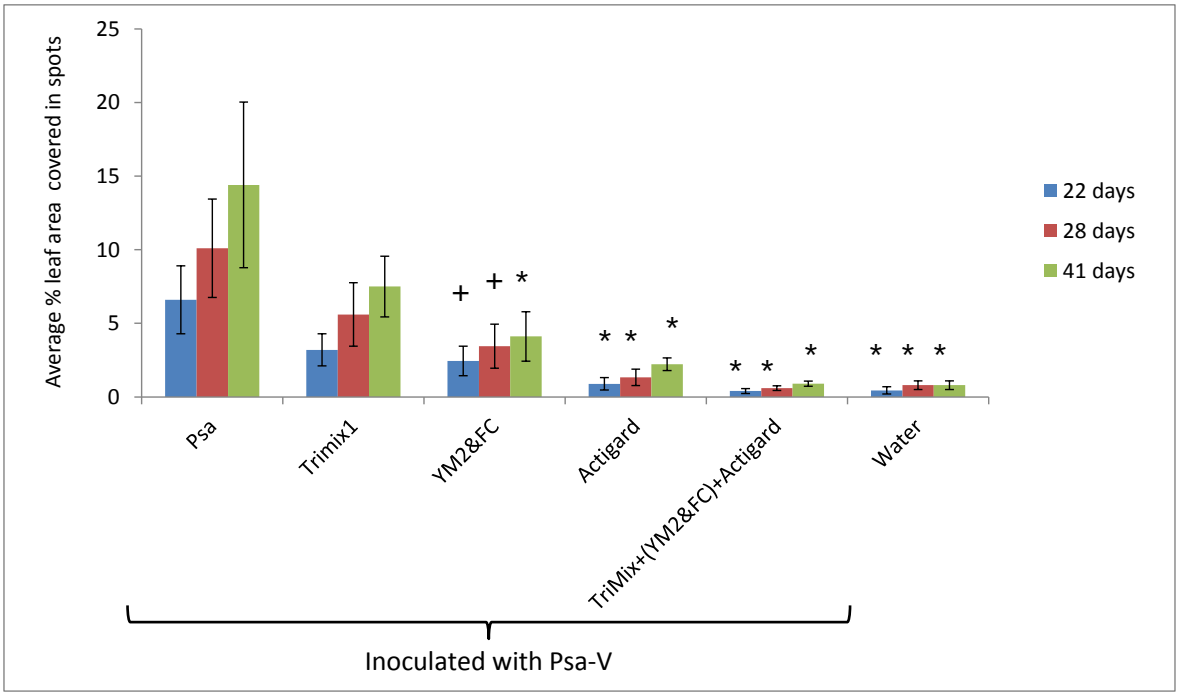
+ Statistically significant from the Psa-only treatment according to a non-parametric (Wilcoxon) test, at the 10% significance level.

Figure 5. 2012/13 Zespri/KVH Potted Plant Trial of *Trichoderma* Mix1 (TriMix1) and Yeast Mix 2 (YM2) on G14 plants. Data are the average total leaf area covered in necrotic Psa-V leaf spots (n = 10), 13 days after inoculation in 2013. Standard error bars are shown.



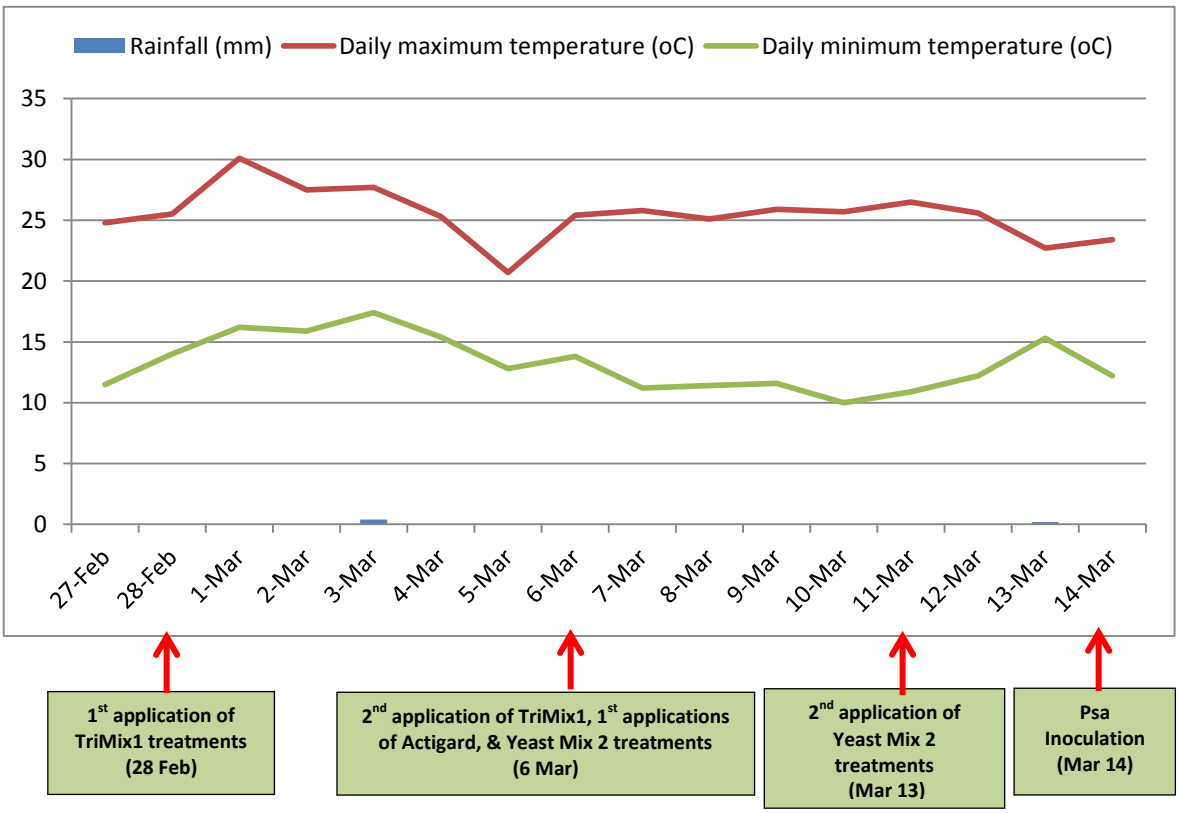
*, + Statistically significant from Psa-only treatment according to a non-parametric (Wilcoxon) test, at the 5% and 10% significance levels respectively.

Figure 6. 2012/13 Zespri/KVH Potted Plant Trial of *Trichoderma* Mix1 (TriMix1) and Yeast Mix 2 (YM2) on G14 plants. Data are the average total leaf area covered in necrotic Psa-V leaf spots, 22, 28 and 41 days after inoculation, for the mature parts of plants (n = 10). Standard error bars are shown.

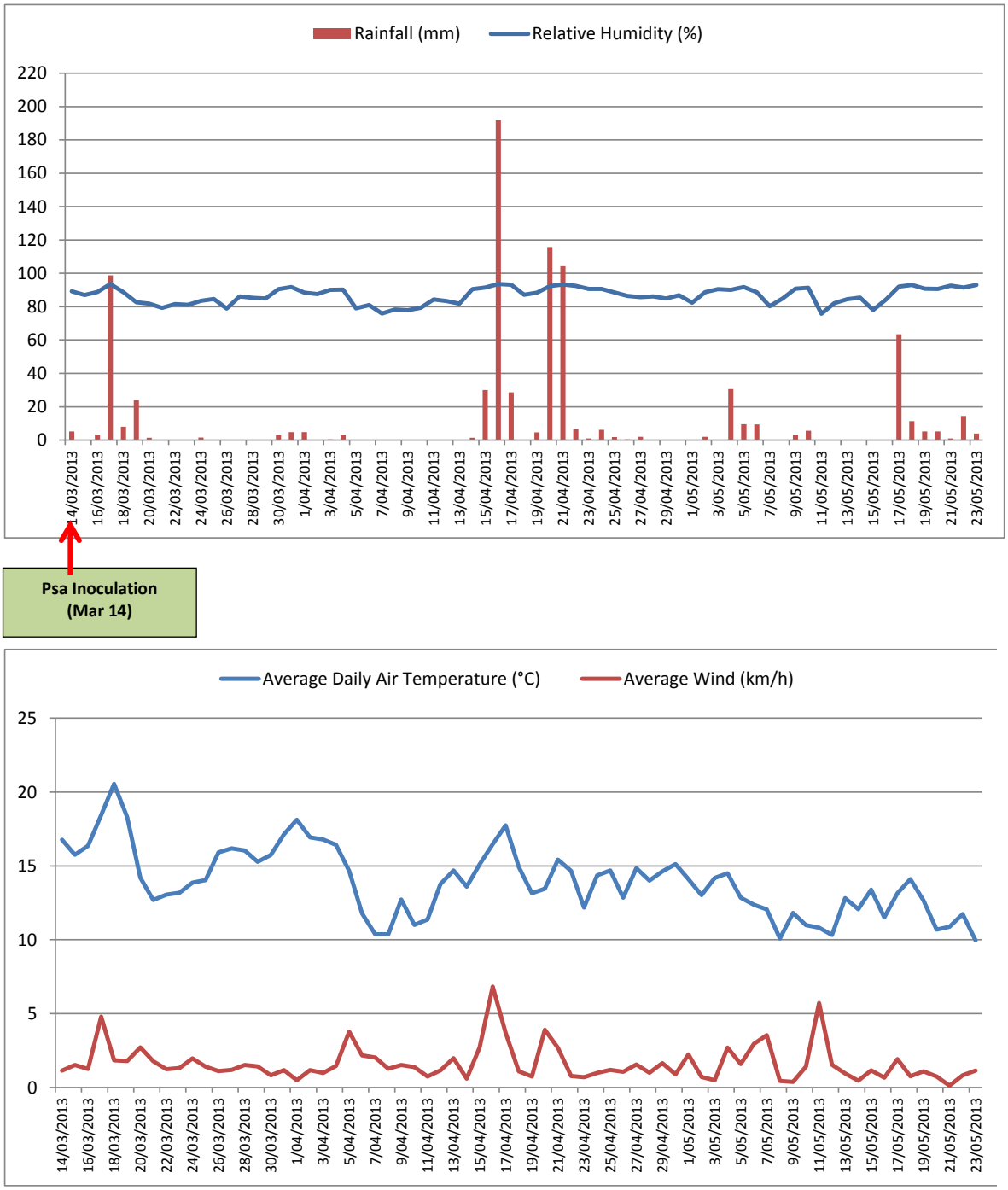


*,+ Statistically significant from the Psa-only treatment according to a non-parametric (Wilcoxon) test, at the 5% and 10% significance levels respectively.

Appendix 1. Weather conditions at Plant and Food Research's Te Puke Research Orchard (TPRO) over the period that treatments were being applied. Source: NIWA Weather Station "Te Puke Ews, Station #12428" – located across the road from site of treatment application.



Appendix 2. Weather conditions at the Zespri/KVH field site during the trial of *Trichoderma* and Yeast Mixes on Bruno and G14 which started in March 2013. Source: Harvest.com (weather station on site).



Appendix 3. YM2 Commercialisation Progress

Summary

YM2 manufacture and scale-up

- A commercial producer can supply the YCom1 and YCom2 components in a number of formats.

- Large quantities of YM2 can be produced more cost effectively. A 3–4 month lead in time for manufacture of large quantities is required and they can package and label as per customer specifications.

YM2 registration and distribution

- Under a non-disclosure agreement (NDA), PFR has shared extensive information about YM2 with a commercial distributor. This company applied their commercialization knowledge and recommended a series of developmental milestones and a plan to address these recommendations with associated timelines was discussed with Zespri/KVH.

- Commercial R&D questions raised are currently being addressed through a PSAF (Pre Seed Accelerator Fund) programme. More applied questions are being addressed in Zespri/KVH – PFR potted vine field trials and the more fundamental questions raised are being addressed in the MBIE – 'Next Generation Biopesticides' programme.

YM2 patenting

- The review completed by an Intellectual Property Company did not identify any Freedom to Operate (FTO) issues with YM2 in New Zealand. However, some prior art (both patent and publications) was identified (e.g. the use of yeasts for control of mammalian diseases caused by bacterial pathogens).

- The search results and PFR data have also been reviewed by AJ Park patent attorneys. Their initial conclusion is that there may be a position to patent the use of YM2 against Psa and other closely related plant diseases. They are now conducting a more detailed review of the data and a decision whether to patent will be made by end of April or early May 2014 depending upon results of the Zespri/KVH and PFR potted vine trials.

In order to advance the commercialisation of YM2 as quickly as possible, a list of potential commercial partners were evaluated by the PFR business manager for the Biological Control and Natural Products Team. A non-disclosure agreement (NDA) was subsequently signed between PFR and a selected commercial distributorand all relevant data on YM2 was shared with the company representatives. After reviewing the YM2 data package, a conference call was scheduled to discuss any outstanding queries. After further consideration a summary document was circulated to PFR, which included a list of commercially-based questions (Section 3.8 page 49). PFR reviewed this document and determined the project area that each question belonged to (e.g. KRIP, MBIE or Zespri/KVH). Some questions did not fall into any of these categories and a separate grant was used (Pre-Seed Accelerator Fund) to specifically address these questions.

A Gantt chart was prepared for Zespri to summaries the key steps leading towards full commercialisation of YM2 and this is presented in Appendix 4.

Manufacture and scale-up

PFR put in place an NDA with the commercial supplier of YM2 in February 2013 which has enabled open discussions about the prototype product, manufacture, formulation and pricing. They can supply the yeast in a number of formats and the preferred format has been identified. One of the issues raised by the commercial partner was the cost of goods and cell viability. This has largely been addressed and is part of the ongoing PSAF project. The supplier has provided smaller product samples with greater yeast cell viability and this now falls within a commercially viable range. Benchmark costings shared have been based on overseas production.. Very large quantities can be produced off-shore however, we have calculated that even with high grower uptake, we may not reach these volumes. A 3–4 month lead in time is required for manufacture of large quantities. They can package and label as per customer specifications.

Registration and Distribution

Given that YM2 can be manufactured and packaged to specification, an agricultural distribution partner is required with expertise in registration and support of these products, plus good kiwifruit industry knowledge. Following assessment of several potential partners, we selected a company to commence detailed discussions, and confirmed this with Zespri.

Under a non-disclosure agreement (NDA), PFR has shared extensive information about YM2. The company were able to apply their knowledge of commercialization of Psa control products, biological control agents and the registration process, to propose a series of questions to be resolved in order to assist commercialisation. These questions included aspects such as cost-viability, adjuvant combination and field trial data. These questions are set out in detail in Section 3.8 (page 49) “YM2 Commercial Questions”. In November 2013, a plan to address these questions and the associated time lines was documented and discussed with Zespri (Appendix 4). These R&D questions are being addressed through PSAF (Pre-Seed Accelerator Fund) programme, the MBIE Novel Biopesticides programme and Zespri field trialling.

PFR acknowledges that we have to balance risks and the need for a fully developed product with the need for timely product release to assist industry. In doing so, PFR has highlighted this to the company who have indicated a willingness to re-assess current data and priorities and timeframes. This will be completed by late April 2014. In addition, we have commenced high-level discussion with other parties, who have indicated a desire to find out more.

Patenting

PFR engaged an Intellectual Property Company to complete a review of yeasts and their application for bacterial disease control. The review did not identify any Freedom to Operate (FTO) issues in NZ. The search did identify some prior art (both patent and publications) that document the use of yeasts for control of bacterial diseases. The majority of this prior art was in relation to medicine/mammalian diseases. The search results have been reviewed, alongside PFR data, by AJ Park patent attorneys. Their initial conclusion is that there may be a position to patent the use of yeasts against Psa and other closely related plant diseases. They are now conducting a more detailed review of the data and a decision whether to patent will be made by the end of May 2014.

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