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Surveying orchards to identify resistance to Psa-V

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Executive summary

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Background

A proof-of-concept study has been undertaken to assess the ability of the woody stem bioassay, a test developed by Plant & Food Research, to detect differences in response to Psa-V inoculation within 'Hort16A' and 'Hayward' kiwifruit vines from different orchards. Whilst the bioassay has been shown to detect differences in Psa-V resistance between genotypes and cultivars, it has yet to be tested as a determinant of varying resistance within a cultivar. Several orchards have been identified as part of this study that are free of Psa-V symptoms. The primary objective of this study was to determine if orchard-specific resistance to Psa-V can be detected using the woody stem bioassay.

Methodology

The woody stem bioassay was performed using 30 canes from each of 14 orchards (numbered 1 to 14; five 'Hayward' and four 'Hort16A' orchards within the Bay of Plenty; five orchards outside the Bay of Plenty). Canes were collected and cool-stored (0°C) for approximately 24 h. Excised kiwifruit canes were surface sterilised, wounded and inoculated with either a high dose (~10^9 CFUs per mL), a low dose (~10^6 CFUs per mL), or a sterilised distilled water (SDW). The cane segments were held in humid plastic (sushi) trays and maintained in the laboratory at 20-23°C. After a three-week incubation time, wound specific lesions were measured, and after an additional 24 h the degree of bacterial ooze scored. The lesion and ooze assessments were combined to calculate an overall index of susceptibility (woody stem bioassay index = WSBI). Analysis of variance and binomial analysis were used to explore differences between orchards, inoculum dose and cultivar.

Key results

This proof-of-concept study has detected some orchards with significantly different woody stem bioassay index (WSBI) values from the PFR standard and from other orchards included in the sample.

There was a range of responses to Psa-V inoculation, ranging from examples of relative Psa-V susceptibility, e.g. orchards 3 and 11, to examples of relative Psa-V resistance, e.g. orchards 2 and 5.

In particular, the 'Hort16A' orchards 1 and 2 showed significantly lower WBSI values than several other orchards and the 'Hayward' orchard 5 also showed significantly lower WBSI values compared to several other orchards.

A higher proportion of dead canes were seen in Psa-V infected orchards.

Canes were tested from the orchards 1, 3 and 4, which had various vigour and rootstock differences within each orchard, and no statistical differences were found.

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Recommendations

There is evidence to suggest the woody stem bioassay can detect within-genotype differences in resistance to Psa-V using kiwifruit vines sourced from different orchards. This is true for both 'Hort16A' and 'Hayward' cultivars.

We recommend sampling of at least some of the same orchards during Stage 2 to confirm if these results are repeatable.

Because of the incidence of cane collapse, it is recommended that extra care is taken to avoid vines that may have Psa-V infection.

Further orchard sampling should also consider factors that may influence the variability observed in these results.

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1 Introduction

Several orchards within and outside the Bay of Plenty region have been identified as part of this study that are free of Psa-V, potentially because of resistance and/or use of unique management techniques (e.g. a Pukekohe orchard using large compost additions) that may confer resistance.

Tolerance to bacterial diseases such as those caused by the various pathovars of *Pseudomonas* spp. has been shown to be affected by plant management (Cao et al. 2006). In the case of Psa-V, there is little information in the published literature to indicate that certain management practices may increase the resistance of kiwifruit to this pathogen. It is possible that certain management techniques already deployed in orchards may reduce the risk of infection or delay the onset of Psa-V related dieback. Management practices such as soil structure amelioration, nutrient application rates and methods, microbe applications, chemical spraying, timing and intensity of pruning and shelter density could potentially influence resistance within a cultivar. Some growers may have coincidentally developed management techniques that enhance vine resistance and some of these orchards may be outside the Psa infection zone.

In this project, several orchards thought to be more tolerant to Psa-V have been identified. These are orchards that show fewer symptoms or slower progression of symptoms, or which have management or orchard features that may confer resistance. A test (woody stem bioassay) for disease resistance has been developed by Plant & Food Research, where canes are inoculated with sufficient Psa-V inoculum to cause lesions. The degree of resistance can be determined by the size of lesion and degree of bacterial oozing that develops after incubation. Whilst the bioassay has been shown to detect differences in Psa-V resistance between cultivars, it has yet to be tested as a determinant of varying resistance of individual vines within a cultivar.

This proof-of-concept study has the primary objective of assessing the ability of the woody stem bioassay to detect differences in Psa-V resistance within 'Hort16A' and 'Hayward' vines from different orchards. This has been addressed by:

- Identifying orchards nominated by growers, packhouses, technical staff and others in the industry with apparently less severe symptoms of Psa-V
- Screening plant material from identified orchards using the woody stem bioassay.

Should the woody stem bioassay be successful in detecting differences in resistance then the project is expected to continue to stage two.

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2 Methods and materials

2.1 Cane sampling

Thirty cane samples (1 from each of 30 vines) were collected from four commercial 'Hort16A' orchards and five 'Hayward' orchards within the Bay of Plenty, and a further five orchards located outside the Bay of Plenty (Table 1 and Appendix 1). Locally sourced canes were collected by Craig Mowatt and ZESPRI area managers, or regional orchardists collected canes from orchards outside the Bay of Plenty. Canes were provided to Plant & Food Research and held in cool-storage (0°C) for approximately 24 h prior to the bioassay setup. Experimental standards included field collected 'Hort16A' (two vines) and 'Hayward' (eight vines) canes (Plant & Food Research, Te Puke Research Orchard (TPRO)), and cold-stored 'Hort16A' (~20 vines) and 'Hayward' (~20 vines) (Plant & Food Research, Ruakura).

2.2 Psa-V inoculum

Psa-V inoculum was prepared from fresh cultures grown for two days and made up to a concentration of at least 1 x 10^9 colony forming units (CFUs) per mL. This was used for all high dose inoculations. This stock was diluted 1000-fold (at least 1 x 10^6 CFUs per mL) and used for all low dose inoculations. The viable cell density was confirmed for each batch of inoculum by serial dilution plating, enabling the quantification of CFUs per mL.

2.3 Bioassay setup

Each replicate cane was cut into 10-cm long segments and placed into labelled orchard-specific onion bags and surface sterilised, followed by a sterile distilled water (SDW) rinse. Canes were then blot dried with sterile paper towels and transferred into labelled plastic (sushi) trays containing a moistened sterile paper towel (15 mL of SDW/tray). Each cane was placed in an ordered position within the labelled tray, enabling accurate identification of each cane segment (Figure 1). Each tray contained no more than seven cane segments.

Figure 1. Standard tray set-up for the woody stem bioassay.

Each cane segment was prepared for inoculation by creating two notch wounds at right angles to the cane. For each of the negative controls, a droplet of SDW (10 μL) was placed directly into each wound using a micro-pipette. For each treated cane, a droplet of high or low dose of Psa-V inoculum (10 μL) was placed directly into each wound. Each tray was then closed and incubated in the laboratory for three weeks at 20-23°C. After seven and 14 days, 5–15 mL of SDW was added to the paper towel in the base of the tray to maintain high humidity.

2.4 Cane assessments

Three weeks after inoculation, canes were prepared for lesion measurements and ooze scoring. Using flame-sterilised blades, cuts were made toward the direction of the wound to expose the lesions at both wound sites (Figure 2). A separate blade and section of the blade was used for each cane and new cut, respectively. The full length of any clearly visible lesion that extended beyond the wound area was measured using digital callipers; this measurement therefore also included the width of the wound site. Each cane segment was returned to the same position within the tray and incubated for a further 24 h.

In some instances, canes deteriorated during the three-week incubation period and did not show healthy green tissue beneath the bark away from the wound site and lesion area. These canes are recorded as dead and are treated as missing data in the initial analysis.

Figure 2. Excised kiwifruit canes cut to expose possible lesions, three weeks after inoculation with Psa-V.

Using a binocular microscope (20 x magnification), each wound/lesion area was observed and given a bacterial ooze score. The scoring system ranged from 0 (no ooze on the lesion area or on the wound site) to 4 (moderate to profuse ooze on the lesion area and evidence of ooze appearing beyond the extent to the lesion).

The lesion and ooze measurements were corrected against the SDW controls; the figures were then converted to a value out of 50 and combined to give an overall woody stem bioassay index or WSBI, with a theoretical maximum of 100. All data were summarised using Microsoft® Excel 2007 and statistical analysis was carried out using GenStat, 13th edition.

Each cultivar was analysed separately by ANOVA, fitting orchard, and by a combined split-plot ANOVA fitting dose, orchard and their interaction. The interaction was highly significant, so doses were considered separately. The proportion of canes that died, had low WBSI (<=10) or high SI (>10) were analysed with a regression analysis of binomial data for each dose, separately fitting orchard, with an over-dispersion parameter where appropriate.

Brief notes on orchards used in survey.

Hort 16 A orchards

Orchard 1 Alexander Rd Te Puke
Hort16 A 3 yrs on Kaimai cloned rootstocks. Surrounded by Psa-V and tested positive. Sprayed 3xStrepto, 3xActiqard, 3xCu and Sporekill. Est. yield 2012 - 9,000try/ha

Orchard 2 Opotiki
Hort 16 A 3 yrs on Bounty 21 cloned rootstocks. Psa on Gold next door. Sprayed Alexin before flowering, then Actiqard, Cu, Spotless. An intensively sprayed orchard Est. yield 2012 3,000 tray/ha

Orchard 3 Paengaroa.
Hort 16 A 8 yrs on Bruno rootstocks. Sixth orchard to get Psa. Sprayed H_2O_2 onto infected plants after cutting out disease. After personal injury couldn't keep up. Orchard looks good, last year (2011) yield was 62,000 trays, est. 2012 25,000 trays

Orchard 4 South Paengaroa
Very

Orchard 8 Pukekohe
16 A grafted 8 yrs on 27 yr Bruno. Closest Psa 2 km. Applied RPR, Mg, humic acid, 15 t compost, 4t gypsum + Fishfert. Last year yielded 10,000 this year 13,000 trays

Orchard 12 Pukekohe
16 A on unknown rootstock Sprayed Actiguard, Movento, Serenade Max. Gypsum and 30t/ha compost applied. Last year produced 25,000 and 2012 est. 25,000 trays/ha.

Orchard 14 Gisborne
16 A on 5yr Bruno. No Psa. Biostart program 2xKelp Green Logic. Last year produced 3,700 and this year 8,300 trays

Hayward

Orchard 5 No 1 Rd
Hayward 30+ yr on Bruno. Psa in Gold 30 m away but now cut out. Two positive Psa tests, no vines, shoots cut out. Sprayed Cu in Dec but stopped contractor. Last year yield 10,200 and this year 11,000 trays/ha

Orchard 6 Upper Te Matai Rd
Hayward 3yrs on Bruno. Psa 30m away in Gold (now cut out). Sprayed Actigard, 3xPlant Shield and Liqu Cu and Sporekill. Last year yield 10,500 and this year 6,000 trays/ha

Orchard 7 Mid Te Matai Rd
Hayward 30+ yr on Bruno. Surrounded by Psa, near one stumped. Sprayed 18xKocide Opti + Seaweed Foliar + 2x Actiqard + 2xSeranade Max + 7 tonne compost. Last year fruit dropped to save vines 2012 est. yield 12,900 trays/ha

Orchard 9 Mid Rangiuru Road/Te Puke.
Hayward on 32 yr Bruno. Three Psa positive tests, Psa at least 1 km on 3 sides. Sprayed Actiqard. Was going to abandon orchard but it suddenly improved. Last year 10,000 this year 4,000 trays/ha with no hives.

Orchard 10 Left of Welcome Bay/Te Puke Highway
Hayward on 25 yr Bruno. Psa in Hayward 25 m away, Psa + but hasn't lost any plants. Sprayed 2xCu, 1 Serenade Max, oils, BT. 5t/ha compost, fish fertiliser. Orchard has improved. Last year 32,000 and this year 20,000 but had very poor pollination.

Orchard 11 Waikato
Hayward on 27 yr Bruno. No Psa. Applied vermicast, RPR, Fishmeal, roots and shoots organic program. Last year due to frost produced 5,300 and this year 7-8,000 trays/ha

Orchard 13 Gisborne
Hayward on 27+yr Bruno. Fe chelate applied to ground and 2x kelp Always has done about 8,000 trays /ha

3 Results and discussion

Examples of lesion development and bacterial ooze are shown in Figures 3a, 3b & 3c.

Figure 3a. Example of lesion development on 'Hort16A'.

Figure 3b. Example of lesion development and cream coloured bacterial ooze exuding from lesion area on 'Hort16A'.

Figure 3c. Example of red coloured bacterial ooze (white circles) exuding from lesion area on 'Hort16A' – relatively uncommon.

The high inoculum dose applied to canes across all bioassays ranged from 1.9 to 3.9 x 10^9 CFU/mL (mean 2.2 x 10^9 CFU/mL, SE = 2.8 x 10^8).

This proof-of-concept study detected significant differences in the woody stem bioassay index (WSBI) between orchards, using high (Figure 4) and low (Figure 5) inoculum doses. A range of responses was observed following Psa-V inoculation, including examples of relative Psa-V susceptibility, e.g. orchards 3 and 11 ('Hort16A') Orchards, and examples of relative Psa-V resistance, e.g. orchard 2 ('Hort16A') and orchard 5 ('Hayward') Orchards.

More specifically for 'Hort16A' canes receiving a high inoculum dose, six orchards had a significantly (P<0.05) lower WBSI than the PFR 'Hort16A' sampled from TPRO vines (Figure 4). The orchard 2 orchard had a significantly (P<0.05) lower WBSI than all other orchards, while orchards 8 and 4 had significantly (P<0.05) lower WBSI than orchard 3. Orchard 2 is the only 'Hort16A' block that is grafted onto 'Bounty' rootstock (Table 1).

For 'Hayward' canes receiving a high inoculum dose, three orchards (6, 7 and 5) had a significantly (P<0.05) lower WBSI than the PFR 'Hayward' sampled from TPRO vines (Figure 4). Five orchards (10, 13, 6, 7 and 5) all had a significantly (P<0.05) lower WBSI than orchard 11 and 9 orchards and were not significantly different from one another.

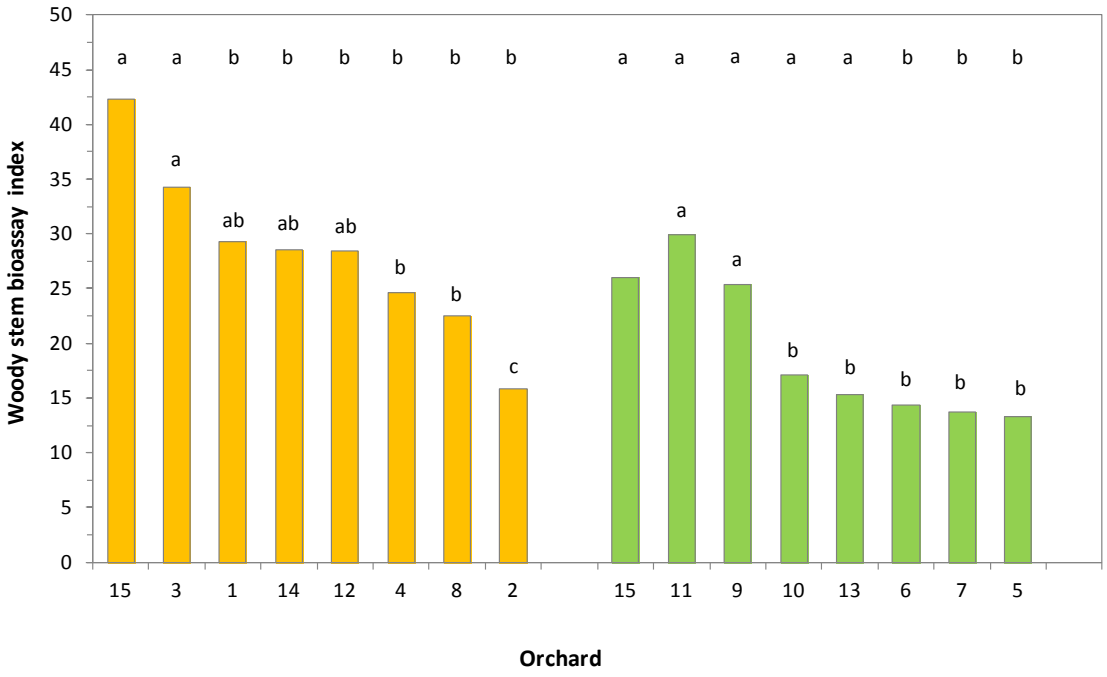

Figure 4. Woody stem bioassay index resulting from the inoculation of a high dose of Psa-V (~10^9 bacteria/ mL) on seven 'Hort16A' orchards (gold coloured bars), seven 'Hayward' orchards (green coloured bars), and the respective Plant & Food Research standards.

The left hand bar for each cultivar is the Least Significant Difference (LSD) for comparisons against the PFR standard and the right hand bar is the LSD for comparisons between any two orchards, other than the PFR standard.

For 'Hort16A' canes receiving a low inoculum dose, five orchards had a significantly (P<0.05) lower WBSI than the PFR 'Hort16A' sampled from TPRO vines and these were also significantly lower than orchards 12 and 3 (Figure 5). Orchard 1had significantly (P<0.05) lower WBSI than orchards 4 and 8.

For 'Hayward' canes inoculated with a low dose of Psa-V, only orchard 5 had significantly lower WSBI than the PFR 'Hayward' sampled from TPRO vines and was also significantly lower than those from orchards 13, 11 and 6 (Figure 5).

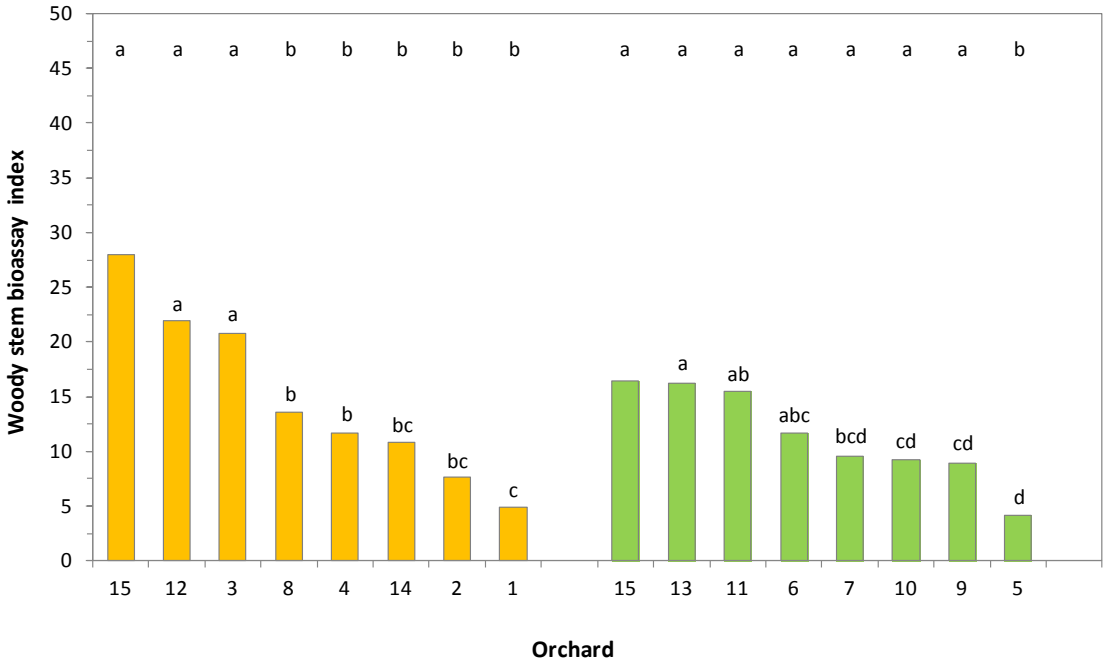

Figure 5. Woody stem bioassay index resulting from the inoculation of a low dose of Psa-V (~10^6 bacteria/ mL) on seven 'Hort16A' orchards (gold coloured bars), seven 'Hayward' orchards (green coloured bars), and the respective Plant & Food Research standards. Note the y-axis scale is the same as for Figure 4.

The left hand bar for each cultivar is the Least Significant Difference (LSD) for comparisons against the PFR standard and the right hand bar is the LSD for comparisons between any two orchards, other than the PFR standard.

For both cultivars, there was a significant interaction between orchard and proportion of canes recorded as dead. An increased proportion of dead canes was evident from orchards with Psa-V present i.e. number 4, 3 and 1 ('Hort16A', Table 3), and orchards 6, 10 and 9 ('Hayward', Table 4). Dead canes were possibility related to the cane becoming overwhelmed by the pathogen or could be due to Psa-V infection of the cane at the time of sampling.

Table 3. Percentage of dead canes from seven 'Hort16A' orchards three weeks after inoculation with Psa-V.

	Orchard							
	4*	3*	1*	14	8	12	2	sed
% dead cane (High)	10.0	16.7	20.0	0.0	3.3	10.0	0.0	8.02
% dead cane (Low)	30.0	36.7	0.0	3.3	3.3	13.3	9.7	8.30

Where * = Psa-V present in orchard, (High) = high inoculum dose and (Low) = low inoculums dose, sed = standard error of the difference.

Table 4. Percentage of dead canes from seven 'Hayward' orchards three weeks after inoculation with Psa-V.

	Orchard							
	13	6*	5	7	10*	11	9*	sed
% dead cane (High)	0.0	43.3	3.3	3.3	10.0	3.3	10.0	7.03
% dead cane (Low)	0.0	0.0	0.0	3.3	3.3	0.0	6.7	5.24

Where * = Psa-V present in orchard, (High) = high inoculum dose and (Low) = low inoculum dose, sed = standard error of the difference.

Canes were tested from orchards 1, 3 and 4 which had been categorised into groups with different vine vigour and rootstock within each orchard. No significant differences were found within orchard 1 (P=0.188 high dose; P=0.875 low dose), orchard 3 (P=0.534, P=0.4), and orchard 4 (P=0.673, P=0.557) after inoculation with both high and low inoculum.

4 Recommendations

There is evidence to suggest the woody stem bioassay can detect orchard differences in the relative response of woody stems of kiwifruit vines to inoculation with Psa-V, for both 'Hort16A' and 'Hayward' cultivars.

We recommend sampling of at least some of the same orchards during stage 2 to confirm if these results are repeatable.

Because of the incidence of cane collapse, it is recommended that extra care is taken to avoid vines that may have Psa-V infection.

Further orchard sampling should also consider factors that may influence the variability observed in these results.

5 References

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6 Acknowledgements

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Appendix 1 Additional comments on cane collection.

Orchard Name	Comments
Orchard 1	Not bagged after collection
Orchard 3	Cane quality was much reduced compared to other orchards at time of assessment
Orchard 4	Cane quality was much reduced compared to other orchards at time of assessment
Orchard 8	Canes were received in 2 lots (a) and (b)
Orchard 13	Canes in transition between orchard and cool-store for more than 48 h
Orchard 14	Canes in transition between orchard and cool-store for more than 48 h