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Woody Stem Bioassay – Copper testing

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December 2012

A report prepared for

ZESPRI® Group Limited

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Plant & Food Research, Te Puke

SPTS No. 7824

PUBLICATION DATA

Mauchline N, Stannard K. 2012. Woody Stem Bioassay – Copper testing. A report prepared for: ZESPRI Group Limited.. Plant & Food Research Contract No. 29084. SPTS No. 7824.

This report has been prepared by The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research), which has its Head Office at 120 Mt Albert Rd, Mt Albert, Auckland.

This report has been approved by:

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Executive summary

Woody Stem Bioassay – Copper testing

Mauchline N, Stannard K, December 2012, SPTS 7824

A woody stem bioassay that enables the assessment of a cultivar's tolerance to *Pseudomonas syringae* pv. *actinidiae* (Psa-V) has been developed by Plant & Food Research (PFR). The objective of this study was to determine the susceptibility of vines from two 'Hort16A' orchards to Psa-V. The Roan Orchard received copper a application seven weeks prior to cane sampling and the Nola Orchard received a copper application nine weeks prior to cane sampling.

On 1 November 2012, fruiting canes were collected from two 'Hort16A' orchards located in Te Puna, Bay of Plenty. The last copper treatments applied prior to cane sampling were on the 14 September 2012 and the 29 August on the Roan and Nola Orchards, respectively. Two canes were collected from four vines from each orchard. The canes were collected, tagged and bagged by PFR staff and immediately returned to the Te Puke Research Centre. Canes were cut into 10 cm lengths (segments), surface sterilised, wounded and inoculated with either Psa-V or sterile water (SW). 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 data were combined to calculate an overall index of susceptibility (woody stem bioassay index = WSBI). The differences in WSBI between orchards were examined. Cane samples were sent to Hill Laboratories, Waikato, and the total copper level from each orchard was determined.

The Nola and Roan Orchards had woody stem bioassay indices (WSBI) of 65 and 62, respectively, indicative of susceptibility to Psa-V. There was no statistically significant difference in the bioassay indices between the two orchards (P=0.39). The Nola and Roan Orchards recorded total copper levels of 11.1mg/ kg and 89 mg/kg of dry weight, respectively (Appendix I). The difference in copper level had no bearing on the susceptibility of canes to Psa-V.

This bioassay was performed using non-dormant kiwifruit canes. This result is an indication of the relative tolerance of vines to Psa-V at a specific physiological age under laboratory conditions. It is important to note that the woody stem bioassay provides only part of the information needed to assess susceptibility.

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1 Introduction

A woody stem bioassay that enables the assessment of a cultivar's tolerance to *Pseudomonas syringae* pv. *actinidiae* (Psa-V) has been developed by Plant & Food Research (PFR). The objective of this study was to determine the susceptibility of vines from two 'Hort16A' orchards to Psa-V. The Roan Orchard received copper a application seven weeks prior to cane sampling and the Nola Orchard received a copper application nine weeks prior to cane sampling.

2 Methods

2.1 Cane sampling

On 1 November 2012, fruiting canes were collected from two 'Hort16A' orchards located in Te Puna, Bay of Plenty. The last copper treatments applied prior to cane sampling were on the 14 September 2012 and the 29 August on the Roan and Nola Orchards, respectively. Two canes were collected from four vines from each orchard. The canes were collected, tagged and bagged by PFR staff and then immediately returned to the Te Puke Research Centre.

2.2 Psa-V inoculum

Psa-V inoculum was prepared from fresh cultures grown for two days and made to a concentration of approximately 8.6 x 10^9 bacteria per mL. The viable cell density was confirmed by serial dilution plating, enabling the quantification of colony forming units per mL.

2.3 Bioassay

Each cane (n = 8 canes) was cut into seven to ten 10-cm-long segments, placed into labelled onion bags and surface sterilised, followed by a sterile water (SW) rinse. Cane segments were then blot dried with sterile paper towels and petroleum jelly applied to each end of the cane to limit desiccation. Cane segments were transferred into labelled plastic (sushi) trays each containing a moistened sterile paper towel (10 mL of SW/tray). Each segment 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 kiwifruit 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 (n = 25 cane segments), a droplet of SW (10 μL) was placed directly into each wound using a micro-pipette. For each treated cane segment (n = 50 cane segments), a droplet 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 within 80 L plastic bins.

2.4 Copper testing

On 1 November cane samples from both orchards were provided to Hill Laboratories, Waikato. Total copper tests were performed on whole cane samples.

2.5 Cane assessments

Three weeks after inoculation, canes were prepared for lesion measurements and ooze scoring. Using flame-sterilised blades, cuts were made to expose the lesions at both wound sites (Figure 2). 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 were recorded as dead and were treated as missing data in the analysis.

Figure 2. Excised kiwifruit canes cut to expose possible lesions, three weeks after inoculation with *Pseudomonas syringae* pv. *actinidiae* (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 edges of the lesion).

The lesion and ooze measurements were corrected against the SW controls. The figures were 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 an unbalanced ANOVA was carried out using GenStat, 13th edition.

3 Results and discussion

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

Figure 3a. Example of *Pseudomonas syringae* pv. *actinidiae* (Psa-V) lesion development on a 'Hort16A' cane.

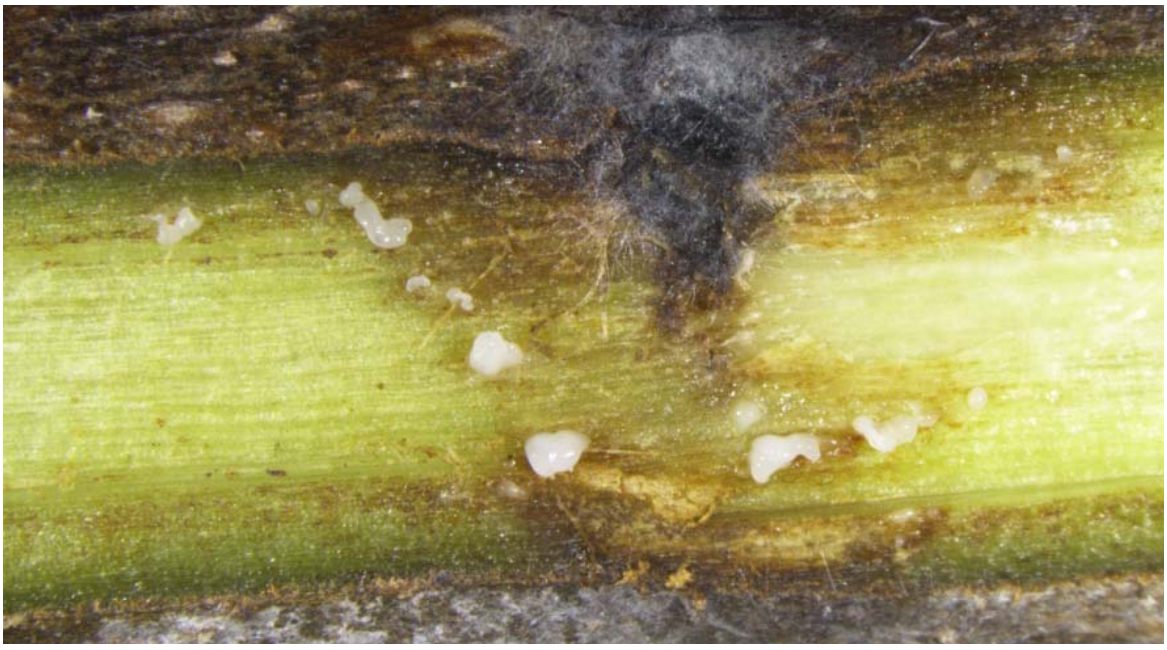

Figure 3b. Example of *Pseudomonas syringae* pv. *actinidiae* (Psa-V) lesion development and cream-coloured bacterial ooze exuding from a lesion area on a 'Hort16A' cane.

The inoculum dose applied to canes was determined at 8.6 x 10^9 bacteria per mL. Approximately 30% of the cane segments deteriorated prior to assessment so these segments were removed from the analysis.

The Nola Orchard had a woody stem bioassay index (WSBI) of 65, indicative of susceptibility to Psa-V (Table 1, Figure 4). The Roan Orchard had an index of 62, also indicative of susceptibility. A WBSI greater than 30 is regarded as moderately susceptible, with level of susceptibility increasing as the index value increases. There was no statistically significant difference between the indices of the two orchards (*P*=0.39).

The Nola Orchard recorded a total copper level of 11.1 mg/ kg of dry weight, and the Roan Orchard recorded 89 mg/ kg of dry weight (Appendix I). The result herein showed the level of copper in the Roan sample was greater than that of the Nola sample; this difference had no bearing on the susceptibility of canes to Psa-V. The copper levels detected were regarded as typical; the average organic copper content in a kiwifruit cane has been reported at 14 mg/ kg (Buwalda & Smith 1987).

This result is an indication of the relative tolerance of specific vines to Psa-V at a certain physiological age under laboratory conditions. It is recommended that dormant winter wood is also screened to verify this result. It is also important to note that bioassays provide only part of the information needed to assess Psa-V susceptibility.

Table 1: Woody stem bioassay indices for vines within the Nola and Roan Orchards after inoculation with *Pseudomonas syringae* pv. *actinidiae* (Psa-V).

Vine	Woody Stem Bioassay Index	SEM
Nola Orchard	65	2.2
Roan Orchard	62	2.3

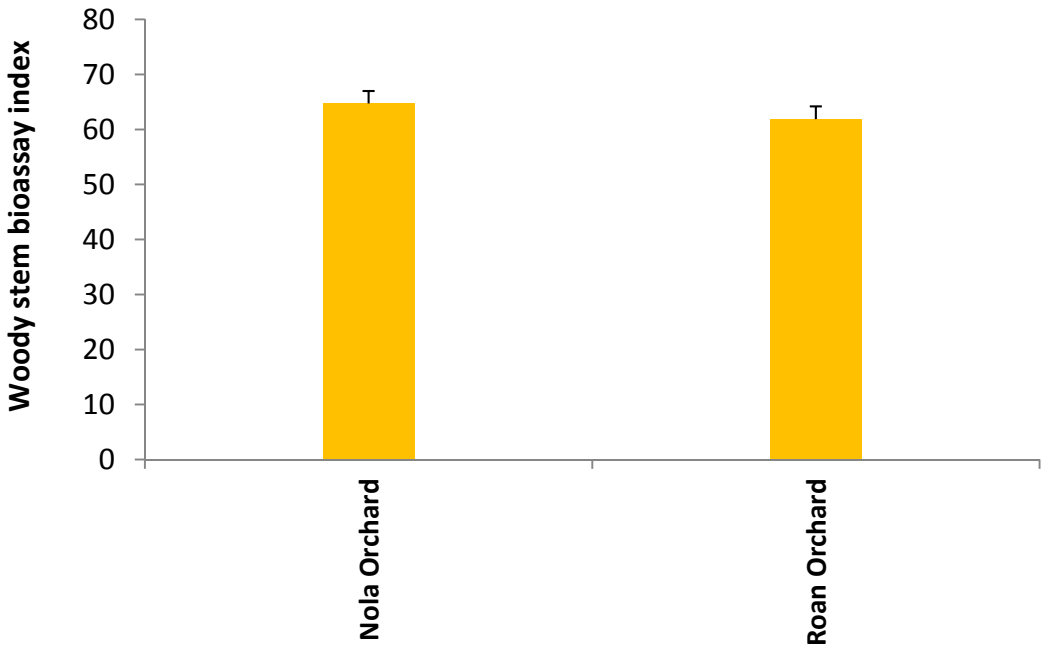

Figure 4. Woody stem bioassay indices for vines within the Nola and Roan Orchards after inoculation with *Pseudomonas syringae* pv. *actinidiae* (Psa-V). Bars are Standard Errors of the Means (SEM).

4 Acknowledgements

The authors would like to acknowledge Jo Stirling and Brenda Anderson for their technical assistance in performing the woody stem bioassay. Furthermore we would like to thank Doug and Robyn Roan of Roan Orchard, and Alan Mecurah, Orchard Manager of Nola Orchard, for providing kiwifruit canes and background information for this bioassay.

5 Reference

Buwalda JG, Smith GS 1987. Accumulation and partitioning of dry matter and mineral nutrients in developing kiwifruit vines. Tree Physiology 3: 295–307.

Appendix I

Hill Laboratories
BETTER TESTING BETTER RESULTS

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ANALYSIS REPORT

Client:	Plant & Food Research	**Lab No:**	1065296 SPv2
Contact:	Nicola Mauchline	**Date Registered:**	02-Nov-2012
	C/- Plant & Food Research	**Date Reported:**	10-Dec-2012
	412 No 1 Road	**Quote No:**	
	RD 2	**Order No:**	TP9385
	TE PUKE 3182	**Client Reference:**	PFR NMauchline
		Submitted By:	Nicola Mauchline

Amended Report

This report replaces an earlier report issued on the 16 Nov 2012 at 2:00 pm
The copper results have changed. See Analyst's Comments.

Sample Type: Kiwifruit cane

	Sample Name:	Kiwifruit 1 01-Nov-2012 11:00 am	Kiwifruit 2 01-Nov-2012 11:00 am			
	Lab Number:	1065296.1	1065296.2			
Dry Matter	g/100g as rcvd	51	46	-	-	-
Copper	mg/kg dry wt	11.1	89	-	-	-

Analyst's Comments

This report has been amended due to a correction of the copper results. For further details see QOWQ 46957.

SUMMARY OF METHODS

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively clean matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis.

Sample Type: Kiwifruit cane

Test	Method Description	Default Detection Limit	Samples
Homogenise	Mincing, chopping, or blending of sample to form homogenous sample fraction. Analysis performed at Hill Laboratories - Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton.	-	1-2
Dry Matter	Drying for 16 hours at 103°C, gravimetry. Analysis performed at Hill Laboratories - Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton. AOAC (OMA) 934.01, 18th Edition (modified - 103 ± 3°C for 16 hours at ambient pressure).	0.10 g/100g as rcvd	1-2
Biological Materials Digestion	Nitric and hydrochloric acid micro digestion, 85°C for 1 hour. Analysis performed at Hill Laboratories - Food & Bioanalytical Division, Waikato Innovation Park, Ruakura Lane, Hamilton.	-	1-2
Copper	Oven dried at 62°C overnight (residual moisture typically 5%) by the Agriculture Division. Biological materials digestion, ICP-MS.	0.010 mg/kg dry wt	1-2

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Samples are held at the laboratory after reporting for a length of time depending on the preservation used and the stability of the analytes being tested. Once the storage period is completed the samples are discarded unless otherwise advised by the client.

Mark Bryant, NZCS (Chemistry)
Senior Technologist - Food & Bioanalytical Division