The Role of Protectants for Reducing Psa Infection via Leaf Scars

PROJECT CODE Zespri 11
V11320

Confidential Report Prepared For Zespri International Limited

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February 2013

1.0 Executive Summary

Zespri engaged HortEvaluation Ltd to evaluate a number of elicitor and copper products that may reduce Psa infection over the leaf fall period.

The trial was established at two sites with suitable Gold 3 and Hayward vines in the Bay of Plenty, within Psa recovery zones.

The elicitor products included single applications of Actigard, BioAlexin and Spotless prior to leaf fall. The copper products included Nordox or Cuprofix Disperss, each applied twice over the leaf fall period.

The trial was carried out as intended, except that air frosts voided the opportunity to include copper sulphate to force leaf fall on some treatments. No other leaf fall protectant products were applied by the respective growers over the leaf fall period.

Buds and leaf scars were excised at the end of leaf fall, laboratory plated and cultured to detect Psa presence. Treated vines were assessed while dormant in late winter; and in spring after bud break, for presence of Psa symptoms ooze, cankers and cane dieback.

Components of yield counts of winter buds, shoots and flower buds were used to measure yield effects.

Leaf samples were also retained frozen for metabolite analysis if any treatment effects were observed.

For Hayward, all of the bud and leaf scar samples were returned as negative for Psa presence. For Gold 3, there were no significant differences between treatments for Psa presence in bud and leaf scar samples, except for the anomalous result that the untreated samples had no Psa detected.

For Hayward and Gold 3, there were insufficient cankers or ooze on trunks, leaders and canes and insufficient cane dieback on Hayward, to provide data for analysis.

For Gold 3, dieback affected between 43-63% of all canes. However, there was no significant difference in the percentage cane dieback across all treatments.

For Hayward and Gold 3, there were no significant differences between the treatments for percentage bud break, percentage fruitful bud break, king flowers per shoot, total flowers per/shoot, king flowers per bud, total flowers per bud or number of shoots.

There was not enough inoculum pressure in the Hayward orchard to observe Psa and obtain possible treatment effects. In the Gold 3 orchard, Psa symptoms were seen but given the unquantifiable nature of natural inoculum distribution, we were not able to obtain significant differences in treatment effects.

None of the treatments had any adverse effects on components of yield.

Given the importance of Psa infection risk during leaf fall, further thought needs to be devoted to methodology which balances the effective evaluation of treatments in a field environment against the risks of high levels of loss in and beyond that environment.

2.0 Introduction

Our knowledge of the Psa infection process is increasingly indicating that all wounds are a risk for infection. Research in Italy and New Zealand has shown that the leaf abscission zone is also a potential entry site. Options for protecting any wounds in kiwifruit are at this time limited.

The use of protectants around the timing of leaf drop warrants investigation and may provide answers that are transferrable to other practices, such as pruning and harvest.

There are also concerns about possible effects of repeat copper applications on bud-break and return bloom.

3.0 Objective

This study aims to

- Compare product options for protecting leaf scars from infection with Psa-V
- Quantify effects of repeat copper application on bud break and return bloom

4.0 Materials and Methods

Trials were carried out in two orchards, one Hayward and one Gold 3 in Bay of Plenty Psa recovery zones.

Table 1: Site Information

Location	**Hayward** David and Lesley Jensen Half Way Orchard, Pyes Pa KPIN 6451	**Gold 3** Paul and Lucy Edkins Golden Meadows Orchard, Pukehina KPIN 3683
Site Details	Block 9 Post-harvest service supplier is Satara	Block 9 Post-harvest service supplier is EastPack
Plants	Conventional Mature, Strip male Good Performing Blocks Low vigour Pergola trained	Conventional Grafted 2011; first production year Near full canopy Pergola trained
Spacing	Bays are 4.5 m between rows and 5.0 m between posts, single planted Plots are single vines two bays wide, or 45 m^2/plot	Bays are 3.5m between rows and 6.0m between posts, double planted Plots are single vines two bays wide or 10.5 m^2/plot
Water Rate	1000 litres/ha	
Treatments	Refer Table 2	
Sprayer	FruitFed trailed motorized handgun sprayer See application method at http://www.youtube.com/watch?v=czCOfC6H-Y8	

Treatments included three elicitor products, Actigard, BioAlexin and Spotless to be applied before leaf fall and two protectant copper products, Nordox and Cuprofix Disperss, to be applied twice during leaf fall, plus an untreated control.

It was initially proposed that there would be a further six treatments, looking at forced leaf drop with the same treatments as listed above. For the elicitor treatments, this would include copper sulphate applied 7- 10 days after the elicitor and for the copper treatments, copper sulphate would be applied 7-10 days before the first copper protectant treatment. The intention of these six additional treatments was to explore whether reducing the length of the leaf fall period by the application of copper sulphate to force leaf fall, had any effects different from leaf fall occurring naturally. However, for both the Hayward and G3 sites, air frosts occurred on 13, 16, 17 and 18 June 2012 between the time elicitors had been applied and the time proposed for the copper sulphate application. These frost events resulted in very rapid natural leaf fall, and therefore the opportunity to apply copper sulphate was missed. Effectively, this meant there were twice as many replicates for each of the elicitor, protectant and untreated control treatments available for assessment, than had originally been proposed.

At each site, 8 -10 additional vines were marked at the end of rows and these vines did not receive any of the trial treatments or any of the growers copper sprays over winter. These vines acted as a “true control” for looking at the effect of copper sprays on bud break.

At both the Hayward and Gold 3 trial sites, the trial areas did not receive any other Psa protectant products or leaf abscission products during the leaf drop period. This extended from 9 June 2012 until after 27 July 2012.

No pruning was carried out within this timeframe.

Table 2: Hayward and Gold 3 Treatments

Treatment	Product	Rate (/100L)	Water rate
1	Untreated in trial area		
2	Untreated in trial area		1000L
3	Actigard	20g	1000L
4	Actigard	20g	1000L
5	BioAlexin	300ml	1000L
6	BioAlexin	300ml	1000L
7	Spotless	350ml	1000L
8	Spotless	350ml	1000L
9	Nordox	110g	1000L
10	Nordox	110g	1000L
11	Cuprofix Disperss	400g	1000L
12	Cuprofix Disperss	400g	1000L
13	Untreated outside trial area		

Layout
Each treatment was replicated 10 times. Replicated treatments were laid out in a randomized basis.

Refer **Appendix 1** Hayward Trial Layout and **Appendix 2** Gold 3 Trial Layout.

Applications

Treatments were applied by Tayah Johnston of FruitFed Supplies using a small plot sprayer. The application method can be seen at http://www.youtube.com/watch?v=czCOfC6H-Y8 Applications were made at targeted times with treatment dates as summarised below.

Table 3: Hayward and Gold 3 Application Times and Conditions

Treatment No.	Treatments	Hayward	Gold 3
1	untreated in trial area	-	-
2	untreated in trial area	-	-
3	Actigard	12/06/2012	13/06/2012
4	Actigard	12/06/2012	13/06/2012
5	BioAlexin	12/06/2012	13/06/2012
6	BioAlexin	12/06/2012	13/06/2012
7	Spotless	12/06/2012	13/06/2012
8	Spotless	12/06/2012	13/06/2012
9	Nordox	17/06/2012, 29/06/2012	17/06/2012, 29/06/2012
10	Nordox	17/06/2012, 29/06/2012	17/06/2012, 29/06/2012
11	Cuprofix Disperss	17/06/2012, 29/06/2012	17/06/2012, 29/06/2012
12	Cuprofix Disperss	17/06/2012, 29/06/2012	17/06/2012, 29/06/2012
13	untreated out of trial area	-	-

5.0 Assessments

5.1 Psa

At the end of the leaf fall period, buds and leaf scars were excised and plated for Psa bacterial presence by Verified Laboratory Services.

For both Hayward and Gold 3, three buds per vine were excised for every vine in treatments 1, 3, 5, 7, 9 and 11.

The intention of this assessment method was to determine if symptom development could be attributed to infection via the leaf scars.

Figure 1: G3 Bud Excision for laboratory analysis

Vines were visually assessed at two timings in late winter / spring to determine the level of Psa symptom expression.

At each assessment time, the number of lesions on trunks, the number of lesions on leaders and the number of lesions on canes, were counted on each vine. The term lesion covers both the reddish/brown ooze from affected tissue and the underlying affected tissue itself, which may or may not have the appearance of a canker.

At each assessment time, the numbers of canes with dieback were counted on each vine.

The total number of canes per vine was also counted at the first assessment.

5.2 Growth

Return bloom was monitored in spring. Assessments were carried out by AgFirst, doing components of yield and bud counts for five canes on each vine in treatments 2, 4, 6, 8, 10, 12 and 13.

5.3 Metabolites

For Hayward, samples of leaves were taken from control vines and vines receiving Actigard, BioAlexin and Spotless pre-treatment and at three days post-treatment.

For Gold 3, samples of leaves were taken from control vines and vines receiving Actigard, BioAlexin and Spotless post-treatment only.

Samples were collected and stored in liquid Nitrogen by Analytica Laboratories. Samples will remain in the freezer until a decision is made as to whether or not to analyse these samples.

5.4 Data Analysis

All data were analysed using a REML (restricted maximum likelihood) model fitting orchard row as a random effect.

6.0 Results

6.1.1 Buds and Leaf Scars

For Hayward, there were not enough Psa growth results from the excised buds and leaf scars, to be analysed. Almost all of the samples were returned as negative for Psa presence.

For Gold 3, a low number of samples were returned positive for Psa presence and there were no significant differences between the treatments for average Psa score. Psa growth percentage is the Psa score multiplied by 25% to give the amount of coverage of the plate in the test. The results of the analysis are presented in Table 4 below.

Table 4: Psa in Gold 3 Buds and Leaf Scars

Gold 3	Psa Score	Psa Growth Percentage
residual degrees of freedom	53	53
Untreated	0.00	0.0
Actigard	0.74	18.4
BioAlexin	0.67	16.7
Spotless	0.64	15.9
Nordox	0.66	16.6
Cuprofix	0.63	15.7
Standard Error of the Difference (s.e.d)	0.363	9.08
Least Significant Difference 5%	0.728	18.20
P-value	0.348	0.348
Treatment Significance	Not Significant	Not Significant

6.1.2 Presence of Visual Symptoms

In the Hayward orchard there was no symptom expression during the trial period. Symptom expression was evident in the Gold 3 orchard and progressed during the trial, with symptoms largely expressed as cane dieback and some cankers and/or ooze seen on trunks, leaders and canes and.

Psa lesions on the trunks, leaders and canes
For Hayward and Gold 3, there were not enough Psa lesions on the trunks, leaders and canes, to be analysed. Almost all the data was zero.

Psa cane dieback
For Hayward, there was insufficient cane dieback data to compare statistically. All the data were zero.

For Gold 3, there were no significant differences between the treatments for percentage cane dieback. The results of the analysis are presented in Table 5 below.

Table 5: Psa Cane Dieback in Gold 3

Gold 3	Percentage Cane Dieback
residual degrees of freedom	113
Untreated	43.2
Actigard	63.5
BioAlexin	53.1
Spotless	54.7
Nordox	53.1
Cuprofix	51.5
Untreated Outside	52.0
Standard Error of the Difference (s.e.d)	10.39
Least Significant Difference 5%	20.59
P-value	0.666
Treatment Significance	Not Significant

6.2 Growth

For Hayward and Gold 3, there were no significant differences between the treatments for

- percentage bud break
- percentage fruitful bud break
- king flowers per shoot
- total flowers per/shoot
- king flowers per bud
- total flowers per bud
- number of shoots.

The results of the analyses are presented in Tables 6 and 7 below.

Table 7: Hayward Growth Results

Hayward	% Bud Break	% Fruitful Bud Break	King Flowers /shoot	Total Flower s/shoot	King Flowers/ winter bud	Total Flowers / winter bud	No. Shoots
residual degrees of freedom	62	62	63	62	62	62	63
Untreated	46.9	44.3	3.87	4.09	1.85	1.94	6.45
Actigard	47.7	43.7	3.68	3.81	1.77	1.83	6.70
BioAlexin	47.7	43.5	3.55	3.64	1.70	1.75	6.99
Spotless	48.7	45.3	3.79	3.88	1.83	1.88	6.43
Nordox	48.6	44.9	3.70	3.87	1.80	1.89	6.77
Cuprofix	45.3	41.4	3.60	3.68	1.63	1.66	6.53
Untreated Outside	47.8	45.7	3.92	4.02	1.88	1.93	6.82
Standard Error of the Difference (s.e.d)	1.98	1.99	0.193	0.216	0.123	0.134	0.334
Least Significant Difference 5%	3.97	3.98	0.386	0.432	0.246	0.267	0.666
P-value	0.667	0.390	0.432	0.336	0.410	0.327	0.585
Treatment Significance	NS	NS	NS	NS	NS	NS	NS

Table 8: Gold 3 Growth Results

Gold 3	% Bud Break	% Fruitful Bud Break	King Flowers /shoot	Total Flower s/shoot	King Flowers/ winter bud	Total Flowers / winter bud	No. Shoots
residual degrees of freedom	58	58	58	58	58	58	58
Untreated	74.8	65.3	3.18	3.65	2.37	2.73	29.4
Actigard	73.7	61.8	3.05	3.42	2.24	2.50	26.6
BioAlexin	75.9	64.8	3.07	3.39	2.32	2.56	25.7
Spotless	74.4	59.9	2.79	3.11	2.08	2.30	25.5
Nordox	76.5	67.3	3.20	3.59	2.44	2.73	28.6
Cuprofix	74.4	65.5	3.43	4.00	2.49	2.91	26.3
Untreated Outside	75.9	65.3	3.48	4.65	2.60	3.46	25.6
Standard Error of the Difference (s.e.d)	5.05	4.93	0.317	0.467	0.292	0.415	3.55
Least Significant Difference 5%	10.11	9.86	0.635	0.935	0.585	0.830	7.10
P-value	0.997	0.755	0.377	0.056	0.651	0.194	0.873
Treatment Significance	NS	NS	NS	NS	NS	NS	NS

7.0 Discussion

For Hayward, there was clearly a very low level of natural inoculum pressure. Psa was barely found in the excised bud and leaf scars, so the lack of visual Psa symptoms in winter and spring was not unexpected.

Similarly, as Psa was apparently barely present around leaf fall, it is not unexpected that there were no visual symptoms expressed in winter and spring, even on vines in the untreated plots.

Neither the treatments themselves nor the very low level of Psa present at the time of treatment as measured by the bud excision and plating has had any positive or negative effect on bud break and floralness, by comparison with the untreated vines.

For Gold 3, Psa was not detected from bud excision in the untreated control vines. By contrast, Psa was found from bud excision in all of the treatments, although there were no significant differences in the levels detected. There is no obvious explanation for this result.

Cane dieback was expressed as a secondary Psa symptom, with the lowest level of dieback at 43% of canes affected, being on the untreated controls, which also yielded the lowest level of Psa, zero, from bud excision. Either, Psa is very variably distributed, or the bud excision sampling plan may have been insufficient to detect the typical presence of Psa in buds, or both, that such data as we collected did not result in useful assessment of treatment effects.

The lack of significant difference between treatments, for percentage of canes with dieback, at around 50% on average, may again relate to the unknown real distribution of Psa on the vines we used. The same comment applies for the Gold 3 growth results.

Investment in field trial work to evaluate product efficacy against Psa, reliant on natural inoculum distribution, is fraught, because of the unknown and difficult to quantify nature of that inoculum distribution.

The opportunity to protect against Psa infection during leaf fall deserves further investigation because this is known to be a high risk infection period. Further thought needs to be given to methodology which balances the effective evaluation of treatments in a field environment against the risks of high levels of loss in and beyond that environment.

8.0 Acknowledgements

The author would like to thank

- David and Lesley Jensen, Half Way Orchard, for the use of their orchard, materials and support for the Hayward trial.
- Ken, Paul and Lucy Edkins, Golden Meadows Orchard, for the use of their orchard, materials and support for the Gold 3 trial
- Tayah Johnston, Technical Research Assistant (Northern region), PGG Wrightson FruitFed Supplies for carrying out the spray applications.
- Peter Sanders, HortEvaluation Ltd, for carrying out the spray applications, assisting with trial layout and review of the trial report.
- Fred Phillips, HortEvaluation Ltd, for review of the trial report.
- Catherine Cameron, AgResearch Ltd, for statistical analysis.
- Dr Mary Black, Zespri for project leadership and management.

Appendix 1: Layout Hayward Site, Pyes Pa

bay													
23													
22							126,ut						
21			123,ut		125,ut				128,ut				
20			122,ut		124,ut		69,12		127,ut		129,ut		130,ut
19													
18			33,11		51,6		68,7		87,1		105,6		
17	121,ut		32,3		50,11		67,10		86,8		104,1		
16			31,4		49,2		66,6		85,5		103,2		
15	15,12		30,9		48,10		65,1		84,8		102,7		120,7
14	14,8		29,5		47,12		64,3		83,11		101,11		119,3
13	13,6		28,12		46,2		63,9		82,7		100,5		118,8
12	12,7		27,2		45,4		62,4		81,9		99,12		117,2
11	11,10		26,1		44,7		61,8		80,1		98,4		116,1
10	10,2		25,6		43,1				79,5		97,9		115,9
9	9,8		24,7		42,11		60,4		78,10		96,6		114,11
8	8,3		23,3		41,9		59,7		77,3		95,11		113,5
7	7,11		22,10		40,6		58,12		76,12		94,9		112,10
6	6,4		21,1		39,5		57,9		75,4		93,3		111,6
5	5,12		20,5		38,3		56,1		74,2		92,10		110,12
4	4,6		19,9		37,8		55,5		73,6		91,2		109,4
3	3,9		18,4		36,8		54,3		72,11		90,12		108,8
2	2,1		17,11		35,7		53,10		71,2		89,7		107,10
1	1,5		16,2		34,10		52,8		70,5		88,4		106,3
bay	F	m	F	m	F	m	F	m	F	m	F	m	F
row	1	2	3	4	5	6	7	8	9	10	11	12	13

Cryptomeria shelter
P radiata shelter
first number is plot number
second number is treatment number

Appendix 2: Layout Gold 3 Site, Pukehina

	M			**B**			**M**	
	30,8	**M**	60,4		90,2		120,3	**M**
	29,1		59,1		89,9	**F**	119,7	
	28,12		58,5	**M**	88,5		118,10	
	27,9	**B**	57,7		87,4		117,6	**B**
	26,4		56,10		86,8	**M**	116,4	
	25,11		55,2	**B**	85,11		115,12	
	24,8	**M**	54,6		84,11		114,5	**M**
	23,5		53,3		83,1	**B**	113,11	
	22,3		52,11	**M**	82,3		112,8	
	21,4	**B**	51,9		81,5		111,2	**B**
	20,7		50,12		80,9	**M**	110,9	
	19,2		49,8	**B**	79,8		109,1	
	18,6	**M**	48,10		78,10		108,4	**M**
	17,1		47,12		77,2	**B**	107,1	
	16,9		46,9	**M**	76,12		106,12	
	15,12	**B**	45,4		75,7		105,9	**B**
	14,11		44,11		74,4	**M**	104,6	
	13,10		43,7	**B**	73,6		103,8	
	12,4	**M**	42,3		72,2		102,11	**M**
	11,2		41,8		71,9	**B**	101,2	
	10,7		40,2	**M**	70,12		100,3	
	9,10	**B**	39,6		69,4		99,10	**B**
	8,8		38,1		68,11	**M**	98,7	
	7,11		37,5	**B**	67,6		97,5	
	6,1	**M**	36,3		66,3		96,1	**M**
	5,6		35,6		65,8	**B**	95,3	
	4,3		34,2	**M**	64,1		94,12	
	3,5	**B**	33,7		63,7		93,7	**B**
	2,9		32,5		62,5	**M**	92,10	
	1,12		31,10	**B**	61,10		91,6	
		M		123,13	**B**	127,13	128,13	
			M	122,13	125,13	126,13		
	B			**M**	124,13			
		B		121,13				
			B					
	M			**Plot**	first number is plot number			
		M		**Plot**	second number is treatment number			
Row	**32**	**31**	**30**	**29**	**28**	**27**	**26**	**25**