Crop Safety of Late Leaf Fall Copper Applications on Gold 3 PROJECT CODE Zespri 20 VI1452

Report Prepared For Zespri International Limited

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June 2014

1.0 Executive Summary

HortEvaluation Ltd were engaged by Zespri to undertake a study to determine whether copper applied with a superspreader or a penetrant adjuvant at late leaf fall causes phytotoxicity to buds. A second objective of this project was to evaluate efficacy for Psa disease control.

The study was carried out on an undercover Gold 3 block, where leaves were still falling under the protective cover.

Four treatments were tested, being a single application of each of: Nordox applied dilute, Nordox applied in a concentrate spray with the superspreader DuWett, Nordox applied in a concentrate spray with the penetrant Engulf, and an untreated control.

Treatments were replicated fifteen times and fully randomised. For each of the first three treatments above, an additional vine was sprayed with the same spray mix and brilliant blue dye added to the mix, to allow for investigation of movement of dye, and presumably active ingredient, into leaf scar tissue.

Applications were made with a gas powered knapsack sprayer to achieve even spray output and coverage.

Assessment of dye penetration was made by cutting and sectioning multiple buds on sprayed canes, twenty four hours after application.

Assessment of yield effects was made by counting buds, shoots and flowers pre thinning and flowering.

No dye penetration was observed into the leaf scar vascular tissue for any of the three treatments sprayed with brilliant blue dye added to the spray mix. It is therefore not surprising that there were no significant differences between treatments for yield data, as measured by winter buds, bud break and floralness.

How long leaf scars remain open to penetration of applied chemicals or direct Psa infection after natural leaf fall is not clear. In this trial, leaf fall was not only delayed but also prolonged, undercover.

The visualisation of spray coverage using brilliant blue dye showed both Nordox applied concentrate with DuWett and Nordox applied concentrate with Engulf had enhanced coverage on the cane and leaf scar, with a continuous and even blue film of spray seen, by comparison with Nordox applied alone dilute.

There was no evidence of phytotoxicity effects on buds, for copper products applied with the surfactants DuWett and Engulf. This result gives more confidence about the safe use of DuWett.

Psa canker and cane dieback assessment was either too low(canker) or analysis of variance (Psa cane dieback) showed no significant differences between treatments. This would have been expected, given the photographic evidence with Brilliant Blue dye, that the dye and therefore the applied copper did not penetrate into the vascular tissue of the leaf scar and associated bud.

2.0 Introduction

Copper applied over the leaf fall period is considered best spray application practice to minimise Psa infection of recently exposed leaf scars.

Recent work using food dye added to copper and superspreader spray mix and applied to Hayward vines within twenty four hours of leaf fall, showed penetration of the spray dye into the leaf vascular tissue.

If copper could move similarly into the leaf scar vascular tissue when applied soon after leaf fall, then it may provide better protection from without and within the vascular tissue, against Psa infection.

The risk is that copper moving into the leaf scar vascular tissue could have phytotoxic effects on that tissue.

3.0 Objective

This study aims to determine whether copper applied with a superspreader or a penetrant adjuvant at late leaf fall causes phytotoxicity to buds. A second objective of this project was to evaluate efficacy for Psa disease control.

4.0 Materials and Methods

The trial was carried out in an orchard which had been grafted to Gold 3 in winter 2011.

Table 1: Site Information

Location	Te Puke, Bay of Plenty
Site Details	Rows 70 -74
Plants	Double planted, every row. Matrix male vines.
	Previously Hort16A, cut off and regrafted winter 2012, Pergola trained

This trial was commenced later in the season than is ideal to test leaf fall Copper applications. Therefore, an undercover area of Gold 3 vines was selected for this trial, where the cover had moderated the temperatures and delayed leaf fall.



Figure 1: Leaf Fall outside looking into the covered area (left) and undercover (right) 11 July 2013

The orchard block used had only low presence of Psa symptoms immediately prior to the commencement of the trial. Vines were selected that were free from Psa symptoms.

Refer Appendix 1: Trial Location and Appendix 2: Trial Layout

Treatments were replicated fifteen times and laid out in a randomised block.

Application was made with a gas assisted knapsack sprayer, to facilitate even application on randomised single vine plots. Refer **Appendix 3: Treatment Application**

The grower Psa protection programme was carried out on the whole orchard including the trial area, except that no leaf fall sprays were applied in this area.

The copper product applied was Nordox. Treatment one was Nordox applied dilute at 1000L/ha alone at the winter rate of 70g/100L. Treatment two and three were applied at the same effective rate of copper but in a concentrate spray, based on proprietor recommendations for both DuWett and Engulf.

DuWett is an organosilicone superspreader which is recommended with copper products to enhance coverage of the vine surfaces. Engulf is a super penetrant surfactant for use post-harvest to promote agrichemicals into difficult to penetrate situations.

Table 2: Treatments; 11 July 2013

Treatment	Product	Rate (g/100L)	Surfactant Product	Surfactant Rate (ml/ha)	Surfactant Rate (ml/100L)	Blue Dye/100L	Water Volume (L/ha)		
1	Nordox	70			0	1%; 1L plot 61 only	1000		
2	Nordox	140	DuWett	750	150	1%; 0.5 L plot 62 only	500		
3	Nordox	140	Engulf	750	150	1%; 0.5 L plot 63 only	500		
4	no application at leaf fall								

For each of treatments one to three, an additional undercover plot outside the trial area was sprayed with the above treatment plus the addition of brilliant blue food colouring.



Figure 2: Treatment One, Nordox Dilute Spray Coverage on Canes and Leaves using Brilliant Blue Dye

5.0 Weather Data

An on-site weather station recorded both undercover and outside weather data. The trial vines were not exposed to rain and less exposed to wind because they were under cover.

The environment in which the trial vines were growing was different to that experienced by vines growing outside, because the cover was in place for the 2012/13 growing season.

Treatments applied on 11 July 2013 were protected from rainfall of 26mm on the day, while relative humidity averaged 89% for inside and outside environments through the application period.

The covered structure protected the trial site from south east to east southeast outside winds of up to 10km/hour, with winds from the same directions moderated by the covered structure to speeds of up to 2.8km/hour during application of treatments.

6.0 Assessments

6.1 Dye Penetration

On 12 July 2013, approximately twenty four hours after spraying, canes were sampled from these plots, removed to a laboratory and buds dissected to examine the extent of dye penetration through the leaf scar.

The purpose of these additional treatments was to explore, by visualising the location of the brilliant blue dye in excised leaf scars, if and to what extent spray mix penetrated leaf scar tissue.

6.2 Growth Effects

Four individual canes were tagged within each plot, subsequent to the completion of winter pruning by the grower.

Tagged canes within plots had winter bud counts (20 August 2013), shoot and flower counts (16 October 2013) undertaken, pre bud thinning and flowering, and fruit counts (12 November 2013); to measure treatment effects on buds.

6.3 Psa Effects

Although not specifically a trial to evaluate treatment effects for Psa disease control, the expression of symptoms was assessed in case treatment effects existed. Vines were assessed once prior to any remedial action being undertaken by the grower to remove symptomatic plant tissue.

Leader cankers were counted as these were evident in some plots. The number of dieback canes per plot was also counted, as these were more widely evident.

6.4 Data Analysis

The data captured on four canes per vine was analysed as a split plot design.

Cankers were present in too low numbers to analyse.

Dieback cane data were log-transformed to meet the normality assumptions of the analysis. Actual treatment means and standard error of the differences are presented with the probability and standard error of the means from the transformed analysis.

7.0 Results

7.1 Brilliant Blue Dye Penetration

No dye penetration was observed into the leaf scar vascular tissue for any of the three treatments sprayed with brilliant blue dye added to the spray mix.



Figure 3: External Leaf Scar and Bud (left) and Cross Section Leaf Scar and Bud (right); in descending order; Nordox dilute, Nordox+DuWett concentrate, Nordox+Engulf concentrated

7.2 Growth Effects

Bud break was low overall on Gold 3 under the canopy, ranging from 32-36 percent. This is thought to be a result of the modified environment being warmer and vines receiving lower winter chill.

Flowers per winter bud were also low (0.58-0.68) on Gold 3 under the canopy. In outdoor Gold 3 crops, a ratio of 1.3 flowers per winter bud is more typically expected.

There were no significant differences between treatments for yield data.

Table 3: Effects on Yield

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Gold 3 Per Cane	Winter Buds 20/08/13	Flowers 16/01/13		Fruit 12/11/13	Flowers / Shoot	Flowers / Winter Bud	Budbreak %	Fruit % of flowers	
residual df	42	42	42	42	42	42	42	42	
Copper only (Trt 1)	22.9 a	15.1 a	8.13 a	9.48 a	1.74 a	0.68 a	36.0 a	62.2 a	
Copper plus DuWett (Trt 2)	23.1 a	12.9 a	8.17 a	7.42 a	1.64 a	0.58 a	36.0 a	54.9 a	
Copper plus Engulf (Trt 3)	22.2 a	15.0 a	7.12 a	8.06 a	2.05 a	0.67 a	32.0 a	53.8 a	
No application (Trt 4)	23.7 a	15.3 a	8.02 a	9.75 a	1.91 a	0.65 a	34.2 a	59.7 a	
Trt s.e.d	1.14	2.58	0.836	1.615	0.252	0.118	3.82	5.78	
LSD 5%	2.30	5.20	1.687	3.259	0.509	0.237	7.71	11.66	
Trt P-value	0.626	0.776	0.550	0.418	0.377	0.827	0.683	0.423	
Trt Significance	NS	NS	NS	NS	NS	NS	NS	NS	
Trt 1 sem	0.60	2.87	0.753	1.763	0.254	0.135	3.59	4.81	
Trt 2 sem	0.79	1.79	0.797	1.471	0.169	0.077	3.51	3.39	
Trt 3 sem	0.72	2.26	0.741	1.430	0.162	0.102	3.28	3.18	
Trt 4 sem	1.02	1.83	0.683	1.684	0.156	0.082	3.18	4.53	

7.3 Psa Effects

Table 4: Psa Treatment Effects

Gold 3	Dieback Canes/Plot
residual df	42
Copper only (Trt 1)	0.47 a
Copper plus DuWett (Trt 2)	0.20 a
Copper plus Engulf (Trt 3)	0.73 a
No application (Trt 4)	0.60 a
Trt s.e.d	0.294
LSD 5%	0.593
Trt P-value	0.387
Trt Significance	NS

Trt 1 sem	0.193
Trt 2 sem	0.108
Trt 3 sem	0.326
Trt 4 sem	0.189

There were no significant differences between treatments for Psa symptoms.

8.0 Discussion

Canes were sectioned across multiple buds. Using this dye on one additional vine for each of treatments one, two and three and sectioning to determine the extent of dye movement, did not demonstrate uptake of the dye in any of the treatments.

It is therefore likely that the treatments as applied to the trial vines did not result in copper penetration into the leaf scar vascular tissues.

Work undertaken by Zespri Orchard Productivity team has shown that for leaf scars where the ready-to-drop leaf was removed by light tapping and then the freshly exposed leaf scar immediately sprayed with dye, uptake of the dye into the leaf scar was much more rapid than for leaves where the leaf dropped naturally and the leaf scar was sprayed with dye within twenty four hours.

The Zespri trial work showed a reduced rate of dye uptake when sprayed within twenty four hours of natural leaf fall, suggesting that ability of the spray solution to penetrate the wound declines rapidly after leaf abscission. The rate of leaf scar wound healing by callus production at or after leaf fall is not well understood. Vascular tissue at the recently exposed leaf scar may be more difficult to achieve spray penetration into, even using a specifically formulated penetrant in the spray mix such as Engulf; after a short time after the scar is exposed.

Leaf fall was not only delayed but also prolonged, undercover. It is not known if the modified environment under cover, affecting both time to commencement of leaf fall and length of leaf fall period, would also affect leaf scar formation, healing rate after leaf fall and susceptibility to spray penetration.

For example, the undercover environment is likely to be drier than the outside environment. Relative humidity on the date of the treatment applications was the same under and outside the canopy (89%), but leaf wetness was not because of the 26mm of rain that fell outside.

In other trials where brilliant blue dye has been shown to move in through 24 - 48 hour old leaf scars, the dye was "pooled" in the leaf scar itself and infiltrated in through the leaf scar by suction pressure of 40-50KPa. This pressure was used as it equates to the xylem tension of kiwifruit vines with active transpiration occurring (N. Gould, Plant and Food Research, pers.comm).

Vines at leaf fall are unlikely to be actively transpiring, so no active plant uptake of dye would be expected. Evidently, even fresh leaf scar wounds are not comparable to other types of wood wounds such as Cicada egg masses laid in wood, where it is possible to achieve dye and therefore insecticide penetration with penetrant products such as Engulf.

How long leaf scars remain open to penetration of applied chemicals or direct Psa infection after natural leaf fall is not clear, but these trial results indicate this period may be relatively short.

What is clear from the visual effects observed on treated wood and leaf scars is that there is a very significant difference in coverage achieved, between the three treatments. In both Figure 2 and the top set of photographs of Figure 3, Nordox applied dilute resulted in coverage as discrete drops, with gaps of untreated tissue between drops, on the cane and leaf scar. The individual blue droplets of spray deposit are clearly distinct.

Both Nordox applied concentrate with DuWett and Nordox applied concentrate with Engulf showed enhanced coverage on the cane and leaf scar, with a continuous and even blue film of spray seen.

The poor budbreak and low number of flowers per bud for shoots that did grow, as a result of the modified environment, combined to deliver much below average productivity, by comparison with the outdoor Gold 3 vines immediately adjacent and in the same rows as the covered Gold 3.

None of the treatments affected growth performance either positively or negatively by comparison with the untreated control, under cover. This would have been expected, given the photographic evidence with Brilliant Blue dye, that the dye and therefore the applied copper did not penetrate into the vascular tissue of the leaf scar and associated bud.

This result supports the safe use of the superspreader DuWett to improve coverage on a hard to cover target, the leaf scar, without risk of phytotoxicity on healed leaf scars.

Psa symptoms were low level in the trial area and there was no evidence that the treatments tested impacted on disease expression.

It is possible that the overhead cover intended to modify the Gold 3 environment to be drier and therefore less favourable for Psa multiplication and infection, was having the desired effect.

9.0 Acknowledgements

The author would like to thank

- The owner and manager of the orchard, for the use of their orchard, materials and support for the trial.
- 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.
- Mary Black, Zespri for project leadership and management.

Appendix 1: Trial Location



Appendix 2: Trial Layout

		Block 1	, Rov	vs 70-74							
				Acce	ess T	rack					
Row	75		74		73		72		71		
Bay	i										
1											
	İ										
2	ļ	1,4		16,3		31,2		46,3			
		2,2		17,3		32,1		47,2			
3	ļ	3,3		18,2		33,1		48,1			
		4,1		19,1		34,4		49,4			
4	į	5,2		20,4		35,3		50,2			
		6,1		21,2		36,2		51,1			
5		7,3		22,1		37,4		52,3			
		8,4		23,3		38,2		53,2			
6		9,1		24,4		39,3		54,4			
		10,2		25,2		40,1		55,1			
7	į	11,3		26,1		41,1		56,3			
	ļ	12,4		27,4		42,3		57,3			
8		13,1		28,3		43,2		58,1			
	į	14,2		29,3		44,4		59,4			
0		15,4		30,4		45,4		60,2			
9											
40	ļ	61,1		62,2		63,3					
10		.,,		J-,-		23,0					
	ļ										
Bay											
	undervine shelter										
		covered area									

Appendix 3: Treatment Application

