Ambitious 10SL Efficacy on Psa Disease on Hayward Kiwifruit 2016 2017

Confidential Report Prepared for Grochem Limited

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1.0 Executive Summary

Grochem engaged HortEvaluation Ltd to undertake field trials to evaluate efficacy of Ambitious 10SL™ (Ambitious) on Hayward kiwifruit; in the Te Puke area of Bay of Plenty in 2016/17, and in Edgecumbe in 2014/15 and 2015/16.

Prior to the commencement of the trial in 2016/17, very few canker or ooze symptoms were present at the new trial site, to provide a visible source of Psa inoculum, but despite this, leaf spot symptoms were very evident by assessment in November 2016.

In both 2014/15 and 2015/16, there were six treatments, with treatments two to four each being a single application of Ambitious, early after bud break (treatment two), near the middle of the period between bud break and flowering (treatment three), and towards the end of the period (treatment four). Treatment five comprised two applications of Ambitious, one applied early and one applied mid, at the same timings as treatments two and three. Treatment six comprised two applications of Ambitious, one applied mid and one applied late, at the same timings as treatments three and four. Treatment one was the untreated control with no Ambitious application.

In 2016/17, later treatments were excluded, as application at the later timing in 2014/15 and 2015/16 was not found to contribute to Psa leafspot disease control (infection typically occurred much to somewhat earlier than flowering). Treatment one was the untreated control (no Ambitious application). Treatment two was a single application of Ambitious, early/mid after bud break. Treatment three was near the midpoint between bud break and flowering and treatment four comprised two applications of Ambitious, one applied early/mid and one applied mid, at the same timings as treatments two and three.

In all three years of trial work, Ambitious was applied at 50ml/100L, sprayed to achieve good coverage. Treatments were replicated eight times and randomised. Plots were single vines.

Different from 2014/15 and 2015/16, where the grower applied their preferred Psa disease control spray programme, excluding Ambitious and any elicitor-type products between budbreak and flowering; in 2016/17, no other Psa disease control products were applied in the trial area. Only the Ambitious treatments above were applied for Psa disease control in the trial area between budbreak and flowering.

On each vine, four typical canes were marked for assessment. On each cane, the total number of leaves per cane and the number of leaves per cane with Psa leaf spot were counted. An assessment of the level of Psa leaf spot severity per cane was made using a 0-5 scale. The percentage of leaves per cane with Psa leaf spot was calculated. On each cane, the total number of flower buds per cane were counted then number of fruit set per cane was counted post flower and pre-thinning.

Key findings in 2014/15 were

- Treatments three, five and six had a significantly lower spot area score (1.2-1.7 per cane) than the control (3.3 per cane)
- Treatments three, five and six also had significantly less percentage spotted leaves (24.6-39.4%) than the control (60.5%)
- Ambitious applied mid-season resulted in significant reduction in Psa leaf spot, as assessed by both severity score and proportion of leaves
- There was no treatment effect on the percentage of reject fruit
- When analysing the causes of reject, treatment three (one application mid) had a significantly higher proportion of reject fruit caused by shape as flat/fan reject

percentage (40.1%) than for other causes of reject, compared with causes of reject for the control (24.2%)
- There were no significant differences between treatments for the final fruit number pre-harvest as a percentage of flower buds originally counted
- Treatment five (application early and mid between bud break and flowering) had significantly higher average fruit weight (119.8g/fruit) than the control and all other treatments (108.6-112g/fruit)

Key Findings in 2015/16 were

- The leaf spot results showed the same trend for treatment effects as for 2014/15, but because the absolute levels of leaf spot score and percentage spotted leaves were much lower, there were no significant differences between treatments
- Vine vigour was not significantly different between treatments, as measured by number of new canes developed in the leader zone, per metre of leader length
- Components of Yield analysis showed no return bloom gain or loss in productivity, from Ambitious applied in the previous season
- Repeat applications of Ambitious in 2015/16 did not adversely affect harvest 2016 fruit maturity characteristics

Key Findings in 2016/17 were

- The level of symptoms observed across all treatments was intermediate between 2014/15 (high levels of leaf spot frequency and severity) and 2015/16 (low levels of leaf spot frequency and severity)
- Both the mid (15.8%) and early plus mid treatments (12.8%) resulted in significantly lower Psa leaf spot percentage, than the untreated control (34.4%)
- Both the mid (0.74) and early plus mid treatments (0.53) resulted in significantly lower Psa leaf spot score, than the untreated control (1.44)
- There was no significant difference in the percentage reject fruit between any of the treatments (4.8-6.5%) compared with the untreated control (4.8%)
- Within the reject fruit, a significantly higher percentage (26.3%) of reject early application fruit (treatments two and four) were rejected for being flat by comparison with no mid application (treatment one; 14.8%) or mid application (treatments three and four; 18.7%).

Psa leaf spot is thought to be an important source of inoculum during the growing season, so Ambitious, which enables a reduction in the expression of leaf spot symptoms, can play a role in the reduction of in-orchard inoculum in spring.

The current spring Psa spray control options all have drawbacks and risks as well as advantages.

Risks of environmental copper accumulation, Psa copper or bactericide resistance, Zespri brand intolerance to the use of animal or plant purposed bactericides applied before flowering and plant growth regulators applied to fruit; and elicitor application timing to avoid unwanted checks to plant growth, are all long-term factors to weigh in considering the place of Ambitious in a crop protection programme.

Ambitious can play an important role in spring protection of leaves, allowing for a break from copper spray application or optimizing the limited bactericide options application timing to highest risk periods.

These results reinforce the findings of the 2014/15 and 2015/16 trials that a single application between bud break and flowering provides a further opportunity for growers to enhance control of the leaf symptoms of Psa disease, without the risk of adverse effects.

2.0 Introduction

Growers have been able to use Ambitious 10SL, (Ambitious; 10g/L forchlorfenuron or CPPU active ingredient) on cropping kiwifruit vines for Psa disease control as permitted by the Zespri Crop Protection Standard since 2014/15.

Ambitious has an ACVM full label claim for this use.

The Zespri 2016/17 Crop Protection Standard (CPS) permitted Ambitious use as follows

- Use rate of 50ml/100L
- Not to be used on Gold varieties

A single application only is permitted to be used during the bud phase; that is between bud break and flowering.

Ambitious has shown efficacy in potted plant studies, has shown good suppression of Psa-leaf spot and no efficacy on bud rot in field trials to date, in studies undertaken by HortEvaluation Ltd.

Ambitious is reliant on good coverage of tissue to achieve effects on target canopies. The active ingredient, forchlorfenuron mode of action is not well understood, but it is clear that application in the period between bud break and flowering results in physical changes to the vegetative canopy.

Such changes have been readily observed and documented. Many growers have reported improved Psa control as a result of inclusion of forchlorfenuron in their spring spray programmes and the control of Psa leaf spot symptoms has been demonstrated by robust trial work.

Some growers have expressed concern about the effect of forchlorfenuron products used in the bud phase, on subsequent fruit shape.

3.0 Objectives

The objectives of the trial were to:

- further evaluate field efficacy of Ambitious on Psa disease suppression on Hayward kiwifruit
- to investigate causes of reject, with a focus on fruit shape, prior to hand thinning.

4.0 Materials and Methods

The trial was located at a site in the Te Puke area of the Bay of Plenty region of New Zealand.

(Appendix 1: Site Location)

The trial block selected had moderate Psa infection levels in 2015/16. In addition, the orchard owners apply a minimal programme of crop protection products for Psa disease control.

Copper products, bactericides and other products listed on the Zespri CPS were not applied during the growing season, with the exception of Ambitious.

The trial site was established in Block 2 with a large buffer zone.

Table 1: Property Details

Location	
	KPIN 9138
	, Te Puke
	Part Block 2
Manager	
Trial Manager	Lynda Hawes
	Email lynda.hawes@kcl.kiwi
Post-Harvest Provider	EastPack Ltd
Plants	Mature Hayward orchard, full canopy strip male, pergola canopy
Spacing	Bays 4.5m, Rows 3.0m,
	Single Planted
Plot Size	2 bays = 27m2
Replication	8
Water Rate	Dilute, spray to wet, expanding canopy cover
Application	Treatments as below
Sprayer	Solo Pressure Knapsack Sprayer, Model 433, Nozzle setting 3

Layout was randomised block with eight replications for each treatment. Each plot was marked with tags showing a top and bottom number. The top number was the plot number and the bottom number was the treatment number.

(Appendix 2: Trial Layout)

The grower programme was applied in addition to the treatments. No other Psa products, including other CPPU products or Actigard, were applied by the grower during the trial application period, in the trial area.

(Appendix 3: Grower Spray Programme)

There were four trial treatments.

Treatment one was the untreated control (no Ambitious application). Treatment two was a single application of Ambitious, early/mid after bud break (referred to as early from hereon). Treatment three was near the midpoint between bud break and flowering (referred to as mid from hereon), and treatment four comprised two applications of Ambitious, one applied early/mid and one applied mid, at the same timings as treatments two and three.

Application was made at 50ml Ambitious/100L, sprayed to achieve good coverage and at increased spray volume for application of treatment three and second application of treatment four, as canopy cover had further developed.

All applications were made with a Solo 433 motorized knapsack sprayer, resulting in spray coverage similar to that achieved by an orchard airblast sprayer.

Table 2: Application Dates

ISO week	39		40	41	42	43	44
Date	30/09/2016				13/10/2016		26/10/2016
Growth Stage	budbreak						
Treatments					T2, T4		T3, T4

Figure 1: Growth Stage at Application timing one (treatments two and four)

Application details were recorded and weather data at application sourced from the nearest weather station.

Table 3: Application Details

Date	**13/10/2016**	**26/10/2016**
Time	**3.55 - 4.35pm**	**10.30am - 12.00pm**
Temperature ^{0}C	18.8 - 19.6	17.9 - 20.0
Wind Direction	SSW	NW to WNW
Average Wind speed km/hour	5.4	6.6
Rain mm	0	
Coverage	Spray to wet	
Drying Conditions	Good; fine & warm	Showery; resprayed plots 2,3,4,6,11,13

Weather data source: Harvest.com/Longview Trust/Last Chance block 6

Grower application of Ambitious on 15 October 2016 was not applied to or oversprayed within the trial area.

(Appendix 4: Psa Risk Model and Spray Applications 2016)

6.0 Assessments

6.1 Psa

Four typical canes were tagged on each vine (plot).

On each cane, Psa leaf spot was assessed once, pre-flower.

- Number of leaves per cane were counted
- Number of leaves with Psa leaf spot per cane were counted, to enable calculation of the percentage of leaves with spot

- Leaf spot severity on each cane was assessed by categorising between 0=no spot and 5= covered in spot/leaf failing.

(Appendix 5: Leaf Spot Scoring System)

On each cane, number of flower buds per cane were counted. Fruit set was determined at early fruit set by counting the number of fruit set. Fruit set as a percentage of flowers was calculated.

Pre-harvest final fruit number was assessed, shortly before harvest after final crop grooming had been completed.

Number of fruit per cane were counted and number of class 1 fruit at harvest as a percentage of flowers was calculated.

6.2 Causes of Reject

After fruit set, all reject fruit was separately harvested from each tagged cane. Cause of reject for each fruit was then categorised as being reject for shape or reject for other reasons.

Shape reject fruit categories were flat, fan, dropped shoulder, square, misshapen and ridging.

Other reject fruit categories were Hayward mark, leafroller damage, Sclerotinia scarring, wind rub, joined fruit and undersize.

7.0 Data Analysis and Results

Analyses were run on the four treatments and on the two main effects; that is early season treatment and mid-season treatment and their interaction.

All analyses were run averaged over the four canes assessed per plot (vine).

For fruit reject data, only those causes of reject where at least one-third of the vines had fruit rejected for that cause were analysed.

Data were also analysed by treatment time, to determine if there were any interactions between treatment timings.

Analysis of variance was carried out on raw data. No data transformation was required for the analyses.

7.1 Psa Assessment

In 2016/2017, treatment effects were again clear on Psa leaf spot.

Table 4: Psa Leaf Spot

	Psa Leafspot Severity	Percentage of Leaves with Psa Leaf Spot
residual df	21	21
Control (Trt 1)	1.44 a	34.4 a
Early (Trt 2)	1.06 ab	24.5 ab
Mid (Trt 3)	0.74 b	15.8 b
Early + Mid (Trt 4)	0.53 b	12.8 b
Trt s.e.d	0.255	7.1
LSD 5%	0.53	14.77
Trt P-value	0.01	0.026
Trt Significance	*	*

For percentage of leaves with Psa leaf spot, early Ambitious application resulted in a reduction in the percentage of leaves with leaf spot (24.5%) compared with the untreated control (34.4%), although not to the same degree as mid (15.8%) or early plus mid (12.8%) Ambitious application, so was not found to be significantly different from the untreated control and from the other treatments.

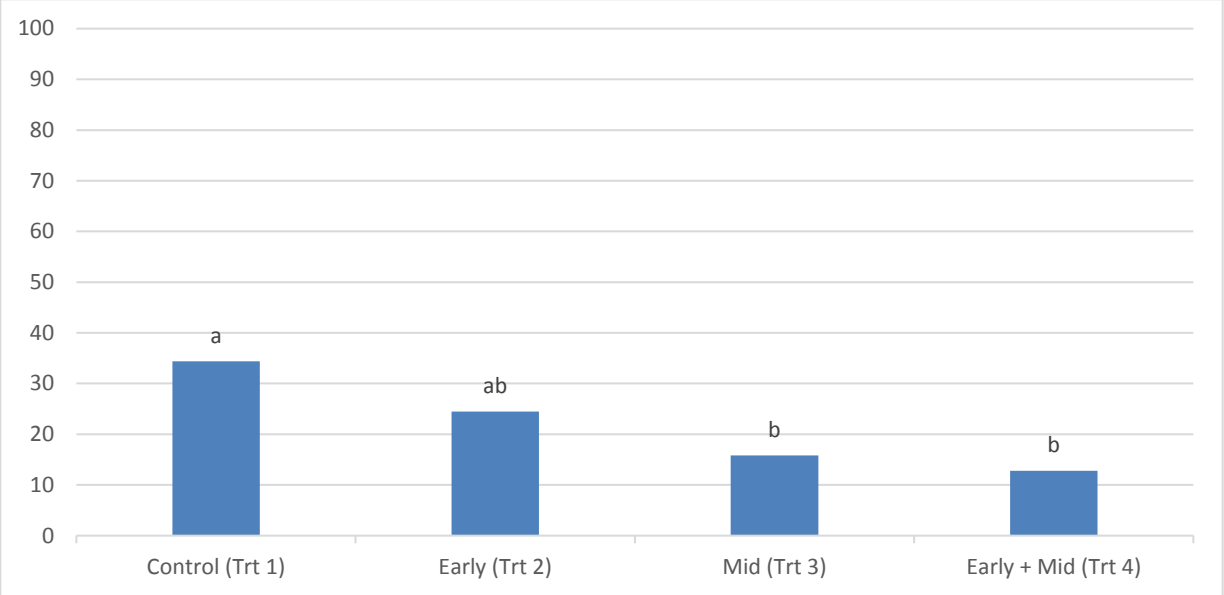


Figure 2: Percentage of Leaves with Psa Leaf Spot 24 November 2016

Both the mid (15.8%) and early plus mid (12.8%) treatments resulted in significantly lower Psa leaf spot percentage, than the untreated control (34.4%).

For Psa leaf spot score, early Ambitious application resulted in a reduction in the Psa leaf spot score (1.06) compared with the untreated control (1.44), although not to the same degree as mid (0.74) or early plus mid (0.53) Ambitious application, so was not found to be significantly different.

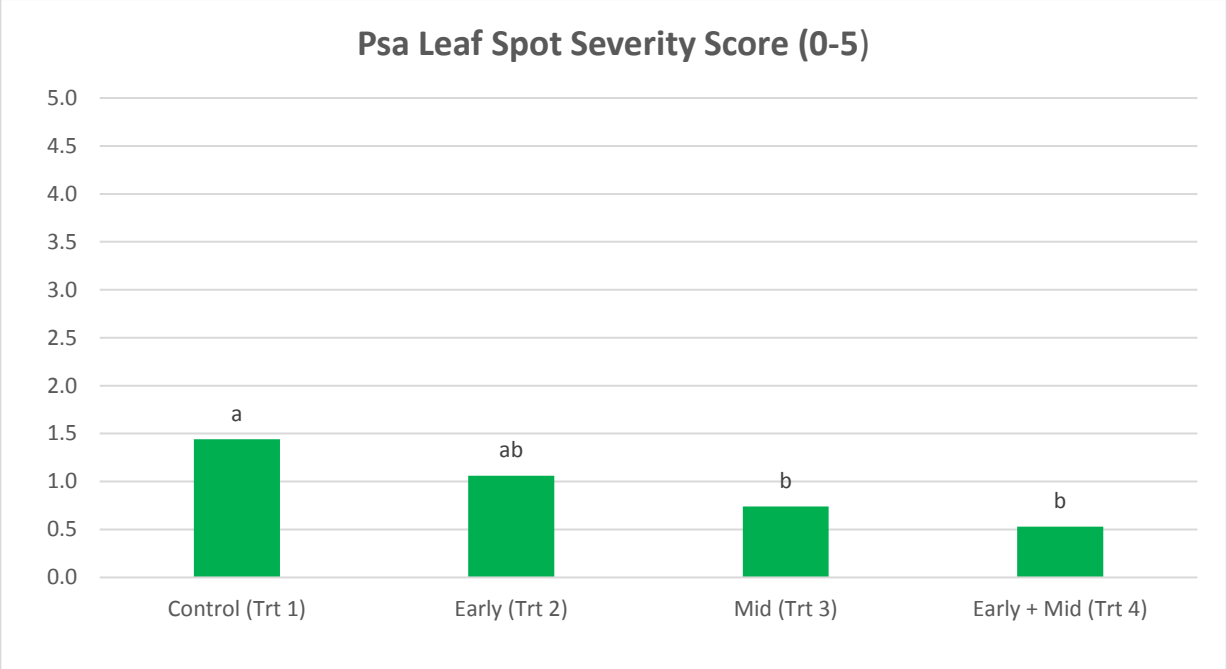


Figure 3: Psa Leaf Spot Severity Score

Both the mid (0.74) and early plus mid treatments (0.53) resulted in significantly lower Psa leaf spot score, than the untreated control (1.44).

There was no significant interaction between the times of application. (data not presented)

The 2014/15 and 2015/16 trials were carried out on the same site as each other, with other non-elicitor type Psa products applied during the spring period, in addition to Ambitious treatments in both years.

The 2016/17 trial was carried out on a different site to the previous years, with no other Psa products applied during the spring period, in addition to Ambitious treatments.

Assessment methods and replication were the same across all three years.

In 2016/17 the trends followed the same pattern as for both 2014/15 and 2015/16.

The percentage of leaves with Psa leaf spot and Psa leaf spot score were intermediate between 2014/15 (high levels of Psa leaf spot frequency and severity) and 2015/16 (low levels of Psa frequency and severity).

In 2015/16, although the trends followed the same pattern as for 2014/15, the absolute levels of leaf spot score and percentage spotted leaves were much lower. There were no significant differences between treatments.

In 2014/15, treatments three, five and six had a significantly lower spot area score (1.2-1.7 per cane) than the control (3.3 per cane); and treatments three, five and six also had significantly less percentage spotted leaves (24.6-39.4%) than the control (60.5%).

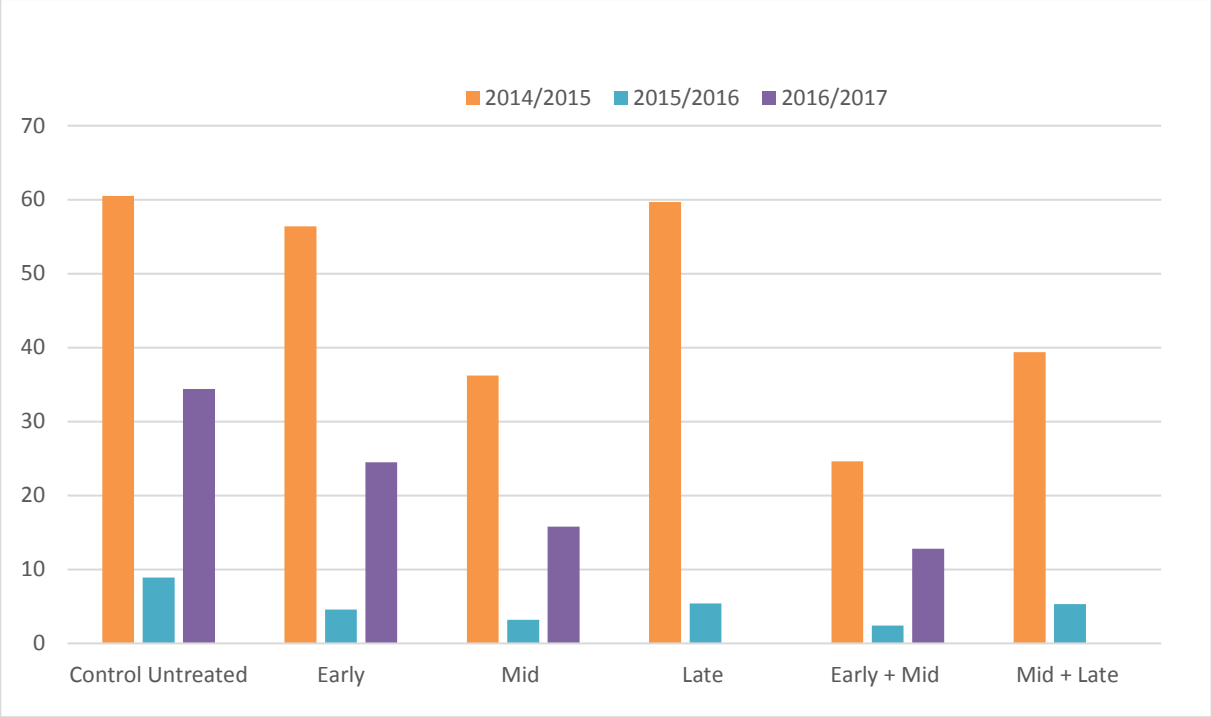


Figure 4: Psa Percentage Spotted Leaves 2014/15, 2015/16 and 2016/17

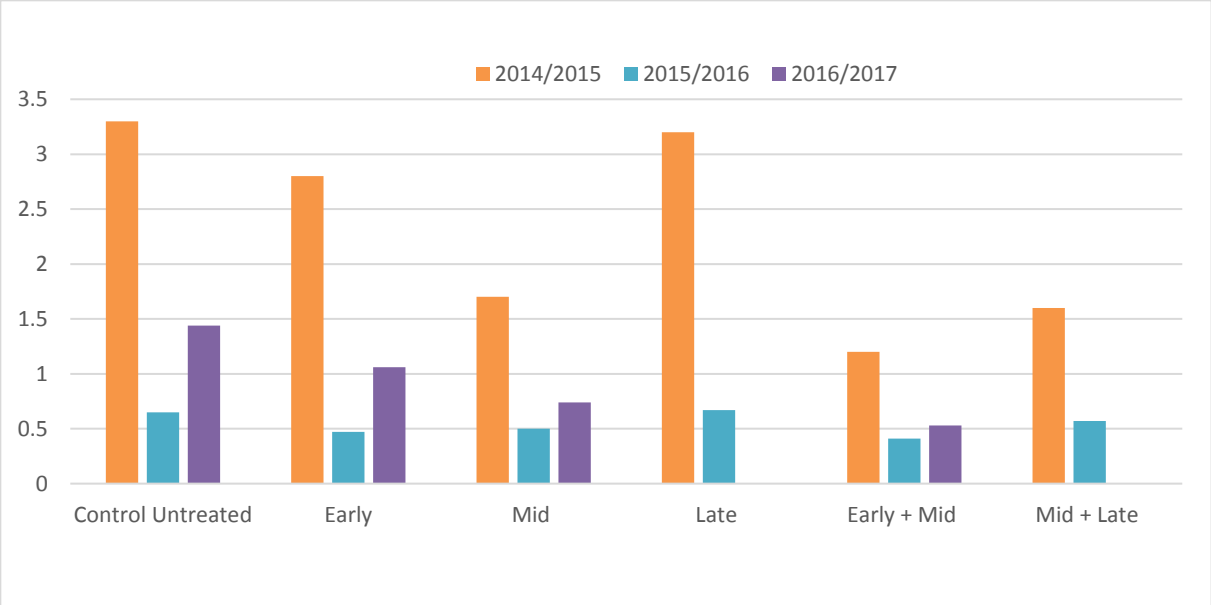


Figure 5: Psa Leaf Spot Score (0-5) 2014/15, 2015/16 and 2016/17

7.2 Reject Assessment

In 2016/17, the percentage reject associated with each treatment was as per the findings of 2014/15; that is, that there was no significant difference in the percentage reject fruit between any of the treatments (4.8-6.5%) compared with the untreated control (4.8%).

In 2016/17, there was no significant difference in the reject rate as assessed post fruit set and prior to hand thinning, between any treatment and the untreated control.

Table 4: Percentage Reject of Total Fruit

	Percentage Reject Fruit Pre-Thin
residual df	21
Control (Trt 1)	4.8 a
Early (Trt 2)	4.8 a
Mid (Trt 3)	6.5 a
Early + Mid (Trt 4)	5.5 a
Trt s.e.d	1.97
LSD 5%	4.09
Trt P-value	0.801
Trt Significance	NS

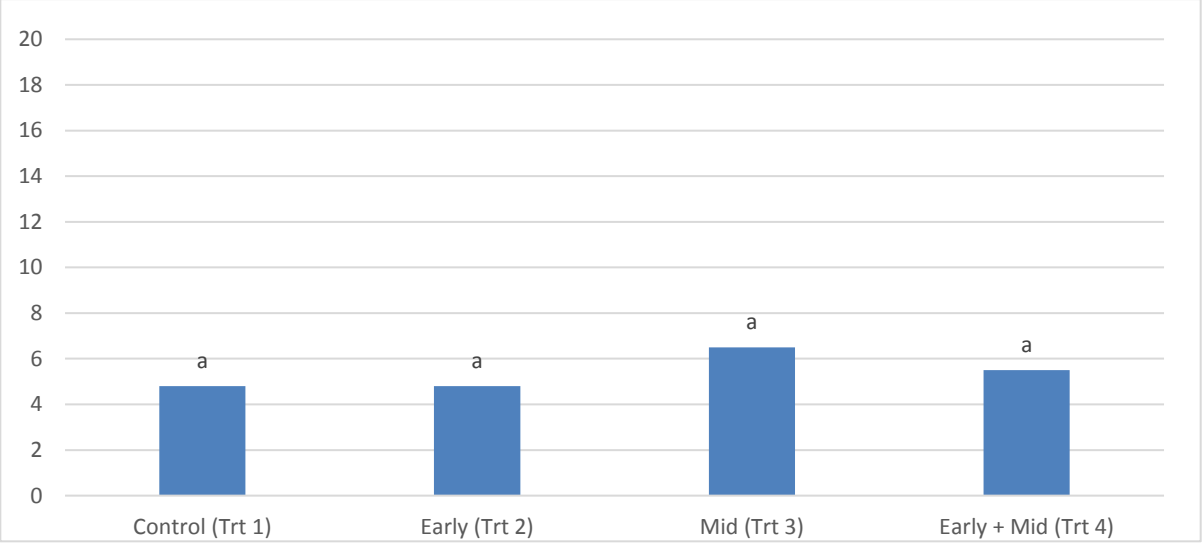


Figure 6: Percentage Reject of Total Fruit Post Fruit Set and Pre-Hand Thinning

For causes of reject fruit in each case, for all reject fruit which was thinned from the four assessed canes per vine, only causes of reject which were found on at least one third of the vines were analysed separately.

Some causes of reject were insufficiently present across all vines to be analysed separately, including dropped shoulder, misshapen, ridging, leafroller damage, Sclerotinia scarring, wind rub, joined fruit and undersize.

Table 5: Causes of Reject (Percentage of Total Reject Fruit)

	Shape				Other	
	Flat	Fan	Square	Total Reject % for Shape	Reject % Hayward Mark	Total Reject % for Other
residual df	18	18	18	18	18	18
Control (Trt 1)	0.0 a	10.2 a	26.0 a	63.2 a	13.1 a	36.8 a
Early (Trt 2)	31.0 a	19.4 a	20.1 a	79.7 a	9.3 a	20.3 a
Mid (Trt 3)	15.8 a	5.6 a	26.1 a	47.5 a	15.0 a	52.5 a
Early + Mid (Trt 4)	21.7 a	22.9 a	22.2 a	69.7 a	13.1 a	30.3 a
Trt s.e.d	12.10	12.91	16.27	17.50	10.65	17.50
LSD 5%	25.16	27.12	34.18	36.77	22.37	36.77

Trt P-value	0.088	0.529	0.977	0.341	0.959	0.341
Trt Significance	NS	NS	NS	NS	NS	NS
No early application	7.2 b	7.9 a	26.0 a	55.4 a	14.1 a	44.6 a
Early application	26.3 a	21.1 a	21.1 a	74.7 a	11.2 a	25.3 a
Early Trt sed	8.56	9.13	11.51	12.37	7.53	12.37
Early Trt P-value	0.038	0.165	0.676	0.135	0.710	0.135
Early Trt Sig.	*	NS	NS	NS	NS	NS
No mid application	14.8 a	14.8 a	23.0 a	71.5 a	11.2 a	28.5 a
Mid application	18.7 a	14.3 a	24.1 a	58.6 a	14.1 a	41.4 a
Mid Trt sed	8.56	9.13	11.51	12.37	7.53	12.37
Mid Trt P-value	0.650	0.952	0.924	0.313	0.712	0.313
Mid Trt Sig.	NS	NS	NS	NS	NS	NS
Early x Mid P-value	0.137	0.663	0.932	0.821	0.900	0.821
Early x Mid Sig.	NS	NS	NS	NS	NS	NS
Trt 1 sem	0.78	8.92	12.58	13.46	8.80	13.46
Trt 2 sem	10.17	9.36	13.91	11.35	5.04	11.35
Trt 3 sem	7.63	4.15	13.55	13.86	9.04	13.86
Trt 4 sem	10.60	8.66	13.44	11.68	7.71	11.68

Overall, within the reject fruit, a significantly higher percentage (26.3%) of early application fruit (treatments two and four) were rejected for being flat by comparison with no mid application (treatment one; 14.8%) or mid application (treatments three and four; 18.7%).

There were no other significant differences between treatments for cause of reject.

8.0 Discussion

In 2016/17, the level of symptoms observed across all treatments was intermediate between 2014/15 (high levels of leaf spot frequency and severity) and 2015/16 (low levels of leaf spot frequency and severity).

Prior to the commencement of the trial in 2016/17, vines were observed for the presence of cankers and ooze. Very few symptoms were present, to provide a visible source of Psa inoculum within the trial site (0.2565ha including buffer zone), the orchard block (3.54ha) and within the orchard (9.95ha).

Despite the low visible level of inoculum prior to trial commencement, leaf spot symptoms were very evident by assessment in November 2016.

Spring 2016 was characterised by very frequent periods of rain and these were accompanied by wind.

From 20 September when there was some bud movement with slow growth rate to achieve bud break by 30 September, to 1 December 2016, there were fifteen separate infection events, occurring on thirty-four days of the seventy-two-day period.

There was severe infection risk on nine days, moderate infection risk on twenty-four days and light infection risk on one day, during the spring to summer period in 2016.

The interval with no infection risk, between days when infection risk occurred from 20 September to 1 December 2016, was never more than four days and averaged 2.26 days.

The events are summarised in Appendix 4: Psa Risk Model and Spray Applications and show the high level of infection pressure that existed at the time of year when emerging buds and the developing leaves were very susceptible to Psa infection.

In considering that no other Psa products were applied on the trial area throughout this period, the results showed that Ambitious applications achieved good results on Psa leaf spot, both frequency and severity, as expressed by percentage of leaves with leaf spot symptoms and leafspot score.

It is possible that the timing of the early Ambitious applications for treatments two and four on 13 October 2016, was slightly too late to have a significant effect on the number of early growing leaves which became infected with leaf spot and the severity of leaf spot infections, given the severity and frequency of infection risk periods that occurred in the early part of the spring growing season in 2016.

The timing of the mid-season Ambitious application appeared to have a good effect on reducing the number of leaves with leafspot and the severity of leafspot which developed. While there was no significant difference between a single mid-season application and both the early and mid-season application, the trend was for an additive control effect for early plus mid timing, over mid-season alone.

It is therefore likely that application timing to obtain beneficial effects on Psa leafspot control could be less critical than was previously thought to be the case.

A single application is likely to give useful control of Psa leafspot when applied before the majority of the leaf canopy is developed and before infection periods occur, once there is a sufficient area of leaf canopy to respond to application.

Use of Ambitious during the period between bud break and flowering represents an important alternative tool to control Psa leaf spot disease.

The other spray control options all have drawbacks and risks as well as advantages. The 2016/17 trial results were obtained in the absence of any other Psa disease control products being applied in the period between bud break and flowering.

It is important to ensure growers have choice of options, including Ambitious, for effective Psa disease control in spring. Ambitious can play an important role in mid spring protection, allowing for a break from copper spray application or optimizing bactericide application timing to highest risk periods.

These results reinforce the findings of the previous two years trial results that a single application between bud break and flowering provides a further opportunity for growers to enhance control of the leaf symptoms of Psa disease, without the risk of consequential return season effects.

9.0 Acknowledgements

HortEvaluation Ltd would like to thank

- The grower, for hosting the trial
- Iain Latter, GroChem for trial design
- Catherine Cameron, AgResearch Ltd, for statistical analysis

Appendix 1: Site Location

Appendix 2: Trial Layout

Bay																	
20 etc																	
19																	
18							31 4			32 3							
17										30 1							
16										29 2							
15							28 1			27 2							
14							26 4			25 3							
13							24 4			23 1							
12							22 3			21 2							
11							20 1			19 3							
10							18 4										
9							17 2			16 2							
8							15 3			14 1							
7							13 4			12 2							
6							10 1			11 4							
5							9 3										
4							8 2			7 1							
3							6 3			4 4							
2							4 2			3 4							
1							2 3			1 1							
Row	18	18	19	20	20	21	22	22	23	24	24	25	26	26	27	28	28
	F	F	M	F	F	M	F	F	M	F	F	M	F	F	M	F	F

Buffer Zone marked with hanging yellow flag tape, both end every row; every 4th bay both side

Appendix 3: Grower Spray Programme

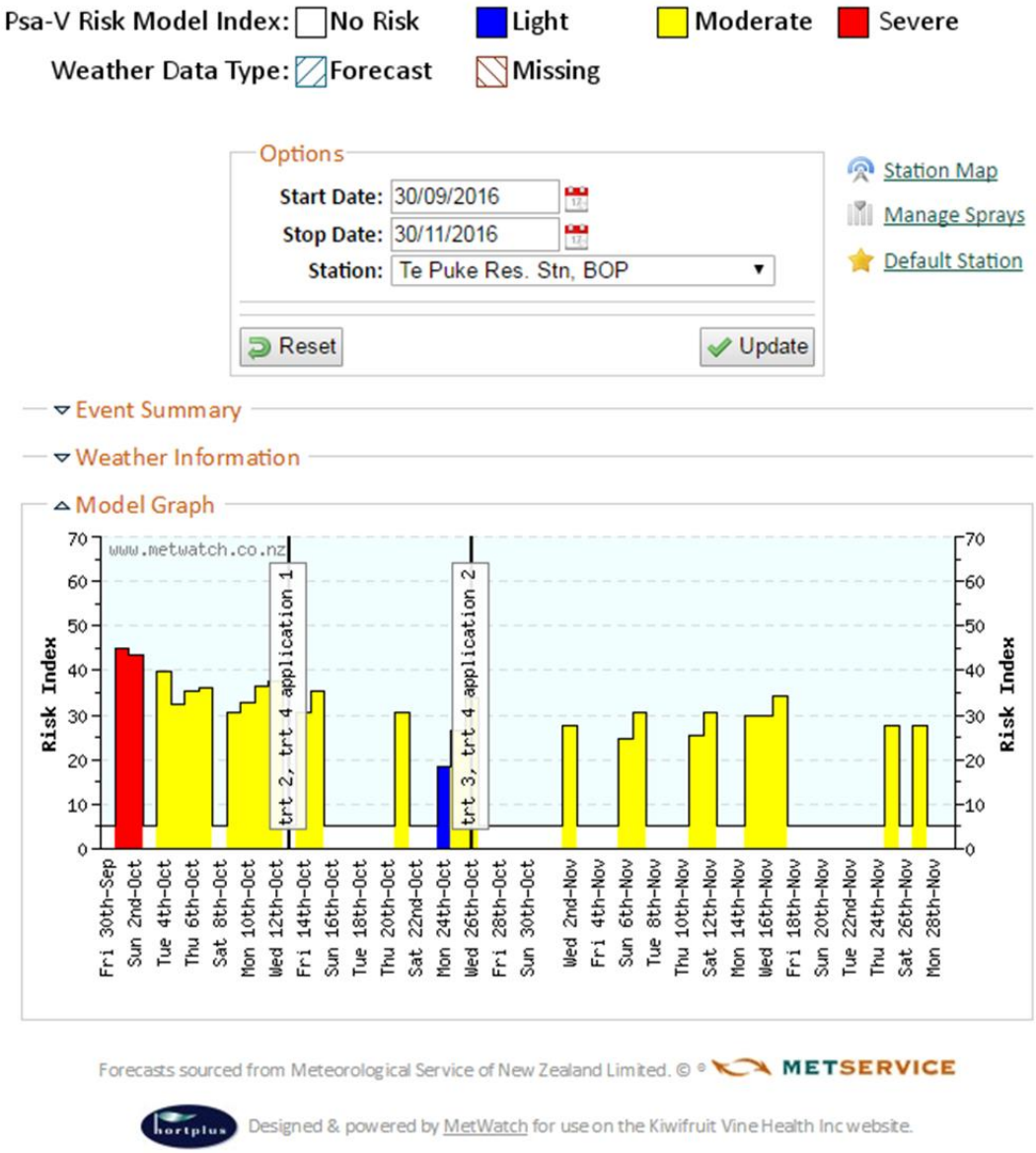
2016/2017

Spray Date	Block	Variety	GM	Product	Product rate per 100L (g or ml)	Product rate per hectare (g or ml)	Justification
7/07/2016	2, 3,1	HW	CK	Copper Sulphate	600	6030	Psa Management
8/08/2016	2, 3,1	HW	CK	Cyan	6000	42211	Improved Budbreak
15/10/2016	2, 3,1	HW	CK	Ambitious 10SL	50	352	Psa Management
1/11/2016	2, 3,1	HW	CK	Movento 100 SC	50	503	Scale Control
6/01/2017	2, 3,1	HW	CK	Dipel DF	50	503	Leafroller Control

NB: Ambitious 10SL applied 15 October 2016, was not applied to the trial area

Appendix 4: Psa Risk Model and Spray Applications

Spring 2016



Appendix 5: Leaf Spot Scoring System