POSTHARVEST PROTECTION OF KIWIFRUIT VINES

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Confidential Report Prepared For Zespri International Limited

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1.0 Executive Summary

Zespri engaged HortEvaluation Ltd to evaluate products with demonstrated efficacy against Psa leaf spot over the leaf fall period.

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

The trial included two foliar applications over the leaf fall period of KeyStrepto, KeyStrepto with the penetrant Slikka, KeyStrepto with the penetrant Engulf, and Actigard. A small plot sprayer was used to apply treatments.

The trial was carried out as intended, except that for the Hayward orchard, it was difficult to find enough male vines not yet infected with Psa to satisfy the number of suitable trial vines required. In that case, the KeyStrepto plus Slikka treatment was not included, and replication was reduced from 15 to 11 plots.

Vines were assessed prior to treatment, in late winter and again in spring, for lesions and dieback canes. The late spring assessment of Matua male vines was for the percentage effective canopy, as it was difficult to discern lesions and cankers and therefore count them.

For Hayward, there were almost no Psa symptoms observed and therefore no treatment effects, despite the obvious presence of Psa inoculum on adjacent male vines in the same plots. Clearly, Hayward female is much less susceptible to Psa infection than Matua male.

For Matua male, we were not able to demonstrate significant treatment effects because of the high degree of variability in the data. It is noteworthy that the two treatments which had the highest percentage effective canopy were the two treatments where we might have expected some systemic effect; that is KeyStrepto+Engulf and Actigard.

For Gold 3 and M91 male, there were no significant differences between the treatments for number of dieback canes per vine at the final assessment in spring on 17 September 2012.

For the Hayward orchard only, vines were sampled for streptomycin. Residues were detected at low levels in leader and trunk phloem and in bark and peeled canes from the male (Matua) vines – females were not sampled. It cannot be said that the streptomycin found in the peeled canes and in phloem tissues were as a result of penetrant action of the Engulf as residues were also detected in the streptomycin only treatment samples. It is possible that contamination occurred from wood to phloem while the core samples were being collected; and again when bark was peeled from the cane samples. No streptomycin was detected in pollen or fruit samples. The applied streptomycin either did not persist in the plants sufficiency to be found, or was not been systemically moved within the plant. No streptomycin residues were detected in Hayward fruit collected 267 days after second application.

Further thought needs to be devoted to methodology which allows for the effective evaluation of treatments in a field environment, where natural inoculum distribution and pre-existing vine infection status is not easily quantified.

2.0 Introduction

Our knowledge of the Psa infection process is increasingly indicating that all wounds are a risk for infection. The period after harvest until winter dormancy is a high risk period for Psa infection of vines.

A high number of wounds are created by picking leaving exposed fruit stalks; and leaf fall leaving exposed leaf scars. At this time, generally favourable environmental conditions exist for Psa multiplication and spread. This combination of factors creates the high risk period for Psa infection.

Spray applications of agrichemicals such as KeyStrepto and Actigard during this period may confer some protection, so that in spring vines have a better chance of coping with Psa.

Male and female vines and female varieties may have differential susceptibility to Psa. This trial therefore set out to protect vines, especially males, from Psa infection over the autumn leaf fall period.

3.0 Objective

This study aims to test if spray applications of agrichemicals to kiwifruit vines postharvest in the late autumn to early winter period confers protection against Psa, on

- M91 male vines and Gold 3 female vines in a commercial orchard
- Matua male vines and Hayward female vines in a commercial orchard. Matua male is considered to be towards the susceptible end of Psa-V susceptibility spectrum

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** Doreen Smallridge Old Coachway Orchard, Paengaroa KPIN 2093	**Gold 3** Paul and Lucy Edkins Golden Meadows Orchard, Pukehina KPIN 3683
Site Details	Blocks 13,15,20,21 Post-harvest service supplier is Aerocool	Block 9 Post-harvest service supplier is EastPack
Plants	Conventional Mature, Matrix male Moderate Performing Blocks Low vigour Pergola trained Matua Male	Conventional Grafted 2011; first production year Near full canopy Pergola trained M91 Male
Spacing	Bays are 4.85 m between rows and 6.0 m between posts, single planted Plots are single vines two bays wide, or 58.2 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 an elicitor product, Actigard, KeyStrepto alone, or mixed with either Engulf or Slikka penetrant, to be applied twice over the leaf fall period, plus an untreated control.

Air frosts which occurred on 13, 16, 17 and 18 June 2012 hastened natural leaf fall

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.

No pruning was carried out within this timeframe.

Table 2: Hayward and Gold 3 Treatments

Gold 3 and M91 Male			**Hayward and Matua Male**		
Treatment	**Product**	**Rate/100L**	**Treatment**	**Product**	**Rate/100L**
1	KeyStrepto	60g	1	KeyStrepto	60g
2	KeyStrepto	60g	2	KeyStrepto	60g
	Slikka	100ml		Engulf	100ml
3	KeyStrepto	60g	3	Actigard	20g
	Engulf	100ml	4	Untreated	
4	Actigard	20g			
5	Untreated				

At both orchards, all treatments were applied to non-symptomatic vines.

At the Hayward orchard, there were already male vines showing symptoms of Psa infection. Male vines used for trial purposes in this orchard were distributed over four blocks, to ensure that enough non-symptomatic vines were used to provide replication of treatments.

While the primary target of the treatments was male vines, the nearest adjacent female vine in the same rows also received the same treatment as each male, at the same time.

Therefore, each treated plot consisted of one male and one female vine.

Layout
On the Hayward orchard, each treatment was replicated 11 times. On the Gold 3 orchard, each treatment was replicated 15 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 or Peter Sanders of HortEvaluation using a small plot sprayer. The application method can be seen at http://www.youtube.com/watch?v=czCOfC6H-Y8

Each treatment was applied twice, with the first application made post-harvest and the second application about 1 month later.

Applications are summarised below.

Table 3: Hayward and Gold 3 Application Dates and Conditions

Date	Treatments	Block	Start Time	Drying Conditions	Wind	Temp	Cloud	Leaf fall %
					km/hour	0C	% cover	
15/06/2012	1,3,4	HW	10 am	Good	2 to 5	14	0	25%
9/07/2012	1,3,4	HW	10 am	Good	6 to 12	12	0	100%
13/06/2012	1,2,3,4	G3	11 am	Good	0	14	0	0
2/07/2012	1,2,3,4	G3	11 am	Good	15 to 20	14	20	100%

5.0 Assessments

5.1 Psa

All vines in the trial were visually assessed for Psa symptoms on three occasions: before treatment, about one month after treatment and finally in early spring.

The purpose of the pre-treatment assessment was to confirm that vines did not have symptoms of Psa infection.

The post treatment and spring assessments were carried out by counting the number of secondary symptoms per vine. At each assessment, the number of lesions on leaders and the number of dieback canes were counted on each male and each female 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.

The exception was the spring assessment of the Matua male vines. On these vines, it was difficult to see both lesions and cankers as these had largely dried out and were not easy to discern.

Instead, a visual assessment was made of the effects of Psa on the Matua male vines. This was recorded as percentage effective canopy, being the amount of leaf canopy present in relation to potential leaf canopy, based on the total amount of wood present which should have produced leaf canopy. i.e. wood tissue affected by Psa did not break bud to produce canopy and this was visually evident.

5.2 Streptomycin Residues

In this trial, tissue samples were collected from the Hayward orchard only for streptomycin analysis. This is because the Gold3 orchard was oversprayed with KeyStrepto subsequent to the treatments being applied and therefore would have masked any treatment effects.

Wood

At the Hayward site, wood samples were collected from the males (Matua) only. This is because sampling of this type and not been undertaken before and was difficult. Therefore sample size needed to be constrained to a 'proof of concept' scale. The samples were collected on 18 June 2012; three days after the first treatments were applied. Samples were collected as follows:

- For each male vine sampled, four pieces of cane, each 10cm long were collected as one sample
- For each male vine sampled, four cores were cut and collected from the trunk at equal spacing around the trunk, as one sample
- For each male vine sampled, four cores were cut and collected from the leader at even spacing along the leader, as one sample
- Nine male vines had the above samples collected for each of treatments 1, 3 and 4. That is KeyStrepto, KeyStrepto plus Engulf and untreated control. Only 4 of these samples were analysed due to the lack of a clear trend in their results and also the difficulty in preparing and analysing the samples.

All wounds created by sampling were treated immediately with Greenseal Ultra. Samples were kept frozen until despatch for laboratory analysis.

The purpose of the wood samples was to provide material for analysis of streptomycin residues in phloem conductive tissue, soon after product application.

Pollen

Composite male and female samples, each comprising about 500g flower fresh weight were harvested from treatments 1, 3 and 4. That is KeyStrepto, KeyStrepto plus Engulf and untreated control.

Samples were collected over a period of several days, as there was insufficient flower available on treated vines to reach the threshold of 500g combined flower weight per treatment, in just one or two days. During the flower collection period, flower samples were stored refrigerated between collection rounds, until the 500g threshold was reached.

At the day all samples reached the 500g threshold, the samples were delivered to Philippa Wright for pollen extraction, then subsequent delivery of the extracted pollen to Asure Quality for residue testing.

Fruit

A composite sample was collected from the Hayward vines trial site, from trunk/trunk, mid/mid and end/end positions from 10 of the vines of treatments 1, 3 and 4. That is KeyStrepto, KeyStrepto plus Engulf and untreated control. Samples were collected 267 days after second treatment applications.

Samples were delivered to Hill Laboratories for residue testing.

5.5 Data Analysis

Data from each property were analysed separately as a randomised block ANOVA. Only variables with more than 40% non-zero data were analysed; non-analysed variables all had less than 10% non-zero data.

A Fishers exact chi-square test was also done for differences between female and male vines, ignoring treatment.

6.0 Results

6.1 Psa Assessment

Hayward - Prior to treatment, Hayward female and Matua male vines elected for treatment were free of apparent Psa symptoms.

During assessment of Hayward female vines, only one vine showed two lesions on canes, so there were insufficient data for analysis of differences of treatment effects.

There were no significant differences between the treatments for Matua male leader lesions in late winter on 7 August 2012; and in spring for male percentage effective canopy at the final assessment on 2 October 2012.The results of the Matua male analysis are presented in Table 4 below.

Table 4: Psa symptoms in Matua Males, in Spring 2013

	Leader Lesions 07/08/12	**% Effective Canopy 02/10/12**
residual degrees of freedom	30	29
KeyStrepto	2.91	57.7
KeyStrepto + Engulf	2.00	77.3
Actigard	1.82	72.7
Untreated	3.82	52.7
Standard Error of the Difference (s.e.d)	1.708	14.49
Least Significant Difference 5%	3.488	29.64
P-value	0.632	0.289
Treatment Significance	Not Significant	Not Significant

Gold 3 - Prior to treatment, almost no Psa symptoms were observed. There were too many zeros in the Gold 3 female data for analysis of differences and in the M91 male data for assessment in late winter on 6 August 2012.

There were no significant differences between the treatments for M91 male and female number of dieback canes per vine at the final assessment in spring on 17 September 2012. The results of the Gold 3 male analysis are presented in Table 5 below.

Table 4: Psa symptoms in Gold 3 and M91 in Spring 2013

	M91 Dieback Canes 17/09/12	**Gold 3 Dieback Canes 17/09/12**
residual degrees of freedom	54	55
KeyStrepto	0.67	2.20
KeyStrepto + Slikka	1.15	2.67
KeyStrepto + Engulf	1.13	3.27
Actigard	1.13	2.92
Untreated	0.87	2.53
Standard Error of the Difference (s.e.d)	0.293	0.876
Least Significant Difference 5%	0.588	1.756
P-value	0.381	0.793
Treatment Significance	Not Significant	Not Significant

Fishers exact chi-square test results showed:

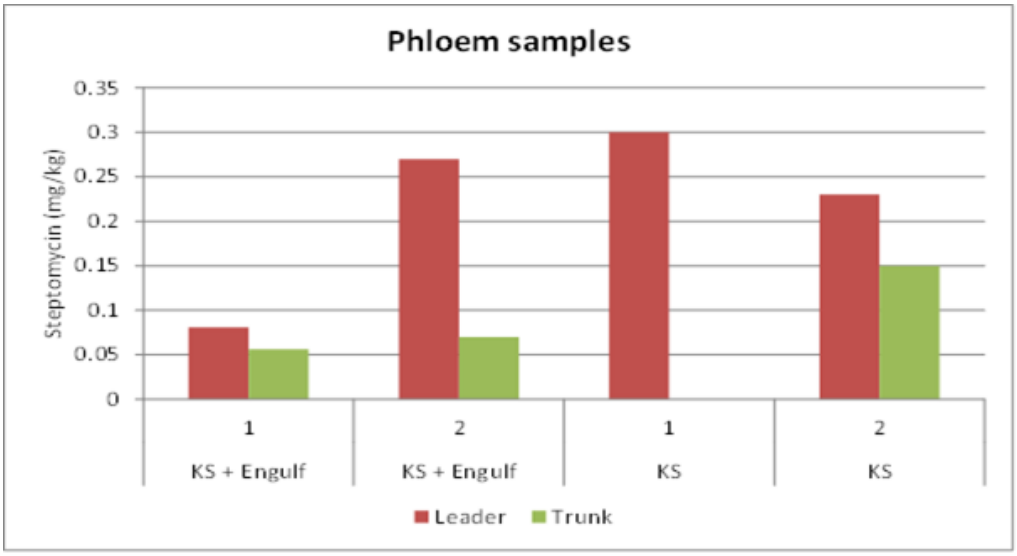
- Comparing lesions on leaders in August, Matua males had more lesions (43%) than Hayward females (0%) (P-value<0.001).
- Comparing lesions on leaders at final assessment in September, Gold 3 females had more lesions(11%) than M91 males (3%) (P-value=0.098).

6.2 Streptomycin Residues

Wood Samples (Hayward orchard - males)

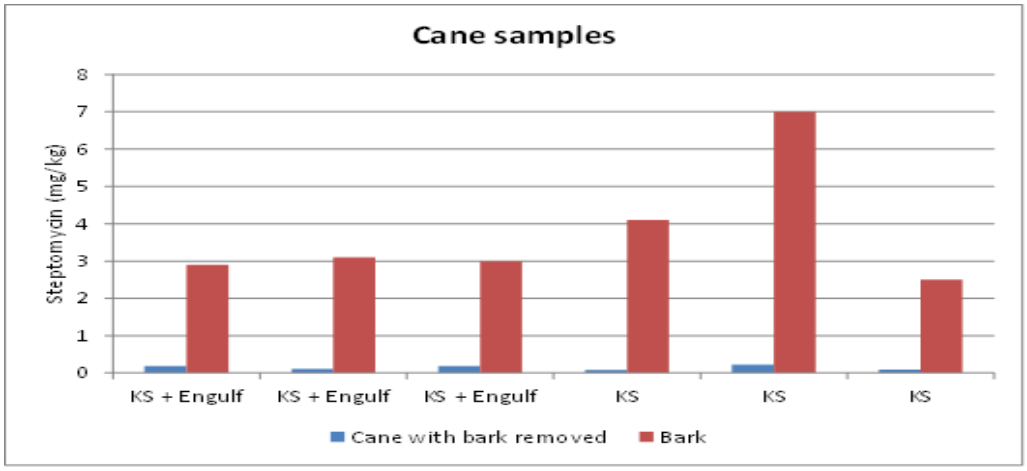
Streptomycin residues were detected in phloem collected from the leader and the trunk of the Matua (male) vines, as shown in Figure 1. The sample size was too low and the individual sample results were too variable to conclude that the addition of Engulf made a difference to the levels detected.

Figure 1: Phloem Streptomycin Residues – Matua Males



Streptomycin residues were also detected in the bark removed from cane samples and at much lower levels in the tissues underlying the peeled cane samples, as shown in figure 2.

Figure 2: Cane and Peeled Cane Streptomycin Residues– Matua Males



6.3 Streptomycin Residues in Pollen Samples (Hayward orchard)

No streptomycin residues were detected in any of the pollen samples.

6.4 Streptomycin Residues in Fruit Samples (Hayward orchard)

No streptomycin was detected in any of the fruit samples taken from the KeyStrepto treatments.

7.0 Discussion

For the Hayward and Matua, there were very few symptoms of Psa recorded in our female vine assessments, despite the not infrequent situation where Hayward female canopy was directly under male canopy literally dripping Psa bacterial ooze during winter.

The time at which such male vines in the same plots became infected with Psa is not clear.

However, given the lack of symptoms in the Hayward vines, Hayward susceptibility to Psa infection is clearly different and much lower than that of Matua male. It is also possible that Matua male vines used in this orchard were already infected with Psa, but were not yet showing symptoms at the time treatments were applied.

We were not able to demonstrate significant difference between our treatments.

It is noteworthy that the two treatments which had the most successful male canopy as measured by percentage effective canopy were the two treatments where we might have expected some systemic effect; that is KeyStrepto+ Engulf and Actigard.

For the Gold 3 and M91, dieback on canes was a significant symptom of Psa. While there were fewer dieback canes per vine on M91 male vines, the effect of these on orchard productivity could be significant because the males were trained as cross or East-West males, with cane present in leaders only. Dieback on male cane affects one of the two productive parts of the plant.

While Gold 3 vines had more dieback canes per plant than M91, the loss of productive canopy is proportionally less because the vines had multiple canes filling 60% or more female canopy.

The lack of significant treatment effects on male and female vines in this trial may relate to the unknown real distribution of Psa on the vines we used.

Streptomycin residues (Hayward orchard)
In the Matua males in the Hayward orchard, streptomycin residues were detected at low levels in leader and trunk phloem and on bark and peeled canes. It cannot be said that the streptomycin found in the peeled canes and in phloem tissues were as a result of penetrant action of the Engulf as residues were also detected in the streptomycin only treatment samples.

It is possible that contamination occurred from wood to phloem while the core samples were being collected; and again when bark was peeled from the cane samples.

Streptomycin has either not persisted in the plants sufficiency to be found in pollen, or has not been systemically moved within the plant to the developing flower and pollen within. Likewise for the fruit.

8.0 Acknowledgements

The author would like to thank

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- Ken, Paul and Lucy Edkins, Golden Meadows Orchard, for the use of their orchard, materials and support for the Gold 3 trial
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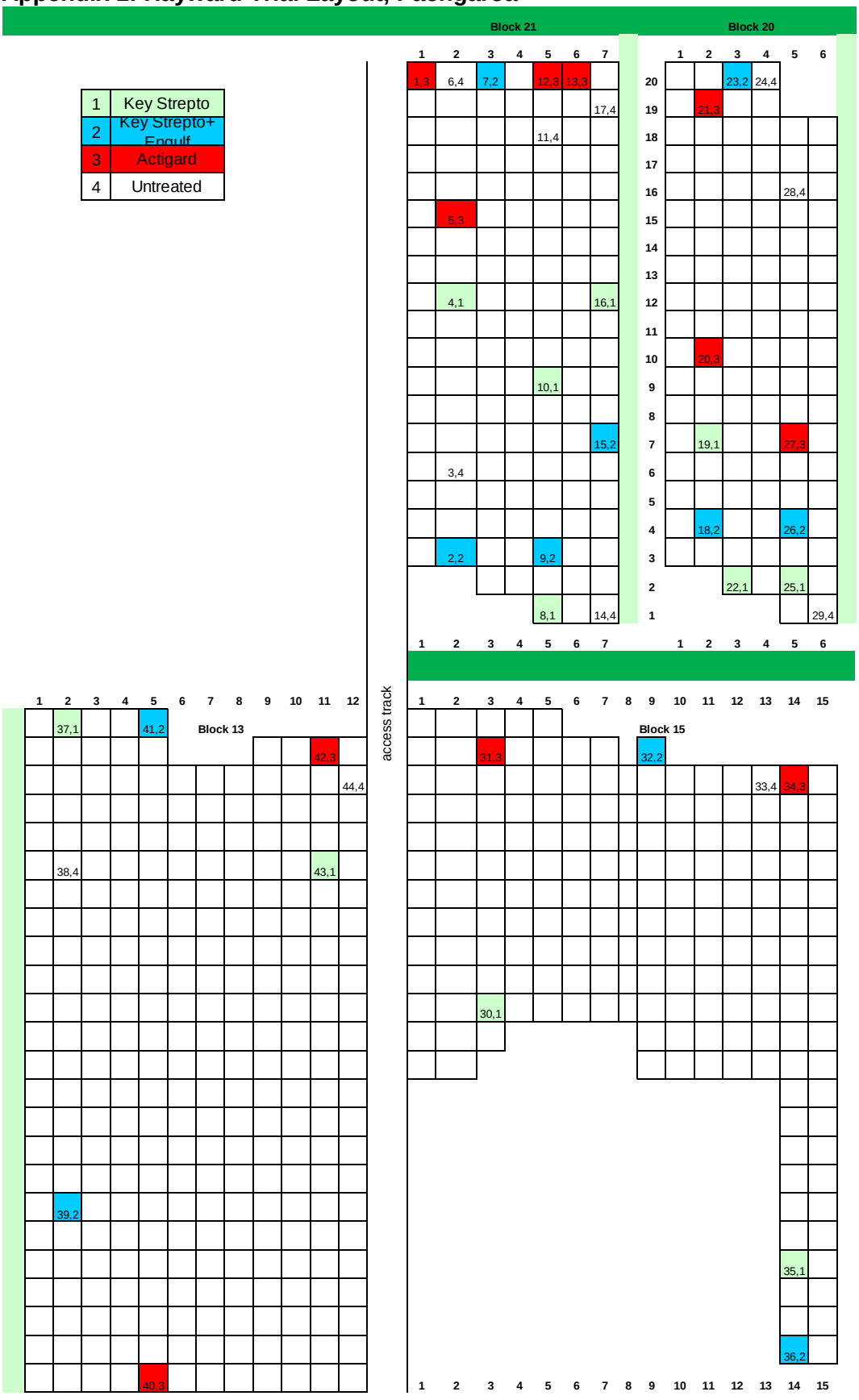
- Fred Phillips, HortEvaluation Ltd, for review of the trial report.
- Catherine Cameron, AgResearch Ltd, for statistical analysis.
- Dr Jayson Benge, Zespri for project leadership and management.

Appendix 1: Hayward Trial Layout, Paengaroa



Appendix 2: Gold 3 Trial Layout, Pukehina

Row	23	22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
										mid block turning area													
								75,1						74,4						46,2			
B			65,5			B			42,5			B			22,1			B			6,2		
	B			60,3			B			38,1			B			18,5			B				
		F			56,2			F			34,1			F			15,4			F			
M			B			52,4			B			31,3			B			12,2			F		
	73,2			B			48,5			B			28,2			B			9,4				
		69,1			B			45,4			B			25,3			B			5,1			
B			64,4			B			41,1			B			21,4			B			2,2		
	B			59,1			B			37,2			B			17,1			B			36,5	
		B			55,3			B			33,2			B			14,5			B			
M			B			51,1			B			30,5			B			11,3			B		
	72,5			B			47,1			B			27,3			B			8,3				
		68,2			B			44,3			B			F			B			4,5			
B			63,3			B			40,4			B			20,4			B			1,3		
	B			58,5			B			F			B			16,3			B			24,2	
		B			54,2			B			32,5			B			13,1			B			
M			B			50,3			B			29,4			B			10,1			B		
	71,3			B			F			B			26,1			B			7,5				
		67,5			B			43,2			B			23,5			B			3,4			
B			62,1			B			39,3			B			19,2			B					
	B			57,4			B			35,4													
		B			53,5			B															
M			B			49,4																	
				B																			
		70,3																					
B			66,4																				
	B			61,2																			

First number is plot number, Second number is treatment number

Treatment	Product
1	KeyStrepto
2	KeyStrepto
	Slikka
3	KeyStrepto
	Engulf
4	Actigard
5	Untreated