The efficacy of various products on Mature Kiwifruit (*Actinidia deliciosa* cv. Hayward) Vines for the control of *Pseudomonas syringae actinidiae* (Psa)

A report for Zespri: 13th June, 2014

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Study sponsor: Zespri

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1.0 Trial information

1.1 General details

Trial code	FSEXP21314-12
Trial title	The efficacy of various products on Mature Kiwifruit (*Actinidia deliciosa* cv. Hayward) Vines for the control of *Pseudomonas syringae actinidiae* (Psa)
Location	Benner Road, Pukehina
Crop	Kiwifruit vine (*Actinidia deliciosa* cv. Hayward)
Trial design	Randomised complete block design
No. of replications	6
Plot size	1 female vine (strip male configuration)
Equipment	Solo motorised knapsack sprayer
Pressure	15 bar
Nozzle	Hollow cone
Water rate	Approx. 800-1200L/ha equivalent

1.2 Treatment details

#	Product	Rate/100L	10/10	21/10	25/10	4/11
1	Untreated control	-	-	-	-	-
2	Key Strepto	60g	✔	✔	-	-
3	Kocide Opti	70g	✔	✔	-	✔
4	Actigard Kocide Opti	20g 70g	✔ ✔	- ✔	- -	✔ ✔
5	Actigard foliar	20g	✔	-	-	✔
6	Actigard soil	0.5g/plant	✔	-	-	✔
7	Kasumin	5L/ha	✔	✔	-	-
8	Ambitious	75ml	✔	-	-	✔
9	Cane girdle	-	-	-	✔	-

2.0 Methodology

2.1 Spray methodology

On the 8th October, 4kg/ha of Serenade Max was applied to all treatments to provide protection during the forecast rainfall until trial commencement on the 10th October. All protectant treatments were sprayed prior to significant rain events. Both Key Strepto and Kasumin were unable to be applied at the final application timing (4-Nov) as Zespri regulations stipulated it was too close to flowering for the use of antibiotics. Actigard and Ambitious were applied at 3-weekly intervals. For the soil drench treatment, Actigard was dissolved in 1L of water and poured around the circumference approximately 20cm away from the base of the trunk. An additional 5L of water was then applied in the same way in order to encourage movement of the active through the soil profile.

The cane girdle was carried out manually by orchard staff. The cane treatment was a single girdle applied toward the base of all major fruiting canes within a plot.

2.2 Assessment methodology

The incidence and severity of Psa infection, noted by characteristic dark brown to black irregular spotting on the leaf surface, was recorded. A severity score of 0 to 5 was used, where 0 indicated 0% leaf area covered in spots, and 5 indicated 75% or more leaf area covered in spots. A severity scoring sheet can be found in appendix 5.6. From these scores, the severity value was calculated using the method of Townsend and Heuburger (1943), described in Kremer & Unterstenhofer (1967), whereby;

$$P = \frac{\text{sum of } (n \times v)}{Z \times N} \times 100$$

where;
P=percentage of infection
n=number of leaves in each category
v=numerical values of categories
Z=numerical value of highest category
N=total number of plant parts

Within each plot, 20 shoots were assessed for leaf spotting, 10 each side of the leader. On each shoot, the first 5 leaves were scored for severity of leaf spot. In addition to the leaf assessment, percentage fruitset was also determined at the second assessment. The number of successful fruitlets versus the total number of flower stalks (i.e. potential fruitlets) were counted to obtain a percentage fruitset.

The fruit within the trial area was thinned soon after fruitset as per normal grower practice.

At harvest, a 100 fruit sample was taken from the untreated control, a standard (Kocide Opti + Actigard), cane girdle and Ambitious treated plots. Individual fruit weight was recorded for all fruit.

A sub-sample of 25 fruit from all of these treatments except for the cane girdle was then randomly selected from the 100 fruit sample and assessed for the following fruit quality parameters;

1. Incidence of russet: non-exportable (>1cm^2) and exportable (<1cm^2)
2. Incidence of square fruit (where width is equal to or greater than length)
3. Incidence of flat fruit (ratio of the minimum diameter/maximum diameter of the cross section is less than 0.8)
4. Incidence of fruit with a “dropped shoulder” (one shoulder above the level of the calyx)
5. Colour: A score between 0-2 (0 - a normal green-light brown colouration; 2 - red/orange colouration)

Square and colouring examples are shown in appendix 5.5.

Symptoms of phytotoxicity were also noted if observed.

2.3 Data analysis

Due to a strong relationship between % fruitset and individual fruit weight, an arbitrary yield/ha was extrapolated based on the mean percentage fruitset and individual fruit weights from each treatment for the purposes of comparison. The following arbitrary assumptions/estimates were used;

- Plot size of $50m^2$
- Hayward yield potential of 10,000 trays/ha. This equates to approximately 1700 fruit/plot (with an average count size of 34)
- A tray weighs approximately 3.8kg

Using these assumptions, the following calculation was performed on each plot;

$$\textbf{Yield (trays/ha)} = \frac{(1700 \times \text{fruitset \%} \times \text{mean fruit weight} \times 200m^2)}{3.8kg}$$

There were a total of 16 treatments in this trial; only 9 are reported on here. Data means were entered into ARM 9 Statistical Software and subjected to Bartlett's test for homogeneity of variance, followed by a suitable transformation if required (as stated in the text where appropriate). A one way ANOVA was then carried out, followed by the Duncan's New MRT test. Raw data has been presented in Appendix 5.7.

2.4 Additional data

Cloud cover, wind, temperature, humidity and drying condition observations were made subjectively at the time of application (Appendix 5.2).

3.0 Results and discussion

3.1. Severity of leaf spotting

A severe increase in leaf spotting occurred, starting 21st October, suggesting a significant infection event in the weeks prior. There was little increase in leaf spotting from the first to the second assessment. The untreated control scored the highest overall at both assessment dates, with an average severity of 47% and 53%, respectively.

Actigard as a soil drench and the cane girdle appeared to be ineffective at reducing the level of spotting, and were statistically equivalent to the untreated control. However it must be noted the cane girdle was carried out after the appearance of leaf spotting, so this result is not altogether surprising. Actigard applied as a foliar spray was effective at reducing the severity of leaf spot at the first, but not the second assessment date. In comparison, when Actigard was combined with 3 applications of Kocide Opti, this treatment was highly effective at reducing leafspot, and on the 16th December, was matched only by Key Strepto.

Ambitious appeared to perform well at the first assessment, and didn't differ from Actigard applied as a foliar or Kasumin. It was however outperformed by Kocide Opti + Actigard, Key Strepto and Kocide Opti alone (although only at the first assessment).

Kocide Opti, Key Strepto and Kasumin applied as protectant sprays all performed reasonably similarly, and were statistically equivalent at both assessment dates. There was a weak trend to suggest that by the second assessment, Key Strepto was more effective at reducing leaf spot compared to Kocide Opti and Kasumin, however the differences were slight, and not statistically different from one another.

Table 1: *Severity of leaf spotting (%)*

#	Product (Rate/100L)	# of applications (starting)	**11-Nov**	**16-Dec**
1	Untreated control	-	47.0 ^{a}	53.0 ^{a}
2	Key Strepto (60g)	2 (10/10)	15.1 $^{f g}$	25.5 $^{g h}$
3	Kocide Opti (70g)	3 (10/10)	19.7 $^{f g}$	31.3 $^{e f g}$
4	Actigard (20g) Kocide Opti (70g)	2 (10/10) 3 (10/10)	14.10 ^{g}	20.83 ^{h}
5	Actigard foliar (20g)	2 (10/10)	35.17 $^{b c}$	45.47 $^{a-d}$
6	Actigard soil (0.5g/plant)	2 (10/10)	38.67 $^{a b c}$	48.57 $^{a b c}$
7	Kasumin (5L/ha)	2 (10/10)	23.83 $^{d-g}$	31.83 $^{e f g}$
8	Ambitious (75ml)	2 (10/10)	29.80 $^{c d e}$	39.80 $^{b-e}$
9	Cane girdle	(25/10)	38.80 $^{a b c}$	44.53 $^{a-d}$
		n	*20*	*20*
		F-value	*10.5****	*8.9****
		LSD (p=0.05)	*9.0*	*9.3*

Statistical results were based off analysis of all 15 treatments
***<0.001

3.2 Fruitset

Average percentage fruitset across the nine treatments (Table 2) failed to conform to Bartlett's, and therefore did not satisfy the criteria for ANOVA, even after transformation. The untreated control suffered severe Psa infection, and only set 46% of available flowers. All treatments trended toward improving fruitset compared to the untreated control, however some performed significantly better compared to others. While the cane girdle was ineffective at reducing the severity of leaf spot (Table 1), it resulted in the highest fruitset after flowering at 89%. Actigard + Kocide Opti achieved the second greatest fruitset at 81%, followed thereafter by Kocide Opti (73%), Kasumin (69%) and Key Strepto (68%). Actigard foliar, Actigard soil and Ambitious achieved greater fruitset compared to the untreated control by between 10% and 15%.

Table 2: *Percentage fruitset*

	Product (Rate/100L)	# of applications (starting)	**16-Dec**
1	Untreated control	-	45.72
2	Key Strepto (60g)	2 (10/10)	67.57
3	Kocide Opti (70g)	3 (10/10)	72.93
4	Actigard (20g) Kocide Opti (70g)	2 (10/10) 3 (10/10)	81.11
5	Actigard foliar (20g)	2 (10/10)	57.44
6	Actigard soil (0.5g/plant)	2 (10/10)	60.28
7	Kasumin (5L/ha)	2 (10/10)	69.37
8	Ambitious (75ml)	2 (10/10)	56.17
9	Cane girdle	(25/10)	88.73
		n	*20*
		F-value	-
		LSD (p=0.05)	-

Statistical results were based off analysis of all 15 treatments
Data did not conform to requirements of ANOVA

3.3 Fruit weight and quality

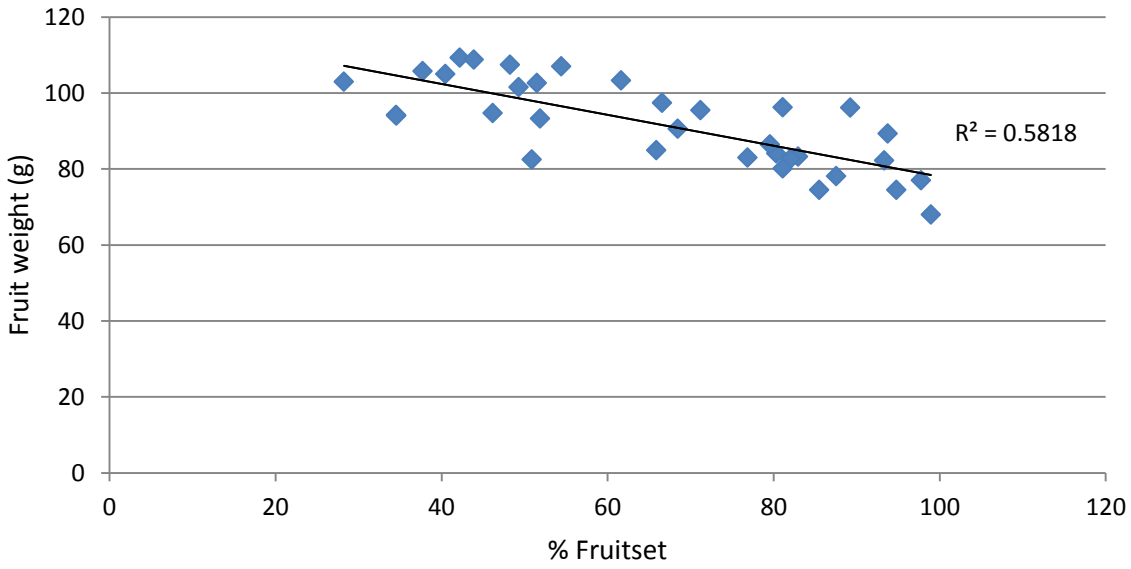


Figure 1.0: Individual fruit weight versus % fruitset

As shown above, individual fruit weight is negatively correlated to percentage fruitset. The fruit weight results (Table 3) must therefore be interpreted with caution, as the percentage fruitset likely impacted on the final fruit weight. Based on a number of assumptions (see methodology), the percentage fruitset and average fruit weight were then used to extrapolate out an arbitrary total yield (trays/ha) for the purposes of reference and comparison. The untreated control had an average individual weight of 92.09g, significantly heavier compared to the standard of Actigard + Kocide Opti. However, Actigard + Kocide Opti set on average 35% more fruit compared to the untreated control, so the arbitrary yield was higher by 2184 trays/ha. Ambitious significantly increased individual fruit weight compared to the standard, but not the untreated control, averaging 97.32g, therefore resulting in greater overall yield (4832 trays/ha) compared to the untreated, but was still not as high as the standard. The cane girdle provided the greatest yield advantage out of all the treatments with 6369 trays/ha. The arbitrary yield/ha values couldn't be analysed using ANOVA as the data did not conform to the requirements for homogeneity.

Table 3: *Mean individual fruit weight (g) and arbitrary comparative yield (trays/ha)*

#	Product (Rate/100L)	# of applications (starting)	Weight (g)	Arbitrary yield (trays/ha)*
1	Untreated control	-	92.09 [b]	3753
2	Actigard (20g) Kocide Opti (70g)	2 (10/10) 3 (10/10)	81.20 [c]	5937
8	Ambitious (75g)	2 (10/10)	97.32 [a b]	4798
9	Cane girdle	-	80.33 [c]	6369
		n	*100*	-
		F-value	*15.88****	-
		LSD (p=0.05)	*6.95*	-

Statistical results were based off analysis of 6 treatments
*Data did not conform to requirements of ANOVA. Arbitrary yield calculations detailed in methodology.

Table 4 shows the mean percentage of fruit in each quality parameter. In this trial, Ambitious didn't appear to impact on the incidence of flat fruit, with all treatments statistically equivalent. Ambitious did however significantly increase the percentage of square fruit and highly coloured fruit (score of 2). The change in colour was attributed to an overall increase in the thickness and density of hairs on the fruit skin. Ambitious also trended higher in terms of the percentage of fruit with dropped shoulder, but the result was not significantly different.

Table 4: *Mean percentage of fruit in each quality parameter*

#	Product (Rate/100L)	# of applications (starting)	Flat	Square	Dropped shoulder	Colour score of 2**
1	Untreated control	-	1.2[b]	1.7[c]	8.3 [bc]	1.3 (6.6) [c]
2	Actigard (20g) Kocide Opti (70g)	2 (10/10) 3 (10/10)	3.0[ab]	0.0[c]	10.0 [bc]	0.0 (0.0) [c]
8	Ambitious (75g)	2 (10/10)	1.2[b]	16.7 [a]	18.9 [ab]	34.0 (35.7) [b]
		n	*30*	*30*	*30*	*30*
		F-value	*4.07**	*9.11****	*4.3***	*44.4****
		LSD (p=0.05)	*1.75*	*8.45*	*10.43*	*9.74*

Statistical results were based off analysis of all 15 treatments
*Data did not conform to requirements of ANOVA
**Transformation performed (arcsine squareroot). Weighted (de-transformed) means displayed. LSD value relates to transformed values shown in brackets.

3.3 Crop tolerance

Some light phytotoxicity (light brown darkening of the veins on the underside of the leaf) was observed in the Kocide Opti alone and Kocide Opti + Actigard treatments, predominantly in bays where canopy density was low (which exposes the same leaves to repeated copper applications). A natural reddening of leaves was evident after the first application of Ambitious. This discolouration faded some weeks later. There were no visual negative effects of this reddening. Leaves and flower buds were also noticeably bigger in size and thickness following application. No differences were apparent in the percentage russet at harvest for Ambitious and the standard (Table 5).

Table 5: *Mean percentage of fruit with exportable (<1cm^2) and non-exportable (>1cm^2) russet*

	Product (Rate/100L)	# of applications (starting)	**Russet >1cm^2**	**Russet <1cm^2**
1	Untreated control	-	0.0	3.3
2	Actigard (20g) Kocide Opti (70g)	2 (10/10) 3 (10/10)	1.1	7.8
4	Ambitious (75g)	2 (10/10)	1.7	5.6
		n	30	30
		F-value	*2.11n.s.*	*1.66n.s.*
		LSD (p=0.05)	*1.60*	*5.26*

Statistical results were based off analysis of all 15 treatments
n.s. not significantly different

4.0 Summary

This trial assessed the performance of a range of products on Hayward under very high infection pressure. The untreated control resulted in between 47% and 53% severity of spotting, and only achieved a mere 45% fruitset from available flowers due to Psa bud rot. The vast majority of leaf spot symptoms appeared rapidly over a period of 3-4 days starting approximately 21st October, suggesting a significant infection event soon after application. This confirms the observations made in an identical trial in the previous season, where significant leaf spot symptoms were seen to appear around the same date. This may indicate a short period of high infection susceptibility within the growth cycle of Hayward vines.

Kocide Opti, Kasumin and Key Strepto all performed similarly in this trial when applied as protectants for reducing the severity of leaf spot and improving overall fruitset. When the elicitor Actigard was used in combination with an effective protectant (in this case Kocide Opti), the efficacy was increased somewhat over either product used alone, and nearly doubled fruitset compared to the untreated control. This demonstrates the importance of utilising multiple modes of action in a program.

Actigard alone showed relatively poor efficacy, but appeared to reduce the levels of leaf spot to some extent. The soil drench treatment was less effective compared to the foliar treatment, which was likely owing to limitations with application technique and contact with the root system. It must be noted that testing elicitors under natural field conditions can be challenging, as the product needs to be applied at least 7 days prior to an infection event to be fully effective; a prediction which is difficult in an uncontrolled field environment. In this trial, it is strongly suspected an infection event occurred soon after the first application was made, which wouldn't have allowed sufficient time for the SAR response to activate.

Cane girdling failed to result in any reduction in leaf spotting, which was more likely attributed to timing (after the infection event) rather than the true efficacy of the treatment. However, interestingly, despite showing severe leaf spotting similar to the untreated control, the cane girdle appeared to be the most effective treatment for reducing the levels of budrot and improving fruitset. All other treatments tended to show a correlation, albeit weak, between levels of leaf spot and percentage fruitset. This may indicate one of two possible scenarios; firstly, that the significant

leaf infection event that took place soon after the trial started (but before canes were girdled) was earlier than the infection event that affected the flower buds, or, secondly, that the infection occurred at the same time as leaf spot, but may take longer to express in the flower bud. The latter would suggest that the cane girdle prevented the infection from progressing to the flower bud.

Under the methodology used in this trial, Ambitious appeared to perform similarly to Actigard applied alone in reducing the levels of leafspot in Hayward kiwifruit, but did little to reduce the levels of bud rot. Neither of these treatments performed to the same standard as Actigard + Kocide Opti in reducing leaf spotting and bud rot caused by Psa. It must be noted that rapid leaf expansion following the application of Ambitious likely resulted in a dilution effect of leaf spotting. Therefore, given that the first assessment was some 2-3 weeks following the appearance of infection, the scores shown in this report may not be a true representation of Psa control at the time of infection. In spite of this, it must be said that the condition of the canopy following the application of Ambitious was visually superior to other treatments in terms of overall leaf colour, canopy density, and leaf thickness, despite the initial high levels of spotting.

Ambitious increased the individual fruit weight over that of the untreated control and the standard, but due to a comparatively low fruitset percentage, it is unlikely to have increased overall yield above Actigard + Kocide Opti. Ambitious resulted in some fruit quality issues at harvest, most notably by increasing the percentage of square fruit. Ambitious also caused a change to the outer colour of fruit to a darker orange. These two quality characteristics were generally associated with one another. The extent of this fruit shape effect needs to be investigated further, as the sample size was small in this trial.

5.0 Appendices

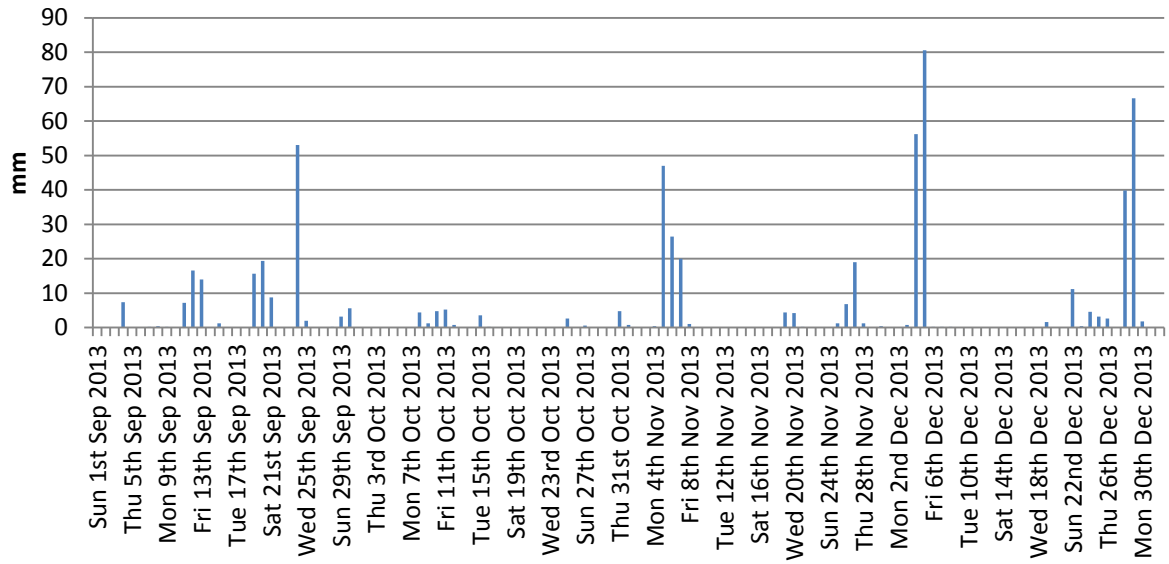
5.1 Acknowledgements

Thanks to Strathboss Kiwifruit for the use of the trial site, and to Alastair and the crop monitoring team for assistance with assessments.

5.2 Application conditions

Date	Time	Wind	Temperature	Drying conditions	Comments
10/10/2012	1400-1800	15	18	Good	
21/10/2012	1200-1500	15	17	Good	
4/11/2012	1100-1400	15	21	Good	

5.3 Rainfall data



Rainfall data generated from Metwatch Online.

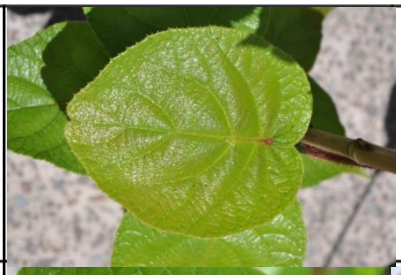
5.4 Products used

Active ingredient	Rate of AI	Trade name	Formulation	Source
Streptomycin	170g/kg	KeyStrepto	WP	Key Industries
Copper hydroxide	300g/kg	Kocide Opti	WDG	DuPont
Acibenzolar-s-methyl	500g/kg	Actigard	WDG	Syngenta
Forchlorfenuron	10g/L	Ambitious	SC	Grochem
Kasugamycin hydrochloride	20g/L	Kasumin	SC	ETEC

5.5 Fruit quality assessment scoring system examples (colour and square fruit)

Figure 2.0: Examples of fruit within the fruit quality scoring system (colour and square fruit).
From left;
1. Colour score of 0
2. Colour score of 2
3. Colour score of 2, square fruit
4. Colour score of 1, square fruit

5.6 Leaf spot scoring system

0: 0 leaf spots	
1: <5 small spots	 
2: <25% leaf area covered in spots	 
3: >25% but <50% leaf area covered in spots	 
4: >50% but <75% leaf area covered in spots	 
5: >75% leaf area covered in spots, necrosis	 

5.7 Raw data

Leaf spotting and fruitset data					Severity 11/11	Severity 16/12	Fruitset 16/12
No.	Name	Rate	Unit	Plot	1	3	4
1	Untreated control			a	49.00	44.60	46.17
				b	48.40	43.80	51.88
				c	60.60	65.40	50.89
				d	42.40	61.00	34.54
				e	35.20	59.60	28.25
				f	46.20	43.40	62.58
				Mean =	46.97	52.97	45.72
2	Kocide Opti	70	g/100 l	a	17.00	27.00	67.48
				b	21.20	19.00	71.62
				c	21.40	43.40	58.47
				d	23.40	41.60	67.22
				e	29.00	45.40	58.87
				f	6.40	11.60	81.75
				Mean =	19.73	31.33	67.57
3	Key Strepto	60	g/100 l	a	17.40	20.20	76.32
				b	20.80	23.20	63.53
				c	18.00	33.80	71.04
				d	15.80	30.40	66.81
				e	12.80	36.60	78.77
				f	5.60	8.80	81.11
				Mean =	15.07	25.50	72.93
4	Actigard Kocide Opti	200 70	g/ha g/100 l	a	21.60	21.00	65.01
				b	22.60	21.00	76.86

				c	9.80	23.60	80.32
				d	17.20	33.20	87.54
				e	8.60	21.20	82.96
				f	4.80	5.00	94.00
				Mean =	14.10	20.83	81.11
5	Actigard foliar	200	g/ha	a	29.60	34.20	46.50
				b	44.20	44.80	62.42
				c	43.60	56.80	43.73
				d	42.00	58.60	63.58
				e	31.00	56.00	51.93
				f	20.60	22.40	76.50
				Mean =	35.17	45.47	57.44
6	Actigard soil	0.5	g/plant	a	40.40	37.60	59.67
				b	45.60	50.80	62.76
				c	42.20	57.60	56.17
				d	42.60	49.20	58.70
				e	20.40	46.20	79.49
				f	40.80	50.00	44.88
				Mean =	38.67	48.57	60.28
7	Kasumin	5	l/ha	a	20.00	31.20	58.05
				b	22.40	29.80	69.01
				c	23.00	29.40	72.15
				d	24.40	44.40	67.50
				e	16.20	24.60	80.54
				f	37.00	31.60	68.98
				Mean =	23.83	31.83	69.37
8	Ambitious	75	ml/100 l	a	37.20	33.80	40.44

				b	26.20	27.60	66.57
				c	33.80	44.20	71.21
				d	26.60	55.60	42.20
				e	32.20	60.40	34.57
				f	22.80	17.20	82.04
				Mean =	29.80	39.80	56.17
9	Cane girdle			a	33.00	34.00	81.09
				b	39.20	50.00	93.75
				c	38.20	40.40	94.77
				d	41.40	61.60	79.54
				e	36.60	45.40	97.75
				f	44.40	35.80	85.50
				Mean =	38.80	44.53	88.73

Fruit weight and quality data					Colour 0	Colour 1	Colour 2	Square	Flat	Dropped	>cm²	<cm²	fruit weight
No.	Name	Rate	Unit	Plot									
1	Untreated control			a	63.33	33.33	3.33	0.00	1.00	6.67	0.00	6.67	94.70
				b	36.67	63.33	0.00	0.00	0.00	3.33	0.00	3.33	93.29
				c	80.00	20.00	0.00	6.67	2.00	26.67	0.00	0.00	82.48
				d	23.33	66.67	10.00	0.00	4.00	10.00	0.00	3.33	94.18
				e	53.33	43.33	3.33	3.33	0.00	3.33	0.00	6.67	102.95
				f	50.00	50.00	0.00	0.00	0.00	0.00	0.00	0.00	84.93
				Mean =	51.11	46.11	2.78	1.67	1.20	8.33	0.00	3.33	92.09
4	Actigard Kocide Opti	200 70	g/ha g/100 l	a	90.00	10.00	0.00	0.00	2.00	6.67	0.00	10.00	90.63
				b	46.67	53.33	0.00	0.00	6.00	6.67	3.33	3.33	83.02
				c	80.00	20.00	0.00	0.00	1.00	16.67	0.00	10.00	84.09
				d									78.15
				e									83.25
				f									68.03
				Mean =	72.22	27.78	0.00	0.00	3.00	10.00	1.11	7.78	81.20
8	Ambitious	75	ml/100 l	a	33.33	56.67	10.00	20.00	2.00	20.00	3.33	16.67	104.98
				b	6.67	56.67	36.67	30.00	2.00	6.67	3.33	3.33	97.46
				c	6.67	46.67	46.67	16.67	0.00	16.67	0.00	6.67	95.51
				d	10.00	63.33	26.67	10.00	1.00	33.33	0.00	3.33	109.30
				e	0.00	63.33	36.67	10.00	0.00	23.33	3.33	3.33	94.01
				f	3.33	43.33	53.33	13.33	2.00	13.33	0.00	0.00	82.66
				Mean =	10.00	55.00	35.00	16.67	1.20	18.89	1.67	5.56	97.32
9	Cane girdle			a									80.18
				b									89.30
				c									74.50

				d									86.44
				e									77.07
				f									74.47
Mean =													80.33