PFR SPTS No. 17502

VI1860: Advancing Emix

Reglinski T, Elmer P, Hoyte S, Spiers M, Taylor J, Parry F, AhChee A, Phipps J

February 2019















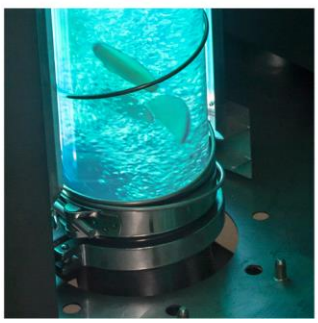


Confidential report for:
Zespri Group Limited

Zespri information:	
Milestone No.	**VI1860-30-E**
Contract No.	**VI1860**
Project Name:	**Reducing dependency upon copper and antibiotic sprays for Psa control by advancing Emix**

PUBLICATION DATA

Reglinski T, Elmer P, Hoyte S, Spiers M, Taylor J, Parry F, AhChee A, Phipps J. February 2019. VI1860: Advancing Emix. A Plant & Food Research report prepared for: Zespri Group Limited. Milestone No. 76172. Contract No. 35327Var. Job code: P/310118/01. SPTS No. 17502.

Report approved by:

Tony Reglinski
Scientist, Plant Pathology
February 2019

Beccy Ganley
Science Group Leader, Plant Pathology
February 2019

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EXECUTIVE SUMMARY

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Plant & Food Research Ruakura

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Background

A new developmental elicitor of kiwifruit host defences ('Emix') has shown efficacy against *Pseudomonas syringae* pv. *actinidiae* (Psa, biovar 3) comparable to that of Actigard™ (Syngenta) in glasshouse assays. The aim of this project was to perform dose response assays on outdoor potted kiwifruit plants (*Actinidia chinensis* var. *deliciosa*, 'Hayward' and *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3)) in order to establish an appropriate field rate for orchard studies. The assay on 'Hayward' plants was completed as planned, however, in October 2018 a contract variation was agreed between Zespri Group Limited (Zespri) and The New Zealand Institute for Plant and Food Research Limited (PFR), whereby the funds assigned for the Gold3 assay was transferred to support the orchard evaluation of Emix, and validate the potential benefits of Emix plus Aureo® Gold combinations, on 'Hayward' vines in commercial orchards. The orchard study was jointly supported by Arysta, Zespri and PFR.

Materials and methods

Potted plant trial

The experiment was performed on clonal 'Hayward' plants at the Te Puke Research Orchard (TPRO, block 20), exposed to natural Psa inoculum. Treatments were: 1) untreated; 2) Kocide® Opti™ (0.7 g/L + Actigard™ (0.2 g/L); 3) Emix (0.2 g/L); 4) Emix (0.5 g/L); 5) Emix (0.8 g/L); 6) Aureo Gold (0.5 g/L); 7) Aureo Gold (0.5 g/L) + Emix (1 g/L). Treatment 7 was applied as a tank mix and all treatments were applied in water containing BOND® Xtra (0.6 mL/L) on 17 October, 30 October, 9 November and 21 November 2017 (Note – for treatment 2, Kocide Opti was applied on 17 October, 30 October and 21 November, and Actigard on 9 November 2017). Plant height measurements were made on 17 October, 9 November, and 21 November 2017. Psa leaf spotting (percentage area of the leaf with necrosis) was recorded on 6 December 2017.

Orchard evaluation

Orchard studies were conducted on mature 'Hayward' vines at two sites in the Waikato region (sites A and B). The treatments were: 1) control; 2) Emix (0.5 g/L); 3) Emix (1.0 g/L); 4) Emix (2.0 g/L); 5) Aureo Gold (0.25 g/L) + Emix (1 g/L); 6) Aureo Gold (0.5 g/L) + Emix (1 g/L); 7) Aureo Gold (0.5 g/L); 8) Kocide Opti (0.7 g/L); 9) Actigard (0.2 g/L). All treatments were applied in water containing BOND Xtra (0.6 mL/L) using a hand gun and motorised pressure sprayer on 11 October, 23 October and 8 November, 2018 at site A, and on 15 October, 25 October and 7 November, 2018 at site B.

Key findings

Potted Hayward trial

- Emix applied at 0.5 g/L and 0.8 g/L reduced Psa leaf spotting by c. 45% compared with the control. However, this was not statistically different to the control.
- The tank mix of Aureo Gold + Emix reduced Psa leaf spotting by 74% and was comparable with Actigard (efficacy = 71%). The most effective treatment was Kocide Opti/Actigard which reduced leaf spotting by over 90%. None of the treatments significantly affected plant growth.

Orchard evaluation

- Emix applied at 1 g/L significantly reduced leaf spot incidence, compared with the control, at site B (efficacy = 51%), but not at site A (efficacy = 46%). Emix applied at 2 g/L significantly reduced leaf spotting at both sites by 58% and 49% at Site A and B respectively. There was no statistical difference in the incidence of leaf spotting for Emix applied at 1 g/L and 2 g/L. Emix applied at 0.5 g/L did not significantly reduce leaf spotting.
- The tank mix of Aureo Gold (0.5 g/L) + Emix (1 g/L) significantly reduced the incidence (efficacy = 62%) and severity (efficacy = 64%) of bud browning at site A and this degree of Psa disease control was similar to Kocide Opti for bud browning incidence (efficacy = 70%) and bud browning severity (efficacy = 80%).
- None of the treatments, including Kocide Opti and Actigard, reduced bud browning compared with the control at site B where disease pressure was higher than at site A (average incidence of bud browning in the controls was 12.4% at site A and 25.8% at site B).
- The combination of Aureo Gold (0.5 g/L) and Emix (1.0 g/L) was generally more effective than the individual components alone. For example, at site B Emix and Aureo Gold reduced Psa incidence on leaves by 51% and 40%, respectively, while the combination reduced incidence by 77% (see efficacy summary in Appendix).

Conclusions

The primary aims of this study were 1) to obtain dose response data for Emix, and, 2) to compare efficacy of Aureo Gold + Emix tank mixes with the individual components. Studies were conducted on potted plants and in orchard vines.

The efficacy of Emix against Psa is dose dependent. In earlier glasshouse studies concentrations of Emix between 0.1 and 0.2 g/L reduced Psa leaf spotting by at least 50% in plants exposed to a single inoculation event (unpublished data). However, the results in this study show that higher concentrations of Emix are required to control Psa in more complex environments where plants are exposed to natural inoculum. In outdoor potted Hayward plants Emix at 0.2 g/L had no effect on leaf spotting whilst Emix at 0.5 and 0.8 g/L reduced Psa leaf spotting by 45%. However, this level of control was not statistically significant because of the variability within the trial. Three repeat applications did not affect plant growth suggesting that these higher rates are plant safe.

In the orchard, Emix was not effective at 0.5 g/L. However, Emix at 1g/L significantly reduced Psa leaf spotting and was not significantly different to Emix at 2g/L. The efficacy of Emix was generally equal to Actigard.

The combination of Aureo Gold (0.5g/L) + Emix (1 g/L) was more effective against Psa than the individual components alone. In the orchard, the tank mix reduced both incidence and severity of bud browning and leaf spot, and was equal in efficacy to Kocide Opti. The superior performance of the combination treatment compared with the individual components in the outdoor potted plants and in the orchard confirm results from glasshouse efficacy trials.

Recommendations

1. A rate of 1g/L Emix is recommended for future field trials.
2. Further studies are recommended to optimise the potential benefits of the Aureo Gold + Emix combination. Studies to optimise timing and application frequency are recommended, for example, comparing tank mix applications at 14-day intervals with alternating applications of the individual components at 7-day intervals. During spring there is prolific growth and alternating the treatments at shorter intervals may offer greater protection of newly emerging tissue.
3. Studies to confirm the crop safety aspects of the Aureo Gold + Emix combination are also necessary for product registration.

For further information please contact:

Tony Reglinski
Plant & Food Research Ruakura
Private Bag 3230
Waikato Mail Centre
Hamilton 3240
NEW ZEALAND

Tel: +64 7 959 4430
DDI: +64 7 959 4546
Fax: +64 7 959 4431
Email: Tony.Reglinski@plantandfood.co.nz

1 INTRODUCTION

A new developmental elicitor of kiwifruit host defences ('Emix') has shown efficacy against *Pseudomonas syringae* pv. *actinidiae* (Psa, biovar 3) comparable to that of Actigard (Syngenta) on potted glasshouse plants (*Actinidia chinensis* var. *deliciosa*, 'Hayward') and in small plot trials on 'Hayward' vines (~50% reduction in leaf spotting and bud rot). Furthermore, it has also been shown that the use of Emix in combination with the newly registered biological control product Aureo Gold (Arysta Lifescience Ltd) resulted in significantly greater control of Psa on potted 'Hayward' plants (average efficacy = 70–80%) compared to either treatment when used alone (average efficacy = 40–50%) (PFR internal KRIP report no. 16995).

Before advancing to large scale field trials it is important to establish an appropriate field rate for Emix. In glasshouse trials, Emix has routinely demonstrated good efficacy against Psa at 0.2 g/L, when applied between 7 and 14 days before pathogen challenge. However, recent evidence suggests that the use of Emix at higher concentrations (e.g. 0.5–0.8 g/L) can also offer protection of new leaves that are emerging at the time of treatment. This could be of particular importance during early spring when there is rapid shoot extension growth and environmental conditions are favourable for Psa infection.

The original aim of this project was to perform experiments using Emix on outdoor potted kiwifruit plants in 'Hayward' and *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3). The assays were to establish a dose-response for Emix and investigate if multiple Emix applications (e.g. four) affected extension shoot growth. This information was intended to guide field evaluation of Emix.

The 'Hayward' plant assay was completed in early 2018. However, at a project review meeting with Dr Sonia Whiteman (Zespri Innovation Team leader) in August 2018 held at Ruakura it was proposed to amend the project goals by diverting funds from the Gold3 potted plant assay to support a field evaluation of Emix on 'Hayward' vines. The reasons were two-fold, 1) the expression of Psa symptoms on Gold3 can be very variable and therefore the potted plant assay may be a poor use of funds, and 2) field data are critical to satisfy the regulatory requirements for Agricultural compounds and veterinary medicines (ACVM) registration and may therefore accelerate Emix development. The proposed variation to the contract (project VI1860) was agreed in October 2018.

The revised project goals were:

1. Potted Plant trial (original contract)
    a. Obtain efficacy data for Emix on outdoor 'Hayward' plants to support establishment of field application rates
    b. Determine if multiple Emix applications (e.g. four) adversely affected extension shoot growth
2. Orchard Trials (contract variation)
    a. Generate dose response data for Emix in orchard vines
    b. Field validate two combination rates of Aureo Gold and Emix.

The field trials, held at two sites, were jointly supported by Arysta Lifescience, Zespri and The New Zealand Institute for Plant and Food Research Limited (PFR).

2 MATERIALS AND METHODS

2.1 Hayward potted plant trial

Clonal tissue-cultured 'Hayward' plants were obtained from Multi-flora Laboratories Limited (Auckland, New Zealand). After arrival as rooted plantlets in agar growth medium in plastic tubs, each plantlet was individually potted into a 1-L pot two-thirds filled with Daltons GB mix (Daltons, Matamata, New Zealand) and topped up with a 50:50 ratio mix of potting mix and perlite. The plants were transferred to a glasshouse and placed in high humidity tents with gradual acclimatisation to ambient conditions. The glasshouse was maintained at 16–24°C, with a day length of approximately 16 h (supplemented with high pressure sodium lamps when necessary). A flood and drain system was used once daily to water the plants. Plants were approximately 30 cm tall with at least 3–4 fully expanded leaves when the first treatments were applied at Ruakura on 17 October 2017. There were 10 replicate plants per treatment.

Treatments:

1. Untreated
2. Kocide Opti (0.7 g/L) + Actigard (0.2 g/L)
3. Actigard (0.2 g/L)
4. Emix (0.2 g/L)
5. Emix (0.5 g/L)
6. Emix (0.8 g/L)
7. Aureo Gold (0.5 g/L)
8. Aureo Gold (0.5 g/L) + Emix (1 g/L) as a tank mix

All treatments were prepared in water containing BOND Xtra (0.6 mL/L) and were applied using a hand held pressurised sprayer.

On 18 October 2017 all plants were transferred to Block 20 at Te Puke Research Orchard. The plants were arranged in a randomised block design under shade cloth with two Psa-infected 'Hayward' plants placed between each replicate as an inoculum source. Repeat treatment sprays were applied on 30 October, 9 November and 21 November, 2017. Treatment 2 in the list above comprised of Kocide Opti on 17 October, 30 October and 21 November, and Actigard on 9 November. Leaf spotting was recorded on 6 December 2017 by estimating the percentage area of Psa necrosis on seven leaves per plant. Height measurements were made on 17 October, 9 November, and 21 November 2017.

2.2 Orchard trials

2.2.1 Trial locations

Two field trials were established in separate kiwifruit orchards in the Waikato, one located near Ohaupo (site A) and the other located near Cambridge (site B). Vines in all blocks were pergola trained with a single vine per bay. There were eight single vine replicates per treatment, arranged in a randomised block design (see Appendix 7.1 and 7.2 for layout).

Treatments:

1. Control
2. Emix (0.5 g/L)
3. Emix (1.0 g/L)
4. Emix (2.0 g/L)
5. Aureo Gold (0.25 g/L) + Emix (1 g/L)
6. Aureo Gold (0.5 g/L) + Emix (1 g/L)
7. Aureo Gold (0.5 g/L)
8. Kocide Opti (0.7g/L)
9. Actigard (0.2/L)

All treatments were applied in water containing BOND Xtra (0.6 mL/L) using a motorised pressure sprayer and hand gun, at a water rate of 1000 L/ha.

Treatment dates:

Site A – 11 October, 23 October and 8 November 2018 (Nordox 75 WG™ was applied to the whole block on 5 October 2018, just after bud-burst).

Site B – 15 October, 25 October and 7 November 2018 (Key Strepto™ was applied to the whole block on 8 October 2018, just after bud burst).

2.2.2 Disease assessments

The incidence (%) of bud infection was determined at both sites on 15 November 2018 by recording symptoms on 150 buds per vine (75 on each side of the vine). Bud browning severity was recorded using an index of 0–5 where 0 = no infection, 1 to 4 equates to the number of infected sepals per bud and 5 = all 5 or 6 infected sepals. Psa leaf spotting was recorded at site B on 15 November 2018 and at site A on 15 and 16 November 2018. Leaf spotting was estimated as the percentage of necrotic leaf area on each of 150 leaves per vine (75 per side). Treatment efficacy was calculated using the following formula: (x-y)/x * 100% where x is the control value and y is a treatment value.

2.2.3 Statistics

Disease data and height data from the potted plant trial were analysed by ANOVA performed on the log (Mean Psa lesion size, and height increment) with Treatment as fixed effect. The mean and confidence limits were then back transformed to the linear scale. The calculations were implemented by GenStat 17.

Generalised Linear Mixed Model (GLMM) was used with the number of buds or leaves scored greater than zero as the binomial count out of 150 total. The fixed effect was Treatment and random effect was Rep. The analysis was performed in GenStat 17th at logit scale. The mean and confidence limits were back transformed. The analysis was performed for all treatments per site, as well as comparisons between Emix concentration and Nil, and the Emix/Aureo Gold combinations versus the individual component parts.

Linear Mixed Models (LMM) was used with REML estimates on the averaged severity scores. The scores were averaged including zero scores. The fixed effect was Treatment and random effect was Rep. The analysis was performed in GenStat 17th. The data were log transformed to satisfy the model assumptions. The back transformed means and confidence limits are reported. The analysis was performed for all treatments per site, as well as comparison between Emix concentration and Nil, and the Emix/Aureo Gold combinations versus the individual component parts.

3 RESULTS

3.1 Outdoor Potted Hayward trial

Only Actigard, Kocide Opti + Actigard tank mix, and the Aureo Gold + Emix tank mix significantly (p<0.05) reduced Psa leaf spotting compared with untreated controls (Table 1, Figure 1). Emix treatments applied at 0.5 g/L and 0.8 g/L reduced Psa leaf spotting by 43% and 48%, respectively and were statistically similar to the Actigard treatment (efficacy = 71%). However, such was the level of variation in the dataset that these Emix concentrations were also not statistically (p>0.05) different to the control. Aureo Gold alone and Emix alone at 0.2 g/L did not significantly (p>0.05) reduce Psa in this assay.

Table 1. Mean *Pseudomonas syringae* pv. *actinidiae* (Psa) leaf spot severity (area of leaves with necrosis) on potted *Actinidia chinensis* var. *deliciosa* 'Hayward' outdoor potted plants in December 2017.

Treatment	*Leaf spotting (% leaf area)	95% Confidence intervals
Untreated	4.2 c	2.41–7.39
Kocide® Opti™/Actigard™	**0.3** a	0.17–0.52
Actigard® only	**1.2** b	0.68–2.08
Emix (0.2 g/L)	4.1 c	2.33–7.15
Emix (0.5 g/L)	2.4 bc	1.35–4.14
Emix (0.8 g/L)	2.2 bc	1.28–3.94
Aureo® Gold (0.5 g/L)	3.9 c	2.23–6.84
Emix (0.5 g/L) + Aureo® Gold (0.5 g/L)	**1.1** b	0.65–2.0
p-value	**<0.001**	

All treatments were applied on 17 October, 30 October, 9 November and 21 November, 2017 except for Kocide® Opti™/Actigard treatment where Kocide® Opti™ was applied on 17 October, 30 October and 21 November, and Actigard on 9 November 2017. Psa leaf spotting was assessed on 6 December 2017. *The data are presented as back transformed means. Values with different letters are significantly different (p<0.05) based on log transformed analysis. Values in bold are significantly different to the untreated control.

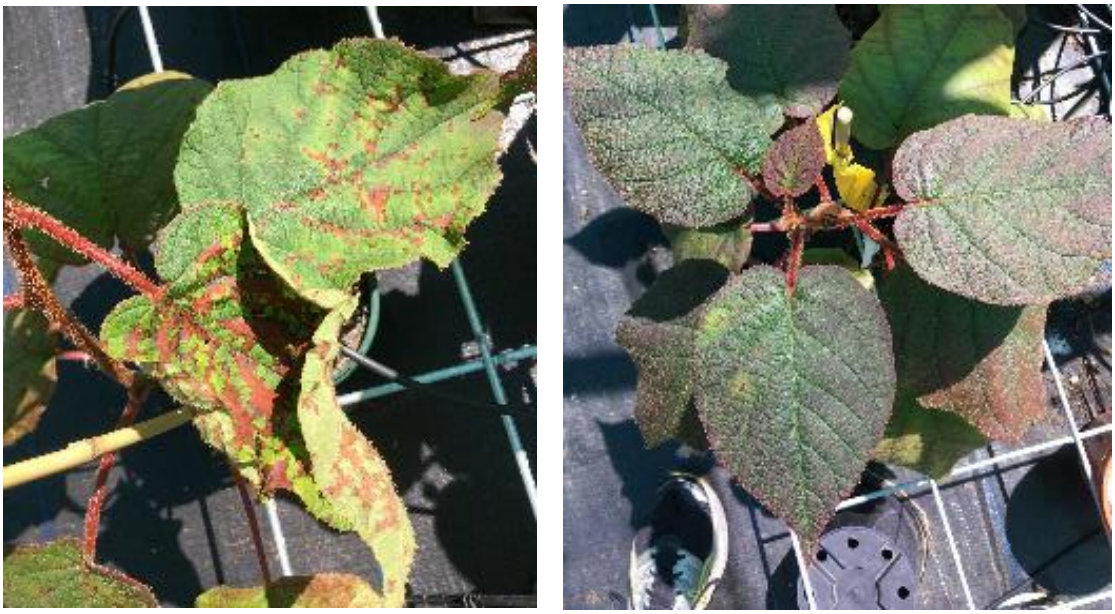


Figure 1. *Pseudomonas syringae* pv. *actinidiae* (Psa) leaf spot symptoms on potted *Actinidia chinensis* var. *deliciosa* 'Hayward' outdoor potted plants on 6 December 2017. Typical Psa leaf spots on potted 'Hayward' plants on 6 December 2017: left = untreated, right = Aureo Gold + Emix (applied as a tank mix on 17 October, 30 October, 9 November and 21 November 2017).

The height of the potted 'Hayward' plants was measured immediately before the first treatment application and then again immediately before the fourth spray. None of the treatments resulted in a statistically significant reduction (p=0.256) in height increment, compared with untreated plants (Table 2).

Table 2. Mean increase in height of *Actinidia chinensis* var. *deliciosa* 'Hayward' potted plants measured between 16 October and 21 November 2017. Sprays were applied on 17 October, 30 October, 9 November and 21 November 2017.

Treatment	*Height increment cm	95% Confidence intervals
Untreated	14.8	8.36–25.83
Kocide® Opti™/Actigard™	13.6	7.6–23.66
Actigard™ only	6.6	3.45–11.79
Emix (0.2 g/L)	10.8	5.98–19.02
Emix (0.5 g/L)	17.1	9.67–29.57
Emix (0.8 g/L)	16.7	9.47–28.98
Aureo® Gold (0.5 g/L)	17.9	10.14–30.93
Emix (0.5 g/L) + Aureo® Gold (0.5 g/L)	11.8	6.58–20.72
p-value	0.256	

All treatments were applied on 17 October, 30 October, 9, November and 21 November 2017 except for the Kocide® Opti™/Actigard treatment where Kocide® Opti™ was applied on 17 October, 30 October and 21 November, and Actigard® on 9 November 2017.

3.2 Orchard trials

3.2.1 Bud browning

The mean incidence of bud browning in the controls at site A and B was 12% and 26%, respectively (Table 3). At site A, only three treatments, Aureo Gold (0.5 g/L) (efficacy = 47%), Aureo Gold (0.5 g/L) + Emix (1 g/L) (efficacy = 62%), and Kocide Opti (efficacy = 70%), significantly (p<0.05) reduced bud browning incidence, compared with the control (see Appendix for efficacy values). None of the treatments had a significant effect on bud browning incidence at site B.

Table 3. Mean incidence (%) of bud browning in two *Actinidia chinensis* var. *deliciosa* 'Hayward' kiwifruit orchards where sprays have been applied from budburst to prior to flowering. Buds were assessed on 15 November 2018.

	Site A		Site B	
Treatment	*Incidence (%)	95% confidence intervals	*Incidence (%)	95% confidence intervals
Control	12.4 c	8.10-18.49	25.8	18.37-34.99
Emix (0.5 g/L)	11.9 c	7.72-17.88	27.5	19.81-36.78
Emix (1 g/L)	10.7 bc	6.86-16.45	23.6	16.46-32.54
Emix (2 g/L)	9.8 bc	6.19-15.32	22.7	15.76-31.62
Aureo® Gold 0.25 g/L + Emix 1 g/L	11.1 bc	7.10-16.86	19.7	13.27-28.3
Aureo® Gold 0.5 g/L+ Emix 1 g/L	**4.7** a	2.51-8.59	17.5	11.45-25.76
Aureo® Gold 0.5 g/L	**6.5** ab	3.78-11.05	21.2	14.51-29.97
Kocide® Opti™	**3.7** a	1.87-7.29	20.0	13.48-25.58
Actigard™	9.4 bc	5.83-14.71	16.5	10.65-24.63
p-value	**0.006**		0.459	

Where: Treatments were applied at site A (Ohaupo, Waikato) on 11 October, 23 October and 8 November, 2018 and at site B (Cambridge, Waikato) on 15 October, 25 October and 7 November, 2018. * The data are presented as back transformed means. Value with different letters are significantly different (p<0.05) based on logit scale analysis. Values in bold are significantly different to the control.

The mean severity of bud browning in the controls at site A and B was 0.15 and 0.47, respectively (Table 4). At site A, only two treatments, Aureo Gold (0.5 g/L) + Emix (1 g/L) (efficacy = 64%) and Kocide Opti (efficacy = 80%), significantly (p<0.05) reduced bud browning severity compared with the control. Actigard and Emix were not effective at reducing the severity of bud browning.

At site B, the best treatment (Aureo Gold (0.5 g/L) + Emix (1 g/L)) reduced Psa bud browning severity by 44%, compared with the control. However, such was the level of variability at this site that none of the treatments had a significant effect on bud browning severity.

Table 4. Mean severity of bud browning in two *Actinidia chinensis* var. *deliciosa* 'Hayward' kiwifruit orchards where sprays have been applied from budburst to prior to flowering. Buds were assessed on 15 November 2018.

	Site A		Site B	
Treatment	Severity	95% confidence intervals	Severity	95% confidence intervals
Control	0.15 c	0.08-0.31	0.47	0.29-0.75
Emix (0.5 g/L)	0.17 c	0.08-0.34	0.47	0.30-0.75
Emix (1.0 g/L)	0.14 c	0.07-0.28	0.39	0.24-0.62
Emix (2.0 g/L)	0.12 bc	0.06-0.24	0.35	0.22-0.55
Aureo® Gold (0.25 g/L) + Emix (1.0 g/L)	0.11 bc	0.05-0.21	0.28	0.18-0.45
Aureo® Gold (0.5 g/L) + Emix (1.0 g/L)	**0.05** ab	0.03-0.12	0.26	0.17-0.42
Aureo® Gold 0.5 g/L	0.08 abc	0.04-0.15	0.32	0.20-0.51
Kocide® Opti™	**0.03** a	0.02-0.06	0.31	0.20-0.5
Actigard™	0.12 bc	0.06-0.25	0.29	0.18-0.46
p-value	**0.006**		0.453	

Where: Treatments were applied at site A (Ohaupo, Waikato) on 11 October, 23 October and 8 November, 2018 and at site B (Cambridge, Waikato) on 15 October, 25 October and 7 November, 2018. * Severity was measured on a 0–5 scale which relates to the number of infected sepals per bud. The data are presented as back transformed means. Values with different letters are significantly different (p<0.05) based on log transformed analysis. Values in bold are significantly different to the control.

3.2.2 Psa leaf spotting

The mean incidence of Psa leaf spotting in the controls at site A and B was 19.3% and 43.6%, respectively (Table 5). In site A, the incidence of leaf spotting was significantly reduced (p<0.05) on vines treated with Emix (2 g/L) (efficacy = 58%), Aureo Gold + Emix (efficacy averaged over the two rates = 65%), and Kocide Opti (efficacy = 72%), compared with the control. The efficacy of Emix (1 g/L), Aureo Gold, and Actigard was 46%, 43% and 47% respectively, but these treatments were not statistically different (p>0.05) to the control.

At site B, all of the treatments except Emix at 0.5 g/L significantly (p<0.05) reduced the incidence of leaf spotting compared with the control. The lowest incidence of leaf spotting (9.9%) was recorded with Aureo Gold (0.5 g/L) + Emix (1 g/L) and this was significantly lower (p<0.05) than with Aureo Gold alone (26%) and lower, but not significantly different than Emix (1 g/L) alone (21.5%).

Table 5. Mean percentage incidence of leaves with *Pseudomonas syringae* pv. *actinidiae* (Psa) spotting in two *Actinidia chinensis* var. *deliciosa* 'Hayward' kiwifruit orchards where sprays were applied from budburst to prior to flowering, and leaves were assessed in orchard A on 15 and 16 November and in orchard B on 15 November 2018.

	Site A		Site B	
Treatment	Incidence (%)	95% confidence intervals	Incidence (%)	95% confidence intervals
Control	19.3 b	12.85-28.02	43.6 d	31.93-56.1
Emix (0.5 g/L)	18.6 b	12.24-27.17	27.9 cd	18.29-40.09
Emix (1.0 g/L)	10.3 ab	5.88-17.57	**21.5** abc	13.13-33.1
Emix (2.0 g/L)	**8.2** a	4.34-14.93	**22.1** abc	13.65-33.84
Aureo® Gold (0.25 g/L) + Emix (1.0 g/L)	**7.4** a	3.76-13.9	**13.5** ab	7.17-23.94
Aureo Gold® (0.5 g/L) + Emix 1.0 g/L	**6.3** a	3.04-12.54	**9.9** a	4.73-19.63
Aureo Gold® (0.5 g/L)	11.0 ab	6.37-18.37	**26.1** bc	16.79-38.12
Kocide® Opti™	**5.7** a	2.51-11.47	**17.7** abc	10.26-28.88
Actigard™	10.2 ab	5.76-17.37	**24.7** bc	15.71-36.68
p-value	**0.007**		**0.002**	

Where: Treatments were applied at site A (Ohaupo, Waikato) on 11 October, 23 October and 8 November, 2018 and at site B (Cambridge, Waikato) on 15 October, 25 October and 7 November, 2018. * The data are presented as back transformed means. Values with different letters are significantly different (p<0.05) based on logit scale analysis. Values in bold are significantly different to the control.

The mean severity of Psa leaf spotting (% of leaf area) in the controls at site A and B was 0.056% and 0.39%, respectively (Table 6). At site A, only Aureo Gold (0.25 g/L) + Emix (1 g/L) and Kocide Opti had significantly (p<0.05) lower mean severity of Psa spotting than the control. At site B, only the Aureo Gold + Emix combinations and Kocide Opti had significantly (p<0.05) lower mean severity than the control. Emix at 2 g/L reduced leaf spot severity by 77%, compared with the control, but such was the variability of the data that this was not statistically significant.

Table 6. Mean severity of leaf spotting caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) in two *Actinidia chinensis* var. *deliciosa* 'Hayward' kiwifruit orchards where sprays were applied from budburst to prior to flowering, and leaves were assessed in orchard A on 15 and 16 November and in orchard B on 15 November 2018.

	Site A		Site B	
Treatment	Severity (% leaf area)	95% confidence intervals	Severity (% leaf area)	95% confidence intervals
Control	0.056 cd	0.020-0.158	0.39 d	0.014-1.043
Emix 0.5 g/L	0.082 d	0.029-0.230	0.16 bcd	0.060-0.428
Emix 1.0 g/L	0.042 bcd	0.014-0.125	0.19 bcd	0.044-0.318
Emix 2.0 g/L	0.030 abcd	0.010-0.089	0.11 abcd	0.042-0.304
Aureo® Gold (0.25 g/L) + Emix 1.0 g/L	**0.014** ab	0.005-0.039	**0.057** abc	0.021-0.152
Aureo Gold® (0.5 g/L) + Emix 1.0 g/L	0.018 abc	0.006-0.054	**0.029** a	0.011-0.077
Aureo Gold® 0.5 g/L	0.029 abcd	0.010-0.082	0.18 cd	0.067-0.481
Kocide® Opti™	**0.010** a	0.004-0.030	**0.04** ab	0.015-0.107
Actigard™	0.019 abc	0.007-0.053	0.15 bcd	0.056-0.400
p-value	**0.034**		**0.015**	

Where: Treatments were applied at site A (Ohaupo, Waikato) on 11 October, 23 October and 8 November, 2018 and at site B (Cambridge, Waikato) on 15 October, 25 October and 7 November, 2018. * The data are presented as back transformed means. Values with different letters are significantly different (p<0.05) based on log transformed analysis. Values in bold are significantly different to the control.

3.3 Key findings

Potted plant trial

- Emix at 0.5 g/L and 0.8 g/L reduced Psa leaf spotting severity by 43% and 48% respectively, compared with the control. However, this was not statistically different to the control. Emix at 0.2 g/L did not reduce Psa leaf spotting.
- Aureo Gold (0.5 g/L) did not reduce Psa leaf spotting.
- The tank mix of Aureo Gold + Emix reduced Psa leaf spotting by 74% and was comparable with Actigard (efficacy = 71%).
- Integrated applications of Kocide Opti and Actigard were the most effective against Psa in potted Hayward plants and reduced leaf spot severity by 93%.
- None of the treatments significantly affected growth of the potted 'Hayward' plants.

Orchard trials

- Emix did not reduce bud browning.
- Emix at 0.5 g/L did not reduce leaf spotting.
- Emix at 1 g/L significantly reduced leaf spot incidence, compared with the control, at site B but not at site A.

- Emix at 2 g/L significantly reduced leaf spotting at both sites.
- Aureo Gold significantly reduced bud browning incidence at Site A but not Site B. Furthermore, Aureo Gold significantly reduced leaf spot incidence at Site B but not Site A.
- Aureo Gold reduced Psa leaf spot severity by 48% and 54% at site A and site B respectively, compared with the control. However, this was not statistically different to the control.
- Aureo Gold (0.5 g/L) + Emix (1 g/L) significantly reduced both the incidence and severity of bud browning at Site A, compared with the control.
- The Aureo Gold + Emix combinations reduced the incidence of leaf spotting at both sites. Aureo Gold (0.25 g/L) + Emix (1.0 g/L) also reduced the mean leaf spot severity at both sites.
- The efficacy of Aureo Gold (0.5 g/L) + Emix (1 g/L) was generally equal to Kocide Opti across both orchards.

4 CONCLUSIONS

The primary aims of this study were 1) to obtain dose response data for Emix, and, 2) to compare efficacy of Aureo Gold + Emix tank mixes with the individual components. Studies were conducted on potted plants and in orchard vines.

The efficacy of Emix against Psa is dose dependent. In earlier glasshouse studies concentrations of Emix between 0.1 and 0.2 g/L reduced Psa leaf spotting by at least 50% in plants exposed to a single inoculation event (unpublished data). However, the results in this study show that higher concentrations of Emix are required to control Psa in more complex environments where plants are exposed to natural inoculum. In outdoor potted Hayward plants Emix at 0.2 g/L had no effect on leaf spotting whilst Emix at 0.5 and 0.8 g/L reduced Psa leaf spotting by 45%. However, this level of control was not statistically significant because of the variability within the trial. Three repeat applications did not affect plant growth suggesting that these higher rates are plant safe. In the orchard, Emix was not effective at 0.5 g/L. However, Emix at 1g/L significantly reduced Psa leaf spotting and was not significantly different to Emix at 2g/L. The efficacy of Emix was generally equal to or better than Actigard.

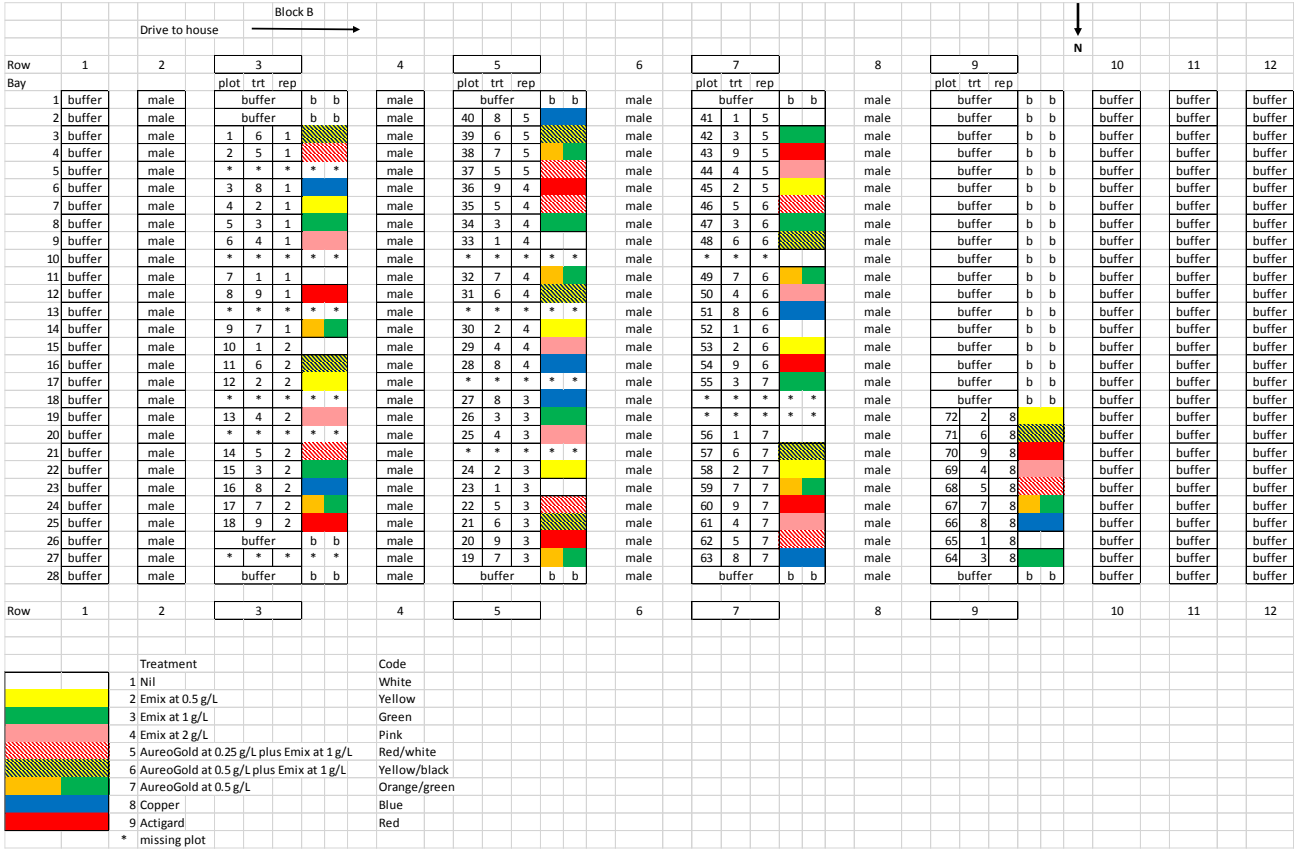
The combination of Aureo Gold (0.5g/L) + Emix (1 g/L) was more effective against Psa than the individual components alone. In the orchard, the tank mix reduced both incidence and severity of bud browning and leaf spot, and was equal in efficacy to Kocide Opti. The superior performance of the combination treatment compared with the individual components in the outdoor potted plants and in the orchard confirm results from glasshouse efficacy trials.

5 RECOMMENDATIONS

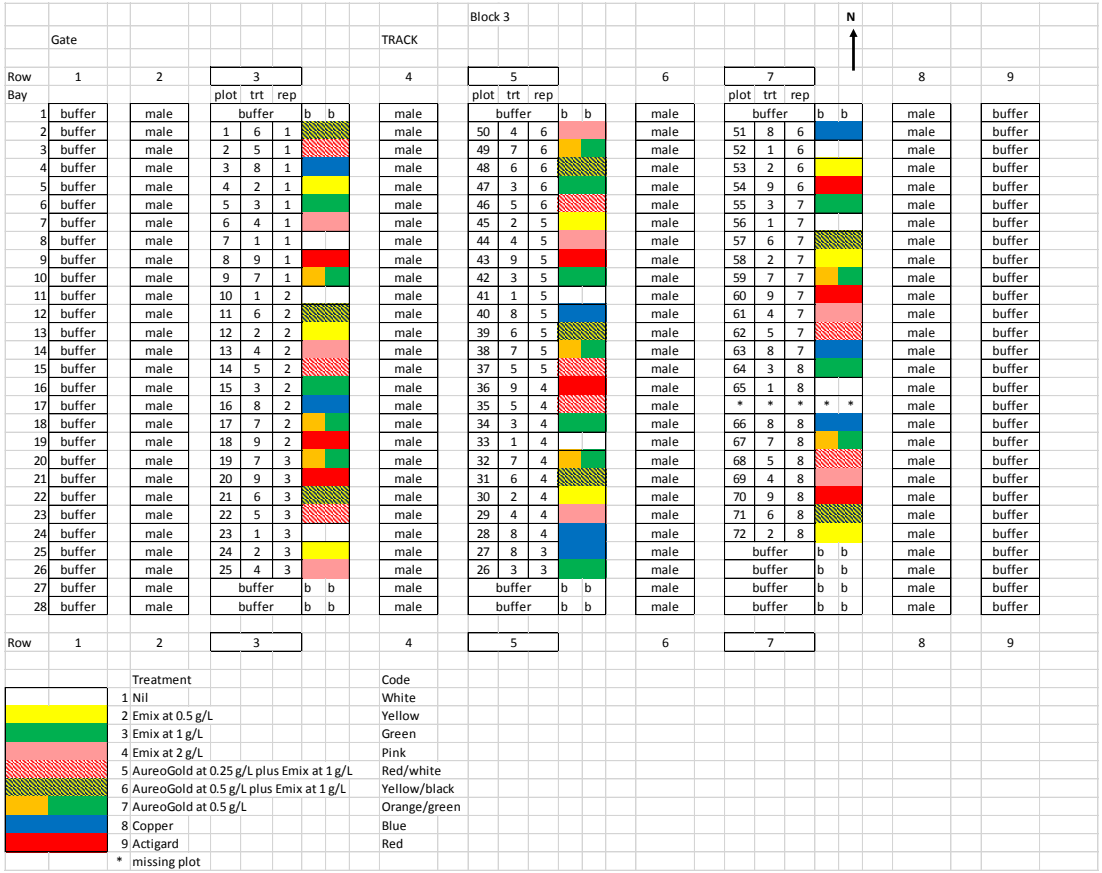
- A rate of 1g/L Emix is recommended for future field trials.
- Further studies are recommended to optimise the potential benefits of the Aureo Gold + Emix combination. Studies to optimise timing and application frequency are recommended, for example, comparing tank mix applications at 14-day intervals with alternating applications of the individual components at 7-day intervals. During spring there is prolific growth and alternating the treatments at shorter intervals may offer greater protection of newly emerging tissue.
- Studies to confirm the crop safety aspects of the Aureo Gold + Emix combination are also necessary for product registration.

APPENDIX

Site A



Site B


Block 3
N
Gate
TRACK
Row 1 2 3 4 5 6 7 8 9
Bay
plot trt rep
buffer
male
b b
Row 1 2 3 4 5 6 7 8 9
Treatment
Code
1 Nil White
2 Emix at 0.5 g/L Yellow
3 Emix at 1 g/L Green
4 Emix at 2 g/L Pink
5 AureoGold at 0.25 g/L plus Emix at 1 g/L Red/white
6 AureoGold at 0.5 g/L plus Emix at 1 g/L Yellow/black
7 AureoGold at 0.5 g/L Orange/green
8 Copper Blue
9 Actigard Red
* missing plot

Summary of the efficacy (%) of Emix (1 g/L) and Aureo Gold alone and combined against Psa on buds and leaves at two trial sites in the Waikato region.

Psa measurements	Site A	Site B
Psa incidence – buds		
Emix	13	9
Aureo Gold	47	18
Aureo Gold + Emix	62	32
Psa incidence – leaves		
Emix	46	51
Aureo Gold	43	40
Aureo Gold + Emix	67	77
Psa severity – buds		
Emix	7	17
Aureo Gold	46	32
Aureo Gold + Emix	64	44
Psa severity – leaves		
Emix	25	51
Aureo Gold	48	54
Aureo Gold + Emix	68	85

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