Development of a ‘lure and kill’ technique for apple leafcurling midge, *Dasineura mali*

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Abstract Apple leafcurling midge (ALCM) is an important quarantine pest of New Zealand apples. Current methods for ALCM control have difficulty meeting the strict phytosanitary standards of some export markets. ‘Lure and kill’ is a new strategy against this pest, in which males are attracted to an insecticide-treated substrate where they collect a lethal dose. Trials were conducted using a gel matrix “Specialized Pheromone & Lure Application Technology” (SPLAT™) to dispense and release the ALCM sex pheromone and the insecticide permethrin. Lures using SPLAT™ were placed within plastic cylinder traps that collected the dead midges so they could be counted to determine treatment efficacy. The size, shape and age of SPLAT™ spots influenced midge catches. Higher catches occurred with spots having a larger quantity of pheromone, greater surface area of SPLAT™, or with fresh material. The implications for the development of a lure and kill strategy for ALCM are discussed.

Keywords Apple leafcurling midge, *Dasineura mali* ALCM, pheromone, insecticide, lure and kill, SPLAT.

INTRODUCTION

Apple leafcurling midge (ALCM, *Dasineura mali*, Diptera: Cecidomyiidae) is an important quarantine pest of New Zealand apples (Shaw & Wallis 2012). This pest requires multiple control strategies that extend from the field to the packhouse and are consistent with the sector’s low residue Pipfruit Apple Futures programme (Walker et al. 2015).

Trials investigating the potential to mass-trap male ALCM started in 2005 (Suckling et al. 2007); however, developing this technology into an effective control strategy for ALCM has proved challenging. Lo et al. (2015) demonstrated that mass-trapping using oil traps could reduce trap

catch of male ALCM by 97% and with 48% fewer larvae in shoots, although their study also concluded that for such a numerous insect the technique required a great deal of refinement to become economically feasible. Therefore, in these underpinning trials we sought to investigate an alternative control system based on ‘lure and kill’ systems. Although it is a similar strategy to mass trapping, lure and kill avoids the need for traps. Instead, pests are simply attracted to an insecticide-treated substrate where they collect a lethal dose. Trials were conducted using a gel matrix “Specialized Pheromone & Lure Application Technology” (SPLAT™, ISCA

Technologies, Riverside, California, USA) to dispense and release the ALCM sex pheromone and the insecticide. The SPLAT™ matrix is composed of waxes, vegetable oils, water and other food-grade materials that are non-toxic and bio-degradable. Trials were conducted using SPLAT™ to dispense and release the ALCM sex pheromone and the insecticide.

MATERIALS AND METHODS

The SPLAT™ technology was tested with pheromone and insecticide within cylinder traps that collected the dead insects so they could be counted to determine efficacy. The ALCM sex pheromone was identified as (Z)-13-acetoxy-8-heptadecen-2-one and has been synthesised and developed for research trials since 2004 (Hall & Cross 2006; Cross & Hall 2009).

Clear plastic mailing tubes with end caps (Visipak®, 52 x 305 mm PETG, Hi-Q Electronics Auckland) were used for testing different lure and kill treatments. The tubes were modified by cutting a 19-mm hole in the endcaps, fitted with 100 x 50 mm Mylar® rain covers. A 50-mm square of lightweight, durable fluted polypropylene (Corflute, 3 mm thick, Sign Sheet Distributors, Auckland) was inserted to hold the substrate, pheromone and insecticide horizontally in the tube. Traps were examined weekly and the numbers of dead midges were counted or estimated by weight.

Trial 1

This trial compared different configurations of SPLAT™ and amounts of pheromone (Table 1). The efficacy of six treatments (T1–T6) was determined in a commercial integrated fruit production (IFP) orchard with high populations of ALCM. Each replicate (n=5) was a trap placed about 300 mm above the ground and 10 m apart, using a randomised block design, in a mature block of ‘Royal Gala’ apple trees. A semi-spherical “dollop” approximately 6 mm in diameter (~60 mm²) containing different amounts of insecticide was used in T1 and T2. A dollop with no insecticide was used as a control, T4. In T3, a “smear” of SPLAT™ was spread over

the entire Corflute area (2500 mm²), whereas wooden tongue depressors (Surgical and Medical Products, Medisport, Cambridge) treated with deltamethrin were used as T5. This treatment was used in a lure and kill field trial in the previous season (unpublished data) so was included here to quantify the efficacy of this system. A control with no substrate nor insecticide was included (T6). The trial began on 18 December 2015 and ran for 8 weeks.

Trial 2

This trial compared fresh and 9-week-old lures in dollop and smear configurations with the aim of understanding more about the longevity of the product as a lure and kill medium. Trial 2 continued using the same lures used for treatments T1 and T3 in Trial 1, at a new location. They were compared with fresh versions (T7 and T8) of the same formulation and delivery shape as T1 and T3 respectively. Each replicate (n=5) was a trap placed about 300 mm above the ground and 10 m apart, using a randomised block design, in a mature block of ‘Royal Gala’ apple trees at the PFR Hawke’s Bay research apple orchard. Trial 2 commenced on 11 February and finished on 11 March 2016.

Trial 3

Two candidate insecticides were compared for their ability to kill ALCM within the clear plastic tubes. The aim was to compare bifenthrin, which could be easier to register for this purpose, with deltamethrin, which has been used previously in some larger field trials to control ALCM. Permethrin was not included in this trial because of observed repellency to ALCM (Liezl Thalwitzer, PFR, pers. comm). The same square of fluted polypropylene (Corflute; described above) was used to support a 42.5-mm filter paper disc. Either 0.5 mL of bifenthrin (100 g/L emulsifiable concentrate) or deltamethrin (25 g/L emulsifiable concentrate) had been applied to the paper previously and allowed to dry. The filter paper was attached to the Corflute by inserting a rubber septum with 100 µg of ALCM pheromone centrally through a punched hole in both the

Table 1. Trial 1 lure and kill treatments against apple leafcurling midge deployed in a commercial apple orchard from 18 December 2015 to 4 February 2016.

Treatment	Substrate	Pheromone (µg)	Insecticide and concentration	Delivery shape	Quantity (mg)
T1	SPLAT™	100	6% permethrin	dollop ²	200
T2	SPLAT™	200	6% permethrin	dollop	400
T3	SPLAT™	100	6% permethrin	smear ²	200
T4	SPLAT™	100	none	dollop	200
T5	Tongue depressor ¹	100	0.67 mL deltamethrin	cap	n/a
T6	none	100	none	cap	n/a

¹ tongue depressors treated with deltamethrin were one-year old (stored in a freezer)

² shapes defined in the text

corflute and filter paper. Each replicate (n=5) was a trap placed about 300 mm above the ground and 10 m apart, using a randomised block design, in a mature block of ‘Granny Smith’ apple trees from 7 to 21 March 2017 in a commercial Hawke’s Bay orchard. The ALCM caught during this period were collected and counted.

Statistics

Analysis of variance (ANOVA) was conducted on log(x+1) transformed data using Genstat (Genstat® 17.1). Results from multiple measurement dates on the same trap were incorporated by treating them as subplots in a split-plot design, where the traps were plots (a ‘split-plot over time’ design). Model adequacy checks were carried out by examining various plots (scatter, histograms and normal probability) of the residuals.

RESULTS

Trial 1

The 400-mg dollops (T2) and smeared SPLAT™ (T3) resulted in the highest mean trap catches, despite T3 having half the quantity of pheromone (Table 2). The 200-mg dollops (T1) caught about half as many midges as T2 and T3. The two controls T4 and T6 (no insecticide) had similarly very low catches, although similar sized swarms of ALCM, to those in the other treatments, were observed hovering around T4 and T6. Last

season’s tongue depressors (T5) produced very low ALCM catches compared with the other pheromone/insecticide combinations.

The three Trial 1 treatments with largest catches of ALCM had similar discrete peaks of activity (Figure 1). The use of ANOVA with a split-plot model that accounted for the variation between sampling dates demonstrated a significant treatment effect ($P<0.001$). Further separation with LSD showed the trap catches of T2 and T3 were not significantly different, but that catches in these two treatments were significantly different from those in all the other treatments ($p<0.05$). At the conclusion of the trial, the catch was comparatively low for all treatments compared with peak emergence in early January, although the smear treatment (31 ALCM /day) was catching twice as much as each of the other treatments.

Trial 2

The fresh smear treatment caught significantly more ALCM ($P=0.004$) than the other three treatments, which were all similar with low catches.

Trial 3

The differences in numbers of ALCM caught in the traps with deltamethrin (11.1 ± 4.2 , mean $\pm$ SEM) or traps with bifenthrin (5.4 ± 2.2) were not statistically significant ($P=0.25$).

Table 2. Mean apple leafcurling midge trap catch per day ($\pm$ SEM) for the duration of Trial 1 on a Hawke's Bay commercial apple orchard and Trial 2, on the PFR research orchard during 2015–16. Means not followed by the same letter are significantly different ($\alpha=0.05$).

Treatment	Substrate type, fresh, aged etc.	Trial 1	Trial 2
T1	SPLAT™ dollop fresh/aged	90.6 $\pm$ 19.8b	5.63 $\pm$ 0.94b
T2	SPLAT™ dollop $\times 2$	173.0 $\pm$ 35.1a	-
T3	SPLAT™ smear fresh/aged	189.0 $\pm$ 36.9a	2.32 $\pm$ 0.86b
T4	SPLAT™ dollop no insecticide	2.1 $\pm$ 0.61d	-
T5	Tongue depressor	18.5 $\pm$ 5.5c	-
T6	Pheromone cap only	3.0 $\pm$ 0.88d	-
T7	SPLAT™ dollop fresh	-	4.36 $\pm$ 0.98b
T8	SPLAT™ smear fresh	-	19.55 $\pm$ 3.91a

DISCUSSION

The shape of SPLAT™ lures had a major influence on catches of ALCM. About twice as many ALCM were caught when the same quantity of SPLAT™ (including pheromone and insecticide) was presented as a smear, instead of a dollop, in the cylinder traps. The surface area of each smear was about 42 times greater than a dollop. The amount of pheromone the SPLAT™ lures contained also had a major influence on catches of ALCM.

Figure 1. Trial 1, mean apple leafcurling midge (ALCM) weekly trap catch with different quantities of pheromone and presentation of SPLAT™, on a Hawke's Bay commercial apple orchard during 2015–16. Error bars denote SEM.

Dollops with twice the quantity of SPLAT and pheromone caught twice the number of ALCM than standard dollops. These results together suggest that the amount of pheromone and the area from which it was released contributed to the attraction of ALCM. However, it is hard to separate the potential effects of various confounding factors. For example, the smears presenting a greater killing surface and the dollops becoming encrusted with dead ALCM, which may have potentially reduced contact time with the insecticide for newly attracted ALCM. The previous season's tongue depressors were included as a treatment to provide a comparison with previous studies where 100 µg of ALCM pheromone was delivered using a red rubber septum (cap). Although the tongue depressors soaked in deltamethrin had been stored at -18°C for a year, it appears the insecticide had degraded, because catches were low despite good observed attraction of ALCM. Catches of ALCM were also very low in the two controls, which verified that the insecticide was killing the midges rather than simply trapping them inside the plastic cylinder where they may have died from other causes.

Trial 2 compared the existing aged treatments with fresh pheromone and insecticide. Unfortunately, Trial 2 was partly compromised by a low ALCM population (beyond peak emergence). Despite this, the fresh smear treatment was much better than the old smear, suggesting the old

smear was either less attractive (lower release rate of pheromone) or that the insecticide had degraded. Observations of the ALCM attracted suggest it was the former situation. Smears were more effective than dollops in Trial 1 but a key question remains about how suitable a smear may be for season-long control of ALCM.

The results of Trial 3 were inconclusive and the trial had to be ended because of bad weather. It is hoped that this trial will be repeated next season, because the choice of insecticide can influence toxicity and longevity as well as commercial suitability. Synthetic pyrethroids are fast acting but also can have repellent activity (Elliott et al. 1978).

Clear plastic tubes appear to provide a suitable trap in which to test lure and kill treatments, although they do need to be protected not only from rain but also from ants. Previous studies have tested the use video to observe the midge (D Rogers, unpublished data) or large fabric sheets beneath the lure and kill substrate to collect the dead insects (P Lo, unpublished data) but both these methods were unsatisfactory for quantifying results. One possible criticism of using these tubes is that they are more effective at killing midges than without a trap because the exposure time to the insecticide is increased if movement from the tube is impeded by the hole size and the presence of a trap. Clearly the midges can still escape, because the control treatments with no insecticide caught very few ALCM. Observations of the midges coming and going from the control tubes suggest this is not a major issue.

In conclusion, SPLAT™ appears to be a suitable substrate for releasing ALCM pheromone as it provides a suitable killing surface when permethrin is incorporated. A smear is more effective than a dollop, at least over the short 8-week period used in this study. Further research is required to determine the suitability of SPLAT™ as a lure and kill substrate on a larger scale and to determine the longevity of the product in terms of the release rate of the ALCM pheromone and the efficacy of the killing surface with the appropriate insecticide incorporated. Finally, to be feasible in commercial apple orchards, lure and kill using SPLAT™ must be cost effective and easy to dispense.

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