Cotton Catchment Communities CRC

FINAL REPORT

(due within 3 months on completion of project)

Part 1 - Summary Details

Cotton CRC Project Number: **1.5.02**

Project Title: Chemical ecology of insects in Australian cotton fields

Project Commencement Date: 1/7/05 **Project Completion Date:** 30/6/08

Cotton CRC Program: **Farm**

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Part 3 – Final Report Guide (due within 3 months on completion of project)

(The points below are to be used as a guideline when completing your final report.)

Background

1. Outline the background to the project.

Pest management remains a key problem concern for the cotton industry, despite recent advances in IPM and the widespread adoption of transgenic cotton. Although previous years had seen reductions in the use of insecticides, *Helicoverpa* pressure has been high during the last two seasons. New insecticides are expensive, and the problems associated with older (cheaper) insecticides such as endosulfan and pyrethroids have again become apparent. Similarly, the danger of excessive reliance on the transgenics has been reinforced. We still need new tools for IPM in cotton, and semiochemicals are among the most promising.

This project derived from the AC-CRC project, "Plant-based attractants for *Helicoverpa* moths and sucking pests of cotton" (Project 2.2.9), which finished on 30/6/06. It included work on further refining and commercialising our current product, Magnet®, for attract-and-kill of *Helicoverpa* moths. More generally it investigated ways in which the behaviour of *Helicoverpa* spp. and the sucking pests, the green mirids, can be manipulated in a cotton landscape which includes transgenic and conventional cotton, refuges for resistance management, beneficial nurseries and non-crop vegetation.

Objectives

2. List the project objectives and the extent to which these have been achieved.

   (a) Develop field bioassay methods for mirids and other insects

   This objective was fully achieved for mirids, but not for other insects although the equipment developed for mirids should be applicable to a range of other insects

   (b) Identify plant volatile compounds attractive to mirids

   This objective was partially achieved. Although we tested 21 volatile chemicals, only two were significantly attractive to mirids. The attraction was only moderate and was somewhat unpredictable. Further, in contrast to what we found with Helicoverpa *when developing Magnet®, combining these chemicals in blends with other, less attractive, chemicals did not enhance attractiveness. We also found two volatiles significantly repellent to mirids.*

   (c) Investigate potential for mating disruption, attract-and-kill and monitoring using mirid pheromones

   This objective was partially achieved. We demonstrated trap shutdown, the first condition for successful use of a pheromone in mating disruption. We also made progress in developing slow-release formulations for the highly volatile mirid pheromones, but

further advances are needed before it is commercially feasible. We found that in some sites, mirid pheromone trap catches predicted field populations, but in others they did not, and further research leading to an understanding of the reasons for the differences is required

(d) Identify plant volatiles which attract or repel key beneficial insects

This objective was dropped when it was found that a new formulation of Magnet® developed in response to regulatory difficulties did not appear to exhibit the same attractiveness to beneficials that the old formulation did.

(e) Develop formulations with improved slow-release and rain fastness

This objective was partially achieved. We found formulations that would slow the release of mirid pheromones and the volatiles in Magnet®. We also found additives that show potential for increasing the rain fastness of Magnet®. However further work is required in both cases.

(f) Identify IP and commercial opportunities

This objective was achieved. We submitted a new patent application for the revised formulation of Magnet®, and we assessed the attractiveness of the new formulation to some important pest species in New Zealand, USA and south east Asia.

(g) Publish and communicate results

This objective was achieved, within the limits imposed by the need to maintain commercial confidence and protect IP. We presented 13 conference papers and posters including many at national and international conferences. We have submitted one paper to a refereed journal and will be submitting more shortly. We also prepared many reports for Ag Biotech, some of which were used in submissions to the APVMA for registration of Magnet®.

(h) Revise Magnet® formulation

This objective was added after regulatory difficulties arose with the old Magnet® formulation, and ended up occupying a large proportion of our research efforts. It was achieved, with all requirements for registration of a revised Magnet® formulation being achieved, and registration now imminent.

Methods

3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

The methodology used for this research has been developed in the previous AC-CRC project (2.2.9) and preceding projects, such as laboratory studies using olfactometers, collection of plant volatiles by solid phase micro extraction (SPME) technique, and methods for small-scale field trials including trapping. Because the methods for each objective/milestone were quite different they will be described in detail under each objective in the following section.

Results

4. Detail and discuss the results for each objective including the statistical analysis of results.

Objective 1: Develop bioassay techniques for mirids

Laboratory rearing of green mirids proved difficult. While cultures can be maintained, it is difficult to generate sufficient numbers of surplus insects for olfactometer experiments. Unlike *Helicoverpa* spp., mirids have low fecundity, hence it is difficult to continuously produce the numbers required for the experiments. Also, nymphs are cannibalistic in mass-rearing containers, and maintaining large numbers in individual containers would have been very labour-intensive and impractical. There is a need to improve culturing methods for mirids, including the development of an artificial diet.

Instead of having an olfactometer set up in the UNE laboratory in Armidale, a field Y-tube olfactometer (Fig. 1) with the appropriate light and temperature controls and airflow system was operated in a caravan based in Narrabri, so that field-collected mirids could be brought from nearby sites. This approach has the difficulty that the history of the mirids (mated status, oviposition status for females, past host associations etc) is not known and not standardised, but it was the only way the project could be advanced.

The olfactometer was made of glass tube measuring 20mm in diameter with a total length of 30 cm. Each Y-arm was 15cm long and the main arm was 15cm long. It was held in an 80 x 30 x 20cm light-tight wooden box painted black. At each end of the box, a slot which could be covered by varying levels of grey translucent plastic was provided. This enabled us to control the light level between total darkness and ambient light intensity. Both ends of the box had holes to connect the Teflon tubing (Sigma-Aldrich) to the olfactometer for airflow. Each end of the Y-arm of the olfactometer was fitted with a 200-ml glass collecting chamber for mirids. Air was supplied by a double-header diaphragm pump (Barnant, Australian Scientific Pty Ltd, Kotara, NSW) passing through an air purifier and humidifier (ARS Research, Florida, USA). Filtered and humidified air passed through the Y-arms at the upwind end of the box at the rate of 1.0 litre/min. One arm of the olfactometer was connected to a glass container holding the test attractant (outside the box, and therefore not visible to the insects) whilst the other end was blank or clean air.

For each olfactometer run, a total of 15 mirids were held in a release chamber at the downwind end which was separated from the main arm of the olfactometer by a meshed gate. Individual female mirids were first briefly immobilised by CO_2 before putting them in the release chamber. The meshed gate was not opened until a total of 15 mirids were held in this chamber. For experiments to be done in low light intensity, the box was closed immediately the gate was opened. Each olfactometer run lasted 3 hrs, after which numbers of mirids in each collecting chamber and Y-

arm (test and control), in the main arm and including those left in the release chamber (downwind), were recorded. Four runs were done for each test attractant and placement of the test attractant (left or right olfactomter arm) was swapped for each run. Mirids were collected using a sweep net from a lucerne field ("Yarral", Narrabri), sexed, held in ventilated individual containers provided with moist dental wick, and then acclimatised in the caravan for no less than 24h before use in the experiments.

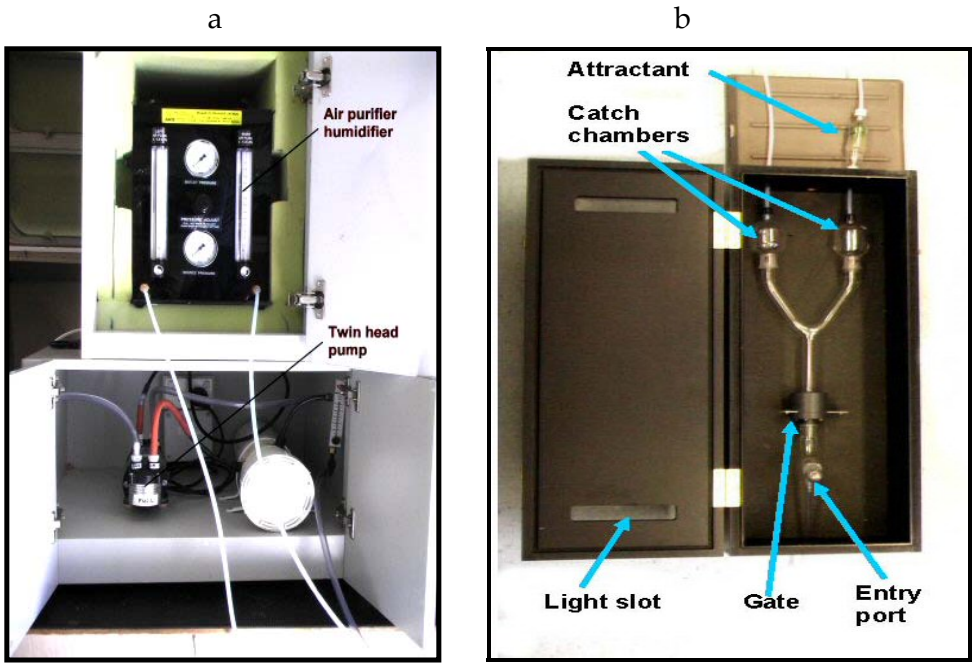

Fig.1. Y-tube olfactometer used in bioassays.(a) air delivery system (b) olfactometer

A series of preliminary olfactometer experiments using fresh lucerne bouquets (about 20 g, collected 30 min prior to the experiments) was first conducted to determine suitable bioassay conditions, eg, temperature, time of day or night and light intensity. Mirids were significantly attracted to lucerne volatiles at light intensity between 0.1-0.5 lux ($p<0.001$) (Fig. 2). At light intensities higher than 0.5 lux (ie about twilight) most mirids remained immobile in the downwind end of the olfactometer, while at lower light intensities they moved upwind, with more going into the arm which held the lucerne (test) than the control arm. There was a suggestion that discrimination between the test and control was better when some light (0.1 to 0.5 lux; moonlight to twilight levels) was present.

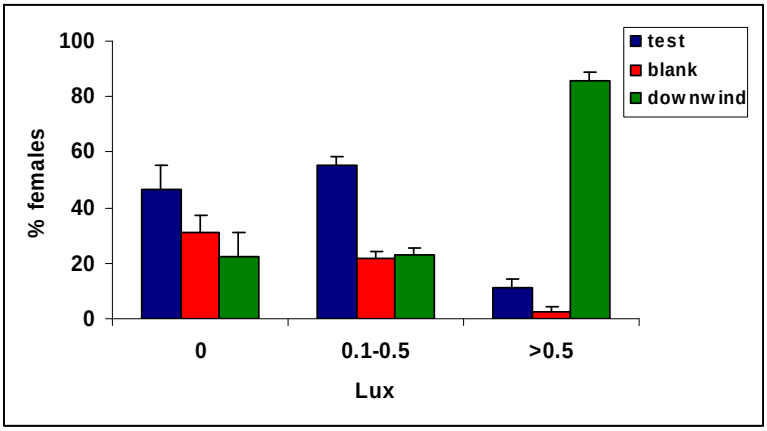

Fig. 2. % response of female mirids to lucerne volatiles at different light intensities. Bars are standard errors. N=6 (0 lux); N=26 (0.1-0.5 lux); N=7 (>0.5 lux).

The trials summarised in Fig. 2 were conducted at various times of the day, using the ability of the olfactometer to regulate light conditions. To examine the possibility of a circadian rhythm in the response to lucerne volatiles, we regressed the percentage of mirids entering the test chamber against the time the experiment started. Results are shown in Fig. 3. Although there was considerable variability, the regression was significant ($F_{1,31}$=8.06, p<0.01). Mirids showed higher % test response to the volatiles in experiments started between 1700-2300h. This suggests that in addition to the direct effect of high light intensity inhibiting the response to volatiles, there is an inherent circadian rhythm which produces higher responsiveness at about the time when twilight would occur.

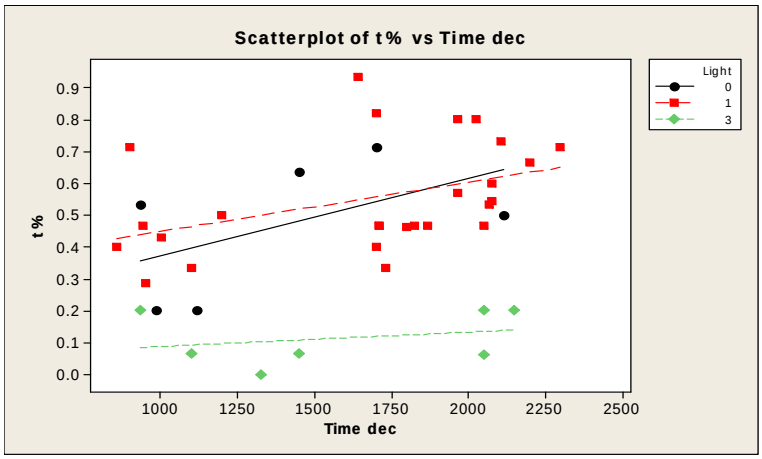

Fig. 3. Percentage of mirids entering the test chamber (t%) in experiments started at different times (time dec) for three different light intensities: 0= 0 lux, 1= 0.1 to 0.5 lux, 3= > 0.5 lux. There were no significant differences between the regressions for light regimes 0 and 1, but both were significantly different from regime 3. Regression equation for light regimes 0 and 1 combined was : t% = 0.248 + 0.000178 time dec; R-sq = 21.2%, R-sq (adj) = 18.6%.

Mirids were also tested under reverse-cycle light conditions to see if we could do the experiments in the daytime, which would have been more convenient. For this purpose, mirids were collected about 1500h and were entrained to be under full light until 0900h next morning, then under dark between 0900-1800h, full light again between 1800-0900h then tested in the olfactometer at 0900h in the dark. This experiment was designed to determine whether the apparent circadian rhythm in response to volatiles could be disrupted by entrainment in reverse cycle lighting. Mirids that were entrained and tested under reverse-cycle condition gave poorer responses in the olfactometer than those obtained under natural cycles (Fig. 4).

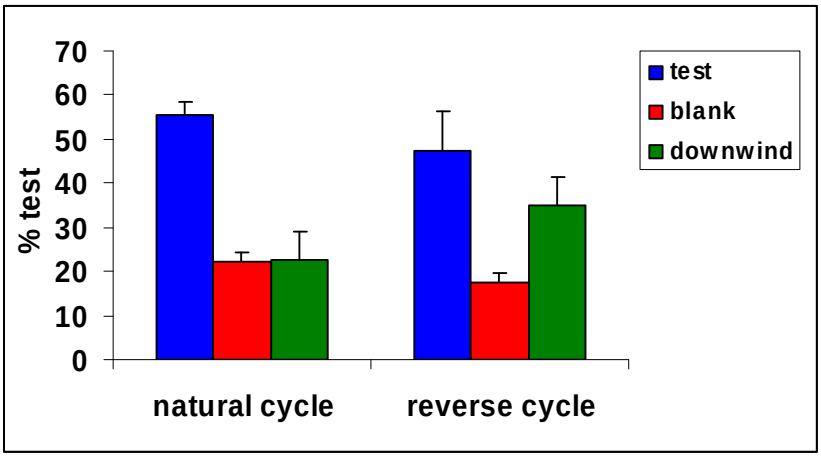

Fig. 4. Response of female mirids entrained under natural and reverse-cycle lighting, to lucerne volatiles at 0.1 to 0.5 lux. N=26 (natural cycle); N=4 (reverse cycle).

Results from these preliminary experiments suggested that mirids are nocturnal in their host-finding behaviour. High light intensity inhibited upwind movement in the olfactometer. Consequently, it was necessary to do subsequent experiments under low light (0.1 to 0.5 lux). Also, entrainment under reverse-cycle conditions did not work well and thus all subsequent olfactometer experiments were restricted to the late afternoon and in the evening (starting times in the range of 1700h - 2200h).

Objective 2: Identify plant volatile compounds attractive to mirids

Lucerne, *Medicago sativa* (L.) is a preferred host of green mirids and was thus used as the test plant attractant in the olfactometer. Volatile emissions from lucerne (alfalfa) in the US have been identified by Blackmer *et al* (2004). Fresh lucerne bouquets as well as volatiles collected from flowering lucerne in the field were tested. Synthetic equivalents of individual lucerne volatiles, sourced from Sigma-Aldrich (Australia), were tested singly and as blends. Chemicals found in the new and old formulations of Magnet® were also tested in the olfactometer.

2.1 Lucerne and volatiles from flowering lucerne

To test responses to natural lucerne volatiles, fresh lucerne bouquets were collected within 30 min before the start of the experiments (before 1730h). About 20g of the bouquet was placed in one of the Y-arms of the olfactometer and the other arm was left blank as the control.

To test responses to volatiles derived from lucerne, volatiles were collected from the plants in the field using Super-Q traps (ARS Research, USA) connected to a 12-cm glass cylinder enclosing the plant. Air was drawn through the traps by a diaphragm pump (Barnant, Australian Scientific Pty Ltd, Kotara, NSW) powered by a 12-v battery and operating in suction mode. Collection was done for 3 hours. The Super-Q trap was then washed with 500-800 µl hexane and the first 3 drops collected in a 1.5ml glass vial. The hexane washing was mixed with 200 µl canola oil, pipetted onto a small piece of dental wick and used as the test attractant in the olfactometer. Volatiles from flowering lucerne plants in the field were collected at different times (am - 0900-1200h; pm-1400-1700h and night-1800-2000h) were tested. Numbers of mirids that went into the test, blank and downwind were recorded. Experiments were also done using a control olfactometer in which both arms were blank (no attractant), but one was arbitrarily designated as the test. Numbers of mirids in the test and blank chambers for fresh lucerne, morning volatiles, afternoon volatiles and night volatiles were compared with those in the control olfactometer using the R statistical package (Dalgaard 2002). Analyses involved the GLM procedure in R with a quasibinomial distribution. Response was calculated as % of mirids in test chambers out of the total numbers used in the olfactometer. A oneway analysis of variance (Minitab v14) followed by Fisher's pairwise comparisons was also done to determine significant differences between treatments.

Figure 1 shows the test% for olfactometer studies using lucerne bouquets and lucerne volatile washings. The GLM analysis showed that fresh lucerne bouquet ($p<0.001$) and volatiles collected in the afternoon ($p<0.05$) were significantly attractive to female mirids compared to the blank olfactometer, and volatiles collected either in the morning or at night were not significantly attractive. Results of the Fisher's pairwise comparison of means are shown in Table 1.

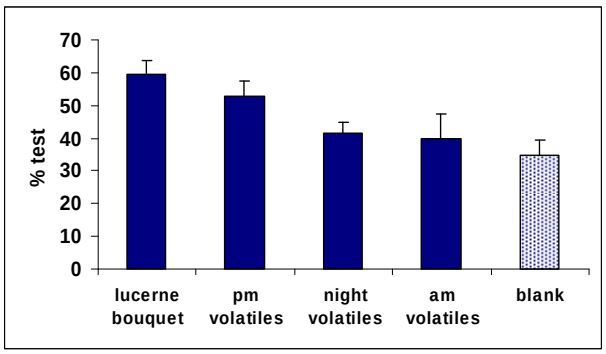

Fig. 5. % test response of female mirids to fresh lucerne bouquet and lucerne volatiles collected at different times. N= 16 (lucerne bouquet), N= 5 (pm volatiles), N= 4 (night volatiles), N= 4 (am volatiles), N= 8 (blank).

Variable	P	Order and significance
% test	0.003	Lucerne bouquet^{a} < pm volatiles^{ab} < night volatiles^{bc} < am volatiles^{bc} < blank^{c}

Table 1. Summary of Fisher's pairwise comparison tests. Treatments with different superscript letters are significantly different ($P < 0.05$).

Another common host plant of green mirids is the tropical verbine *Cullen* (=*Psoralea*) *cinerea* (Lindl.) J.W Grimes which is found in drier inland part of Australia. Potted plants were grown in the glasshouse from seeds obtained from inland plants (seeds supplied by James Hereward from collections made as part of Project 1.1.04). Volatiles from this plant were collected by SPME and SuperQ-trap methods and analysed by GC-MS. Olfactometer experiments were conducted using hexane extracts of these volatiles and their synthetic equivalents, but the results yielded no significant attraction to female mirids.

2.2 Single chemicals and blends

Single chemicals were tested in the olfactometer using 0.1% concentration in 200 µl canola oil pipetted into a small piece of dental wick. A total of 21 single chemicals were tested (Fig. 6). Two, (*E*)-2-hexenal and cineole, were significantly attractive to mirids, and another two, nerol and trans-2-hexenol, were repellents.

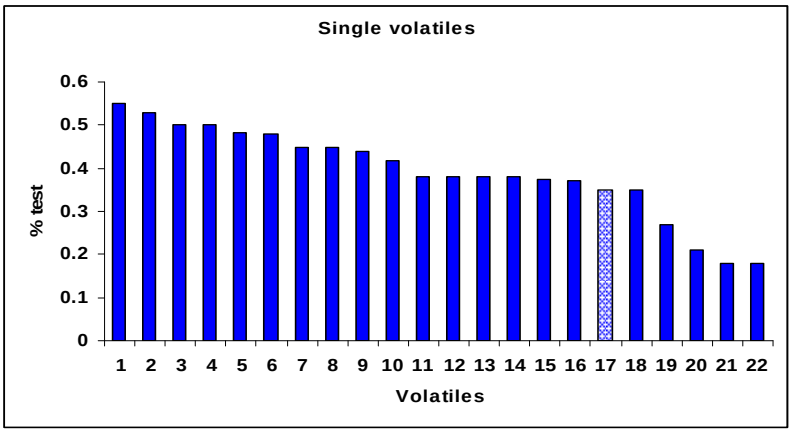

Fig. 6. % test response of mirid females to single volatiles in the olfactometer. Volatiles 1 ($p<0.05$) and 2 ($p<0.01$) significantly more attractive, and volatiles 21 ($p<0.05$) and 22 ($p<0.05$) significantly less attractive compared with blank. Volatiles are: 1=cineole, 2=(*E*)-2 hexenal, 3=4-methoxybenzyl alcohol, 4=geraniol, 5=(*Z*)-3-hexenol, 6=limonene, 7=β-caryophyllene, 8=(*Z*)-3-hexenyl butyrate, 9=(*Z*)-3-hexenyl acetate, 10=butyl salicylate, 11=α-farnesene, 12=α-pinene, 13=myrcene, 14=ocimene, 15=(*E*)-2-hexyl hexanoate, 16=linalool, 17=blank, 18= hexyl hexanoate, 19=phenylacetaldehyde, 20=hexyl acetate, 21=nerol, 22=trans-2-hexenol.

A total of 8 blends were tested in the olfactometer, and none of these was significantly attractive to female mirids (Fig. 7). Components of these blends are given in Table 2.

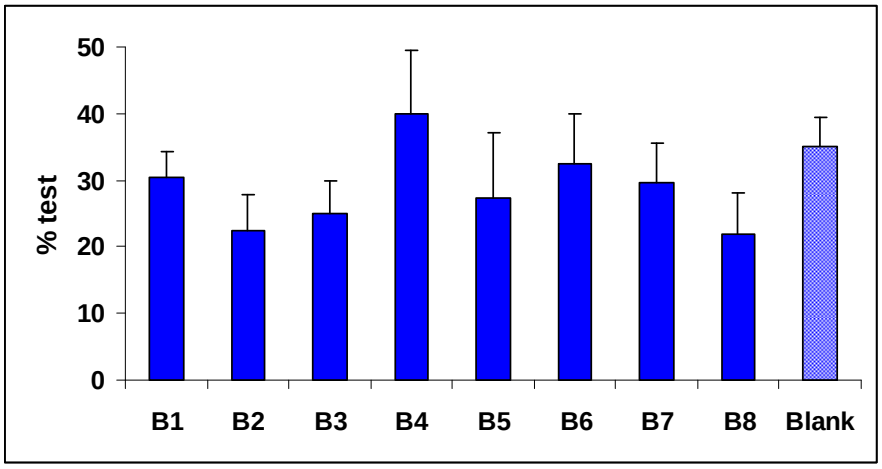

Fig. 7. % test response of mirid females to volatile blends in the olfactometer. Blend components are given in Table 2.

Blend	Components
1	60 υl limonene + 10 υl(*E*)-2-hexenal + 10υl (*Z*)-3-hexenol
2	40υl limonene + 10υl linalool +10υl (*Z*)-3-hexenyl butyrate + 10υl α-farnesene + 5υl (*E*)-2-hexenal + 5υl (*Z*)-3-hexenol
3	16υl phenylacetaldehyde (PAA) + 16υl linalool + 12υl cineole + 8υl (*Z*)-3-hexenol + 8υl α-farnesene + 4υl (*E*)-2-hexenal
4	36υl (*E*)-2-hexenal + 24υl (*Z*)-3-hexenyl acetate + 8υl limonene + 12υl PAA
5	40υl (*E*)-2-hexenal + 24υl (*Z*)-3-hexenyl acetate + 16υl PAA
6	4υl (*E*)-2-hexenal + 2.4υl (*Z*)-3-hexenyl acetate + 1.6υl PAA
7	8υl (*E*)-2-hexenal + 8υl β-caryophyllene
8	8υl (*E*)-2-hexenal + 4υl (*Z*)-3-hexenyl acetate

Table 2. Components of the different volatile blends tested in the olfactometer.

We investigated another olfactometer technique using low-level green LED light. Mirids were observed to be strongly attracted to low-level green LED light in the absence of plant volatiles, and we thought this might have been a more sensitive method to measure attractiveness than our normal bioassay method. We tried to measure the attractiveness of a given volatile or blend by the numbers of mirids choosing the test chamber (volatile) when the control chamber had the LED light. However, this method was not significantly better than our normal olfactometer method without the LED light.

In summary, our olfactometer work showed that only two of the 21 volatiles tested were found to be significantly attractive to mirids, and two volatiles might act as repellants. Combining volatiles into blends did not increase attractiveness to mirids. Field testing of volatiles and blends was restricted by the availability of a field crop with high mirid numbers for this purpose in the last year of the project.

Objective 3: Investigate potential for mating disruption, attract-and- kill, and monitoring using mirid pheromones

3.1 Mating disruption and attract-and-kill

This work was done in collaboration with a CRC post-graduate student, Sam Lowor, as part of his PhD research project.

Methods

A field trial was done on dryland cotton at "Prospect", Warra, Qld, to investigate the potential of mating disruption and attract-and-kill using a sprayable formulation of the mirid pheromone in Magnet® base (without the Magnet® volatiles). There were two sites, about 400 metres apart - field S3 (treatment blocks) and field WL4 (control blocks). Treatment blocks were demarcated as 290 m x 300 m (8.7 ha) for field S3 (Fig. 8). Buffer zones of 300 m were created between treatments. For field WL4, no treatments were imposed but pheromone traps were laid out in a similar pattern to field S3, except that the dimensions of this field were slightly smaller (Fig. 9). Each treatment plot had 4 pheromone traps (AgriSense® design) containing mirid lures (2 mg loading). The assumption of the experiment was that if mating disruption occurred, catches would be lower in the traps in the treated areas than those in the control areas, or in the control field WL4, because the males would not be able to locate the traps in an environment saturated by pheromone (trap shutdown). In the attract-and-kill treatment areas, lower catches would result because the mirid density would be reduced.

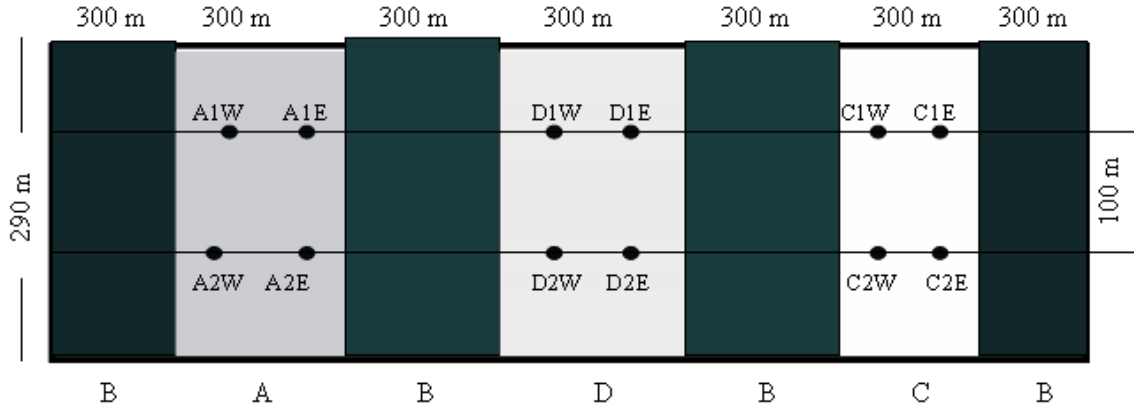

Fig. 8. Layout of field S3 (treated) showing location of pheromone traps. ● – pheromone traps, A - attract-and-kill, D – mating disruption, C - control , B – buffer zones.

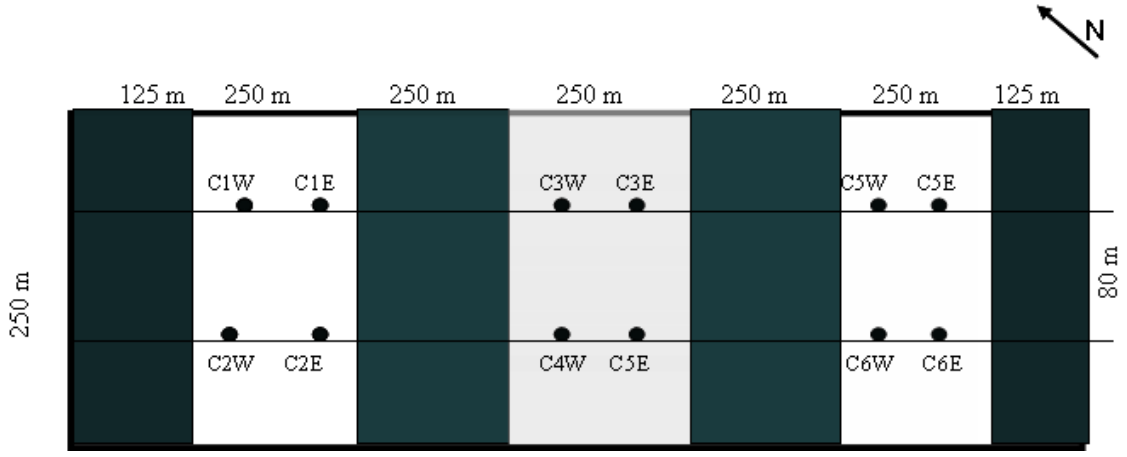

Fig. 9. Layout of field WL4 (control) showing location of pheromone traps. ● – pheromone traps

For the attract-and-kill treatment, one row in every 32 (including skip rows) was treated with 500ml/100m of 1.2% mirid pheromone mix in Magnet® base. Fipronil (Regent®, Bayer Australia Ltd, Pymble, NSW, Australia) was the insecticide sprayed onto the treated rows as a cover spray, after the pheromone, at a rate of 1.25 ml a.i./100m. For mating disruption, one row in every 16 (including skip rows) was treated with 500 ml/100m of 2.4% mirid pheromone in the base described above. The pheromone quantity applied per hectare was therefore four times that in the attract-and-kill treatment (twice as concentrated, and applied to twice as many rows). However, there was no insecticide present. Formulations were applied by spraying through a low pressure electric pump using a nozzle designed for liquid fertiliser application. The pump was mounted on a modified motor cycle.

The treatments in field S3 were sampled using a large backpack suction sampler (D-Vac). Four samples were taken from 50 m strips of cotton that did not have any treatment application within the treatment blocks. Treatments were sampled at 0 day (pre-treatment), 1, 2, 3, 4 and 7 days post application.

Results

Figure 10 shows mean pheromone trap catches in field S3 (treated), both before and after the treatments. Trap catches were not significantly different ($P = 0.658$) between the sites marked for the various treatments from day1 to day 12 (that is, during the pre-treatment phase). Differences however, occurred between the control and the treatments after the application of the mating disruption and attract-and-kill formulations to field S3 on the 12th day. Catches of male GM in the control plot were significantly higher compared to the mating disruption and attract-and-kill plots for day 14 ($P = 0.003$, when no GM were found in any trap from either the attract-and-kill or the mating disruption treatment), day 16 ($P < 0.001$) and day 18 ($P = 0.004$). There was no significant difference between the treatments on day 23 ($P = 0.06$).

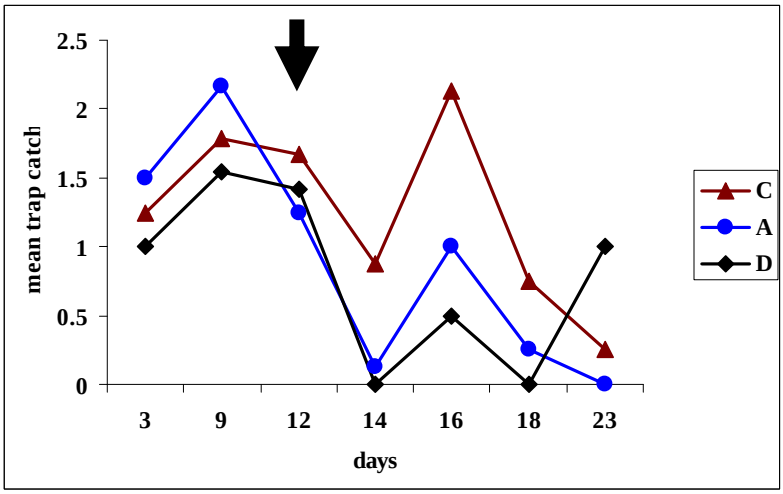

Fig. 10. Mean pheromone trap catches for the treated plots in field S3, "Prospect", Warra, Qld. D – mating disruption treatment A - attract-and-kill treatment C - control. Black arrow indicates day of treatment application.

Figure 11 shows catches in the nearby field WL4, where no treatments were applied. No significant spatial differences existed between the summed trap catches from days 1 to 23 (P = 0.358). Further analysis revealed no significant spatial variation, either along or across the field, on any day. This further strengthens the inference that the differences between plots within field S3 on days 14, 16 and 18 could have been due to the treatments applied.

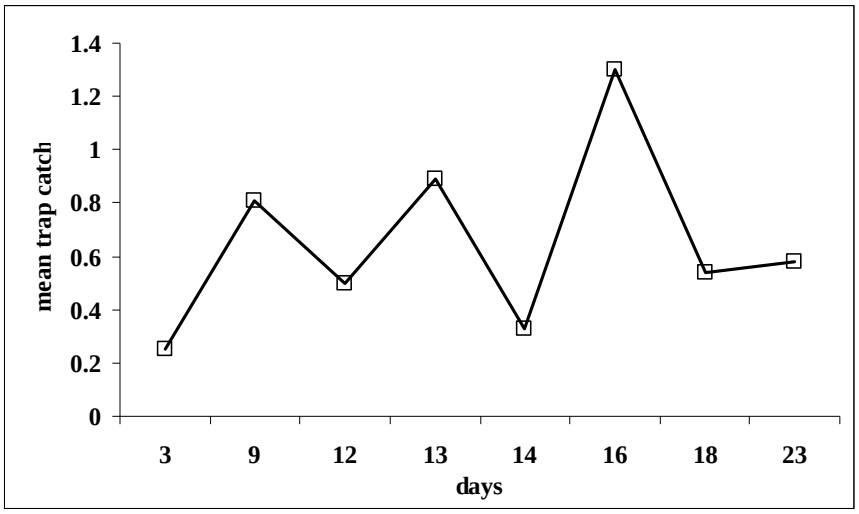

Fig. 11. Mean pheromone trap catches for the untreated field WL4, "Prospect", Warra, Qld.

There was a complete trap shutdown for two days in the mating disruption treatment and a further partial shutdown for at least another 4 days (Fig. 10). A possible explanation why trap shutdown did not last longer was the quick dissipation of the pheromone spray. The results suggest that mating disruption **mi**ght be feasible provided the problem with pheromone dissipation is resolved by a slow release method.

Lower trap catches were also recorded from the attract-and-kill treatment, after the formulation was applied (Fig. 10). This could have been because some had been killed by the insecticide applied to the treated rows. It could also have been because there was sufficient pheromone in the formulation to produce mating disruption, independently of any killing effect. An attempt was made to assess the impact of the insecticide by placing horticultural plastic along 5 m of treated row in three locations within the attract-and-kill plot and examining it at regular intervals for dead insects. No GM (males or females, or nymphs) were found. This may have been because numbers were so low, or because the fipronil did not kill mirids quickly enough to prevent them moving away from the treated area before dying. It is therefore not possible to be sure of the mechanism for trap reductions in this treatment. However, the attract-and-kill formulation seemed to work over the same time frame as the mating disruption formulation, suggesting mating disruption as the mechanism.

3.2 Mirid pheromone and Magnet® for attract-and-kill

Methods

The primary aim of this trial was to compare the attractiveness to green mirids of formulations of the mirid pheromone volatiles both alone and in combination with Magnet®. The trial was set up in a field of flowering faba beans at "Carbucky", near Goondiwindi, Qld. There were 4 treatments:

- Magnet® base alone (without the Magnet® volatiles)
- Magnet® (with Magnet® volatiles)
- Magnet® base containing the GM pheromone volatiles
- Magnet® base with Magnet® volatiles plus GM pheromone volatiles

The Magnet® volatiles comprised a mixture of the 5 components (of the old Magnet formulation) giving a total volatile content of 3.2%. GM pheromones consisted of 5:1 ratio of hexyl hexanoate and (*E*) 2-hexenyl hexanoate, at a final concentration of 1.2% volatiles. No insecticide was added to any treatment. Treatments were applied to 50m strips of faba beans, arranged in a square pattern of four rows each containing one replicate of each treatment, with 50 m buffer strips between them. Rows were separated by 50m. Formulations were applied to the tops of plants by hand (shaken from a plastic bottle) at 500 ml per 50 m.

The treatments were sampled using a large backpack suction sampler (D-vac), based on a Solo Mist Blower Port 423. The nozzle was moved over the top of the plants at a slow walking speed and insects collected in a nylon bag, then transferred to plastic bags and frozen prior to counting. Treatments were sampled at 20, 31, 78 and 123 h post application. The 20 h sample was done in mid-morning, on the day following application, but all subsequent samples were done at night, around 2200-2300h. This was because prior trapping studies showed that most male mirids came to the pheromone in the early evening. On each sampling occasion, four control (untreated) 50m sections were sampled from randomly chosen locations between the treated rows. The controls therefore represented sections from which insects had not been previously removed, whereas for the treated sections, the numbers present represented mostly new arrivals.

Results

Green mirids (GM)

Mean numbers of male and female GM from each treatment on each sample occasion are shown in Table 3. GM numbers were very low. In the control sections they were always below 1 per 50 m. At the 20h sample, there were no significant differences between treatments. This may have been because males which approached the pheromone during the night left again the next morning, before the sample. For the 31 and 78 h treatments there were significant differences between the treatments in the case of males ($F_{4,15}$ = 3.52, P = 0.03 for 31 h, and $F_{4,15}$=3.31, P=0.04 for 78h). For the females, however, there were no significant differences. For the males, the differences were mostly due to higher numbers in the pheromone-only treatment. This treatment was significantly different from all the others using LSD tests. In particular, the Magnet® + pheromone treatment was not significantly different from the control and Magnet® only treatments. This suggests that Magnet® base and Magnet® with volatiles are not strongly attractive to male GM, and that the Magnet® volatiles interfere with the response to pheromones.

At the final sample time, 123 h, the trends were similar to earlier samples, but the differences were not statistically significant ($F_{4,15}$ = 1.73, P=0.19). In the 78 and 123 h samples, there was a tendency for the Magnet® + pheromones treatment to yield more male GM than the control, base only or Magnet®. The differences were not statistically significant, but the results suggest that perhaps the inhibitory effects of Magnet® volatiles on responses to pheromones were beginning to wear off, and extending sampling for longer periods in future trials might be worthwhile.

When the catches were summed over all sample intervals, there was a highly significant difference for males ($F_{4,15}$ = 6.25, P=0.004). Most of this was due to the pheromone-only treatment, which was significantly different from all others using LSD tests. Overall, this treatment yielded about 12 times the number of male GM in control. The Magnet® + pheromone treatment yielded about 4 times the number in the controls, though the difference was not statistically significant.

Helicoverpa spp.

For *Helicoverpa* moths, there were significant differences at 20 h ($F_{4,15}$=5.58, P=0.006), 31h ($F_{4,15}$=3.50, P=0.033) and but not at 78h ($F_{4,15}$=2.84, P=0.06) or at 123h (results not analysable because of low numbers). When the data for the all sampling times were summed, there were highly significant differences between the treatments ($F_{4,15}$=7.16, P=0.002). In all cases, the trend was for the treatments containing Magnet® volatiles to have much higher counts than those without. No moths were ever caught in the control sections, and only a few (presumably responding to the sugar in the Magnet® base) were caught in the treatments with Magnet® base alone, or Magnet® base + mirid pheromones. These results provide further evidence that Magnet® is attractive to *Helicoverpa* moths, and that the attraction is primarily due to the presence of the plant volatiles rather than the sugar. Overall, treatments which contained Magnet® volatiles had about 10 times the number of *Helicoverpa* moths than those which had sugar only.

Treatment	Hours post spray	Male GM	Female GM	Total GM	*Helicoverpa* moths
Control	20	0.00a	0.00a	0.00a	0.00a
Base	20	0.00a	0.50a	0.50a	0.75a
Magnet	20	0.75a	0.00a	0.75a	7.00b
Pheromones	20	0.75a	0.00a	0.75a	0.00a
Magnet + pheromones	20	0.25a	0.00a	0.25a	7.75b
Control	31	0.25a	0.25a	0.50a	0.00a
Base	31	1.00a	1.25a	2.25ab	0.50ab
Magnet	31	0.25a	0.75a	1.00a	4.50c
Pheromones	31	3.50b	0.75a	4.25b	0.50ab
Magnet + pheromones	31	0.75a	0.75a	1.00a	3.75bc
Control	78	0.25a	0.00a	0.25a	0.00a
Base	78	0.75a	0.00a	0.75a	0.50a
Magnet	78	0.00a	0.25a	0.25a	2.00ab
Pheromones	78	3.00b	0.75a	3.75b	0.50a
Magnet + pheromones	78	1.00a	0.00a	1.00a	2.75b
Control	123	0.25a	0.50a	0.75a	0.00a
Base	123	0.00a	0.00a	0.00a	0.00a
Magnet	123	0.00a	0.50a	0.50a	0.00a
Pheromones	123	2.00a	0.00a	2.00a	0.00a
Magnet + pheromones	123	1.25a	0.25a	1.50a	0.25a
Control	Total	0.75a	0.75a	1.5a	0.00a
Base	Total	1.75a	1.75a	3.5a	1.75a
Magnet	Total	1.00a	1.50a	2.5a	13.50b
Pheromones	Total	9.25b	1.50a	10.75b	1.00a
Magnet + pheromones	Total	3.25a	0.50a	3.75a	14.50b

Table 3. GM males and females collected by suction sampling from 50m sections of treated rows.

The numbers of moths (predominantly *H. punctigera*) present during this trial were high. The large D-vac is an extremely inefficient method of collecting moths, which usually fly off well before the nozzle gets close to them. It is unlikely that the efficiency of the sampling was more than about 10%. The data therefore indicate that very large numbers of moths were being attracted to the Magnet® treatments in this trial. There was a general tendency for moth numbers to decline with successive samples, and virtually none were present at 123 h, which is about the time when Magnet® with added insecticide stops killing moths in the field. This result suggests that it is the volatiles rather than the insecticide which have run out in our previous field trials of Magnet®, and that research on slow-release formulations for the volatiles would be worthwhile.

The treatment containing mirid pheromones alone gave catches which were very similar to the Magnet® base alone, indicating that these compounds are not

attractive to *Helicoverpa* spp. Similarly, the Magnet® volatile treatments with and without pheromones yielded similar moth numbers, indicating that the mirid pheromones neither synergise nor inhibit the attractiveness of Magnet®.

Conclusions

The experiment was done under unfavourable conditions, early in the season, before GM numbers had built up. The actual numbers of GM are probably an underestimate, since the suction sampler was only 50-60% efficient (Stanley 1997). Nevertheless the experiment showed a clear tendency of male (but not female) GM to accumulate in the rows treated with pheromone only. If a contact foliar insecticide had been applied to these rows, it could have killed them. The use of a separate foliar insecticide appears necessary because GM do not seem to contact the formulations directly, or ingest them. Rather, they perch in foliage close to the formulations. We did not use any insecticide in this trial because dead mirids are very hard to find, especially at the densities present in this experiment. Instead we collected them live by suction sampling. These results are consistent with the attract-and-kill trial described in Section 3.1, but do not confirm that the mechanism of reduced mirid trap catches in that experiment was attract-and-kill rather than mating disruption. We still need to do experiments with fast-killing insecticides, and find dead mirids, to determine whether attract-and-kill will really work.

Insecticides used to control mirids damage natural enemy populations, and the ability to control GM by treating only occasional rows with them and allowing natural enemies to survive in the other rows would be a considerable advance in cotton IPM. Killing male GM would reduce damage to cotton directly (since the males themselves feed on the crop), and indirectly by removing potential mates for the females, thus reducing the next generation. The magnitude of the indirect effect would depend on the extent of multiple mating, and the ability of mated female GM to move into the crop from outside sources. Both of these factors are not well understood for GM at present.

The experiment also suggested that pheromone-based attract-and-kill for male GM would work best if it was not combined in the same formulation with Magnet® for *Helicoverpa* spp., since Magnet® volatiles appear to inhibit responses to pheromones. However, simultaneous application of different formulations in alternate row spacings might work. Similarly, the results suggest that the pheromones might work after the activity of the Magnet® volatiles wears off, so in situations where the immediate problem was *Helicoverpa* rather than GM, adding mirid pheromones might be useful if it did not significantly increase the cost of the material (the pheromone volatiles are cheap, and not used in large quantities). The results suggest that addition of the mirid pheromones to Magnet® would not affect the attractiveness of Magnet® to *Helicoverpa* moths, either positively or negatively, so it might be possible to develop a blend which was just as effective on *Helicoverpa* spp. but with the added bonus of some residual effect on mirids. However, this possibility requires further research, and it is likely that such a blend would not be suitable in situations where the immediate problem was mirids, since it might take a few days to work.

3.3 Pheromone aerosol dispensers for mating disruption or attract-and-kill

We investigated the feasibility of using aerosol dispensers of pheromones for mating disruption or attract-and-kill. These dispensers can be programmed to release the pheromone at desired time intervals. The aerosol cans were sourced from a supplier in New Zealand and filling the cans with pheromone was done by NU-CANS in South Australia.

Small mating disruption trials using these dispensers were done on pigeon peas and mung beans. Three pheromone-filled aerosol cans were programmed to release pheromone every 30 min between 1800 and 0600h. The pheromone traps were placed in the middle of the field next to the dispensers, and another three traps were located on the edges of the field without dispensers. Trap shutdown in the middle traps was expected if mating disruption worked. Traps were checked for 4 nights. A total of 1, 2, 0 and 0 mirids in the three middle traps, and 1, 2, 1 and 0 mirids in the three outer traps, for the 1st, 2nd, 3rd and 4th night, respectively, were caught. A similar experiment was done on mung beans for 3 nights, but no mirids were caught in any of the six traps. Aerosol dispensers might have potential for mating disruption, but further testing is needed under high mirid population.

The feasibility of using the aerosol dispensers with sticky traps for attract-and-kill was also tested. Four 80cm x 60cm yellow sticky traps (two with dispensers which sprayed the pheromone onto a felt pad at intervals of 30 min, and two without dispensers) were run for 4 nights on a lucerne field at "Yarral" in Narrabri. Although mirid numbers were low, there were more mirids in the sticky traps with the pheromone dispensers than the sticky traps alone. Sticky traps with the dispensers caught a total of 20 mirids, whilst those without the dispensers caught a total of 7. Further trials on mating disruption or attract-and-kill were not conducted as we were constrained by lack of a field crop with high mirid numbers.

3.4 Pheromone trapping for monitoring mirids

Pheromone trapping trials were conducted at 8 locations in NSW and Queensland in collaboration with the Cotton CRC extension officers and researchers. The main objective of these trials was to establish whether pheromone catches are correlated with mirid numbers in the field. This information will help evaluate whether pheromone traps could be useful as monitoring tools for mirids.

Trapping trials were done on Bollgard II® cotton at the following locations: Auscott (Narrabri), Brigadoon (Boggabri), Avalon (Warren) and Hillston in NSW, and Korolea (Goondiwindi), Mayfield (Dalby), Brookglen and Thomas Cotton (St. George) in Qld. The trials were conducted between October 2007 and February 2008, ie, from the 2-3 leaf stage to the boll stage of the cotton plants. Results for three sites (Auscott, Brigadoon and Korolea) were used for the Honours project of Suzette Argent (Project 5.10.07.08)

Methods

Four pheromone traps (AgriSense) were set up at each site using pheromone lures coated with araldite glue to slow down pheromone dissipation (Figs. 12a and 12b). Traps were cleared at least twice a week and lures changed every 5 weeks. Each trap was provided with a 2 x 4 cm block of pest strip (dichlorvos) to kill the mirids. Field sampling for mirids was done when traps were cleared using either one or a combination of two or more of these methods – 6 x 1m visual, 6 x 20 sweep net, 6 x 1m beat sheet and 6 x 20m Dvac or suction (Fig. 13). Mirid samples were dissected to determine sex ratio and mated status of females.

a b

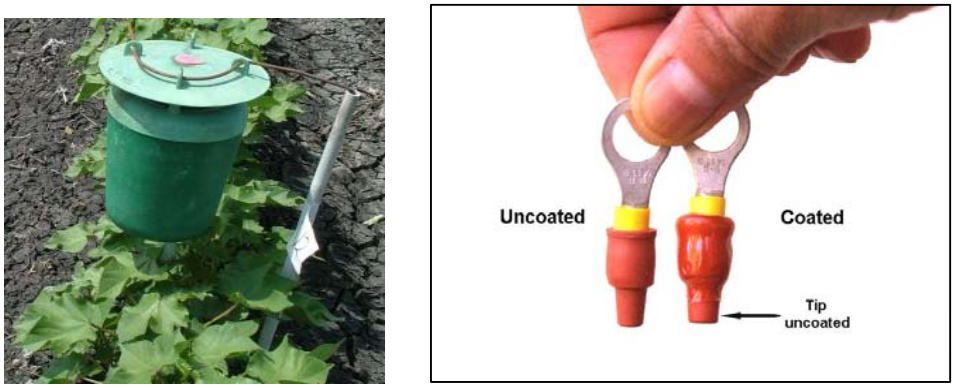

Fig. 12. Pheromone trap (a) and coated lure (b) used in the trials.

Visual Dvac or suction

Sweep net Beat sheet

Fig. 13. Sampling methods for mirids.

To determine whether the efficiency of the different sampling methods varied, mirid catches of adults and nymphs using visual, sweep net, suction and beat sheet methods, were compared at three locations with three operators (Auscott, Brigadoon and Korolea), on three sampling dates. Data were log-transformed to meet assumption of normal distribution. Analysis of covariance and GLM analysis (Minitab v14) showed that the relative efficiency of each sampling method varied considerably between operators, so correction factors were applied to each site to produce a population density index for adults and nymphs. Data on trap catches and mirid numbers were analysed by regression analysis using Minitab v14. In most cases, normality tests required these data to be either square-root or log-transformed.

Results

The relationship between pheromone trap catches and field mirids varied between locations. Significant correlations between pheromone trap catches and both mirid adults and nymphs were shown at Korolea and Brookglen, between trap catches and mirid nymphs at Brigadoon and between trap catches and mirid adults at Thomas Cotton. No correlations with either adults or nymphs were observed at Auscott, Mayfield, Avalon and Hillston.

Pheromone trap catches were positively correlated with both mirid adults and nymphs at Korolea (Figs. 14 and 15). Peak trap catches appeared to coincide with peak numbers of mirids in the field.

a b

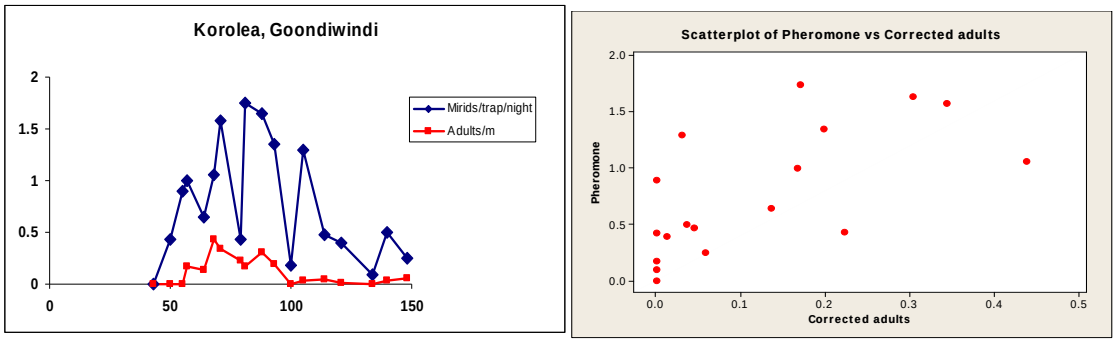

Fig. 14. (a) Pheromone trap catches per night and corrected numbers of adult mirids (Y-axis) plotted against time (days after October 1) and (b) scatter plot of pheromone trap catches and corrected numbers of adult mirids at Korolea ($p<0.01$).

a b

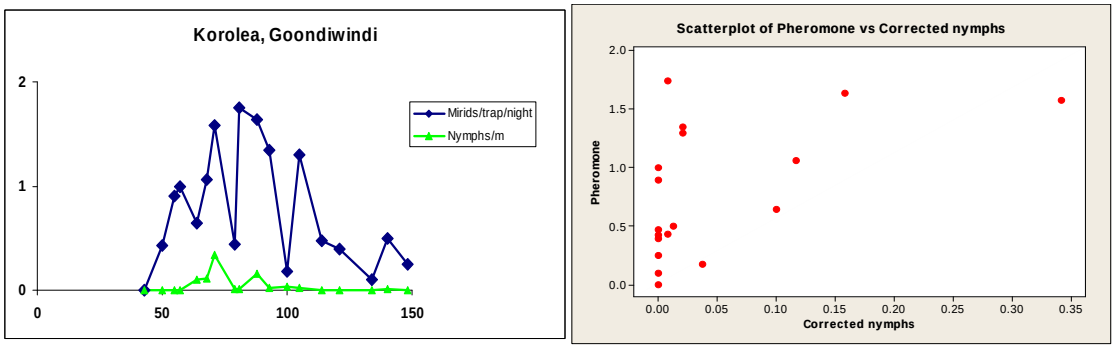

Fig. 15. Pheromone trap catches per night and corrected numbers of mirid nymphs and scatter plot for Korolea ($p<0.05$). Axes as for Fig. 14.

Results at Brookglen were similar to those at Korolea. Pheromone trap catches were positively correlated with both mirid adults and nymphs, and peak trap catches coincided with peak mirid numbers in the field (Figs. 16 and 17).

a b

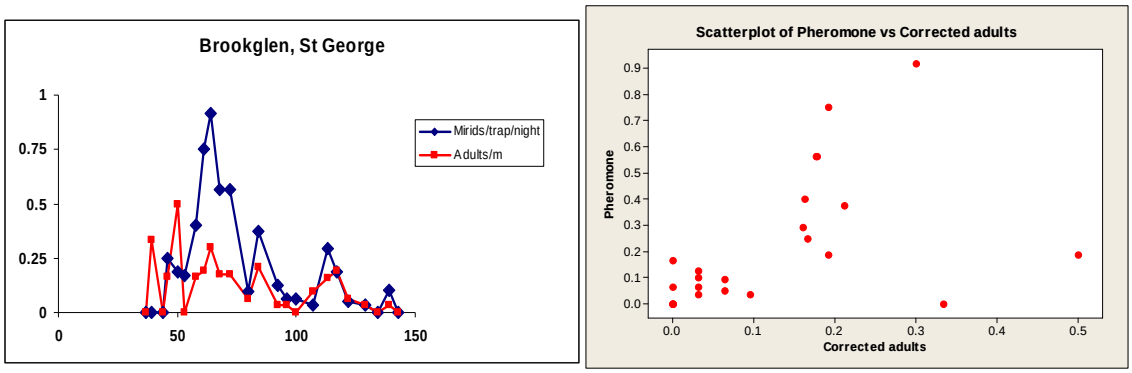

Fig. 16. Pheromone trap catches per night and corrected numbers of adult mirids and scatter plot for at Brookglen ($p<0.001$). Axes as for Fig. 14.

a b

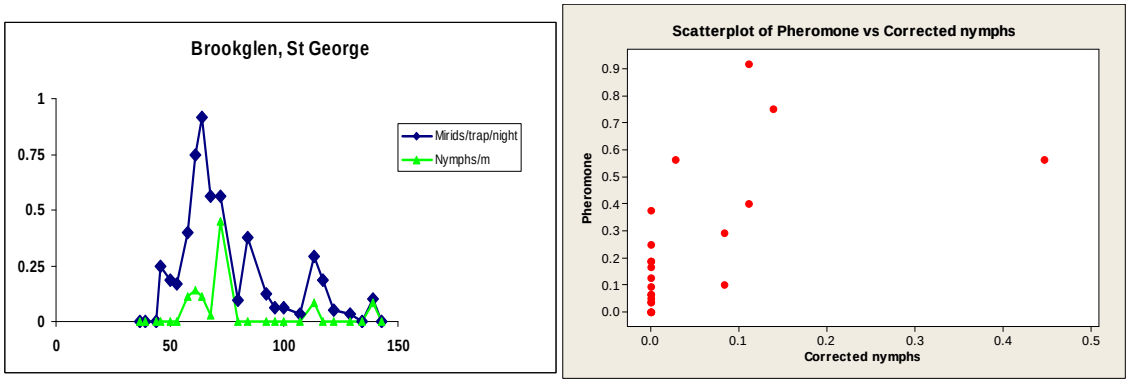

Fig. 17. Pheromone trap catches per night and corrected numbers of mirid nymphs and scatter plot for Brookglen ($p<0.001$). Axes as for Fig. 14.

At Brigadoon, pheromone trap catches were not significantly correlated with adult mirids, but there was a significant negative correlation with mirid nymphs (Figs. 18 and 19). Earlier in the season, pheromone traps caught mirids when there were no adults in the field as indicated by visual sampling. Later in the season, there was a decline in trap catches when mirid adults and nymphs were still found in the field.

a b

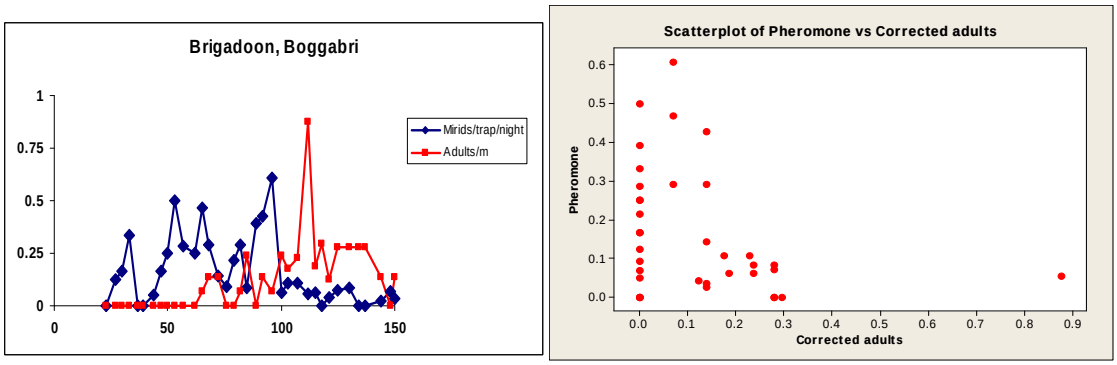

Fig.18. Pheromone trap catches per night and corrected numbers of adult mirids and scatter plot for at Brigadoon ($p=0.105$). Axes as for Fig. 14.

a b

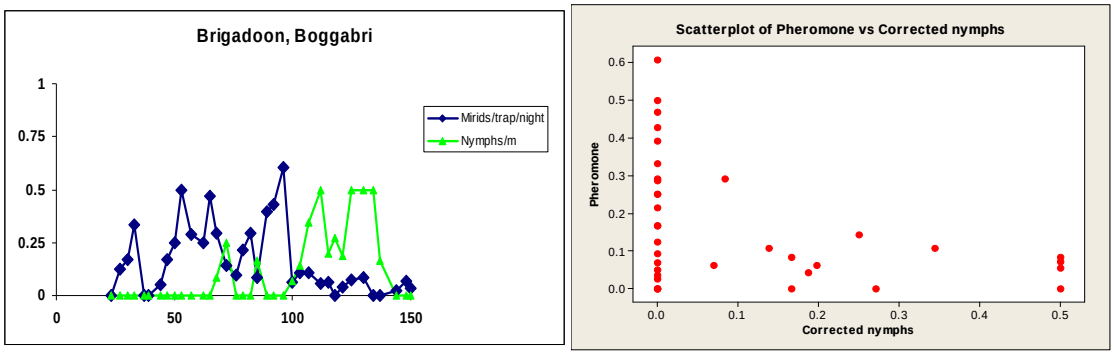

Fig. 19. Pheromone trap catches per night and corrected numbers of mirid nymphs and scatter plot for Brigadoon ($p<0.05$). Axes as for Fig. 14.

At Thomas Cotton, there was a significant positive correlation of pheromone catches and corrected adult mirid numbers but not with mirid nymphs ($p=0.094$) (Fig. 20). Peak trap catches appeared to coincide with peak mirid numbers in the field.

a b

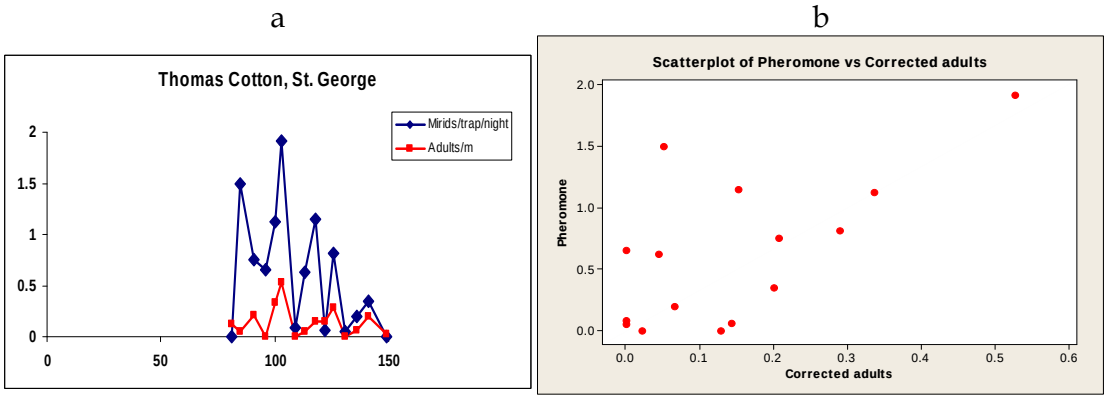

Fig. 20. Pheromone trap catches per night and corrected numbers of adult mirids and scatter plot for at Thomas Cotton ($p<0.01$). Axes as for Fig. 14.

There were no significant correlations between trap catches and mirid adults or nymphs at Auscott, Mayfield, Avalon and Hillston (Figs. 21-24).

a b

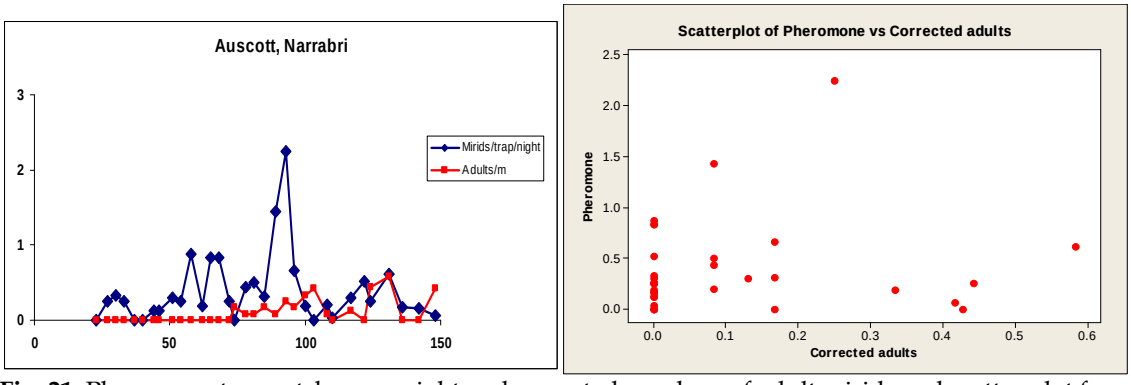

Fig. 21. Pheromone trap catches per night and corrected numbers of adult mirids and scatter plot for Auscott ($p=0.0532$). Axes as for Fig. 14.

a b

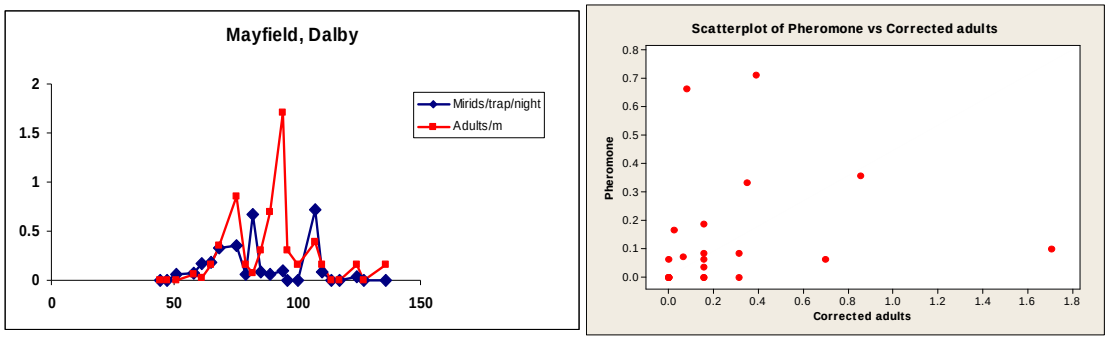

Fig. 22. Pheromone trap catches per night and corrected numbers of adult mirids and scatter plot for Mayfield (p=0.052). Axes as for Fig. 14.

a b

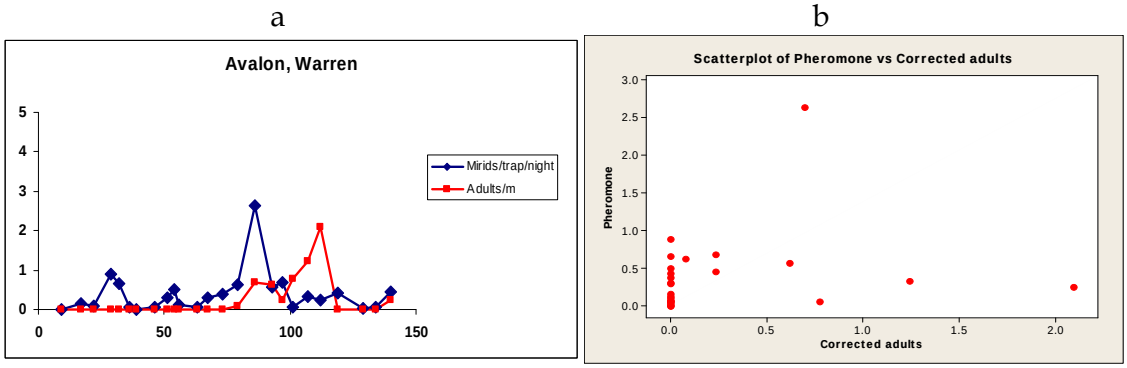

Fig. 23. Pheromone trap catches per night and corrected numbers of adult mirids and scatter plot for Avalon (p=0.105). Axes as for Fig. 14.

a b

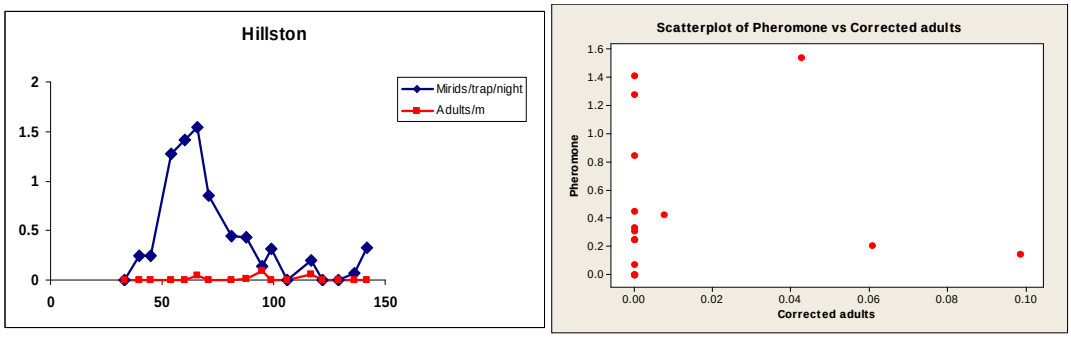

Fig. 24. Pheromone trap catches per night and corrected numbers of adult mirids and scatter plot for Hillston (p=0.612). Axes as for Fig. 14.

One of the deficiencies of the trials was that at some locations, clearing of traps and mirid sampling were not done regularly, hence the big gaps between some data points. In addition, some of the sampled mirids were not collected and thus, data on % females and mated status of females were not available on some sampling dates.

Adult mirids were dissected to determine sex and mated status of females. Female mated status was determined according to the description by Strong *et al* (1970) for the American mirid, *Lygus hesperus*. A female was scored as mated when the seminal depository appeared enlarged and genital pouch inflated and whitish in colour.

Regression analyses were done on pheromone catches versus per cent mated females for each location except Hillston, where there was only 1 data point and Thomas

Cotton, where there were only five data points. There was a significant positive correlation between pheromone catches and % mated females at Brigadoon indicating that the only times the pheromone traps worked was when majority of the females were mated (Fig. 25). No significant correlations between trap catches and % mated were observed at Korolea (p=0.546), Auscott (p=0.252), Brookglen (p=0.067), Mayfield (p=0.230) and Avalon (p=0.816).

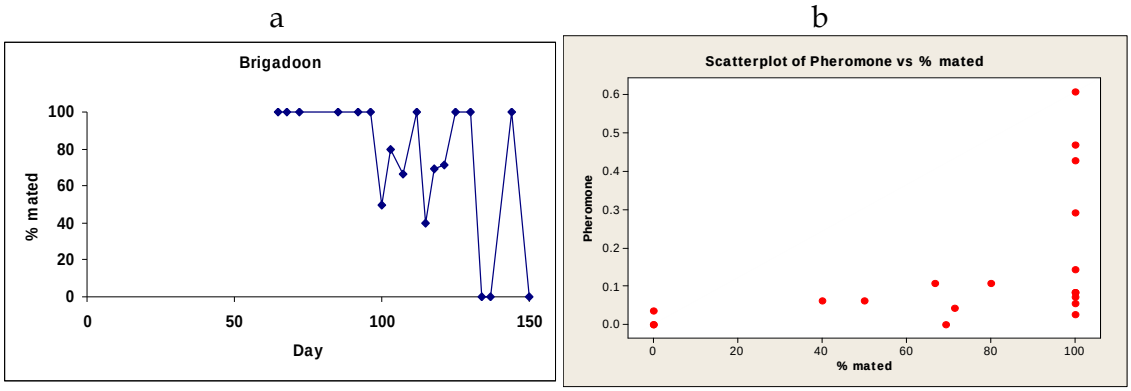

Fig. 25. Per cent mated females (a) and scatterplot of pheromone trap catches vs % mated females (b) at Brigadoon (p<0.01).

Conclusions

The pattern of pheromone trap data was not consistent in all the trial sites. In some locations such as Auscott and Brigadoon in the Namoi Valley, Avalon in the Macquarie Valley, and Hillston in southern NSW, pheromone traps caught mirids early in the season when visual or sweep net sampling yielded no mirid adults in the field. On the other hand, at other locations like Korolea, Goondiwindi and Brookglen and Thomas Cotton, St George, the trap catches correlated with mirids found in the field. These results suggest that pheromone traps can detect the presence of mirids in the field before field sampling can.

The trapping studies suggest that the population dynamics of mirids between locations were different. At some locations, there was a good correlation of trap catches with mirid numbers in the field (eg, Korolea), whilst at other sites, such correlation is poor (eg Brigadoon). At Brigadoon, variation might be explained by the difference in the pattern of % mated females, which was very variable (Fig. 25), suggesting possible immigration of unmated females from a nearby source. When this happens, pheromone traps might not work well because of the 'female competition" effect, ie, males can detect the pheromones from real females and therefore will not respond to synthetic lures in the traps.

Further trials in the 2008-2009 season might be helpful to get some more information to evaluate further the usefulness of pheromone traps as monitoring tools for mirids. Current data suggest that pheromone traps on their own might not be reliable monitoring tools to indicate presence or absence of mirids in the field. Nevertheless, they could be valuable tools for studying the population dynamics of mirids, hence a better understanding of mirid ecology.

3.5 When do mirids come to pheromone?

A field trial was done to determine when mirids come to pheromone traps. Four AgriSense traps were set up on lucerne at "Yarral", Narrabri. These traps were checked every 4 hrs over 3 days, except between 2200h and 0600h.

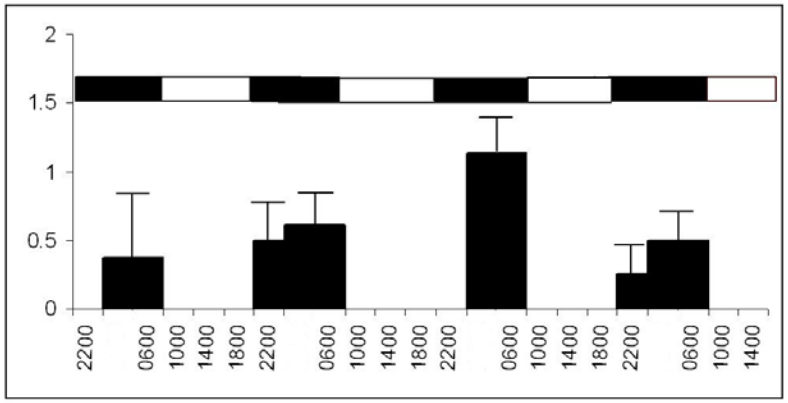

Fig. 26. Time of night when male mirids come to pheromone traps. The black and white horizontal bars above the graph represent night and day periods. Vertical bars are mean mirid numbers, ± s.e.

Although mirid numbers were low during the experiment, Figure 26 shows that mirids came to the pheromone traps only between 1800 and 0600h, with most of them coming between 2200 and 0600h. These results indicate that mirids are primarily nocturnal in their mate-finding behaviour, which is similar to our earlier olfactometer experiments showing that mirids are nocturnal in their host-finding behaviour.

Objective 4: Identify plant volatiles which attract or repel key beneficial insects

This objective was originally intended to improve Magnet® by making it less attractive to beneficial insects and therefore more selective. However, it had to be modified when the original formulation had to be revised in order to meet APVMA registration requirements. One of the volatile components was replaced with two new ones (see Objective 8). The priority became one of determining the attractiveness of the new formulation to beneficial insects.

Experiments using suction sampling on cotton rows to which Magnet®, without insecticide, was applied, were done to repeat the work on attraction of Magnet® to non-target insects, including beneficial species, in cotton. Experiments were conducted on a field of approximately 60 ha of cotton (cv Sicot 71BR) at the ACRI in 2007. Four replicates were established. A section of 50m of row was treated with Magnet®. Treated rows were separated by 50m buffer zones from the edge of the field (head ditch end), and from each other. In addition to the treated rows, untreated rows at 5m, 10m and 20m from the treated rows were selected for sampling. For two replicates these rows were to the south of the treated row, and for

the other two they were to the north (this was done in order to confound any possible effects of wind direction). At the tail-ditch end of the field, four distant control (untreated) rows were selected. These were about 300m from the nearest treated section, and were used to determine whether Magnet® application might be affecting the entire region around treated rows. No distant control samples were taken on the last night of the third experiment, because the field had been irrigated, rendering the rows inaccessible. Prior to each experiment baseline samples were taken from 50m sections of eight randomly selected rows from within the main experimental area, but avoiding sections which would later be used for sampling in the main experiments.

4.1 Methods

Magnet® was applied to the treated areas at the commercial rate (200 ml per 50m). Subsequent sampling compared the numbers of insects on treated versus nearby and more distant untreated sections. Higher numbers on treated sections indicate that the insects are being attracted to Magnet®; lower numbers indicate repellence. A large suction sampler consisting of a Solo® backpack style mist blower, with the fan reversed so the machine sucked instead of blowing, was used to collect insects from each 50 m section of row (Fig. 27). Insects collected in a muslin bag inserted in the mouth of the sampler were then transferred to a plastic bag and killed by placing them in a portable freezer. They were later sorted under a dissecting microscope and classified to taxa at various levels ranging from species to order, depending on the numbers present and the importance of each taxon as pests or beneficials in cotton.

Fig. 27. The suction sampling machine

Three separate experiments were run. The first experiment sampled treated and untreated rows during the daytime. Magnet® was applied at approximately 1900h (EDST) on 8/1/07, and sampling took place at approximately 1300h EDST on 9/1/07 (18h), 1300h EDST on 10/1/07 (42h) and 0800 EDST on 12/1/07 (85h). The second experiment was intended to repeat the first one but using night-time sampling to determine whether any nocturnally active insects might be attracted to Magnet® in a way which would not be detected by sampling during the daytime. The same areas of treated row were used as for the first experiment, because approximately three weeks had elapsed and several rain events had washed all the old Magnet® from the treated sections. Magnet® was applied at approximately

1900h EDST on 2/2/07 and sampling took place at 0100h on 3/2/07 (6h). It had been intended to continue sampling on subsequent nights, but a rain event in the afternoon of 3/2/07 forced us to abandon the experiment. The third experiment used night-time sampling at longer intervals after Magnet® application in order to complete the observations planned for the second experiment. The treated sections were moved another 50m towards the tail-ditch end of the field to avoid the possibility of effects from remnants of the Magnet® left from the second experiment. Magnet® was applied at approximately 1900h on 4/2/07 and samples were taken at approximately 0100 on 6/2/07 (30h) and 8/2/07 (78h).

Data were transformed $\log_{10}(x+1)$ in order to better meet the assumptions of normal distribution. For experiments 1 and 3, where there were three and two sampling intervals respectively, a two-way analysis of variance for each taxon was performed. Row (treated, row 5, row 10, row 20 and distant control) and sampling interval were factors, and the dependent variable was the transformed number of insects. When either treatment or the treatment x interval interaction was significant ($P<0.05$), further analyses were conducted to investigate the reasons for the significance. For each sample interval in such cases, a one way analysis of variance with treatment as a factor, followed by Fisher's multiple means comparison procedure was used. For the baseline sampling performed prior to sampling during the second experiment, a one way analysis of variance on similarly transformed data was performed for each taxon, with sampling time (day or night) as a factor.

4.2 Results

Experiment 1 – daytime sampling

In experiment 1, the daytime experiment, there were several taxa which showed significant effects of treatment or the treatment x sampling interval interaction. These included *Graptostethus servus*, flea beetles, red & blue beetles, transverse ladybirds and mosquitoes. There were many cases where the effect of sampling interval was significant, and this was usually due to fewer insects being collected on the last sampling interval. This may be because it was cooler since the sampling time had to be advanced due to an impending irrigation of the field. Differences between sampling intervals may also reflect depletion of insect populations through repeat sampling. Figure 28 shows the differences between rows for four of the six taxa for which either the main effect of treatment or the interaction between treatment and sampling interval were significant.

Flea beetles were clearly repelled by Magnet®. The numbers on treated rows were lower than on untreated rows at all sampling intervals, and the difference was statistically significant for all except Row 20 at 18h. There was evidence of weaker repellence for red and blue beetles and transverse ladybirds. For these species, the treated rows usually had the lowest numbers, and there were some statistically significant differences between treated and untreated rows, though there were many other comparisons which were not statistically significant. For mosquitoes, the statistical significance of treatment depended mostly on a significant difference at 18h between the distant control rows and all other treatments. It is possible that this indicates repellence from the general area of Magnet® treatment, but since all the

distant control replicates were at the one end of the field it is not possible to conclude that this is the case. The effect might have been other differences between the two ends of the field, eg proximity to the tail ditch or the adjacent sorghum crop.

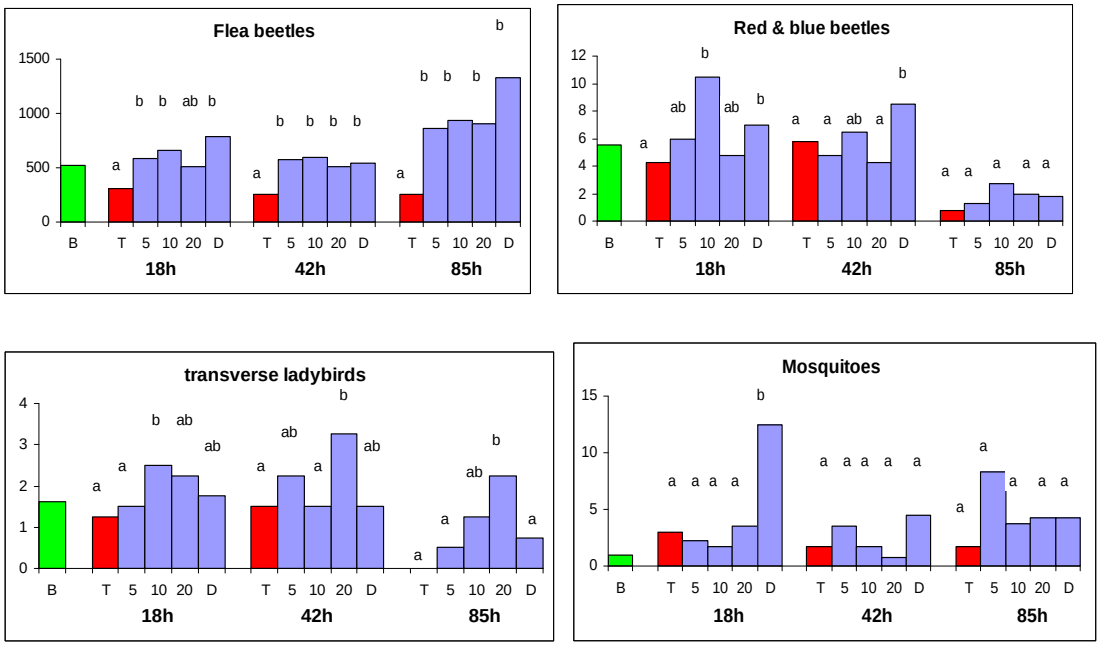

Fig. 28. Mean numbers of four taxa for which the main effect and/or the interaction involving treatment were significant. B= baseline sampling (54h before Magnet® application), T=treated rows, 5, 10 and 20 = rows 5, 10 and 20 away from the treated row, D=distant control (other end of the field). Numbers below each set of five bars are hours after Magnet® application. Bars with the same letter indicate rows which were not significantly different, within each sampling interval.

There were significant effects for two other taxa. The first was for weevils, for which there was a significant treatment x day interaction, but no significant main effect of treatment. Further analyses showed that this was mostly due to differences between the distant controls and the main experiment, which shifted over time. At the first sampling interval, the distant controls had more weevils than any other treatment, while at the last sampling interval they had the fewest. For similar reasons to those given above for mosquitoes, it is not possible to conclude that this effect was associated with Magnet®. The final case was the seed bug *Graptostethus servus*, for which there was evidence of attraction. Only 12 of these insects were found in the entire experiment (three at 18h, six at 42h and three at 85h). However, all of them were on treated rows. In such cases the large number of zeros means that analysis of variance is unreliable, so a more conservative non-parametric test (Kruskal-Wallis) was used. The results indicated that the difference was significant (H = 12.59, DF = 4, P = 0.013).

Baseline sampling: day vs night

Baseline sampling was conducted immediately before the first night experiment. The diversity of the insect community at this time was broadly similar to that of the daytime experiments even though three weeks had elapsed between the two

experiments, the cotton was older, and several management practices (irrigation and insecticide applications) had occurred in that time. However, the numbers of some species such as flea beetles had declined dramatically, and the numbers of others such as flies had increased. A few species which had not been previously found, or only recorded in such small numbers were present. They included predatory shield bugs, brown smudge bugs and flower beetles. The latter specifically inhabit the flowers of the cotton plant, and these were more abundant by late January than in the early flowering cotton of the daytime experiment, done in early January.

The baseline sampling indicated that the same insects were collected at night as in the daytime. No taxon was found exclusively at night, and the only taxa which were found exclusively in the daytime were those present in very low numbers. There were many taxa for which the numbers found at night were significantly lower than those in the daytime. This suggests that the same insect community is present during the night and day, but sampling is more efficient for some insects during the day. This may be because those insects are inactive during the night time (ie they are diurnal, perhaps resting in sheltered places at night), or because temperatures were about 11^{o}C cooler at night, so insects were less active and less liable to collection by the suction sampler.

Experiment 2 - First night sampling

Since there was only one sampling interval, one way analysis of variance was used and there was no interaction between treatment and sampling interval. Results for which treatment was significant were further examined using Fisher's multiple comparison of means procedure. There were five taxa for which a significant result was obtained. These included flea beetles, red and blue beetles, mosquitoes, brown lacewings and Lepidoptera (moths other than *Helicoverpa* spp.). The first three of these were the same taxa for which significant differences were recorded in daytime in Experiment 1. Figure 29 shows the results of further analyses on these taxa.

The three taxa for which significance was obtained in both experiments, night and day, (ie flea beetles, red and blue beetles and mosquitoes) all showed similar patterns to those obtained in Experiment 1. There was clear evidence of repellence for flea beetles, with the Magnet® treated row having significantly less than any other row. For red and blue beetles there may have been weak repellence, with the row 20m away from the treated row having more than any other row. If this was the case, the repellence appeared more diffuse than in the daytime, because the numbers in rows 5 and 10 were not significantly different from the treated row. For mosquitoes, the same pattern appeared as in the previous experiment, with more present in the distant control (tail ditch) end of the field, which might or might not be associated with Magnet®. For brown lacewings, the lowest numbers were found in the treated row, but the difference was only statistically significant when compared with Row 10. This may indicate repellence but for such a conclusion to be robust, larger numbers and a clearer pattern (eg higher numbers in row 20) would be required. For Lepidoptera other than *Helicoverpa* spp., significantly greater numbers were found on the treated row than any other row. While the primary target of Magnet® is *Helicoverpa* spp., it is well known from many previous experiments that other moths are attracted to it. In the experiments described here, *Helicoverpa* was extremely rare. This was because pest pressure at the time was

exceptionally low, and because suction sampling is an inefficient method of capturing large, strong-flying moths like *Helicoverpa* spp. Most of the moths collected were small pyralids and other microlepidoptera, though there were a few other large noctuids.

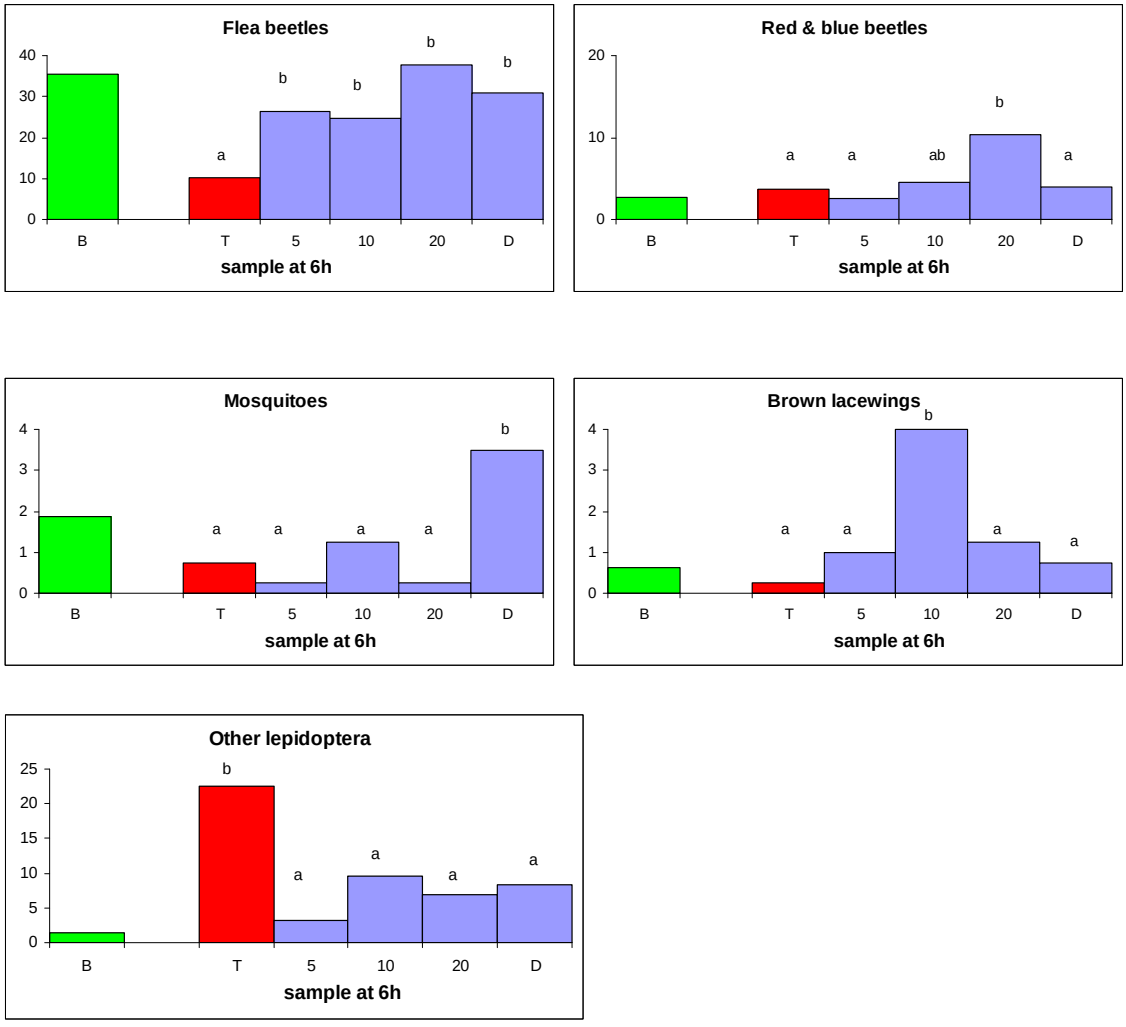

Fig. 29. Mean numbers of five taxa for which the effect of treatment was significant in Experiment 2. B= baseline sampling at night (54h before Magnet® application), T=treated rows, 5, 10 and 15 = rows 5, 10 and 15 away from the treated row, D=distant control (other end of the field). Sampling was done 6 h after Magnet® application. Bars with the same letter indicate rows which were not significantly different, within the 6h sampling interval.

Experiment 3 - Second night sampling

Since there were two sampling intervals in this experiment, the analysis was as for Experiment 1, with a two-way analysis of variance followed by separate one-way analyses in cases where either treatment or the treatment x sampling interval was significant. The distant controls were not sampled at 78h, because that area of the field was wet following irrigation. In this experiment there were seven taxa for which significant main effects of treatment, and/or interaction effects, were recorded. Further statistical analyses were performed for all these taxa, but only five

are described here: *Graptostethus servus*, flea beetles, variegated ladybirds, brown lacewings and wasps. For the other two, (minute two-spotted ladybirds and cotton seed bugs) the numbers were very small, and the patterns of interaction did not primarily involve treated rows. These were considered to be chance events.

For *G. servus*, only four insects were collected in the entire experiment, but all of these were on Magnet® treated rows. A Kruskal-Wallis test indicated that this result was not statistically significant, but the pattern was similar to that recorded in the daytime sampling experiment (Experiment 1). For flea beetles, the pattern was similar to those found in both the two previous experiments. Significantly lower numbers were found on treated rows than on most untreated rows. This confirms the strong repellent effect of Magnet® for this species, whether sampled during the day or night. For brown lacewings, this experiment provided further evidence for a repellent effect at 30h with significantly fewer found on the treated row than on rows 10 and 20. At 78h, no brown lacewings were found on the treated rows whereas there were a few on the untreated rows, but the numbers were too low to show statistical significance. For wasps there was a suggestion of a weak repellent effect at 30h, but not at 78h. For variegated ladybirds there was no evidence of attraction or repellence at 30h. However, at 78h there were significantly more of these ladybirds on the treated row and row 5 than on rows 10 and 20 (Fig. 30).

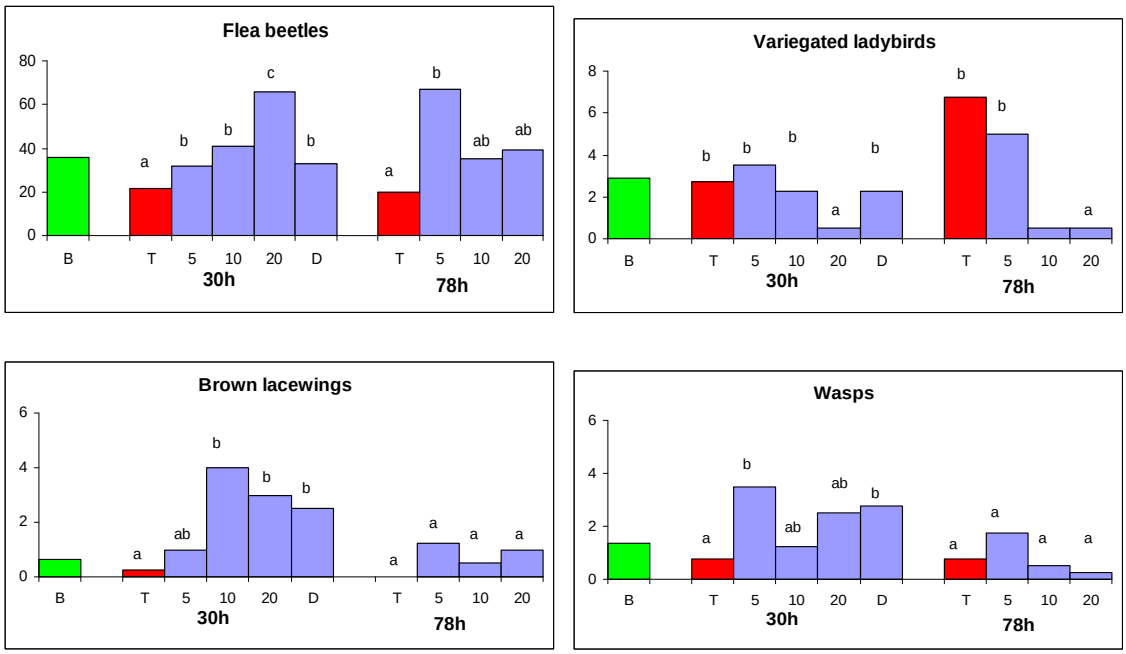

Fig. 30. Mean numbers of four taxa for which the main effect and/or the interaction involving treatment in Experiment 3 were significant. B= baseline sampling (54h before Magnet® application), T=treated rows, 5, 10 and 15 = rows 5, 10 and 15 away from the treated row, D=distant control (other end of the field). Numbers below each set of five bars are hours after Magnet® application. Bars with the same letter indicate rows which not significantly different, within each sampling interval.

4.3 Summary

A summary of the taxa which were affected (attracted or repelled) by Magnet® in these experiments is presented in Table 4. There were three taxa attracted to

Magnet®, and five taxa repelled by it. In addition there were 15 taxa (spiders, green jassids, brown jassids, planthoppers, big-eyed bugs, pirate bugs, Rutherglen bugs, green mirids, apple dimpling bugs, brown smudge bugs, thrips, weevils, flower beetles, other flies, green lacewings) for which numbers should have been adequate to detect an effect had one existed, but no such effect could be found.

Taxon	Time affected	Strength of effect
Attracted		
Lepidoptera (other than *Helicoverpa*)	Night, after 6h Night, after 78h	Strong Moderate
Variegated ladybirds *Graptostethus servus*	Day and night, from 18 to 85h	Strong
Repelled		
Flea beetles	Day and night, from 6 to 85 h	Strong
Red and blue beetles		Weak
Transverse ladybirds	Day and night, from 6 to 42h	Weak
Brown lacewings	Day, from 18 to 85h	Moderate
Wasps	Night, from 6 to 30h Night, 30h	Weak

Table 4. A summary of the taxa found to be affected (attracted or repelled) by Magnet®.

There were six taxa (damsel bugs, cotton seed bugs, broken back bugs, minute two-spotted ladybird, three-banded ladybird, predatory shield bugs) for which the numbers were too small to detect any effect, and one for which a possible effect was not readily interpretable (mosquitoes).

In summary, these experiments revealed little to suggest that the use of new formulation Magnet® will seriously impact on non-target species, including beneficial insects, in cotton. Only three non-target groups were attracted to it, and two of these were exotic species of no conservation significance. The third group was non-target Lepidoptera, for which previous studies (on old Magnet) have indicated no conservation concerns. Only one beneficial species, the exotic variegated ladybird *Hippodamia variegata*, was attracted to it. That attraction was weak, and other evidence suggests that for ladybirds the behavioural effects of Magnet® and one of its constituent volatiles, phenaylacetaldehyde, are complex and may well include repellence at certain times or in certain concentrations. In contrast, there was evidence that new Magnet® was repellent to four native beneficial groups (red and blue beetles, transverse ladybirds, brown lacewings and wasps), so impacts on these species are likely to be even more limited than for the many species for which no attraction or repellence could be demonstrated. Conclusions from experiments such as these are limited to the taxa that are present in sufficient numbers at the time of the experiments, and there were several taxa that were either so uncommon that no conclusions could be drawn, or altogether absent.

Objective 5: Develop formulations with improved slow-release and rain fastness

5.1 Slow release formulations for mating disruption using mirid pheromone

Five types of carriers mixed with mirid pheromone were evaluated for slow release of the pheromone for mating disruption. These were Magnet® base, Sirene®, Bond®

sticker, microcrystalline wax and wax/oil mixture. Magnet® base is the carrier for the attract-and-kill product without the volatile components. Sirene® is a matrix consisting of antioxidants and UV protectants used in attract-and-kill with pheromones of orchard pests like codling moth in North America, which we sourced from Philip Kirsch (IPM Technologies, USA). Bond® sticker is a latex-based adjuvant and sticker (Bond®, Nufarm Aust. Limited, Laverton, Victoria, Australia). Microcrystalline wax (Techniwax 20003) was sourced from H & R GSP Pty Ltd, Vic. Such wax has been used as a slow-release matrix for moth pheromones (SPLAT™, ISCA Technologies Ltd, http://www.iscatech.com/exec/index.htm). Canola oil was used in the wax/oil mixture. Pheromone formulations were manually sprayed on the leaves of potted cotton plans in the glasshouse. Samples of these formulations were collected from 0 hr (application time) and at regular intervals, dissolved in hexane and analysed in the GC-MS to determine levels of the major component, hexyl hexanoate. Results are shown in Figure 31.

Hexyl hexanoate was almost gone after 24 hours with the Magnet® base and wax. Less than 50% of it remained after 48hrs with Sirene®, Bond® sticker and wax/oil carriers. These results indicate that while some of these carriers were significantly effective in slowing down the release of the pheromone, none gave slow-release effects likely to be sufficient for commercial use.

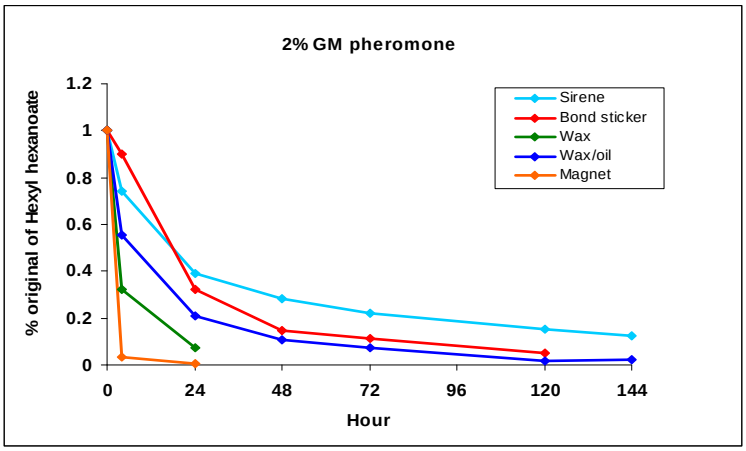

Fig. 31. Weathering of hexyl hexanoate using various carriers.

5.2 Slow release of mirid pheromone from lures in traps for monitoring

Weathering studies were conducted to determine pheromone dissipation over time. Pheromone volatiles were collected by solid phase micro extraction (SPME) technique and lures were weathered over time using the apparatus illustrated in Figure 32b, and at specified intervals volatiles were collected from lures transferred to the apparatus illustrated in Figure 32a for 10 min periods. Filtered and humidified air passed through both systems at a rate of 5L/min through an air compressor connected to the outside of the building.

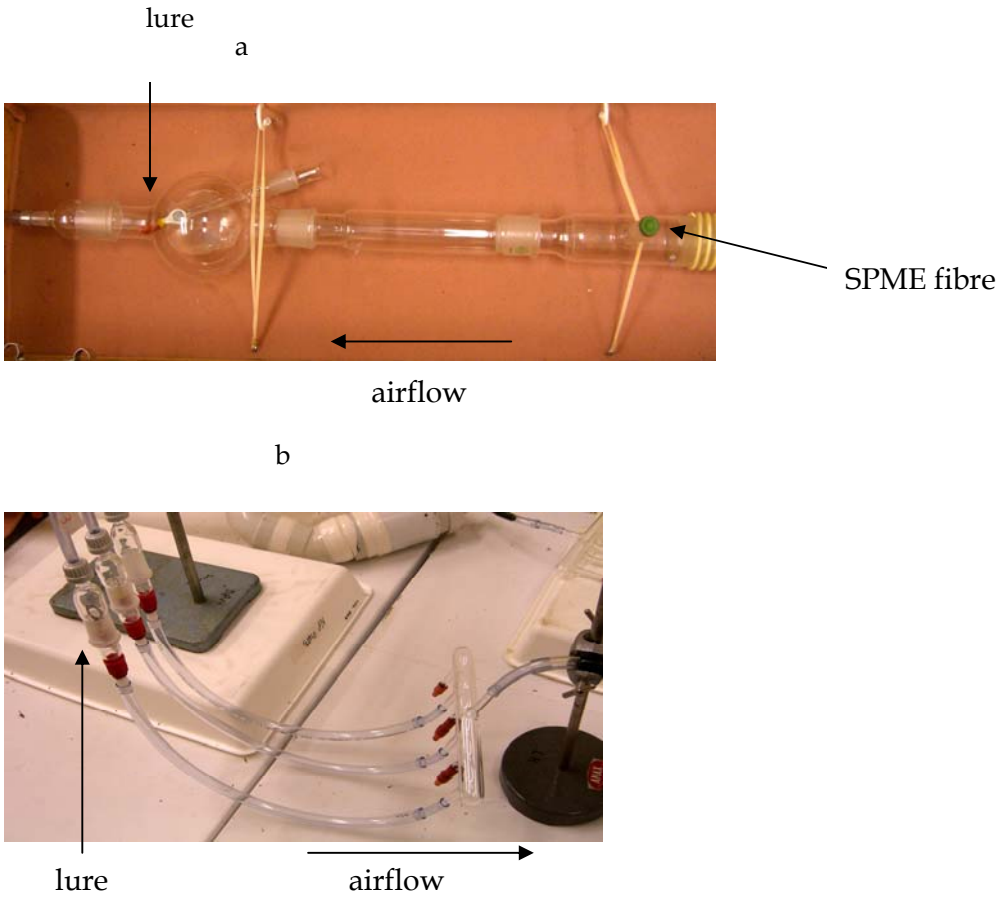

Fig. 32. Volatile collection (a) and weathering (b) apparatus for studying mirid pheromone loss from lures.

Pheromone volatiles from rubber septa were easily lost. The amount of hexyl hexanoate was less than 40% after the 3rd day, and was nearly gone in 2 weeks (Fig. 33).

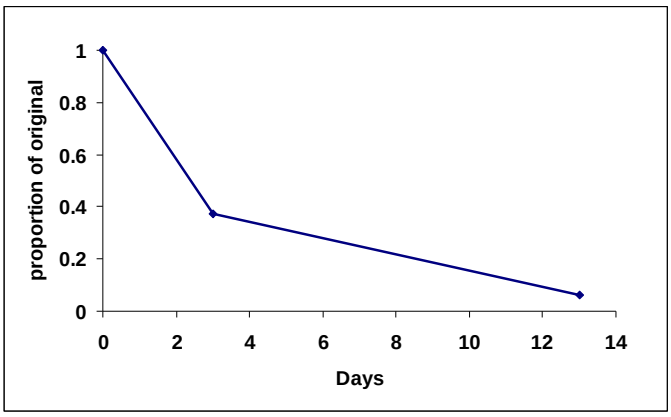

Fig. 33. Weathering of hexyl hexanoate in hexane.

We investigated the feasibility of coating the rubber septa to slow down pheromone release. The procedure for araldite coating was easier and more convenient than

resin coating. The tip of the rubber septum was left uncoated to ensure continuous slow release whilst the coated part served as a 'reservoir' of the pheromone (Fig. 34).

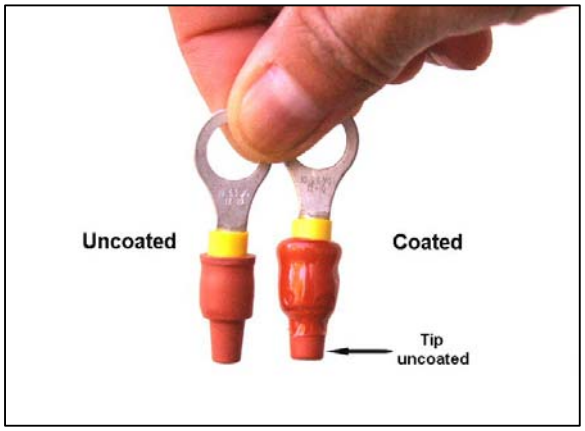

Fig. 34. GM pheromone lures coated with araldite glue (right) and uncoated (left).

A comparison of the release rate of resin-coated, araldite (glue)-coated and uncoated lures is shown in Figure 35. Pheromone dissipation in septa with the 24-h araldite glue and resin coating was similar, and as the former was easier, it was adopted for field trials.

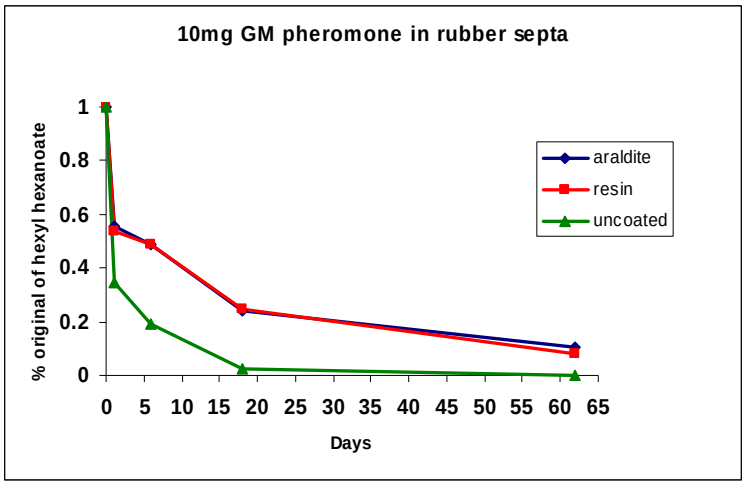

Fig. 35. Weathering of uncoated GM pheromone lures, and lures coated with araldite and resin.

A small trapping trial was conducted at ACRI in 2006-2007 season to test the longevity of coated pheromone lures in the field. Six AgriSense traps were set up on Bollgard cotton and were cleared twice a week. Field sampling for mirids by beat sheet method was also done when traps were cleared. Results showed that coating the lures improved the longevity of the lures in the field (Fig. 36). Coated lures (solid blue line) continued to catch mirids for a much longer period than the uncoated one (red line). At day 30, the uncoated lures were replaced with newly coated ones (dashed lines), and numbers of mirids caught with these lures were similar with those caught in the older coated lures.

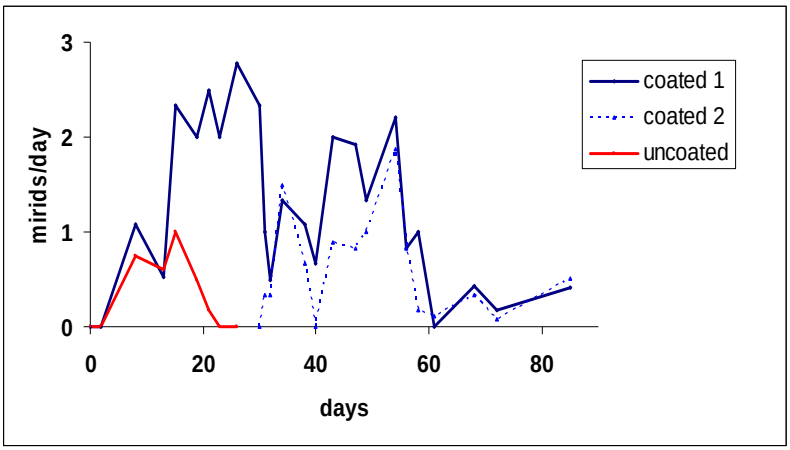

Fig.36. Mirid numbers with coated and uncoated lures in pheromone traps. Coated 1 is the original lure unchanged since Day 1, and Coated 2 is a fresh coated lure applied to half the traps on Day 30

Figure 37 shows pheromone trap catches vs field samples collected by beat sheeting. Although the numbers were low, the results suggest that pheromone traps might be a more sensitive method to monitor mirid numbers compared with the current sampling method.

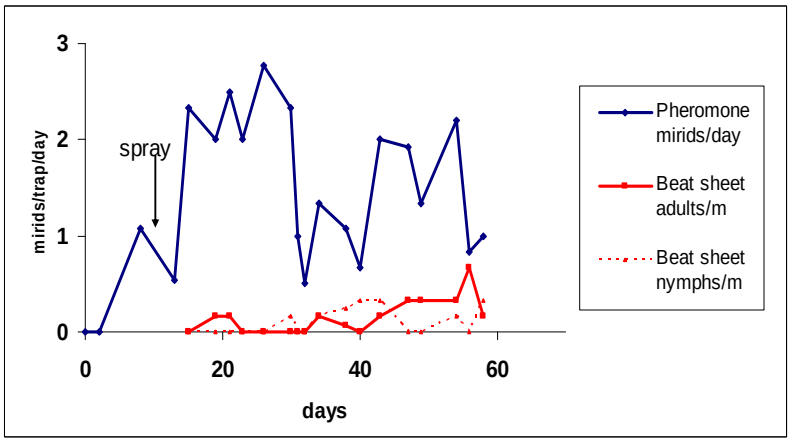

Fig. 37. Pheromone trap catches vs field mirid numbers sampled by beat sheet method.

5.3 Magnet® formulated in microcrystalline wax

A field trial on faba beans was conducted at "Carbucky", Goondiwindi to determine whether Magnet® volatiles formulated in emulsified microcrystalline wax would attract and kill *Helicoverpa* moths. The trials were carried out in conditions of low night time temperatures. Minimum temperatures were around 5^{o}C, and most activity by *Helicoverpa* moths had ceased within an hour of sunset. Moths were, however, very active in the daytime, and large numbers were present. Flight, oviposition and responses to pheromone traps and to plant volatile attractants were all observed throughout the daylight hours. This behaviour is unusual in cotton crops, but has been observed in other situations where night temperatures are low

(Coombs 1992, Gregg *et al.* unpublished). It is presumably a compensatory response to the night being unavailable for activity due to low temperature.

Methods

A field of about 100 ha of faba beans, in full flower, was used. Small-scale experiments involving treatment of 50m strips of row were conducted. Strips were laid out in a line along two edges of the field. This was because the beans were lower and the rows had less trash on the edges than in the middle of the field, so dead moths would be easier to find. Strips (50m) of the 4th row in from the road were treated alternately with the two formulations, with 50 m buffer strips between. There were 4 replicates of each treatment. Pheromone traps, one for each *Helicoverpa* species, were placed in the northwest and southwest corners of the field. High numbers of *Helicoverpa* spp. were present during the experiment, as indicated by pheromone trap catches and general activity. The dominant species was *H. punctigera.*

The conventional (oil) Magnet® was supplied by AgBiotech. The emulsified wax formulation was made by emulsifying 15% microcrystalline wax (supplied by UNE's Chemistry Department) in water containing 40% sugar, with 2.2% kemotan as the emulsifying agent and 0.5% BHT as an antioxidant. Sunflower oil at 4% was also included to make the mixture less friable when it dried. These proportions were based on information in Atterholt *et al.* (1998, 1999). Old Magnet volatiles were added to a total volatile concentration of 3.2% and blue food dye (Queen Fine Foods) was added at 2.2%, as in our earlier lab formulations of Magnet®. The insecticide for all treatments was methomyl at 0.5% a.i., supplied as Electra 225®. Since we were only intending to compare treatments rather than assess total moth kills, we counted only the furrows adjacent to the treated rows. The first collection was made early in the morning after treatment, but it soon became apparent that most of the moths were being killed during the day, so subsequent counts were done later in the morning, or in the afternoon.

Results

The results for the Magnet® in oil (O) vs Magnet® in wax (W) are given in Table 5. There were two unusually low replicates, W1 and O2 (one in each treatment). There were 14/108 (13.0%) *H. armigera* in the wax treatment and 19/94 (20.2%) in the oil treatment, and this difference was not statistically significant. The main purpose of the experiment was to determine if *Helicoverpa* would feed on the wax formulation and be killed. If so, emulsified waxes offer potentially slower release and more rain-fastness than the current oil-based formulation. This experiment indicated that moths do feed on the wax formulation, and are killed at a rate comparable to the current formulation. The wax formulation did not liquify to the extent the current formulation did, following dew, but it softened to the extent that it was probably ingestible.

Replicate	4/9 am	5/9pm	6/9am	Total
W1	5	0	1	6
O1	13	4	5	22
W2	4	0	13	17
O2	1	3	4	8
W3	3	7	12	22
O3	7	9	14	30
W4	1	38	24	63
O4	13	15	6	34
Mean Wax	3.3 (0.9)	11.3(9.1)	12.5(4.7)	27.0 (12.5)
Mean Oil	8.5 (2.9)	7.8 (2.8)	7.3 (2.3)	23.5 (5.8)
Pheromone traps	127.5, 15.5	117,46.5	-	

Table 5. Total *Helicoverpa* counts in the wax and oil formulations of Magnet. Figures in brackets after the means are standard errors of the means. Pheromone trap catches are given for the day, with *H. punctigera* first then *H. armigera.*

5.4 Improving rain fastness of Magnet®

Magnet® is not rain fast. Unlike conventional insecticide formulations, which are mostly oil-based, Magnet® is based on an oil-in-water emulsion which has to be liquid (or at least semi-liquid) for moths to ingest it. It also needs to be present as relatively discrete "puddles", rather than thinly spread over the foliage like a conventional insecticide. These considerations mean that it will always be a challenge to develop genuinely rain fast Magnet® formulations. However, it may be possible to improve rain fastness by adding certain materials to the formulation.

Magnet® generally dries after a few hours exposure to sun or low humidity on the foliage. It then liquefies, at least partially, during the night when humidity rises and the hygroscopic sugar it contains absorbs water. In its dry state it is slightly more rain fast than when wet. However, even in this state any more than a few mm of moderately intense rain will remove most of it from the foliage. Even very heavy dew, to the extent where water runs freely from the foliage during the night, will smear the material across the foliage and reduce its effectiveness. These conditions are rare in most Australian cotton areas, but were encountered during our trials of Magnet® in Georgia, USA (see Section 6.2). This report describes trials that investigated the effects of six candidate additives on rain fastness of new Magnet®.

Methods

Trial site

The trials were conducted on the property of Mulgowie Farms, about 25 km west of Home Hill in northern Queensland, in June 2008. The plant was corn in the vegetative stage, approximately 60 cm high. "Rainfall" was supplied by an overhead centre pivot irrigation system with a 400 m radius (Fig. 38) which was set to provide 6 mm for every pass over the target plants. Since the area covered by the pivot was greater towards the outside and the irrigator therefore travelled faster near the

outside than near the centre pivot, the intensity of the "rain" could be varied by placing the trial at different distances away from the centre pivot. Also, conditions were very humid within the crop, and heavy fogs occurred during the night which resulted in heavy dews on the foliages. This provided the opportunity to test the effects of the additives in providing resistance to smearing in these conditions.

Formulations

New Magnet® was supplied by Ag Biotech. No insecticide was included. The six candidate additives were:

1. PCT 13
PCT 13 is an experimental non-ionic polyacrylamide product from Ciba Specialty Chemicals (Basel, Switzerland). 2g was soaked for 30 min in 5 ml of methylated spirits, evaporated on a stirring hotplate and then dissolved in 10 ml of warm water on a stirring hotplate. The solution was then added to 150 g Magnet. The final concentration was 1.3% w/w. It was not possible to get higher concentrations because the Magnet® turned to gel.

2. Magnafloc 156
Magnafloc 156 (Ciba Specialty Chemicals) is an anionic polymer used for flocculation in water treatment. 0.6g was soaked in 5ml of methylated spirits, evaporated on a stirring hotplate and then dissolved in 10 ml of warm water, on a stirring hotplate and the final solution was added to 150 g Magnet. The final concentration was 0.4% w/w. It was not possible to get higher concentrations because the Magnet® turned to gel.

3. Magnafloc 351
Magnafloc 351 (Ciba Specialty Chemicals) is a cationic polymer used for flocculation in water treatment. 0.3g was soaked with 5 ml of methylated spirit for 30 min, evaporated on a stirring hotplate then dissolved in 10 ml of warm water, on a stirring hotplate. The final concentration was 0.2%. It was not possible to get higher concentrations. The Magnet did not turn to gel, but it became very viscous and sticky if higher amounts were used.

4. Silicone sealer in limonene
5g of silicone sealer, from a hardware store, was dissolved in 25g of limonene. 15g of this mixture was added to 150g of Magnet® (10%w/w).

5. Aquatain
Aquatain **(**Ultimate Products (Aust) Pty Ltd, 522 Princes Highway, Noble Park Vic) is a silicone-oil based product designed to reduce evaporation from farm dams and swimmimg pools. We used the swimming pool product, bought from a local pool supply store. 7.5g direct from the bottle was mixed with 150g of Magnet® (5%w/w)

6. Bond latex-based sticker
Bond (Nufarm Ltd, 103-105 Pipe Road, Laverton North, Victoria) is a latex-based sticker designed to stick pesticides, especially herbicides, to foliage. It contains 450g/l of synthetic latex and 100g/l surfactant. 7.5g was added directly from the bottle to 150g Magnet® (5% w/w).

A control consisting of Magnet® with no additives was provided for each trial.

Trial design

Three replicates of each treatment were established, marked by flags of different colors (Fig. 39). Around each flag, approximately 20 ml of each formulation was applied by shaking from a "pop-top" plastic bottle, over approximately one meter of corn. Before and after the passage of the irrigator, the treatments were scored subjectively on three criteria: the quantity of Magnet® remaining, the liquidity of the Magnet®, and the integrity of the Magnet® (ie whether it had remained in puddles, or had been smeared over the foliage). Each criterion was scored 0-5 on the following basis (Table 6).

Score	Quantity	Liquidity	Integrity
0	None present	NA	NA
1	<10% of original present	Completely dry	Completely smeared
2	10-30% of original present	Mostly dry (tacky)	Mostly smeared
3	30-60% of original present	Slightly dry	Slightly smeared
4	60-90% of original present	As wet as control*	As discrete as control*
5	90-100% of original present	More dilute than control*	More discrete than control*

Table 6. Description of the criteria and scoring for evaluating rain fastness. * for liquidity and integrity, the comparisons were with the control (Magnet® alone) treatment immediately after application. Rainfall often increased the dilution, and some additives increased the integrity by increasing the stickiness and tendency of the material to puddle when first applied. Therefore it was necessary to have scores which were higher than the control (at application) scores, for these criteria.

Fig. 38 Centre pivot supplying rain"

Fig. 39 Trial sites marked by flags

Results

Trial 1 - Heavy rain soon after application

This trial was located 270 m from the centre pivot, and the 6 mm of rain fell at a rate of 1 mm per minute (equivalent to an intense thunderstorm). The formulations were

applied at approximately 1500 h, and the irrigator passed over the trial at 1615 h. The formulations were still quite wet when the rain was applied. This combination of heavy rain soon after application represents the most demanding test for rain fastness. The results were scored about 1 hour after the passage of the irrigator.

Scores prior to the rain are given in Figure 40, and after the rain in Figure 41.

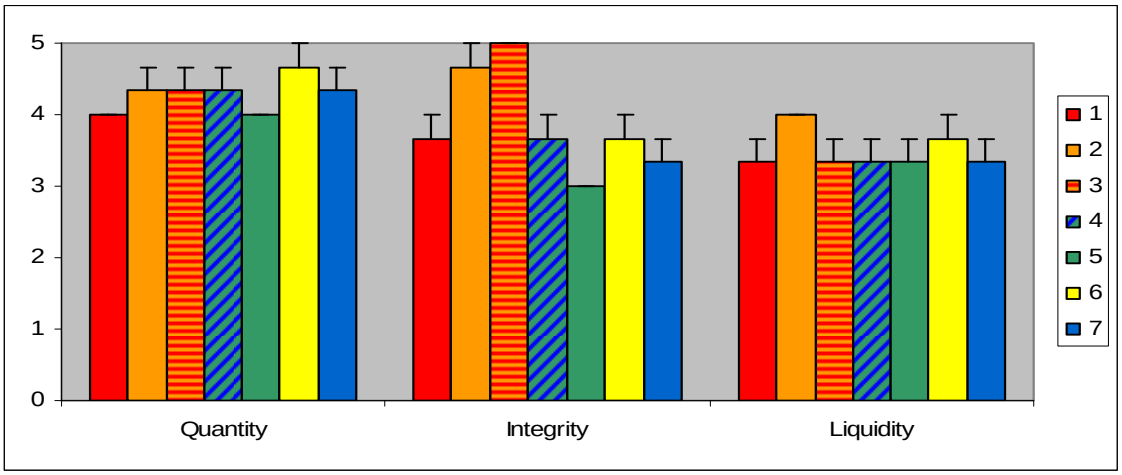

Fig. 40. Trial 1, prior to rain. 1= PCT 13, 2= Magnafloc 156, 3= Magnafloc 351, 4=silicone/limonene, 5=Aquatain, 6=Bond sticker, 7=control. Bars are standard errors of the mean; where no bar is present, all three reps had the same score.

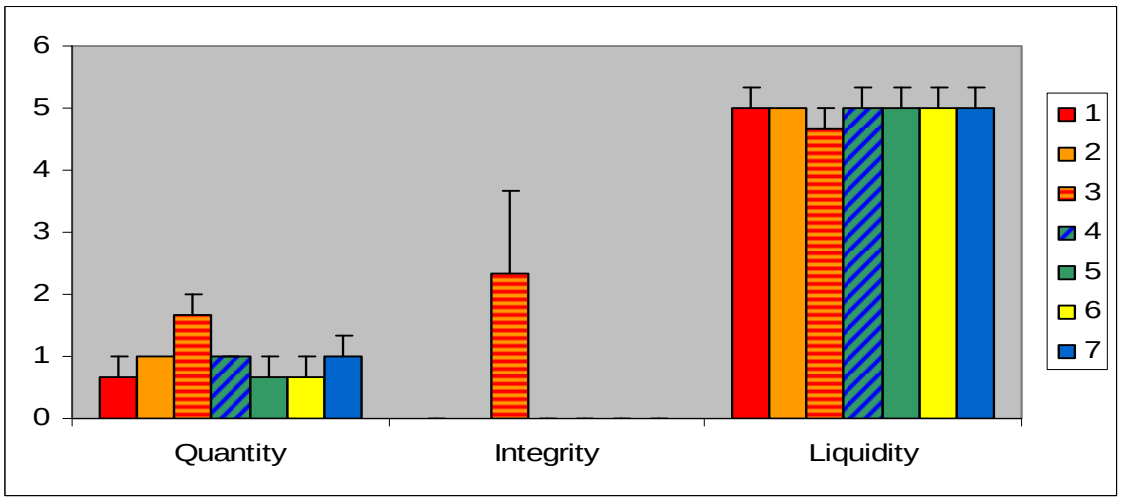

Fig. 41. Trial 1, after rain. 1= PCT 13, 2= Magnafloc 156, 3= Magnafloc 351, 4=silicone/limonene, 5=Aquatain, 6=Bond sticker, 7=control. Bars are standard errors of the mean; where no bar is present, all three reps had the same score.

Prior to the rain, all the formulations had only slightly dried (scores 3-4), and the material was still almost all present (scores 4-5). There were no significant differences between the treatments in these criteria. However there were significant differences in regard to integrity ($p=0.002$). The two Magnafloc additives made the formulations very sticky and stringy. They tended to remain on the plant in very discrete pools or lines. The integrity scores for these formulations were therefore significantly ($P<0.05$ by Fisher's least significant differences test) higher than all the other formulations. The lowest integrity score was for the Aquatain formulation, but it was not significantly different from the control.

After the rain, all formulations performed poorly. Very little material remained, and most of that was present in very dilute form in the whorls of the leaves, where is had been washed from the leaf surface. The only exception was for the Magnafloc 351 treatment, where in some replicates a small quantity remained on the leaf surface. However the differences were not statistically significant (p=0.134). As a result of dilution, liquidity scores were higher (ie more dilute) than the for the original application, and integrity scores were zero except for the Magnafloc 351 treatment. In this treatment there was considerable replicate variation, with one replicate was scored as 4, another as 3, and the third as 0. Consequently the standard error was high, and the effect was not quite statistically significant (p=0.06).

The conclusion from this experiment was that none of the additives would provide much protection from the extreme situation of heavy rain falling soon after application. A small degree of protection might be provided by Magnafloc 351.

Trial 2. Moderate rain applied after 6h drying time

This experiment was located 50 m from the centre pivot, and the 6 mm of rain fell at the rate of 0.2 mm/minute. The formulations were applied at approximately 1000 h, and the irrigator passed over the trial 6.5 h later, at 1630 h. Pre-rainfall scores were made 30 min prior to the passage of the irrigator, and post-rainfall scores were made within 30 min afterwards.

Pre-rainfall scores are shown in Figure 42, and post-rain scores in Figure 43.

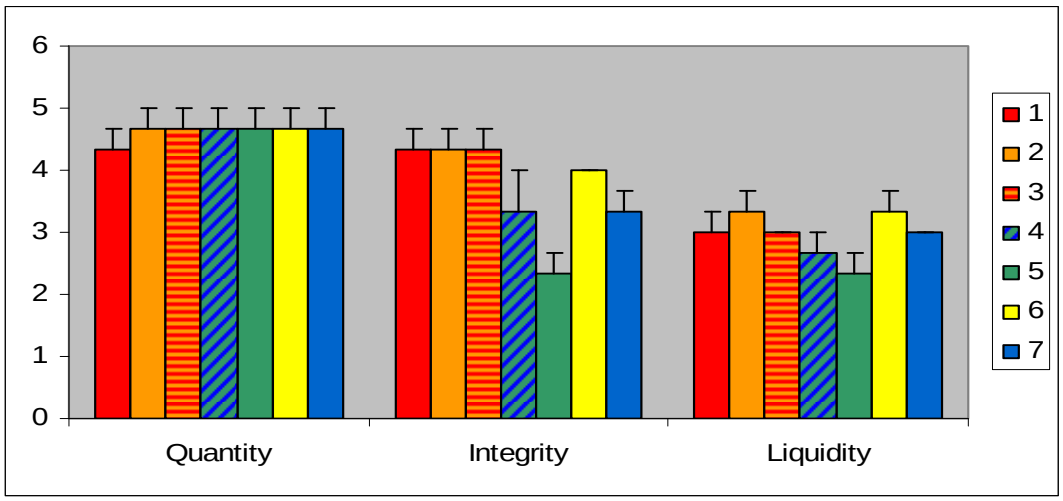

Fig. 42. Trial 2, prior to rain. 1= PCT 13, 2= Magnafloc 156, 3= Magnafloc 351, 4=silicone/limonene, 5=Aquatain, 6=Bond sticker, 7=control. Bars are standard errors of the mean; where no bar is present, all three reps had the same score.

Prior to the rain, there were no significant differences in quantity and liquidity between the treatments. Almost all the material was still present in all treatments, and most replicates of all treatments were scored in the 2-3 range (slightly dry to tacky). However there were significant differences in integrity. The treatments which retained most integrity were the two Magnafloc treatments (as in Trial 1), but also PCT 13 and Bond sticker. These treatments however were not significantly different from the control. They were significantly different ($p<0.05$) from the

Aquatain treatment, which as in Trial 1 showed the lowest integrity scores. However the Aquatain was not significantly lower than the control.

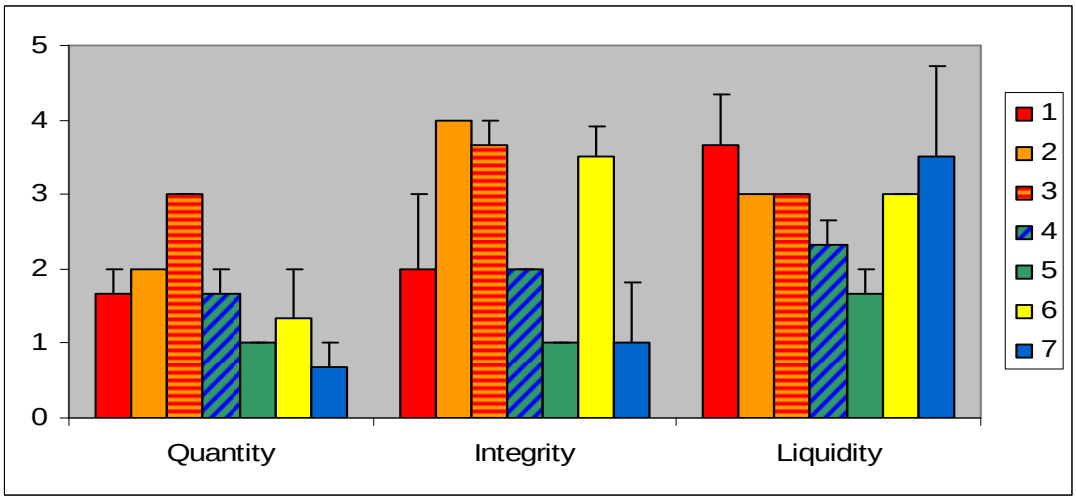

Fig. 43. Trial 2, after rain. 1= PCT 13, 2= Magnafloc 156, 3= Magnafloc 351, 4=silicone/limonene, 5=Aquatain, 6=Bond sticker, 7=control. Bars are standard errors of the mean; where no bar is present, all three reps had the same score.

After the rain, there were significant differences in scores for quantity ($p=0.005$) and integrity ($p=0.006$), but not for liquidity ($p=0.164$). The highest quantity and integrity scores were recorded for the two Magnafloc treatments, both of which were significantly different ($p<0.05$) from the control for both criteria. No other treatments were significantly different from the control for quantity. However the Bond treatment also had significantly higher scores for integrity than did the control. As in Trial 1, the Aquatain treatment performed poorly in regard to both quantity and integrity, being very smeared across the foliage. However it was the driest of the treatments, possibly because the smeared material resisted dilution by the rain.

Trial 3 - Heavy dew

This trial arose from an attempt to leave the formulations to dry for at least 24 h before rain. Heavy dew affected the formulations to the extent that this attempt had to be abandoned. However the trial provided insights into the effects of dew and the ability of the additives to mitigate them.

The formulations were applied at about 1500 h. On inspection at about 0700 h the next morning, a very heavy fog (visibility < 20 m) was encountered. The corn foliage had been saturated, with water running freely from the leaves. No scores were recorded on the afternoon prior to the dew. Scores recorded the next morning at 1030 h are illustrated in Figure 44. There were no significant differences in quantity ($p=0.652$) or integrity ($p=0.463$). The dew had substantially smeared mot of the treatments, with the scores for integrity being mostly 1 and 2. However there was considerable variation between replications, with some replicates of the PCT 13, silicone and Bond treatments scoring 3 or 4. For liquidity there were significant differences ($P=0.024$), with PCT13, Magnafloc 156, silicone and Bond tending to prevent the drying out that occurred with the other treatments (including the control) from 7 am until 1030 h when the experiment was scored. Aquatain again performed poorly on all three criteria.

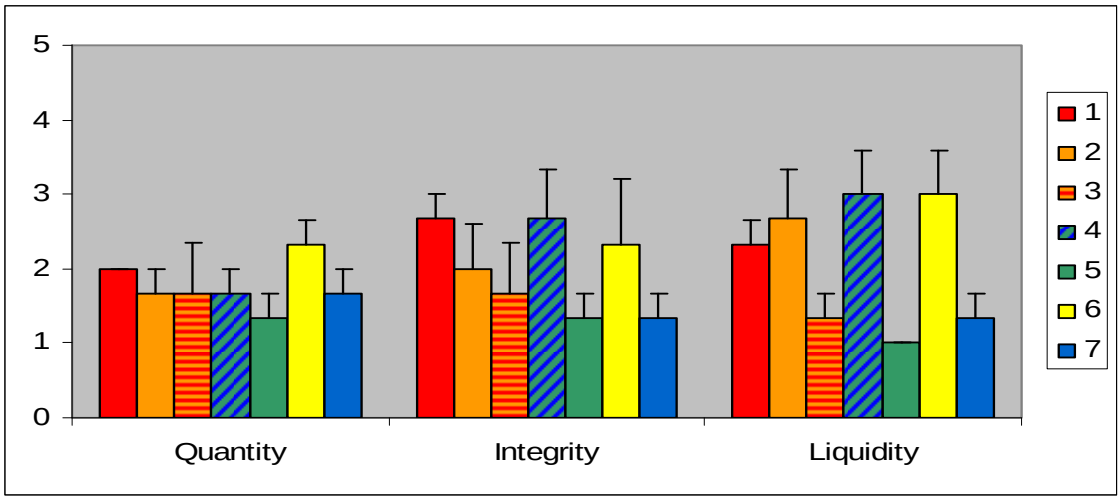

Fig. 44. Trial 3, after heavy dew. 1= PCT 13, 2= Magnafloc 156, 3= Magnafloc 351, 4=silicone/limonene, 5=Aquatain, 6=Bond sticker, 7=control. Bars are standard errors of the mean; where no bar is present, all three reps had the same score.

Trial 4 - Moderate dew

This trial was conducted over the night following Trial 3. Formulations were again applied at about 1500 h. However the night was less humid, with some wind, and at 0700 h the next morning when the trial was scored, only patchy fog was present. The leaves were quite wet, but there had not been extensive run-off from them, unlike the previous experiment. Scores are shown in Figure 45.

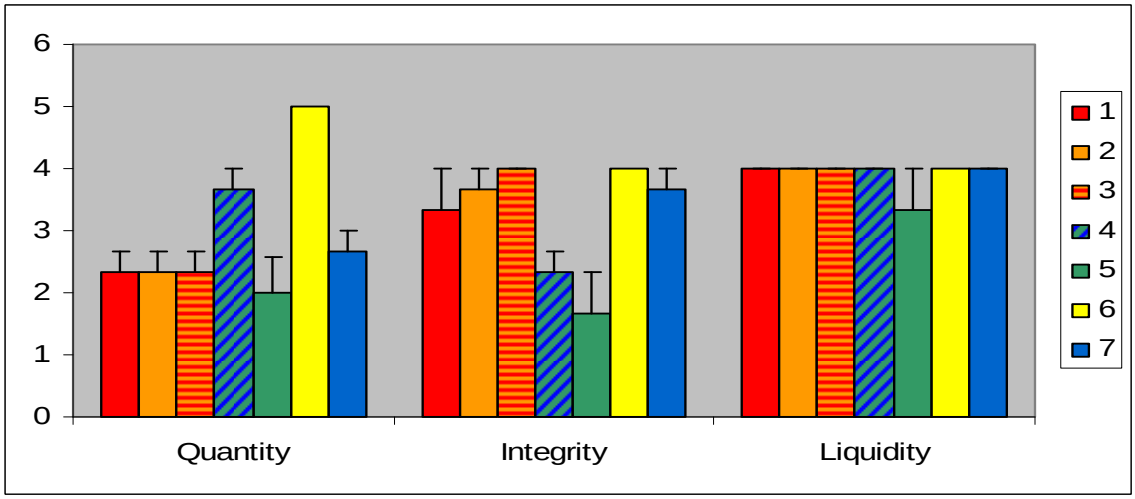

Fig. 45. Trial 3, after moderate dew. 1= PCT 13, 2= Magnafloc 156, 3= Magnafloc 351, 4=silicone/limonene, 5=Aquatain, 6=Bond sticker, 7=control. Bars are standard errors of the mean; where no bar is present, all three reps had the same score.

There were significant differences for quantity ($p<0.001$), integrity ($p=0.009$) but not for liquidity ($p=0.463$). All formulations were about as liquid as when originally applied. In regard to quantity, the silicone and (especially) the Bond treatments had the most material remaining. However, in the silicone treatment the material had become significantly more smeared (lower integrity score) than the control or PCT 13, Magnafloc 156, Magnafloc 351 and Bond treatments. By contrast the Bond treatment retained integrity. The deposits were however noticeably whiter than for

the control, PCT 13 or both Magnafloc treatments. This suggested that the blue dye had been leached from the deposits by the water in the dew. Whether other components, especially the volatiles, had also been lost is not clear. We attempted to determine by smell whether the volatiles were still present, but could not detect any difference between the deposits from the Bond treatment and any other treatment. All smelled weaker than when applied the previous night.

Conclusions

All of these experiments were done under difficult conditions, when the Magnet® never had a chance to fully dry out either because the rain was applied before it could do so, or because high overnight humidity prevented it from happening. Under these conditions, none of the treatments gave much protection from heavy rain. However, the Magnafloc treatments, particularly Magnafloc 351, appeared to give some protection from moderate rain. This warrants further investigation, especially since, because of the high night-time humidity, we were not able to simulate a situation where the formulations had been allowed to dry for 24 h or more before exposure to rain. However, a potential disadvantage of Magnafloc 351 is that it makes the formulation very sticky and stringy. This may cause difficulties with application, eg blocked nozzles.

In regard to dew, Bond sticker warrants further investigation for potential protection against smearing. However, work is needed to determine whether volatile components, sugar and insecticides are being leached from the deposits in the same way that the water-soluble blue dye appears to be. This would involve GC-MS analyses for the volatiles. The same type of work is needed for potential rain fastness additives such as Magnafloc 351. There is no point in retaining deposits on the foliage if key ingredients have been leached out.

The combination of Magnafloc 351 and Bond sticker, perhaps in smaller quantities than we used in this experiment, also warrants investigation. Since they seem to protect against different kinds of exposure to water, combination of the two may give better overall protection, and reducing the amount of each may avoid difficulties with blocking nozzles.

Silicone formulations, whether of silicone sealer in limonene or the silicone oil in Aquatain, seem to be deleterious. They appear to reduce surface tension and lead to more smearing than occurs with normal Magnet® formulations.

Pictures illustrating the effects of various additives are shown in Figure 46.

Control Magnet drops on plants, pre-rain

Magnafloc 351 drops on plants, pre-rain
(note well-defined but stringy appearance)

Silicone/limonene treatment after heavy dew
Note extreme smearing

Bond sticker treatment after moderate dew. Note integrity, but white colour suggests dye leaching

Magnafloc 351 treatment after light rain – the best treatment providing some rain fastness

Fig. 46 Illustrations of the effects of additives on rain fastness of Magnet®

Objective 6: Identify IP and commercial opportunites

6.1 Patent for new Magnet® blend

The necessity to change components in the Magnet® blend has had significant implications for our IP strategy. A new application for registration of the revised formulation of Magnet® has been submitted to APVMA in 2006 and we are currently awaiting its approval. At the time of writing we expect the application for registration to be gazetted for public comment (meaning it has passed all APVMA requirements) on 4 November 2008.

The requirement for re-formulating Magnet® has also meant that we have had to abandon our patent for the original Magnet® blend. This patent had a hierarchy of claims from very broad ones (which would have protected the new blend as well as the old one) to more specific claims for particular blends. National phase patent examinations in several countries indicated that only the narrow claims were likely to be accepted, and those claims had been rendered irrelevant by the APVMA decision. It was therefore necessary to file a new provisional patent application and this has been done (Australian Patent Application P78841.AU, *Insect Attractant Composition*, inventors P.C. Gregg, A.P. del Socorro and A. J.Hawes, filed in the name of the Cotton CRC on 1 October 2008).

6.2 Magnet® trials in the US

These trials were conducted to determine the effectiveness of Magnet® on American heliothine moths. There are two species in the USA, *Helicoverpa zea* which closely resembles *H. armigera* both morphologically and ecologically, and *Heliothis virescens* which is somewhat different to *H. punctigera*. These two species dominate the New World heliothine complex, and if Magnet® was ever to be useful in north or south America it would have to attract them. The trials also included some of the early attempts to find substitutes for problematic volatile in regard to Australian registration.

The aims of the field trials were: (1) to test the attractiveness of Magnet against American heliothine moths and other noctuid pests, and (2) to compare standard Magnet with variant formulations in which Z-3 hexenyl salicylate was substituted by another volatile (benzyl alcohol, Z-3 hexenyl benzoate, ethyl salicylate, 2-methoxybenzyl alcohol) and in which benzyl alcohol was added to the standard Magnet® formulation. The trials were done in July 2005, in collaboration with Dr. John Ruberson from the Dept. of Entomology, University of Georgia (UGA). Trial sites were at Chula, on private land, Plains, the University of Georgia Experimental Station at Plains, and the UGA's CM Stripling Irrigation Research Park.

All experiments were conducted in squaring to early flowering cotton (Bollgard). Cotton fields in this region were not irrigated (except at Stripling). They were usually small and irregularly shaped, which meant we were forced to use replicates

of only 30m of treated row rather than the 50m we would normally use. There were four replicates per treatment. We searched the three rows adjacent to the treated row, on either side, and identified all moths to species where possible. All *H. zea* and *H. virescens* moths were dissected to determine sex and mated status. Otherwise, methods were the same as we would use in Australia for small-scale Magnet® trials.

The weather was hot and very humid. Heavy dew every night has meant that both our trials only yielded useful data on the first day because of the smearing effects of dew described in Section 5.4. There were also many scavengers, notably fire ants, which made collecting and identifying dead moths difficult.

Chula

Two experiments were conducted at Chula, with the following treatments:

Experiment 1: Red – Magnet (standard); Orange – MagnetB (benzyl alcohol substituted for Z-3 hexenyl salicylate); Yellow – MagnetHB (Z-3 hexenyl benzoate substituted for Z-3 hexenyl salicylate)

Experiment 2: Red – Magnet (standard); Orange – MagnetBA (standard Magnet + benzyl alcohol); Yellow – ModTexas (modified Texas blend); Green – Control (no volatiles)

Experiment 1

Numbers of moths in various groups are shown in Figure 47 for the first day after treatment, and Figure 48 for the second day.

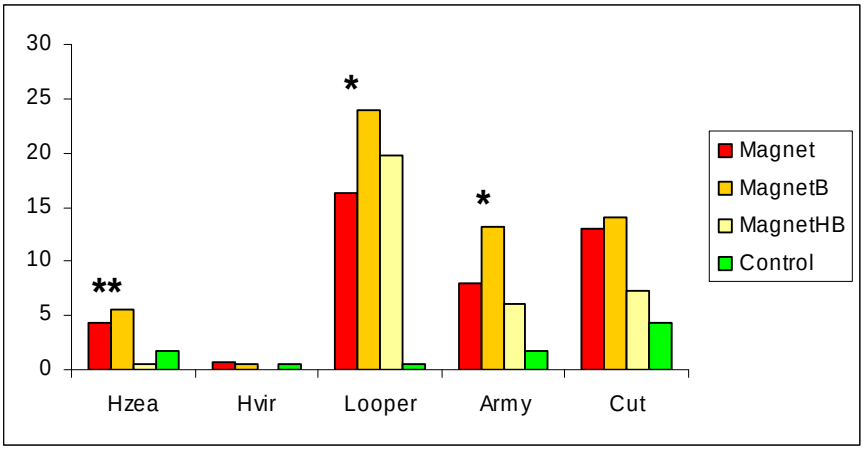

Fig. 47. Numbers of heliothine moths, loopers, armyworms and cutworms at Chula, Day 1, Experiment 1. Stars indicate statistical significance level of oneway AOV's with log-transformed data.

On the first day, Magnet was significantly more attractive to corn earworm, *Helicoverpa zea*, than the control (no volatile) treatment. The difference was about threefold, which is about what we would expect for *H. armigera* in Australia. Numbers of *Heliothis virescens* were too small to make any comparison. There were large numbers of loopers present, and they were strongly attracted to Magnet. There was also significant attraction of armyworms to Magnet. Although there were

higher cutworm numbers in the Magnet treatment than the control, the difference was not statistically significant – there was much variation between replicates.

For all moth groups, the numbers were higher for MagnetB than for Magnet. However, Magnet HB had lower numbers than Magnet. Again, high levels of variation between replicates made it impossible to show that these differences were statistically significant. However, the overall impression was that MagnetB was at least as good as the standard Magnet.

On day 2, the numbers of moths collected were much smaller, and there were no significant differences between treatments, though there was a tendency for the three Magnet treatments top have slightly more loopers, armyworms and cutworms than the control, no-volatile treatment (Fig. 48).

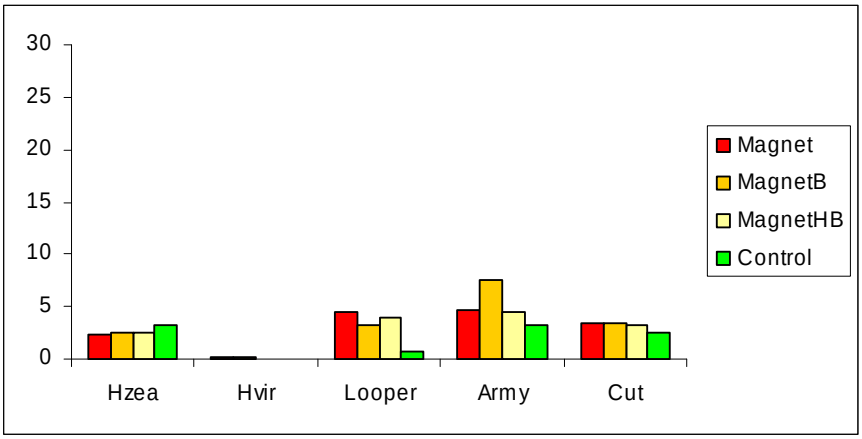

Fig. 48. Numbers of heliothine moths, loopers, armyworms and cutworms, Chula, Day 2, Expt 1.

The treatments on Day 2 were in very poor condition due to smearing. Extremely heavy dew had smeared most of it thinly across the leaves, and diluted or washed off the remainder. No smell was detectable.

Other noctuids

Numbers of other noctuid species (loopers, armyworms and cutworms) in the first day are shown in Figure 49. The most common loopers were the non-pest species, *Agrapha oxyygramma* (sharp stigma looper) but there were also significant numbers of the pest species, *Trichoplusia ni* (cabbage looper). Other looper species included *Pseudoplusia includens* (soybean looper) but the numbers were low. There were several species of armyworms present, including *Spodoptera frugiperda* (fall armyworm), *S. ornithogalli* (yellow-striped armyworm) and *S. dolichos.* Of these species, only *S. frugiperda* showed significant attraction to Magnet, although numbers were low. Other armyworm species included *S. exigua* (beet armyworm), a few *Leucania* spp. and *Pseudaletia unipuncta* (true armyworm). We did not identify the cutworms to species, but many were *A. ipsilon* (greasy cutworm).

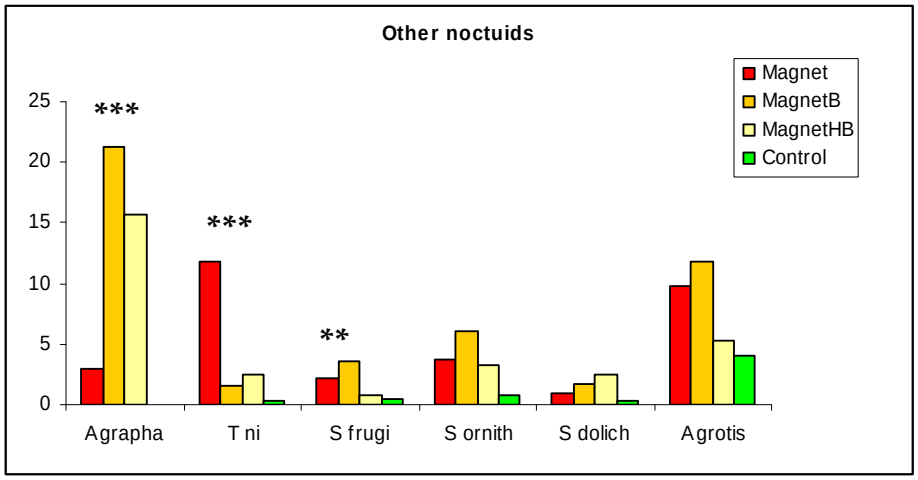

Fig. 49. Numbers of other noctuids, Day 1, Chula, Experiment 1. Stars indicate statistical significance level of oneway AOV's with log-transformed data.

% H. zea females

The percentages of *H. zea* females for the different treatments (data pooled over 2 days) were 82, 80, 50 and 60%, respectively for Magnet, MagnetB, MagnetHB and the control. Of the very few *H. virescens* killed in all the treatments, 40% were females.

Experiment 2

In experiment 1, Magnet B (benzyl alcohol substituted for Z-3 hexenyl salicylate) was as good as the standard Magnet. So, in this experiment, we added benzyl alcohol to the standard Magnet to see if this volatile enhanced attractiveness. We also included a modified Texas (ModTex) blend (ie, *Gaura*-based blend patented by Lopez *et al* and described in Shaver *et al* 1998, which is a potential competitor for Magnet®) for comparison with Magnet.

There were more moths killed in experiment 2 than experiment 1 at Chula. Numbers of heliothine moths and other noctuids in the different treatments are shown in Figure 50. Magnet, Magnet BA as well as ModTexas caught significant numbers of *H. zea* compared with the control. Again, the numbers of *H. virescens* were very low. There were also significantly higher numbers of loopers and cutworms in the three blends than those in the control. For armyworms, numbers in the two Magnet treatments were significantly higher than ModTexas and the control. As in experiment 1, moth numbers in the second day were much lower (Fig. 51). Significant differences between the blends and control were observed only in the loopers.

Other noctuids

As in experiment 1, loopers were the most common other noctuids, which included *A. oxygramma, T. ni* and *P. includens* (Fig. 52). All three volatile treatments were significantly different from the control for both *A. oxygramma* and *T. ni*. The three common armyworm species were *S. ornithogalli, S. dolichos* and *S. frugiperda*. Magnet and MagnetBA killed significantly higher numbers of *S. ornithogalli* than ModTexas

and the control. Numbers of *S. dolichos* killed with Magnet BA were significantly higher than those with the other two blends and the control.

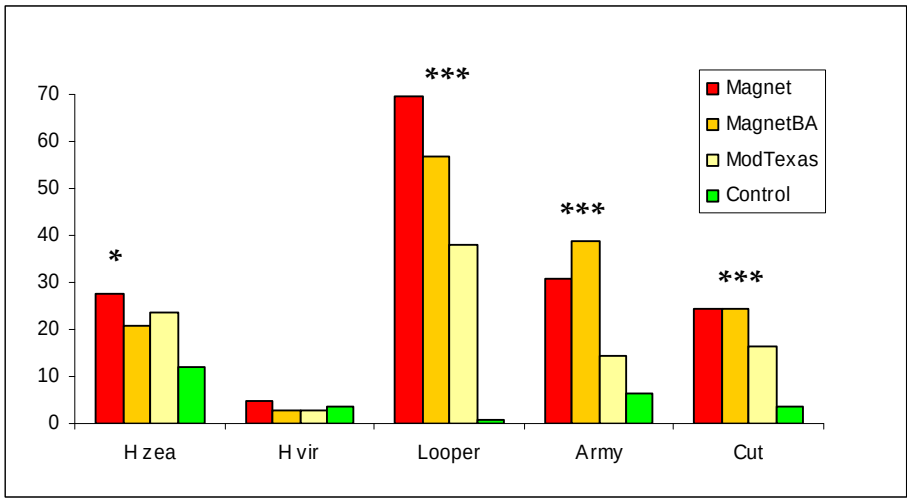

Fig. 50. Numbers of heliothine moths and other noctuids, Day 1, Chula, Experiment 2. Stars indicate statistical significance level of oneway AOV's with log-transformed data.

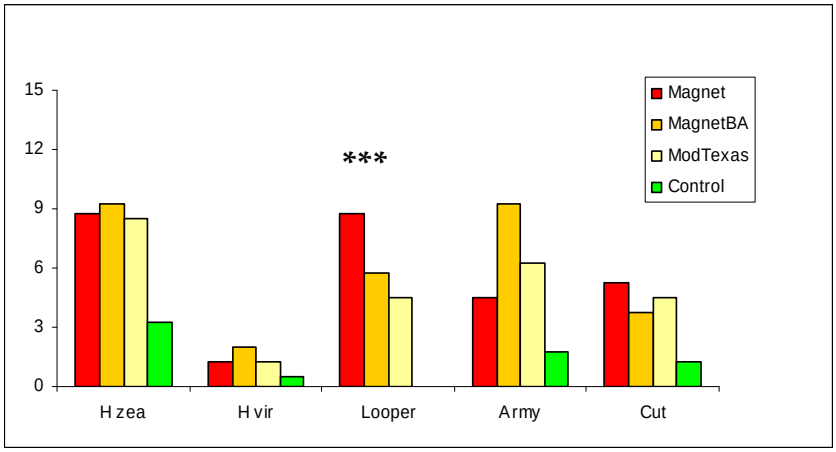

Fig. 51. Numbers of heliothine moths and other noctuids, Day 2, Chula, Experiment 2. Stars indicate statistical significance levels of oneway AOV's with log-transformed data.

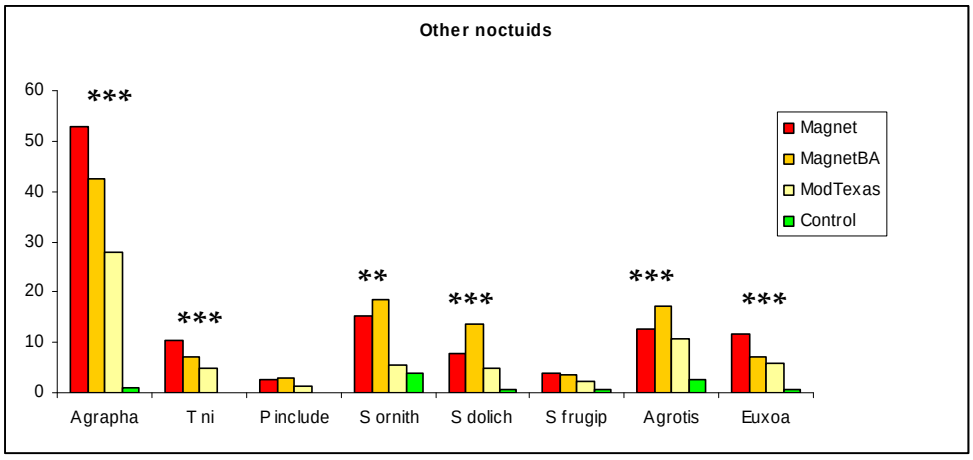

Fig. 52. Numbers of other noctuid species at Chula, Day 1, Experiment 2. Stars indicate statistical significance levels of oneway AOV's with log-transformed data.

% Heliothine females

The percentages of females in the heliothine moths are shown in Table 7. Data were pooled for the two days.

	Magnet	MagnetBA	ModTexas	Control
H. zea	54.6	58.7	54.9	52.5
H. virescens	60	44.4	60	47.4

Table 7. Percentages of *H. zea* and *H. virescens* females, Chula, Experiment 2.

Plains

There were 3 treatments: Red – standard Magnet; Orange – MagnetB (benzyl alcohol substituted for Z-3 hexenyl salicylate); Green – no volatiles (control). Results on day 1 at Plains are shown in Figure 53.

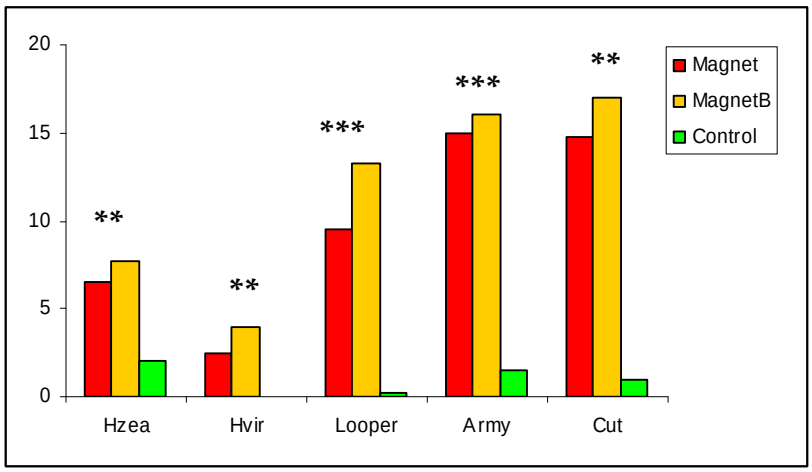

Fig. 53. Numbers of heliothine moths, loopers, armyworms and cutworms, Plains, Day 1. Stars indicate statistical significance levels of oneway AOV's with log-transformed data.

There were more heliothines at Plains than at Chula, experiment 1, especially *Heliothis virescens*. We found highly significant differences between treatments for all major moth groups, with both standard Magnet and MagnetB having more than the no-volatile control. Trends for loopers, armyworms and cutworms were also similar to those in Chula. Results from Day 2 were similar in experiment 1 at Chula, ie much lower numbers and few differences between the treatments (Fig. 54). The condition of the Magnet deteriorated on the second day at Plains, as it did at Chula.

Other noctuids

Other noctuid species killed in significant numbers in the volatile-treated rows were the looper, *A. oxygramma*, the armyworm, *S. ornithogalli*, and the cutworms, *Agrotis* and *Euxoa* spp. (Fig. 55).

Fig. 54. Numbers of heliothine moths, loopers, armyworms and cutworms, Plains, Day 2.

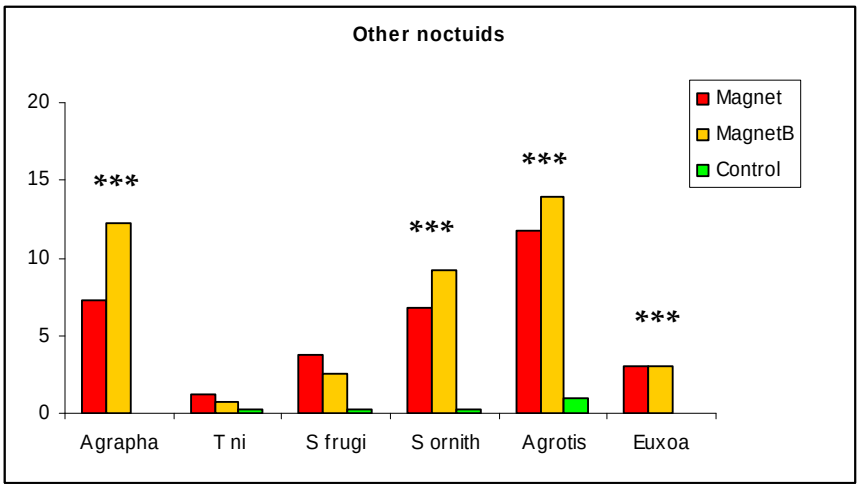

Fig. 55. Numbers of other noctuid species, Plains, Day 1. Stars indicate statistical significance levels of one-way AOV's with log-transformed data.

% Heliothine females

The percentages of heliothine females in the three treatments are shown in Table 8. Data were pooled for the two days.

	Magnet	MagnetB	Control
H. zea	69	61	67
H. virescens	33	35	(0 killed)

Table 8. Percentages of *H. zea* and *H. virescens* females at Plains.

Stripling

The treatments were: Red – standard Magnet; Orange – MagnetE (ethyl salicylate substituted for Z-3 hexenyl salicylate); Yellow – MagnetMBA (2-methoxybenzyl alcohol substituted for Z-3 hexenyl salicylate); Green – no volatile (control).

In this experiment, only 3 rows on both sides were searched due to the intervening rows being too trashy with stubbles which made it difficult to search for moths. Moth numbers were low, and there were no significant differences between volatile and no-volatile treatments, except in the cutworms (Fig. 56).

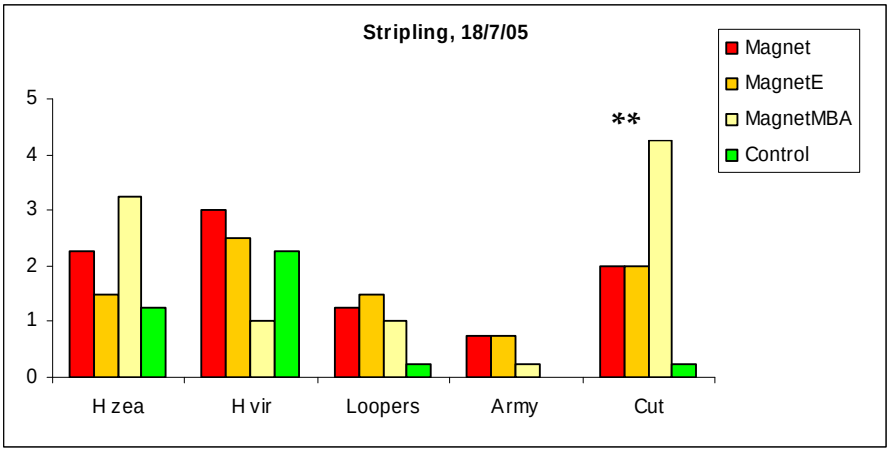

Fig. 56. Numbers of helitohine moths and other noctuids, Stripling. Stars indicate statistically significant levels of one-way AOV's with log-transformed data.

Conclusions

1. In contrast to what we experience in Australia where Magnet® works for 4-6 days, we only had useful data from the first day of our trials due to the hot and humid conditions as well as heavy dew at night in Tifton.

2. Magnet® worked for the American heliothines, *Helicoverpa zea* and *Heliothis virescens*, as well as other looper, armyworm and cutworm species. The most common species killed were the loopers, *Agrapha oxygramma* and *Trichoplusia ni,* the armyworms, *Spodoptera ornithogalli, S. dolichos* and *S. frugiperda*, and the cutworms, *Agrotis* spp.

3. MagnetB (benzyl alcohol substituted for Z-3 hexenyl salicylate) appeared to be as attractive as the standard Magnet. One consideration though in using benzyl alcohol is that it is more volatile than Z-3 hexenyl salicylate. Residue studies in the glasshouse indicated that it is gone after 48hrs.

4. The addition of benzyl alcohol to standard Magnet did not significantly increase the attractiveness of the latter.

5. Small-scale field trials using other Z-hexenyl substitutes (eg, ethyl salicylate, 2-methoxybenzyl alcohol, etc) when moths are more abundant should be repeated to obtain more conclusive data.

6. Of the heliothine moths killed at Chula and Plains, females ranged from 50-82% for *H. zea* and 33-60% for *H. virescens*.

6.3 Magnet® trials in New Zealand

Magnet® field trials in New Zealand were initiated following discussions with Graham Walker, Crop and Food Research, Auckland, after his attendance at the combined conference of the Australian and New Zealand Entomological Societies in Adelaide, September 2006. The main objective was to determine the effectiveness of Magnet® on pests of New Zealand crops, especially horticultural crops. These pests included *Helicoverpa armigera*, the recently introduced oriental soybean looper *Thysanoplusia orichalcea* and *Phthorimaea operculella* (potato tuber moth).

The trials were conducted near Pukekohe, about 50 km south of Auckland, in March 2008. The first trial was established at the Pukekohe Research Station on corn planted between experimental plots of brassica crops, onions and potatoes. The second site, on the property of Allen Fong, was established when it became clear, after two nights work on the Research Station site, that the numbers of moths being killed were low. It was felt that a site on a commercial lettuce farm might provide more moths, especially of *T. orichalcea*. The site consisted of a partially harvested lettuce field, about 2 ha in area. In the harvested area, which occupied the centre of the field, many lettuce plants which had been rejected at harvest were still standing. There were also many lettuce leaves on the ground. The Magnet® was applied to these substrates. At each site, four replicates of each of two treatments, new Magnet® and blank, was used. Both formulations had methomyl 0.5% a.i. (as Lannate®) included and the application rate was 300 ml per 30m applied using plastic water bottles with "pop-tops". Dead moths were collected early in the morning for 4 days, by searching within 2m of the treated rows. They were then identified to species where possible, and dissected to determine the presence of spermatophores in females, and blue dye (indicating ingestion of the formulations) in both sexes.

The cumulative numbers of moths collected after 4 days during the Research Station trial and the cumulative mean numbers for each day are shown for *H. armigera* and *T. orichalcea* are shown in Figure 57.

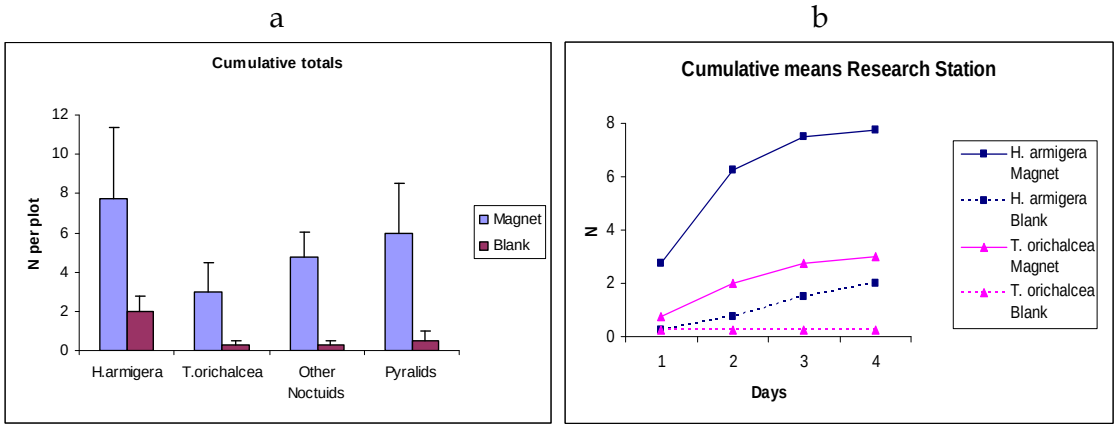

Fig. 57. Total cumulative numbers of various moth types (a) and daily cumulative numbers of *H. armigera* and *T. orichalcea* in the Research Station trial (b).

The mean number of *H. armigera* moths collected in the 30m Magnet® replicates was 7.25 ± 3.61, while for the blank replicates it was 2.0 ± 1.41. The difference was not significant (p=0.19) due to the high standard errors, which reflected a positional effect in that replicates near the southwest (closest to a windbreak of trees) had very low numbers of moths. However the relative difference (about 4 x) was very similar to that which we expect for Australian *H. armigera*. Pooling all replicate x day data points in a single analysis (giving 31 instead of 7 degrees of freedom) gave a significant difference between the Magnet® and blank treatments (p=0.038).

For *T. orichalcea*, the mean cumulative number in Magnet® replicates was 3.0 ± 1.47, and for the blank it was 0.25± 0.25 (ie only one *T. orichalcea* was ever found in the blank replicates), resulting in a 12 x difference. However the difference was not statistically significant (p=0.115), again because a positional effect similar to that found for *H. armigera* resulted in high standard errors. As with *H. armigera*, when all the day x replicate data were pooled, a significant difference was found (P=0.008)

For other noctuids (mainly *Agrotis ipsilon*, greasy cutworm, and *Mythimna separata*, oriental armyworm) a statistically significant difference (4.75 ± 1.32 vs 0.25 ± 0.25, P =0.015) was recorded. *A ipsilon* is among the species known to be attracted to Magnet® from Australian work, and *M. separata* is closely related to the Australian species *M. convecta*, which is also attracted by Magnet®. For pyralid moths (species not recorded, but no significant pests). 6.0 ± 2.5 moths were found in the Magnet® treatment and 0.5 ± 0.5 in the blank treatment. Again there was a strong positional effect, but it was opposite to that found for *H. armigera* and *T. orichalcea*, ie more moths were found near the windbreak. No potato tuber moths were found in either the control or the blank treatments, even though large numbers were collected in the pheromone traps and the moths could readily be seen flying about the remnant potatoes in the trial site.

For *H. armigera*, approximately 55% of the moths killed in the Magnet® treatment were females, and most of them had mated at least once. This is consistent with experience in Australia, though the proportion of mated females often varies considerably in space and time. Similarly, slightly more than half of the *T. orichalcea* killed in Magnet® treatments were female. For both species, the proportions of males and females, and the mated status of females, was not significantly different between Magnet® and blank treatments using χ^2 tests, mostly reflecting the low numbers in the blank treatments.

The cumulative numbers of moths collected after 3 days during the Fong experiment, and the cumulative mean numbers for each day are shown for *H. armigera* and *T. orichalcea* are shown in Figure 58.

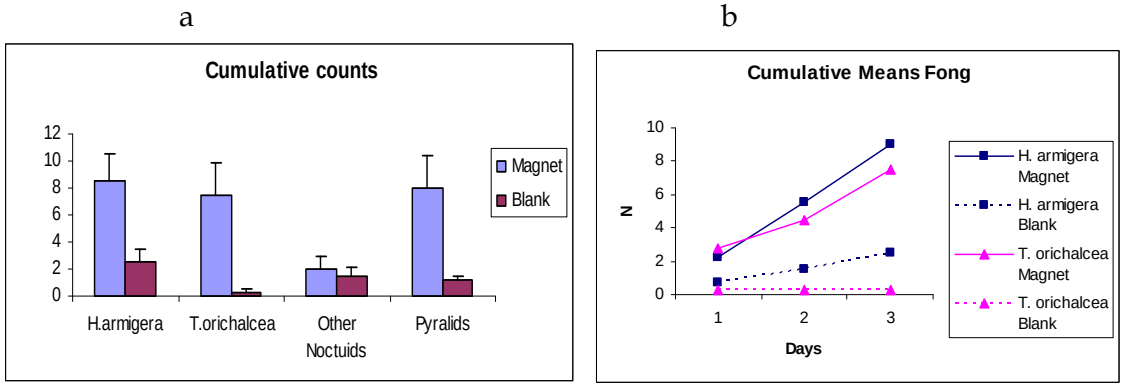

Fig. 58. Total cumulative numbers of various moth types (a) and daily cumulative numbers of *H. armigera* and *T. orichalcea* in the Fong trial (b).

The Fong trial was less influenced by positional effects than the Research Station trial, and significant differences between Magnet® and blank treatments were found for the cumulative counts of *H. armigera* (8.5 + 2.02 vs 2.5 + 1.45, p=0.036, a difference of 3.4x) and *T. orichalcea* (7.5 + 2.38 vs 0.25 + 0.25, p= 0.024, a difference of 30x). A significant difference was also found for pyralids, but not for other noctuids (of which numbers were low).

The Fong trial was characterized by remarkably low numbers of male moths, compared to the Research Station trial. Over 75% of the *H. armigera* killed, and over 95% of the *T. orichalcea*, were females. Males were only found in the Magnet® treatment. Most females were mated, many more than once for the *H. armigera,* but only once for the *T. orichalcea*. For *H. armigera,* there was a significant difference between the Magnet® and the blank treatments ($\chi^2_{4} = 4.98$, $p < 0.05$), which was mostly due to the relative lack of males and virgin females in the control compared to the Magnet® treatment. There were no significant differences for *T. orichalcea*, due to very low numbers in the blank treatment.

These trials have clearly demonstrated the attractiveness of Magnet® and its volatile components to *H. armigera* and *T. orichalcea* in New Zealand. In the case of *H. armigera*, the results are very similar to those we typically obtain in small scale field trials in Australia: a difference of 3-4x in the number of moths killed in the Magnet® compared to the blank treatments, with a slight to moderate preponderance of females, and females of all types, from virgins through to multiply mated, being attracted. The strong positional effects are also typical of this species in small scale trials, and the period that the material remained attractive is typical.

For *T. orichalcea*, the difference between Magnet® and blank was even stronger (12-30x). This difference probably reflects low attractiveness of sugar for *T. orichalcea*. Sugar alone is known to be attractive to heliothine moths, but is less so for loopers.

The numbers of moths we killed (around 6-8 per 30 m Magnet® replicate, or 0.25/m, for both *H. armigera* and *T. orichalcea*) were substantially lower than we typically get in small scale trials against *H. armigera* in Australia. However, a number of factors should be considered:

- we searched only about 2m away from each row, and in Australian trials this has been shown to recover only about a third of moths actually killed, as the remainder can move some distance before the insecticide takes effect.
- searching conditions were poor because of much trash and lettuce foliage in the Fong site, and because of extensive weed growth away from the 1m cleared zone in the Research Station site.
- There was evidence that moth numbers were quite low. No moths could be flushed (using the dirt-throwing technique) from the vegetation around the Research Station site, and only about one moth per $100m^2$ at the Fong site. Only low numbers of moths could be seen flying in car headlights and by torchlight around sunset. Against this, there was significant oviposition by *T. orichalcea* on lettuce at the Fong site. And pheromone traps operated after the trial was finished produced substantial catches.
- Temperatures were relatively low. Daily maxima were around 25^oC, and minima were 8-10^oC. Temperatures around sunset were probably in the region of 15-20^oC. This may account for the lack of general moth activity. On such nights in Australia high kills with Magnet® would be unusual.

There is a suggestion in the data that for both sites we were dealing with residual populations without much recruitment, especially for *T. orichalcea*. In the Research Station trial, pheromone trap catches declined greatly after two days of the trial. This could be due to population reductions within the immediate area (few ha). While we recovered only 39 *H. armigera* and 13 *T. orichalcea* in total from this trial, these figures should be multiplied by at least three to give an estimate of the total kill, and the resulting figures may represent a significant proportion of the local population, given the small size of the site and the lack of moths in flush counts. In the Fong trial, the lack of males, especially for *T. orichalcea*, suggests an ageing female population remnant of a recent larger population (possibly reduced by the insecticides which presumably killed the old dead moths we found on the site).

These considerations suggest that it would be useful to conduct further trials when either or both of *H. armigera a*nd *T. orichalcea* are known to be present in large numbers (using criteria other than pheromone traps, which are known to be susceptible to female competition effects, and to attract males at lower temperatures than are required for general moth activity including feeding).

Magnet® showed no attraction to potato tuber moth. Although the potatoes had been removed from the experimental plots prior to our trial, some remnant plants had been left and PTM was still present on them. A 2 ha field of potatoes was located about 100m from the Research Station site. Although there were no potatoes close to the Fong site, PTM were still present, as indicated by the attraction of moths to the pheromone-contaminated Scentry® trap installed at the end of the experiment. Although PTM are small and would be hard to find once killed, they are no smaller than some of the pyralids we readily found. It seems likely that Magnet® has no attraction to this species, which is not be surprising since they are taxonomically quite distant from the noctuids for which Magnet® was developed.

6.4 Potential Southeast Asian markets

Work by AgBiotech in Taiwan and Thailand has demonstrated the attractiveness of Magnet® to the diamondback moth, hence, the potential for SE Asian market for the product. This work is commercial-in-confidence and will not be described here.

Objective 7: Publish and communicate results

It has been difficult to publish in refereed journals because of commercial—in-confidence considerations and IP protection. Apart from the Provisional patent referred to in Section 6.1, we have written numerous reports for Ag Biotech, many of which have been used in reports to APVMA for registration purposes. Research findings have been presented in various conferences/meetings locally and overseas, Australian Cotton Conference and in the annual Cotton CRC Science Reviews.

Gregg PC, Del Socorro AP, Hawes AJ & Grundy PR. 2005. Area-wide effects of a plant volatile-based attract and kill formulation against *Helicoverpa armigera* and *H. punctigera* in Australia. *Proceedings of the 21st Annual Meeting of the International Society of Chemical Ecology, Washington DC, USA, July 2005.*

Lowor ST, Del Socorro AP & Gregg PC. 2005. Sex pheromones of *Creontiades dilutus* (Stål) (Hemiptera: Miridae): identification and field tests. *Proceedings of the 21st Annual Meeting of the International Society of Chemical Ecology, Washington DC, USA, July 2005.*

Gregg P, Del Socorro A & Lowor S. 2006. Pheromones for mirids. *Proceeedings of the 13th Australian Cotton Conference, Broadbeach, Qld. 7-11 August.*

Del Socorro A, Gregg P & Lowor S. 2006. Semiochemicals for green mirids in Australia. *Proceedings of the 37th Australian Entomological Society Scientific Conference, Adelaide, South Australia, September 2006.*

Gregg PC, Del Socorro AP, Hawes A & Grundy P. 2006. Area-wide impacts of attract-and-kill formulations for noctuid moth pests based on plant volatiles. *Proceedings of the 37th Australian Entomological Society Scientific Conference, Adelaide, South Australia, September 2006.*

Gregg PC 2007. Biology, ecology and management of the green mirid, *Creontiades dilutus* (Stål) in Australia. Proceedings of the 2nd Lygus Symposium, Pacific Grove, CA, USA, April 2007. Published in Goodell, PB and Ellsworth, PC (2008) Second International Lygus Symposium, Asilomar. 27 pp, *Journal of Insect Science* 8:49, available on-line: insectsscience.org//8.49

Grundy P, Short,S., Hawes,A., Zalucki, M. & Gregg,P. (2006) Moth busting for Bt resistance management. *Proceedings of the 13th Australian Cotton Conference, Broadbeach, Qld. 7-11 August.*

Del Socorro A, Lowor S & Gregg PC. 2007. Sex pheromones of the green mirid, *Creontiades dilutus*. Proceedings of the 2nd Lygus Symposium, Pacific Grove, CA, USA, April 2007. Published in Goodell, PB and Ellsworth, PC (2008) Second International Lygus Symposium, Asilomar. 27 pp, *Journal of Insect Science* 8:49, available on-line: insectsscience.org//8.49

Del Socorro A & Gregg P. 2007. Responses to plant volatiles by the green mirid, *Creontiades dilutus. Proceedings of the 2nd Lygus Symposium, Pacific Grove, CA, USA, April 2007.*

Del Socorro A & Gregg P. 2007. Green mirid pheromone. *Mirid Review Meeting, Qld DPIF, Toowoomba, Qld. July 2007.*

Del Socorro A & Gregg P. 2008. Mirid pheromone update. *Proceedings of the Northern Farming Systems IPM Researchers Forum, Qld DPIF, Toowoomba, June 2008.*

Del Socorro A, Gregg P, Ruberson J & Hawes, A. 2008. Field trials of attract-and-kill (Magnet®) on American heliothine and other noctuid pests in cotton. *Proceedings of the XXIII International Congress of Entomology, Durban, South Africa, July 2008.*

Gregg, PC and Wilson, LJ (2008). The changing climate for entomology. *Proceedings of the 14th Australian Cotton Conference, Broadbeach, Qld. 11-14 August*

Del Socorro AP, Gregg PC, Alter D & Moore CJ. Bioassays of plants as potential sources of attractants for adults of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Manuscript submitted to *Australian Journal of Entomology.*

Objective 8: Revise Magnet® formulation for registration.

This objective was not part of our original application, but became necessary due to regulatory obstacles which emerged after the application was submitted. It distracted us from fully completing some of the objectives discussed above.

Our initial submission for registration of Magnet® in August 2004 was rejected because insufficient data were available on the toxicity of one component (Z)-3 hexenyl salicylate. We were faced with a choice of expensive and time-consuming work to generate toxicological data for this component, or re-formulating Magnet® by substituting components for which FEMA (Flavor and Extract Manufacturers Association; http://www.femaflavor.org/) data were available. We opted for the second approach but this required demonstrations that the efficacy of any new formulation be equivalent to the old Magnet. Hence, we ran over 20 trials testing various modifications of the Magnet® volatile mixtures. The trials in the USA (Section 6.2) provide early examples of some of these experiments. Much additional work was done on cotton, corn and beans, at Bowen and the Darling Downs in Queensland and Bellata, NSW. Results of two of these trials on cotton in the Darling Downs (OLE3 and NASS), testing what eventually became the new Magnet® formulation, (described as Magnet B4 in these trials) are discussed below.

8. 1 OLE3 field trial

Four blends including the old Magnet formulation were tested. Mean numbers of *H. armigera, H. punctigera* and other pest and non-pest species, including small lepidopterans collected for 3 days, per 30m row section, are given in Table 9. As the aim was to compare among blends, no control (no volatiles) treatment was included.

Data were tested for normality using the procedure in Minitab v14. Most of the moth counts differed significantly from a normal distribution (Ryan-Joiner test, $P < 0.05$. A $\log_{10}(x+1)$ transformation was applied, and this effectively normalized the data. Data on % male, %mated (for both species) and %target did not differ significantly from normal (Ryan-Joiner $p > 0.1$), so no transformation was applied.

Treatment	Total Ha	Total Hp	Other pests	Other nonpests	Small leps	% target
Magnet B4	161.8 (24.1)	42.0 (7.2)	30.8 (4.9)	3.5 (0.6)	21.5 (6.3)	79.0 (9.2)
PF3 + 4-meth BA	106.8 (16.2)	21.0 (3.2)	24.0 (3.8)	2.3 (0.5)	9.5 (2.2)	78.0 (2.6)
Old Magnet	121.0 (31.3)	21.8 (4.4)	19.8 (3.8)	3.0 (0.7)	15.0 (6.6)	79.3 (2.3)
PF3 + But Sal	181.5 (25.6)	50.8 (8.1)	34.5 (6.1)	4.0 (0.8)	21.5 (7.6)	80.0 (4.0)

Table 9. Means and standard errors (in brackets) for all treatments over all three days. % target figures are calculated as (*H. armigera* + *H. punctigera)*/total of all moths collected.

Generalised linear models were fitted to the data using the GLM procedure in Minitab v14. In all cases the data were orthogonal and balanced, so these analyses are equivalent to two-way AOV's. A summary of the GLMs is given in Table 10.

Source	Df	Tot Ha	Tot Hp	Other pests	Other non-pests	Small Leps	% target
Treatment	3	*	***	Ns	Ns	Ns	Ns
Date	2	***	***	**	Ns	*	***
Treatment x Date	6	ns	ns	Ns	Ns	Ns	Ns

Table 10. Summary of GLM analyses; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

There were significant differences between treatments in counts of *H. armigera* and *H. punctigera*, but not for other pests, non-pests and small lepidopterans. There were also significant differences between days for most moth types; these are generally due to lower kills on the second day than the first, then a resurgence in kills for the third day in the case of the two *Helicoverpa* species. For the other moths, kills generally declined steadily from day 1 through to day 3. This resulted in a highly significant increase in %target from days 1 to 3. The results suggested that most of the resident moths were killed in the first two days, and there was immigr.ation of *Helicoverpa* species (but not the other moths) on the third night. There were no significant interactions between treatments and days, for any moth type.

For total *H. armigera* and total *H. punctigera*, where the GLM analyses indicated significant effects of treatment, Fisher's pairwise comparison of means procedure

was applied to a one way AoV of the cumulative data, using Minitab. Results are shown in Table 11.

Variable	P	Order and significance
Total Ha	0.124	PF3+ButSal^{a}<MagnetB4^{a}<OldMagnet^{a}<PF3+4Meth^{a}
Total Hp	0.002	PF3+ButSal^{a}<MagnetB4^{a}<OldMagnet^{b}<PF3+4Meth^{b}

Table 11. Summary of Fisher's pairwise comparison tests. Treatments with different superscript letters are significantly different ($P < 0.05$)

For *H. armigera,* the significant effect of treatment in the GLM analysis was not repeated in the cumulative AoV. However, the effect was repeated in the case of *H. punctigera*, where the two best treatments were PF3+Butyl salicylate, and the Magnet B4 formulation. For this species the new Magnet B4 was significantly better than the old Magnet. The percentage of target moths was not significantly affected by treatment, varying from 78 to 80%. However, most of the non-target moths were other noctuid pest species.

8.2 NASS field trial

Two blends, the old and new Magnet® formulations (Magnet B4) and a new single volatile, butyl salicylate (ButSal) were compared with a blank (no volatile) treatment. Mean numbers of *H. armigera, H. punctigera* and other pest and non-pest species, including small lepidopterans collected for 3 days, per 30 m row section, are given in Table 12.

Treatment	Total Ha	Total Hp	Other pests	Other nonpests	Small leps	% target
Blank	28.8 (5.7)	0.3 (0.3)	0.3 (0.3)	0.5 (0.5)	0.5 (0.5)	96.8 (2.4)
ButSal	44.5 (12.4)	0.5 (0.5)	0.5 (0.5)	0.3 (0.3)	0.3 (0.3)	98.3 (1.0)
Old Magnet	63.3 (8.1)	0.3 (0.3)	1.5 (0.9)	4.8 (1.4)	12.3 (5.8)	79.0 (4.9)
Magnet B4	137.6 (23.2)	3.3 (1.3)	4.8 (0.5)	3.0 (0.4)	15.3 (2.6)	85.5 (1.7)

Table 12. Means and standard errors (in brackets) for all treatments over all three days. % target figures are calculated as (*H. armigera* + *H. punctigera)*/total of all moths collected.

Data were tested for normality using the procedure in Minitab v14. Most of the moth counts differed significantly from a normal distribution (Ryan-Joiner test, $P < 0.05$. A $\log_{10}(x+1)$ transformation was applied, and this effectively normalized the data. Data on *H armigera* % male, %mated and %target did not differ significantly from normal (Ryan-Joiner $p > 0.1$), so no transformation was applied.

Generalised linear models were fitted to the data using the GLM procedure in Minitab v14. In all but one case (% mated females, where one data point was absent due to lack of females), the data were orthogonal and balanced, so these analyses are equivalent to two-way AoV's. A summary of the GLMs is given in Table 13.

Source	Df	Total Ha	Total Hp	Other pests	Other non-pests	Small Leps	% target
Treatment	3	***	***	***	***	***	***
Date	2	***	***	Ns	*	**	*
Treatment x Date	6	*	*	Ns	Ns	*	Ns

Table 13. Summary of GLM analyses * P < 0.05, ** P < 0.01, *** P < 0.001

There were significant differences between treatments in counts of all moth types, and significant differences between days for most moth types; the latter were generally due to lower kills on the third day. A significant treatment x date interaction means that the relative effectiveness of treatments changed over time. For Ha, Hp and small leps, most of the significance arises because of a jump in the catches on the second day in the Magnet B4 treatment, which did not occur in the other treatments.

There were more non-target moths, especially small leps, in the old Magnet and Magnet B4 treatments. However, when expressed as a percentage of total catch, there were still significantly more such moths in these treatments. Hence the %target figures for those treatments are down. This suggests that there is attraction of *H. armigera* to sugar only, but this is not so for many other lepidopterans.

Where the GLM analyses indicated significant effects of treatment, Fisher's pairwise comparison of means procedure was applied to a oneway AOV of the cumulative data, using Minitab. Results are shown in Table 14.

Variable	P	Order and significance
Total Ha	< 0.001	Blank^{a}<ButSal^{ab}<Old Magnet^{b}<MagnetB4^{c}
Ha % mated	0.002	Blank^{a}<ButSal^{a}<Old Magnet^{ab}<MagnetB4^{b}
Total Hp	0.014	Old Magnet^{a}<Blank^{a}<ButSal^{a}<MagnetB4^{b}
Other pests	0.004	Blank^{a}<ButSal^{a}<Old Magnet^{a}<MagnetB4^{b}
Other non-pest	0.001	ButSal^{a}<Blank^{a}<Old Magnet^{b}<MagnetB4^{b}
Small leps	<0.001	ButSal^{a}<Blank^{a}<Old Magnet^{b}<MagnetB4^{b}
%target	0.002	Old Magnet^{a}<MagnetB4^{a}<Blank^{b}<ButSal^{b}

Table 14. Summary of Fisher's pairwise comparison tests. Treatments with different superscript letters are significantly different (p< 0.05).

Notable results from these comparisons include: (1) Magnet B4 was significantly more effective on *H. armigera* than old Magnet; (2) Magnet B4 got significantly more *H. punctigera* and other pests than did the blank or butyl salicylate. Old Magnet did not. *H. punctigera* was quite uncommon, though; (3) there is no evidence that butyl salicylate was different from the blank in any respect, though there was a trend for greater *Helicoverpa* kills with butyl salicylate; (4) for non-pest species and small leps, both old Magnet and Magnet B4 got significantly more than the blank, but did not differ significantly from each other; (5) for the percentage of target species, both old Magnet and Magnet B4 were significantly lower than the blank, but did not differ significantly from each other.

Outcomes

5. **Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.**

In the application we identified the likely outputs from the project as follows:

Output 1: A better Magnet®.

This objective morphed in "a Magnet® which will get through the regulatory hoops" because of the obstacles raised by APVMA. Nevertheless we have not only achieved this, but have a new Magnet® which is better than the old one in four respects:

(i) it is at least as attractive, and significantly more attractive in some trials, to *H. armigera* and *H. punctigera* (see Sections 8.1 and 8,2)

(ii) it appears to be less attractive to important groups of beneficial insects (see Section 4)

(iii) we have some leads on how to make it more rain fast (see Section 5.4)

(iv) we know it works on some important pests of cotton and other crops in New Zealand, the USA and south-east Asia (see Sections 6.2 to 6.4)

Outcomes: Large-scale field trials in our previous AC-CRC project (2.2.9) demonstrated that Magnet® could be a useful tool in area-wide management. Magnet® is the first attract-and-kill technology for female *Helicoverpa* moths that has been commercialised, and to our knowledge it will be the first synthetic chemical attractant for moths to be registered anywhere in the world. This will provide Australian cotton growers with a new tool for use in IPM, and an Australian SME (Ag Biotech) with market opportunities both domestically and overseas, with royalty flows to the CRC.

We originally envisaged Magnet® as a tool for reducing moth pressure on an area-wide basis, primarily for conventional cotton, to facilitate the action of other IPM components (Del Socorro & Gregg 2003). Since then, Bollgard II® has been widely adopted, and the area of conventional cotton has been greatly reduced. Bollgard II® is to some extent a competing technology for *Helicoverpa* management, and this together with reduced cotton area due to drought has reduced the potential market for Magnet®. However it has been argued (Gregg & Wilson 2008) that the current level of Bollgard II® adoption (90% or more) is not in the best interests of the industry and that alternatives are required. Magnet® will facilitate a more sustainable balance between Bollgard II® and conventional cotton, and the wider use of conventional cotton, managed under IPM, as a refuge option. Further, we are

now exploring ways in which Magnet® can be used in Bollgard II®/refuge systems, increasing the efficiency of refuges and thus enhancing the resistance management plans for Bollgard II® (see Section 8c below).

Output 2: A pheromone formulation which can be used for mating disruption of green mirids

We are still some way from delivering this output, in part because of the time required to meet the requirements to register Magnet®. We have a pheromone which can produce trap shut down in the field (see Section 3.1), and will attract male mirids to particular areas of the field (Section 3.2), where it should be possible to kill them with insecticides. However, the pheromone (though cheap) is quite volatile, and while we have made some progress towards slow-release methods (see Section 5.1) we need to make further improvements before this approach is likely to be commercially viable for controlling mirids.

We have also investigated the potential of the mirid pheromone as a monitoring tool which might overcome some of the problems growers and consultants have in sampling for this pest. The pheromone can detect mirids earlier in the season than any other method of sampling (see Section 3.4) and so might be useful where presence/absence information is required. However as a quantitative sampling tool its value is uncertain. It works at some sites but not others, and we do not understand the reasons. We need further information on basic mirid biology, including reproductive behaviour and movement. The pheromone may be a useful research tool for generating this information.

Output 3. A product similar to Magnet® which works on green mirids

In spite of a great deal of work, we have been unable to deliver this output. We found only two volatiles which were significantly attractive to mirids in laboratory olfactometer studies (see Figure 6, Section 2.2). One of these, (*E*)-2 hexenal is a generic green leaf volatile which is present in many host plants and may therefore be of limited value for use in the field. The other, cineole, requires confirmation. Even more seriously, in contrast with our experience in developing Magnet® for *Helicoverpa* moths, where volatile blends yielded higher attraction than single chemicals, combining volatiles into blends did not enhance attractiveness to mirids.

These results suggest that the responses of mirids to host plant volatiles may differ from those of *Helicoverpa*, in ways which will make it hard to develop a semiochemical attractant for them. A further problem is that there is no good method for rearing mirids in the lab. While it is possible to maintain a culture, generating the numbers of surplus insects required for olfactometer experiments is not possible with existing techniques. This meant we had to rely on field-collected insects. We did not know their age, mated status and crop origins (other than that they were collected from lucerne). These factors may explain much of the inconsistency we found in olfactometer results.

Output 4: Plant volatile formulations which do not attract beneficial species

We have delivered one output in this category, the new Magnet® formulation which has only limited attractiveness to beneficial species (see Table 4, Section 4). The original intention of this objective was to identify specific volatiles which could be added to products like old Magnet to render them less attractive to beneficials. However, the relative lack of attractiveness of new Magnet® to beneficials, and the regulatory complications which could arise from adding new volatiles, suggest that this line of research is unlikely to produce further outcomes of commercial value.

Outcomes: If this work had not been done, the outcomes listed under Output 1 above (a better Magnet®) might have been jeopardised because Magnet® would not be a "soft" option and therefore not compatible with IPM. We have avoided this.

Output 5: Knowledge of how to use semiochemical tools in managing resistance

We have made considerable advances in this respect, including preliminary studies of refuge enhancement using Magnet® without insecticides to increase oviposition, and the use of Magnet® with insecticide in "moth busting" for central Queensland. These areas are being continued in our current project 1.05.10, and will be described in detail in reports for that project. The moth busting research has already been described by Paul Grundy in the Final Report for Project 1.04.07, and published (Grundy *et al* 2006) and will not be further discussed here.

6. ***Please describe any:-***

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);

Australian Patent Application P78841.AU, *Insect Attractant Composition*, inventors P.C. Gregg, A.P. del Socorro and A. J. Hawes, filed in the name of the Cotton CRC on 1 October 2008

b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and

Although we have not produced commercially viable mirid pheromones or plant volatile attractants for mirids, we have developed many techniques useful for further research in these fields, for example, bioassay methods such as the mirid olfactometer, and slow-release formulations for both pheromones and plant volatiles.

c) required changes to the Intellectual Property register.

The provisional patent for the new Magnet® should be added to the register.

Conclusion

7. **Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?**

Semiochemicals such as the volatiles used in attract-and-kill for *Helicoverpa* (Magnet®) and mirid pheromones are less persistent, more selective and less damaging to non-target organisms, including beneficial insects, than conventional insecticides. They can work synergistically with other components of area-wide management to reduce overall pesticide use.

The imminent registration of Magnet® will allow unrestricted commercial use of the product in Australia in attract-and-kill for conventional cotton and possibly in resistance management for transgenic cotton. Overseas trials demonstrated that the product will work for heliothine and other noctuid pests in other countries, and this might lead to potential international markets.

We cannot yet recommend pheromone traps as reliable monitoring tools for mirid populations in the field, or as tools for mating disruption or attract-and-kill in mirid control. However, advances in slow-release technology might make the use of pheromones for control commercially feasible, and pheromone trapping coupled with regular field sampling for mirids might provide a better understanding of the population dynamics and mirid ecology in general, to properly manage this pest.

In spite of considerable work, we have not been able to develop plant volatile formulations which attract mirids. Lack of a method for rearing large numbers of mirids in the laboratory has been a major problem.

Extension Opportunities

8. **Detail a plan for the activities or other steps that may be taken:**
   (a) **to further develop or to exploit the project technology**.

Negotiations are in progress with Ag Biotech in relation to international rights for the new Magnet® product. Our current project, 1.05.10, is developing applications for Magnet® in Bollgard II® dominated cotton systems, and this, together with ongoing work overseas, will enhance market opportunities for Magnet®.

(b) for the future presentation and dissemination of the project outcomes.

Following the registration of Magnet®, we will be free to publish much of the research which led to its development in the refereed scientific literature. We have submitted one such paper, and more will follow. Marketing of Magnet® will be primarily the responsibility of Ag Biotech, and extension of trial results will need to be treated with caution because of the risk of perceived bias on the part of the CRC.

However, we will discuss the options with the Crop Protection National Priority Team.

(c) for future research.

We will be continuing research on the applications of Magnet® in resistance management for Bollgard II® through Project 1.05.10. This will include the placement and timing of Magnet® applications to cause relatively greater mortality in moths emerging from Bollgard compared to refuges, and the use of Magnet® in "moth busting" as an alternative to pupae busting. The latter is becoming particularly important with the growing imperative for minimum till agriculture in cotton farming systems, for soil management, crop rotations and water conservation.

Although funding for this work has ceased, we will also try to continue work on mirid pheromones, especially on their potential for enhancing understanding of the basic population biology and ecology of mirids, and potentially their ultimate use in mirid control. We will aim to do this through Summer and Honours scholarships.

We strongly recommend (though we do not intend to undertake this ourselves) that research on rearing mirids in the laboratory be undertaken, with the aim of developing a system which will generate large numbers of surplus insects for research purposes. Until this is done, further work on plant volatiles for mirids is not recommended.

Publications

9. A. List the publications arising from the research project and/or a publication plan. (NB: Where possible, please provide a copy of any publication/s)

See Objective 7 above.

B. Have you developed any online resources and what is the website address?

No

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

This project derived from a previous Australian Cotton CRC project, "Plant-based attractants for *Helicoverpa* moths and sucking pests of cotton" (Project 2.2.9). It investigated ways in which the behaviour of *Helicoverpa* spp. and the sucking pests, the green mirids, can be manipulated in a cotton landscape which includes transgenic and conventional cotton, refuges for resistance management, beneficial

nurseries and non-crop vegetation. The project generally aimed to develop plant-based attractants or repellents for cotton pests, including further refining and commercialising our current product, Magnet®. Specific objectives were: (1) to develop field bioassay methods for mirids, (2) identify plant volatiles attractive to mirids, (3) investigate potential for mating disruption and attract-an-kill using mirid pheromones, (4) identify plant volatiles which attract or repel key beneficial insects, (5) develop formulations with slow-release and rain fastness, (6) to investigate the potential of using Magnet® to enhance refuges for transgenic cotton.

This work showed that mirids are nocturnal in their host-finding (response to plant volatiles) and mate-finding (response to pheromone traps) behaviours. Field olfactometer bioassays using fresh lucerne bouquets, hexane washings and synthetic equivalents of volatiles collected from lucerne were done to evaluate potential plant volatiles as attractants or repellents for mirids. Lucerne bouquets were significantly attractive to female mirids but only two chemicals appeared to be attractive and two were repellents to mirids. Combining volatiles into blends did not increase attractiveness to mirids.

A technique to improve longevity of mirid pheromone in the field was developed which involved coating the rubber septa with araldite glue but leaving the tip of the septa uncoated. In collaboration with CRC extension officers and researchers, pheromone trapping trials were conducted at 8 locations in NSW and Qld to evaluate the usefulness of pheromone traps for monitoring mirid populations. Results suggested that population dynamics of mirids were different between locations. Pheromone catches were significantly correlated with field mirids at some sites, whilst such correlation was poor at other sites. In some cases, pheromone traps appeared to catch male mirids only when female mirids in the field were mostly mated, suggesting that the effectiveness of pheromone traps might be influenced by the "female competition" effect in the field. Results from this work suggest that pheromone traps on their own may not be reliable monitoring tools, but may be valuable in providing a better understanding of the population dynamics and mirid ecology in general. Mating disruption using mirid pheromones might be feasible provided a method for slow release of pheromone is developed.

A regulatory difficulty was encountered with one of the volatile components of the Magnet® formulation and we decided to replace this component with two others, to avoid the need for expensive toxicological research. Many trials of potential alternatives were conducted, and these resulted in the development of a new Magnet® formulation which was at least as attractive as the old one to Helicoverpa spp., and less attractive to beneficial insects. Registration of this new formulation is imminent, and we have conducted trials on its attractiveness on a range of other species in the USA, New Zealand and south east Asia, as well as on applications for improving resistance management for transgenic cotton in Australia.

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