FINAL REPORT

Part 1 - Summary Details

Cotton CRC Project Number: CRC Project 1.05.10

Project Title: Improving the efficiency of Bollgard II® refuges with semiochemicals

Project Commencement Date: 01/07/08 **Project Completion Date:** 30/06/12

Cotton CRC Program: 1 (The Farm)

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Part 3 – Final Report Guide (due at 31st May 2012)

Background

1. Outline the background to the project.

The Australian cotton industry is now heavily reliant on genetically engineered Bt (Bollgard II®) cotton for the control of *Helicoverpa* spp. and other Lepidopteran pests. Over 95% of the area is Bollgard II®. The introduction of this technology has been associated with a reduction in insecticide use of around 80%. There are clear economic benefits to growers using Bollgard II®, at least in some seasons. The lifestyle improvements and the reduced environmental impacts of pesticides have considerably benefited growers and the community. The industry's social license to operate depends on maintaining these environmental and community credits. In turn, this depends on maintaining the effectiveness of Bollgard II® technology, in particular by avoiding resistance.

The industry has a rigorous and widely adopted resistance management strategy, in which the planting of refuges is a key component. However, refuges are costly and are often associated with management difficulties. They usually produce little economic return to offset these problems. Thus, it is important that refuge efficiency is maximized. We need refuges which produce the highest numbers of susceptible moths relative to potentially resistant ones emerging from Bollgard II®, and produce them when and where they are required. Considerable research has gone into the types of crops, the areas required, and the location of refuges to achieve these aims. Very little attention has been given to the possibility of improving the effectiveness of refuges by chemical modification of insect behaviour. Research on semiochemicals such as Magnet® (CRC Project 1.05.02 and predecessors) has shown that we now have the capacity to move *Helicoverpa* populations around on a diverse landscape of Bollgard II® and refuge crops, over large distances. We should also be able to manipulate moth population density and oviposition on similar scales. This project aimed to translate that capacity into more effective refuge management, to give reductions in areas required for refuges, or more robust resistance management strategies, or both. It aimed to explore new approaches with an existing product (Magnet®), and develop new products specifically to enhance refuges.

The project focused on developing methods for selective attract-and-kill using Magnet® to increase the efficiency of refuges and to identify host plant volatiles which stimulate host location for oviposition, and develop new formulations which can be applied in the field for oviposition enhancement of refuges. The project originally proposed to have two components: (a) "moth-busting" on a selective basis to improve the effectiveness of refuges generally and (b) developing volatile mixtures which selectively attracted mated female moths and/or stimulated them to lay eggs.

For moth-busting we aimed to build on work (e.g. Grundy *et al* 2006) which showed that Magnet® can manipulate moth populations on a significant scale, and the impacts vary according to distance from the treated area. Since Magnet® remains active for < 1 week, selective timing or placement could kill proportionately more moths emerging from Bollgard® than from refuges.

For oviposition enhancement, we aimed to improve on results showing that Magnet®, applied without insecticide, could produce modest increases in egg laying in refuges (Addison 2010, Gregg *et al* unpublished data 2005, Whitehouse unpublished data 2010) . We believed there was scope for improvement because Magnet® was thought to be primarily a feeding attractant because it was developed from olfactometer studies of virgin female moths, whose first priority is energy for flight, mating and egg development and which they obtain mostly by feeding on nectar from flowers (Del Socorro *et al* 2010a, Gregg *et al* 2010a). Similar studies using mated females might show that different volatiles, perhaps not floral in origin, are required to attract those mated females and persuade them to lay. Identification of such volatiles might lead to the development of a new blend for enhancing oviposition.

Objectives

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

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During the course of the project, the objectives were changed. Some milestones were dropped, and new ones introduced, in consultation with the CRC. A major disruption early in the project was caused by the rejection by the regulatory authority APVMA (Australian Pesticides and Veterinary Medicines Authority) of an application to register the original formulation of Magnet® This was expected to be achieved during the previous project, CRC 1.05.02, but APVMA advised that one volatile component of the original formulation required extensive mammalian toxicology studies. Rather than meet this time consuming and expensive requirement, we opted to re-formulate the product with volatiles which would not require that testing. This meant that we had to repeat many efficacy and non-target impact studies with the new formulation, which distracted the project team from the objectives of Project 1.05.10 in its first year. The registration of Magnet® is discussed further in the section on Outcomes.

The objectives of Project 1.05.10 are described below:

1. *Laboratory identification of volatiles which enhance or suppress oviposition by H. armigera*

This objective was not achieved. It had two components: identifying volatiles which might attract mated females more effectively than those already in Magnet®, and identifying volatiles which stimulated oviposition. We tested a total of 12 individual volatile compounds, including the 6 components of Magnet®, and the Magnet® blend itself, in the olfactometer to compare test responses in unmated and mated moths. We found that all the volatiles we tested were more attractive to mated females than unmated ones, and some of the volatiles currently in Magnet® were among the most attractive. We therefore considered that the initial premise of this work, that because we used unmated moths in the development of Magnet® there might be better volatiles for mated moths, was not correct, and that it would not be possible to greatly improve the blend for mated females. We negotiated the dropping of milestones around this objective. We also negotiated the dropping of milestones around the identification of volatiles suppressing or enhancing oviposition because, despite extensive efforts, we were unable to find a bioassay method that gave consistent results.

2. *Development of methods for testing the impact of selective timing and placement of Magnet® attract-and-kill formulations*

This objective was achieved. We developed methods and protocols for the four field trials conducted (see next objective, and detailed Methods section)

3. *Conduct field trials on selective placement and timing of Magnet® attract-and-kill for refuge manipulation*

This objective was achieved. A field trial was conducted at Auscott, Narrabri to determine whether treating Bollgard II® cotton with Magnet® would reduce the number of eggs laid on the treated area, and larvae surviving in the Bollgard II® and thus moths emerging from the crop, in comparison with moths emerging from conventional cotton refuge, thus increasing the efficiency of the refuge. Another trial was done at “Lynora Downs”, Rolleston, CQ on six contiguous conventional cotton fields along a transect of about 3kms to try to find a gradient from one end of the transect to the other which might indicate the range of the effect of Magnet®. A third trial was done on spring wheat at ACRI.

4. *Initial field trials on modified Magnet® formulations for enhancing oviposition*

This objective was achieved, though not in the form originally proposed. Despite the fact that we were unable to identify volatiles which were significantly more attractive to mated females in the laboratory, we conducted a trial to determine whether exclusion of the volatiles least attractive to mated females would produce the same effect. This was done in mung beans in Narrabri ("Yarral") in 2010 using modified Magnet® formulations. We were not looking at whether oviposition was increased, but at the proportion of mated females in the moth kill. This trial also investigated whether modifications to the Magnet® blend might be worth pursuing for other reasons e.g., better efficacy, lower cost and better rainfastness.

5. *Identification of one or more new volatile blends for oviposition enhancement, which are worth patenting and submitting for registration with APVMA*

This objective was not achieved. Our laboratory work and the results of the “Yarral” trial suggested that mated moths are both more active in the olfactometer and more responsive to most plant volatiles than unmated ones, and therefore it would be difficult to improve much on the existing Magnet® formulation as an attractant for mated moths. In consultation with commercial partners AgBitech and the CRC, we decided that this, combined with the difficulty of registering products containing new compounds and the limited commercial market for refuge enhancement products, meant that it was not worthwhile pursuing this objective.

6. *Development of bioassays for oviposition stimulations, and preliminary identification of non-volatile compounds which enhance oviposition*

This objective was not achieved. We spent several months testing various bioassay methods (including some which are reported to work in the literature), and never found one which provided consistent results. This work was described in our progress reports, and the CRC agreed to remove the corresponding Milestone (2 March 2012).

7. *Development of novel techniques for identifying the host origin of moths killed by Magnet®*

This objective was introduced late in the project, as a substitute for the dropped objectives relating to oviposition enhancement. It was partially achieved. We spent several months on this, and although we obtained some promising leads, our expertise in chemistry was not good enough to convert them to reliable techniques. We therefore enlisted the aid of a chemist, Ben Greatrex, and this Milestone has become the focus of his project, 1.05.13, in which we are collaborating. In view of this the CRC agreed to drop the relevant milestone.

8. *Conduct further field trials on selective placement and timing of Magnet® attract-and-kill for refuge manipulation*

This objective was also achieved, though not in the form originally proposed. This was due to criticism by other researchers that using Magnet® to reduce moth numbers in Bollgard cotton might increase oviposition because the volatiles might attract more moths than the insecticide could kill, or remain active after the insecticide wore off, leading to more eggs and thus inadvertently increasing selection pressure for resistance. We have never seen such effects in many farm-scale research and commercial trials, but to answer the criticism we conducted a field trial near Bellata, NSW to determine the residual activity of Magnet® in the field. . In addition to the Auscott and Rolleston Magnet® trials, a field trial on wheat was done at Chico, ACRI to determine whether applying Magnet® to the wheat when the overwintering moths emerged in spring can be effective as a substitute for pupae busting or an ameliorant for poor pupae busting.

9. *Data on selective placement and timing of attract-and-kill analysed, recommendations available*

This objective was achieved. All data have been analysed, and reported on in Progress Reports and in presentations to REFCOM.

10. *Recommendations for improving refuge efficiency with semiochemicals are ready for inclusion in 2012-13 RMPs*

This objective has been partially achieved. Peter Gregg served on the Bt Technical Panel of TIMS, advising on resistance management and the potential role of Magnet® in this. He made recommendations for the use of Magnet® in contingency plans to be adopted in the event of further increases in resistance frequency. While these have not yet been adopted, and there are no requirements around Magnet® use in 2012/13 RMPs, TIMS and the industry remain interested and CRDC has funded a further three year project to examine the use of moth busting as a potential substitute for pupae busting.

Methods

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

Some of the methodology used for this research was developed in previous CRC projects, such as laboratory studies using olfactometers and methods for small-scale field trials. Other methods were developed during the course of this project. Because the methods for each objective/milestone were quite different they will be described in detail under each objective in the following section.

Objective 1: Development of methods for testing new volatiles for oviposition enhancement and suppression using olfactometers and oviposition bioassays

A fresh *H. armigera* culture was established in the insectary using larvae collected from chickpeas in Cecil Plains, Qld. Mated females were needed in the olfactometer bioassays. Several methods of having mated females to be used in the olfactometer were tested. Preliminary work was done to determine if mated females can be marked by using fluorescent dyes in the abdominal tips/claspers of males, which would be transferred to the females on mating. However, this method did not work, because the transfer of dye was not consistent enough.

Another method was setting up individual mating containers with one 2-day old female and two 1-day or older males, using our lab moths, and left to mate for two nights. After two nights, females were separated from the males, and used in the olfactometer. This method yielded very low mating success, and hence, insufficient numbers of mated females for bioassays. We have seen this problem previously in cultures which have not had a long history of laboratory rearing. We therefore decided to use moths from pupae supplied by AgBitech from a long-term lab culture. These had much higher mating success (>85%). The new protocol involved setting up individual pairs of two-day old female and male of any age in mating cups and allowing them to mate for 2 nights, after which females were separated from males.

For each olfactometer run, 50 females (30 paired with males for 2 nights and 20 unmated) with ages ranging from 2-6 days, were used. Test volatile attractants were formulated at 10% in canola oil, and for each run, 200 µl of the formulation in dental wick was used which was replaced every 2hrs. Each test volatile was tested 4 times (replicate runs) in the olfactometer. These methods were identical to those used on unmated females (Del Socorro *et al.* 2010a, Gregg *et al.* 2010a). After each run, all moths were collected and kept in 70% alcohol until dissection to check mated status.

A total of 12 volatile compounds including the 6 individual components of Magnet®, and the Magnet® blend were screened using a two-choice olfactometer. As control, blank (no volatiles in both test and control chambers) and canola blank were also tested in the olfactometer. The volatile compounds tested included α-pinene, phenylacetaldehyde, limonene, cineole, butyl salicylate, anisyl alcohol, β-caryophyllene, geraniol, linalool, 2-phenylethanol, Z-3-hexenyl acetate and E-2-hexenal.

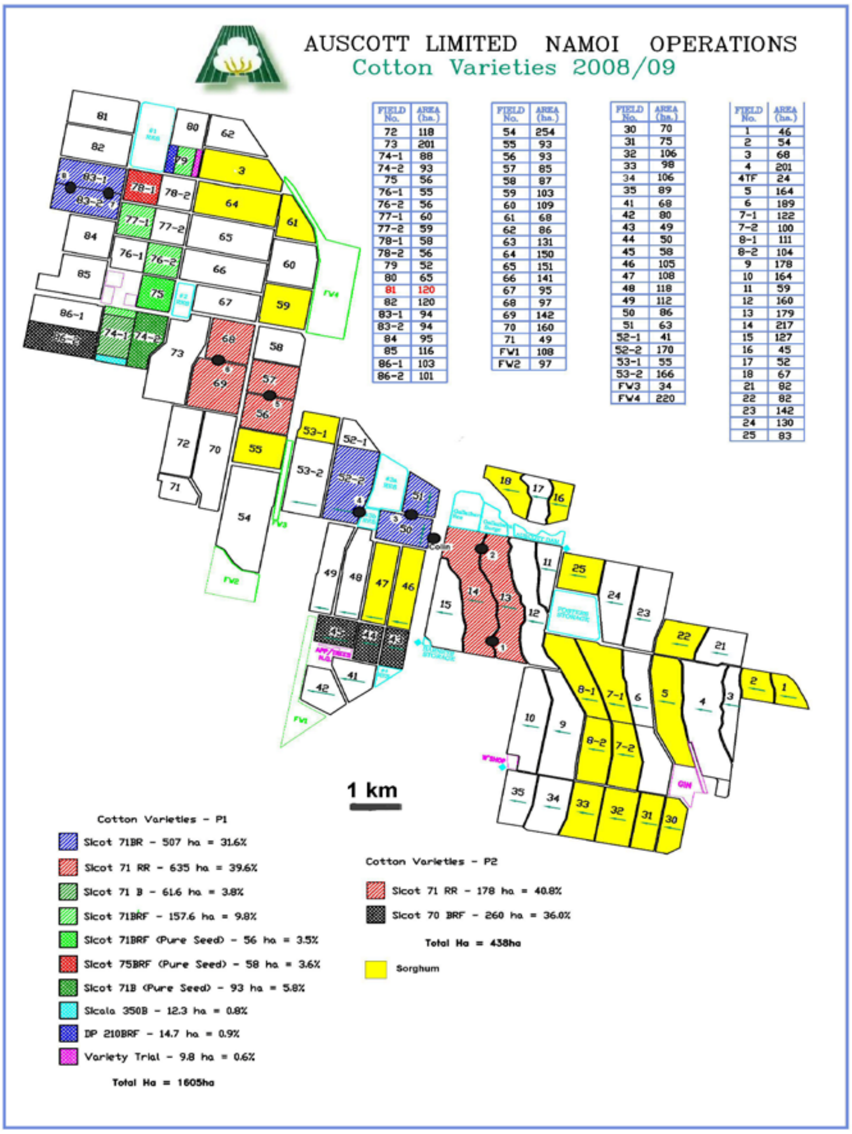
Objective 2: Development of methods for testing the impact of selective timing and placement of Magnet attract-and-kill formulations

Protocols developed for the field trials are presented in the next section (Objective 4).

Objective 3: Conduct field trials on selective placement and timing of Magnet attract-and-kill for refuge manipulation

Auscott trial

A field trial was conducted at Auscott, Narrabri to determine whether treating Bollgard II® cotton with Magnet® will proportionately reduce the number of moths emerging from the crop, in comparison with nearby conventional cotton refuge, thus increasing the efficiency of the refuge. The trial was run on the extended properties of Auscott Limited, Narrabri, on a transect of approximately 16 kms from NW to SE, with both Bollgard II® and conventional cotton grown in various fields as refuge, along the transect (Fig. 1).



FIELD No.	AREA (ha.)
72	118
73	201
74-1	88
74-2	93
75	56
76-1	55
76-2	56
77-1	60
77-2	59
78-1	58
78-2	56
79	52
80	65
81	120
82	120
83-1	94
83-2	94
84	95
85	116
86-1	103
86-2	101

FIELD No.	AREA (ha.)
54	254
55	93
56	93
57	85
58	87
59	103
60	109
61	68
62	86
63	131
64	150
65	151
66	141
67	95
68	97
69	142
70	160
71	49
FW1	108
FW2	97

FIELD No.	AREA (ha.)
30	70
31	75
32	106
33	98
34	106
35	89
41	68
42	80
43	49
44	50
45	58
46	105
47	108
48	118
49	112
50	86
51	63
52-1	41
52-2	170
53-1	55
53-2	166
FW3	34
FW4	220

FIELD No.	AREA (ha.)
1	46
2	54
3	68
4	201
4TF	24
5	164
6	189
7-1	122
7-2	100
8-1	111
8-2	104
9	178
10	164
11	59
12	160
13	179
14	217
15	127
16	45
17	52
18	67
21	82
22	82
23	142
24	130
25	83

Fig, 1. Trial site at Auscott Narrabri. Treated field (Field 50) is in the centre of the farm. Black dots represent monitoring sites.

The fields monitored for the trial (starting from the SE) were: 13 and 14 – conventional cotton, 50 – Magnet® treated Bollgard II®, 51 and 52-2 – nearby Bollgard II®, 56, 57, 68, 69 – conventional cotton, and 83-1 and 83-2 – distant Bollgard II®.

Field 50 (Bollgard II®) was treated with Magnet® with methomyl (0.5% a.i.) five times during the season. The impact of treatments was measured by dead moth counts following each application. At twice-weekly intervals, the following were also conducted: collections from 8 pheromone traps for *H. armigera* spread along the transect, 50m moth flush counts, 1m visual bugchecks, 20 sweep net samples in each field. Additional data (bugchecks) were also sought from Auscott.

“Lynora Downs” trial

Another trial was done at “Lynora Downs”, Rolleston, CQ on conventional cotton. This was intended primarily as a commercial evaluation of Magnet® for conventional cotton, but the spatial layout of the farm provided an opportunity to examine potential spatial variation in the impact of Magnet®.

The treated farm had seven contiguous cotton fields along a transect of about 3kms (Fig. 2). The four fields at one end of this transect were treated 8 times with Magnet®, at approximately weekly intervals between mid December and early February. Eggs and larvae were counted in all fields by a consultant employed by the grower. A distant control farm was located about 20 km away. The aim of this trial, from the perspective of the grower and AgBiTech, was to examine the impact of Magnet® treatment at a whole farm level, by comparison with the distant control farm. From the perspective of the objectives of this project, the aim was to try to find a gradient in egg density from one end of the transect to the other which might indicate the range of the effect of Magnet®.

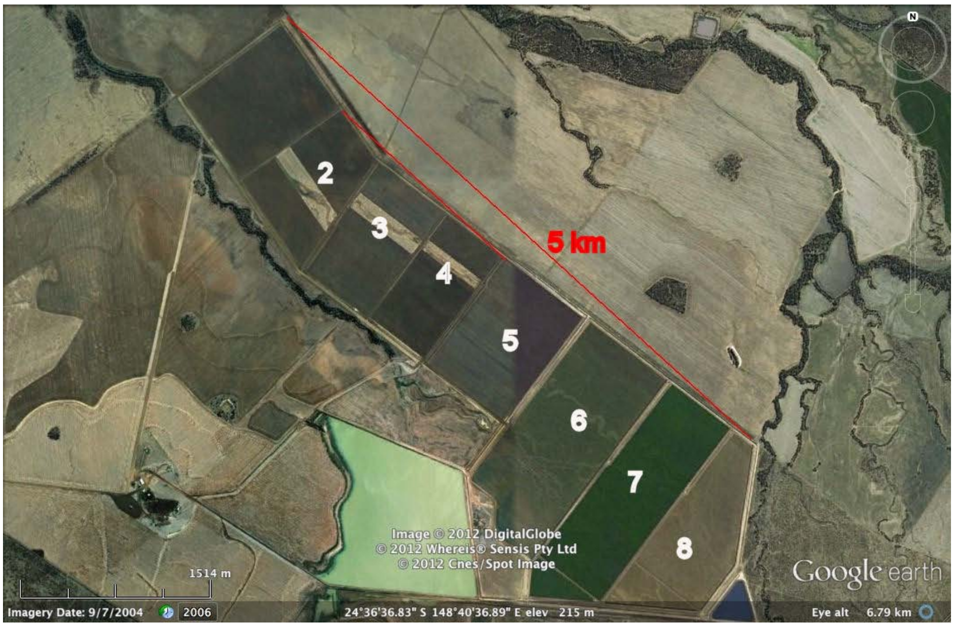


Fig 2 Google Earth® image of the treated farm, "Lynora Downs”, Rolleston, Qld. Fields 5-8 were treated with Magnet® on eight occasions between 11 Dec 2008 and 3 Feb 2009. Fields 2, 3 and 4 were not treated. The field to the northwest of Field 2 did not have cotton. Egg and larval densities were monitored in all fields.by consultants 2-3 times per week.

Objective 4: Initial field trials on modified Magnet® formulations for enhancing oviposition

“Yarral” trial

We conducted a trial in mung beans in Narrabri ("Yarral") in 2010 using modified Magnet® formulations. We were not looking at whether oviposition was increased, but at the proportion of mated females in the moth kill. This trial also investigated whether modifications to the Magnet® blend might be worth pursuing for other reasons e.g., better efficacy, lower cost and better rainfastness. There were five treatments: Magnet®; Magnet® without α-pinene; Magnet® without cineole; Magnet® with 2-phenylethanol substituted for phenylacetaldehyde, and Magnet + 0.2% Magnafloc® . The rationale for the treatments other than the Magnet® control was: α -pinene was the least attractive compounds to mated moths (Fig. 4 in this report), so deleting it might increase relative attraction to mated moths; cineole was the least attractive to unmated moths (and also problematic in formulating Magnet®); 2-phenylethanol was very attractive to mated females (see Fig. 4 in this report, and also Gregg *et al.* 2010b) and might have substituted for phenylacetaldehyde, which is the most expensive and difficult to supply of the Magnet® volatiles, and Magnafloc® is a polymer reported in our earlier work to provide some rainfastness, but its effects on attractiveness have not been tested.

Each treatment was replicated 4 times, on a 50 m section of row. Dead moths were collected over 4 days, identified to species, and for *Helicoverpa* moths, dissected to determine sex and mated status. Moths other than *Helicoverpa* spp. were also recorded, but not dissected. Due to personnel limitations, we searched only the adjacent furrows on the treated rows, which in the past we have found retrieves about a third of the total moths killed.

Objective 5: Identification of one or more new volatile blends for oviposition enhancement, which are worth patenting and submitting for registration with APVMA

Methods for this are described under Objective 1, above.

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Objective 6: Development of bioassays for oviposition stimulants, and preliminary identification of non-volatile compounds which enhance oviposition

We spent several months testing various bioassay methods for oviposition stimulants, and never found one which provided consistent results. The methods included:

(1) Suspending strips of blotting paper on glass walled aquarium cages in which one or more mated females were confined overnight. Strips on alternating walls were soaked in ethanol extracts of leaves of cotton and pigeonpeas, and eggs were counted the next morning.

(2) Treating alternate strips of paper towelling with plant extracts, marked out with marking pens, which lined the walls of mass oviposition cages containing 10-20 females, as described by Teakle & Jensen (1985), and counting eggs the next morning.

(3) We adapted Robert Mensah's method (CRC Project 1.05.01) which involved four filter papers suspended from the sides of 30cm buckets and treated alternatively with fractions of Plant X extract being tested for deterrent or stimulant properties. For our purposes alternate blank and ethanol extract treated filter papers were used.

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Objective 7: Development of novel techniques for identifying the host origin of moths killed by Magnet®

This work involved rearing moths on known host plants in the laboratory, extracting their various body parts with organic solvents and analysing the extracts for compounds which might serve as markers of host origin. Leaf extracts were also analysed from host plants to guide the search for possible markers. We focused on alkanes (waxes) in the cuticle because these compounds are known to vary considerably between different host plants, and are relatively stable, which means they would be preserved in dead insects and they might survive the transition from larval stages to the adult (through histological re-organisation during the pupal stage). Piskorski *et al.* (2010) have recently shown that the alkane composition of the cuticle of codling moth larvae reflects that of the host plants on which they fed.

Moth rearing

Moths came from *H. armigera* larvae reared on potted plants (cotton, pigeon pea, soybean and mungbean) in the glasshouse. Ten to twelve pots of each host plant were maintained in the glasshouse. At reproductive stage (i.e., squaring, flowering, podding) a voile bag measuring 30cm x 22cm closed with Velcro seal on the top opening and string on the bottom end was placed in each plant and seeded with three 2nd instar larvae. At the large larval stage, a handful of peat moss was put in each bag for the larvae to pupate in. Pupae were collected, sexed and held in individual 35-ml plastic cups with moist vermiculite. Upon emergence, moths were collected and frozen until use for analysis.

Leaf extraction

About 100 grams of leaves were collected in a paper bag and individually dipped in 100ml hexane for about 10 sec. The hexane solution was filtered using Whatman # 1 filter paper, transferred to a round bottom flask and concentrated to dryness using a rotary evaporator. The concentrated sample was mixed with 3ml hexane and the solution was filtered again using a glass fibre filter. Samples were kept frozen until analysis.

Extraction of moths

We tested two methods of moth extraction for analysis - using either pair (left or right) of wings or the whole body without wings. Either the left or right fore and hind wings were clipped and soaked in 1ml hexane for 1 hr. After soaking the sample was homogenised using a tissue grinder with a glass rod, filtered into a sample vial using a glass fibre filter, then concentrated in nitrogen. About 100µl of hexane was added to the concentrated sample, then pippeted into a 1.5ml sample vial with a 100µl insert. The whole body method was similar to the wing method except the sample was not crushed or homogenised so that internal body contents were not included.

GC-MS analysis

GC-MS analysis was done using a Hewlett Packard 6890 under conditions similar to those described by Del Socorro *et al.* (2010) for plant volatiles, except that the initial temperature was 80°C and a final temperature of 300°C, with a run time of 42 min. These higher temperatures were needed to elute relatively non-volatile components such as high molecular weight alkanes.

Objective 8: Conduct further field trials on selective placement and timing of Magnet attract-and-kill for refuge manipulation

Bellata trial

We conducted a field trial near Bellata, NSW to determine the residual activity of Magnet® in the field. This has been necessitated by criticism that using Magnet® to reduce moth numbers in Bollgard II® cotton might inadvertently increase selection pressure for resistance. Answering that question is critical for future trials of Magnet® in resistance management.

The concept of the trial was to apply Magnet®, then follow it at various times with blank Magnet® formulation, containing feeding stimulant, insecticide and all excipients except the attractant volatiles. This would be compared with treatments which had only ever received blank formulation, and any increase in moth kill could be attributed to residual attraction from the original Magnet® treatment.

The trial was done on rain-fed Bollgard II® cotton, planted in the super-single configuration which facilitated finding dead moths in mature cotton, at "Boundary Well", 14 km east of Bellata. A small-scale trial using four replicates of 50m treated rows was conducted with the following treatments (Table 1) at weekly intervals.

Treatment	Initial application	Subsequent applications
1	Magnet®	Magnet®-Magnet®-Magnet®
2	Magnet®	Magnet®-Blank-Blank
3	Magnet®	Blank-Blank-Blank
4	Blank	Blank-Blank-Blank

Table 1. Treatments in the Bellata experiment on residual activity of Magnet®

Before Magnet® application, both sides of treated rows were raked clear of stubble to facilitate moth collection. Magnet® was applied at the normal commercial rate (250ml/50m) and methomyl (0.5%) was added to all treatments. Dead moths were collected on one side of the row (alternating between replicates) for the first day only in the first application, for two days on both sides of the treated row in the second and third applications, and on both sides for one day in the final application. Prior to each subsequent application, old dead moths were removed from the treatment areas so that they would not be confused with those killed by the new application. All noctuid moths were identified to species, and *Helicoverpa* spp. moths were sexed and dissected to determine mated status.

The purposes of the treatments were as follows:

Treatment 1 – Fresh Magnet® on each occasion, to indicate the numbers of moths available to be killed and to provide an upper reference for the extent to which activity of the volatiles had declined in Treatments 2 and 3. If similar numbers of moths were available to be killed and if the volatiles were persisting more than 1 week and having cumulative effects, this treatment should show progressive increases in kills relative to the blank control (Treatment 4).
Treatment 2 – similar to Treatment 1 in the first application, with Magnet® being re-applied in the second week, following 16 mm of rain which had removed much of the Magnet® from Treatments 1-3.
Treatment 3 – The key treatment that measured the decline in activity of volatiles. Since fresh insecticide but not added volatiles were applied on each occasion, the comparison with Treatment 4 should indicate how long the volatiles persisted.
Treatment 4 – The blank, to provide a lower estimate of the reduction in the activity of volatiles. We know that blank treatments in small-scale trials typically kill 20-25% of the moths that Magnet® with volatiles does. This is because sugar alone is attractive to

Helicoverpa spp. moths. If the moth kills in Treatments 2 and 3 exceeded those in Treatment 4, it would be likely due to residual attraction from the earlier Magnet® applications.

Chico wheat trial

The aim of this trial was to determine whether Magnet® sprayed on wheat in spring would attract and kill *H. armigera* moths. This was a first step in investigating whether selective placement of Magnet® in spring might be useful in resistance management. This small-scale Magnet® trial was conducted on wheat (head-filling stage) at Chico, ACRI (Field A2). The crop was sprayed at a rate of 250ml per 30m + 0.5% a.i. methomyl on 19 October 2010. There were two treatments – Magnet® and blank (Magnet® base only + methomyl, without volatiles), replicated 4 times. Each treatment was applied on 30m rows with 30m buffer between rows. The field was sown with wheat on 40” rows (equivalent to 3 rows of wheat per cotton row (Fig. 3). Treatments were applied by sprinkling from a “pop-top” plastic bottle, across two of these wheat rows. The field was previously planted to cotton as shown by the cotton trash on the ground. We searched the three rows plus the adjacent furrows for dead moths early in the morning, over the next two days. Moths were sorted to species. *Helicoverpa armigera* and *Mythimna convecta* moths were dissected to determine sex, mated status and presence of blue dye.

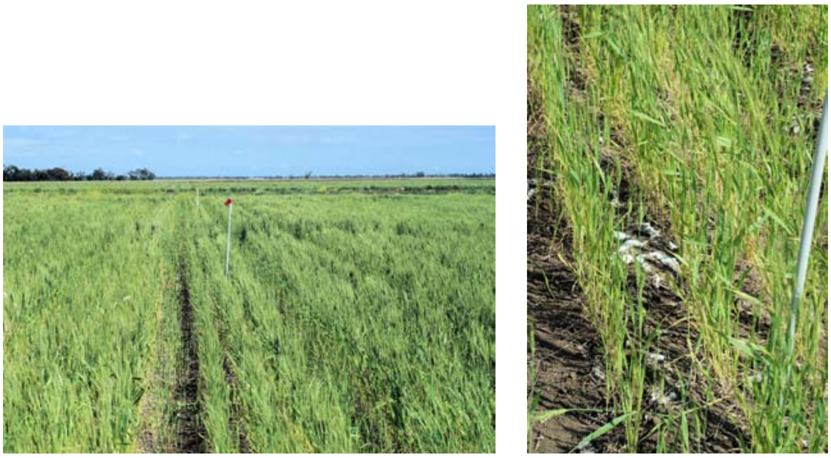


Fig.3. Trial site showing a control (blank) plot marked by red flags, and treated rows showing cotton trash on the ground. It was an old cotton field (though pupae busted

Results

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

Objective 1: Laboratory identification of volatiles which enhance or suppress oviposition by H. armigera

Data were analysed using Minitab® and R-analysis (R Development Core Team 2009). The latter measured the % test response (test/total) as well as the % total test response ((test+control)/total), for mated and unmated female moths for each test attractant in comparison with either of the two controls, blank or canola blank. This analysis was similar to olfactometer experiments done to test the attractiveness of unmated *H. armigera* females to plants, individual compounds and blends (Del Socorro *et al.* 2010, Gregg *et al.* 2010b). Each test attractant was compared with the two controls, blank (no volatile) and canola blank.

The mean % test responses of all test attractants for mated and unmated moths are shown in Fig. 4. All of the 12 volatiles tested, plus the Magnet® blend, were more attractive to mated than unmated moths. Even in a blank olfactometer (i.e., no volatile) or where only the carrier canola oil (canola blank) was present, there was a trend for more mated than unmated moths in the test chamber, although the differences were not statistically significant. These results suggest that mated moths are simply more active in the olfactometer than unmated ones, and perhaps respond more strongly to any volatile.

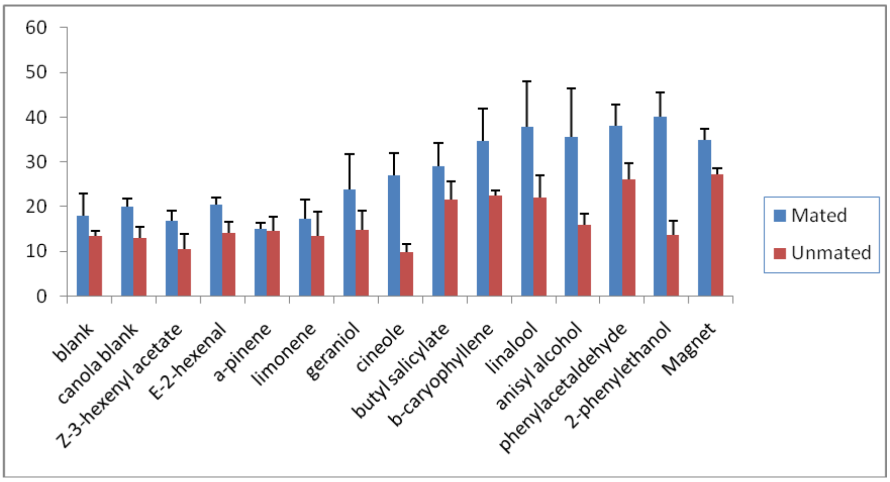


Fig. 4. % test response of mated and unmated *H. armigera* females to Magnet® and individual plant volatile compounds in the olfactometer. Bars are s.e's. of the means.

Overall, both factors (mated status and volatile type) were highly significant (p=0.000) but their interaction was not (p=0.669). Further analysis using R compared each volatile to the blank control (Table 2) and the canola blank (Table 3).

Chemical	**% Positive Response***	**Attractant**	**Mating**	**AxM**	**% Total Response***	**Attractant**	**Mating**	**AxM**
α-Pinene	15.8, 14.0	ns	ns	ns	26.9, 31.7	ns	ns	ns
Anisyl alcohol	18.8, 32.6	ns	0.041	ns	32.9, 49.8	ns	ns	ns
Butyl salicylate	22.0, 28.8	0.02	ns	ns	35.3, 42.9	ns	ns	ns
β-caryophyllene	23.7, 33.7	0.0017	0.05	ns	35.1, 53.8	ns	0.0036	ns
Cineole	12.9, 23.8	ns	0.01	ns	24.3, 39.1	ns	0.19	ns
Geraniol	16.3, 23.7	ns	ns	ns	54.2, 54.8	ns	ns	ns
Limonene	13.1, 17.3	ns	ns	ns	23.1, 35.8	ns	0.032	ns
Linalool	24.0, 36.2	0.017	ns	ns	40.3, 63.2	0.025	0.0052	ns
Phenylacetaldehyde	29.0, 41.1	<0.0001	0.001	ns	38.2, 54.3	0.0303	0.0046	ns
2-Phenylethanol	17.9, 36.0	0.00452	0.00023	0.03274	30.8, 56.8	ns	0.00029	0.02596
Z-3-hexenyl acetate	11.3, 16.0	ns	ns	ns	27.2, 35.5	ns	ns	ns
E-2-Hexenal	14.3, 19.5	ns	ns	ns	36.1, 43.2	ns	ns	ns
Magnet®	27.6, 34.7	<0.0001	ns	ns	36.7, 49.6	ns	0.03	ns
Canola blank	12.8, 18.0	ns	ns	ns	20.6, 30.9	ns	ns	ns

Table 2. Summary of R-analysis, test chemical vs blank control. *The format x,y are for unmated (x) and mated (y) female moths.

Chemical	**% Positive Response***	**Attractant**	**Mating**	**AxM**	**% Total Response***	**Attractant**	**Mating**	**AxM**
α-Pinene	13.2, 16.6	ns	Ns	ns	25.6, 32.9	ns	ns	ns
Anisyl alcohol	17.9, 33.4	ns	0.01	ns	30.7, 51.6	0.037	0.11	ns
Butyl salicylate	21.1, 29.6	0.01	0.03	ns	33.8, 43.9	0.01	ns	ns
β-caryophyllene	22.8, 34.5	0.001	0.01	ns	32.9, 55.8	<.0.0001	<0.0001	ns
Cineole	12.1, 24.5	ns	0.00011	ns	22.4, 40.9	ns	<0.0001	ns
Geraniol	15.5, 24.4	ns	Ns	ns	53.1, 55.9	0.0035	ns	ns
Limonene	12.4, 18.0	ns	Ns	ns	24.4, 37.5	Ns	0.00032	ns
Linalool	23.0, 37.1	0.011	0.03	ns	37.8, 65.4	<0.0001	<0.0001	ns
Phenylacetaldehyde	28.2, 41.8	<0.0001	<0.0001	ns	36.3, 55.8	<0.0001	<0.0001	ns
2-Phenylethanol	17.0, 36.9	0.0003	<0.0001	0.004	28.1, 59.3	<0.0001	<0.0001	0.0032
Z-3-hexenyl acetate	10.6, 16.6	ns	0.011	ns	25.6, 36.9	ns	0.02	ns
E-2-Hexenal	13.4, 20.2	ns	0.00017	ns	34.6, 44.6	0.0037	0.05	ns
Magnet®	26.6, 36.5	<0.0001	<0.0001	ns	35.0, 56.4	<0.0001	<0.0001	ns
Blank	13.8, 19.4	ns	Ns	ns	28.6, 40.9	ns	ns	ns

Table 3. Summary of R-analysis, test chemical vs canola blank control. *The format x,y are for unmated (x) and mated (y) female moths.

In comparison with both the the blank and canola blank controls, the two factors, test attractant and mated status, and their interaction were statistically significant with 2-phenylethanol only. Compared to the blank control, % positive responses were significantly different between mated and unmated moths tested to anisyl alcohol, β-caryophyllene, cineole and phenylacetaldehyde. On the other hand, such responses were significantly different between mated and unmated moths tested to anisyl alcohol, butyl salicylate, β-caryophyllene, cineole, linalool, Magnet®, phenylacetaldehyde, Z-3-hexenyl acetate and E-2-hexenal when compared with the canola blank control.

We also compared the responses of mated females with unmated females which had been confined with another moth for an equivalent period to that which the mated females spent with males, to determine whether it was mating *per se* that influenced responsiveness in the olfactometer, or just confinement with another moth. Four groups of females were tested in the olfactometer using canola blank only – mated paired with male, unmated paired with female, unmated paired with male, and unmated not paired. There was no significant difference between these groups of females, suggesting it is mating which is the crucial factor influencing their responses in the olfactometer (Fig. 5).

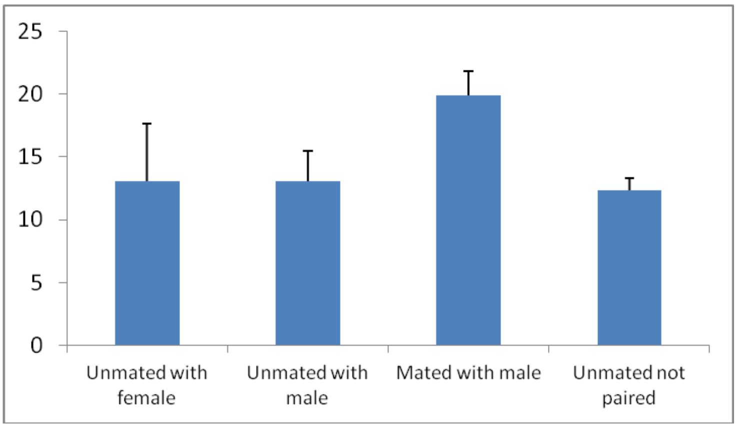


Fig. 5. % test response to canola blank of females confined with other moths.

These results suggest that mated moths are both more active in the olfactometer and more responsive to most plant volatiles than unmated ones, and therefore it may be difficult to improve much on the existing Magnet® formulation as an attractant for mated moths, and hence, probably little scope for improving on Magnet® for enhancing oviposition in refuges. In consultation with AgBitech, we decided that this, combined with the difficulty of registering products containing new compounds, and the limited market for refuge enhancement, meant that it was not worthwhile continuing the work to get the targeted 20 volatiles. We did however test in the field some variations on the Magnet® formulation which would not pose major regulatory difficulties (see Objective 5).

Objective 3: Conduct field trials on selective placement and timing of Magnet® attract-and-kill for refuge manipulation

Auscott trial

Moth numbers were lower in the Magnet®-treated and adjacent untreated fields compared with those in the distant untreated fields (Fig. 6). Similarly, there were lower egg counts in both the Magnet®-treated (Field 50) and adjacent Bollgard II® fields compared to control Bollgard II® fields located approximately 10kms away (Fig. 7). The number of Bollgard II® survivors (medium and large larvae) in Field 50 and adjacent fields was approximately a third of that in the distant Bollgard II® control fields (Fig. 8). However, the application of insecticides which killed moths to conventional fields has complicated spatial analysis of the data. This suggests that in future it will be difficult to conduct these experiments where there is a mosaic of conventional and Bollgard II® cotton.

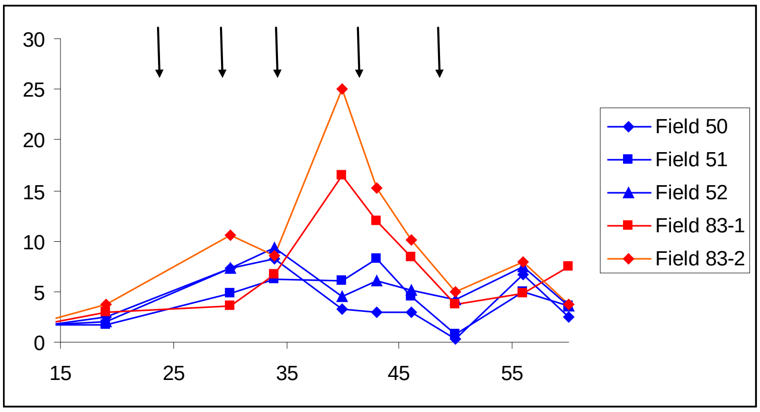


Fig. 6. Moth counts per 50m (by flush counts), in Magnet®-treated (Field 50), untreated adjacent fields (Fields 51 and 52) and distant untreated fields (Fields 83-1 and 83-2), Auscott, 2008. Black arrows = Magnet® sprays.

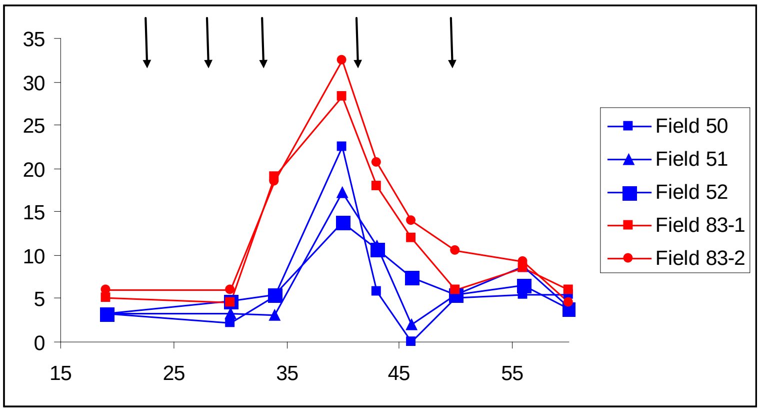


Fig. 7. Egg counts per meter in Magnet®-treated (Field 50), untreated adjacent fields (Fields 51 and 52) and distant untreated fields (Fields 83-1 and 83-2), Auscott, 2008. Black arrows = Magnet® sprays.

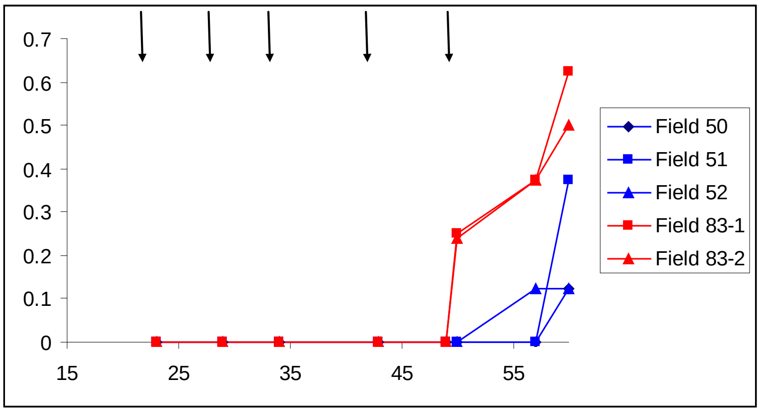
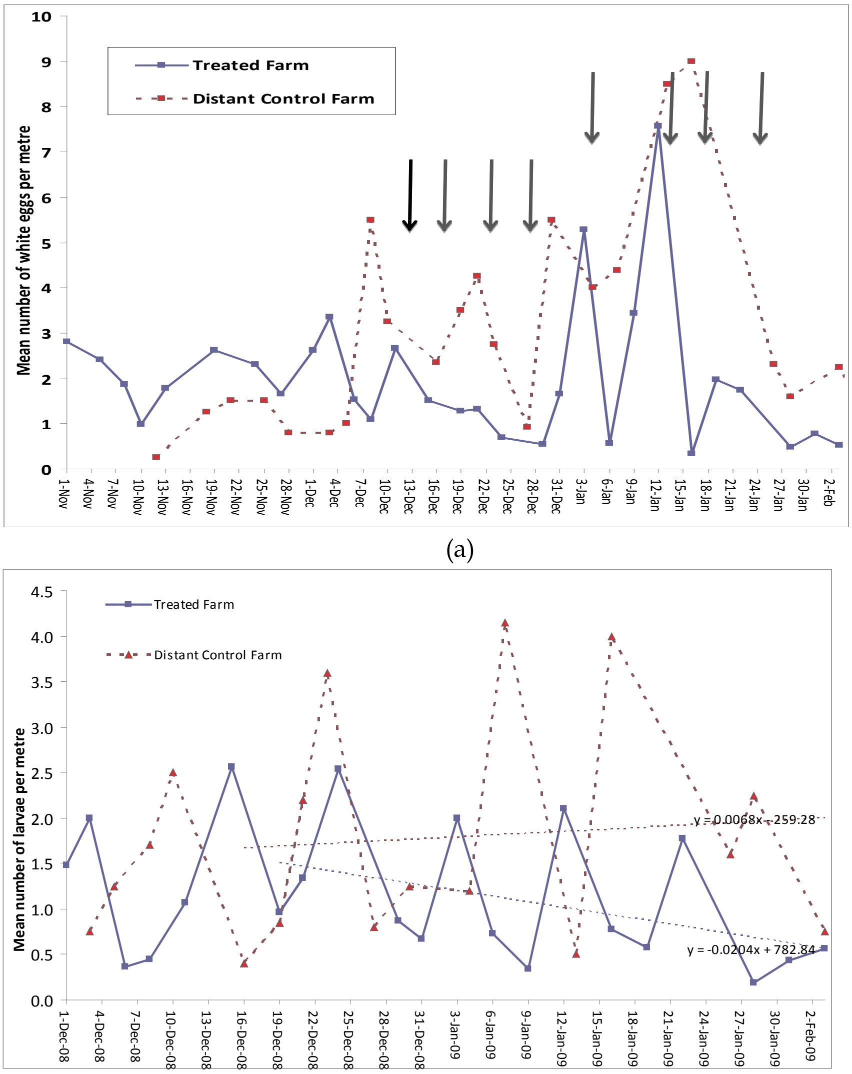


Fig. 8. Surviving larvae per meter in Magnet®-treated (Field 50), untreated adjacent fields (Fields 51 and 52) and distant untreated fields (Fields 83-1 and 83-2), Auscott, 2008. Black arrows = Magnet® sprays
Red arrow = pyrethroid spray on conventional cotton

"Lynora Downs" trial

While successful as a commercial trial of Magnet® on conventional cotton, as were many similar trials in the previous Project 1.05.02, the "Lynora Downs" trial did not provide much information on spatial variation in impacts of Magnet®. Counts of eggs and larvae for the treated farm compared to the distant control farm are shown in Fig. 9.



(a)

(b)

Fig. 9. Counts of eggs (a) and larvae (b) per metre of row on the treated farm, "Lynora Downs" compared to an untreated control located 20 km away.

From the perspective of a commercial trial the results were encouraging. Egg and larval numbers were consistently lower on the treated farm compared to the distant control farm. There also appeared to be a pattern in which egg numbers on the treated farm declined immediately after each Magnet® application (Fig. 9a), and the only occasions when egg numbers on the treated farm approached those on the distant control farm were on 3 January and again on 12 January. Both of these occasions were when planned Magnet® applications were delayed by rain. This suggests that there was a population of moths moving into the treated farm on frequent occasions but that they were being killed by Magnet® before they could lay many eggs, except on occasions when the residual effect of Magnet® was wearing off and re-application was delayed. The numbers of larvae, despite considerable fluctuations were also higher in the treated farm, and regression analyses showed that the data were best described using two separate regression lines, the trend of which was up in the control farm, but down in the treated farm.

These results are consistent with earlier commercial trials of Magnet® (Project 1.05.02), and provide further support to the hypothesis that numbers of larvae surviving in Bollgard II® might be suppressed by Magnet® (through reduction in egg lay), as shown in the Auscott trial. They also support the conclusions that the residual effect of Magnet® is only about 1 week, as indicated by the Bellata trial.

Attempts to find a gradient in egg lay from one end of the untreated area on "Lynora Downs" to the other were not successful. As shown in Fig. 10, there were no clear trends in egg numbers on the gradient from Field 4 (nearest the Magnet®) to Field 2 (furthest away). The only occasion on which such a trend could have been present was following Day 75 (15 January) after the 6th - 8th applications. Unfortunately we were not provided with replicate egg counts for each field, only the field means that were given to the grower, so we were not able to statistically test this result.

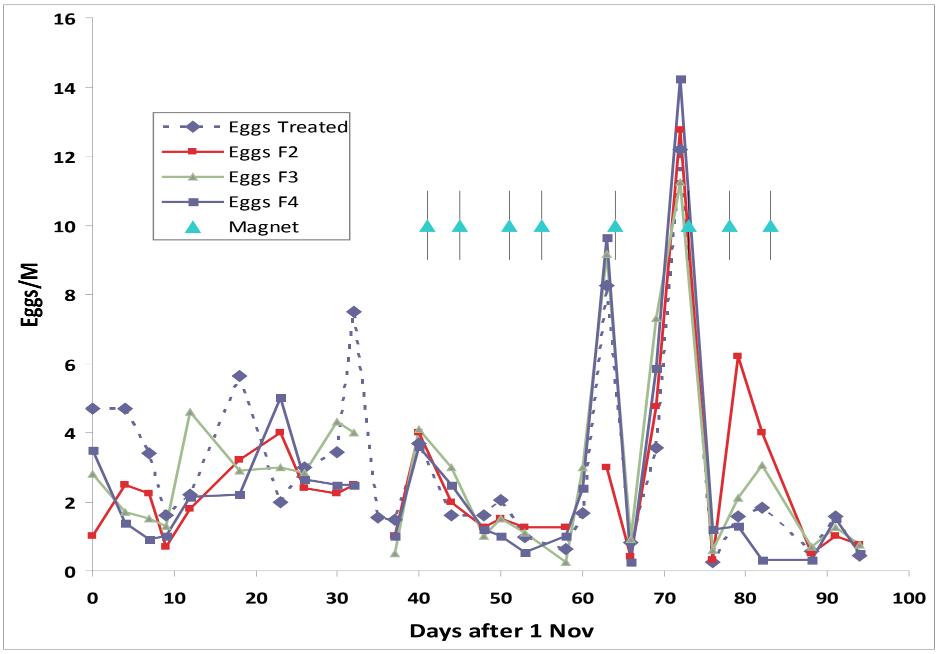


Fig. 10. Egg numbers on different fields in the treated farm at "Lynora Downs". The treated area is the mean of four treated fields, F2-F4 are untreated fields, with F4 the closest and F2 the furthest (about 1.0 - 1.5 km) from the treatment.

Comparing this result with the comparison between the treated farm overall and the distant control farm, it appears likely that, with the possible exception of late in the experiment, the frequent and large applications of Magnet® produced area-wide impacts that swamped any potential spatial differences within the treated farm.

Objective 4: Initial field trials on modified Magnet® formulations for enhancing oviposition

"Yarral" trial

Moth numbers were very low during this trial (as also found in our work on the Summer Scholarship project of Joel Eulenstein (Project 5.10.03.27), done nearby at a similar time). Moth numbers killed in the various treatments are shown in Fig. 11.

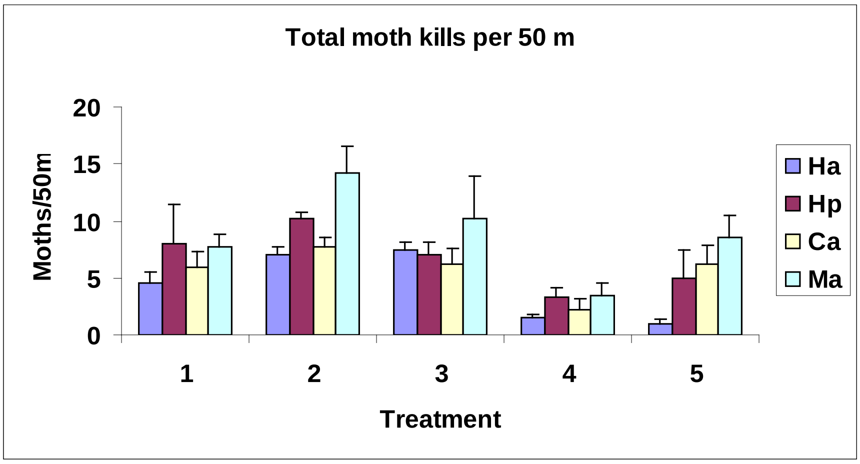


Fig. 11. Moth kills in the "Yarral" mung bean trial. Ha = *Helicoverpa armigera*, Hp = *Helicoverpa punctigera*, Ca = *Chrysodeixis* spp. (mostly *C. argentifera*), Ma = *Mocis alternata.* Treatments: 1 = Magnet®, 2 = Magnet® without α-pinene, 3 = Magnet® without cineole, 4 = Magnet® with 2-phenylethanol instead of phenylacetaldehyde, 5 = Magnet® plus Magnafloc®. Bars are s.e's. of the means.

There were slightly more *H. punctigera* than *H. armigera*, and similar numbers of *Chrysodeixis* spp. (loopers; minor pests of both cotton and grain legumes). There were also significant numbers of the bean looper, *Mocis alternata* (Walker), which is a new record as a potential Magnet® target. Among these species, the only statistically significant difference was for *H. armigera*, where treatments 4 (2-phenylethanol) and 5 (Magnafloc®) performed significantly worse than the three other Magnet® variations. There was however a trend for the 2-phenylethanol formulation to kill fewer moths of all species. A possible reason for this is that, although 2-phenylethanol is very attractive in the olfactometer (Fig. 4 and Gregg *et al.* 2010b), in the field it is highly volatile and rapidly lost from Magnet® deposits on leaves. Similarly, the reason for lower numbers in the Magnafloc® treatment might be the formation of a "skin" which, while increasing rainfastness, also affects the release rate of the volatiles. If so, it is curious that only *H. armigera* seems to have been affected.

The composition of the *Helicoverpa* spp. killed, in relation to sex and mated status, is shown in Fig. 12.

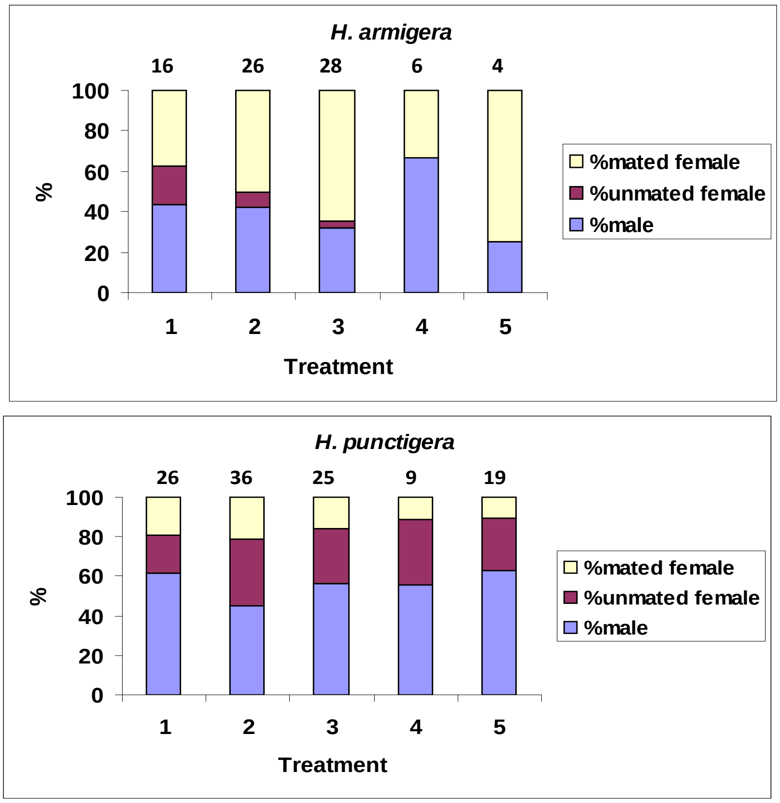


Fig. 12. Percentages of males, unmated females and mated females in dissected moths from the mung bean trial. Treatment numbers are as for Fig. 2. Numbers above the columns are total number dissected.

In all treatments, the female *H. armigera* population was dominated by mated moths, while that of *H. punctigera* was dominated by unmated ones. Males were generally more abundant in the *H. punctigera* population. Chi-square analyses, from which total catches < 10 were excluded (Treatments 4 and 5 for *H. armigera*, and Treatment 4 for *H. punctigera*) did not reveal statistically significant differences in the proportion of mated vs unmated females.

These results provide further support for the belief that it will not be easy to obtain formulations which are better than Magnet® at attracting mated female moths for refuge enhancement (although it may be worth trying Magnet® without cineole again, when more moths are present). They also suggest that 2-phenylethanol cannot be substituted for phenylacetaldehyde, and that further work is necessary to evaluate Magnafloc® for rainfastness and attractiveness. Finally, Magnet® formulations lacking either cineole or α-pinene performed at least as well as conventional Magnet®, and may warrant further trials in conditions where moth numbers are higher.

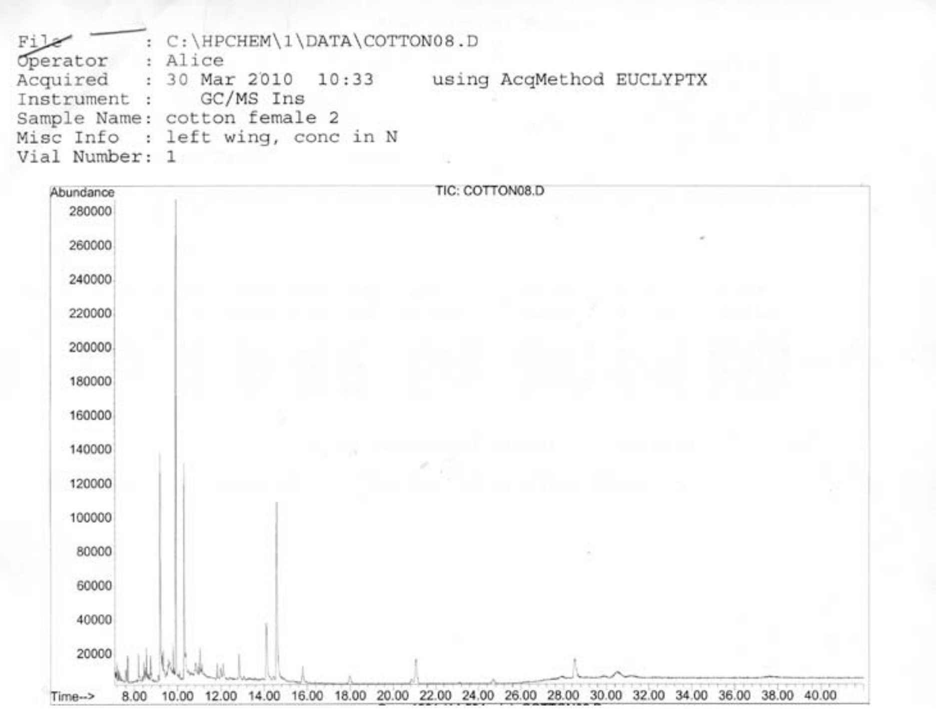
Objective 6: Development of bioassays for oviposition stimulants, and preliminary identification of non-volatile compounds which enhance oviposition

Despite conducting about 20 different experiments, we found no consistent and statistically significant differences in oviposition on blotting paper, filter paper or paper towel treated with extracts of plants compared to blank paper. We considered that this reflects changes in oviposition behaviour that occur when female moths are confined in small cages or oviposition arenas, compared to natural environments. In these situations moths will lay on a variety of highly unnatural surfaces, including glass and metal. This appears to be the case whether the moths are single or in groups. Dominic Cross (CRC Project 1.01.35) has recently reported the same problem in small field cages. It may also reflect laboratory adaptation to laying on unnatural surfaces in long term cultures. We note that Robert Mensah (CRC Project 1.05.01) got consistent oviposition preferences in 30 cm buckets, but in our hands this was not successful, and in subsequent projects (CRC 1.05.04) Mensah moved to whole plant studies in mesh houses, an option which was not available to us. Hence we dropped this aspect of the work.

Objective 7: Development of novel techniques for identifying the host origin of moths killed by Magnet

This work was begun late in the project, and was intended as a preliminary investigation which might reveal any obvious biochemical markers of host origin, but which might have to be continued if the question proved complex. We analysed more than 100 samples of moth and leaf extracts. Each extract typically revealed 30-50 peaks on the GC-MS output (e.g. Figs. 13 and 14). These peaks were often identified as alkanes, both saturated and unsaturated, and alkane derivatives, usually in the C_{24} to C_{38} range. Such compounds typically occur in the cuticular waxes of insect wings and bodies (Piskorsky *et al.* 2010). Despite extensive searches, we were not able to find any compound which always occurred in moths reared on one host but not on another. The same compound could often be identified in some but not all moths from every host. We also tried using ratios between pairs of the more prominent compounds, but no consistent trends could be found.

We identified peaks using the NIST library of reference compounds included in the GC-MS software, but often the matches were poor. Also, compounds with different retention times were frequently identified as the same chemical. These problems are often overcome by alternative means of analysis, and by more sophisticated sample preparation, but this requires the expertise of organic chemists. With our very limited organic chemistry background, it was deemed necessary to seek expert assistance to continue the work. In collaboration with Ben Greatrex (Chemistry Dept, UNE) this work has been continued through another one-year CRC project (Project 1.05.13) A separate Final Report will be submitted for this project.



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Acquired    : 30 Mar 2010  10:33     using AcqMethod EUCLYPTX
Instrument  :    GC/MS Ins
Sample Name: cotton female 2
Misc Info   : left wing, conc in N
Vial Number: 1
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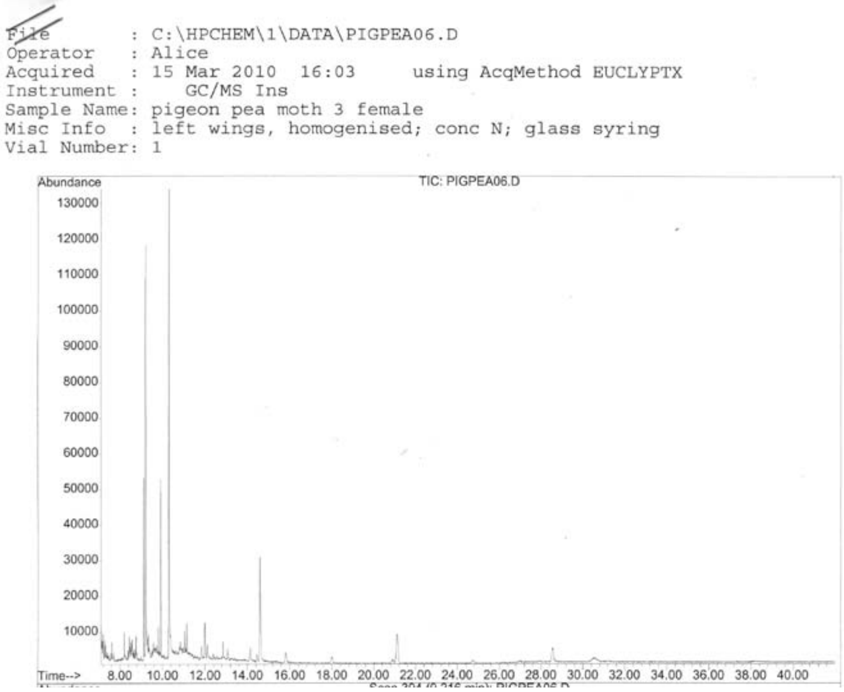
Fig. 13. GC-MS output from an extract of the wings of an *H. armigera* moth reared on cotton. Each peak represents a separate compound, and there are many additional peaks that are too small to be revealed in this vertical scale



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Instrument  :    GC/MS Ins
Sample Name: pigeon pea moth 3 female
Misc Info   : left wings, homogenised; conc N; glass syring
Vial Number: 1
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Fig. 14. As for Fig. 13, but for a moth reared on pigeon peas. Some compounds are in common with the cotton moth above. Others are unique in this specimen, but were not consistently found in all pigeon pea moths.

Objective 8: Conduct further field trials on selective placement and timing of Magnet attract-and-kill for refuge manipulation

Bellata trial

About 90% of the *Helicoverpa* spp. moths killed were *H. armigera*, and there were no clear trends between treatments or dates of application in species composition. Similarly there were no clear trends in sex ratios or mated status between treatments, so for analysis they were all pooled as "*Helicoverpa*". Data were transformed $\log_{10}(x+1)$ and subjected to analysis of variance followed by Fisher's Pairwise Comparison Tests, using Minitab®.

A summary of the mean numbers of moths killed after the initial and subsequent treatments is shown in Tables 4-7. Treatments followed by the same letter are not significantly different (Fisher's Pairwise Comparison of Means, $P > 0.05$).

Treatment	**Total *Helicoverpa***	**Total Others**
1. Magnet®	52.5 ± 22.5 a	18.8 ± 1.9 a
2. Magnet®	54.5 ± 18.2 a	20.8 ± 2.0 a
3. Magnet®	56.5 ± 10.9 a	22.5 ± 3.0 a
4. Blank	8.3 ± 1.0 b	0.5 ± 0.3 b

Table 4.. Mean numbers of total *Helicoverpa* and other moths per 50 m killed after initial treatment (January 8). One side of the row counted on one day.

The first application (Table 4) killed large numbers of moths. We know that searching both adjacent furrows recovers about a third of the total moths killed, and that Magnet® continues killing for 4-6 days. Since we only counted moths on one side of the treated row, for one day only, it is likely that total kills were of the order of 1000 moths per replicate in each Magnet® treatment and 150 in each blank, i.e. about 12,000 *Helicoverpa* spp. moths and 4,000 other moths over the whole experiment. The latter were mostly other pest noctuids such as cutworms (*Agrotis* spp.), common armyworm (*Mythimna convecta*), sugarcane armyworm (*Mythimna loreyimima*) cluster caterpillar (*Spodoptera litura*), Asian soybean looper (*Thysanoplusia orichalcea*) and semi-looper (*Trigonodes hyppasia*) .

As expected, Treatments 1-3, which were all fresh Magnet®, killed similar numbers of moths, and many more than Treatment 4, the blank. Dead *Helicoverpa* moths were 6-7 times more abundant in these treatments than in the blank, and in the case of other species (most of which do not respond to sugar alone) the ration was approximately 40 x. In summary, the results of this application indicate that Magnet® performed as would be expected when large numbers of moths were present.

Moth kills from the second application are shown in Table 5.

Treatment	**Total *Helicoverpa***	**Total Others**
1. Magnet®-Magnet®	46.3 ± 10.3 a	20.3 ± 1.4 a
2. Magnet®-Magnet®	43.8 ± 7.3 a	19.3 ± 2.9 a
3. Magnet®-Blank	20.5 ± 1.7 b	4.8 ± 0.5 b
4. Blank-Blank	13.5 ± 2.7 c	3.5 ± 1.0 b

Table 5. Mean numbers of total *Helicoverpa* and other moths per 50 m killed after second treatment (January 15). Both sides of the row counted for two days.

Moth kills were lower than after the first application. Considering that both sides of the rows were counted for two days, it is likely that total kills in the fresh Magnet® treatments (1 and 2) were of the order of 400 *Helicoverpa* spp. moths per replicate, and about 150 other moths.

As expected, there were no differences between the two fresh Magnet® treatments (1 and 2), but both were significantly different from the blank (Treatment 4), with kill ratios of around 3.5 for *Helicoverpa* spp. and 6 for other moths. The key result was for Treatment 3, which involved blank formulation applied 1 week after Magnet®. It killed a little less than half the moths killed by the fresh Magnet® in treatments 1 and 2, and about 50% more than the blank control (Treatment 4). However, the difference from the blank was not statistically significant. This indicates that there may have been some residual attraction from the volatiles of the previous week, but the lack of statistical significance makes it hard to be sure. There were no significant differences in other moth species killed between Treatments 3 and 4, indicating that residual volatiles were not influencing them.

Moth kills following the third application are shown in Table 6. There was a further decline in the total kill from the previous week, in all treatments.

Treatment	**Total *Helicoverpa***	**Total Others**
1. Magnet®-Magnet®-Magnet®	10.5 ±2.3 a	14.8 ± 4.0 a
2. Magnet®-Magnet®-Blank	6.0 ±1.2 ab	2.8 ± 1.1 b
3. Magnetü-Blank-Blank	6.3 ±1.0 ab	1.5 ± 0.9 bc
4. Blank-Blank-Blank	5.8 ±1.4 b	0.3 ± 0.3 c

Table 6. Mean numbers of total *Helicoverpa* and other moths per 50m killed after third treatment (January 22). Both sides of the row counted for two days.

For *Helicoverpa* spp. the only treatment to be significantly different from the blank was the fresh Magnet® treatment, and even in this case the kill ratio was less than two to one, which is lower than we normally expect from Magnet® trials. For other moths, both the fresh Magnet® and Magnet® one week old (Treatment 2) killed significantly more moths than the blank. Kill ratios were much higher than for *Helicoverpa* spp. However, these comparisons should be treated with extreme caution because the numbers were very small, especially in the blank (Treatment 4) where only one moth was found from all four replicates combined. There appeared to be no attraction from Magnet® applied two weeks ago (Treatment 3 vs Treatment 4) for either *Helicoverpa* spp or other moths.

Moth kills following the fourth application are shown in Table 7. The numbers killed fell further from the third application, to very low levels. Hardly any *Helicoverpa* were found, and numbers were too low to show a statistically significant difference from the blank for any treatment, even for fresh Magnet®. Slightly higher numbers of other moths were found, and there was a significant difference from the blank for Treatment 1 (fresh Magnet®), but not for Treatment 2 (Magnet® 2 weeks ago) or Treatment 3 (Magnet® 3 weeks ago).

Treatment	**Total *Helicoverpa***	**Total Others**
1. Magnet®-Magnet®-Magnet®-Magnet	1.5 ± 0.5 a	5.0 ± 0.7 a
2. Magnet®-Magnet®-Blank-Blank	0.8 ± 0.3 a	0.5 ± 0.3 b
3. Magnet®-Blank-Blank-Blank	0.3 ± 0.3 a	0.5 ± 0.3 b
4. Blank-Blank-Blank-Blank	0.8 ± 0.5 a	0.5 ± 0.3 b

Table 7. Mean numbers of total *Helicoverpa* and other moths per 50m killed after final treatment (January 28). Both sides of the row counted for one day.

The results are difficult to interpret in relation to the main question of this experiment - do residual volatiles from Magnet® application remain attractive after the insecticide wears off, i.e. for a week or longer? This is because of two confounding trends: the rapid decline in moth numbers, and the decline in relative attractiveness of Magnet®, as the experiment progressed. The decline in moth numbers is shown in Fig. 15. Note the log scale on the Y axis - the declines are not linear.

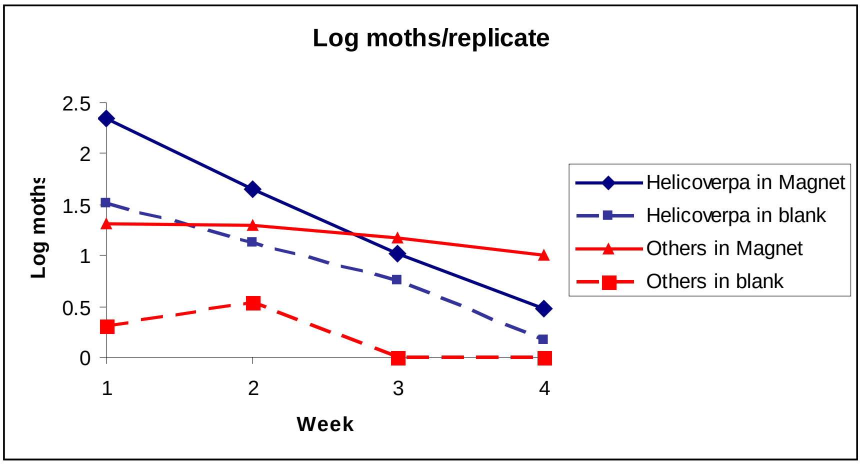


Fig. 15. Log_{10} of moth numbers per replicate. Data from Tables 1-4, corrected for the number of days of sampling and sides of the row collected, for *Helicoverpa* spp. and other moths, in fresh Magnet® and blank treatments.

The decline in numbers was more marked for *Helicoverpa* spp. than for other moths. The most likely explanation is a regional decrease in moth numbers, since at around the same time consultants throughout the Namoi and Gwydir regions were reporting major reductions in egg pressure. However, given the large numbers of moths killed in the first, and to a lesser extent the second application, the possibility that the trial itself depleted local moth populations cannot be excluded.

The decline in relative attractiveness of Magnet® can be seen in the narrowing of the gap between fresh Magnet® and blank as the experiment progressed (Fig. 15). The kill ratios for *Helicoverpa* started at about 7:1 (better than usually seen in Magnet® trials) and finished at less than 2:1 (worse than usual). There appeared to be no such decline for other moths, which suggests that the product itself was performing as usual (though very low numbers in weeks 3 and 4 in the blank mean that this conclusion is tentative). It seems more likely that the effect involved some change in the *Helicoverpa* moths (e.g., progressive ageing of the population) or their environment (e.g., progressively increasing competition from volatiles released by cotton flowers as the crop developed).

While these trends cloud the effects of time on the attractiveness of Magnet® volatiles, it is possible to draw some comparisons of kill ratios between blank and Magnet® after 1, 2 or 3 weeks (Table 8). In making these comparisons it is necessary to discard some kill ratios because they were based on extremely small numbers (one moth only in either the blank or old Magnet® treatments). When this is done it can be seen that all the kill ratios were close to one, indicating lack of residual activity of the volatiles. Only for the one week separating the first and second applications was there any suggestion of increased kills in the old Magnet® treatments, and even this increase (40-50%) was not statistically significant.

Age of Magnet®	**Treatment Comparison**	**Week**	**Kill ratio *Helicoverpa***	**Kill ratio others**
1 week	Treatment 3 vs Treatment 4	2	1.5	1.4
1 week	Treatment 2 vs Treatment 4	3	1.0	9.3a
2 weeks	Treatment 3 vs Treatment 4	3	1.1	5.0a
2 weeks	Treatment 2 vs Treatment 4	4	1.0	1.0
3 weeks	Treatment 3 vs Treatment 4	4	0.4a	1.0

Table 8. Kill ratios for aged Magnet® and blank treatments following different applications. a = estimate unreliable due to very low numbers. In no case were the treatments different, indicating that no kill ratios were significantly different from 1:1.

This trial was conducted primarily to address the claims of Mensah and Macpherson (2010) that Magnet® can continue to attract, but not kill, moths up to 3 weeks after application, and that these moths might lay eggs on treated fields, leading to higher egg pressure. This claim has the potential to affect Magnet® usage, both for conventional cotton and in resistance management for Bt cotton.

While the results are difficult to interpret due to declining kills and an apparent decline in the responsiveness of *Helicoverpa* spp. moths to the Magnet® as the trial progressed, the results provided no evidence to support the hypothesis of residual attractiveness for periods longer than one week, and only inconclusive evidence to support the hypothesis of a relatively weak residual attraction after just one week.

We used moth kills from feeding on blank (no-volatile) treatments as the measure of residual attraction, whereas Mensah and Macpherson (2010) used egg lays. Given that Magnet® is a feeding rather than oviposition attractant, and that previous studies have indicated only modest increases in oviposition immediately following treatment with Magnet® without insecticide (Addison 2010, Gregg *et al.* unpublished), it seems likely that feeding would be a much more sensitive indicator of residual attractiveness than oviposition. It therefore appears highly unlikely that Magnet® with insecticide might inadvertently increase oviposition on Bollgard II® cotton and so increase selection pressure for resistance, and that attract and kill with Magnet® should be considered as a tool for managing resistance in Bt cotton.

Chico wheat trial

This was a small-scale trial in which short strips of wheat were treated with Magnet® and blank formulations. Searching conditions for finding dead moths were poor due to extensive stubble on the ground. Hence total moth kills were low. A total of 47 *H. armigera*, 29 *Mythimna convecta* (common armyworm), 22 *Mythimna loryemima* (sugarcane armyworm), 18 *Agrotis infusa* (common cutworm), 10 *Thysanoplusia orichalcea* (soybean looper) and 28 *Spodoptera exigua* (beet armyworm) were collected across all the treated plots. There were no *H. punctigera* and a small number of other moths including *Agrotis munda* (brown cutworm) and pyralids were also collected. While relatively low, these kills should be interpreted in the light of very low general noctuid moth activity in the region at the time.

Mean numbers of moths collected per 30m for each treatment are shown in Fig. 16. For most species there were highly significant differences between the Magnet® and blank treatments. No *H. armigera* or *M. convecta*, and only very small numbers of *M. loryemima* and *A. infusa* were found in the blank-treated rows.

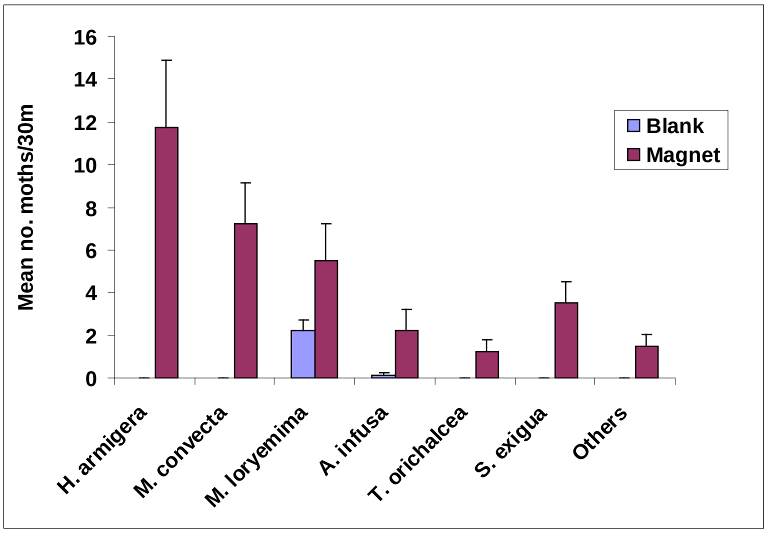


Fig. 16. Mean + SE number of moths collected per 30m.

Data analysis was done by Kruskal-Wallis nonparametric tests, because of the large number of zeros from the blank treatments. Results of the Kruskal-Wallis tests for the different species are given in Table 9. Except for *M. loryemima*, the Magnet® treatment was significantly different from the blank treatment for all species. Thirty one per cent of *H. armigera* and 57% of *M. convecta* killed were females.

Species	**P value**
H. armigera	0.001
M. convecta	0.012
M. loryemima	0.248
A. infusa	0.024
T. orichalcea	0.036
S. exigua	0.001
Other moths	0.036

Table 9. P-values of Kruskal-Wallis tests for different species.

Droplets of Magnet® were retained well on wheat, despite an apparently unfavourable leaf shape. . After two days, it was observed that flag leaves of the plant, which were bent horizontally, could hold large Magnet® drops, smaller droplets were held well on vertical leaves and stems, and leaf auricles can act as reservoirs for Magnet® droplets (Figs. 17-18).

Results of this trial indicate that Magnet® should work well on spring wheat, and an application for registration would be feasible. Overall, the Magnet® treatment killed significantly more moths, of several species, than the blank. Whether Magnet® has a role in pest management in wheat *per se* is uncertain. *M. convecta* is a sporadic but sometimes serious pest of winter cereals. Farmers often do not realise damaging numbers are present until lopping of the wheat heads is seen, by which time substantial damage may have occurred and control by larvicides is not feasible. If combined with a system of monitoring adult armyworms, such as pheromone or fermentation traps, Magnet® might be a way of pre-empting armyworm damage.

From the cotton perspective, the results of this trial indicate that Magnet® applied to wheat will kill significant numbers of *H. armigera*, and this could be valuable in Bt resistance management. Whether the moths killed in this trial emerged from old cotton fields, including the trial field, or whether they moved into the field from other locations is not known. We need a marker of crop origin in moths to answer this question, as with many other questions in resistance management. However, it would seem likely that Magnet® applied to wheat in the spring is likely to disproportionately kill moths emerging nearby, which in the case of non-compliant fields would mostly derive from pupae overwintering from the previous cotton crop, which are potentially resistant to Bt.

Fig. 17. Large droplets of Magnet® on the flag leaf (left) and smaller droplets on vertical leaves and stems (right) two days after application.

Fig. 18. Magnet® pool held around the leaf auricles of a wheat plant, two days after application.

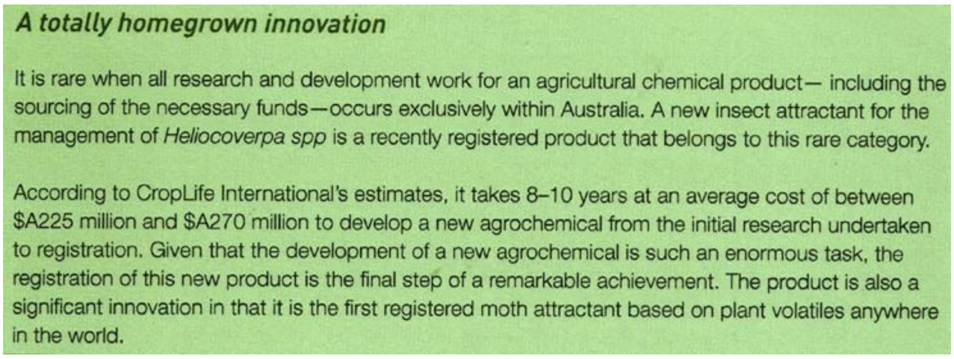
Outcomes

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.

Registration of Magnet®

The major outcome during this project was not listed as one of the objectives, because it was intended to be achieved in the previous Project 1.05.02. This was the registration of Magnet®. The original formulation of Magnet® included five plant volatile components, including the green leaf volatile (*Z*)-3 hexenyl salicylate. All had alternative uses in the food and cosmetics industry, and early discussions with APVMA indicated that they would probably be acceptable. However, unlike the other four, (Z)-3 hexenyl salicylate does not have approval as a food additive by the Flavouring and Extract Manufacturer's Association (FEMA), and this caused APVMA to advise that they would reject it unless we provided extensive mammalian toxicity data. Since this costs over $1 million and takes 3 years, we decided in consultation with commercial partners AgBiTech that we would search for alternative volatiles which might be substituted for (*Z*)-3 hexenyl salicylate, focusing on those which were chemically analogous and had FEMA accreditation. We conducted about 20 small-scale field comparisons of candidate blends in the Burdekin and Darling Downs regions. When we settled on an alternative formulation which had efficacy at least equal to the original formulation, we also conducted suction sampling trials to assess the impacts on general insect fauna of cotton fields, especially beneficial insects, and repeated studies of residues and storage stability. The outcome was a new submission to APVMA for a blend in which (*Z*)-3 hexenyl salicylate was replaced by butyl salicylate and anisyl alcohol. This blend was granted registration in 2009. It is currently registered in cotton, corn and beans, with the addition of small quantities of any of three insecticides, methomyl, thiodicarb or spinosad.

On the basis of this achievement, we were invited to write an Overview paper for *Australian Journal of Entomology* (Gregg *et al.* 2010a). The registration was also highlighted in APVMA's 2009 Annual Report, as shown in Fig. 20:



A totally homegrown innovation

It is rare when all research and development work for an agricultural chemical product— including the sourcing of the necessary funds—occurs exclusively within Australia. A new insect attractant for the management of *Heliocoverpa spp* is a recently registered product that belongs to this rare category.

According to CropLife International's estimates, it takes 8–10 years at an average cost of between $A225 million and $A270 million to develop a new agrochemical from the initial research undertaken to registration. Given that the development of a new agrochemical is such an enormous task, the registration of this new product is the final step of a remarkable achievement. The product is also a significant innovation in that it is the first registered moth attractant based on plant volatiles anywhere in the world.

Fig. 20 Highlight extracted from the Annual Report of APVMA, 2009.

Though most of the trial work necessary for registration of the new formulation was done towards the end of our previous project, 1.05.02, writing reports and liaising with APVMA, our commercial partners and their registration consultant represented a significant distraction for the research team from the objectives of the new project in its first year.

Outcomes relating to the objectives of Project 1.05.10

The outputs we sought from this project included potential new semiochemical products targeting mated female moths, applications to APVMA for registration of Magnet® for resistance management, and recommendations for the use of Magnet® in refuge management and for Bollgard II®/conventional cotton landscapes generally. All of these outputs would contribute to the outcome of improved Resistance Management Plans for Bollgard II® cotton.

Some of these outputs will take longer to realise than the duration of Project 1.05.10, and some have been shown to be not feasible. We do not have new semiochemical products targeting mated moths, because we have shown that the existing product is hard to improve upon. We do not yet have changes to RMPs which incorporate the use of Magnet®, because it is clear that TIMS, Monsanto, APVMA and the cotton research community in general will require more evidence than can be provided in one project before such changes will be adopted. We have however generated data from this project which indicates the feasibility of moth busting, and suggests that some of the potential risks raised by other researchers may not be of serious concern.

There has been debate over the potential value of Magnet® in management of resistance to Bt cotton in *Helicoverpa* spp. We have suggested that Magnet®, with insecticide added, could be applied to Bt cotton late in the season, to attract and kill potentially resistant moths emerging from the cotton. Some concern has been expressed that this may also attract and kill susceptible moths generated by the refuge. We investigated this in the Auscott and "Lynora Downs" trials. Results in both cases suggested that the impact of Magnet® declined with distance from the treated area, though in the case of "Lynora Downs" this conclusion is weakened by the lack of replication in egg count data. This result is similar to those obtained in other commercial trials in earlier projects (e.g. Gregg *et al.* 2005, Grundy *et al.* 2006). Taken together, these results suggest that with careful placement it should be possible to selectively kill moths emerging from Bollgard II® cotton.

The Chico wheat trial suggested the potential for another form of moth busting: by placement on spring wheat on or nearby to old cotton fields , which may be useful either for remediating fields where pupae busting has been inadequate, or as a routine component of RMPs.

Some researchers (S. Downes, M. Whitehouse *et al.*, pers. comm. 2010) have also expressed concern that the volatiles in Magnet® may remain attractive after the insecticide ceases to kill moths, so more moths could be attracted to and lay eggs in Bt cotton than would be the case without Magnet® application. This could increase the pool of larvae exposed to selection pressure, thus accelerating resistance. We have never seen increases in egg pressure (relative to untreated fields) following Magnet® application, either in research trials or commercial applications (e.g. the Auscott and "Lynora Downs" trials in this report). The suggestion that this can happen appears to derive from experiments by Mensah and Macpherson (2010), who found increases in egg lay in Magnet® treated fields compared to controls. These increases occurred 2-3 weeks after treatment. In the Bellata trial we showed that Magnet® did not continue to attract moths beyond about a week, making it likely that the Mensah & Macpherson results occurred by chance in their unreplicated study. Our trial at Bellata failed to find any evidence that would suggest residual attractiveness after the insecticide wears off, a result which is consistent with our greenhouse studies on the dissipation of Magnet® residues, done for registration.

There has been considerable discussion within TIMS and REFCOM on how Magnet® might be used for resistance management in the light of recent trends in resistance allele frequency especially in *H. punctigera*. There has also been interest from grower groups in area-wide Magnet® applications for both resistance management and reducing the *Helicoverpa* pressure in conventional cotton. Peter Gregg and Anthony Hawes are continuing to play active roles in these discussions. On the basis of these results and discussions, CRDC has funded a three year project which will investigate the potential for moth busting in resistance management on an area-wide scale.

6. **Please describe any:-**
   a) **technical advances achieved (e.g. commercially significant developments, patents applied for or granted licenses, etc.);**

   A patent has been taken out on the revised formulation for Magnet® (Gregg PC, Del Socorro AP & Hawes AJ (2008) *Insect attractant composition*. Application No. 2008229734, Filed 1 October 2008.

   b) **other information developed from research (e.g. discoveries in methodology, equipment design, etc.); and**
   c) **required changes to the Intellectual Property register.**

   None

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**?

This project was aimed at determining whether the novel moth attractant technology, Magnet®, can contribute to resistance management plans for transgenic Bt cotton. Progress in the early stages of the project was limited by the need to modify the formulation of Magnet® to overcome regulatory obstacles, and to report on repeated efficacy and environmental impact studies necessary for the registration, but in 2009 registration was obtained. This is the first such product to be registered in the world, and it offers the cotton industry the potential to manipulate moth populations on a broad scale. While most Australian cotton is now Bt, which does not normally require protection from the major lepidopteran pests such as *Helicoverpa* spp., the potential exists to use Magnet® to strengthen current RMPs, or improve new ones.

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In this project we have demonstrated that carefully timed and placed applications of Magnet® in summer can reduce oviposition on Bollgard II® cotton, leading to fewer surviving larvae and this a reduced population of potentially resistant moths. We have also demonstrated that concerns expressed by some researchers that in some circumstances Magnet® might disproportionately attract moths from refuges, or increase oviposition leading to potentially increased selection pressure, are unlikely to be realised.

We have also shown that application of Magnet® to spring wheat can attract and kill *H. armigera* moths, which may allow the use of the product in remediation of poorly pupae-busted cotton fields.

Clarification of the potential for using Magnet® will depend on the development of a method of identifying the host origin of moths killed by the product, or collected from the general population using various trapping methods. We have therefore initiated studies to investigate the potential of biochemical markers, especially cuticular alkanes, to provide this information. This work requires specialised expertise in organic chemistry, and has therefore been continued in another project.

We have also developed the concept of moth busting in late season cotton to directly target potentially resistant moths, and this has been further explored through a one-year preliminary project (reported separately) which studied the technique at the farm scale. This led to the funding of a further three year project to apply the concept on an area-wide scale.

Results from the project are made available to the Transgenic & Insecticide Management Strategies Committee (TIMS) of Cotton Australia, and the industry's scientific forum for resistance management, REFCOM. The project will contribute to improved resistance management for transgenic Bt cotton, but further field testing on area-wide scales over the next three years will be necessary to generate the robust data to support such changes.

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.**
- **(b) for the future presentation and dissemination of the project outcomes.**
- **(c) for future research.**

We have a new three year project funded by CRDC to continue work on moth busting after the end of the CRC. We are also investigating continued funding for the development of markers of host origin, where we have some promising leads but the technology is not yet ready for field application.

The main target audience for adoption of this work will be TIMS, Monsanto and the APVMA, rather than growers directly. Any changes to RMPs that are made as a result of the work will be uniformly adopted throughout the industry, because compliance with RMPs is mandatory. To that end we will continue to advocate for the use of Magnet® in RMPs, to the extent warranted by ongoing research results. This will occur through P. Gregg's membership of the Bt Technical Panel of TIMS, through presentations to REFCOM and through informal discussions with key researchers.

Although growers are not the direct target for extension efforts, we will make the findings of the research widely available to growers, through presentations to the Cotton Conference, articles in industry publications such as *Australian Cottongrower*, and through attendance at field days and other grower meetings. In preparation for the coming area-wide project, which will probably be conducted in the Upper Namoi region, we have made presentations to meetings of the Upper Namoi and Lower Namoi Cotton Growers Associations, and we will be presenting at two field days planned for the Upper Namoi on 5th and 7th June 2012. We are also preparing an article for the next edition of *Australian Cottongrower*.

Publications

9. A. Publications relevant to this project.

Peer reviewed articles / books

Gregg PC, Greive KA, Del Socorro AP and Hawes AJ. (2010a). Research to realisation: the challenging path for novel pest management products in Australia. A*ustralian Journal of Entomology,* **49**, 1-9.

Del Socorro AP, Gregg PC, Alter D and Moore CJ. (2010a). Development of a synthetic plant volatile-based attracticide for female noctuid moths. I. Potential sources of volatiles attractive to *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). A*ustralian Journal of Entomology,* **49**, 10–20.

Gregg PC, Del Socorro AP and Henderson GS. (2010b). Development of a synthetic plant volatile-based attracticide for female noctuid moths. II. Bioassays of synthetic plant volatiles as attractants for the adults of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Australian Journal of Entomology,* **49**, 21–30.

Del Socorro AP, Gregg PC and Hawes AJ. (2010b).Development of a synthetic plant volatile-based attracticide for female noctuid moths. III. Insecticides for adult *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Australian Journal of Entomology,* **49**, 31–39.

Non-peer reviewed articles

Gregg PC & Del Socorro AP (2010) Magnet® - potential roles in management of resistance to Bt. *The Australian Cottongrower*, **31** (3): 22-24.

Anon (2010) The soft attraction of Magnet, *CRDC Spotlight Magazine,* Summer 2010/11 issue.

Presentations (conference, field days, workshops etc)

Del Socorro A, Gregg P & Hawes A (2012). Potential role of a plant-volatile based attractant (Magnet®) for resistance management of *Helicoverpa* spp. in transgenic cotton. *Gordon Conference on Plant Volatiles, Ventura, California USA, 29 Jan- 3 Feb 2012.*

Gregg, P, Del Socorro A & Hawes A. (2012) Development and commercialisation of Magnet® - a plant volatile based attract and kill system for *Helicoverpa* spp. *Gordon Research Conference on Plant Volatiles, 29 January-3 February 2012, Ventura, California.*

Gregg PC, Del Socorro AP & Hawes AJ (2010). A novel attracticide for female noctuid moths, based on plant volatile compounds.*12th IUPAC International Congress of Pesticide Chemistry. Melbourne, Vic. 4-8 July 2010.*

Del Socorro, A. & Gregg P. (2010). Potential role of a new attracticide for resistance management of *Helicoverpa* spp. in Bollgard II® cotton. *The Australian Entomological Society's 41st AGM & Scientific Conference, Perth, WA, September 2010.*

Gregg PC, Del Socorro AP & Hawes AJ (2010) A novel attracticide for female noctuid moths, based on plant volatile compounds. *12th International Congress of Pesticide Chemistry, International Union of Pure and Applied Chemistry, 4-7 July, Melbourne*

Del Socorro A, Gregg P & Hawes A. (2009). Development of an attracticide for heliothine moths: from laboratory bioassay of plants to commercial field trials. *Fifth Asia-Pacific Conference on chemical Ecology. Honolulu, October 26-30.* Abstract O21.

Gregg P, Del Socorro A & Hawes A. (2009). Development of an attracticide for heliothine moths: Regulatory and commercial considerations. *Fifth Asia-Pacific Conference on chemical Ecology. Honolulu, October 26-30.* Abstract O22.

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. *XXIII International Congress of Entomology, Durban, South Africa, July 2008.*

Del Socorro AP & Gregg PC (2008) Semiochemicals for green mirids and *Helicoverpa*: an update. *Northern Farming Systems IPM Forum, Toowoomba, Toowoomba, 25-26 June 2008.* Queensland Department of Primary Industries and Fisheries.

B. All other publications by project team during this period.

Peer reviewed articles / books

Bahar MH, Backhouse D, Gregg P & Mensah R (2011) Efficacy of a *Cladosporium* sp. fungus against *Helicoverpa armigera* (Lepidoptera: Noctuidae), other insect pests and beneficial insects of cotton in *Biocontrol Science and Technology* **21**, 1387-1397

Bahar MH, Stanley JN, Gregg PC, Del Socorro AP, & Kristiansen P (2011) Comparing the predatory performance of green lacewing on cotton bollworm on conventional and Bt cotton. *Journal of Applied Entomology* **136**, 263-270

Lu B, Downes S, Wilson L, Gregg P, Knight K, Kauter G & McCorkell B (2011) Preferences of field bollworm larvae for cotton plant structures: impact of Bt and history of survival on Bt crops. *Entomologia Experimentalis et Applicata* **140**, 17-27

Reddal AA, Sadras VO, Wilson LJ & Gregg PC (2011) Contradictions in host plant resistance to pests: spider mite (*Tetranychus urticae* Koch) behaviour undermines the potential resistance of smooth-leaved cotton (*Gossypium hirsutum* L.) *Pest Management Science* **67**, 360-369

Lowor ST, Gregg PC & Del Socorro AP (2009). Sex pheromones of the green mirid *Creontiades dilutus* (Stal) (Hemiptera: Miridae). *International Journal of Agricultural Research* **4**, 137-145.

Lowor ST, Gregg PC & Del Socorro AP (2009). Potential for pheromone-based attract and kill and mating disruption of the green mirid Creontiades dilutus (Stal) (Hemiptera: Miridae). *International Journal of Agricultural Research* **4**, 153-162.

Khan M., Gregg P & Mensah R. (2009) Effect of temperature on the biology of *Creontiades dilutus* (Stäl) (Heteroptera; Miridae). *Australian Journal of Entomology* **48**, 210-216

Lowor S, Del Socorro AP & Gregg PC (2009) A sex attractant of the rough bollworm *Earias huegeliana* (Gaede) (Lepidoptera: Noctuidae). *Scientific Research and Essays* **4**, 419-425.

Bahar H, Stanley J, Gregg P & Del Socorro A. (2009) Do green lacewings (*Mallada signata*) contribute to the mortality of *Helicoverpa* on transgenic Bt cotton? *Tropentag 2009: Biophysical and socio-economic frame conditions for the sustainable management of natural; resources. October 6-8, 2009.* University of Hamburg, Germany, p.39

Non-peered reviewed articles

Bahar H, Stanley J, Gregg P & Del Socorro A. (2009) Catching *Helicoverpa* survivors on Bollgard II with green lacewings. *Australian Cottongrower* **30** (6) 12-14.

Bahar MH, Stanley JN Backhouse D, Gregg PC**,** Del Socorro A & Mensah R. (2011). Interactions among an entomopathogenic fungus, an insect predator, *Mallada signatus* (Neuroptera: Chrysopidae), and the host, *Helicoverpa armigera* Lepidoptera: Noctuidae), on Bt Cotton . *Entomological Society of America - North Central Branch Annual Meeting,* 14-16 March 2011, Minneapolis, USA. Available on line at http://www.ent.iastate.edu/entsoc/2011/node/521**.**

Gregg P, Downes S, Tann C. & Del Socorro A (2010) *Helicoverpa punctigera* (native budworm) - a puzzle in ecology and resistance management for transgenic cotton. *Australian Entomological Society 41st Annual Conference, 26-30 September, Perth*

Gregg PC (2010) Millimeters to kilometers: insect mobility and its consequences for pest management. *ASSAB 2010 - Annual Conference of the Australasian Society for the Study of Animal Behaviour.* 6-10 April, Narrabri, NSW

Reddal AA, Sadras VO, Wilson LJ & Gregg PC (2010). Predicting spider mite (Acari: Tetranychidae) damage to cotton from a knowledge of their behaviour: a lesson in getting it wrong. *ASSAB 2010 - Annual Conference of the Australasian Society for the Study of Animal Behaviour.* 6-10 April, Narrabri, NSW

Reddall AA, Sadras VO, Wilson LJ & Gregg PC (2010). Choosy mites: preference for water stressed and well watered cotton over two seasons and consequent plant damage. *ASSAB 2010 - Annual Conference of the Australasian Society for the Study of Animal Behaviour.* 6-10 April, Narrabri, NSW

Lu B, Downes S, Wilson L, Gregg P, Knight K & Kauter G. (2010) Do bollworm larvae show behavioural resistance to dual-toxin Bt-cotton? *ASSAB 2010 - Annual Conference of the Australasian Society for the Study of Animal Behaviour.* 6-10 April, Narrabri, NSW

Bahar H, Stanley J, Gregg P & Del Socorro A. (2010) Comparing the foraging behaviour of green lacewing (*Mallada signata)* on transgenic Bt cotton and conventional cotton using *Helicoverpa armigera* eggs or neonate larvae and cotton aphids (*Aphis gossypii*) as prey. *59th Annual Conference of the New Zealand Entomological Society,* 12-14 April, Wellington, NZ.

Bahar H, Stanley JN, Gregg PC & Del Socorro A. (2009) Do green lacewings (*Mallada signata*) add mortality of *Helicoverpa armigera* on transgenic Bt cotton? *Entomological Societies of Canada & Manitoba Conference, Winnipeg, Canada 18-21 October 2009.* A40.

Del Socorro AP Lowor S & Gregg PC (2008) Pheromone trapping for the green mirid *Creontiades dilutus. Australian Entomological Society, 34th Scientific Conference and A.G.M., Orange, 28 September-1 October.*

Gregg PC & Wilson LJ. (2008) The changing climate for entomology. *14th Australian Cotton Conference, Broadbeach, 10-12 August.* Australian Cotton Grower's Research Association, Sydney.

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Addison S. (2010). Enhancement of refuges for *Helicoverpa armigera* (Lepidoptera: Noctuidae) used in the resistance management plan for cotton (*Gossypium hirsutum* L.) containing Bollgard II® traits. *Agriculture, Ecosystems & Environment* 135, 328-335

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

Mensah RK and Macpherson I (2010). Lure-and-kill as reduced-risk strategy for managing *Helicoverpa* spp. on conventional cotton crops within transgenic cotton fields. *Journal of Biocontrol* **24**, 91-103.

Piskorski R, Trematerra P & Dorn S. (2010). Cuticular hydrocarbon profiles of codling moth larvae, *Cydia pomonella* (Lepidoptera: Tortricidae), reflect those of their host plant species. *Biology Journal of the Linnean Society* **101**, 376-384.

R Development Core Team. (2009), *R: A language and environment for statistical computing.* Foundation for Statistical Computing, Vienna, Austria.

Teakle RE & Jensen JM (1985) *Heliothis punctiger*. In Singh P & Moore RF (eds.) *Handbook of insect rearing, Vol 2.* Elsevier, Amsterdam pp. 313-322.

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

No

Part 4 – Final Report Executive Summary

The Australian cotton industry is now heavily reliant on genetically engineered Bt cotton for the control of *Helicoverpa* spp. and other moth pests. The introduction of this technology has been associated with a reduction in insecticide use of around 80%. The lifestyle improvements and the reduced environmental impacts of pesticides have considerably benefited growers and the community. The industry's social license to operate depends on maintaining these environmental and community credits. In turn, this depends on maintaining the effectiveness of Bt, in particular by avoiding resistance in *Helicoverpa* spp.

The industry has a rigorous and widely adopted resistance management strategy, in which the planting of refuges (crops that breed unselected insects) is a key component. It is important that refuge efficiency is maximized. We need refuges which produce the highest numbers of susceptible moths relative to potentially resistant ones emerging from Bt cotton, and produce them when and where they are required. This project focused on the possibility of improving the effectiveness of refuges, or reducing the number of moths emerging from Bt cotton, by chemical modification of insect behaviour. The development by researchers in this project of the novel moth attractant Magnet® means that we now have the capacity to move *Helicoverpa* populations around on a diverse landscape of Bt cotton and refuge crops, over large distances. This project aimed to translate that capacity into more effective refuge management, to enable reductions in areas required for refuges, or more robust resistance management strategies, or both. It aimed to explore new approaches with an existing product (Magnet®), and develop new products specifically to enhance refuges.

In this project we have demonstrated that carefully timed and placed applications of Magnet® in summer can reduce oviposition on Bt cotton, leading to fewer surviving larvae and this a reduced population of potentially resistant moths. We have also demonstrated that concerns expressed by some researchers that in some circumstances Magnet® might disproportionately attract moths from refuges, or increase oviposition leading to potentially increased selection pressure, are unlikely to be realised. We have shown that application of Magnet® to spring wheat can attract and kill *H. armigera* moths, which may allow the use of the product in remediation of cotton fields which have not been adequately cultivated to destroy overwintering pupae, as required by resistance management plans. We have also developed the concept of moth busting in late season cotton to directly target potentially resistant moths, and this has been further explored through a one-year preliminary project (reported separately) which studied the technique at the farm scale.

Clarification of the potential for using Magnet® or similar products for resistance management will depend on the development of a method of identifying the host origin of moths killed by the product, or collected from the general population using various trapping methods. We have therefore initiated studies to investigate the potential of biochemical markers, especially cuticular alkanes, to provide this information. This work requires specialised expertise in organic chemistry, and has therefore been continued in another project. Results from the project are made available to the Transgenic & Insecticide Management Strategies Committee (TIMS) of Cotton Australia, and the industry's scientific forum for resistance management, REFCOM. The project will contribute to improved resistance management for transgenic Bt cotton, but further field testing on area-wide scales over the next three years will be necessary to generate the robust data to support such changes.