Cotton Catchment Communities CRC

FINAL REPORT

Part 1 - Summary Details

Cotton CRC Project Number: 1.01.68

Project Title: Substitutes for pupae busting - targeting larvae or moths: Pilot study

Project Commencement Date: 1/07/2011 (contract signed 14/10/2011)

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Cotton CRC Program: 1 (The Farm)

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

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

Background

1. Outline the background to the project.

The industry now depends on Bt cotton (Bollgard II®, and in future Bollgard III and possibly others). Adoption levels now exceed 95%. It is likely that, if we lost these products due to resistance, societal objections to pesticide use would prevent widespread return to conventional cotton. While there is no immediate crisis, trends in the frequency of resistant alleles for Cry1Ac and Cry2Ab, in both *Helicoverpa* spp, are concerning. We must maintain rigorous and science-based Resistance Management Plans. Pupae busting by tillage has been a central component of RMPs, providing a source of mortality to potentially resistant insects, unrelated to Bt selection. However, it has disadvantages. Its efficacy is questionable in modern Bt cropping systems, where the crop matures early and surviving *Helicoverpa* larvae may emerge as moths before diapause induction. At present we have no means of targeting these potentially resistant insects. Also, pupae busting is expensive and interferes with farming systems, making it hard to follow cotton with winter crops like wheat. It restricts the implementation of minimum till, which can conserve soil moisture and enhance soil carbon. These difficulties are acute in dryland systems, but also affect irrigated cotton, and will do so more as competition for water and carbon farming opportunities develop. Replacing pupae busting with another Bt-unrelated mortality source would have many benefits. The moth attractant Magnet®, developed by the researchers of this project in the Cotton CRC, offers this possibility. It consists of a blend of plant volatile chemicals, to which is added small quantities of insecticide. In conventional cotton, it has been shown to attract and kill many thousands of moths, with consequent area-wide reductions in egg lay, and little impact on beneficial insects. With good timing and placement, it should be able to preferentially kill potentially resistant moths emerging from Bt cotton. We call this approach "moth busting". The aim of this project was to conduct preliminary studies, to be followed by a larger trial, to determine whether potentially resistant moths might be preferentially killed to the extent that moth busting could be a valuable addition to RMPs, perhaps to the point where it might be able to replace pupae busting.

This project derived from a 3 year Full Research Proposal submitted to CRDC in 2010 as an Expression of Interest for the Cotton CRC extension bid. When the extension bid failed, CRDC declined to fund the FRP, but later commissioned a one-year pilot study which was to lead to another 3 year FRP to be submitted in 2011. That FRP was subsequently funded, so this pilot study (Project 1.01.68) has focused on developing methodology to be used in the larger project, rather than attempting to provide definitive answers to the question of whether moth busting can substitute for pupae busting. We are unlikely to be able to provide such definitive answers in one season.

Objectives

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

The broad objective of the project was to conduct preliminary studies to inform a larger project aimed at determining whether Magnet® and/or conventional insecticides can be used to reduce the numbers of overwintering *Helicoverpa* spp. surviving under Bollgard II®, to the next cotton season, to the extent that pupae busting is not needed.

Specific aims, which overlap with and have been extended into the larger project, were:

(1) to determine whether appropriate placement and timing of Magnet ® can selectively remove moths emerging from Bollgard II® late in the season, or kill them in spring,

This aim has been partially achieved. A farm-scale trial of late season moth busting was conducted on "Milchengowrie" in February-March 2012, and is described later in this report. It was not possible to conduct a spring moth busting trial in this project because the contracts for the project were not signed until October 2011, by which time it was too late to establish such a trial. However, a small-scale trial of spring moth busting was conducted under the related Project 1.05.10, and is described in the Final Report for that project.

(2) to determine whether application of Magnet® and/or conventional insecticides can reduce larval populations contributing to overwintering

This aim has not been achieved, though it may be achieved later this winter, within the larger project. We intended to make opportunistic use of commercial Bollgard II® fields which had been treated either with Magnet® or larvicides to prevent or cure above-threshold numbers in January-March. Numbers of overwintering pupae could be compared with nearby untreated fields. We were unable to do this in 2011 because of the late start of the project. In 2012, mid-late season *Helicoverpa* pressure was very light, and this plus the incessant rain meant there was no use of Magnet® on Bollgard II®. Anecdotal evidence suggests that there were very few larvicides used for this purpose either, but we will investigate this further when CCA data become available, and if suitable fields are identified we will compare pupal numbers in them with those in nearby unsprayed fields.

(3) to develop methods to be used in area-wide moth busting trials

This aim has largely been achieved, although scaling up the methods for a series of area-wide trials may still provide challenges. As detailed below, we have modelled moth emergence from Bollgard II® in relation to diapause induction and defoliation timing, and this has identified a suitable window for late-season Magnet® applications to catch emerging moths. We have also modified methods for aerial application of Magnet® to very late season Bollgard II® crops (it has previously been used mostly on early to mid-season conventional cotton crops). We have developed improved methods for light and pheromone trapping to collect moths

from areas within, adjacent and distant from Magnet® applications, and we have assisted in the development of methods for identifying the host origins of moths killed by Magnet® (CRC Project 1.05.13, Ben Greatrex, UNE). One difficulty has been in developing methods for efficiently and safely collecting dead moths killed by Magnet® in late season cotton. Results from the "Milchengowrie" trial suggest that this can only be done in skip-row cotton, since collecting from closed canopies sprayed by air in late-season conditions (when heavy dew is often present) can present unacceptable risks of insecticide exposure to moth collectors.

(4) to help develop RMPs which avoid the need for pupae busting.

This is a long-term aim of the larger project. It is likely that more than one season of data will be needed to convince TIMS and Monsanto that the concept is feasible. However, we have presented the concepts and preliminary data to a meeting o REFCOM (26 July 2011) and informally to members of the Bt Tech Panel of TIMS.

Methods

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

(a) Modelling studies – emergence of moths from surviving larvae on Bollgard® vs diapause induction and defoliation timing.

Methods used for modelling moth emergence in relation to diapause induction, and diapause induction in relation to timing of defoliation, are described below. They contribute to the objective:

(1) to determine whether appropriate placement and timing of Magnet® can selectively remove moths emerging from Bollgard II® late in the season, or kill them in spring

The aim of this work was to determine optimum times for late season Magnet® application for moth busting, i.e. application times that would catch moths emerging from Bollgard II®.

Pupation, emergence and diapause induction

We obtained data from Sharon Downes on the time of accession (approximately the time of collection) of larvae from Bollgard II® in the Namoi Valley, from 2005-06 to 2010-11. Excluding the first year, in which almost all the larvae collected were in the Very Small category and therefore probably not true Bollgard II® survivors, there were 406 larvae. These larvae were categorised by size (VS, S, M, L) in some years, and by instars in other years (I-V), and in other years the length was measured. We therefore converted all these categories into instars.

Then we plotted frequency histograms against date (days or weeks after June 30) for all instars individually, and for the combined total, (Figs 1 and 2) and derived descriptive statistics (especially quartiles) for the distribution curves using Minitab®.

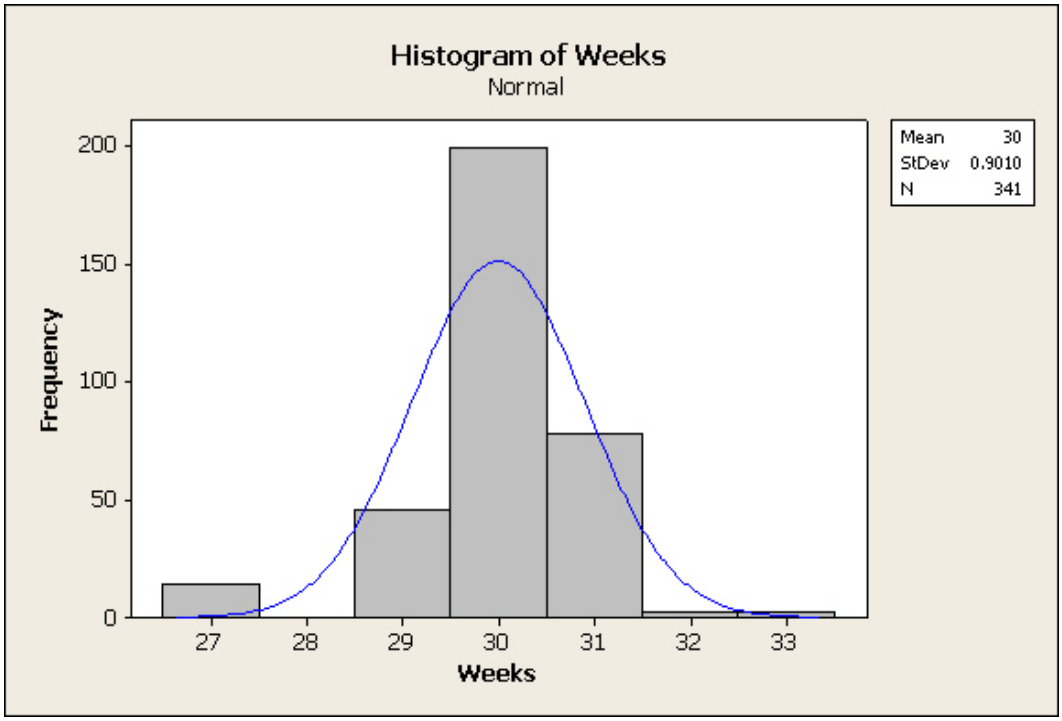


Fig. 1. Histogram of times of collection of Bollgard II® survivors (all instars) in the Lower Namoi Valley, 2006-07 to 2010-11, in weeks after 1 July.

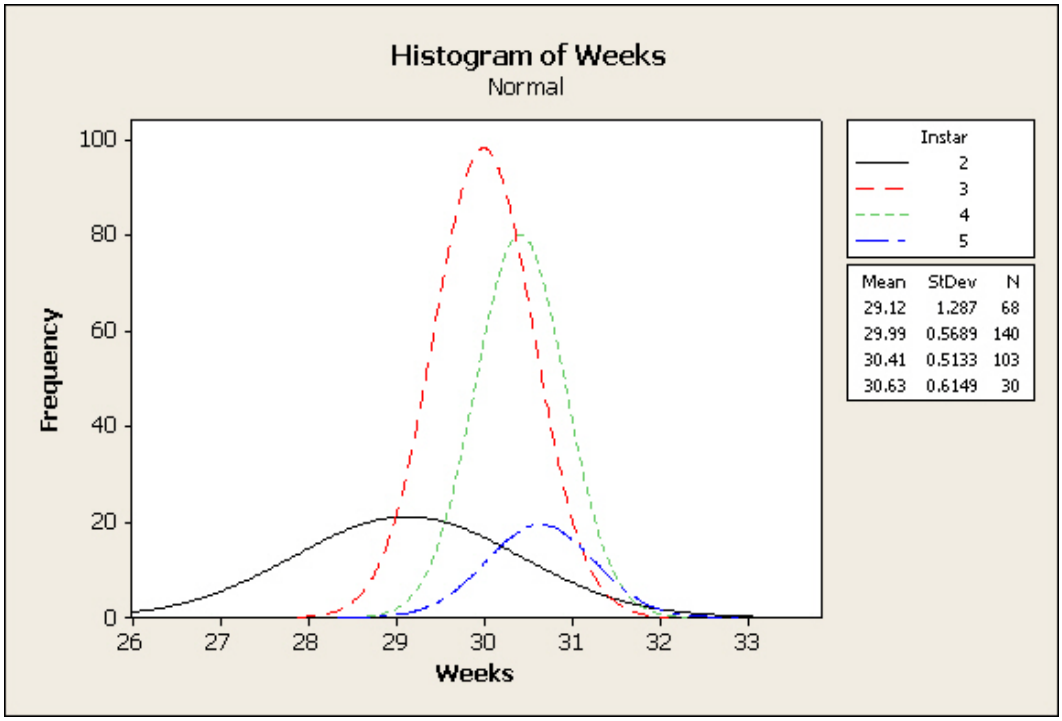


Fig. 2 Distribution curves fitted to the histograms of collection times for individual instars of surviving larvae in the lower Namoi valley, 2006-07 to 2010-11 in weeks after 1 July.

Then we added the following times (Table 1) to allow for completion of development through pupation and moth emergence, based on day-degree modelling using the average day degrees for mid summer in Narrabri:

Instar	Larval time (weeks)	Pupal time (weeks)	Total time (weeks)
II	2.1	2.3	4.4
III	1.7	2.3	4.0
IV	1.3	2.3	3.6
V	0.8	2.3	3.1

Table 1. Additional times to pupation and moth emergence from various instars of Bollgard II® survivors for Narrabri.

This yielded data describing emergence by quartiles (i.e. minimum 0%, first quartile 25%, second quartile 50%, third quartile 75%, and maximum 100% cumulative emergence).

Pupation and defoliation timing

To compare pupation of Bollgard II® survivors with the timing of diapause, we used estimates of cumulative diapause incidence in autumn presented for the Namoi, Macintyre and Darling Downs regions by Dillon (1998), who used the HEAPS model which contains the same algorithms for diapause induction as used in the Diapause and Emergence Tool in CottAssist (this tool is itself not suited for estimating seasonal averages, since it relies on local weather in a particular season).
To compare induction of diapause with the timing of defoliation, we asked leading consultants in the Namoi (Steve Madden), the Macintyre (Iain Macpherson) and the Darling Downs (Murray Boshammer) to estimate the cumulative percentage of dryland and irrigated crops that were defoliated by weekly intervals from the first week in March to the last week in April, in an average year.

b) Preliminary late season moth-busting trail, "Milchengowrie"

Methods used in the farm-scale late season moth busting trial are described below. They also contributed to the objective:

(1) to determine whether appropriate placement and timing of Magnet® can selectively remove moths emerging from Bollgard II® late in the season, or kill them in spring,

The aims of this trial were: (1) to develop methodology for use in larger scale trials, and (2) to collect preliminary data to indicate whether appropriately placed Magnet® could kill proportionately more moths in Bollgard II® cotton than in nearby refuges.

Trial site

The trial was conducted on a relatively isolated part of "Milchengowrie" (a large cotton farm owned by Prime Ag), near Boggabri (Fig. 3).

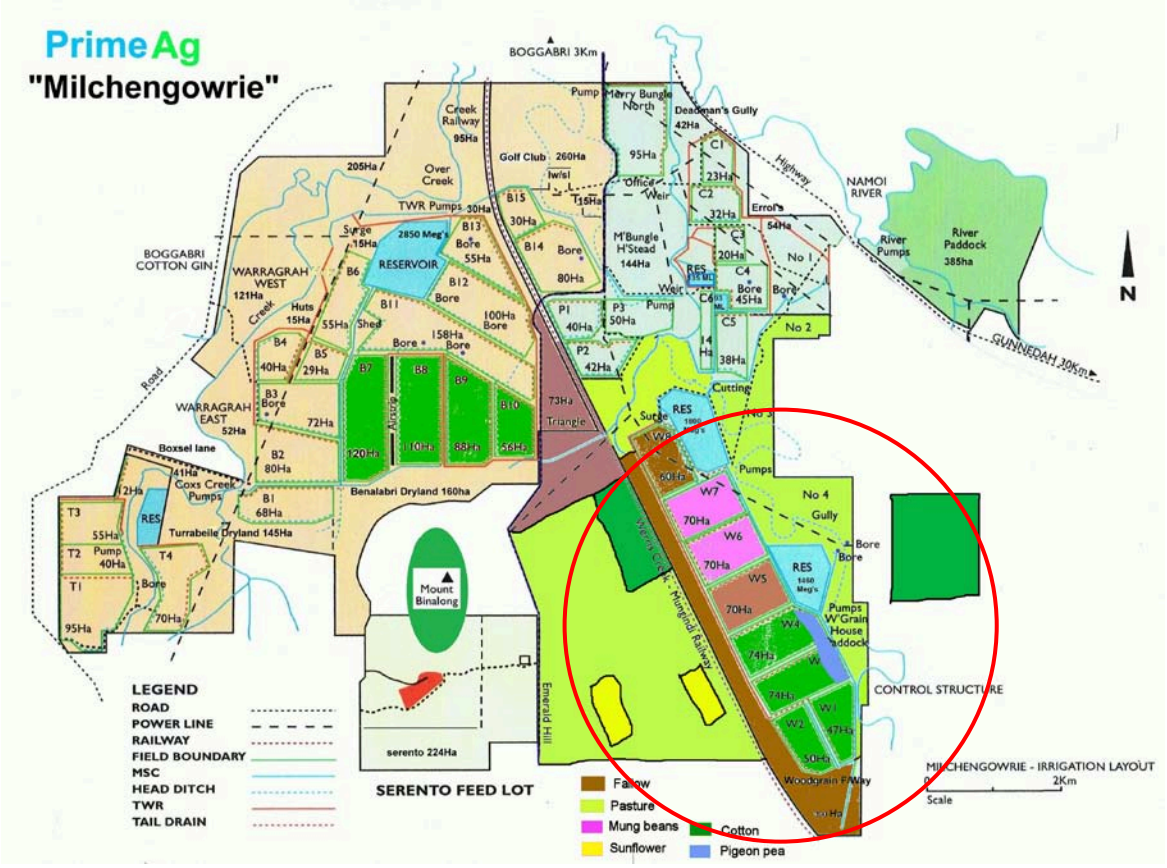


Fig. 3. Farm map of 'Milchengowrie". Red circle indicates boundary of the main study area

All cotton in the study area was Bollgard II®. The treated area comprised Field W2 (skip row, semi-dryland), and the south western halves of Fields W3 and W4 (solid plant, fully irrigated). This meant that the Magnet® was applied as far away as possible from the pigeon pea refuge, which was in the northeast ends of W3 and W4 (Fig. 4).

Moth trapping

During the study populations of adult *H. armigera* and *H. punctigera* were monitored using light and pheromone traps. There were eight trapping sites with examples of each trap type located within the study area (Fig. 4), two in the pigeon pea refuge, two in the solid plant cotton to be treated with Magnet®, two in the untreated solid plant area (adjacent to the refuge), and one each in the treated and untreated skip row areas. An additional four pairs of pheromone traps (but no light traps) were located in more distant areas (2-4 km away, to the northeast, northwest, west and southwest), two in cotton, one in mung beans and one in sunflowers.

Pheromone traps were the conventional Unitrap (canister) design (Gregg & Wilson 1991; Fig. 5a) baited with laminate pheromone lures obtained from Entosol Pty. Ltd. (PO Box 28, Roselands, NSW). Light traps were modified from a design developed by P. Gregg for the PhD project of Olivia Kvedaras (Kvedaras 2002). These traps used a 6w, 12v UV fluorescent tube mounted in an inverted fibreglass cone (airport runway marker) set in a plastic garbage bin (Fig. 5b). Since the light shines upwards, these traps should only catch insects flying above them, and therefore the catch

might represent local populations rather than insects which might have been attracted from some distance by the light. For this project, the fluorescent tubes were replaced by strips of waterproof UV LEDS (Volka Lighting, Point Cook Rd, Point Cook, Vic). Advances in LED design have created the potential for designing light traps which consume less power, and are more reliable, than traditional fluorescent tube designs. For these traps a one metre strip containing 60 LEDS was attached using cable ties to a piece of steel rod shaped into a circle, which fitted into the fibreglass cone (Fig. 5c).

Moths were trapped into 70% ethanol in light traps, and killed by exposure to dichlorvos in household pest strips in pheromone traps. Light trap catches were dissected to determine species, sex and mated status. If more than 20 *Helicoverpa* spp. were trapped, a sample of 20 was dissected, and the proportions of the species and sexes in this sample were multiplied by the total catch to estimate numbers. If less than 20 were trapped, all the moths were dissected. Moths have been frozen for possible later investigation with biochemical markers of host origin (Ben Greatrex, Project 1.05.13).

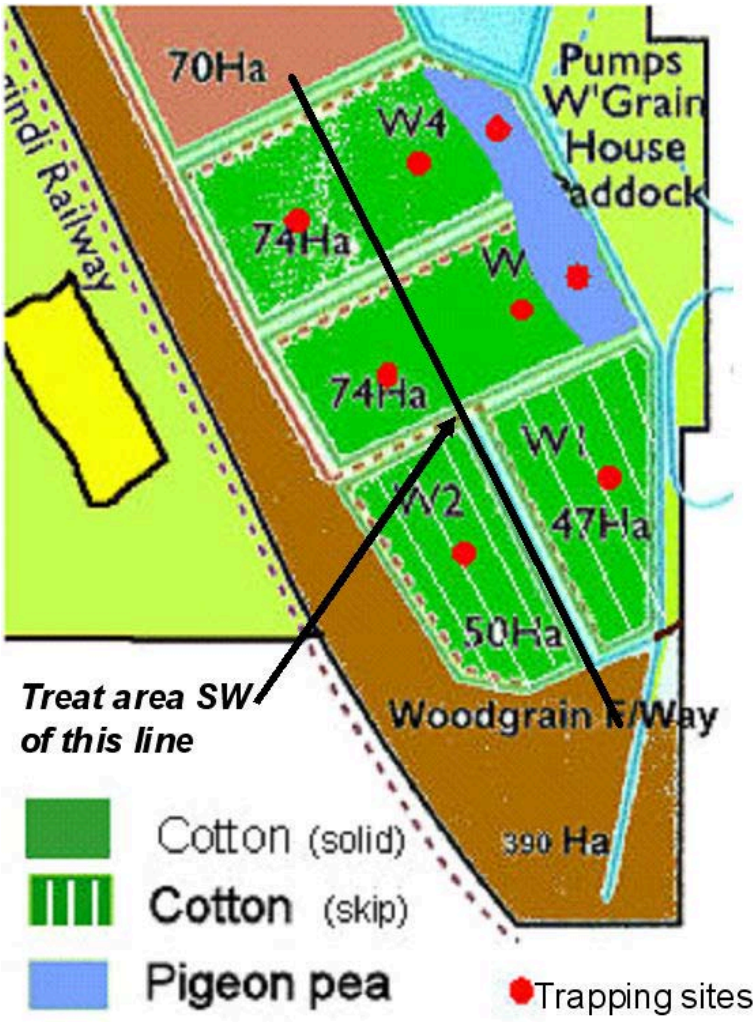


Fig. 4 Detail of study area on "Milchengowrie). Each of the red dot marks a trapping site where one light trap, one *H. armigera* pheromone trap, and one *H. punctigera* pheromone trap were located.

Fig. 5. (a) Unitrap pheromone trap with clear base to show killing agent (dichlorvos strip) - those used in this experiment had opaque green bases). (b) cone type light trap (c) light source for the trap, consisting of a ring of 60 waterproof UV LEDs.

Magnet® application

Magnet® was applied by air on two occasions, late in the afternoon of 24 February and again late in the afternoon of 9 March. It was applied through a pipe attached to the underside of the aircraft (Fig. 6), following methods developed by A. Hawes (AgBiTech), with the assistance of a number of aerial agriculture operators. This produces a band of droplets (1-5 mm diameter) which covers a width of 1-2m, depending on wind and aircraft altitude. For the first application (24 February), the aircraft flew from northwest to southeast across the entire treated area (Fig. 4), which meant that the Magnet® bands were applied parallel to the rows in the solid plant cotton, but across the rows in the skip row, since these were oriented at right angles to those in the solid plant. Five bands, spaced 140 m apart, were applied. For the second application, bands were applied to the solid plant area in the same way, but for the skip row field, the direction of application was changed so as to be parallel to

the rows. This was done to facilitate searching for dead moths (see below). Eight bands, 140 m apart, were applied to the skip row field.
The rate of application was 500 ml per 100 m of band. The insecticide added to the Magnet® was methomyl, in Electra 225® (Farmoz Ltd, St. Leonards, NSW) at a concentration of 0.5% active ingredient.

It had been intended to apply at least three Magnet® applications at weekly intervals, starting about 18 February, as indicated by the modelling described above. However, persistent wet weather prevented this. Magnet® is not rain fast, and since the moth killing effect typically lasts 4-6 days, it is best applied when forecasts indicate several days of dry weather. This rarely occurred during the trial, and in fact heavy rain occurred two days after the first Magnet® application, which probably reduced considerably the impact of that treatment.

Dead moth collection

For early to mid season Magnet® treatments in conventional cotton, the impact of the treatment can be assessed by collecting dead moths from the ground early in the morning, on successive mornings following treatment. Since we know that approximately one third of the total moths killed will be found in the furrows adjacent to the treated row (Del Socorro *et al.* 2010), it is possible to estimate the total moth kill per hectare. However, this proved extremely difficult in late season Bollgard II® cotton. The bulk of the canopy prevented efficient searching of the ground, and high humidity due to both rain and late irrigations meant that the leaves were covered in dew. This made it hard to see the Magnet® droplets, and therefore difficult to identify the location of the band. To ensure the safety of the moth collectors (even though they wore protective clothing), we often had to avoid areas close to the band, which meant that areas which probably had the most dead moths could not be searched. Consequently it was not possible to quantitatively estimate the impact of the treatments, although we were able to collect substantial numbers of moths for later analysis using biochemical markers of host origin. Changing the orientation of the bands on the skip row cotton for the second application made finding dead moths easier and safer, since moth cllectors were able to walk along the side of treated rows without contacting the canopy. However some dead moths which fell between the planted rows would still have been missed.

Statistical analysis

Trap catch data were transformed $\log_{10}(x+1)$ to stabilise the variance, and then subjected to analyses of variance and general linear modelling using the statistical package Minitab®.

(a)

(b)

Fig. 6. Aerial application of Magnet® at "Milchengowrie". (a) Apparatus for spraying consisting of under-wing pipe, (b) spraying in progress.

Results

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

(a) ***Modelling studies – emergence of moths from surviving larvae on Bollgard® vs diapause induction and defoliation.***

Pupation, emergence and diapause induction

Predicted times for pupation and for moth emergence, from each cohort of larvae, are shown in Tables 2 and 3.

Instar	Minimum	1st quartile	2nd quartile	Third quartile	Maximum
II	29.1	31.3	31.1	31.1	32.1
III	30.7	31.7	31.7	31.7	34.7
IV	30.3	31.3	31.3	32.3	32.3
V	30.8	31.8	31.8	31.8	32.8

Table 2 Predicted times (weeks after 1 July) of pupation from larvae surviving on Bollgard II® at Narrabri

Instar	Minimum	1st quartile	2nd quartile	Third quartile	Maximum
II	31.4	33.4	33.4	33.4	34.4
III	33.0	34.0	34.0	34.0	37.0
IV	32.6	33.6	33.6	34.6	34.6
V	33.1	33.1	34.1	34.1	35.1

Table 3 Predicted times of moth emergence from larvae surviving on Bollgard II® at Narrabri

The predicted time for pupation ranged from a minimum of Week 29.1 (or 18th January) for the earliest Instar II larva, to Week 34.7 (or 1 March) for the latest Instar II larvae. The great majority of larvae were predicted to have pupated between Weeks 31 and 33 (4th February - 18th February)

The minimum predicted time of emergence (from the earliest Instar II larvae) was Week 31.4, (or 7th February), and the maximum predicted time of emergence (from the latest Instar III larvae) was week 37.0 (or 28th March). The great majority of moths were predicted to emerge in Weeks 33 to 35 (18th February to 4th March).

The percentage of *H. armigera* larvae entering diapause as pupae is compared with the predicted pupation dates for the Bollgard II® survivors in the Namoi in Fig. 7.

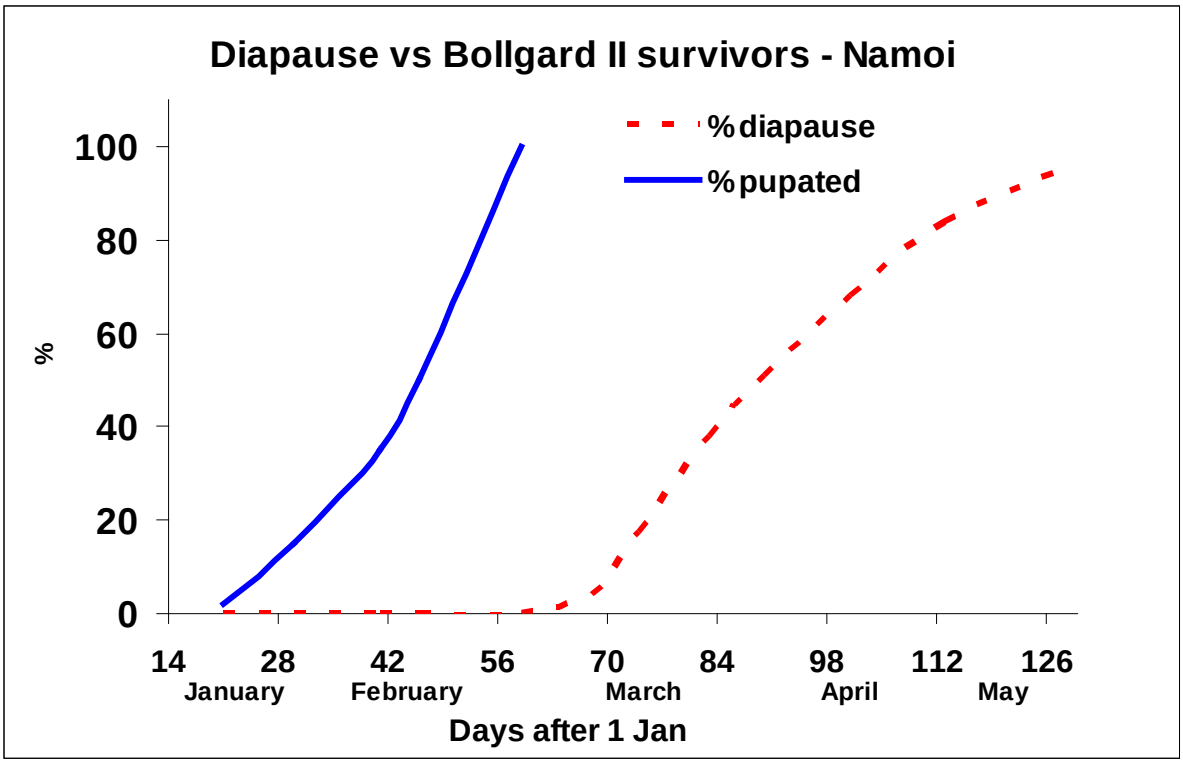


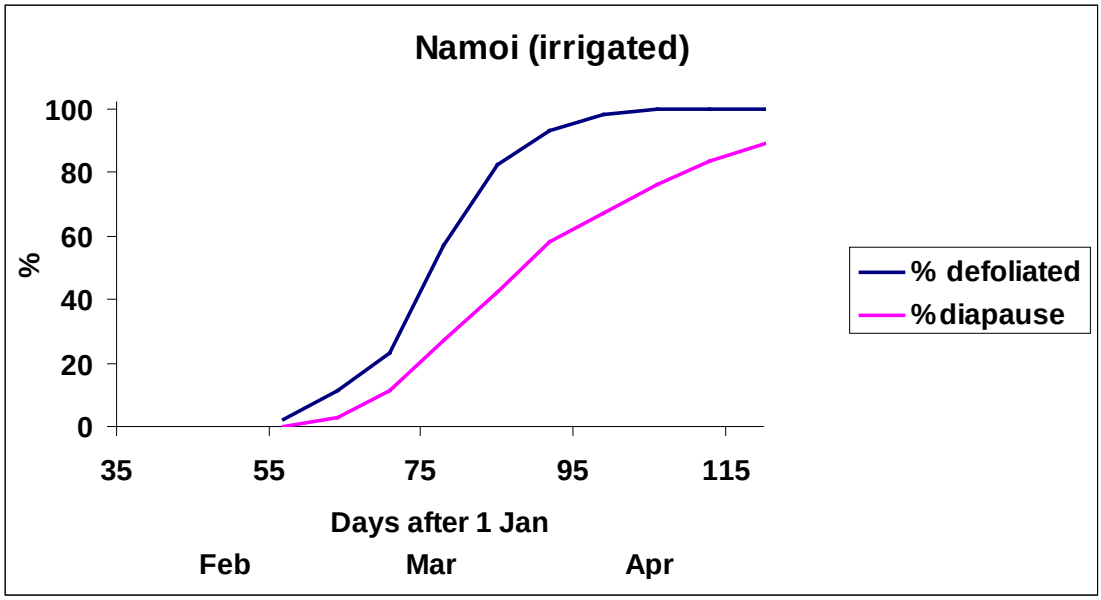
Fig. 7 Predicted cumulative percent pupation compared to diapause induction incidence for the Namoi Valley. Pupation percentages are based on data in Table 2, diapause incidence is from Dillon (1998), for *H. armigera*.

All the larvae from the cohort of survivors were predicted to have pupated before any diapause was induced, and therefore would not contribute to the overwintering diapause population which is targeted by pupae busting.

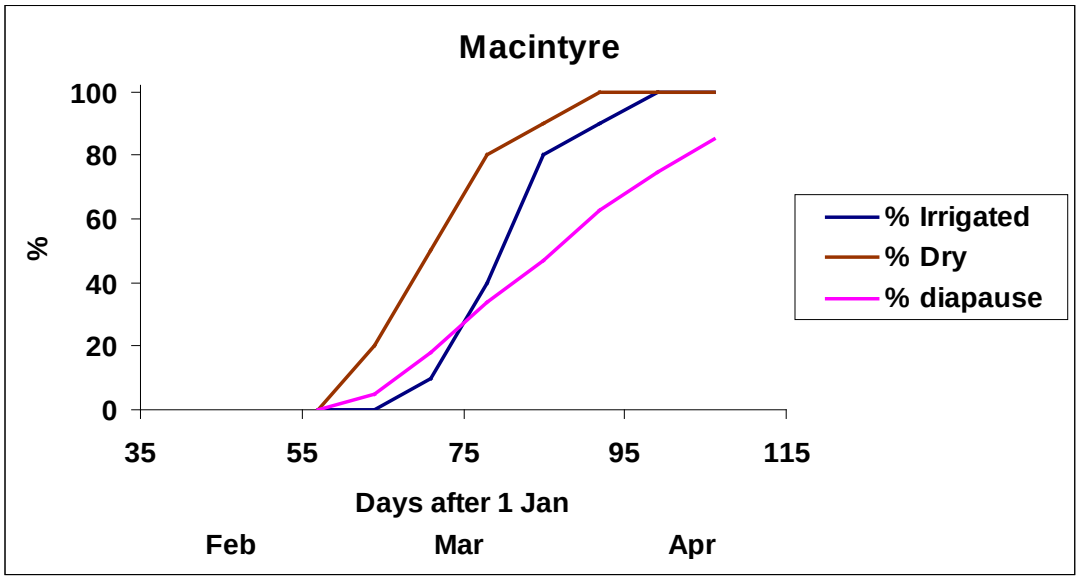
Pupation and defoliation timing

We also compared the timing of defoliation with diapause induction in *H. armigera* for three areas, the Namoi, Macintyre and Darling Downs (Fig. 8). In the areas for which data on dryland defoliation were available (the Downs and Macintyre), defoliation was virtually completed before diapause incidence reached 50%. For all three areas defoliation of irrigated cotton was virtually complete when about 65-75% of larvae were entering diapause.

Consultants who provided the defoliation data commented that these results reflected earlier defoliation of high-retention Bollgard II® crops in modern cotton farming systems, compared with later defoliation when pupae busting requirements were introduced to conventional cotton for the purposes of managing insecticide resistance. They also noted that defoliation times had been later in the two last years, which have been cool and wet, compared with the seasons prior to this, which were generally hot and dry. The dates they gave are approximate averages across all these seasons. Dryland cotton could be defoliated even earlier in very dry seasons.


Namoi (irrigated)
100
80
60
40
20
0
%
35
55
75
95
115
Days after 1 Jan
Feb
Mar
Apr
% defoliated
%diapause

(a)


Macintyre
100
80
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Days after 1 Jan
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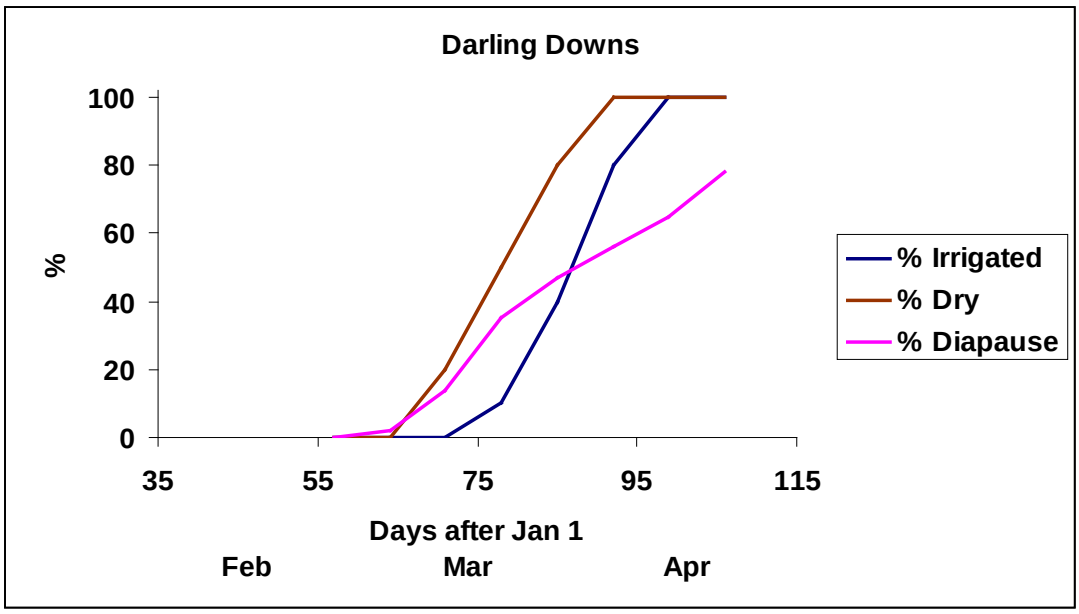
% Irrigated
% Dry
% diapause

(b)


Darling Downs
100
80
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20
0
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35
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75
95
115
Days after Jan 1
Feb
Mar
Apr
% Irrigated
% Dry
% Diapause

(c)

Fig. 8. Defoliation dates in dryland and irrigated cotton in (a) the Namoi (data courtesy of Steve Madden; insufficient data on dryland crops from this region), (b) the Macintyre (data courtesy of Iain Macpherson) and (c) the Darling Downs (data courtesy of Murray Boshammer), compared with diapause induction in *H. armigera* (from Dillon 1998)

Implications of the models

Reports of field damage to Bollgard II® cotton seem to coincide with eggs laid around peak flowering (Whitburn & Downes 2009, Lu, 2010). The timing of larval numbers supports this hypothesis (Fig, 1), and the data also show staggered timing of the successive instars by periods consistent with instar durations (Fig. 2). While the mechanisms of larval survival remain unclear, reduced expression of Bt toxins by some plants around peak flowering seems to be involved (S. Downes *pers. com.* 2011)

While there is as yet no evidence that larvae surviving on Bollgard II® cotton are more likely to be resistant to Bt toxins than the general population, it seems likely that future resistant individuals will come from these cohorts. Thus it would seem advisable to target moth busting applications at the peak period of emergence of these moths, i.e. 18th February to 4th March, in an average year. These are moths from larvae which are unlikely to be entering pupal diapause, because they would be pupating in mid-February (4th-18th), when the diapause induction model shows a very low incidence of diapause in the Namoi valley.

There is a caution surrounding this conclusion, however. We are targeting potentially resistant moths within these cohorts, and because of the fitness costs associated with resistance, these insects may be slower developing. Bird & Akhurst (2007) showed that *H. armigera* larvae resistant to Cry1Ac took on average 4.7 days longer to complete larval development on conventional cotton than did susceptible larvae. There is no evidence of changes to pupal duration, or of delayed development associated with Cry2Ab resistance. It may however be wise to add 5 days to the predicted period of peak moth emergence in order to catch later emerging resistant moths. This would suggest a peak emergence period to be covered by Magnet® applications of 18th February to 9th March, in an average year.

Another approach might be to target moths emerging from larvae which developed later in the season, when Cry1Ac expression may have declined, and larvae are effectively exposed only to Cry2Ab. This would target potentially Cry2Ab resistant moths. The problem with this is that it is not possible to precisely identify this time. Olsen *et al.* (2005) showed that in Ingard® cotton, it could occur as early as mid-December, while Lu (2010) with Bollgard II® cotton showed very little decline in Cry1Ac expression even by mid February. It therefore seems more logical to target moths emerging from the cohort of larval survivors, on the assumption that most resistant moths will be among them. Any later developing larvae are likely to enter diapause, and could be targeted by spring applications of Magnet® to wheat on or adjacent to old cotton land.

Comparisons of diapause induction with survivor pupation dates, and with defoliation times, call into question the effectiveness of pupae busting in modern high retention cotton systems, in which defoliation has been occurring earlier than in the era of conventional cotton. It is clear from Fig. 3 that very few of the cohort of survivors in Bollgard II® will enter diapause. Instead, they will emerge from the

crop and potentially move into refuges, other host crops and non-crop vegetation. Some may migrate substantial distances.

It is also clear from Fig. 4 that many cotton crops, especially dryland ones, will have been defoliated and therefore unable to support continued larval development, at a time when diapause incidence is quite low. This suggests that a substantial proportion of very late season larvae (even later than the main survivor cohort) will also emerge prior to winter, and therefore not be controlled by pupae busting.

It should be noted that these models have used diapause induction data for *H. armigera* only, and *H. punctigera* must also be considered a potential resistance threat in view of recent trends in resistance allele frequency (Downes *et al.* 2010). The seasonal incidence of diapause in *H. punctigera* lags behind that of *H. armigera* (Dillon 1998), so it is likely that pupae busting is even less effective as a resistance management tool for this species than it is for *H. armigera*.

Current Resistance Management Plans have no way of targeting these late season, pre-diapause insects. Growers can apply *ad hoc* larvicide treatments to control above-threshold numbers, which occurs on only 3-18% of the Bollgard II® acreage depending on seasonal pressure (S. Downes, *pers. com.* 2012). In Central Queensland only, late season trap cropping is employed, but is of uncertain efficacy (Grundy *et al.* 2006). While late season refuges provide susceptible individuals to genetically dilute resistance frequencies, this can be a double-edged sword if refuges are being contaminated. It might lead to an increased frequency of heterozygotes which mate to produce resistant homozygotes.

b) Preliminary late season moth-busting trail, "Milchengowrie"

Pheromone and light trap catches

Both the pheromone and light trap catches were dominated by *H. armigera.* In total, 4416 *H. armigera* were caught in pheromone traps across all treatments, and 117 *H. punctigera*, giving 97.4% *H. armigera.* For light traps, the totals were 2528 and 893 respectively, giving 73.9% *H. armigera*. It is likely that the light trap result more closely reflects the ratio in the field. The relative inefficiency of pheromone traps for *H. punctigera*, especially in mid to late season, has previously been noted (P. Gregg, unpublished data). The light trap catches were dominated by males (91.3%, pooled across both species). There were no clear differences in sex ratios between the treatments (refuge, untreated cotton, Magnet®-treated cotton and distant traps), so males and females were combined for subsequent analyses. Pheromone traps, of course, catch only males. The dominance of males in light trap catches has sometimes been noted (e.g, Kvedaras 2002), but is not universal. It is possible that the design of the cone trap, which catches insects flying above it, may bias the catches towards males, because they tend to fly at higher altitudes and cover more area in their search for females, whereas females remain more in the canopy while laying eggs. Since we will need to trap more females in the coming larger project, a critical comparison of this trap with more conventional designs which trap from broader areas, using unshielded light sources, is warranted. However, the relatively high trap catches, low battery drain and reliability of the traps is encouraging. Not a single night's catch was lost from a total of 112 trap nights, despite trying weather

conditions, which compares very favourably with previous experience using fluorescent light sources.

Preliminary analyses showed no significant differences in either pheromone or light trap catches between the solid plant and skip row cotton fields, so traps in these areas were pooled to give three replicates in each of the Magnet® -treated and untreated cotton areas, compared with two in the pigeon pea refuge.

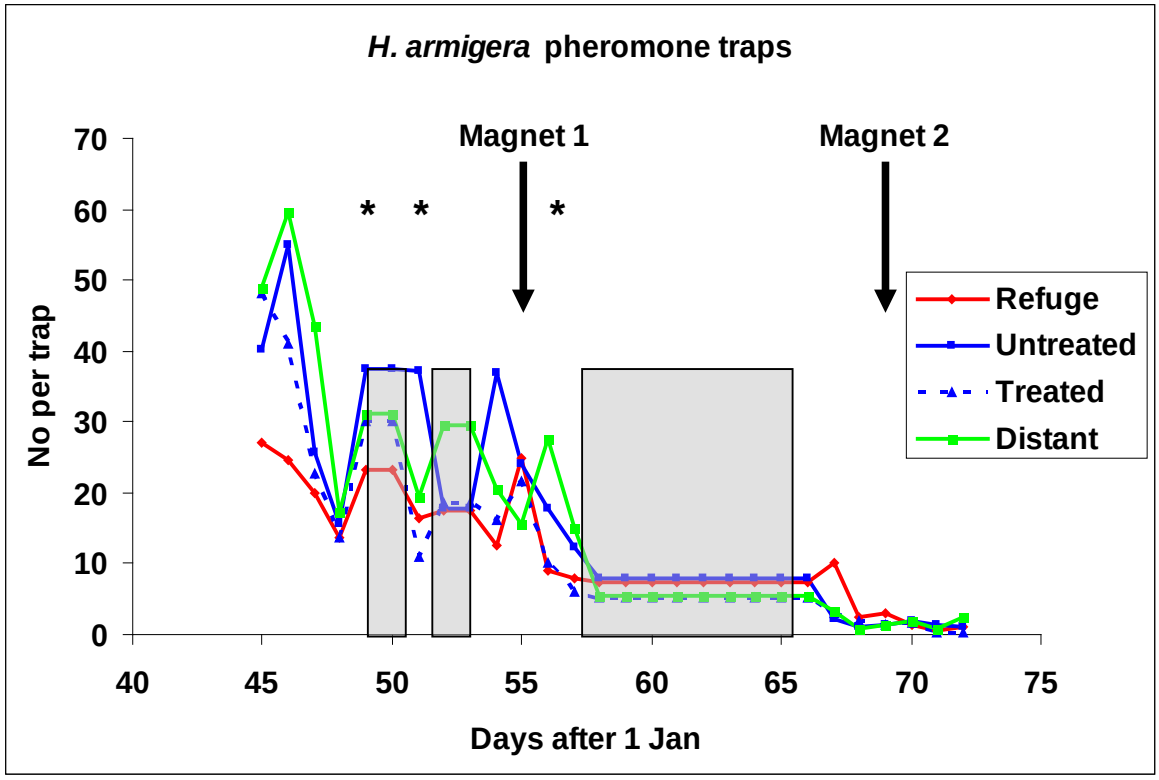

Fig. 8. Pheromone trap catches for *H. armigera* during the "Milchengowrie" trial. Grey areas represent periods when the traps were inaccessible due to rainfall - during these times the traps remained in operation, so the total catch at the next service interval was divided by the number of days since the previous service, and is represented by horizontal lines describing the average catch per day. Asterisks represent individual days on which there were statistically significant differences between treatments. Arrows show dates of Magnet® application.

Pheromone trap catches for *H. armigera* are shown in Fig. 8. A general linear model with treatment (refuge, untreated cotton, treated cotton and distant traps) as a factor and time as a covariate showed that there was a highly significant ($F_{1,188}$ = 245.9, P <0.001) effect of time, due to a decline in numbers trapped as the experiment progressed. There was no significant main effect of treatment ($F_{3,188}$=1.2, P=0.30), and no significant interaction between time and treatment ($F_{3, 188}$=0.9, P=0.44).

Pheromone trap catches for *H. punctigera* are shown in Fig. 9. Although numbers were low and fluctuated considerably from day to day, a general linear model found significant effects of treatment ($F_{3,188}$= 5.6, P=0.001) and time ($F_{1,188}$=42.7, P < 0.001), with an interaction term that was close to significant (P=0.06).

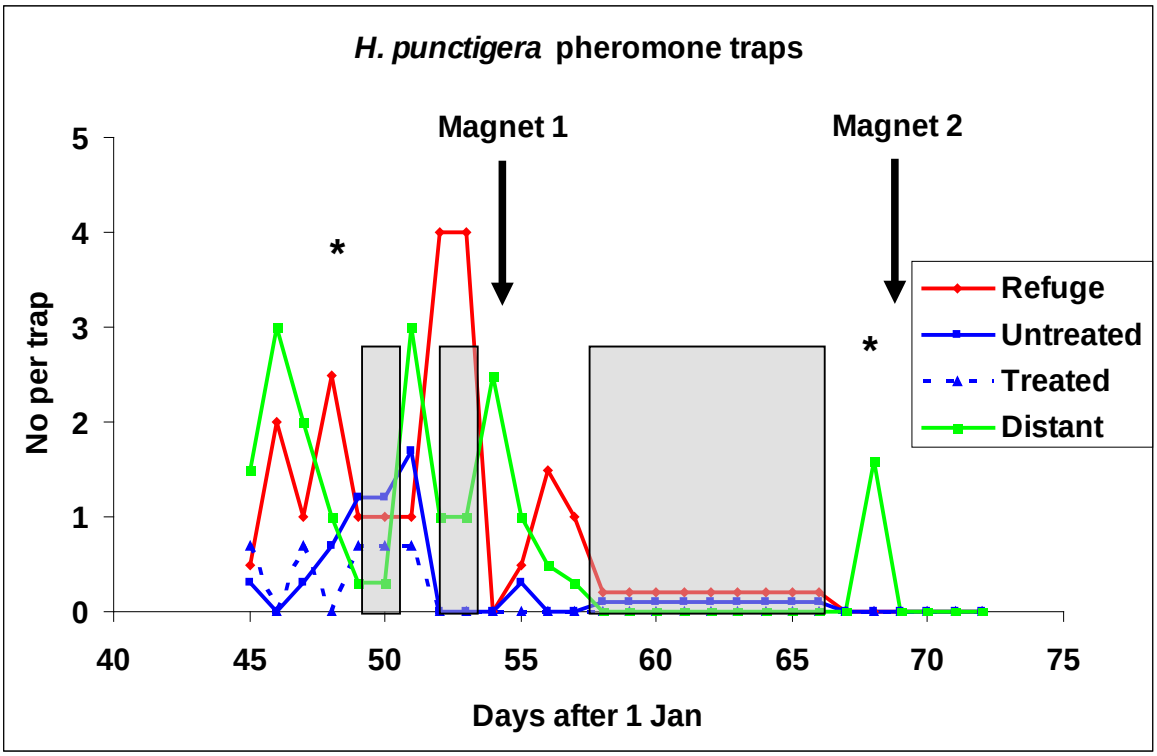

Fig. 9. Pheromone trap catches for *H.punctigera* during the "Milchengowrie" trial. Grey areas represent periods when the traps were inaccessible due to rainfall - during these times the traps remained in operation, so the total catch at the next service interval was divided by the number of days since the previous service, and is represented by horizontal lines describing the average catch per day. Asterisks represent individual days on which there were statistically significant differences between treatments. Arrows show dates of Magnet® application.

Examination of Fig. 9 indicates that these differences were mainly due to higher catches in the refuge and distant traps, especially early in the experiment. These differences were not due to Magnet®, because they were apparent before it was applied. They probably reflect greater attractiveness of the refuges (pigeon pea) and one of the distant trap sites (mung beans) compared to cotton.

Light trap catches for *H. armigera* are shown in Fig. 10. A general linear model revealed significant effects of time ($F_{1,105}$=357.1, P<0.001) and treatment ($F_{2,105}$=3.5, P<0.05), but no significant interaction. Examination of Fig. 10 reveals that the effect of time was due to a decrease in numbers as the experiment progressed (as also shown by pheromone catches), and the effect of treatment was due to higher catches in the refuge compared to the cotton. This effect was likely to be related to greater attractiveness of the crop (pigeon pea) compared to cotton, and not to the Magnet®, since it was apparent before the first application.

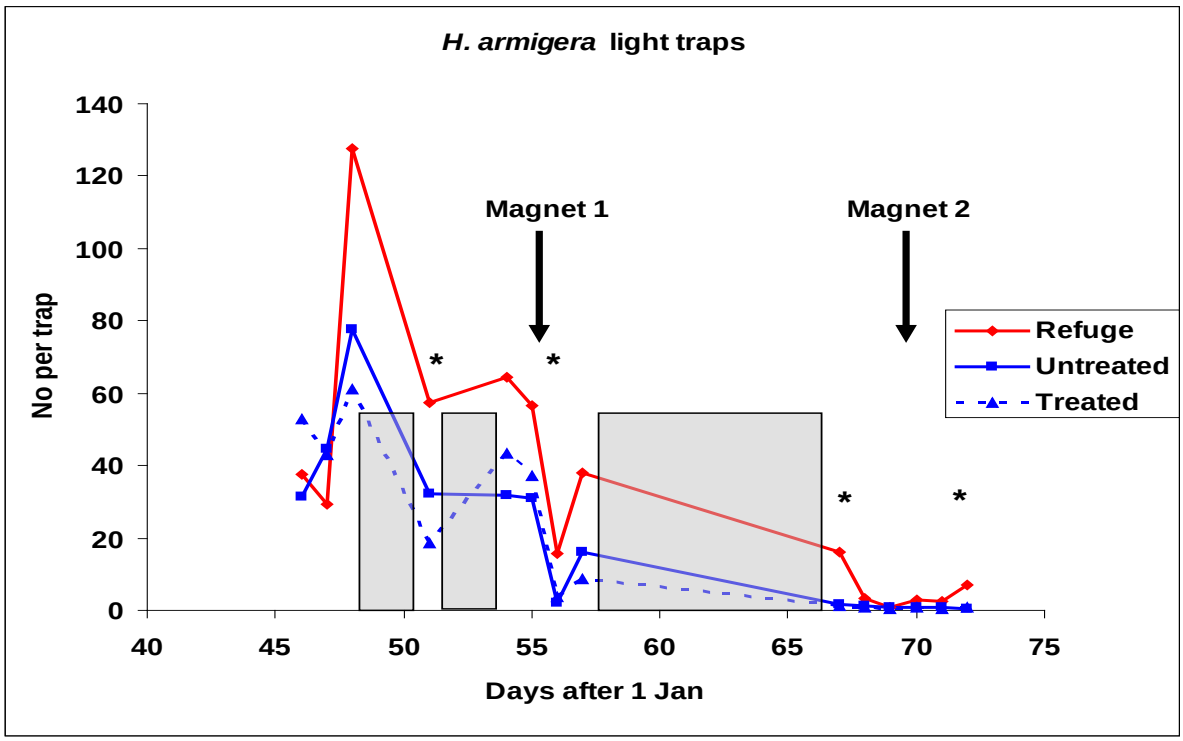

Fig. 9. Light trap catches for *H. armigera* during the "Milchengowrie" trial. Grey areas represent periods when the traps were inaccessible due to rainfall - during these times the traps were switched off. Asterisks represent individual days on which there were statistically significant differences between treatments. Arrows show dates of Magnet® application.

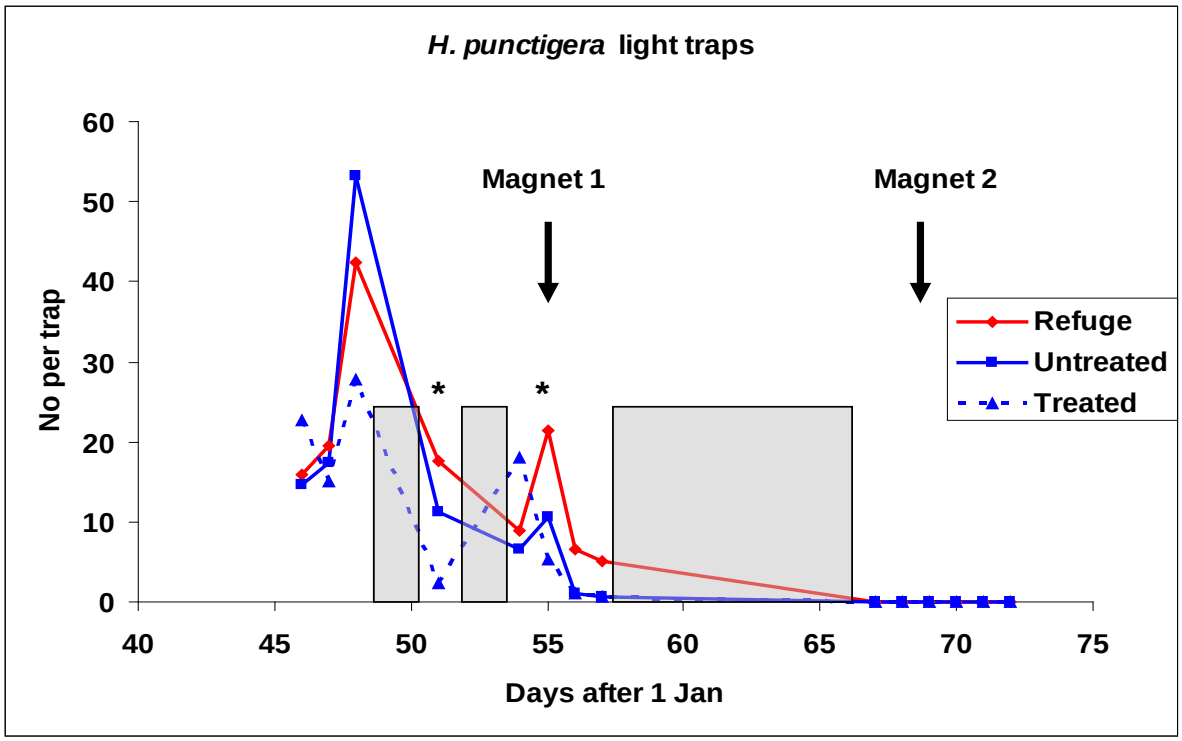

Fig. 10. Light trap catches for *H. punctigera* during the "Milchengowrie" trial. Grey areas represent periods when the traps were inaccessible due to rainfall - during these times the traps were switched off. Asterisks represent individual days on which there were statistically significant differences between treatments. Arrows show dates of Magnet® application.

Light trap catches of *H. punctigera* are shown in Fig. 11. A general linear model showed that there was a significant effect of time ($F_{1,105}$=323.7, P<0.001), but not of treatment, and no significant interaction between treatment and time.

The lack of significant effects attributable to Magnet® in the experiment overall is not surprising because the experiment ran for 38 days, and Magnet® would have been active for only five of these days: two after the first application (Days 55 and 56), before it was washed off by rain, and three after the second application (days 70-72), before the experiment was terminated due to low moth numbers.

To examine potential effects of Magnet® more closely we focused on the period around the first Magnet® application, Days 53-56. This included the two days before the application (Days 53 and 54, 23-24 February) and the two days after it (Days 55 and 56, 25-26 February). These days are sandwiched between two rain periods (Figs. 9 and 10) and therefore represent our best estimates of baseline numbers before Magnet® application, and numbers following application. The catches for these periods were transformed $\log_{10}$ (x+1) and subjected to GLM analyses in which there were two factors. The first factor was treatment (refuge, untreated cotton, treated cotton and distant (in the case of pheromone traps - there were unfortunately no distant light traps due to a shortage of equipment). The second factor was period (before and after Magnet® application). Results of these analyses are summarised in Table 4.

		Statistical summary		
Trap type	**Species**	Treatment	Period	Interaction
Light	*H. armigera*	$F_{2,25}$ = 6.4, P=0.006	$F_{1,2\,5}$= 37.0, P<0.001	$F_{2,25}$ = 1.5, P=0.260
Light	*H. punctigera*	$F_{2,25}$ = 3.4, P=0.049	$F_{1,25}$ = 28.4, P<0.001	$F_{2,25}$ = 0.5, P=0.601
Pheromone	*H. armigera*	$F_{3,38,}$ = 2.2, P=0.100	$F_{1,38}$ = 6.4, P=0.016	$F_{3,38}$ = 3.3, P=0.030
Pheromone	*H. punctigera*	*	*	*

Table 4. Summary of statistical analyses of trap catches before and after the first Magnet® application. Data for *H. punctigera* from pheromone traps were not analysed because of very low catches with many zero values.

There were significant effects of the period (before and after Magnet®) for all except *H. punctigera* in the pheromone traps, for which numbers were very low and the analysis was invalidated by the many zero catches. These effects generally reflected substantial reductions in catch numbers, which were probably due to the Magnet® application, because there were no obvious changes in weather which might have explained them (e.g. lower temperatures or higher wind speeds; Gregg & Wilson 1991). This hypothesis is also supported by the fact that the only exception to the trend for declining numbers after the Magnet® application was in the distant pheromone traps, where Magnet® effects would have been least and where catches actually increased.

While the only statistically significant Treatment x Period interaction was for *H. armigera* pheromone traps, there was a pattern to the extent of the reductions in catches following Magnet® application (Table 5). The pattern for both species in light trap catches was that the percentage reduction following Magnet® application was directly related to how far the traps were from where the product was applied. In the treated area the reduction was 84.6% for *H. armigera* and 92.9% for *H. punctigera*. In the adjacent untreated cotton it was 70.8% for *H. armigera* and 90.8% for *H. punctigera*. In the refuge, which was most distant from the application, the reduction was about 55% for both species.

			Mean trap catch		
Trap type	**Species**	**Treatment**	**Before**	**After**	**Apparent reduction (%)**
Light	*H. armigera*	Refuge	60.50	26.75	55.8
Light	*H. armigera*	Untreated cotton	31.40	9.17	70.8
Light	*H. armigera*	Treated cotton	40.17	6.17	84.6
Light	*H. punctigera*	Refuge	15.25	5.75	55.7
Light	*H. punctigera*	Untreated cotton	9.00	0.83	90.8
Light	*H. punctigera*	Treated cotton	11.67	0.83	92.9
Pheromone	*H. armigera*	Distant traps	18.13	21.50	-18.6
Pheromone	*H. armigera*	Refuge	18.75	8.50	54.4
Pheromone	*H. armigera*	Untreated cotton	30.17	15.00	50.3
Pheromone	*H. armigera*	Treated cotton	18.83	8.00	57.5
Pheromone	*H. punctigera*	Distant traps	1.75	0.33	*
Pheromone	*H. punctigera*	Refuge	0.17	1.25	*
Pheromone	*H. punctigera*	Untreated cotton	0	0	*
Pheromone	*H. punctigera*	Treated cotton	0	0	*

Table 5. Mean trap catches and percentage reductions following Magnet® application. % reductions were not calculated for *H. punctigera* pheromone traps because low numbers would make them unreliable.

This pattern was not seen in pheromone trap catches for *H. armigera*, where the reduction was 50-58% in all areas except the distant traps, which showed a negative reduction (i.e. an increase) in catches. The reason for this may be that light trap catches reflect local populations since the traps can only be seen from above, while pheromone traps can bring males from much further away because the pheromone plumes can be followed for considerable distances. Thus, pheromone traps may be reflecting farm scale changes in numbers, while light traps reflect more local differences.

If reductions in light trap catches reflect the impact of Magnet® on local moth populations, these results indicate that, while Magnet® has area-wide effects, those effects are greatest where it is applied, and progressively decline with distance. This pattern has also been seen with egg densities in numerous trials of Magnet® on conventional cotton. It indicates that, while Magnet® may kill some moths from refuges, if placed correctly it will kill proportionately more from the Bollgard II® cotton. For example, in this experiment approximately 45% of the moths from the refuge survived, while only about 15% of those in the cotton did. This skews the balance in favour of the unselected refuge moths, and would be equivalent to a threefold increase in the productivity of the refuge. This result is encouraging, especially since the impact of the Magnet® would have been limited because it was washed off by the rain after two days.

These calculations are, however, simplistic, and to strengthen the case we need information on the host origins of moths killed by Magnet®, and those in the general population. We also need to repeat experiments like this on a larger scale, and in conditions where the impact of Magnet® treatment can be fully realised. Such experiments are planned in the larger follow-up project beginning next season.

Dead moth collections and host origin analyses

While difficulties were experienced in collecting Magnet®-killed moths from late season cotton (see Methods section), we were able to collect a total of 205 such moths. Of these 138 were collected from the solid plant cotton after the first Magnet® application, and 67 from the skip row cotton after the second application. Most of these moths have been dissected, with 61% and 83% from the first and second applications respectively being *H. armigera*. From the first application, 45% of the moths, across both species, were males. From the second application the male percentage was 42%. The sex ratios are consistent with previous experience with Magnet® in conventional cotton, where typically slightly over half of the kill consists of female moths. They provide further evidence that the light traps are biased towards males, and that the pheromone traps overestimate the proportion of *H. armigera* in the population.

These moths have been frozen and will be subjected to analysis using biochemical host plant origin markers when these become available from Project 1.05.13.

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.

The outcomes of the project can be summarised as:

1. The inadequacy of pupae busting for control of late-emerging moths which have been subjected to Bt selection in quick-maturing high retention Bollgard II® crops pressure has been demonstrated by modelling. In the Namoi, larvae from the main cohort of Bt survivors will pupate before any diapause is induced, and therefore moths will emerge before they are vulnerable to pupae busting. In the Namoi, Macintyre and Darling Downs, substantial areas of the crop will have been defoliated before diapause becomes common, and so most of the pupae from larvae supported by these crops will not be vulnerable to pupae busting. These late emerging moths constitute a threat to resistance management, and point to a need to include additional tactics (such as Magnet®) in RMPs.

2. A preliminary late-season moth busting trial has demonstrated the feasibility of imposing differential mortality on moths emerging from Bollgard II® cotton and moths from refuges and other unselected sources, using strategically placed applications of Magnet®. However the concept needs to be tested on a wider scale, and validated through the use of markers of host plant origin, and comparisons of changes in resistance allele frequency between treated and untreated regions.

3. Better understanding of the technical requirements for an area-wide test of the moth-busting approach has been obtained. The appropriate timing for Magnet® applications to catch late emerging moths from Bollgard II® cotton in an average

year has been identified as 21 day window from 18th February to 9th March. This would require 3-4 applications of Magnet®. The period should be adjusted forward or backwards depending on whether predicted crop development is ahead or behind the average. Methods for collecting moths from treated and untreated areas have been developed and improved. Issues associated with dead moth collection from late season cotton have been identified.

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

The Cotton CRC submitted a patent for the concept of moth busting for resistance management in 2011, but it has been decided that it is in the best interests of the industry if this patent is allowed to lapse and the information is released to the public domain.

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

See detailed description of the methodology and results above for information on development of methods which will facilitate large scale trials of moth busting

   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 has demonstrated through modelling that pupae busting for *Helicoverpa* spp. has limited efficacy in modern high retention cotton systems, and that there is a need to improve late season resistance management tactics, in particular by targeting moths. Moth busting with Magnet® could make a useful contribution to RMPs, perhaps to the extent that it could substitute for pupae busting, which has agronomic and environmental drawbacks. A pilot study conducted during this project has indicated the feasibility of this approach, and has developed techniques which will be applied in a larger study of the area-wide impact of moth busting.

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.**

Area-wide trials need to be undertaken to further validate the concept of moth busting. This will occur under the 3 year project to begin in July 2012. If results from

that project are encouraging, we will advocate in TIMS for the inclusion of moth busting with Magnet® in future Resistance Management Plans. These recommendations, if adopted by Monsanto and submitted to APVMA, will improve RMPs and possibly reduce or eliminate the need for pupae busting.

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

We are planning an article to be submitted to *Australian Cottongrower* in the next month. We are planning to present this material at the Australian Cotton Conference in August 2012. We have had two papers accepted for presentation at the International Congress of Entomology in Korea, also in August 2012.

We will also present the results of this work at two field days being organised by the Upper Namoi Cotton Growers Association, on 5 and 7 June 2012. This will serve to inform growers about the research to date, and also enable us to make contacts which will facilitate the large-scale project which will be undertaken in this region over the next three seasons.

(c) for future research.

The main need is to expand the spatial scale of the research, and to validate the concepts through the use of markers of host origin, and through changes in the frequency of resistance alleles. This will be done as part of the follow-up area-wide project which has now been funded by CRDC.

Publications

9. A. Publications relevant to this project.

Peer reviewed articles / books

None

Non-peered reviewed articles

None

Presentations (conference, field days, workshops etc)

Gregg P (2011) Moth busting and other tactics – update. *Presentation to REFCOM, July 11, Toowoomba*

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.*

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

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. *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.

Non-peer reviewed articles

Bahar MH, Stanley JN Backhouse D, Gregg P**,** 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**.**

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

No.

Part 4 – Final Report Executive Summary

The Australian cotton industry depends heavily on genetically engineered Bt cotton (currently in the form of Bollgard II®) which provides resistance to the key pests of cotton, larvae of the moths *Helicoverpa armigera* and *H. punctigera*. Bt cotton has enabled substantial reductions in the use of insecticides, provided greater flexibility on cotton farming systems, and made the crop easier to grow. However, as with most pest management tactics, there is potential for the pests to develop resistance, and to counter this the Australian cotton industry has developed comprehensive Resistance Management Plans (RMPs), observance of which is mandatory for growers of Bt cotton.

A traditional component of RMPs, dating from resistance management of conventional insecticides, is pupae busting, or cultivation of the soil to destroy overwintering (and potentially resistant) pupae. However, pupae busting incurs financial, agronomic and environmental costs. It restricts the implementation of minimum tillage techniques which can help prevent erosion, conserve soil moisture and enhance soil carbon. Moreover, we have demonstrated through modelling studies in this project that in modern Bt cotton systems, with high fruit retention and early maturation, many potentially resistant insects are emerging before overwintering diapause is initiated, and are thus not vulnerable to pupae busting. There is a need to develop tactics for RMPs that can fill this gap.

One potential tactic is to target moths instead of pupae, using the attract-and-kill technology Magnet® which was developed by the researchers in this project during early work in successive Cotton CRCs. Magnet® consists of a mixture of plant volatile compounds which, when combined with small quantities of insecticide, can attract and kill adult *Helicoverpa* spp. moths. It has impacts beyond the area in which it is applied, but with careful placement it might be able to kill proportionately more potentially resistant moths from cotton than susceptible moths from refuge crops and other sources, thereby enhancing the genetic dilution effect provided by refuge crops which are another component of RMPs. A farm scale trial conducted during this project indicated the feasibility of this approach, and helped develop techniques to be used in a larger, area-wide trial of the approach to be conducted over the next three years. If successful, this trial could lead to the development of more robust RMPs, and the reduction of elimination of the requirement for pupae busting.

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Kvedaras OL (2002) *The influence of host plants on the mating behaviour of* Helicoverpa armigera *(Hübner) (Lepidoptera: Noctuidae.* PhD thesis, University of New England, Armidale.

Lu B (2010) *Thresholds and mechanisms of survival for Bt-susceptible* Helicoverpa *spp. living on Bollgard II® cotton*. PhD thesis, University of New England, Armidale.

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