Cotton Catchment Communities CRC

FINAL REPORT

(due within 3 months on completion of project)

Part 1 - Summary Details

Cotton CRC Project Number: 1.01.52

Project Title: Maximising the efficiency of Bt refuge crops

Project Commencement Date: 01/7/2006 **Project Completion Date:** 30/6/2009

Cotton CRC Program: **The Farm**

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

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

Background

1. Outline the background to the project.

The use of transgenic (Bt) cotton (Ingard® and more recently Bollgard II®) has greatly reduced the use of insecticides in Australian cotton production and the status of key lepidopteran pests such as *Helicoverpa* spp. It has also generated a greater opportunity to manage and capture ecosystem services provided by beneficial invertebrates (predators, parasitoids) as part of IPM strategies for other insect pests that are not controlled by Bt. But the potential for *Helicoverpa* spp. (especially *H. armigera*) to develop resistance to Bt remains a major challenge for the industry. *H. armigera* has consistently developed resistance to synthetic pesticides in the field, and cultures of Bt resistant strains of *H. armigera* have been generated in the lab by CSIRO Entomology staff. These strains are resistant to both Cry1Ac and Cry2Ab, the two toxins present in Bollgard II® cotton. The resistant strains have been derived from field collected insects possessing the resistance alleles.

In addition, since this project began, CSIRO staff monitoring for evidence of the development of Bt resistance (both in *H. armigera* and *H. punctigera*) have indeed found increases in the frequencies of resistance alleles for Cry2Ab in both species – a significant worry for the industry. The trends in resistance in *H. punctigera* were quite unexpected – it has shown little evidence previously of a tendency to develop resistance to conventional pesticides and its traditionally accepted behaviour of invading eastern cropping areas annually from breeding sources in inland Australia (with little over-wintering in cropping regions) was thought to significantly dilute any likelihood of the species developing resistance. Thus during the tenure of this project, we began to suspect that perhaps we didn't understand as much about the ecology of *Helicoverpa* spp as perhaps we thought we did.

A resistance management plan (RMP) has been adopted by the Australian cotton industry, part of which is the mandatory requirement for Bt cotton growers to provide refuge crops (no Bt exposure) as sources of susceptible moths. Such moths should then mate with potentially resistant moths arising from the Bt crops and help reduce the chance of resistance developing more broadly. Prior to and during Ingard® use, the relative merits of various refuge crop options were identified (Projects CSE64C & CSE90C). Pigeon pea proved to be generally the most productive source of *Helicoverpa*. Other alternatives included maize, sorghum and conventional cotton. Subsequent research focussed on demonstrating if, as assumed in the RMP, moths from refuges do in fact mate (at random) with moths from Bt cotton (Project CSE107C). Initial results suggested this may not strictly be the case, but the data were very limited. Some recent overseas research suggests that *H. armigera* moths select mates that have grown as larvae on the same plant host as themselves (implying, that the mating of moths generated from different crop types could be rarer than desired). We needed to be more sure than we were of the degree to which this behavioural bias occurs in Australia. If it were to be substantial, then the efficacy of some refuge crops is questionable.

The farming of cotton and associated grain crops has developed significantly in recent years with technological advances. The introduction of Bollgard II® cotton, with its much greater efficacy in killing *Helicoverpa*, has increased the proportion of Bt cotton being used to approximately 80-90% of all cotton, from a 30% cap under Ingard®. The abundance of beneficial species (predators, parasitoids) is also now perceived to be much greater on the cotton landscape than in previous years. These trends will have shifted the performances (and requirements) of refuges from those documented in the Ingard® era. With these shifts in the cotton production environment, it is important that the efficacy of Bt refuges continues to be evaluated to identify optimal means to grow refuges most productively for minimal costs to the cotton farmer. One possible way to increase the productivity of refuges is to use feeding attractants such as occur in "Magnet" (without the pesticide component) to enhance *Helicoverpa* abundance. Research to identify novel refuge options is also desirable in a changing cotton production environment (driven by e.g. altering financial imperatives for farmers, limitations on available water for irrigation due drought etc). In addition, there needs to be more emphasis placed on the collective efficiency of refuge crops at landscape scale, rather than the hitherto simple one-on-one comparative analyses of the performances of refuge crop options cf Bt cotton crops, in terms of *Helicoverpa* production.

Another key question needing answering is how the abundance of *Helicoverpa* spp is varying at landscape scale, as a result of land management advances, including the advent of transgenic cotton. Will the use of a high proportion of Bollgard II amongst the cotton crop lead to a reduction in the abundance of *Helicoverpa* generally, especially *H. armigera* which are believed to be more locally generated within the cropping regions of eastern Australia than *H. punctigera* ? Through our previous monitoring of early season incidence of *Helicoverpa* spp. on non-cotton host plants in northern NSW, and our networks of pheromone trapping in the Namoi and St George regions (Projects CSE64C, CSE90C, CSE107C), we have established a long-term data set to enable assessment of temporal shifts in *Helicoverpa* abundance. In the past we have shown that the deployment of Ingard® cotton in fact corresponded with a heightened late season abundance at landscape scale of *H. armigera* (possibly related to the relatively poor performance of Ingard® in late season, accompanied nonetheless with reduced pesticide use ?).

We intended to continue such monitoring to provide an on-going assessment of how the abundance of *Helicoverpa* has tracked with the advent of Bollgard II® cotton. Intuitively, we expected that, if anything, the abundance of *H. armigera* should be reduced, as was noted for pink bollworm populations in the USA, when high proportions of the cotton crop became transgenic. Monitoring of the status of *Helicoverpa* populations also has strong relevance to the farming of other crops that are susceptible to these pests, within mixed cropping enterprises in cotton production regions. The monitoring data, in conjunction with climatic data (local in cotton production regions and more inland), also enabled an assessment of weather variables (especially rainfall) as drivers of the population dynamics of these pests.

Objectives

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

The long-term success of Bt cotton relies heavily on effective and cost-efficient strategies to counter the development of Bt resistance in *Helicoverpa* spp. This includes the mandatory deployment of refuge crops to generate Bt susceptible moths, which the Australian cotton industry has established, when Bt cotton is grown by farmers. The overall aim of our work in this project was therefore to develop optimum strategies for refuge crop management. In particular, the project aimed to : 1). Demonstrate the efficiency of coverage of Bt cotton by moths from non-cotton sources, and the degree of cross-mating of moths from different plant hosts; 2). Evaluate methods to enhance the production of *Helicoverpa* within refuge crops; 3). Continue monitoring of landscape-scale changes in the abundance of *Helicoverpa* spp.; 4) Conduct field trials to evaluate novel refuge crop options. The 4th aim was added to the project for its 3rd year, when Dr Mary Whitehouse joined the project from her previous work on mirid pest management.

The Project Plan for this project listed the following Objectives (and Milestones) :

1. Monitor early season abundance of *Helicoverpa* eggs & larvae.

   [Surveys of weed & non-cotton crop plants conducted each spring in Namoi and St George regions.] **Achieved.**

2. Monitor *Helicoverpa* moth abundance in pheromone traps.

   [Traps cleared weekly at grids in Namoi & St George, data filed electronically and running comparisons made with previous year's data on a seasonal basis.] **Achieved.**

3. Identify novel refuge crop options for both dryland and irrigated Bt cotton production.

   [Field trials conducted which evaluate pigeon pea, slashed / not slashed / split planting dates for unsprayed cotton.] **Achieved – but the work is preliminary.**

4. Measure influence of "Magnet" on *Helicoverpa* moth production in refuges.

   [Experiments conducted on-farm in Namoi region, selecting 3 different refuge crop types.] **Mostly achieved, but again the work was preliminary. We had hoped to use more crops than pigeon pea – but seasonal rain / low abundance of moths limited the number of trials we could conduct.**

5. <u>Determine crop origin of moths found in transgenic cotton fields and temporal and spatial coverage of moths from non-cotton sources.</u>

[Range of sites selected in northern NSW, larval *Helicoverpa* monitored in refuges, emergences predicted, collections of moths made in Bt crops, and chemical analyses completed to identify moth origins.] **Mostly achieved. We encountered difficulties separating moths from C3 plant origins (cotton, pigeon pea) in the field – but we backed the work up with laboratory studies.**

6. <u>Extend research results to industry.</u>

[At least one scientific MS and one industry magazine article submitted and one seminar / grower talk presented / year.] **Achieved.**

7. <u>Complete final report for project.</u>

[Submit report by Sept 2009] **This report.**

This report is structured around these Objectives, within the Methods, Results, Outcomes & Conclusions sections below. In addition, we include mention of extra deliverables the project provided.

Methods

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

<u>Monitor early season abundance of *Helicoverpa* eggs & larvae.</u>

Helicoverpa populations were monitored, as in previous years, on weed and non-cotton hosts for eggs and larvae during spring, using periodic visual and sweep-net collections (e.g. usually 100 sweeps / site & / or 6 x 1m of crop row replicates for visual checks). Crops checked during the springs of 2006-08 were mostly faba bean and chickpea, weeds were predominantly Paterson's curse, vetch and medics, and native daisies were often surveyed, when available. We collected mostly from the Namoi & Gwydir valleys. The counts of eggs and larvae were expressed against the relevant sampling effort. The collected material was reduced to a sub-set when prolific and individuals were reared through to maturity or death / parasitoid emergence in the laboratory. As in previous years, the numbers of individuals reared / collection varied greatly, but in most cases were from a few to approx. 50. The main emphasis in this work was to provide early information on the build up of *Helicoverpa* populations (both for our own work later in the season and more broadly for the cotton industry & other researchers). The work also provided ongoing comparisons across years and between vegetation types (crops and non-crops) of the incidence of diseases and parasitoids affecting *Helicoverpa*, and the relative abundance of the two *Helicoverpa* spp. in early season.

Monitor *Helicoverpa* moth abundance in pheromone traps.

Grids of 11 pairs of Agrisense canister pheromone traps (one for each *Helicoverpa* species) were maintained in the Namoi Valley (within 10km radius of ACRI, Narrabri), as in previous projects. The traps were emptied weekly, weather permitting. Lures were changed monthly and pesticide strips were changed bi-monthly. This work was used to track long term, landscape scale changes in the abundance of these key pests (e.g. fluxes in abundance associated with Bt cotton deployment). The research also provided prior and within season guidance to growers of population trends in *Helicoverpa* spp. abundance.

Pheromone traps were also provided to cotton consultants in the cotton growing regions of St George, as done each year since 1996. The consultants maintained pairs of traps and provided weekly counts of the numbers of moths collected throughout the growing seasons (but note not all year round, as near ACRI, but rather just during the cotton growing season).

We continued to analyse the long-term data sets that we have amassed through the ongoing pheromone and light trapping (CSIRO-designed cone light traps) done in the vicinity of ACRI. We report here particularly on our interrogation of the data in relation to inland rainfall. It has been well-accepted previously that moth catches (in particular the abundance of 1st generation *H. punctigera* moths) in the eastern cropping regions (e.g. near Narrabri) can be related to rainfall patterns, in particular inland rainfall in autumn-winter which drives emigration of *H. punctigera* further east. A significant element of support for this view is based upon CSIRO light trap data collected during the 1970's and 80's at Narrabri (e.g. Oertel *et al* 1999, *Aust J. Entomol.* **38**, 99-103). We sought to determine if such relationships still hold with later collections, and with pheromone trap collections as well. Fig. 1 illustrates the locations of meteorological stations and districts we used (essentially in common with Oertel *et al* 1999) for these analyses. We also attempted to relate catches of moths in traps near Narrabri with more general indicators of inland rainfall (indices derived by P. Gregg using Long Paddock, Qld Govt records) (see Fig. 2 for examples).

Whilst it is well recognised that *Helicoverpa* moths mate several times, the extent to which this happens is poorly documented for *H. armigera* and *H. punctigera*. Better understanding of the multiple mating of these species is needed in relation to interpreting our studies (and those of others) on the incidence and outcomes of random mating re refuge crop deployment and the presence of sperm precedence. We therefore took the opportunity to analyse a wealth of information that has been accumulated at ACRI over many years, wherein female moths (caught in light traps) have been dissected to assess reproductive condition (i.e. gravid or not) and the number of spermatophores present (= the number of matings).

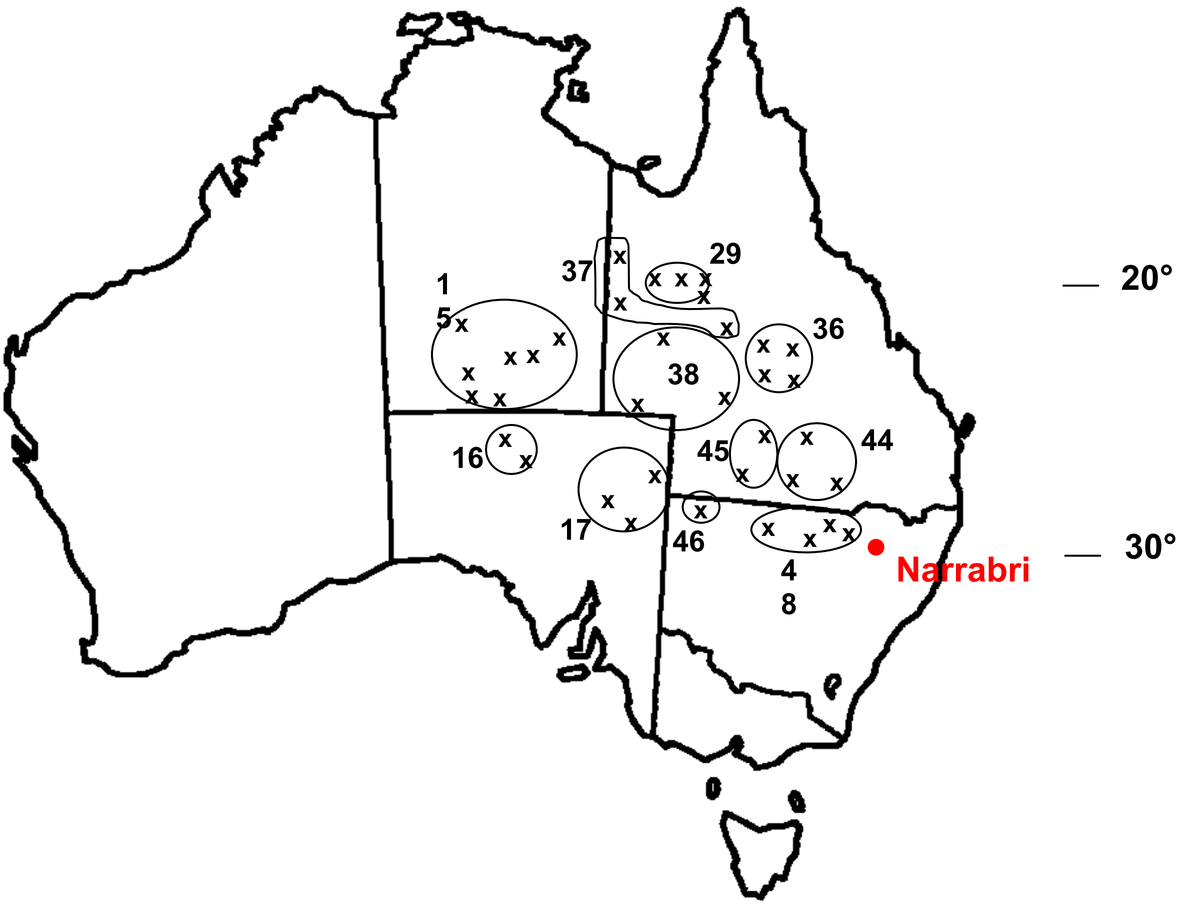

Fig. 1. Locations of Bureau of Meteorology weather stations (x) and recognised Meteorological Districts (circled) used for correlations between rainfall data and trapping records for *Helicoverpa* spp. near ACRI, Narrabri.

Identify novel refuge crop options for both dryland and irrigated Bt cotton production.

During 2008-09, we conducted 3 preliminary field trials (at Getta Getta [Macintyre], Blue Hills & Dobikin [both Namoi]) to evaluate the relative efficiency of pigeon pea cf unsprayed cotton as a refuge crop option for dryland Bt cotton production (Fig 3). We also conducted a preliminary trial to evaluate the relative efficiency of split planting dates cf single planting date for unsprayed cotton as a refuge crop option for irrigated Bt cotton production at Gunedra (Wee Waa, Lower Namoi) (Fig 4). Both visual (for eggs and larvae of *Helicoverpa*) and soil sampling (for pupae) methods were employed to evaluate efficiencies, and were conducted several times throughout the season. The work was done in conjunction with Monsanto, from whom additional funding was obtained.

Getta Getta was sampled for eggs, larvae and pupae on 5 occasions, from early December 2008 to late March 2009. On each occasion, 6 separate 1m rows of crop (canopy) were inspected for *Helicoverpa* eggs and larvae (plus other invertebrates) in both pigeon pea and unsprayed cotton crops. In addition, 14 m^2 of soil (done as individual $1m^2$ samples) was sorted to estimate pupae abundance beneath each crop. The eggs, larvae and live pupae were subsequently reared in the laboratory to moths / parasitoids. Pigeon pea and unsprayed cotton crops at Blue Hills and Dobikin were sampled in a similar way on 6 occasions from early January to early April 2009, at both sites.

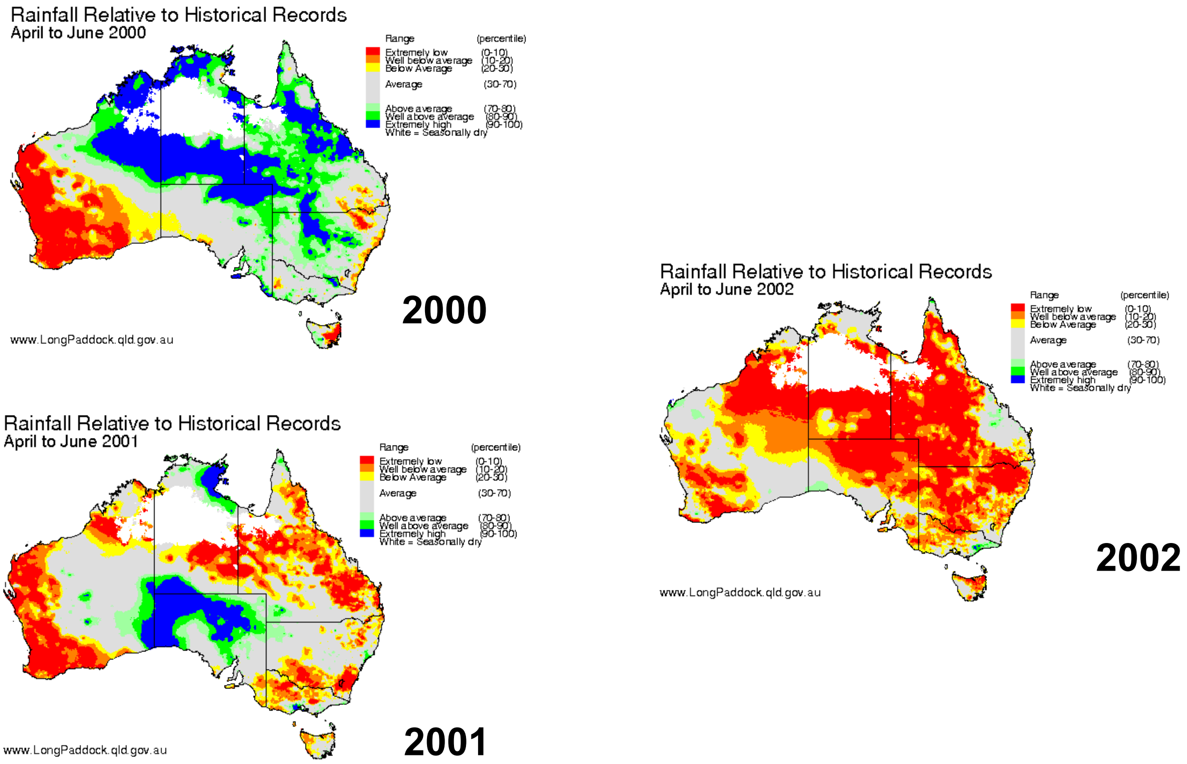

Fig. 2. Examples of autumn - winter rainfall records (specific annual data relative to long-term data sets), as provided on the Long Paddock (Qld Gov't) web site. Such data were ranked from dry (e.g. 2002) through to wet (e.g. 2000), on a scale of 1-10.

Fig. 3. Pigeon pea being trialled as a potential refuge crop in a dryland farming system at Dobikin (Bellata, Namoi), 2008-09.

At Gunedra, visual sampling of the crop canopies and pupae digs were conducted on 8 occasions between late December 2008 and early April 2009, in Bollgard II and unsprayed conventional cotton. On each sampling occasion in each crop, 6 x 1m rows of crop were surveyed visually and 14 x 1 m^2 of soil were hand-sorted. There were two types of conventional cotton grown as refuges. One was sown at the same time as the Bollgard II (October 2008), and the other later (at the first irrigation, late December 2008). Subsets of the eggs and larvae collected within the canopies and pupae in the soil were reared to adult moths (or parasitoids) as described elsewhere in this report.

Fig. 4. Split plantings of unsprayed conventional cotton being trialled as a potential refuge crop in an irrigated farming system at Gunedra (Wee Waa, Namoi), 2008-09. 1st planting on left; 2nd planting on right.

One of the problems with using conventional cotton in dryland refuges is that it gets eaten out by *Helicoverpa* early on, and then isn't attractive to moths later in the season. In the 2008/09 season we piloted two alternative approaches to using just cotton as a refuge in a dryland system, both of which involve planting mixed crops (following on from the promising results reported in Baker *et al* 2008, *Aust. J. Agric. Res*. **59**, 723-32). In experimental plot 1, a mixture of conventional cotton (90%) and mung bean (10%) was planted, while in plot 2 a mixture of conventional cotton (90%) and pigeon pea (10%) was planted. The rationale was that the legumes will initially grow taller than the cotton, and take the first wave of *Helicoverpa* egg-lays, hopefully ensuring that the cotton remained attractive to *Helicoverpa* later in the season. The advantage of the legumes is that they may also enrich the soil by providing nitrogen. The added advantage of the mung bean is that by the end of the season, the mung bean dies away to nothing, so that if the grower wanted to harvest the refuge crop, he could (Fig. 5).

The crops were checked four times. Each time 6 visuals and 6 pupae digs were undertaken in each plot. In each visual we recorded the number and colour of the *Helicoverpa* eggs, and the presence of any larvae. Pupae found in the pupae digs were taken back to the laboratory and left to develop.

At the end of the season we harvested the cotton from the plots to compare raw lint weights, and we sampled 10 plants from each plot for plant mapping, to identify when the plant formed fruit, and where the fruit was retained until maturity. When plant mapping, all bolls and fruit-scars were scored, and their position on the plant noted as either at the bottom (nodes 5-10), middle (nodes 11-15), top (nodes 16-20), vegetative branches; and very top (nodes 21+ - these bolls are unlikely to be harvested).

Work on advantages of slashing cotton to enhance the efficacy of cotton as a refuge was conducted by Monsanto (thus sharing responsibilities re novel refuge options), and thus will not be reported on here.

Fig. 5. Cotton with 10% mung bean (left) and 10% pigeon pea (right). During the season, the mung bean became incorporated in the cotton (a), while the pigeon pea was always taller (b). At harvest time, the mung beans had died away to nothing (c), while the pigeon pea did not respond to two applications of defoliant (d).

<u>Measure influence of Magnet® on *Helicoverpa* moth production in refuges.</u>

We investigated, through field trials with P. Gregg (U.N.E., Cotton CRC), if the feeding attractant, Magnet® (without associated insecticide), can be used to increase *Helicoverpa* production within refuges. We conducted three separate comparisons of egg numbers within refuge crops (pigeon pea) sprayed / not sprayed with Magnet® (at Kangaloon, Lammermore, & Underbri during 2006-07) (Lower Namoi & Macintyre valleys). We designed our field experiments to also determine if such practices reduced egg lays on associated Bt cotton crops concurrently (thus reducing "exposure" of moths to Bt). We sampled both within pigeon pea refuge crops (within plots designated as treated with Magnet®, and matching controls, and in adjacent areas still within the same refuge crop) and within Bollgard II cotton crops adjacent to the refuges crops. Each sampling plot (treated and control) was replicated twice. Each site was sampled on days immediately after Magnet® application. We sampled Kangaloon over 2 days (11 & 12 January 2007), Lammermore over 4 days (23-26 January 2007), and Underbri over 2 days (22 & 23

February 2007). Four separate metres of crop row were sampled for white eggs on each day within each plot. In addition, we conducted flush counts during day and night (twice for Kangaloon, four times for Lammermore, and twice for Underbri) within the treated and control refuge crop plots. Four 50 m walks were taken to flush moths within each plot on each occasion. We also ran light traps (one for each treated and control refuge crop plot) at Lammermore (4 nights) and Underbri (3 nights).

Repeat experiments, in subsequent seasons, were hampered by weather restraints (rainfall at critical times which prevented sensible Magnet® application; insufficient moth activity at previously selected sites to warrant trials).

<u>Determine crop origin of moths found in transgenic cotton fields and temporal and spatial coverage of moths from non-cotton sources.</u>

Several sampling sites (pairs of Bollgard II and associated refuge crops) were established in each season of the project (121 sites overall; 39 in 2006-07, 37 in 2007-08, & 45 in 2008-09), in the Namoi, Macintyre & Gwydir Valleys + St George region, with the aim of setting up subsequent studies of the movements of moths from the refuge crops (and other non-Bt sources) into the Bt cotton, and the degree of cross-mating that occurred there between moths of different plant-host origin. We monitored the seasonal production of *Helicoverpa* from the individual refuges (eggs, larvae, pupae) at approx 3 weekly intervals to determine likely temporal patterns in moth emergence and thus when would be optimal to conduct night surveys for mating pairs in the associated Bt crops (777 site visits across the 3 years). These surveys were based on visual sampling (6 replicate, 1 m rows of crop within each field on each sampling occasion for canopy invertebrates, and soil sampling for pupae). The sampling enabled additional assessments of the frequencies of secondary pests and beneficial species in these crops. Subsets of *Helicoverpa* eggs and larvae were reared through to adult moths in the laboratory, thus giving assessments of species bias, parasitism and disease incidence. As in previous years, this approach inevitably meant that many crops were followed which led to no opportunity to collect mating moths – because the populations of *Helicoverpa* never developed to a sufficient extent to make night collections profitable. This was particularly so during the tenure of this project, when moth populations were relatively low. Nevertheless, we collected moths (both mating and single) from several sites (see Table 1) and analysed them (wings + head) for indicators of their crop origin (using carbon and nitrogen stable isotopes – see earlier reports for a more detailed description of methodology; we used the U.N.E. mass spectrometry laboratory in Armidale for these analyses). We also collected pupae from beneath various crop types (cotton, pigeon pea, maize & sorghum) near Narrabri, reared these to adult moths, and analysed for stable isotope signatures - to expand our data base (and confidence) in expected stable isotope signatures for known crop origins. In particular, we included stable nitrogen isotope analyses in the hope that such would help us differentiate legume (e.g. pigeon pea) from cotton within C3 plant origins. Legumes are expected to have relatively low Delta N values, because of their capacity to fix atmospheric N. In addition, we attempted to have gossypol signatures determined by Monsanto (USA). We provided several hundred samples of moths (those collected in 2007-08 – see Table 1) to Monsanto, linked to stable isotope analyses done on other parts of their bodies. Unfortunately laboratory mishaps in the Monsanto labs, beyond our control, prevented usable data being provided.

Table 1. Numbers of *H. armigera* moths collected at night at various sites and analysed for stable isotope signatures

Year & Property	Month	Pairs	Singles
2006-07			
Morella (Macintyre)	January	4	248
Tucka Tucka (Macintyre)	January	6	20
Warendi (Macintyre)	February	2	60
Kangaloon (L. Namoi)	January	6	35
Careera (L. Namoi)	February	1	8
2007-08			
Keytah (Gwydir)	February	40	44
Long View (L. Namoi)	January	10	140
Iona (St George)	January	9	109
2008-09			
Currawidgen (U. Namoi)	January	6	0
Careera (L. Namoi)	January	1	0
Havana (L. Namoi)	January	1	50
Taratan (L. Namoi)	January	0	32
Tucka Tucka (Macintyre)	January	71	106

We aimed to demonstrate conclusively through this work if cross-mating between moths of different plant host origins is occurring effectively, and if moths of non-cotton crop origins are common throughout Bt cotton fields. We concentrated on *H. armigera*, because this was the species in focus at the start of the project. We did however obtain some preliminary data for *H. punctigera* as well. The field research was mostly based in northern NSW.

In addition (during 2008-09), we conducted 3 field & laboratory rearing room studies with *H. armigera* , wherein moths were reared (as larvae) on either pigeon pea or cotton and then allowed to mate - to determine the degree of cross-mating of moths from these two plant host origins. This work was required because we had difficulties differentiating moths derived from these plants in the field using stable isotopes.

Fig. 6. 400 Material bags on cotton (left) and pigeon pea (right) each containing one developing caterpillar.

In the first experiment, 400 eggs collected from the laboratory colony were used. In the second experiment, 800 eggs collected off a maize crop were used. In the third experiment, 428 eggs collected off Bt cotton, and 356 eggs collected off pigeon pea were used. In each experiment, the eggs were placed on medium and allowed to develop until the larvae reached 3^{rd} instar, at which point they were individually caged on either field growing pigeon pea or cotton until they reached 5^{th} instar (Fig. 6). At this point they were taken off the plants and allowed to develop into pupae on vermiculite in the laboratory. As pupae, they were sexed and their day/night cycle was reversed. Upon emergence they were marked as either cotton or pigeon pea raised, and kept in same-sex groups until they were tested. To test the moths, the same number of male, female, pigeon pea, and cotton moths were put into an observing chamber within an hour of "sunset" and checked every 15 mins for 6 to 7 hours. Any moths found mating were removed, and left until they separated. They were then measured, their crop of origin noted, and the length of time they were mating recorded. The female moths were then kept for a further 5 days, and the number of eggs they laid was recorded.

Further, we conducted some preliminary (opportunistic) work on single-pair matings, using pupae (120 *H. armigera*, 60 *H. punctigera*) collected from beneath particular crops (especially pigeon pea, and to a lesser extent maize and cotton), and reared to moths in the laboratory. Ultimately, we were aiming to test if these moths mate equally well when derived from different plant hosts, as they do when derived from the same plant hosts. The work is still in its infancy. Our measure of effective mating was if at least one fertile egg (i.e. it hatched) resulted from the pairing. Thus far the vast majority of our single-pair matings involve the same plant host origins (pigeon pea) – because this is where most pupae have been found thus far. Pupae have been sourced in several valleys. It may prove possible, in time, to test if moths derived from different valleys also mate effectively.

Extend research results to industry.

[See details below under Publications for meetings addressed, publications etc]

Additional Deliverables

Intensive studies of refuge efficacy at St George

Whilst substantial effort has been made to evaluate the relative productivity of individual refuge crops in the past, little emphasis has been placed on the collective / regional contribution of suites of refuge crops. Ultimately, it will be the area-wide effects of refuge crops that will be crucial in limiting the onset of Bt resistance in cotton production systems. We were alerted to the opportunity of monitoring the moth production of the majority of refuge crops deployed in the St George region of southern Qld during 2008-09. All of these crops were pigeon pea (Fig. 7). We measured (visually) *Helicoverpa* egg and larvae abundance (as well as beneficial species abundance and the incidence of insect disease) on 25 crops (6 m of row in each crop) on 4 occasions (6-8 January, 27-30 January, 24-26 February, & 17-19 March) throughout the season. *Helicoverpa* eggs and larvae were also collected for rearing in the laboratory to assess the incidence of parasitism. In addition, we hand-sorted 6 separate 1 m^2 of surface soil within each crop on the last 3 sampling occasions to measure *Helicoverpa* pupae abundance. Field –collected pupae were also lab-reared to measure the relative abundance of the two *Helicoverpa* species, and the incidence of parasitism. On each visit to the refuge crops in St George, we used Monsanto's mid season audit ranking system to measure the attractiveness of the crops to *Helicoverpa*. We viewed this intensive survey of refuge performance in the St George region as a prelude to our intention in future (new grant just started with CRDC funding & managed through Cotton CRC) seasons to not only measure refuge production, but coverage capacity through dispersal of the emerging moths across the region.

Comment on synchrony of pigeon pea with cotton

To be successful, refuge crops must not only produce large numbers of susceptible moths, but also produce them in a timely way, such that mating with emergent moths from Bt cotton crops is enabled. One way to check that this is likely to be the case was to re-analyse the degree of correlation between the numbers of live pupae found beneath Bt cotton crops and their dedicated refuges nearby, at the same time. The implication is, that if such numbers are correlated, then resultant moth emergences from such pupae are also likely to be correlated. Our largest data-base in this regard is the extensive surveys made beneath Ingard® and refuge crops during 1996-2003. We thus re-analysed this old data, selecting only surveys taken during January, February and March (such correlation data has not been presented previously in reports).

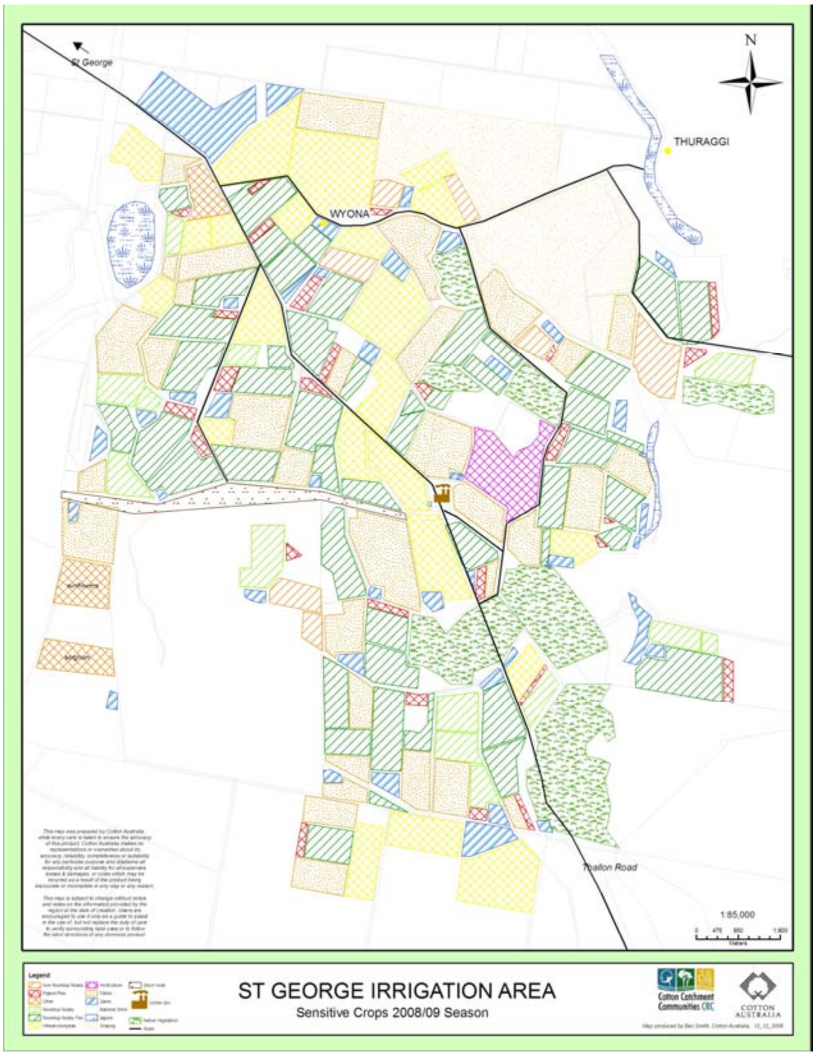

Fig. 7. Locations of various cropping types in the St George region during 2008-09 (map kindly provided by Dallas King). Pink hatched areas depict the 25 areas used for refuge crops (all pigeon pea), and which were all sampled for *Helicoverpa* (eggs, larvae, and pupae).

Measure over-wintering of pupae of Helicoverpa

Surveys of potentially over-wintering pupae were also conducted in one or more of the Namoi, Macintyre and Gwydir Valleys, St George and Darling Downs regions, during late March to early May 2007, 2008 & 2009. Pupae were collected from beneath either pigeon pea (mostly) or cotton crops, and reared through to adult emergence to determine *Helicoverpa* spp.

Results

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

<u>Monitor early season abundance of *Helicoverpa* eggs & larvae.</u>

Virtually no native vegetation was sampled during spring 2006, due to the prevailing drought. Weed hosts for *Helicoverpa* eggs and larvae were restricted to irrigation channel banks and road-side verge areas that received some moisture.

Crops that were sampled included mostly faba bean and chickpea, but lupins, field pea, canola, linseed, coriander and wheat were also sampled. *Helicoverpa* pressure on faba bean and chickpea was such that control sprays were required on most crops. All moth emergences from weeds were *H. punctigera*, but some *H. armigera* also emerged from crop samples (see Fig. 8; data for previous years are included to enable long-term comparisons). Parasitoids were mostly Tachinid flies, *Microplitis* and *Chelonus*. In contrast to spring 2005, *Neocleptria* (= *Heliothis punctifera*) was very rare (in spring 2005 this species was as frequent as *H. punctigera* in the rearings from weeds and native vegetation), comprising 5 & 11% of rearings from crops and weeds respectively.

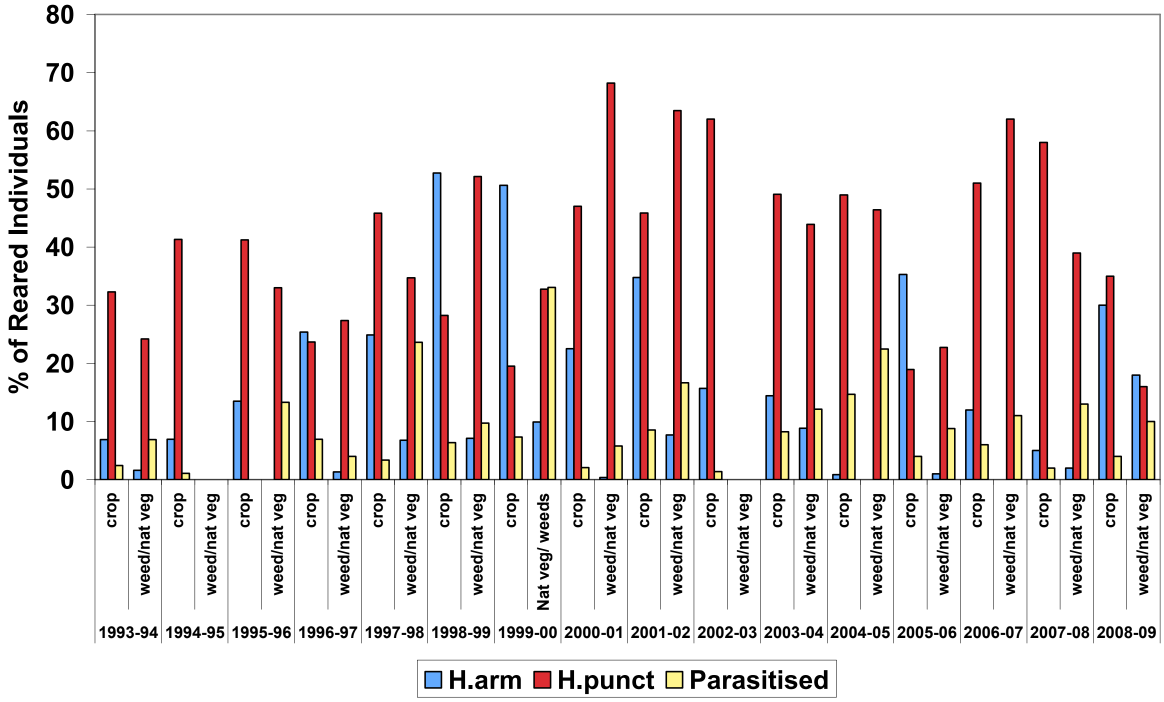

Fig. 8. Incidence of *H. armigera* and *H. punctigera* in eggs and larvae of *Helicoverpa* spp. collected from crops and weeds / native vegetation during July to November and reared through successfully to moths or parasitoids each year from 1993 to 2008. Unaccounted for rearings include disease, emergence as *Neoclepotria* (= *Heliothis punctifera*), and unknown causes of mortality.

In spring 2007, chickpea was the main crop sampled, followed by faba bean, lupins and field peas. Again conditions were very dry. Few weed and native vegetation hosts were available and, similar to the previous year, concentrated along some road verges and soaks. *H. armigera* was present as early as mid September, but generally rare. The vast majority of moths reared were *H. punctigera*. As in 2006, up to 13% of rearings were parasitised. *Neocleptria* comprised 11% of rearings from crops, but wasn't recorded from weeds and native vegetation.

In spring 2008, conditions were moister than the two previous years, but wild plant hosts were still not common. *Neocleptria* was not observed at all. Crop hosts sampled included faba bean, chickpea, field pea and canola. *H. armigera* and *H. punctigera* were reared in equal numbers from crops and weeds (cf earlier years when *H. punctigera* predominated).

Each cotton growing season thus started off with somewhat different moth communities present.

Monitor *Helicoverpa* moth abundance in pheromone traps.

In general, moth catches were lower in the pheromone traps set near ACRI, Narrabri during 2006-2009, compared with previous years (Figs 9-11). This pattern can be attributed, at least in part, to the reduced rainfall experienced in the region in recent years – and hence limited plant hosts for populations to build upon. These low numbers of moths made several aspects of our ecological research quite challenging. In Figs 12-13, trends in pheromone trap catches of both *H. armigera* and *H. punctigera* over time are summarised. Some major fluctuations in the abundance of the two moths are implied. The periods chosen for such analyses (1985-89, 1992-2002, & 2002-09) were selected partly because of availability of data from studies by other authors (1985-89), but also because of association of data sets with other trapping studies at the study site (light trap data recorded for 1992-2002, but not subsequently – data being compared in a publication currently in review).

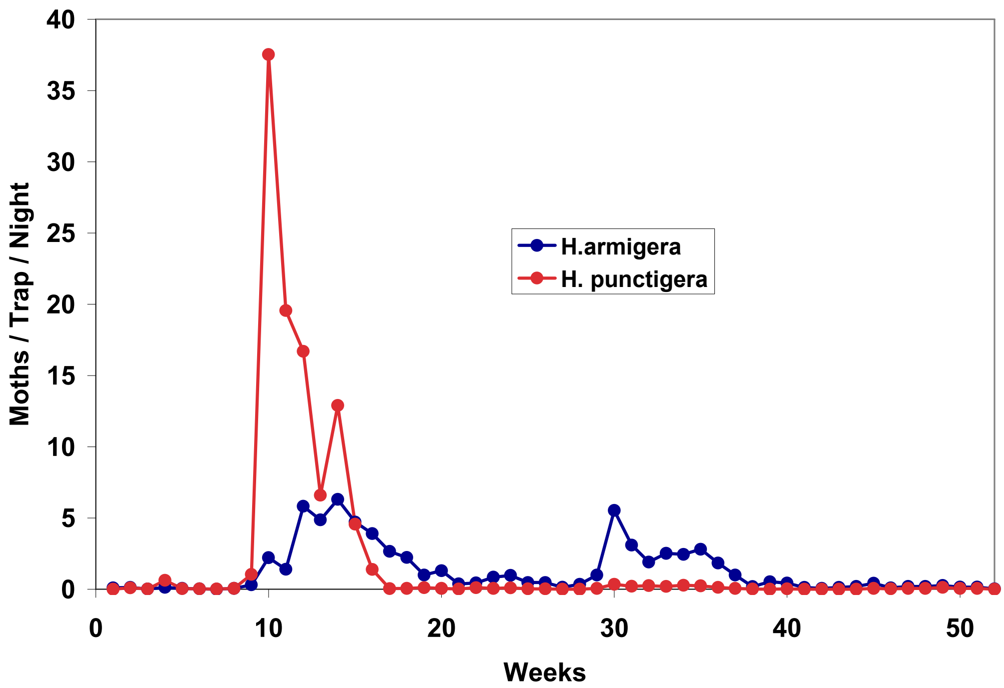

Fig. 9. Abundance of *H. armigera* and *H. punctigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley during 2006-07. Weeks are from 1 July to 30 June.

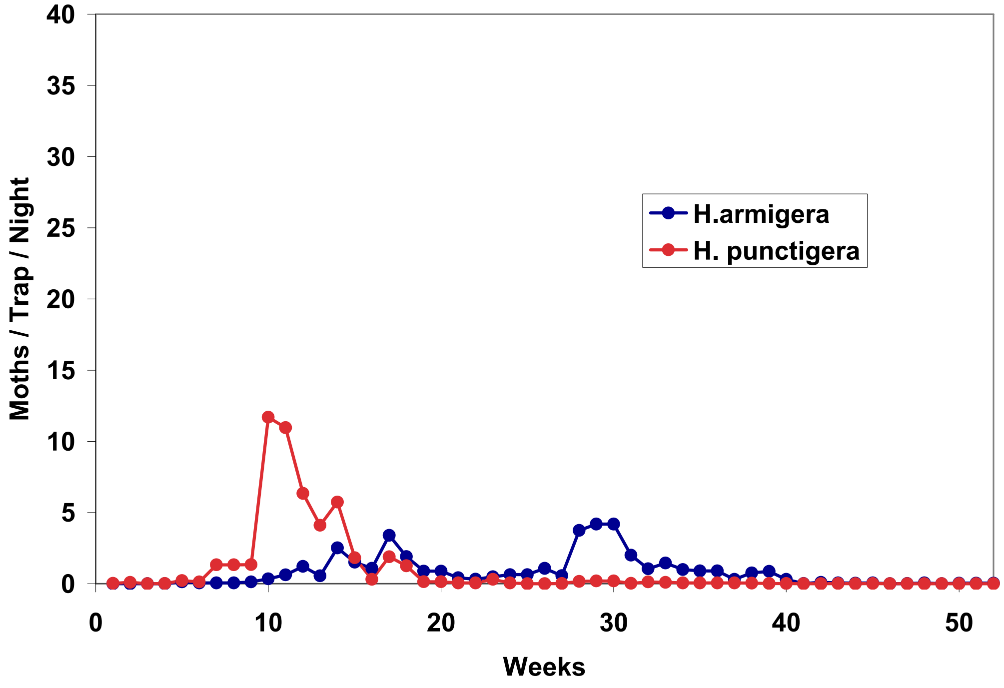

Fig. 10. Abundance of *H. armigera* and *H. punctigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley during 2007-08. Weeks are from 1 July to 30 June.

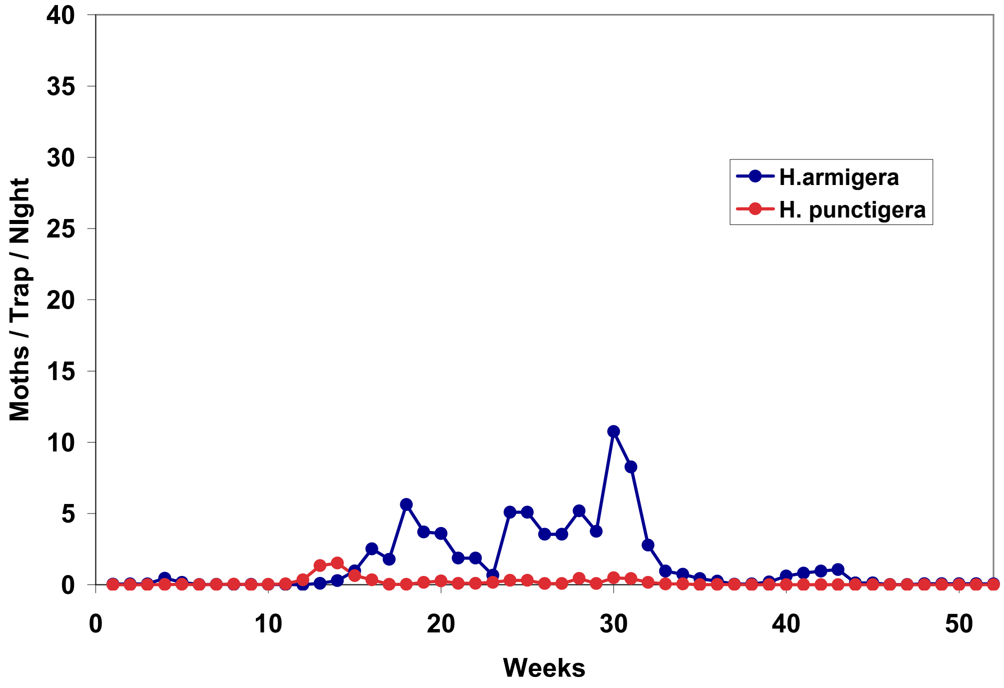

Fig. 11. Abundance of *H. armigera* and *H. punctigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley during 2008-09. Weeks are from 1 July to 30 June.

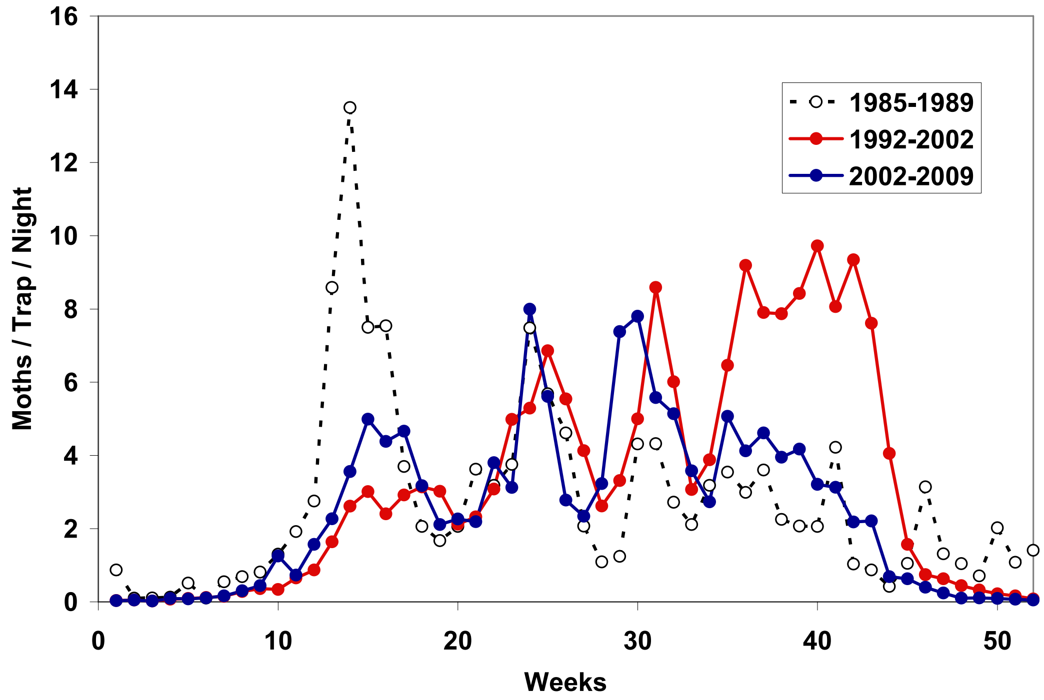

Fig. 12. Abundance of *H. armigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley – average data for 3 periods of time, as shown. Weeks are from 1 July to 30 June.

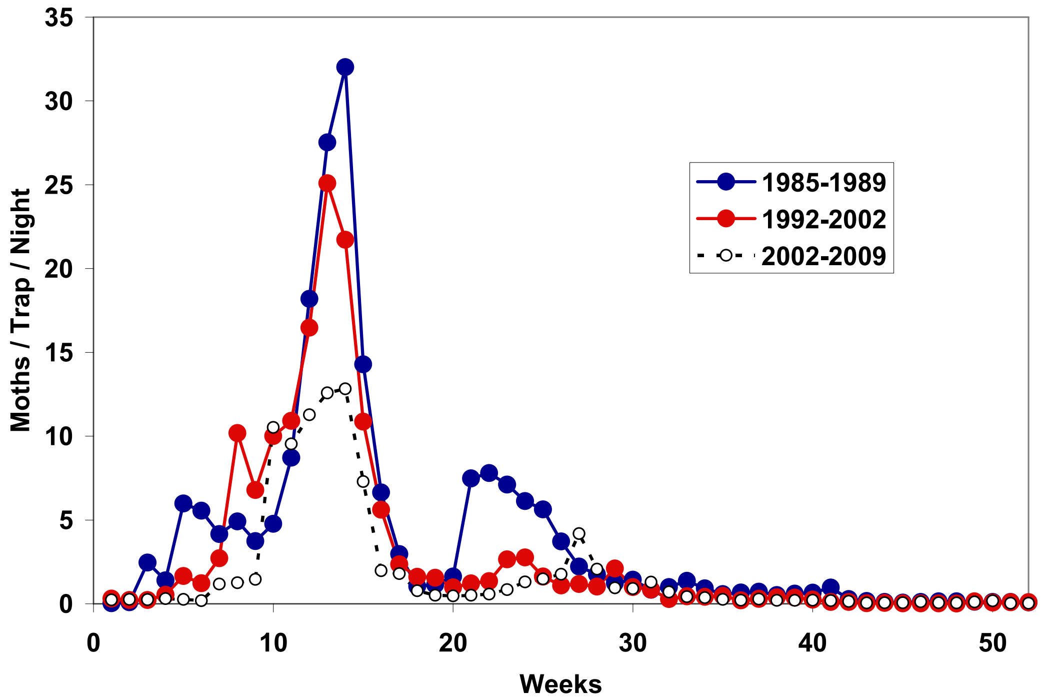

Fig. 13. Abundance of *H. punctigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley – average data for 3 periods of time, as shown. Weeks are from 1 July to 30 June.

Fig. 14 also considers long term trends in the numbers of *H. armigera* collected in pheromone traps near ACRI, Narrabri. Earlier versions of this figure have appeared in reports for previous CRDC projects. What seems to have emerged is a strong tendency for late generations of (male) *H. armigera* to have increased in abundance (or trappability) during the era of Ingard® (single Bt gene cotton) (1996-2004). Since then, numbers of *H. armigera* have generally been low, with the exception of the 2005-06 season. This particular season was rather unusual, in that it was particularly hot and the 3rd generation of *H. armigera* occurred substantially earlier than usual (see Fig. 15). This meant the numbers of moths in Weeks 21-30 were unusually inflated in this particular year – thus leading to a rather misleading appearance in the graph of very high 2nd generation numbers. We will continue to monitor population trends in the same way as before, using our Narrabri pheromone trap grid, for the next 3 years before formulating our thoughts on drivers of observed patterns – and then publishing the results. But for the moment, it is very tempting to speculate e.g. that the high numbers of late season *H. armigera* were related, at least in part, to the poor expression of Cry1Ac Bt in Ingard® cotton, coupled with a reduction in pesticide application at the same time. Of course, many other drivers (e.g. incidence of other crops on the landscape) could also be factors needing consideration.

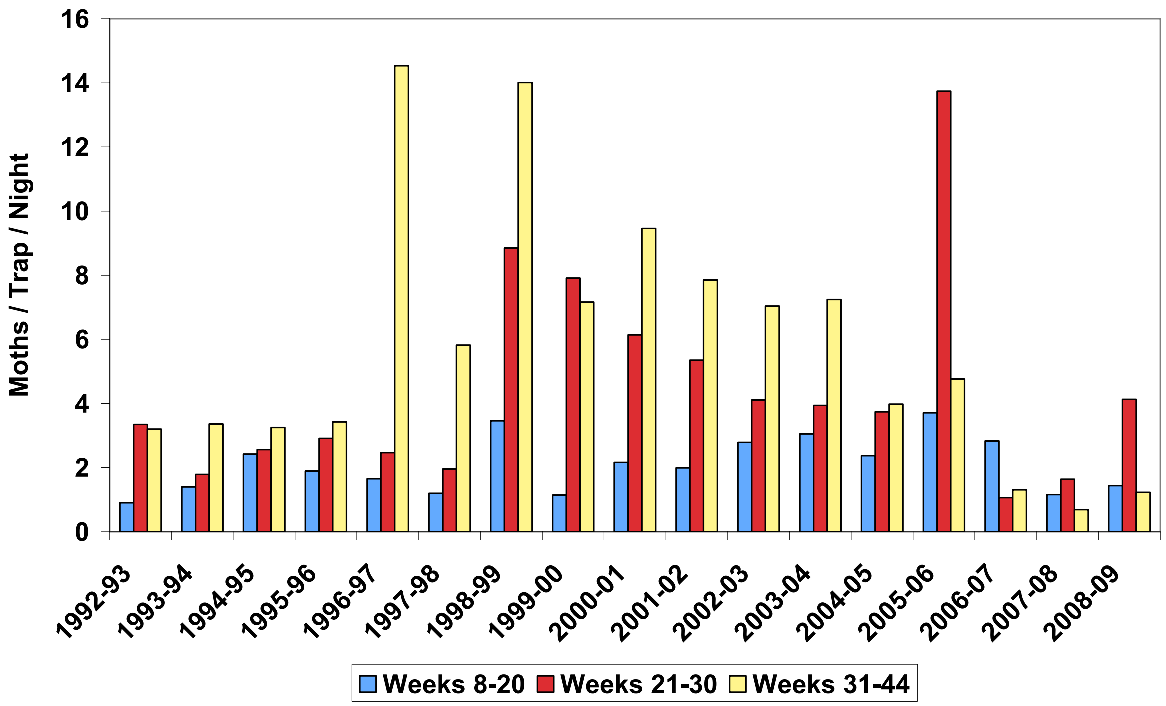

Fig. 14. Abundance of *H. armigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley – for the years 1992-2009. Data are apportioned within each season into 3 groupings : weeks 8-20, 21-30, and 31-44, which approximate the timings of generations of *H. armigigera*. Weeks are calculated from July 1.

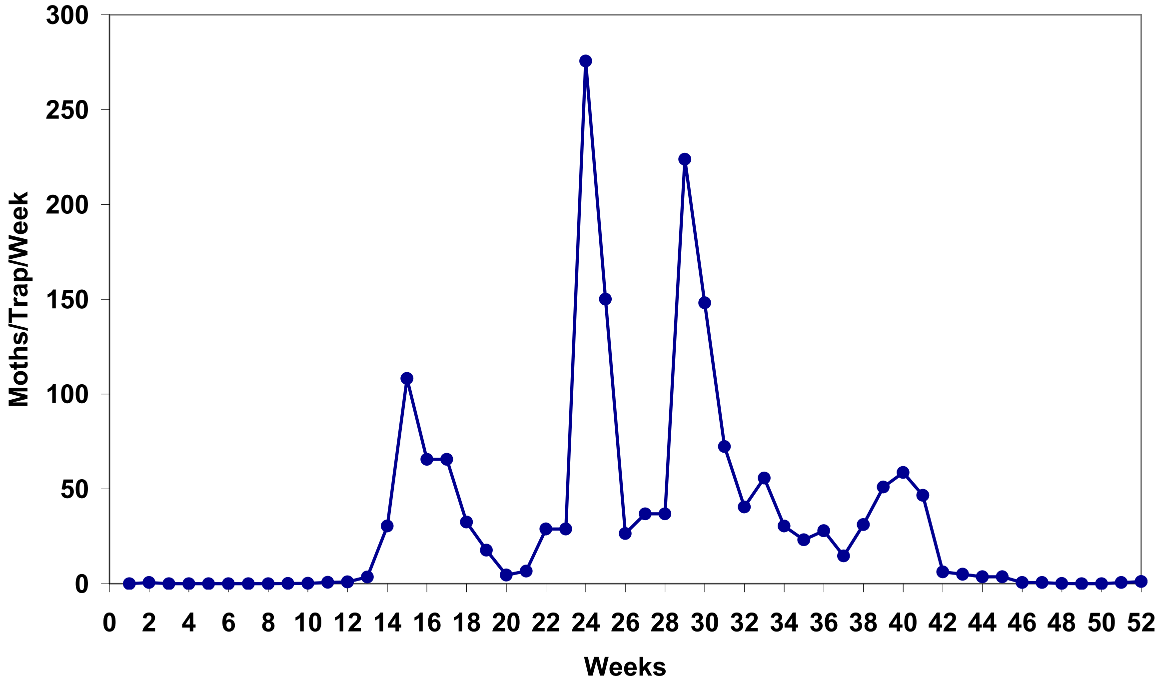

Fig. 15. Abundance of *H. armigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley during 2005-06. Weeks are from 1 July to 30 June.

In contrast, numbers of *H. punctigera* moths in pheromone traps near ACRI, Narrabri do not show any patterns in relation to the deployment of Ingard® cotton (Fig. 16).

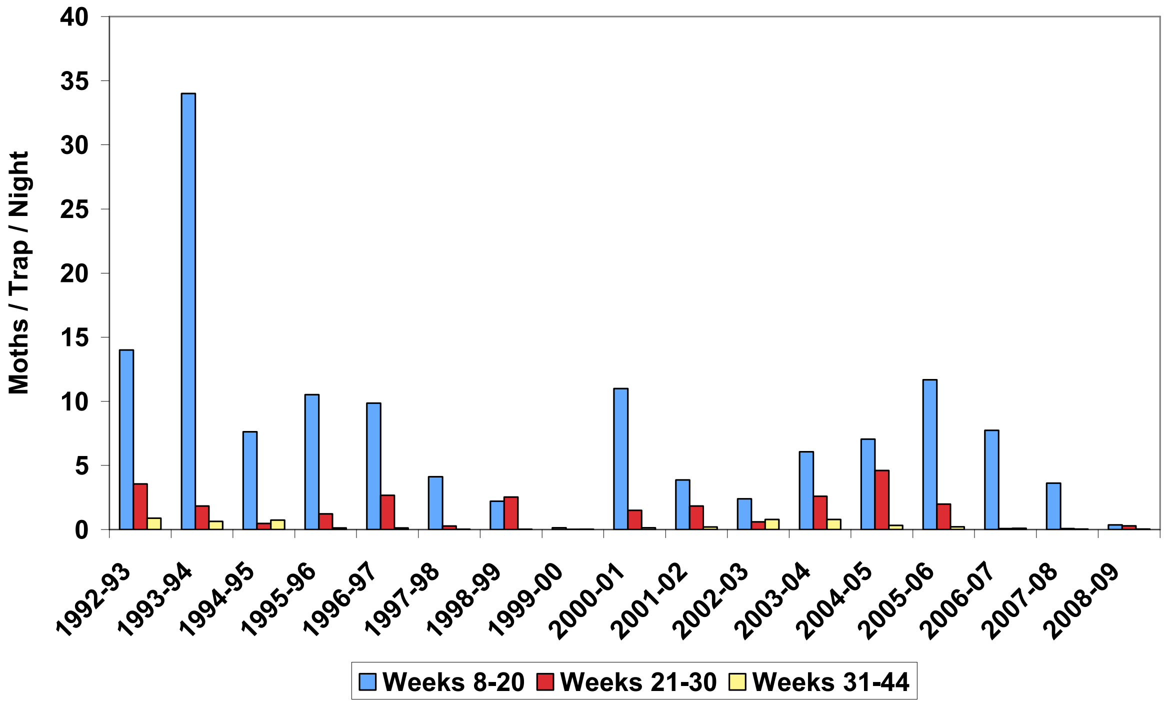

Fig. 16. Abundance of *H. punctigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley – for the years 1992-2009. Data are apportioned within each season into 3 groupings : weeks 8-20, 21-30, and 31-44, which approximate the timings of generations of *H. punctigera*. Weeks are calculated from July 1.

Turning now to relationships between trap catches near Narrabri and inland rainfall, the latter has varied substantially since 1970 (Fig. 17). Oertel et *al*'s (1999) study, which argued for a positive relationship between autumn-winter rainfalls in inland regions and subsequent 1st generation *H. punctigera* abundance in light traps at ACRI, was based on catches collected during the cotton growing seasons of 1973-76 and 1981-86. We have additional data sets for light traps (different design from those in Oertel *et al* 1999), from 1991-2002. In addition, we have the pheromone trapping data sets from 1992-2009 which can also be used to relate abundance patterns, for both moth species (but obviously only for males). Further, Zalucki *et al* (2009)(*Aust. J. Ent.* **48**, 85-96) published a continuous set of light trap abundance data for ACRI (1970-2002), across which they applied a correction factor to enable inclusion of both types of light trap used at ACRI. We have thus related all these data sets to both the autumn-winter rainfalls (April to July) recorded at the meteorological stations depicted in Fig. 1 (we have used total rainfalls as per Fig. 17 below, rainfalls for individual months, and total rainfalls for autumn-winter in particular regions), as well as rankings of the rainfall recorded in inland regions (see Fig. 2).

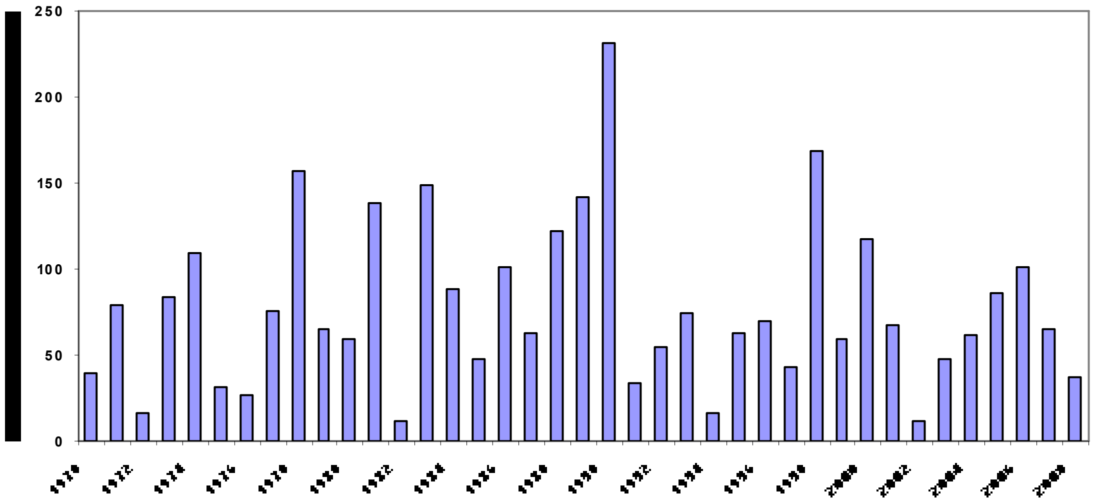

Fig. 17. Average rainfall (mm) between April to July each year across 36 meteorological stations in northern S.A., southern N.T., south-western Qld, and northern N.S.W. Such data (and individual months within these periods) were used to derive correlations with light trap records for *Helicoverpa* moths from 1973-76 and 1981-86 (other authors e.g. Oertel et al 1999) and from e.g. 1991-2002 (ourselves).

No significant correlations were found for either moth species using the light trap data collected from 1991-2002 (sexes were not separated for the analyses). With the pheromone trap data (1992-2002), the following significant correlations were obtained between trap catches of 1st generation moths and preceding inland rainfalls : for *H. armigera*, District 29 (Qld) (June, $r = 0.796$, $p < 0.01$), District 36 (Qld) (June, $r = 0.795$, $p < 0.01$), District 37 (Qld) (June, $r = 0.788$, $p < 0.01$), District 44 (Qld) (June, $r = 0.668$, $p < 0.05$; Total for all 4 months, $r = 0.632$, $p < 0.05$), District 45 (Qld) (Total for all 4 months, $r = 0.673$, $p < 0.05$), All Districts combined (Total for all 4 months, r

= 0.646, $p < 0.05$); and for *H. punctigera*, District 17 (S.A.) (May, $r = 0.832$, $p < 0.01$), District 36 (Qld) (July, $r = 0.670$, $p < 0.05$), District 46 (N.S.W.) (May, $r = 0.858$, $p < 0.01$). The other 51 and 55 possible correlations involving rainfall and pheromone trap records for *H. armigera* and *H. punctigera* respectively were not significant. It seems reasonable to conclude that at least some of the significant correlations we obtained could easily have occurred by chance. Indeed, when a Bonferroni correction was made to p, to take into account the large numbers of potential correlations and chance significance, none of the correlations listed above were sufficiently strong to remain acceptable (p needed to be < 0.001). Similarly, a modified Bonferroni test, which is less stringent, failed to yield any significant correlations. It is however worthy of note that all the significant correlations, obtained before Bonferroni corrections, were positive (10/10).

In addition, we were unable to obtain any significant correlations between the "corrected" light trap data (from Zalucki *et al* 2009) and the inland rainfall data, nor between the ranked inland rainfall data (based on the Long Paddock web site maps) and our light trap data (1991-2002), the "corrected" light trap data, or the pheromone trap data.

Overall, we therefore conclude (whilst not disputing that movements of moths, in particular *H. punctigera* from inland Australia to more eastern cropping regions in late winter – spring, may well occur), there is very little evidence to support that such movements can be predicted using inland rainfall patterns.

Another tenet of our current understanding of the ecology of *Helicoverpa* spp in Australia is that *H. punctigera* rarely over-winters in eastern cropping regions. Rather, it is argued that this species immigrates to such regions in early spring and then its numbers decline over the summer season, for thus far inexplicable reasons. Whether or not *H. punctigera* migrates "back" to inland regions in late summer is not understood. On the other hand, it is well accepted that *H. armigera* does over-winter in eastern cropping regions. This being so, one would expect to find a positive correlation between the numbers of *H. armigera* in late season generations and the 1st generation in the subsequent spring in the vicinity of Narrabari, and not so for *H. punctigera*. An opportunity to test this has arisen, using data from our trapping regimes, in particular the pheromone trap data sets, which represent the most years. Curiously, we found the opposite to what we expected (Fig. 18). There is evidence for a positive relationship between the abundance of *H. punctigera* moths in pheromone traps near ACRI, Narrabri in the 3rd generation and the subsequent 1st generation (for 1993-2008, $r = 0.587$, $p < 0.05$). No similar correlation has however been found for *H. armigera* ($r = -0.137$, $p > 0.05$). We offer no explanation for this puzzle at present, except to say that the results highlight that we probably need to far better understand the over-wintering ecology of *H. punctigera* than we currently do.

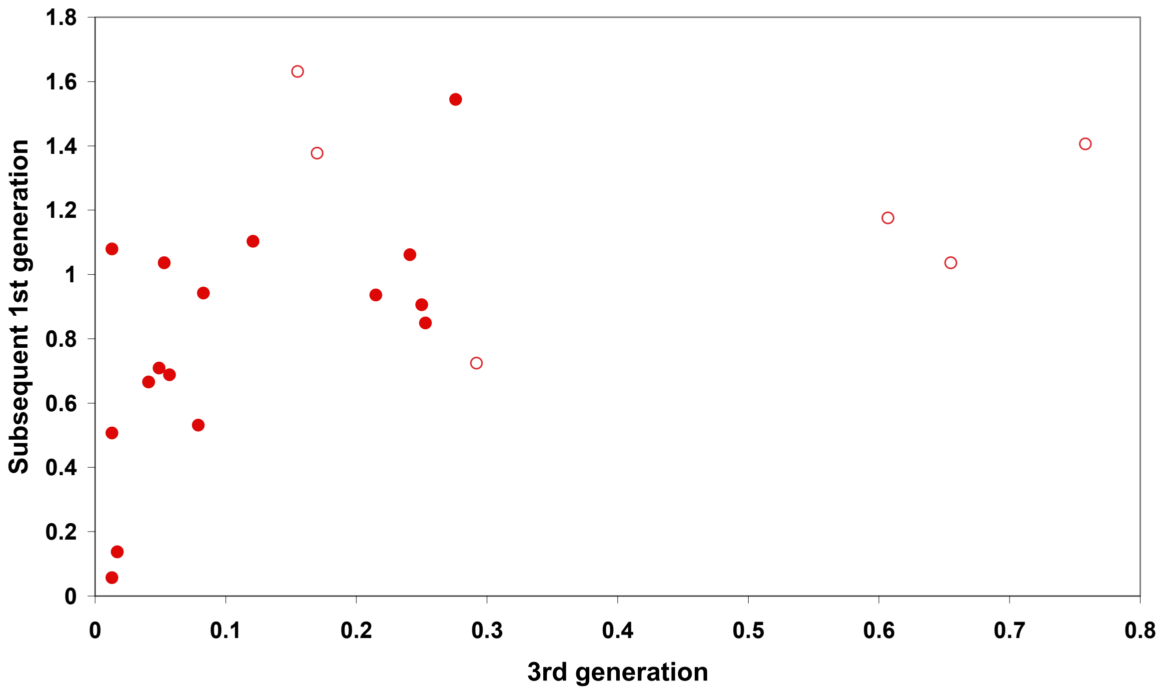

Fig. 18. Log (x + 1) plots for *H. punctigera* in pheromone traps near ACRI (moths / traps / night) (1987-2008). "Open" points are 1987-1992 (when more traps were used, in the same general region – but not in the same specific locations).

Totals of 2,640 female *H. armigera* moths and 4,454 female *H. punctigera* moths, caught in light traps set in cotton crops, were dissected for evidence of reproductive maturity and spermatophores from 1990-2002. The reproductive maturity (% gravid) of female *H. punctigera* caught in light traps was consistently higher than that of *H. armigera* (Fig. 19) (Chi-squared with 1 d.f. = 179.0, $p < 0.001$, for total catches across all years). There was a clear seasonal peak in the reproductive maturity of *H. armigera* (December - February), but this was less evident in *H. punctigera*. The average number of spermatophores / female were 0.74 and 1.03 for *H. armigera* and *H. punctigera* respectively. Overall, 56% of female *H. armigera* had not mated, 24% had mated once, and 20% had mated more than once when they were caught (Fig. 20). Put differently, 45% of mated *H. armigera* had mated more than once. In contrast, 35% of female *H. punctigera* had not mated, 39% had mated once, 26% had mated more than once, and 40% of mated moths had mated more than once. Analysing the frequencies for all spermatophore scores (0-6 / female) for the two species gave Chi-squared with 6 d.f. = 320.6, $p < 0.001$. This suggests that *H. punctigera* was more likely to have mated than *H. armigera*, at the time they were trapped. On the other hand, by analysing only the frequencies of "positive" spermatophore counts (1-6 / female) we obtained Chi-squared with 5 d.f. = 14.7, $p < 0.05$. This suggests that mated *H. armigera* females had mated slightly more often than the mated *H. punctigera* females.

Note : other studies (e.g. Coombs *et al* 1993, *Bull. Ent. Res.* **83**, 529-34) have reported much lower levels of reproductive maturity and spermatophore counts in *H. armigera* and *H. punctigera* caught in light traps over mountain tops in northern N.S.W. (and considered to be long distance migrants) than we observed. It seems unlikely that the moths we trapped were long distance migrants, but relatively local in their movements.

We also dissected many female moths which were caught in light traps at ACRI, Narrabri in the 1990's in association with various crop types. In essence, this work showed there were no obvious differences between degree of mating and reproductive maturity of the moths. We do not present this data here.

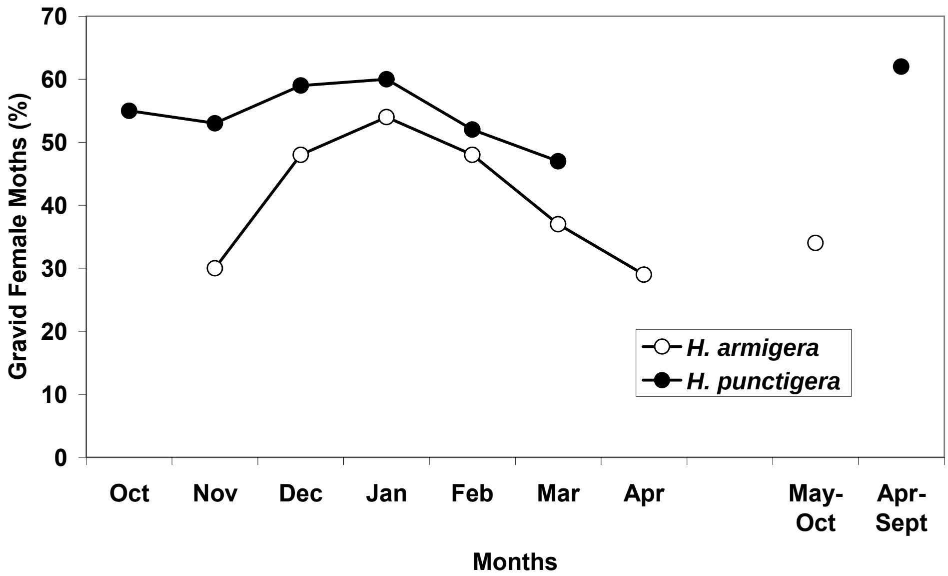

Fig. 19. Reproductive maturity of *H. armigera* and *H. punctigera* female moths caught in light traps set within cotton crops at ACRI, Narrabri, New South Wales from 1990-91 to 2001-02. The number of observations in each month (n), varied between 193-1,058 for *H. armigera* and 157-1,664 for *H. punctigera*. Data for May-October and April-September are based on summations of relatively small catches in months between those times (total n = 122 for *H. armigera* and total n = 68 for *H. punctigera*).

The pheromone traps managed by consultants in the St George region continued to provide us with invaluable data on population trends in *H. armigera* and *H.punctigera*, post the deployment of Bt cotton in the region. The numbers of both species have varied chaotically over recent years near St George, with no obvious trends in moth numbers (Figs 21-22). The abundance of eggs does however seem to have been more consistently high in recent years (Fig. 23). Curiously, the season with highest egg numbers (2007-08) was one of the poorest for moth numbers, especially *H. punctigera*. This could imply strong pressure from natural enemies in that particular season.

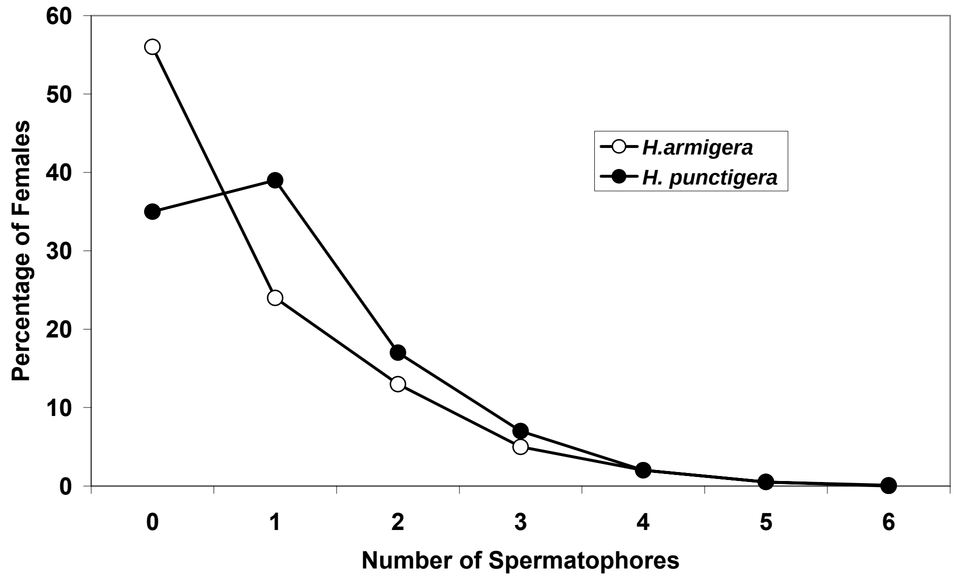

Fig. 20. Average frequency of spermatophores in dissected *H. armigera* and *H. punctigera* female moths caught in light traps set within cotton crops at ACRI, Narrabri, New South Wales over the 11 year period from 1990-91 to 2001-02.

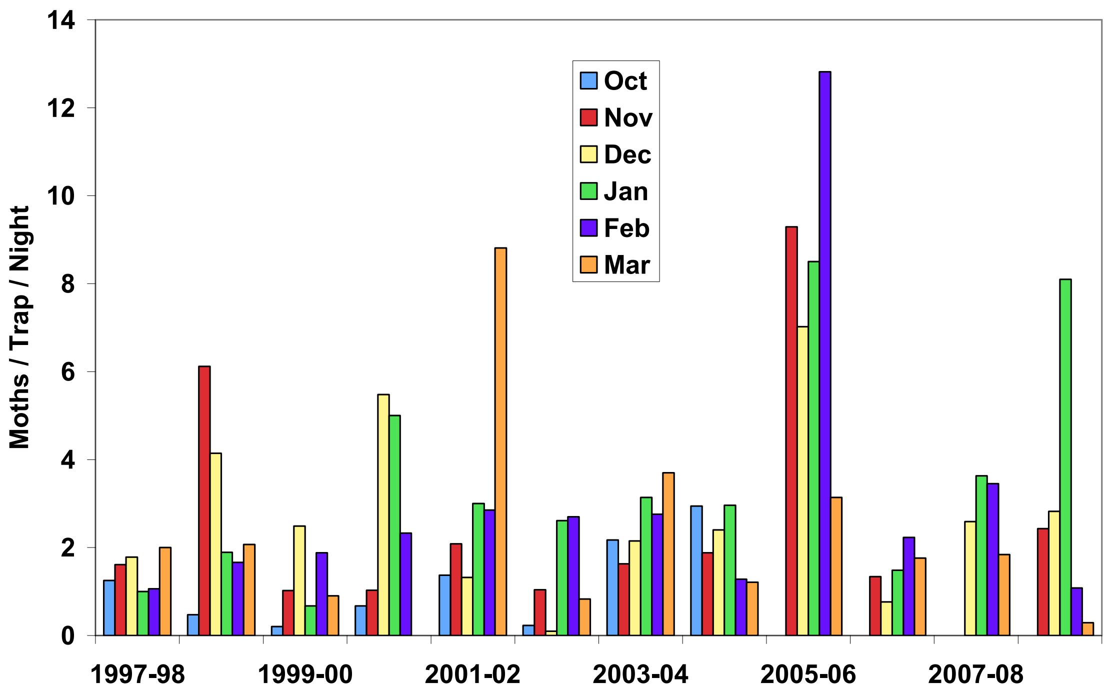

Fig. 21. Abundance of *H. armigera* male moths in pheromone traps set within the St George region between 1997-2009.

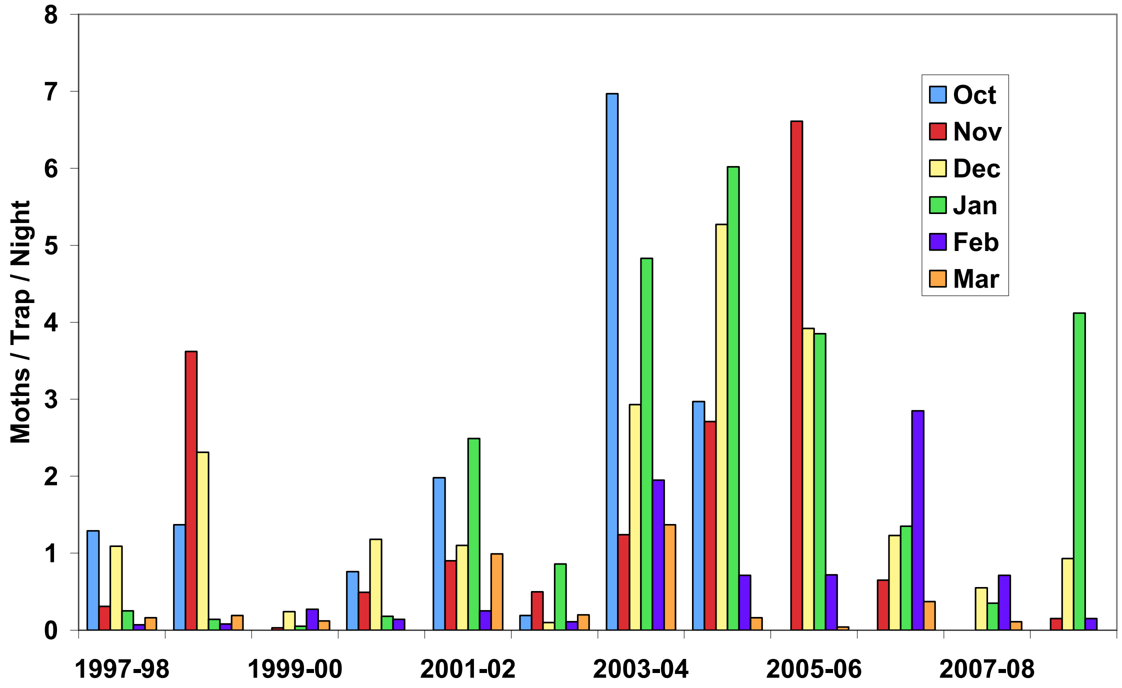

Fig. 22. Abundance of *H. punctigera* male moths in pheromone traps set within the St George region between 1997-2009.

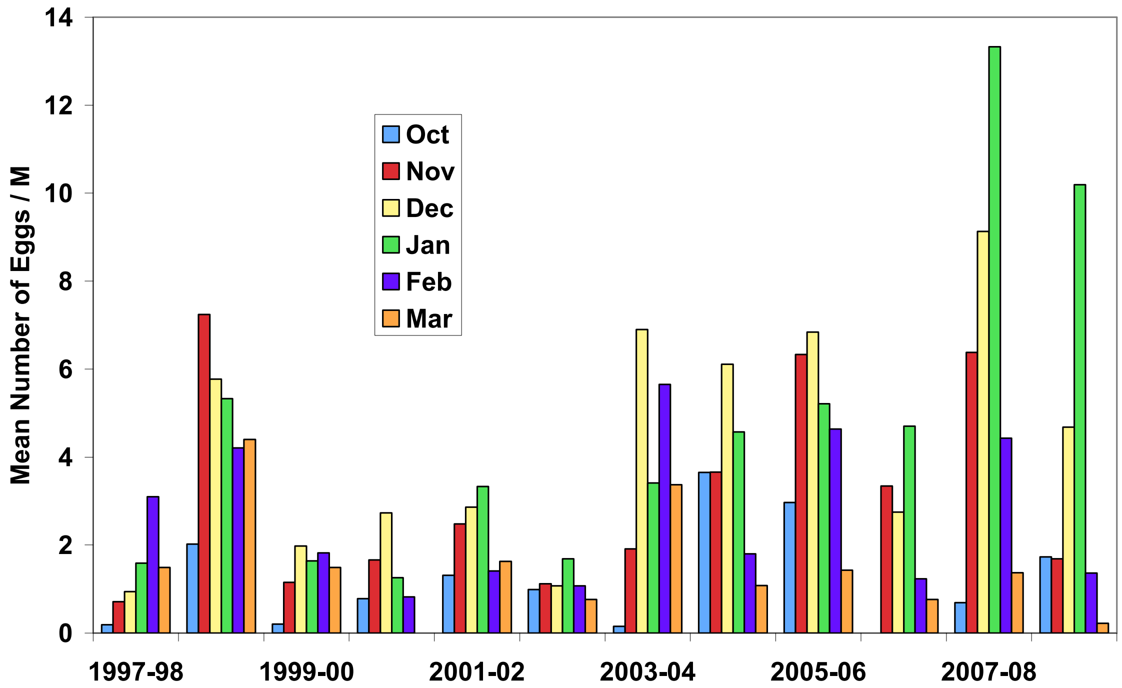

Fig. 23. Average abundance of *Helicoverpa* eggs on Bollgard II cotton within the St George region between 1997-2009.

Identify novel refuge crop options for both dryland and irrigated Bt cotton production.

Pigeon pea as an option for a dryland refuge crop

The abundance of *Helicoverpa* eggs, larvae and pupae was usually higher in and beneath pigeon pea cf cotton at Getta Getta (Fig. 24). The majority of moths reared from field collections at Getta Getta were *H. armigera*. Pupae were especially heavily parasitised (Fig. 27). The vast majority of pupal rearings were of course from pigeon pea soils. Similar results, although not as consistent across all samplings, were obtained at Blue Hills (Figs 25 & 27). Pupal parasitism wasn't as extreme at Blue Hills. Numbers were too low to merit comparisons between pupal parasitism beneath pigeon pea and cotton. At Dobikin, the abundance of *Helicoverpa* eggs and larvae was consistently higher on pigeon pea cf cotton crops (Fig. 26). Pupae were rare beneath both crops.

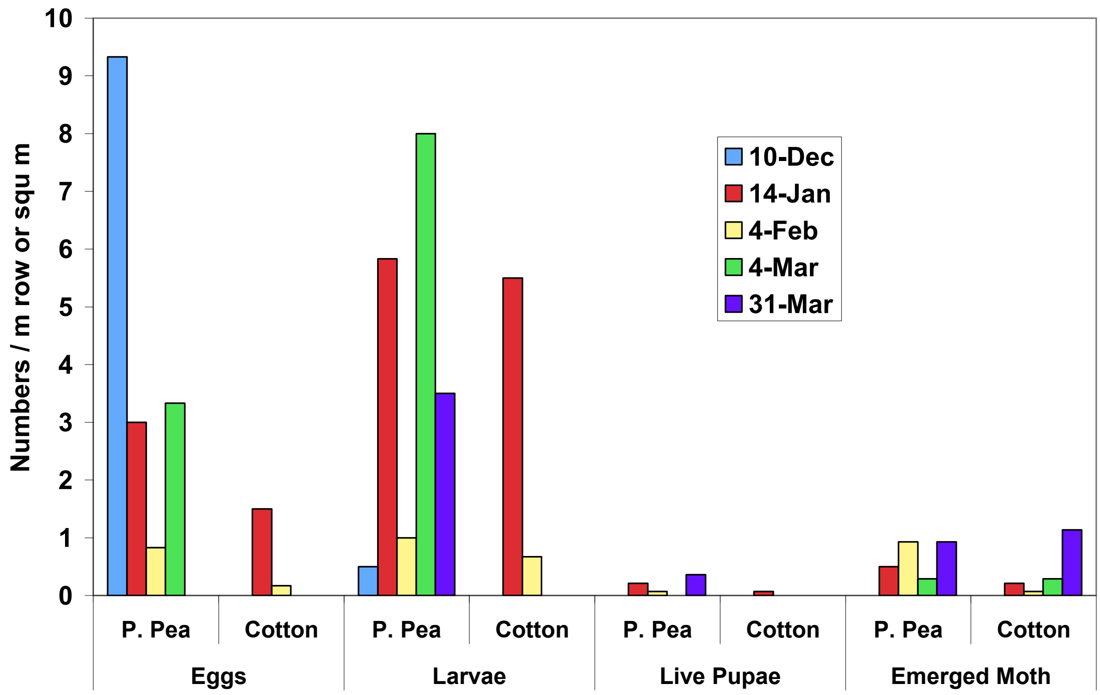

Fig. 24. Abundance of eggs, larvae, live pupae and emerged moths (from pupal case counts) of *Helicoverpa* in pigeon pea and unsprayed conventional cotton crops at Getta Getta during 208-09.

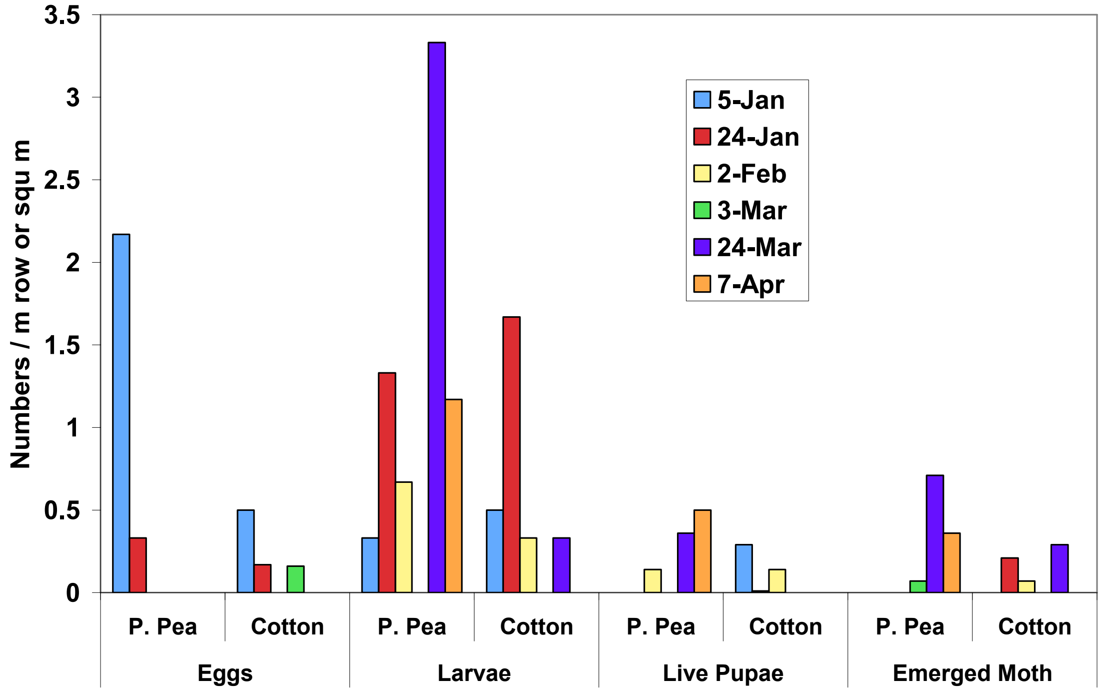

Fig. 25. Abundance of eggs, larvae, live pupae and emerged moths (from pupal case counts) of *Helicoverpa* in pigeon pea and unsprayed conventional cotton crops at Blue Hills during 208-09.

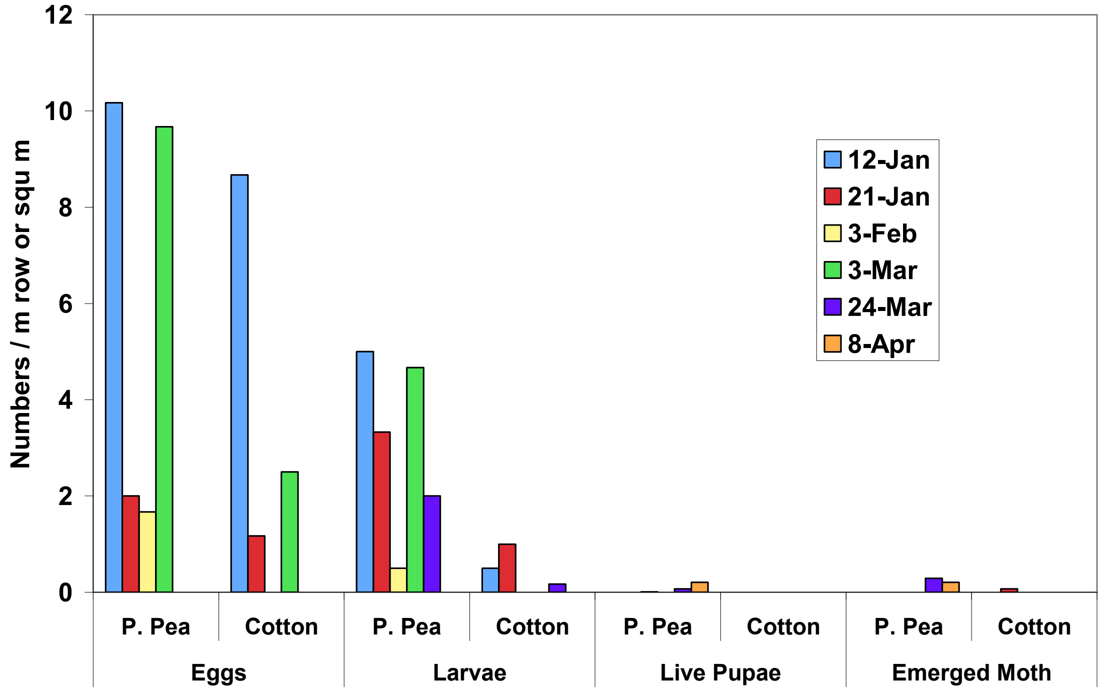

Fig. 26. Abundance of eggs, larvae, live pupae and emerged moths (from pupal case counts) of *Helicoverpa* in pigeon pea and unsprayed conventional cotton crops at Dobikin during 208-09.

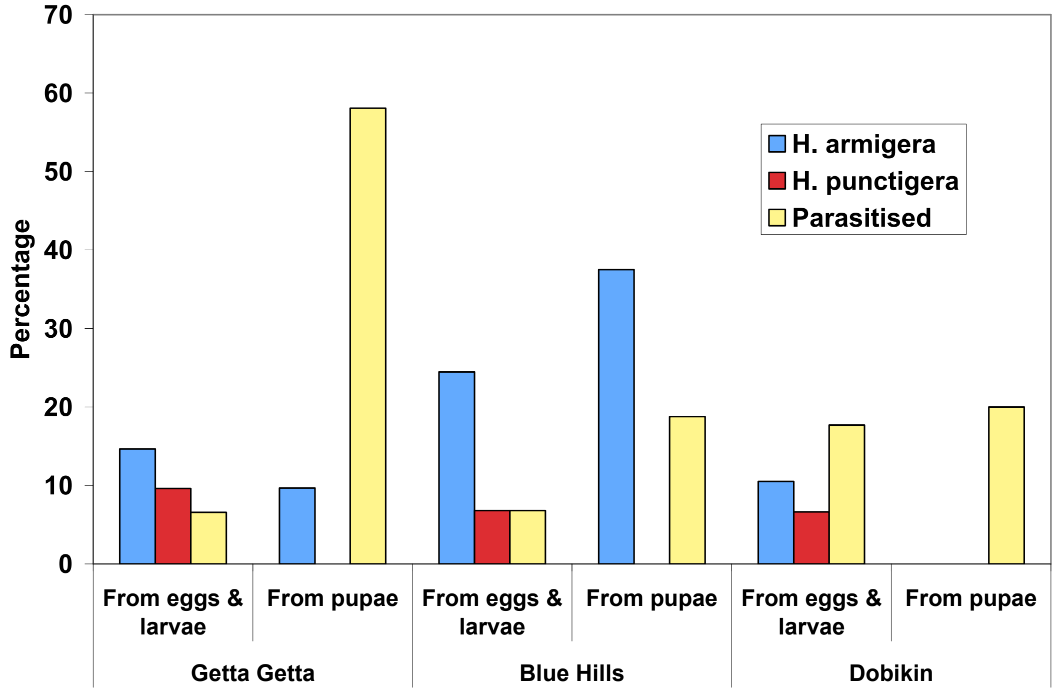

Fig. 27. Rearings of eggs and larvae and live pupae of *Helicoverpa* collected within dryland pigeon pea and unsprayed conventional cotton crops (data combined for the 2 crop types), during 2008-09.

In general, the results suggest that pigeon pea is as productive, if not more so, than unsprayed cotton for *Helicoverpa* as a refuge in dryland situations. The numbers of pupae recorded were surprisingly small, perhaps worryingly so, but the research was hampered in terms of efficiency of sampling for pupae in dry soil (very difficult to sort through cf irrigated soil). We have little doubt we under-estimated pupae abundance in these habitats. We are pursuing the possibility of emergence cages providing alternative estimates of pupal abundance in dryland fields in future years.

Split plantings of cotton for an irrigated refuge crop

The Bollgard II and the 1st planting of unsprayed conventional cotton were first sampled on 31 December 2008, whilst the 2nd planting of unsprayed conventional cotton was first sampled on 10 February. Egg numbers on the Bollgard II and the 1st planted cotton refuge crop were temporally very similar (Fig. 28). Egg numbers of the 2nd planted cotton refuge crop were greater on 10 & 27 February and 12 March, 2009, than the other two crops. [The latter were not sampled on 26 March and 7 April, 2009].

Larvae were rarely found on Bollgard II; they were more common on the 2nd planted cotton refuge crop cf the 1st on 26 March 2009. Live pupae were also more common late in the season beneath the 2nd planted cotton refuge crop cf the 1st. Emerged moths were however consistently higher under the 1st planted cotton refuge.

These results suggest in general that split-planting of cotton refuges may extend the production of *Helicoverpa* from refuges later into the season, but more data (i.e. more seasons surveyed) are needed to confirm such. We are pursuing such studies in our new project.

Overall, parasitism of *Helicoverpa* pupae at Gunedra (as shown through laboratory rearings of field collected samples) was high (56 %), whilst for eggs and larvae it was lower (Fig. 29). The majority of reared moths were *H. armigera*.

A separate trial was conducted at Werrina Downs (near Dalby) in 2007-08 to evaluate split-plantings of conventional cotton as a novel refuge option, but due to lack of irrigation that year, crop performance was poor. We are thus reluctant to report the results here.

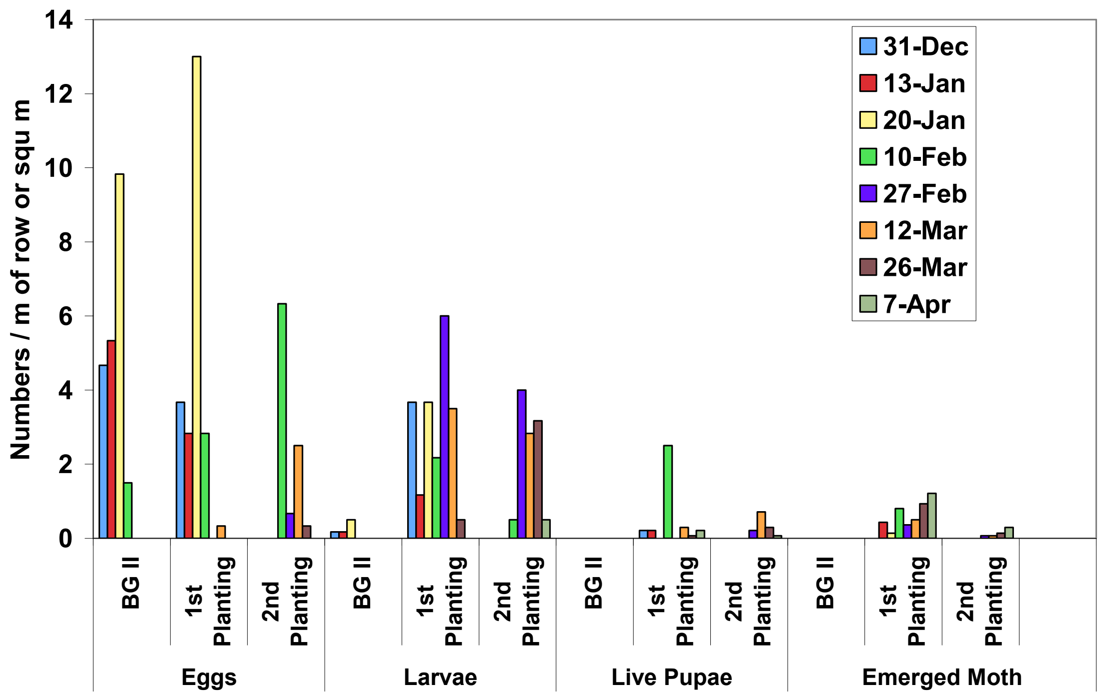

Fig. 28. Numbers of *Helicoverpa* eggs, larvae, live pupae and emerged moths (from pupal case counts) recorded at Gundera (Wee Waa) in the split-planting of cotton refuges trial during 2008-09.

Mixed crop agronomy & moth production

Our aim with growing mixed dryland refuge crops in the 2008/09 season was to test the agronomy of such crops, and to undertake 3 samples for eggs, larvae and pupae throughout the season. As expected, the mung beans grew well early in the season, and had almost rotted away by May, thereby posing no trash problem when we harvested the refuge (Fig. 5 (c). Pigeon pea grew well from the beginning and remained strong throughout the season. By April pigeon pea was out-growing the cotton, but is was impossible to defoliate (Fig. 5 (d) and produced a lot of green trash when the refuge was harvested.

We looked at the boll production and the total lint produced by the mixed crops in comparison to Bt and conventional cotton. Not surprisingly, the Bt cotton produced more bolls than the refuges (Fig. 30 (b) chi sq=44.7,df=12, P<0.001) and more raw lint

(6 bales/ha for the Bt plot, and 3.6, 2.8 and 2.6 respectively for the conventional, 10% pigeon pea, and 10% mung bean plots). Boll production was consistently high for the Bt crop for the middle, top and very top of the plant.

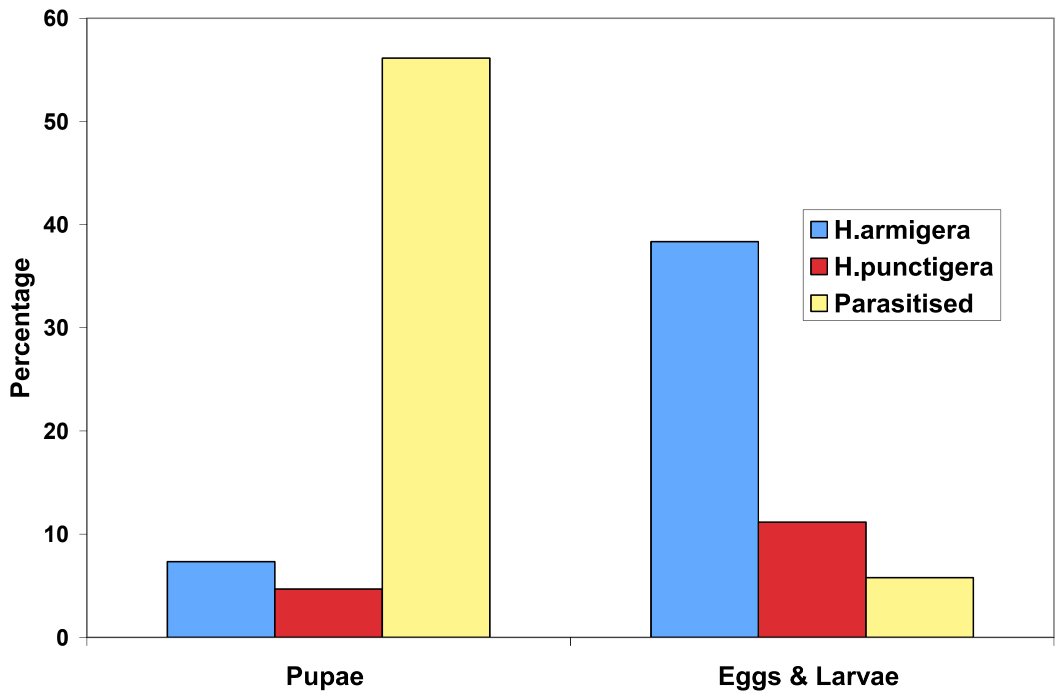

Fig. 29. Results of laboratory rearings of *Helicoverpa* eggs and larvae and pupae collected from the split-planting of cotton refuges trial during 2008-09.

On the other had, there was a significant difference in the number of fruiting sites, with 10% mung beans producing significantly more fruiting sites than expected (348) and Bt cotton producing significantly less (271, goodness of fit test: chi sq= 11.6). Most of the increase in fruiting sites in the mung beans occurred in the bottom and vegetative parts of the plant.

This season we undertook four samples for eggs and larvae, and three samples for pupae (Fig. 31). In December there was no significant difference in the number of eggs laid on any of the treatments (ANOVA; df =3,20; F= 1.65; P=0.21 NS). The samples taken on the 15th of January showed a significant difference between treatments for both egg lays and larvae, with a strong crop by treatment interaction (ANOVA treatment x lifestage; df=4, 20; F= 8.06; P<0.001; Fig. 31 (a)) . None of the treatments had egg lays that were significantly different from the eggs lays on Bt cotton. However, both conventional cotton and the mung bean mix had egg lays that were significantly less than those on the pigeon pea mix. All eggs found in the mung bean mix were found on cotton plants, except 1 in December. In the 10% pigeon pea mix, the load was more evenly spread. Although no eggs in December were found on pigeon pea, 18 or 66% were found on pigeon pea on the 15th of January, and the only 2 found on the 29th of January were on pigeon pea.

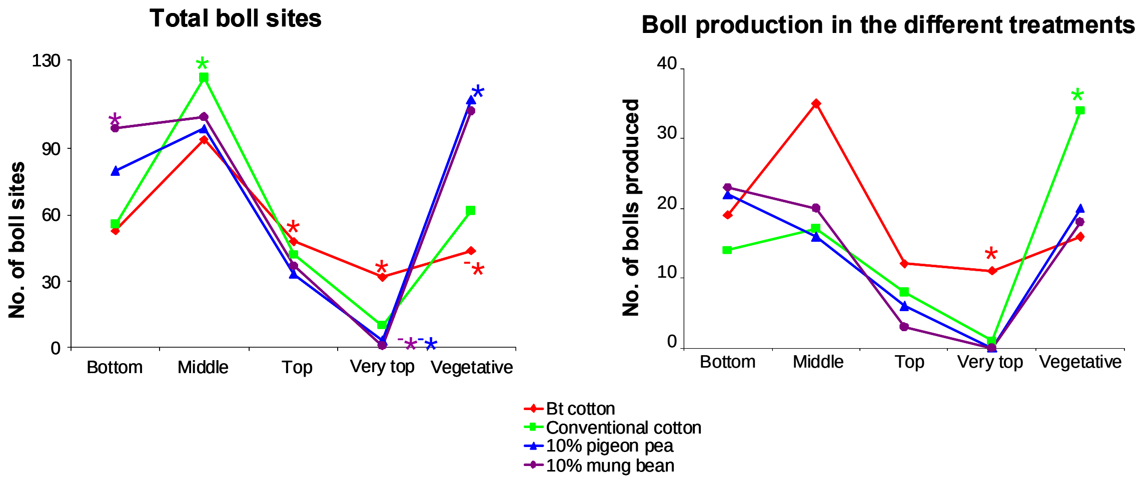

Fig. 30. Total boll sites and bolls produced by cotton in the various treatments. The asterisks above the line indicate a sample which was significantly higher than expected, while an asterisk preceded by a negative sign indicates a sample that was significantly lower than expected.

The 10% Mung bean mix had significantly higher numbers of larvae than any other treatment on the 15th of January (Fig. 31 (a) although again all of these were found on cotton plants (although two larvae were found on mung bean plants on the 29th of January). In the 10% pigeon pea crop, 3 out of 8 larvae, and 4 out of 7 larvae were on the pigeon pea plants on the 15th and 29th of January respectively.

We found 20 pupae, pre-pupae, and pupae husks (which had apparently yielded moths) in total (Fig. 31 (b)). None were found in the 15th of January sample. Of the pupae, 30% were damaged while sampling or failed to emerge, and none yielded moths in the laboratory (although three pupae yielded the wasp *Heterpelma*, and 4 yielded tachnid flies).

The pigeon pea mix appeared to maintain attractiveness later into the season, and produced the highest number of pupae, but most of the pupae were damaged or yielded parasitiods.

These results showed that 10% mung bean and 10% pigeon pea were feasible as a refuge options. However, their presence seemed to mostly enhance early boll production, and not increase later boll production as was predicted.

This was particularly the case with the mung bean mix. The rationale for planting a mung bean mix was to draw early season larvae to the mung beans, sparing the cotton. Because it would have been spared early season damage, the cotton would remain attractive later into the season. This did not occur. Fruit production was particularly high early in the season, but few of these fruit survived, apparently eaten out by *Helicoverpa*. In addition, all eggs and larvae found in the mung bean mix were found on cotton plants (except one egg and two larvae). So the presence of mung beans did not draw the eggs and larvae to these plants, but they did appear to enhance the number of larvae in this treatment early in the season and enhance cotton fruiting, making these refuges more effective. However, the high number of larvae early on in the 10% mung bean mix may have deterred moths from ovipositing on this treatment latter as larvae frass is known to be a deterent to oviposition in *Helicoverpa* (Firempong and Zalucki 1991, *Aust. J. Zool.* **39**, 343-50; although eggs at least 12 hours old are not). The cotton in the 10% mung bean trial also seemed to stop producing fruit earlier than the Bt crop, and would not have been useful for a late season rally of *Helicoverpa*.

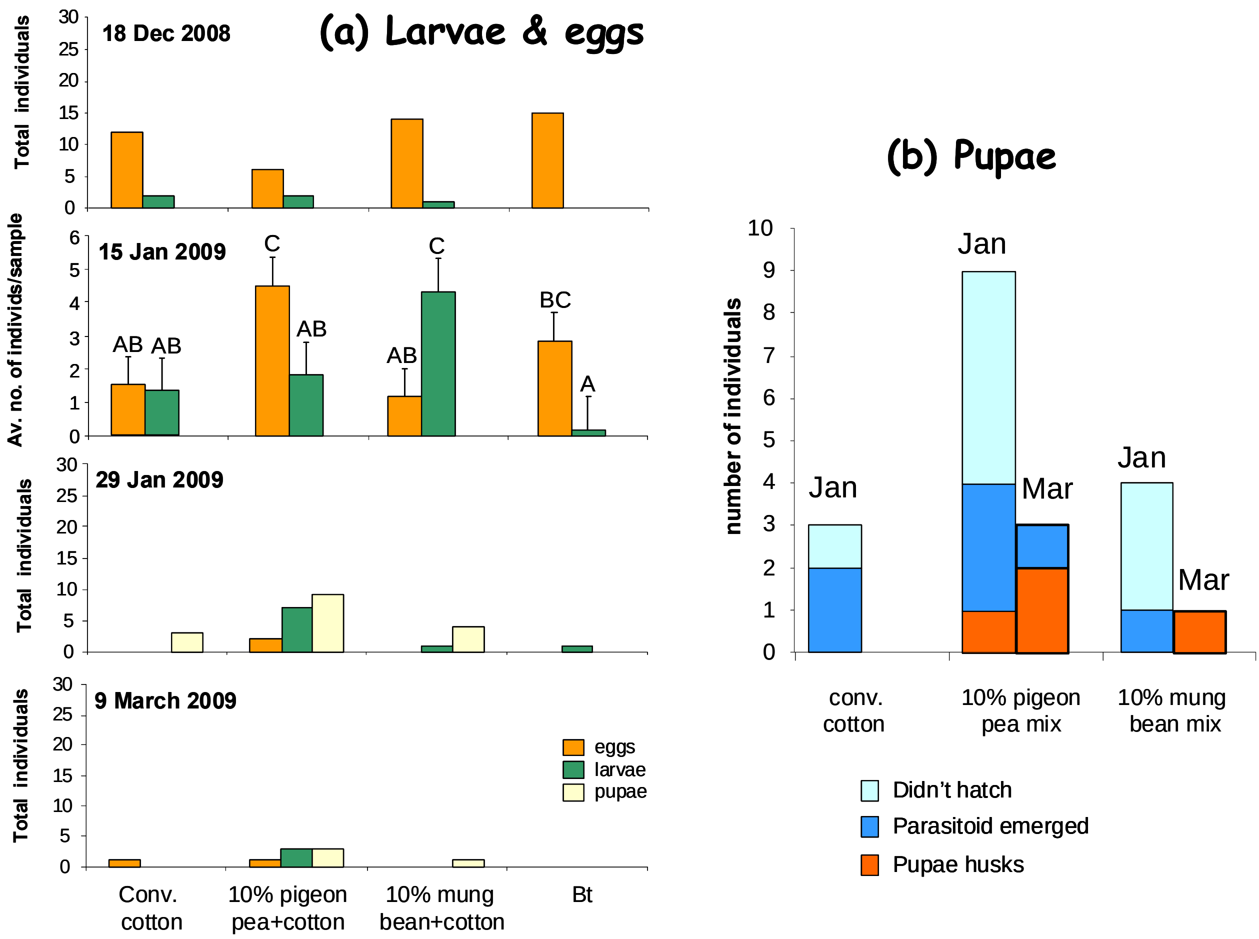

Fig. 31. Overview of eggs, larvae and pupae collected from mixed crop unirrigated refuges at ACRI. No pupae digs were undertaken on Dec 18; no pupae were found on Jan 15.

The 10% Pigeon pea mix, on the other hand, attracted a high number of egg lays, which continued throughout the season, but which were not significantly different from those on Bt crop. The pigeon pea plants were attractive in the crop. Some of this attractiveness may have been due to their height, as relative plant height is known to attract oviposition in *Helicoverpa* (Firempong and Zalucki 1990, *Aust. J.*

Zool. **37**, 675-83). Even though the cotton cut out before the Bt cotton crop, the pigeon pea plants remained attractive to the end, and so this mix could be useful in a late season rally. The pigeon pea mix also produced more pupae, although none of these survived.

As 10% mung bean increased both fruit and larvae production in this preliminary trial, these initial findings suggest that a crop mix could be used to enhance early season attractiveness of a refuge. As such, it could be useful in combination with methods to increase late season attractiveness, such as split planted refuges. In these refuges, the early planted crop is not offering as much moth production as a normal refuge because only half the refuge is operating. By enhancing the early season production of this refuge (perhaps by using a 10% mung bean mix in the first planting) this problem could be overcome. The potential of mixed crops as refuges is being further investigated in the current refuge project (Enhancing the efficiency of Bt refuge crops within a changing cotton environment).

<u>Measure influence of Magnet® on *Helicoverpa* moth production in refuges.</u>

There was some indication that Magnet® increased the presence of *Helicoverpa* in pigeon pea refuge crops, although the effect was not always consistent. White eggs were consistently higher in the presence of Magnet® within refuges (Table 2), more moths were usually flushed from the Magnet® treated plots (Table 3), and more *Helicoverpa* moths were usually caught in light traps in where Magnet® had been applied (Table 4). The results suggest that Magnet® might be used as a means to increase the efficiency of refuge crops, which is in agreement with work concurrently being done by other researchers in Australia.

Table 2. Average numbers of white eggs of *Helicoverpa* / metre of crop.

	Refuge Plot	Within Refuge Crop, adjacent to "treated" & "control" plots	Nearby in Bollgard II
Kangaloon			
Treated	13.38	7.88	1.63
Control	6.63	13.13	1.88
Lammermore			
Treated	1.50	1.44	1.13
Control	0.91	1.13	0.69
Underbri			
Treated	1.00	1.88	0.88
Control	0.00	1.75	0.25

Table 3. Total moths observed when flushing refuge crops

	Kangaloon	**Lammermore**	**Underbri**
Day			
Treated	10	38	15
Control	10	19	3
Night			
Treated	31	-	4
Control	21	-	2

Table 4. Average total catch of moths in light traps / night in refuge crops

	H. armigera Males	*H. armigera* Females	*H. punctigera* Males	*H. punctigera* Females	Other Noctuidae
Lammermore					
Treated	57.0	9.3	66.5	12.5	21.8
Control	37.3	5.8	47.0	6.3	15.0
Underbri					
Treated	4.0	3.0	10.3	4.0	27.3
Control	4.3	1.3	7.7	1.0	35.7

<u>Determine crop origin of moths found in transgenic cotton fields and temporal and spatial coverage of moths from non-cotton sources.</u>

Moths reared from known plant host origins

Whilst the stable carbon isotope signatures for moths reared from pupae collected beneath various crop plants (i.e. known host plant origins for these moths) reflected what had been demonstrated before (see Final Report for Project CSE 107C – 2006), and clearly differentiated C3 and C4 plants, stable nitrogen isotope signatures were not useful in differentiating plant host origins amongst the same moths (Table 5), in particular the C3 plants, cotton and pigeon pea. Apparently (M. Peoples & I. Rochester, CSIRO PI – pers. comm..), pigeon pea is a rather poor fixer of atmospheric N, and this may explain the much higher Delta N for this legume than might otherwise be expected.

Table 5. Stable isotope signatures for *H. armigera* moths of known plant host origin. Moths were collected within the Namoi Valley during 2007 and 2008. Data are means ± S.E. Non-parametric stats used because variances unequal, in particular amongst 2008 data for Delta N, and data couldn't be adequately transformed to permit parametric ANOVAs. Different letters after means indicate significant differences.

Plant Origin	Year of Collection as Pupae	Number of Individuals	Delta C	Delta N
Cotton	2007	20	-29.90 ± 0.30a	14.79 ± 0.46a
Maize	2007	20	-12.64 ± 0.14b	7.94 ± 0.85c
Pigeon Pea	2007	20	-26.06 ± 0.43a	14.30 ± 0.37ab
Sorghum	2007	20	-14.39 ± 0.21b	12.31 ± 0.34bc
Kruskal-Wallis H			71.18, p < 0.001	41.05, p < 0.001
Cotton	2008	5	-26.89 ± 0.49a	15.02 ± 1.17a
Maize	2008	9	-10.73 ± 0.18b	11.57 ± 0.75a
Pigeon Pea	2008	10	-25.62 ± 0.58a	13.88 ± 1.06a
Kruskal-Wallis H			16.58, p < 0.001	4.62, p > 0.05

2006-07 season

During 2006-07, too few mating pairs of *H. armigera* moths were captured to merit reporting here their stable isotope signatures, and testing for random mating. Instead, the isotope signatures of the combined single and mating moths captured over the two most successful Bollgard II crops that year (i.e. where most moths were collected) are plotted in Figs 32-33 (n = 256 for moths from Morella; n = 64 moths from Warendi). As in previous seasons (see Project 107C final Report – 2006), carbon isotope analyses could distinguish two primary peaks in origin of the moths, approximating what would be expected for C3 and C4 plant host origins. However, the discrimination was not as marked as found at other times (also see below for subsequent years). The nitrogen isotope analyses were not useful for discriminating different plant host origins amongst the moths.

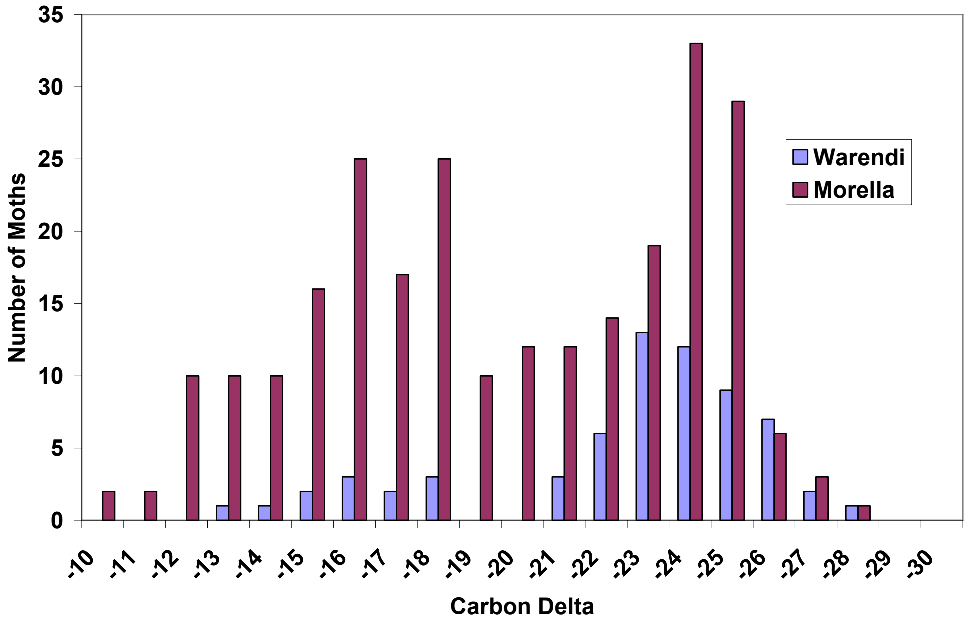

Fig. 32. Stable carbon isotope analyses of *Helicoverpa armigera* moths collected at 2 sites (2006-07)

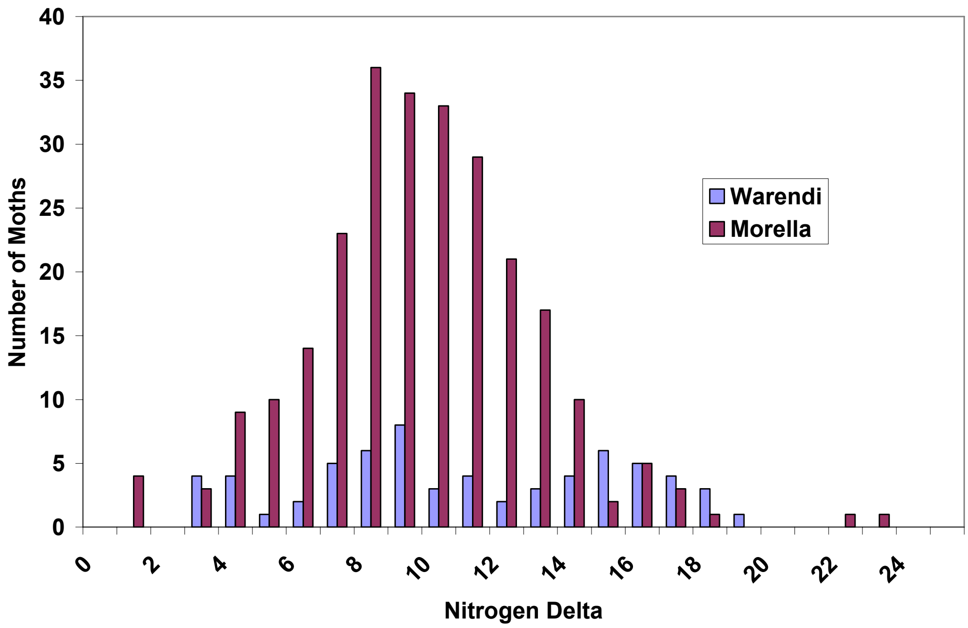

Fig. 33. Stable nitrogen isotope analyses of *Helicoverpa armigera* moths collected at 2 sites (2006-07). Samples were from same moths as in previous Figure.

2007-08 season

The heads and wings of 409 *H. armigera* moths (mating pairs and singles) collected at 3 sites (Keytah, Longview and Iona) during 2007-08 were analysed for C and N stable isotope signatures (Figs 34-35). The results confirmed further that moths can be differentiated using C signatures into those with C4 and C3 plant host origins (Carbon Delta approx -8 to -20 and -20 to -30 respectively). In addition, our analyses of matings of moths from different plant-host origins continued to support the notion that such occur at random (see Final Report for Project CSE 107C – 2006). e.g. X^2 tests of mixed and same-host matings amongst 40 mated pairs of *H. armigera* collected at Keytah in 2007-08 were non-significant ($X^2 = 1.81$, $p > 0.05$), suggesting random mating occurs (in this case there were 5 C3 x C3 matings [E = 4.8], 13 C4 x C4 matings [E = 17.1], and 22 C3 x C4 matings [E = 18.1]). We also were able to test for random mating of *H. punctigera* at the same site and obtained a similar result ($X^2 = 0.23$, $p > 0.05$). However, N signatures are not likely to be useful traits to separate moths with different plant host origins, in particular within C3 plants (such as cotton and pigeon pea) as previously hoped (based on atmospheric N fixation, characteristic of legumes) (Fig. 35). This is further supported by ongoing analyses we have made of stable N isotope signatures of moths from known plant host origins, wherein such moths have not been possible to differentiate (see Table 5).

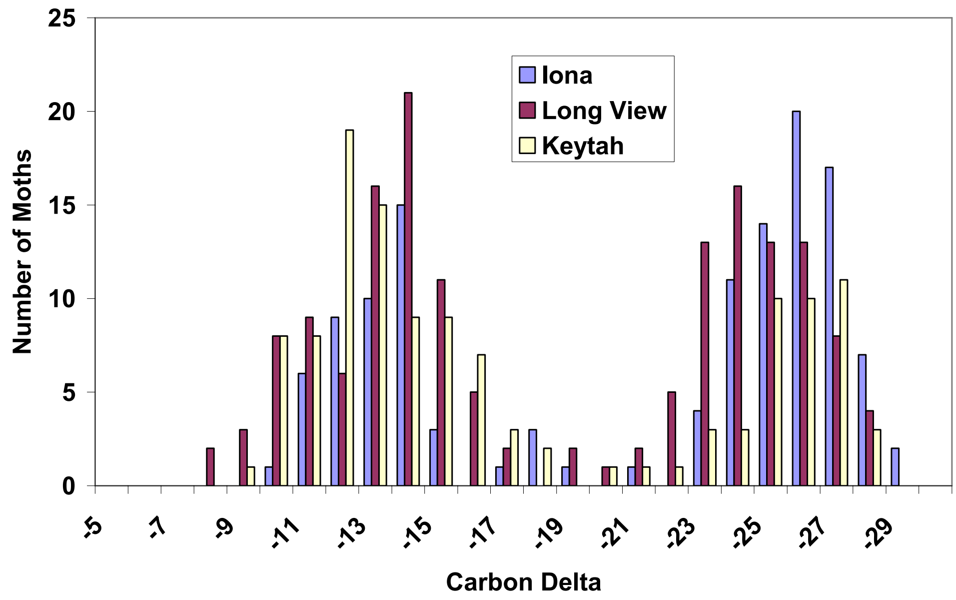

Fig. 34. Stable carbon isotope analyses of *Helicoverpa armigera* moths collected at 3 sites (2007-08)

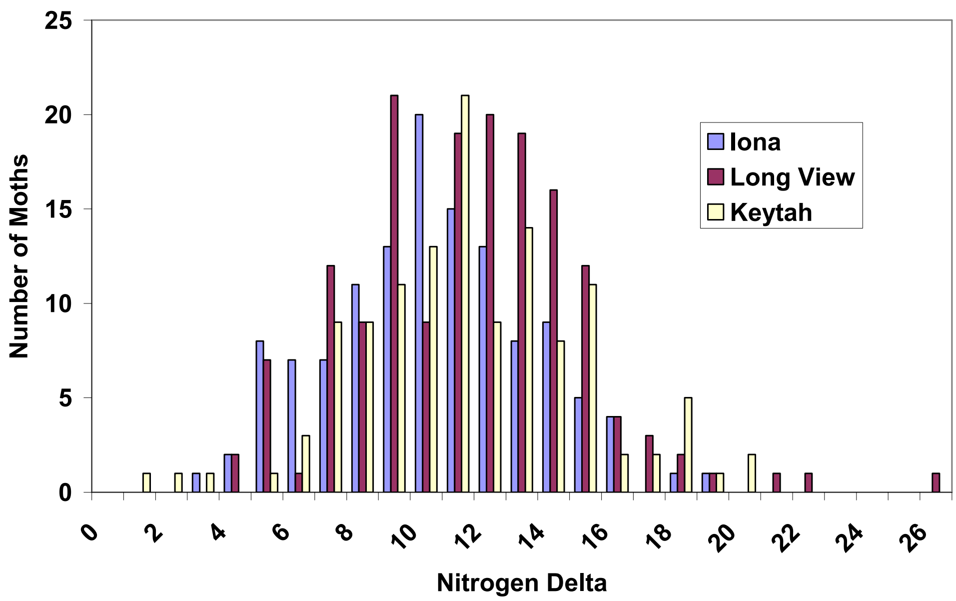

Fig. 35. Stable nitrogen isotope analyses of *Helicoverpa armigera* moths collected at 3 sites (2007-08). Samples were from same moths as in previous Figure.

2008-09 season

Stable isotope measures of the wings and heads of approximately 400 moths (mating pairs and singles) collected at Currawidgen, Careera, Havana, Taratan and Tucka Tucka during 2008-09, plus additional specimens collected from known plant host sources (as pupae) are still being analysed at UNE. Resultant data will be forwarded in due course.

Visual assessments of canopy invertebrates

Various invertebrate species (pests and beneficial) were recorded (presence / absence) when replicated visual inspections were made of the canopies of Bollgard II cotton crops and associated refuges. We present here (Figs 36-38) some data for 3 key taxa : predatory beetles, bugs and spiders. In general, spiders were more commonly recorded than the other taxa. We have yet to refine the data into more specific taxa and search for patterns. There was no apparent difference in frequency of occurrence of any of the three main taxa between crops.

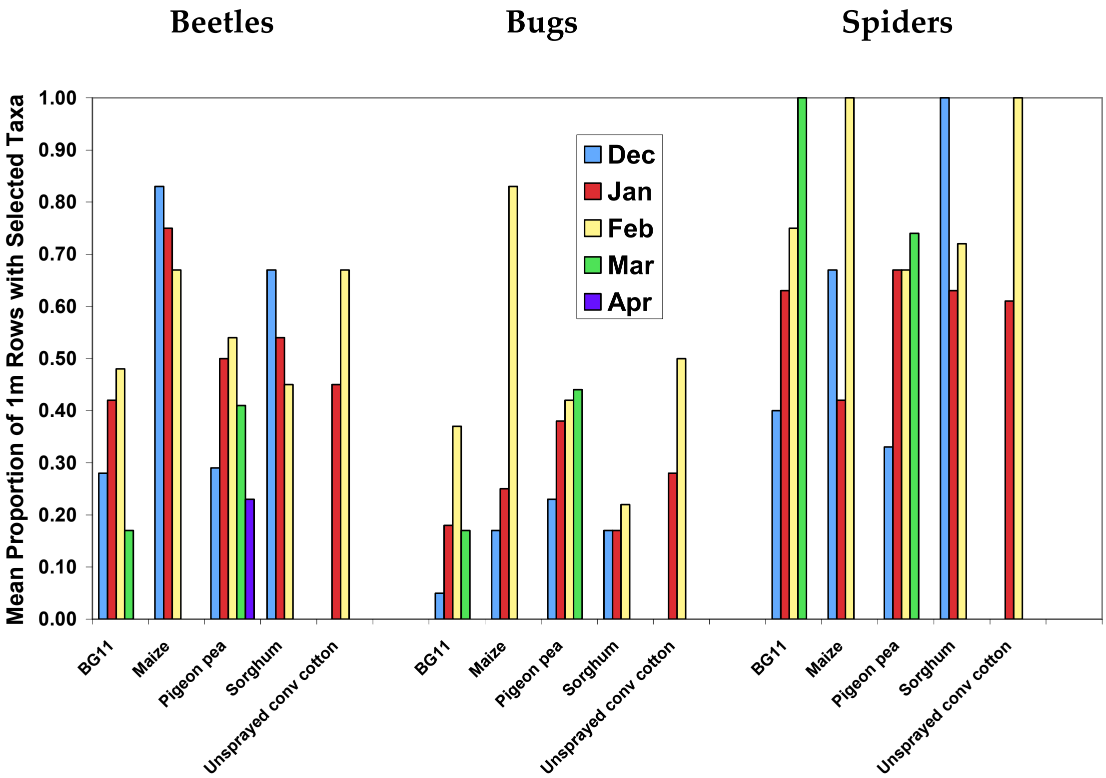

Fig. 36. Incidence of selected beneficial invertebrate taxa, expressed as a proportion of 1m crop rows scoring their presence (2006-07). Note unsprayed conventional cotton was not surveyed in December or March this season. Only pigeon pea was sampled in April, and maize and sorghum were not sampled in March.

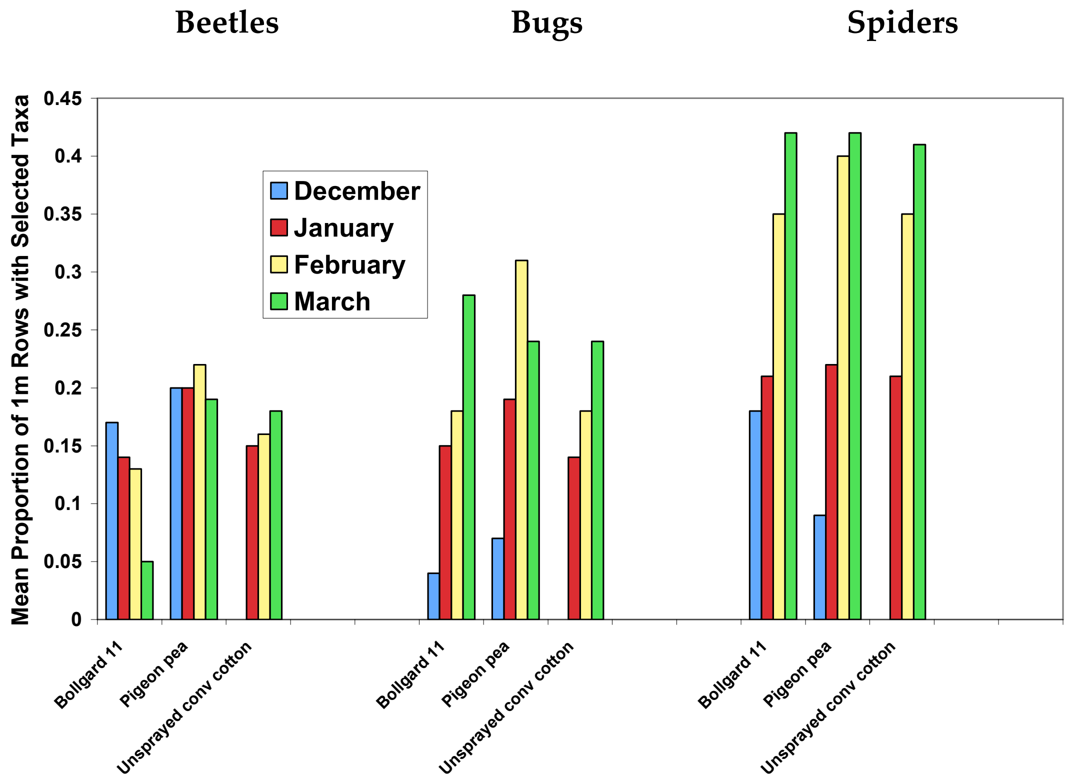

Fig. 37. Incidence of selected beneficial invertebrate taxa, expressed as a proportion of 1m crop rows scoring their presence (2007-08). Note unsprayed conventional cotton was not surveyed in December this season.

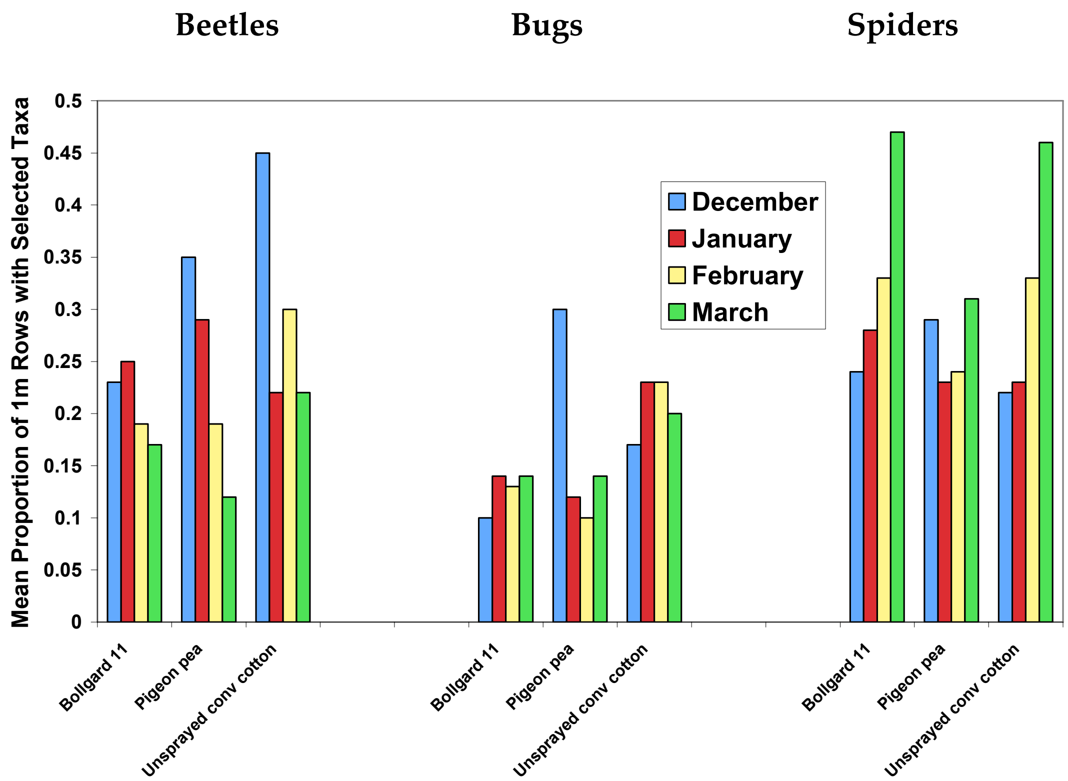

Fig. 38. Incidence of selected beneficial invertebrate taxa, expressed as a proportion of 1m crop rows scoring their presence (2008-09).

The abundance of *Helicoverpa* eggs and larvae were also measured / m of crop row in all 3 years of the project throughout Bollgard II, pigeon pea and unsprayed conventional cotton crops (Table 6). The relative abundance of *Helicoverpa* eggs on the 3 crops varied between years, perhaps reflecting the degree and timing of oviposition and / or natural enemy pressure. However, the relative abundance of larvae was consistent, with most found on pigeon pea and least on Bollgard II.

Table 6. Abundance of eggs and larvae of *Helicoverpa* on 3 key crops, assessed by visual counts in the 3 years of the project.

	Bollgard II	**Pigeon Pea**	**Unsprayed Cotton**
2006-07			
Mean Eggs / M	5.33	11.51	1.67
Mean Larvae / M	0.25	12.60	4.50
N (Reared/ Site Visits)	294 / 47	1479 / 67	46 / 6
2007-08			
Mean Eggs / M	29.52	13.04	9.83
Mean Larvae / M	1.37	16.59	6.98
N (Reared/ Site Visits)	1035 / 46	2194 / 79	888 / 54
2008-09			
Mean Eggs / M	19.4	16.01	10.03
Mean Larvae / M	0.56	16.98	7.83
N (Reared/ Site Visits)	359 / 25	2700 / 150	420 / 29

In all 3 years, the rearings of the *Helicoverpa* eggs and larvae from these visual surveys mostly yielded *H. armigera* moths rather than *H. punctigera* (Fig. 39). Parasitism was generally low in Bollgard II® crops compared with pigeon pea and unsprayed conventional cotton. Disease incidence was consistently lower in Bollgard II® cotton compared with unsprayed conventional cotton and pigeon pea.

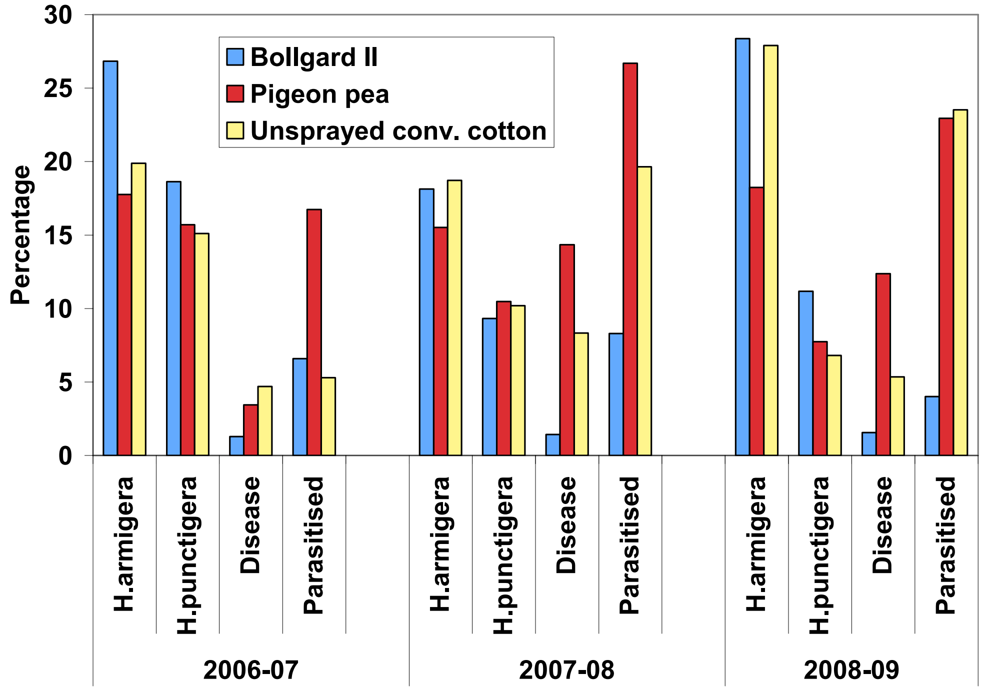

Fig. 39. Rearings of eggs and larvae of *Helicoverpa* collected within 3 crop types throughout the study : 195 site visits in 2006-07, 182 site visits in 2007-08, and 400 site visits in 2008-09. Surveys were done in the Namoi, Macintyre and Gwydir Valleys, plus the St George region.

Laboratory mating experiments

In each of the three experiments involving moths reared from larvae feeding upon pigeon pea and cotton, equal numbers of male and female moths exposed to the two crops were tested in one mating chamber over three nights. 173 moths were tested in experiment 1, 144 in experiment 2, and 156 in experiment 3. Despite the large number of moths used in these mating experiments (n= 473) and obvious calling by the females, across the three experiments a total of only 62 moths (31 pairs) mated (13%). Nevertheless the results (Fig. 40) showed no preference by the moths for partners that had foraged as caterpillars on either the same (n=16 pairs) or a different (n=15 pairs) crop to the one to which they had been exposed as caterpillars.

These results support the assumption that moths do not discriminate partners on the basis of what they ate as caterpillars. This is in agreement with work on preferences of different hosts as oviposition sites in *H. armigera*. Firempong and Zalucki (1991) (*Aust. J. Zool.* **39**, 343-50) found that the diet of the larvae did not influence oviposition preferences of the adult moth. However, they and others (Cunningham *et al* 1998, *Anim. Behav*. **55**, 227-34) did show that the exposure of a moth to a plant in its first few days of emergence biased its preference of the host as an oviposition site. Therefore, while larval experience may not influence mate choice, experience of an emerging moth may do so. In future work it will be important to test whether the crop from which a moth emerges influences its mate choice.

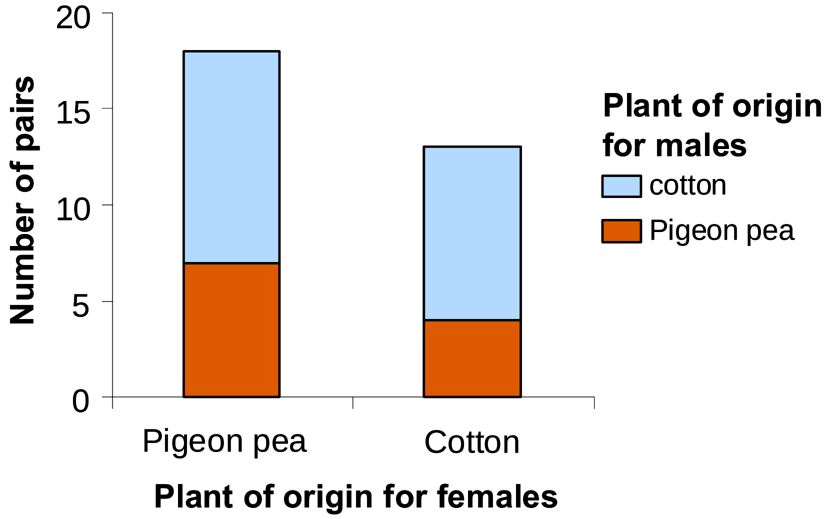

Fig. 40. Plants upon which the mating pairs had been raised. Females raised on pigeon pea or cotton showed no bias to mating with males raised on either pigeon pea or cotton.

The outcomes of the single-pair matings are summarised in Table 7. As explained earlier, the data are very much preliminary and only partially complete. We cannot conclude anything at this stage about similarities / differences between single and different plant host origins. But thus far it does appear that it is easier to establish successful single-pair matings with *H. punctigera* compared with *H. armigera* (of course based on field collections of pupae to initiate such work).

<u>Extend research results to industry</u>

[See details below under Publications for meetings addressed, publications etc]

Table 7. Outcomes from single-pair matings in the laboratory. F = fertile matings, NF = non-fertile matings

Plant hosts of parents	*H. armigera*	*H. punctigera*
Pigeon pea x Pigeon pea	9 F / 33 NF	18 F / 10 NF
Pigeon Pea x Cotton	0 F / 5 NF	1 F / 0 NF
Pigeon Pea x Maize	3 F / 6 NF	-
Pigeon Pea x Pumpkin	-	1 F / 0 NF
Maize x Cotton	1 F / 1 NF	-

Additional Deliverables

Intensive studies of refuge efficacy at St George

The abundance of *Helicoverpa* eggs, larvae and pupae varied greatly between the 25 pigeon pea crops at St George. Fig. 41 illustrates this for the 1st egg sampling, and relates such data to estimates of attractiveness of the crops at the time, based on Monsanto audit indices that we calculated too. There was a significant correlation between the egg counts and the evaluation indices (Spearman-Rank Correlation Coeff. = 0.596, $p < 0.005$). Fig. 42 provides egg count data for all 4 visits made to the refuge crops at St George, and highlights the 5 crops wherein highest egg numbers were recorded during the study. Data for densities of larvae are not presented here, but are available on request. Densities of live pupae are however presented in Fig. 43, and are linked via asterisks to those fields where egg numbers were particularly high. It is clear that there was a poor match between initial attractiveness of the refuge crops and their ultimate production of live pupae. Similarly, numbers of emerged moths (based on evidence from empty pupal cases found in the soil) bore no obvious relationship with crop attractiveness either (Fig. 44). The abundance of pupae beneath pigeon pea refuges at St George in 2008-09 (overall mean = 0.8 / m^2) was relatively low compared with findings in previous years, but not outside the range recorded then (e.g. 1996-2003, mean = 1.4 / m^2; range = 0.8-2.3 live pupae / m^2).

H. punctigera was more common than *H. armigera* in the lab rearings of eggs and larvae collected from St George at the 1st sampling (i.e. early January, 2009), but thereafter *H. armigera* predominated (Fig. 45). Parasitism was modest early in the season, but much higher on the 4th visit (i.e. mid March 2009). Rearings from the pupal collections at St George are not complete at time of writing, and will be reported at a later date.

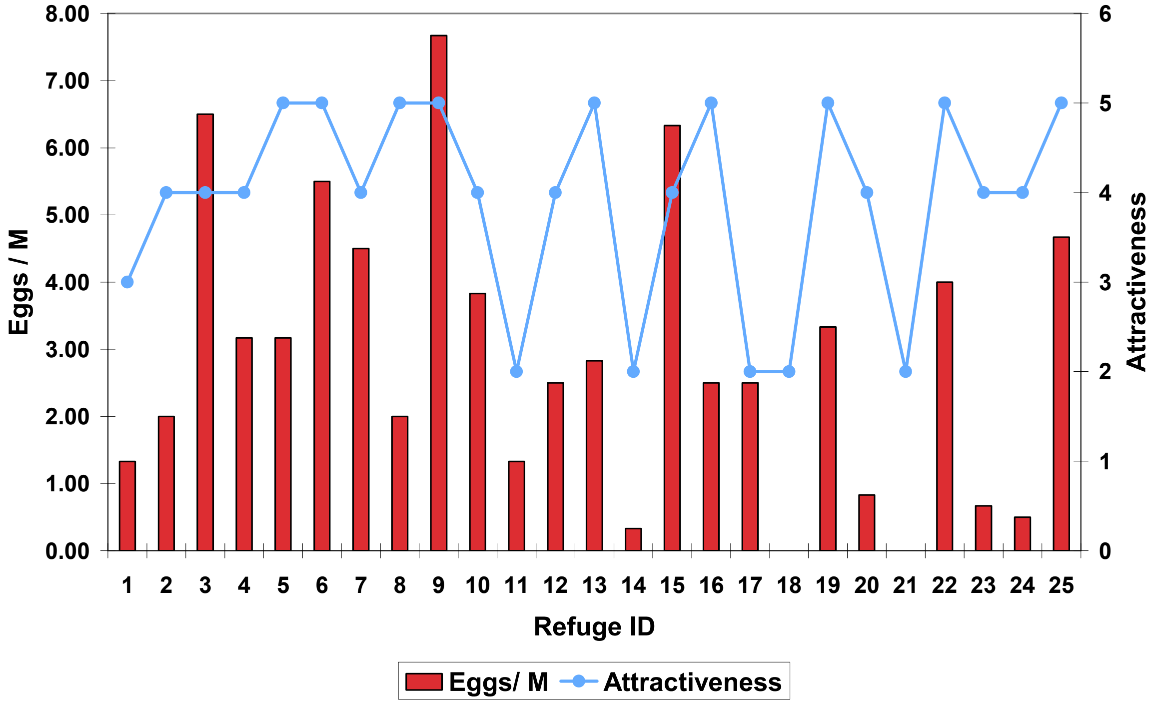

Fig. 41. Numbers of *Helicoverpa* eggs on 25 pigeon pea refuge crops in the St George region at 1st sampling (6-8 January 2009) (bars), and estimates of attractiveness (Monsanto audit / evaluation index) at the same time for each crop.

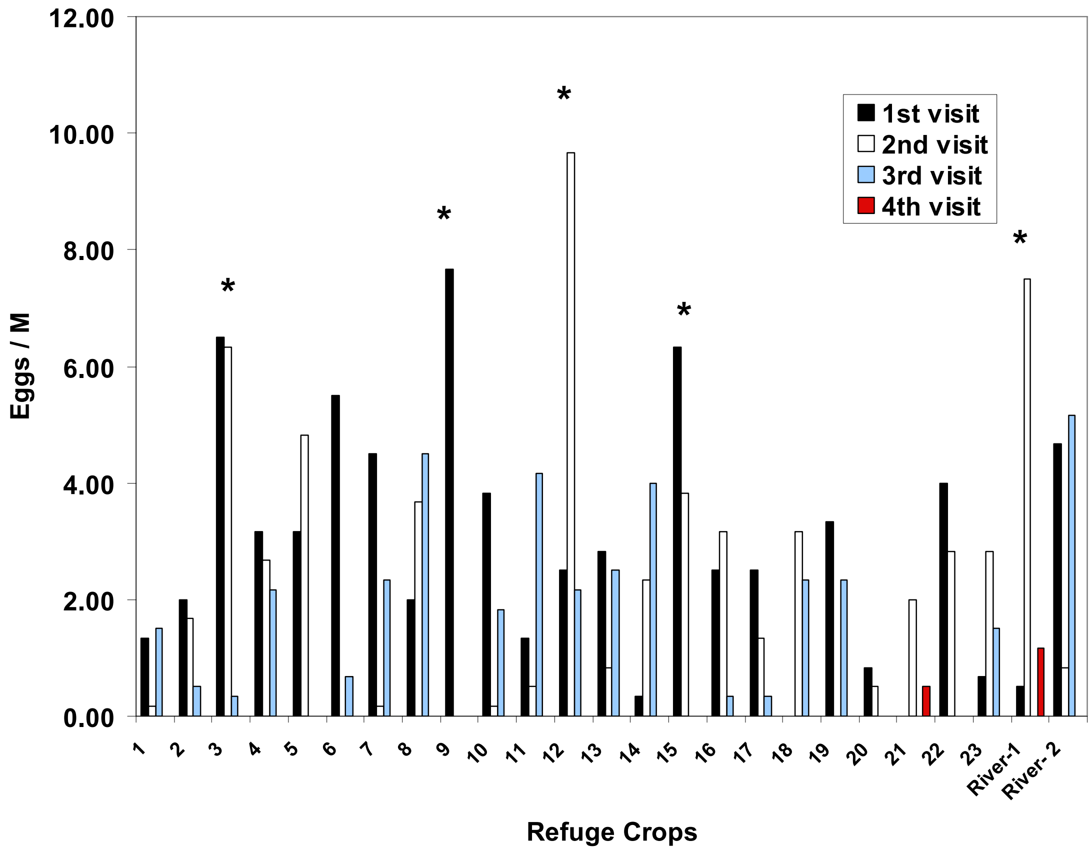

Fig. 42. Numbers of *Helicoverpa* eggs on 25 pigeon pea refuge crops in the St George region at 4 samplings (January to March 2009) (bars). Asterixes simply highlight the five occasions with greatest egg loads.

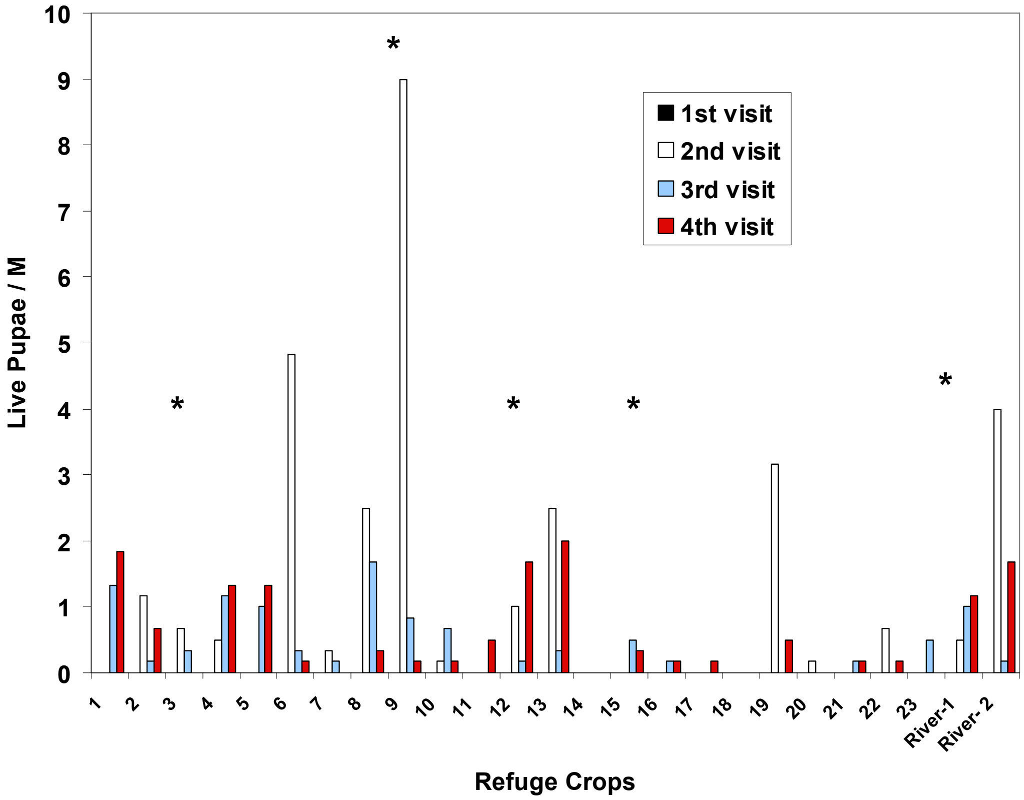

Fig. 43. Numbers of *Helicoverpa* pupae beneath 25 pigeon pea refuge crops in the St George region at 3 samplings (late January to March 2009) (bars) [Note : no pupae sampling occurred on the first visit to St George]. Asterixes simply highlight the five occasions with greatest egg loads (brought forward from previous Figure).

Comment on synchrony of pigeon pea with cotton

The numbers of live *Helicoverpa* pupae recorded in soil beneath unsprayed and sprayed conventional cotton refuges during January to March (1996-2003) (6 valleys) were significantly correlated with those found concurrently beneath Ingard® crops associated with them (Table 8). In contrast, the numbers of pupae beneath pigeon pea refuges were not. The number of cases involving sorghum refuges were probably too few to merit conclusions. At first glance this could be taken to indicate that pigeon pea refuges do not offer useful synchrony for emergence, and hence mating with moths from Bt cotton crops. However, when we plotted the abundance of pupae by week from the start of January (Fig. 46), it became apparent that the numbers of moths below pigeon pea were consistently higher than those below Ingard® cotton. Such a pattern would drive a poor correlation, but nevertheless there would be a consistent supply of susceptible moths being provided by pigeon pea.

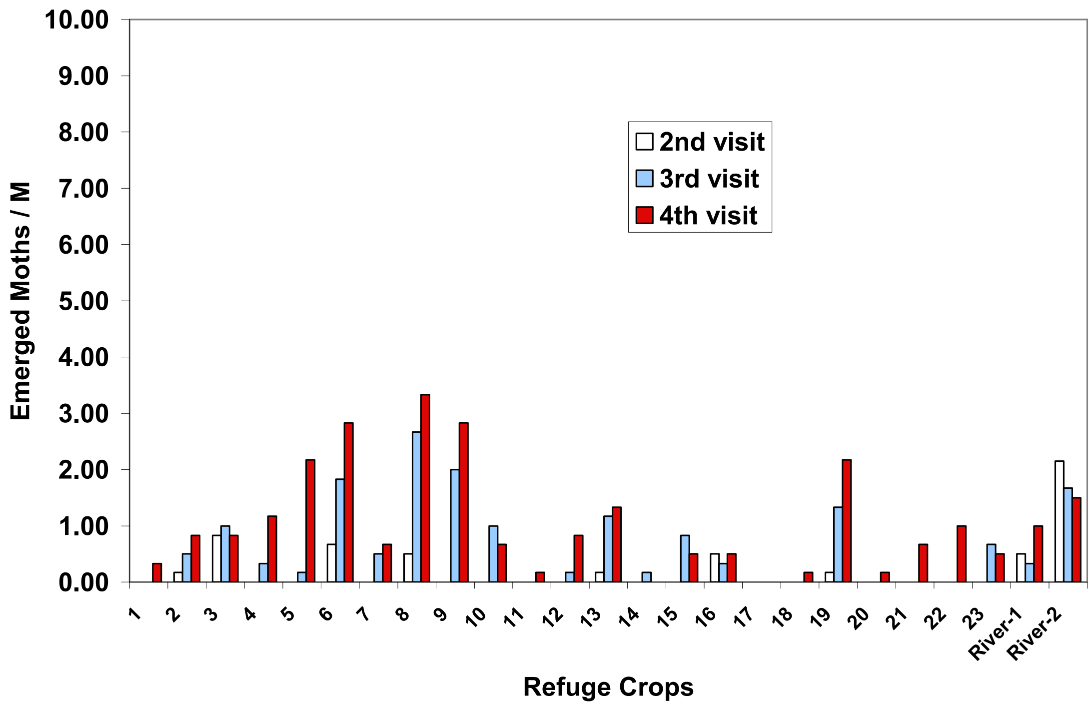

Fig. 44. Numbers of emerged moths of *Helicoverpa* (based on counts of empty pupal cases) pupae beneath 25 pigeon pea refuge crops in the St George region at 3 samplings (late January to March 2009) (bars) [Note : no pupae sampling occurred on the first visit to St George].

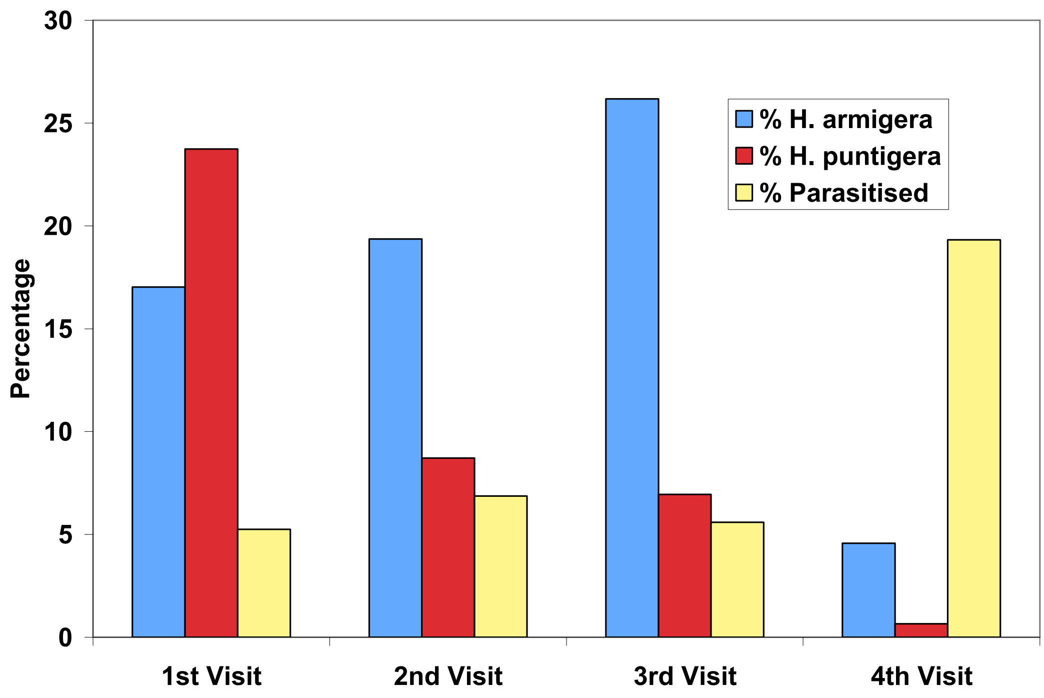

Fig. 45. Results of laboratory rearing of *Helicoverpa* eggs and larvae collected on each visit to sample the 25 pigeon pea refuge crops at St George (2008-09). Note 458 eggs & larvae were reared from the 1st visit, 540 from the 2nd visit, 451 from the 3rd and 131 from the 4th.

Table 8. Pearson Correlations (r) between mean numbers of live *Helicoverpa* pupae recorded in pairs of Ingard® cotton crops and their associated refuge crops during January – March 1996-2003. Numbers of pairs of observations are given as sub-scripts to r.

Refuge Crop	Correlation Coeff.	Prob.
Unsprayed Conv. Cotton	r_{85} = 0.411	< 0.001
Sprayed Conv. Cotton	r_{393} = 0.259	< 0.001
Pigeon Pea	r_{123} = -0.027	> 0.05
Sorghum	r_{44} = 0.015	> 0.05

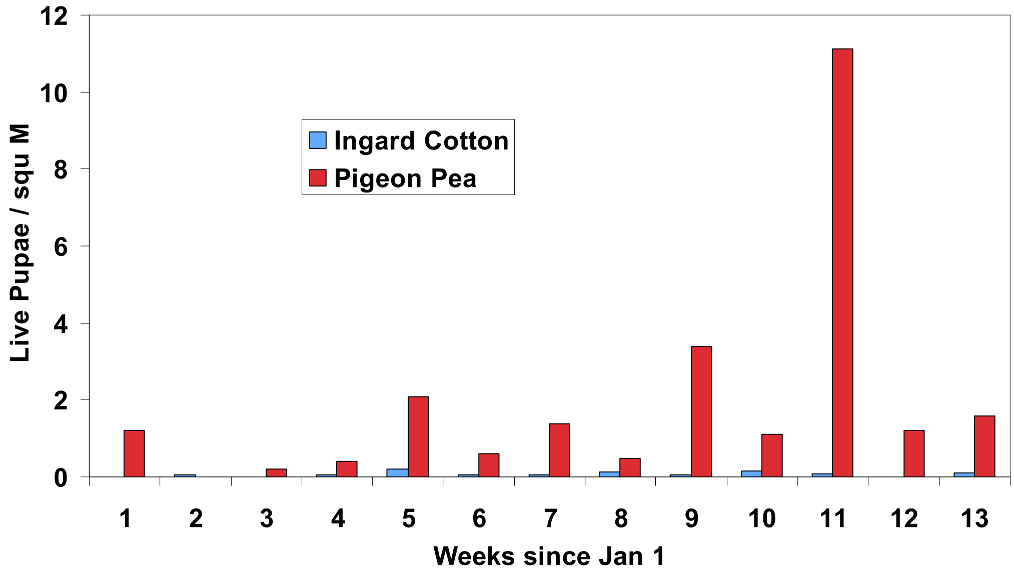

Fig. 46. Abundance of live *Helicoverpa* pupae beneath Ingard cotton crops and their associated pigeon pea refuge crops at weekly intervals (data averaged over 1996-2003).

Measure over-wintering of pupae of Helicoverpa

Of 117 live pupae collected beneath pigeon pea and cotton crops between late March to early May, 2007 and reared in the laboratory, 19 emerged as moths (10 *H. armigera* and 9 *H. punctigera*). Similarly, of 579 reared from collections made in March 2008, 79 emerged as *H. armigera* and 21 as *H. punctigera* moths. Pupae (n = 282) collected from 24 sites in April-May, 2009 yielded 71 *H. armigera* and only 4 *H. punctigera* moths. The losses on each occasion reflected deaths from unknown causes, disease and parasitism (e.g. 126 of the pupae in 2009 were parasitised). More collections and rearings are needed to determine the proportions of *H. punctigera* and *H. armigera* that over-winter in cropping regions, but it seems likely that at least some *H. punctigera* do so.

Long-term Rearings of Helicoverpa eggs & larvae

Many thousands of *Helicoverpa* eggs and larvae that have been collected in the field over the last 15 years or so have been reared through in the laboratory to determine their species. Fig. 47 below illustrates some of the resultant data from these rearings. It is clearly apparent that whilst *H. punctigera* predominates amongst the rearings made from spring-collected material and then diminishes as the cotton season progresses through summer as *H. armigera* becomes more common, it is still present in low numbers in autumn, in most years.

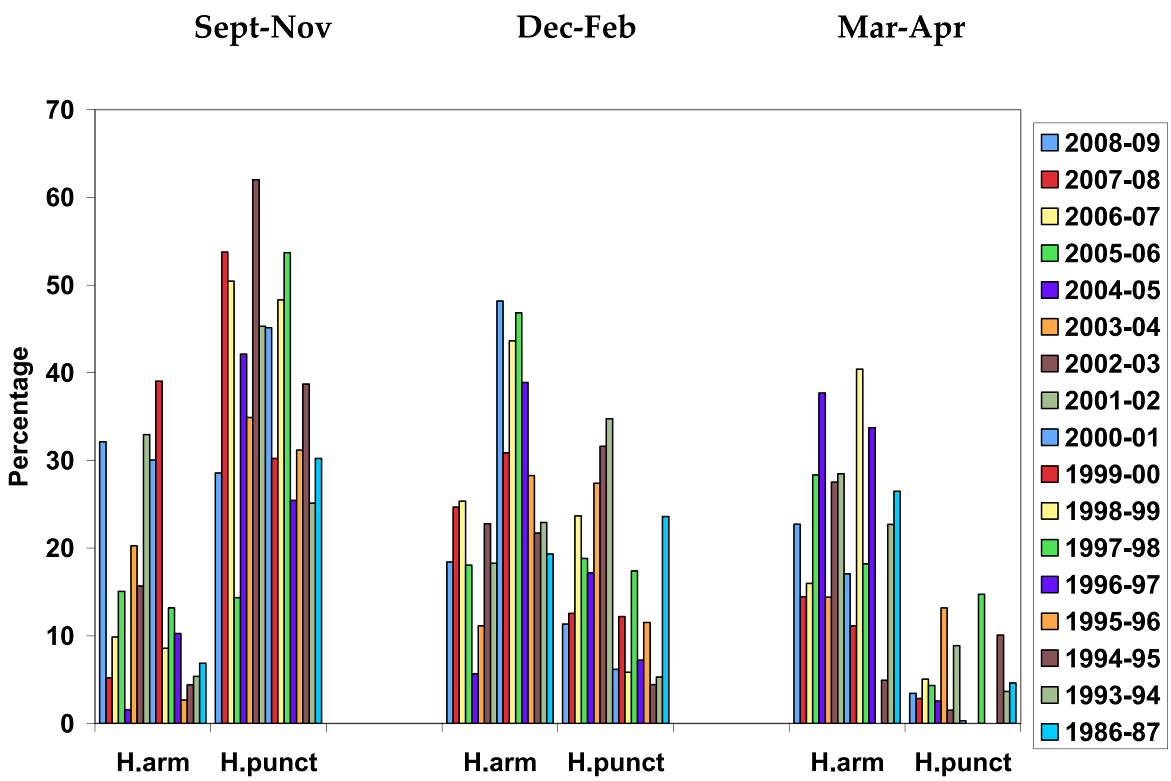

Fig. 47. Rearings of *Helicoverpa* eggs and larvae, collected during various periods of the year, through to moths. Note : in total 19,454 individuals from 1,603 sites were reared from collections made between September-November. Similarly totals for December-February were 23,229 (1,459 sites) and for March-April were 11,831 (648 sites).

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.

Helicoverpa armigera and *H. punctigera* are major pests of cotton in Australia. The biology of the two species is known to differ, and this project reinforced that fact. Both species are controlled to a significant extent by transgenic (Bt) cotton, but effective management strategies such as deployment of refuge crops to produce large numbers of susceptible moths are required to prevent the onset of Bt resistance in the moths. The threat of Bt resistance development has been made even more evident, in both *Helicoverpa* spp., in other concurrent research projects which have monitored the incidence of resistance alleles in field populations. The need for sound understanding of the ecology of both moth species is of ongoing importance, to provide a firm basis upon which to place rigorous, adaptive management practices.

The research in this project sought to help answer 3 core questions :

1). Is the refuge strategy for Bt cotton working ? Do sources outside Bt cotton crops, in particular dedicated refuge crops (with no Bt exposure), provide a sufficient proportion of the *Helicoverpa* moths found mating in Bt cotton crops to support the assumption that development of Bt resistance is likely to be reduced through random mating of moths on the landscape ?

2). What factors influence the production of *Helicoverpa* moths in refuges ?

3). Is the abundance of *Helicoverpa* changing in time at landscape level ?

Through the ongoing demonstration of random cross-mating of moths from different plant host origins (for *H. armigera*, but not as yet for *H. punctigera*), the preliminary identification of novel means to enhance refuge performance / diversify options (e.g. use of Magnet® devoid of pesticide, split-planting of conventional cotton refuges, use of pigeon pea for dryland situations, and mixing of crop types together as refuges) and continued monitoring (trapping and canopy & soil surveys) for temporal and spatial shifts in population numbers, these questions have been effectively explored. The expected science outcomes in terms of an increased understanding of the ecology of *Helicoverpa* at landscape scale (including the cross-mating of moths from different origins and factors driving patterns in abundance) have been fulfilled, as has the delivery of applied outcomes such as a questioning of the validity of refuge deployment as a component of the Resistance Management Plan for transgenic cotton. The research has also identified weaknesses in our current understanding, such as certain aspects of the population dynamics of *H. punctigera*, and the prevalence of pupal parasitism which currently detracts significantly from achieving maximum refuge crop productivity. The research has highlighted the need to move to a more holistic / collective appraisal of refuge performance at landscape scale – which is a core component of the project's successor ("Enhancing the efficiency of Bt refuge crops within a changing cotton environment", 2009-12).

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

No commercially sensitive information / techniques were developed during this project.

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

The most significant technical development related to this project continues to be the use of stable isotopes as markers of plant host origin for the moths. We were disappointed that the utility we found with C isotopes was not mirrored in the N isotopes. We believe this to be related to the poor performance of pigeon pea as a legume and its relatively low atmospheric N fixation. We were also disappointed in the lack of success we had with utilising the gossypol analysis techniques provided by Monsanto (USA). Failures there were beyond our control. We intend to send another supply of specimens to Monsanto to enable another run of testing – hopefully to distinguish pigeon pea and cotton sourced moths.

We anticipate exciting times ahead as we further develop our capacity to analyse the spread of refuge-derived moths across landscapes (AWM study at St George in coming years). We were somewhat surprised by the lack of correlation between inland rainfalls and *H. punctigera* numbers in traps at Narrabri. This result calls in to question the strength of previous conclusions in the literature based on correlations – unless the ecology of the moths has substantially changed in recent years.

Our work with Magnet®, in terms of increasing refuge efficacy, was only preliminary, but nevertheless does lend some support that there is some positive prospects there for the future. The same follows for the preliminary work we conducted on novel refuge options – both for irrigated and dryland cotton production.

   c) required changes to the Intellectual Property register.

None required. The findings from the research are effectively in the public domain. Intellectual property pertaining to the research is essentially in the form of publications produced, preceded by clearance through CRDC and the CRC.

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?

The project demonstrated :

1. In general, the abundance of *Helicoverpa* spp. was relatively low in the last 3 years, as witnessed through spring surveys of non cotton crops, weeds and native vegetation, pheromone trapping, and within season monitoring in Bollgard II® cotton crops and associated refuges. There appears to have been long-term shifts in the seasonal patterns of moths, especially *H. armigera*.

2. Re-analysis of long-term trapping data sets did not support the notion of correlations between inland rainfall patterns and the abundance of *H. punctigera* in eastern cropping regions such as ACRI, Narrabri. Surprisingly, *H. punctigera* abundance in spring in the Narrabri region was correlated with local population numbers present towards the close of the previous cotton growing season, implying that over-wintering may be more common than previously thought for this species. Surveys for *H. punctigera* pupae in cropping soils during autumn however yielded variable results. Better understanding of the ecology of *H. punctigera* in the "off" season is needed, given the recent focus on its capacity to develop Bt resistance.

3. Preliminary studies of novel refuge crop options for irrigated and dryland cotton production systems were promising, with pigeon pea emerging as a possible alternative to unsprayed conventional cotton in dryland situations and split-planting of conventional cotton seeming feasible for irrigated cotton production systems through the provision of extended refuge attractiveness. Some encouraging findings also emerged from mixed cropping options as refuges. But, all these options require further study to be convincing. Other work suggested applications of Magnet® (without the pesticide ingredient) may slightly increase the abundance of *Helicoverpa* in pigeon pea refuge crops.

4. Ongoing research on the mating of *H. armigera* in the field continued to confirm that moths from different plant host origins are likely to mate at random. However, our work was limited primarily to studies of C3 x C4 plant host origins (using C isotope markers) because attempts to use N isotope and gossypol markers proved unsuccessful. Nevertheless, laboratory studies provided support for mating being random amongst moths from different C3 plant host origins (cotton & pigeon pea). Studies now need to focus more on *H. punctigera*. Pigeon pea refuges appear to "deliver" moths in a timely way for cross-mating with moths that emerge from Bt cotton.

5. Natural enemies were common in Bollgard II® cotton and refuge crops. In particular pupal parasitism seemed high, and is likely to be a significant factor impinging on refuge production of *Helicoverpa* moths.

6. Area-wide studies of the impact of multiple refuges within landscapes (cf solitary one-on-one comparisons of Bt cotton with its dedicated refuge crop) began in the St George region and will continue in the new project (2009-12). This work clearly demonstrated how variable refuge performance could be within a cotton producing region. It seems likely that the locations of the most productive refuges within landscapes could be quite critical for overall refuge efficacy. The initial attractiveness of refuges (i.e. for oviposition) seems to bear little relationship to the

ultimate production of *Helicoverpa* moths. Abundance of pupae in St George in 2008-09 was low relative to earlier studies (1996-2003), but not outside the previously observed, between - year variability.

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.

See comments above re AWM of refuges – the St George study in the current project, and further pursuance of novel refuge options and gossypol as a marker for plant host origins of moths.

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

Results from the research will continue to be presented during TIMS meetings and REFCOM, Resistance Road Shows and grower / consultant meetings, CSD Variety Trials booklets, various Insect Pest Ecology & Management workshops (e.g. Toowoomba), IPM Forums etc as in the past. Colin Tann continues to be active in his role on the TIMS technical committee. Project staff are in close contact with several consultants and other advisers throughout the industry, when organising field sites and surveys, and in particular through collaborative work in the St George region. Project results will continue to be communicated through conference presentations, seminars, industry magazines and media articles and scientific publications as in the past (see some related details below).

   (c) for future research.

A new proposal was submitted to CRDC (and on to the CRC), and supported, for funding through 2009-12 : "Enhancing the efficiency of Bt refuge crops within a changing cotton environment". The aims of this work are to :

1. Test new refuge crop options, such as the effectiveness of pigeon pea in dryland systems and establishing the efficacy of split cotton plantings and mixed species refuge crops. This will build upon the preliminary work in irrigated and dryland systems described above in this Final Report, and provide refuge options for southern regions.
2. Continue ongoing monitoring of landscape-scale changes in the abundance of *Helicoverpa* spp., particularly in the Namoi Valley and St George region. Tracking changes in *Helicoverpa* abundance can identify fluxes associated with Bt cotton use and provide guidance to growers and researchers of population trends.
3. Examine the efficacy of refuges. We will continue experiments on the movements and mating patterns (especially degree of randomness) of moths emerging from different types of refuges and Bt cotton, especially *H. punctigera* which hitherto has been poorly studied.
4. Develop a more holistic /AWM approach to refuges. Most research on refuge crops has focussed on the performance of individual crops. We will expand our work to an Area Wide Management approach (as needed for such a mobile pest as *Helicoverpa*) using a regional case study (building upon the 1 year St George study

reported above). We will also evaluate costs and benefits (besides *Helicoverpa* production) of refuges for the grower, such as whether refuges are sources (or sinks) of natural enemies and emerging pests. We will liaise with an agricultural economist to evaluate refuges.

These studies will 1) provide growers with more refuge options; 2) provide information on changes in *Helicoverpa* populations which have ramifications to resistance management; 3) further test underlying assumptions of the refuge strategy and 4) confirm its efficacy in the current cotton growing environment in terms of regional effectiveness and overall farm management.

Project staff met occasionally with Monsanto staff to assist them with interpretations of data sets (e.g. in Goondiwindi, Canberra & other sites). Colin Tann worked collaboratively with Monsanto on various aspects of novel refuge option trials (see notes in text above). We anticipate further such collaborations, so that maximum efficiencies are derived from research efforts.

Publications

9. A. List the publications arising from the research project and/or a publication plan.

Baker, G.H., Tann, C.R. (2007). Mating of Helicoverpa armigera (Lepidoptera : Noctuidae) moths in relation to their plant hosts as larvae within Australian cotton farming systems. In : Proc. 4th World Cotton Research Conf. CD-ROM. Lubbock, Texas, USA.

Baker, G.H., Tann, C.R., Fitt, G.P. (2008). Production of *Helicoverpa* spp. (Lepidoptera, Noctuidae) from different refuge crops to accompany transgenic cotton plantings in eastern Australia. *Aust. J. Agric. Res*. **59**, 723-732.

Baker, G.H., Tann, C.R., Fitt, G.P. (submitted). A tale of two trapping methods : *Helicoverpa* spp. (Lepidoptera, Noctuidae) in pheromone and light traps in Australian cotton production systems. *Bull. Ent. Res*.

Baker, G., Tann, C., Downes, S. & Mahon R. (2006). Research Comments. Entomology. In : "Variety Trial Results 2006". Cotton Seed Distributors, Wee Waa, pp. 95-97.

Baker, G.H. & Tann, C.R. (2006). Mating of *Helicoverpa armigera* moths in Bollgard® cotton. Proc. Aust. Cotton Conf., Gold Coast, Qld. 5 pp.

Lawrence, L., Tann, C. & Baker, G. (2007). Refuge crops provide refuge for more than *Helicoverpa*. *The Australian Cottongrower*, 28, 26-27.

Lawrence, L., Tann, C. & Baker, G. (2007). Refuge crops for *Helicoverpa* in Australian cotton farming systems. *Outlooks on Pest Management*. Feb. 2007, pp. 19-20.

Lawrence, L., Tann, C. & Baker, G. (2007). Refuges harbour pests and beneficial insects. *Farming Ahead* No. 183 : 52-54.

Tann, C., Baker, G., & Downes, S. (2007). Research Comments. Entomology. In : "Variety Trial Results 2007". Cotton Seed Distributors, Wee Waa, pp. 86-88.

Tann, C., Baker, G., Downes, S. & Mahon, R. (2008). Refuges, Bt resistance testing, and *Helicoverpa* populations. In : "2008 Variety Trial Results". Cotton Seed Distributors, Wee Waa, pp. 78-80.

Tann, C., Baker, G., Downes, S., Whitehouse, M., Mahon, R., Whitburn, G. (2009). Bt resistance, refuges and *Helicoverpa* ecology. In : "2009 Variety Trial Results". Cotton Seed Distributors, Wee Waa, pp. ……. ??

Whitehouse, M.E.A., Wilson, L.J., Fitt, G.P., Constable, G.A. (2009). Integrated pest management and the effects of transgenic cotton on insect communities in Australia : lessons from the past and future directions. Proc. 3rd Intern. Symp. Biological Control of Arthropods. pp. 161-172. Christchurch, New Zealand.

Torres, J.B., Ruberson, J.R., Whitehouse, M.E.A. (In press). Transgenic cottons for sustainable pest management. In : Agricultural Development and Sustainability, Ed ….. ?

Manuscripts nearing completion :

Baker, G.H., Tann, C.R. (in prep.) Plant host origins of mating *Helicoverpa armigera* (Lepidoptera : Noctuidae) within Australian cotton farming systems**.** Intended for *Aust. J. Ent.*

Talks given :

Colin Tann and Mary Whitehouse have attended and addressed various grower group meetings during the tenure of this project, including e.g. the Cotton Industry's Big Day Out (at Keytah), refuge discussion meeting with growers and staff of CSD at "Gunedra", and Resistance Road Shows conducted in all major cotton production valleys. The research has been summarised in various media articles (e.g. "Spotlight", May 2008).

Mary Whitehouse presented an invited talk at the Intern. Symp. Biol. Control of Arthropods (N.Z., 2009), and gave a 3rd year lecture at Univ. Tech. Sydney on spiders in Australian cotton.

Colin Tann attended the Intern. Congr. Entomology (Durban, Sth Africa) (2008) and delivered a poster presentation on the project's work (Tann, C. & Baker, G. "Stable isotope analyses to determine plant host origins of mating *Helicoverpa armigera* (Lepidoptera : Noctuidae) within Bt cotton crops in Australia"). The same poster was presented at e.g. the Aust. Cotton Conf. in Qld and Cotton CRC Reviews. GB, CT & MW have been regular attendees, and occasional presenters at these events.

Geoff Baker gave various talks on the ecology and management of *Helicoverpa* at REFCOM meetings, and featured the work of this project in invited talks (e.g. at the Ecol. Soc. Australia Annual Conf., Sydney, 2008, and Intern. Colloq. Soil Zool., Brazil), and in a separate talk at Univ. Darmstadt, Germany. He presented a paper on the random mating of *H. armigera* moths at the 4th World Cotton Conference USA, 2007). Geoff visited China (2008), funded by the Chinese Ministry of Science & Tech (in particular CAAS in Beijing and CAS in Urumchi, Xinjiang) to advise on local cotton pest management research, give talks on Australian *Helicoverpa* research, and discuss research activities in common (especially related to *Helicoverpa* ecology, Bt resistance and management). Geoff presented talks on cotton pest management to various visiting scientific delegations in Canberra and hosted a member of an Australia-China Young Scientist Exchange delegation (Prof Ma Chun-sen, Univ. Beijing).

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

Ongoing results from the network of pheromone traps for *H. armigera* and *H. punctigera* have been posted on the Cotton CRC web site in past years. We intend to continue this service in future years, if the CRC is interested.

Part 4 – Final Report Executive Summary

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

The use of transgenic (Bt) cotton (Ingard® and more recently Bollgard II®) has greatly reduced the use of insecticides in Australian cotton production and the status of key lepidopteran pests such as *Helicoverpa* spp. But the potential for these moths to develop resistance to Bt remains a major challenge for the industry. A resistance management plan has been adopted, part of which is the mandatory requirement for Bt cotton growers to provide refuge crops (no Bt exposure) as sources of susceptible moths. Such moths are expected to mate with potentially resistant moths arising from the Bt crops and help reduce the chance of resistance developing more broadly. During the Ingard® era, the relative moth productions of various refuge crop options were identified. However, only limited research has been devoted to identifying the degree of cross-mating of moths between refuges and Bt cotton crops.

This project's research determined the incidence of cross-mating of *Helicoverpa* in key refuge crop - Bollgard II® cotton combinations. It concluded that mating of *H. armigera* moths from different plant host origins occurs at random – as assumed under the Resistance Management Strategy adopted by the Australian Cotton Industry. However, our knowledge for *H. punctigera* in this regard remains very limited – and *H. punctigera* has emerged through recent surveys of Bt resistance alleles to be surprisingly important too. In addition, we began to identify the degree of coverage achieved spatially (landscape-scale) by refuge-derived, susceptible moths, by surveying intensively all refuge crops within a single cotton production region (St George) throughout a growing season. This work will be continued in a new project (2009-12). Further, we identified means by which the performance of refuges (production of *Helicoverpa*) can be enhanced, in particular through the use of moth attractants such as those in Magnet®, and through novel refuge crop options (e.g. pigeon pea and mixed crops for dryland systems, split-planting of unsprayed conventional cotton for irrigated systems). The ultimate aim of the research is to optimise the efficiency of refuges, and enable maximum, effective production for least input by farmers.

We also maintained our ongoing monitoring programs for *Helicoverpa* moths (pheromone trap grids in the L. Namoi and St George regions) to provide local guidance to growers of population trends, as well as identifying long-term patterns in the abundance of these key pests at seasonal and regional scales. The abundance of *Helicoverpa* spp was in general low during the tenure of this project. Rearing of field-collected eggs, larvae and pupae of *Helicoverpa* also suggested that the incidence of natural enemies (e.g. pupal parasitoids) is currently quite high. This raises concerns for the effective production of susceptible moths from refuges. Whilst the abundance of *Helicoverpa* within refuges was relatively low during 2006-09, it was not outside the range documented in previous years. The performances of refuges will be closely monitored in the new project.

During the project, we re-analysed old data sets to determine e.g. the degree of evidence that exists that supports the notion that rainfall patterns in inland areas of Australia drive the production and migration of *H. punctigera* from there to the eastern cropping regions, such as the L. Namoi. We found little evidence of correlations between inland rainfall patterns and the numbers of *H. punctigera* subsequently trapped near Narrabri since the early 1990's. Rather, we found some evidence of early season abundance of *H. punctigera* being related to numbers at the end of the previous cropping season – suggesting greater local over-wintering than previously thought to occur. Clearly, we still have much to learn concerning the ecology of these key pests.

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