Cotton Catchment Communities CRC

FINAL REPORT

(due 31 May, 2012)

Part 1 - Summary Details

Cotton CRC Project Number: 1.01.65CRC1005

Project Title: Enhancing the Efficiency of Bt Refuge Crops within a Changing Cotton Environment

Project Commencement Date: 01/7/2009 **Project Completion Date:** 30/6/2012

Cotton CRC Program: **The Farm**

Part 2 – Contact Details

Administrator: Ms Sonja Slatter,
Organisation: CSIRO Sustainable Agriculture Flagship
Postal Address: Ecosciences Precinct, Boggo Rd, Dutton Pk, Qld 4103
Ph: 07 3833 5690 **Fax:** **E-mail:** Sonja.Slatter@csiro.au

Principal Researcher: Dr Geoff H. Baker,
Organisation: CSIRO Ecosystem Sciences & S.A.F.,
Postal Address: P.O. Box 1700, Canberra, ACT 2601
Ph: 02 6246 4406 **Fax:** 02 6246 4094 **E-mail:** Geoff.Baker@csiro.au

Supervisor: Dr Geoff H. Baker
Organisation: As above
Postal Address: As above
Ph: **Fax:** **E-mail:**

Signature of Research Provider Representative: ______________________________

Part 3 – Final Report (due 31 May at CCC CRC, but within 3 months on completion of project at CRDC)

Background

1. Outline the background to the project.

The deployment of transgenic (Bt) cotton has greatly reduced the use of insecticides on Australian cotton and the status of key lepidopteran pests, particularly *Helicoverpa armigera* and *H. punctigera*. But the potential for these moths to develop resistance to Bt remains a major challenge for the industry. A Bt resistance management strategy was developed in 1994, part of which was the mandatory requirement for Bt cotton producers to grow non-Bt crops as refuges and thus produce moths susceptible to Bt. Such moths are assumed to mate, at random, with potentially resistant moths arising from the Bt crops and thereby help retard the evolution of resistance. Work elsewhere has shown that refuges help to delay resistance in other moth pests of cotton (e.g. in the USA; Tabashnik et al 2008, Nature Biotechnology 26 : 199-202).

However, farming practices for cotton and associated grain crops in Australia have changed significantly in recent years and these changes have put new focus on the efficacy of refuge management. For example, Bollgard II now dominates (approx 85 % of the cotton crop; cf 30% cap earlier for Ingard), cotton has been far less of a landscape-wide monoculture and more part of a mosaic of various crops during the recent drought, and beneficial species have a more direct role in IPM. In addition, management issues such as water shortage, weed management, and herbicide damage can conflict with current refuge requirements. Growers want more choice and less restriction to their farm profits at a time when cash flow is tight. Nevertheless, refuges are probably even more important now, given a recent increase in the frequency of resistance alleles to Cry2Ab in populations of both *H. armigera* and *H. punctigera* and the dominance of Bollgard 11 on the landscape. With the recent recognition of resistance risk in *H. punctigera* (previously not thought likely because 1. this species hadn't developed resistance to conventional insecticides earlier and 2. perceived reliability of regular influxes of large numbers of susceptible moths from inland recruitment areas), refuge options have been reduced. The cotton industry no longer accepts sorghum and maize as refuge crop options because they are poor hosts for *H. punctigera*.

This project followed on from CRC 1.01.52 ("Maximising the efficiency of Bt refuge crops"). Some elements of 1.01.65 are direct continuations of work done in 1.01.52 (e.g. ongoing long-term monitoring of the abundance of the moths using trapping grids), other aspects expanded preliminary work initiated in 1.01.52 (e.g. regional evaluation of the collective efficacy of refuge crops at St George), and various new research topics were also explored (e.g. trials evaluating mixes of conventional cotton varieties as refuge options).

Objectives

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

The overall aim of this project was to provide research to underpin confidence in and enhance refuge crop efficacy. Firstly, the project aimed 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, and to 2) examine the efficacy of existing refuges. We proposed to continue experiments on the movements and mating patterns (especially degree of randomness) of moths emerging from different types of refuges and Bt cotton for both *H. armigera* and *H. punctigera*. In addition, the project aimed to 3) continue ongoing monitoring of landscape-scale changes in the abundance of *Helicoverpa* spp., particularly in the Namoi Valley and St George regions, thus tracking population trends and especially changes associated with Bt cotton use. Finally, we noted that most research on refuge crops had hitherto focussed on the performance of individual crops. We proposed that we would 4) expand our work to an Area-Wide Management approach to refuges (as needed for such a mobile pest as *Helicoverpa*, using a regional case study, whilst evaluating the costs and benefits (besides *Helicoverpa* production) by considering if refuges act as sources or sinks for natural enemies and emerging pests. We also agreed to liaise with an agricultural economist to further evaluate the costs of refuge options.

We argued these studies would 1) provide growers with more refuge options, 2) provide information on changes in *Helicoverpa* populations which have ramifications to resistance management, 3) 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.

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

Objective 1. Identify new refuge crop options

Milestone 1.1 : Field trials conducted in NSW (including ACRI) to evaluate novel refuge crop options. Some work will be collaborative with Monsanto.

Achieved.

Objective 2. Monitor long-term changes in *Helicoverpa* abundance

Milestone 2.1 : Pheromone traps cleared weekly at grids in Namoi & St George, data filed electronically.

Achieved.

Milestone 2.2 : Surveys of weed & non-cotton crop plants conducted each spring in Namoi region for *Helicoverpa* eggs and larvae. Collections reared to maturity.Achieved.

Objective 3. Identify degree of cross-mating of *Helicoverpa* from different plant host origins

Milestone 3.1 : Sites monitored in northern NSW / southern Qld, emergences predicted, night collections made of mating moths & chemical analyses completed to identify both origins.

Achieved, but work on *H. punctigera* was less than wished for – simply because of the poor availability of this species (and mating couple in particular).

Milestone 3.2 : Laboratory experiments conducted to identify cross-mating of moths from plant hosts proving intractable to discern in field (e.g. pigeon pea & cotton).

Achieved.

Objective 4. Conduct a landscape scale evaluation of refuge crop efficacy

Milestone 4.1: Contributing farms & sampling protocols identified / developed for landscape study, probably in St George.

Achieved.

Milestone 4.2 : Ongoing surveys of *Helicoverpa* eggs, larvae, pupae conducted at 3 weekly intervals throughout cotton season on at least 20 farms (Bt cotton & refuge crops).

Mostly achieved, with limitations due to local flooding in some seasons.

Milestone 4.3 : Capacity for refuge crops to service *Helicoverpa* outputs from Bt cotton crops identified for selected region.

Mostly achieved, but modelling based on the collected data still in progress.

Objective 5. Provide a holistic appraisal of costs/ benefits of key refuge crop options

Milestone 5.1 : Surveys completed for secondary pests & beneficial species in refuge crops (and associated Bt cotton crops) collected while doing other activities detailed in project plan (visual assessments, beat sheets, suctions as appropriate). Available data on other local variables (e.g. soils, water & nutrients applied) sourced; key soil variables (e.g. organic C) measured.

Achieved.

Milestone 5.2 : Protocols developed with an economist for collaboration on broad cost / benefit analyses of refuge crop options.

Not Achieved.

Objective 6. Extend research results to industry and scientific colleagues.

Milestone 6.1 : At least one scientific MS and one industry magazine article submitted and one seminar / grower talk presented / year.

Achieved.

Objective 7. Complete final report for project.

Milestone 7.1 : Report submitted by Sept 2012 & oral presentation of final report delivered as invited by CRDC.

This was altered during project because of CRC closure in June 2012. Thus required to submit final report to CCC CRC by May 2012. i.e. This report. A further report will follow to CRDC, by 30 September 2012, including findings from late in the project that were unable to be completed by the time of the early CRC closure.

Achieved, as required thus far.

This report is structured around these Objectives, within the Methods, Results, Outcomes & Conclusions sections below. To facilitate enquiries, we indicate the names of the researchers responsible for various sections (ahead of relevant text).

METHODS

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

1. Identify new refuge crop options

M. Whitehouse

Background

The aim of this part of the refuge project was to develop new refuge crop options to improve the efficacy of refuges, thereby providing growers with more choice. In irrigated cotton there are currently two refuge options for all Helicoverpa: pigeon pea and non-Bt cotton. In dryland systems only non-Bt cotton can be a non-irrigated refuge. Therefore the aim of this section was to identify new refuge crop options in both dryland and irrigated systems that would improve refuge efficacy. In particular we tested the efficacy of pigeon pea, split cotton plantings, mixed species refuge crops and different varieties of cotton as refuges in irrigated systems; and mixed species refuge crops, pigeon pea, Sicot 80 RRF cotton, and ratoon cotton as refuge crops in dryland cotton. If refuges for cotton could increase their attractiveness and productivity (and thereby their efficacy) they would improve their ability to reduce the risk of Helicoverpa developing resistance to Bt toxins.

In order to improve the efficacy of refuges, we need an effective way of measuring efficacy of refuges relative to the Bt crop. To date, the efficacy of refuges has been largely measured in terms of the number of eggs laid on a crop (attractiveness of the refuge to moths); and the number of pupae or moths emerging from the refuge (productivity). When making the initial calculations, Roush et al 1998 assumed that Bt crops would attract egg lays at the same density as conventional cotton, and Helicoverpa would suffer the same mortality rate in Bt as it does in non-Bt cotton (if the Bt cotton did not contain the Bt toxin). Given this assumption, they calculated that 10% of the Bt crop area should be planted in cotton refuge to delay resistance. This 10% cotton refuge is the gauge upon which other refuges are tested. For example, pigeon pea can be planted at 5% of the Bt crop area because previous work (Baker et al 2008) indicated that it attracts twice the amount of eggs and produces twice the number of pupae, as non-Bt cotton.

However, using non-Bt cotton as the gauge by which refuge efficacy is measured is problematic, largely because it does not account for any changes in efficacy of non-Bt cotton refuges relative to Bt cotton. For example, water stressed and nutrient deprived crops could have quite different Helicoverpa attractiveness and productivity to well irrigated and nourished crops. In addition, high levels of attractiveness in the non-Bt refuge resulting in heavy larvae pressure could reduce the refuge's attractiveness latter in the season through damage caused by the larvae. Bt cotton would not suffer this reduction as is should receive little larval damage. Also, there may be varietal differences between Bt cotton crops and non-Bt refuges in attractiveness and Helicoverpa productivity. It would be better if we could directly calibrate the efficacy of refuges to Bt crops.

Unfortunately, the only way Bt crops and refuges can be directly compared is by measuring the number of eggs per metre laid in each crop (attractiveness). But does attractiveness correlate to Helicoverpa productivity?

Although ultimately the number of eggs laid in a crop must correlate with the number of moths produced from the crop, there are many variables that create noise in this relationship, decreasing the correlation between egg numbers and moth numbers. These can be categorized as sampling noise and survivorship noise.

Survivorship noise is caused by differences in survivorship between and within fields caused by seasonal differences, changes in the number of parasitoids, predators, level of disease, crop nutrition, and the effect of other larvae both as predators and competitors.

Sampling noise is caused by variability in the number of eggs laid per metre across a crop and over time throughout the season. Variability over time is a particular problem because of the different methods used to sample eggs, pupae and moths. As eggs take 2-3 days to hatch, the visual surveys record 2-3 days of egg laying activity. Alternatively, pupae take 2 weeks to develop into moths, and 3 weeks to develop into parasitod wasps. Thus pupae digs sample 2-3 weeks of Helicoverpa pressure. The question is can variance caused by sampling and survivorship noise be overcome in order to obtain meaningful correlations between eggs laid and moths produced?

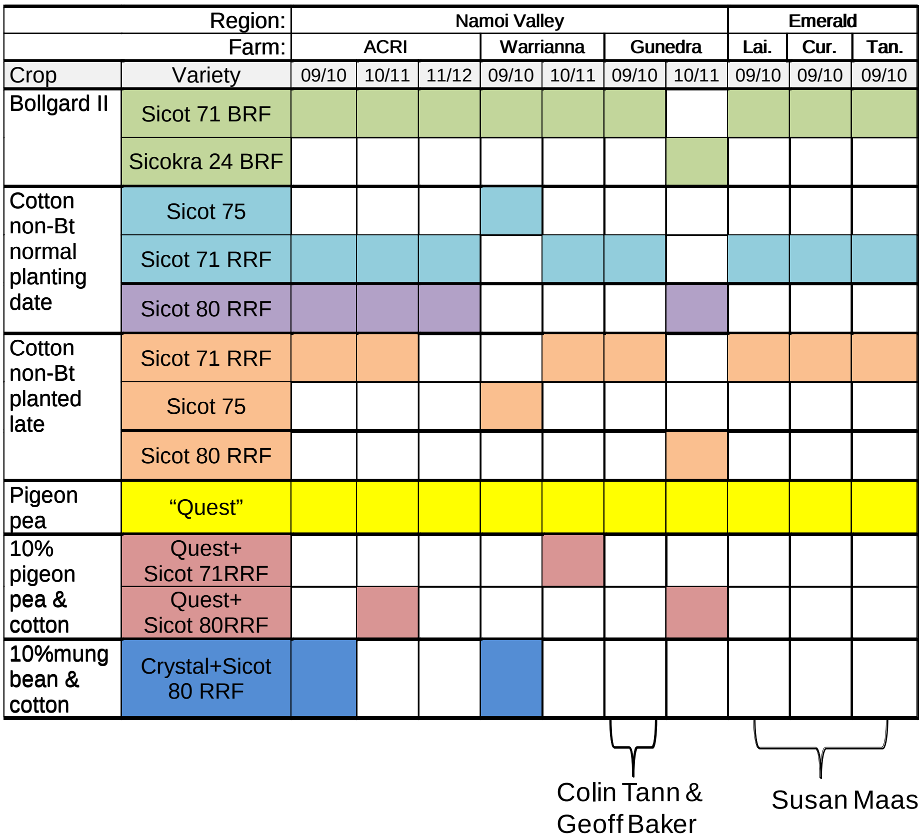
Another problem when testing the efficacy of refuges is that both Bt cotton and its refuge could vary in attractiveness and productivity throughout the season. Should refuges be managed so that they produce many moths in one big bang at the end or the beginning of the season, or should refuges consistently produce moths throughout the season? As resistant moths could emerge at any stage during the season, refuge efficacy needs to be consistent throughout the season.

Thus there were three aims to this section:

- Are there specific types of refuges in irrigated and dry land cotton that attract significantly more egg lays or produce significantly more moths than other refuges, particularly non-Bt cotton refuges?
- Do different refuge types at least match Bt cotton in attractiveness throughout the season?
- Can pupae or moth counts be correlated with egg counts?
- Are there changes over the season in the relationship between egg and pupae or moth counts?

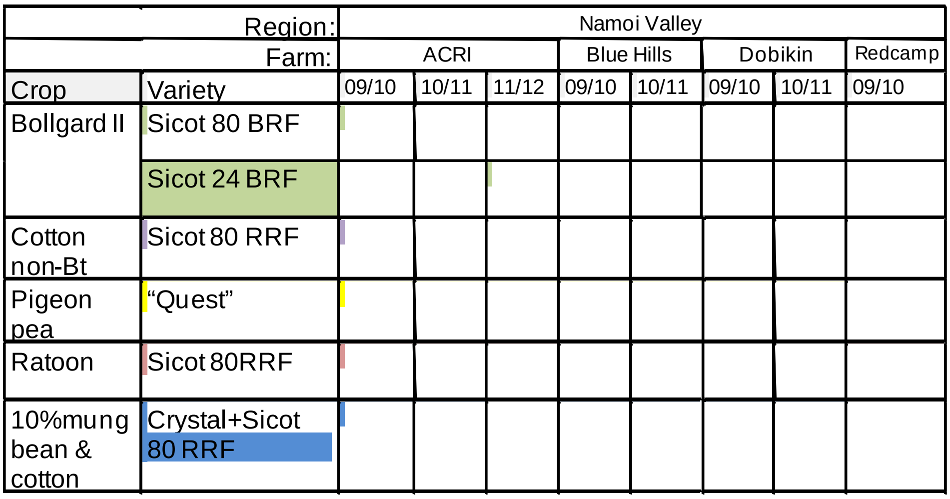
Sampling method

Ten irrigated fields were sampled from 1 experimental station (ACRI) and 5 commercial cotton farms from two regions (Namoi valley and Emerald) over 3 seasons, from 2009/10 to 2011/12 (Table 1). Eight dryland fields were sampled from 1 experimental station and 3 commercial cotton farms in the Namoi Valley over three seasons, from 2009/10 to 2011/12 (Table 2). Fields varied greatly in size and shape, although reps within each field were similar in size. All reps were 24 rows wide (the minimuim size of a refuge crop) and at least 50 m long. Reps were sampled randomly using visual surveys, pupae digs and emergence cages. In visual surveys, 1 metre of a row of crop was visually checked for the presence of Helicoverpa eggs and larvae. To pupae dig, the ground under 1 metre of a row of crop was dug to a depth of 10 cm, on both sides of the crop, to unearth any pupae (Fig. 1). Emergence cages sampled 1 metre row of crop. Plants within the 1 metre were removed, and an emergence cage placed over the area and secured to the ground. Emerging moths were collected from a vial at the top of the cage weekly for two weeks. Between 5 and 3 samples were taken over the season at any given site (see Figs 6, 8 and 9) and between 12 and 6 reps were taken per treatment (Table 3).



Region:		Namoi Valley							Emerald		
Farm:		ACRI			Warrianna		Gunedra		Lai.	Cur.	Tan.
Crop	Variety	09/10	10/11	11/12	09/10	10/11	09/10	10/11	09/10	09/10	09/10
Bollgard II	🟩 Sicot 71 BRF	🟩	🟩	🟩	🟩	🟩	🟩		🟩	🟩	🟩
	🟩 Sicokra 24 BRF							🟩			
Cotton non-Bt normal planting date	🟦 Sicot 75				🟦						
	🟦 Sicot 71 RRF	🟦	🟦	🟦		🟦	🟦		🟦	🟦	🟦
	🟪 Sicot 80 RRF	🟪	🟪	🟪				🟪			
Cotton non-Bt planted late	🟧 Sicot 71 RRF	🟧	🟧			🟧	🟧		🟧	🟧	🟧
	🟧 Sicot 75				🟧						
	🟧 Sicot 80 RRF							🟧			
Pigeon pea	🟨 "Quest"	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
10% pigeon pea & cotton	🟥 Quest+ Sicot 71RRF					🟥					
	🟥 Quest+ Sicot 80RRF		🟥					🟥			
10%mung bean & cotton	🟦 Crystal+Sicot 80 RRF	🟦			🟦						

Table 1. Chart of refuges tested with irrigated Bollgard II on the six farms over three seasons. Colin Tann and Geoff Baker sampled Gunedra in the 2009/10 season, and Susan Maas sampled the Emerald farms in the 2009/10 season.



Region:		Namoi Valley							
Farm:		ACRI			Blue Hills		Dobikin		Redcamp
Crop	Variety	09/10	10/11	11/12	09/10	10/11	09/10	10/11	09/10
Bollgard II	Sicot 80 BRF								
	🟩 Sicot 24 BRF								
Cotton non-Bt	Sicot 80 RRF								
Pigeon pea	"Quest"								
Ratoon	Sicot 80RRF								
10%mung bean & cotton	Crystal+Sicot 🟦 80 RRF								

Table 2. Chart of refuges tested with dryland Bollgard II on the four farms over three seasons.

Irrigated or Dryland	Farm	Year	Sampling method (no. per rep)		
Irrigated	ACRI	09/10	Visual (6)	Pupae dig (10)	
Irrigated		10/11	Visual (12)	Pupae dig (6)	Cage (6)
Irrigated		11/12	Visual (12)	Pupae dig (6)	Cage (6)
Irrigated	Warrianna	09/10	Visual (6)	Pupae dig (10)	
Irrigated		10/11	Visual (12)	Pupae dig (6)	Cage (6)
Irrigated	Gunedra	09/10	Visual (6)	Pupae dig (10)	
Irrigated		10/11	Visual (12)	Pupae dig (6)	Cage (6)
Irrigated	Laikipia	09/10	Visual (6)	Pupae dig (12)	Cage (6)
Irrigated	Currimundi	09/10	Visual (6)	Pupae dig (12)	
Irrigated	Tandawanna	09/10	Visual (6)	Pupae dig (12)	
Dryland	ACRI	09/10	Visual (6)	Pupae dig (10)	
Dryland		10/11	Visual (12)	Pupae dig (6)	Cage (6)
Dryland		11/12	Visual (12)	Pupae dig (6)	Cage (6)
Dryland	Blue Hills	09/10	Visual (6)	Pupae dig (10)	
Dryland		10/11	Visual (12)	Pupae dig (6)	Cage (6)
Dryland	Dobikin	09/10	Visual (6)	Pupae dig (10)	
Dryland		10/11	Visual (12)	Pupae dig (6)	Cage (6)
Dryland	Redcamp	09/10	Visual (6)	Pupae dig (10)	

Table 3. Sampling methods and reps per sample in irrigated and dryland cotton.

To test if egg counts could be correlated with moth emergence, most pupae digs and emergence cages re-sampled metres used in the visual survey, and were timed to capture the cohort of Helicoverpa that had been sampled in the visual survey.

Fig. 1. Pupae dig in progress under a cotton crop

Emergence cages

Usually moths counts are obtained by collecting pupae from pupae digs, and then maintaining these in the lab until they die or hatch. However, pupae digging can be problematic, as the soil may be too hard or too wet to dig, there may be differences in the ease of recovering pupae under different crops, and harvested pupae are protected from subsequent underground attack by predators or parasitoids. Large emergence cages had been used in the past, but we wanted to develop small, easy to move cages that would sample one metre of crop, so we developed the cage in Fig 2.

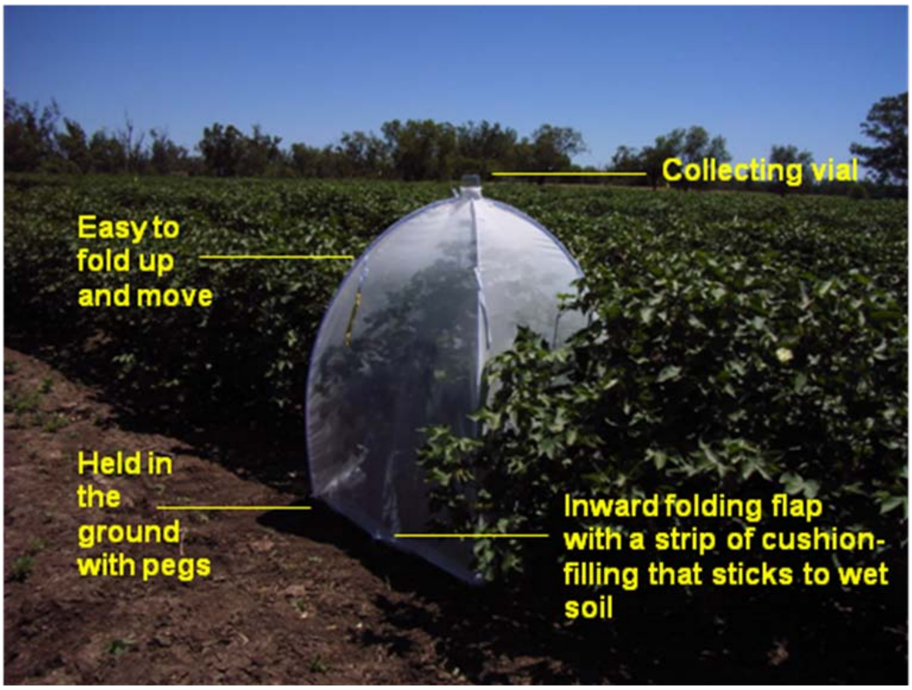


Fig 2. Emergence cage in a cotton field

We considered patenting the cage, but there are similar cages on the market (but without the strip of filling around the base) and we thought that the cost of patenting the cages would not be cost effective, given the likely returns.

Analysis

Results were analysed using the statistical package GenStat (13th edition). In particular results were compared using ANOVA, transforming the data where applicable, non-parametric Kruskal-Wallis one-way ANOVA (when data could not be transformed); and linear and non-linear regression. To compare the effectiveness of different refuges to either attract eggs or produce moths over the season, smoothing splines (Whitehouse et al 2005) were used with the help of the biometrician, Steven Harden (NSW DPI). Egg or moth counts in all irrigated trials were modelled over time using smoothing splines (Verbyla et al 1999) which uses the data to determine the shape of the response. This was done within a linear mixed model using ASREML (Gilmour et al 2000). Each spline curve consists of a linear component (slope and intercept terms) and a non linear component (spline term). The fixed terms in the model are crop and day and their interaction. If the crop term is significant then the spline curves for each crop type have differing intercepts.

G. Baker & C. Tann

2009/10 : Gunedra, Namoi Valley and Neilo, Macintyre Valley

One of the problems faced with using unsprayed conventional cotton as a refuge crop is that it can get eaten out by *Helicoverpa* spp. or other pests such as tip worm, *Crocidosema plebejana*, early in the season and then isn't attractive to moths later in the season. In the last year of CRC Project 1.01.52, we ran a pilot trial (at Gunedra, Namoi Valley) which investigated the potential benefit that might accrue from split planting, i.e. sowing 2 portions of a cotton refuge crop at different times – one part at the same time as the accompanying Bollgard II crop was sown and the other later (at 1st irrigation). This concept followed on from some earlier work (with Monsanto) that sought to find ways to reduce the refuge requirement (Ha's) for unsprayed cotton). We thought this approach could spread the attractiveness of the refuge crop over a longer period of the season, and provide a net improvement in refuge performance. The results suggested a modest improvement at best in terms of extending refuge performance. We decided to repeat the work in this project, to deliver more robust conclusions. We established two split planting trials in 2009-10, one again at Gunedra and another at Neilo, Macintyre Valley. Both trials were accompanied with pigeon pea crops, as points of reference. We had hoped to also establish a trial at Narramine, Macquarie Valley, but because of chronic water shortages and a restrictive permit we were unable to progress with this.

There was no replication of crops at either site. Single fields of each option (Bollgard II, 1st and 2nd cotton refuge crop plantings, and pigeon pea) were simply arranged side by side, but the areas for each "plot" were consequently large. The Neilo trial site was approx. 40Ha and the Gunedra site was approx. 8Ha, with individual plots at least 200 rows wide.

At Gunedra, the Bollgard II, pigeon pea and 1st planting of cotton refuge were sown on the 9th and 12th October 2009 respectively. The 2nd planting of cotton refuge occurred on 7th December 2009, at the time of the 1st irrigation. *Helicoverpa* eggs and larvae (+ beneficial species) were scored on plants in all fields (visual sampling, on 6 well-spaced apart 1 m lengths of crop) on 18 December (except for the 2nd cotton planting), 12 January, 2 & 22 February and 15 March. Pupae were sampled in the soil (within 10 well-spaced apart 1m^2 plots centred on plant rows) on these same dates (except 18 December) and also on 14 April. Note, no attempt was made to sample pupae beneath Bollgard II. Unfortunately, the 2nd planting of cotton was badly damaged by tip worm, which greatly reduced its attractiveness to *Helicoverpa* moths.

At Neilo, the Bollgard II, pigeon pea and 1st planting of cotton refuge were sown during the 1st week of October 2009. The 2nd planting of the cotton refuge occurred in late November, again at the time of the 1st irrigation. *Helicoverpa* eggs and larvae (+ beneficial species) were scored on plants in all fields (visual sampling – as above) on 21 December (except for the 2nd cotton planting), 13 January, 3 & 23 February, 16 March and 7 April. Pupae were sampled in the soil (as above) on these same dates (except for 21 December). Not, again no attempt was made to sample pupae beneath Bollgard II. The 2nd planting of cotton was also damaged by tip worms at this site (but not as badly as at Gunedra), which again reduced its attractiveness to *Helicoverpa* moths.

As in all field trials we conducted, the eggs, larvae and pupae we collected during sampling were returned to the laboratory for rearing through to determine species of moth and parasitism.

2010/11 : Taraba, Gunedra, and CSD (Little Mollee)

The results from the split planting trials in 2009-10 were not supportive of there being clear advantages to be gained by such practices, but the trials were impeded by tip worm damage (as noted above). We decided to repeat such trials, at Taraba, Macintyre Valley and at Gunedra, Cardale, and CSD (Little Mollee), Namoi Valley.

At Taraba, there were two plantings of unsprayed cotton, one planted 26th October and the other planted 17th December (at the 1st irrigation). The trial was placed at the southern end of a Bollgard II cotton field and was comprised of two 9 Ha plots containing the two different plantings of unsprayed cotton. Ten individual metres of crop row, selected at random within each plot, were sampled for pupae on 4 occasions, together with 6 visual 1 m of row samples (on 3 occasions) counting *Helicoverpa* eggs and larvae and any key beneficial species present. Egg lays within the associated Bollgard II cotton crop were also monitored.

At Gunedra, we ran another split planting trial (8 Ha in total), with a pigeon pea comparison. A 1st planting of unsprayed conventional cotton was made on 11 October (along with a crop of pigeon pea), followed by a 2nd planting of cotton on 20 December (associated with the 1st irrigation). Sampling of eggs, larvae and pupae of *Helicoverpa,* plus beneficial species was conducted as per other trials, with 3 survey dates for eggs and larvae (13 January, 8 February & 8 March) and 4 for pupae (as for eggs and larvae, plus 14 April).

We attempted to establish an additional trial at Cardale to again investigate the efficacy of split planting of cotton refuges. However, the site was plagued with problems – flooded by river water & heavily weed infested which interfered with ability to establish timely plantings. We sampled the trial on 4 occasions, but due to the poor plant stand and subsequent very low *Helicoverpa* spp. activity, we feel the data are of little value, and do not present them here.

We also established a "look-see" trial at CSD (Little Mollee) to investigate the potential efficacy of using early, mid and late maturing varieties of conventional cotton as a means of enhancing attractiveness in refuges over time. The trial included a comparison with pigeon pea (1 km distant from trial). The earliest cotton plantings were of conventional varieties Sicot 80 RRF, planted on 9th October, and Sicot 71 RRF, planted on 1st November. These plots were planted side by side, 32 rows each making up approx. 1.5 Ha trial area. A later planting, which was a combination of Sicot 71 RRF and Sicot 43 RRF (in equal amounts) (the latter being an earlier maturing variety that the other two) was then planted next to that on the 15th December. Irrigation of these plots was by overhead central pivot. Sampling for *Helicoverpa* eggs, larvae and pupae was similar to that described above, and occurred within the conventional cotton crops and the pigeon pea. Eggs and larvae were sampled on 4 occasions (14 December, 18 January, 9 February & 10 March) and pupae on 5 occasions (as for eggs & larvae, plus 13 April). An associated Bollgard II crop, which was growing next to the refuge, was sampled for eggs on one occasion (9 February).

2011/12: Brookstead, Darling Downs

Observations we made earlier in the project (see above, and previous projects) suggested differences can occur between conventional cotton varieties in terms of their attraction to *Helicoverpa* spp., but such had not always shown consistency. For example, Sicot 71 appeared to be more attractive and received greater damage than Sicot 80 in two crops sown within an unsprayed refuge at Little Mollee, near Narrabri (see Fig. 1) (note : this was separate to the trial indicated above for 2010-11 at Little Mollee). In addition, maturity lengths and growth habits differ between varieties, and if we are able to take advantage of this in producing a refuge that is composed of > 1 variety and thus remains attractive for a longer time, and is protected from herbicide drift, then unsprayed cotton refuges may become a better option, or at least be offered as a 5% option like pigeon pea.

For the 2011-12 season, we established a large (replicated treatment) trial of approx. 15 Ha at "Thologolong", near Brookstead in the Darling Downs in collaboration with Monsanto and CSD which investigated the advantages of mixing available non-Bt cotton varieties, that have RRF traits, which we expected will be strongly attractive to *Helicoverpa*. Okra leaf RRF varieties would be ideal as refuge options, but such are not presently available – hence we could not include such in this trial. Instead we focussed on mixing Sicot 80 and Sicot 43, two varieties with contrasting development rates. We hypothesised that such could spread attractiveness in time and extend the utility of cotton refuges.

Fig. 3. Contrasting impacts of *Helicoverpa* damage on two conventional, cotton varieties at Little Mollee, Narrabri, in early 2010. Sicot 80 on right, Sicot 71 on left.

The trial near Brookstead had 4 treatments (all RRF conventional cotton) : 1) Sicot 71, 2) Sicot 80, 3) Sicot 43, & 4) an equal mix of Sictot 80 and Sicot 43. These 3 varieties differ in their growth habit and development, Sicot 71 being vigorous (but less so than Sicot 80) & full season, Sicot 80 being very vigorous & full season, and Sicot 43 being compact & short season. There were 3 replicate plots for each treatment (each replicate approx 700 m long & 18 rows wide). The treatments / replicates were arranged in a split plot design.

Surveys for *Helicoverpa* eggs and larvae were taken on 6 occasions (between December 2011 and early April 2012. Counts of eggs and larvae (numbers / m or crop row) were made on 6 separate 1 m or row, located at random within each replicate on each survey occasion. Presence of key beneficial invertebrate taxa (predatory bugs, beetles and spiders), plus selected secondary pests (green vegetable bug, mirids) were noted within the same cotton plants. *Helicoverpa* pupae were sampled on 5 occasions (between January and early April, 2012) by hand-sorting 6 separate 1 m^2 of soil at random within each replicate. The collections of eggs, larvae and pupae were reared in the laboratory to identify moth species and levels of parasitism and disease. The trial site was flood irrigated on two occasions during the study. A pigeon pea crop was located approx 1 km away from the trial site (on an adjoining farm). This crop was regularly watered (more so than the trial site) using lateral move irrigation. Six (random) m of row were sampled for eggs, larvae and pupae within this pigeon pea crop on the same sampling visits to the trial, to provide a crude (admittedly, un-replicated and slightly removed spatially) comparison.

On 5 occasions, (January to early April, 2012), the numbers and heights of cotton plants within 5 separate (random) m of row were measured within each replicate at Brookstead, along with the numbers of flowers, squares and bolls / plant.

We had intended a similar trial in the Namoi Valley (at Little Mollee), but at the "last minute", the area chosen was no longer available due to pressing commercial interests, and an alternative possibility that was offered was considered unsuitable due to potential spray drift issues.

We also had planned to investigate the effects of ratooning pigeon pea in light of some interesting work done in China recently (Z. Lu, CAS, China, pers. comm.). The Chinese have apparently found that pigeon pea performance (Helicoverpa load) can be enhanced through a second season by ratooning. Our proposed work was to take place at ACRI. However, plant survival was very poor over the 2011 winter to warrant conducting the experiment. At that stage, no other pigeon pea refuges remained.

G. Baker & C. Tann

2. Monitor long-term changes in *Helicoverpa* abundance

Pheromone Trapping in the Namoi Valley & St George

Grids of 11 pairs (increased to 14 pairs in 2010-11) 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 sincerely acknowledge Jamie Street and his staff for their amazing efforts in continually running these traps over many years for us.] In addition, these same consultants have provided us with ongoing egg count data from cotton crops in the St George region.

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 up-dates on pheromone trapping of *H. punctigera* at ACRI (Narrabri) in relation to inland rainfall. These relationships were last analysed (see Final Report for CRC Project 1.01.52) for pheromone trap data from 1992-2002 – we now extend the comparisons until 2012. As indicated in previous projects, prior to our recent work, there was general acceptance that levels of abundance of *H. punctigera* in early season (spring) in the eastern cropping regions were responses to migrations of moths from inland Australia, following heavy winter rains there. However, this concept was based on trapping data in a limited number of years during the 1970's and 1980's. Because such movement (and the assumption that moths from inland Australia would be Bt susceptible) has been central to dismissing *H. punctigera* as a likely threat re it developing Bt resistance within Bt resistance management plans, we have continued to keep a "weather-eye" on trapping records and their association with inland rainfall levels. In the past our trapping data have failed to support the notion of spring peaks in *H. punctigera* abundance in pheromone (and light) traps in the eastern cropping region matching high inland rainfalls during the previous winter. Fig. 4 illustrates the locations of the meteorological stations and districts we (and others before us in a similar way : Oertel et al 1999, *Aust J Entomol* 38, 99-103) have used for these analyses.

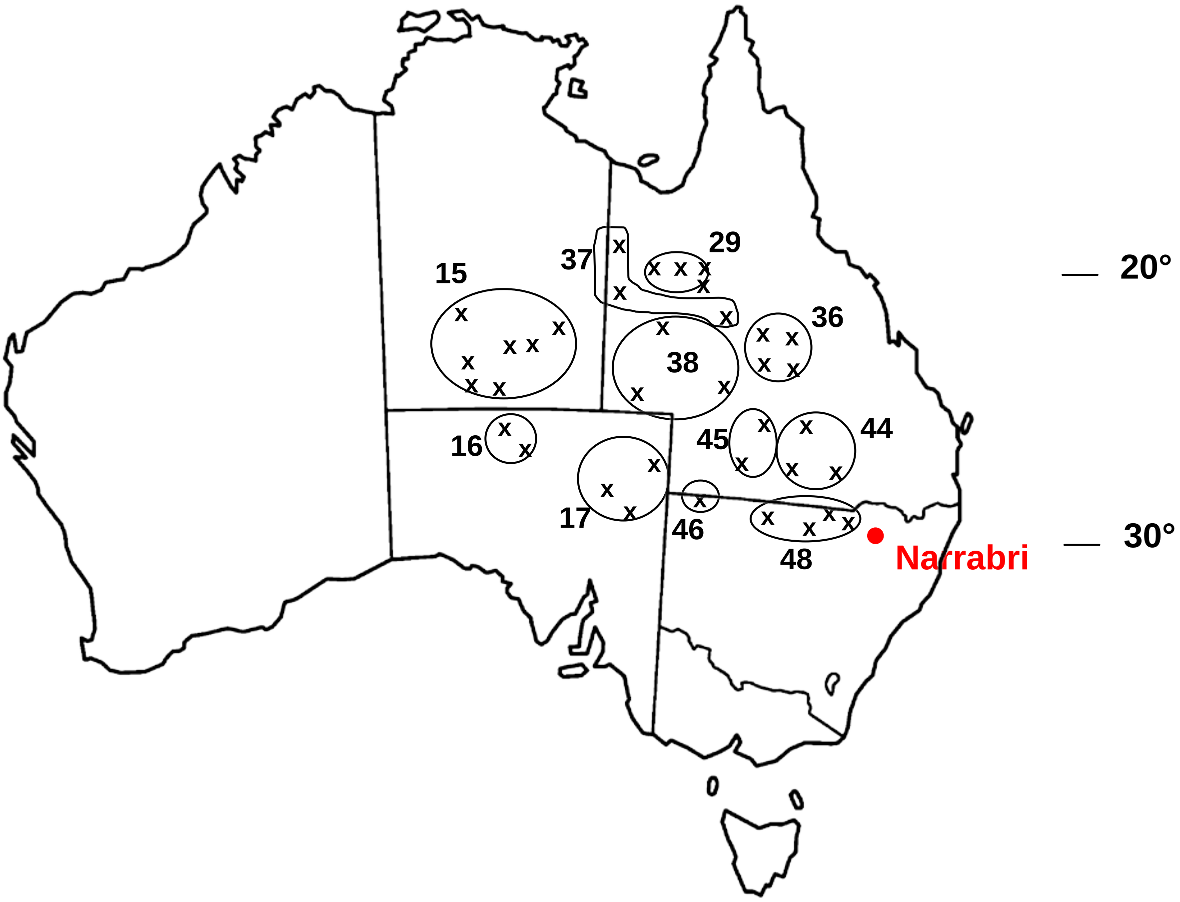


Fig. 4. Locations of Bureau of Meteorology weather stations (x) and recognised Meteorological Districts (circled) used for correlations between rainfall data and trapping records for *Helicoverpa* spp. near ACRI, Narrabri.

Surveys of weed & non-cotton crop plants conducted each spring in the Namoi region for Helicoverpa *eggs and larvae.*

This work was conducted, as in many previous years, on weed and non-cotton crops for eggs and larvae during spring, using periodic visual and sweep-net collections (usually 100 sweeps / site & / or 6 x 1m of crop row replicates for visual checks) and subsequent rearing of collections in order to gain some prediction of the likely seasonal challenges ahead and increased understanding of long term patterns in early season impacts of parasitism and disease. Plant hosts checked during the springs of 2009-2011 were mostly chick peas, lupins, field pea, faba bean, medic, *Echium*, various daisies, *Caspedia* sp. and *Brachycombe*. Collections were made in the Namoi, Macintyre, & Gwydir Valleys, plus St George, Dirranbandi & Walgett. Collections were less intensive during this project (cf earlier ones), as priorities for such information diminished, and reflecting limitations of drought and hence availability of suitable plant hosts (especially in 2009). In all 69 sites were surveyed in spring 2009, 91 in 2010, and 50 in 2011. Totals of 1425, 497, and 327 individuals were reared in the respective years. The counts of eggs and larvae were expressed against the relevant sampling effort.

3. Identify degree of cross-mating of *Helicoverpa* from different plant host origins

G. Baker & C. Tann

Sites monitored in northern NSW / southern Qld, emergences predicted, night collections made of mating moths & chemical analyses completed to identify both origins.

As many sites as possible were established each season which contained a Bollgard II cotton crop and an associated refuge crop within the Namoi, Macintyre, & St George valleys. This worked in with other studies that were also being undertaken in these regions. The aim was to set up subsequent studies of cross-mating between moths of different plant host origin, in particular to test for random mating (as assumed in the RMP), especially for *H. punctigera* which previously had not been assessed (almost all previous effort has been devoted to *H. armigera* because of perceptions at the time that it provided primary risk re Bt resistance development). At these sites we monitored the abundance of eggs, larvae and pupae at regular intervals to predict when and where sufficient moths were likely to be present to enable night collections, especially of mating moths. Large infestations within a defined cohort are required to enable an emergence to be predicted that will be sufficient for a night collection.

Because of the relative scarcity of moths in the past 3 seasons, especially *H. punctigera*, the field collections of mating moths referred to above proved impossible to achieve. We thus devoted much of our time to laboratory analyses of *H. armigera* specimens previously collected & stored (CRC Project 1.01.52). These collections were analysed for stable C and N isotope signatures, following methods reported previously. We did however have a reasonably large set (n = 110) of *H. punctigera* moths, most of which were mating, stored from a collection made within a Bollgard II cotton crop at Shangri-la (Namoi Valley) in the 2004-05 season which were also able to have analysed for stable isotopes, along with smaller numbers of *H. punctigera* collected at Battery Hill (Namoi Valley) (Ingard, 2003-04) (n = 18) and South Callandoon (Macintyre Valley) (Bollgard II, 2004-05) (n = 31).

M. Whitehouse

Laboratory experiments conducted to identify cross-mating of moths from plant hosts proving intractable to discern in field (e.g. pigeon pea & cotton).

A major assumption underlying the use of pigeon pea as refuges for cotton is that there is random mating between moths from both crops. Helicoverpa is a generalist herbivoure which attacks a range of species. However within the Helicoverpa complex there could be cryptic species that specialize on only a few host types, and avoid mating with individuals that associate with other host plants. Such behaviour could be advantageous if it enhanced the development of tolerance to the toxins of the chosen host plant. If moths emerging from pigeon pea are unlikely to mate with those emerging from cotton, then the role of pigeon pea as a refuge is compromised.

Alternatively, Helicoverpa are able to learn and are more likely to oviposit on the host plant to which they are first exposed as adults (Cunningham et al, 1998). If this form of learning is also used to recognize acceptable mating partners, then the crop from which Helicoverpa emerges could influence its choice of partner, and compromise the role of pigeon peas as refuges for cotton.

Consequently, two sets of experiments were undertaken. The first set tested whether the crop on which the larvae were raised influenced their choice of a mating partner as an adult; the second tested if the crop first experienced by newly emerged adults influenced their choice in mating partner.

1. *Larval host experiment*

Three experiments were set up to test if a Helicoverpa's experience as a caterpillar influences its choice of mating partner.

Experiment 1a: Laboratory colony eggs

Fig. 5. Field shot of larvae in individual bags feeding on cotton (left) or pigeon pea (right) as part of Experiment 1a.

In December 2008 400 *Helicoverpa armigera* neonates from the laboratory colony were placed on diet. At third instar half of them were placed in individual bags on either pigeon pea or cotton plants in the field (Fig. 5). At 5th instar they were removed and placed on vermiculite to pupate. When the moths emerged they were sexed, and then marked using twink to distinguish between those raised on pigeon pea or cotton.

Moths were tested when they were at least 4 days old by placing them in a perspex tank (40cmx40cmx40cm) at 25°C and 60% humidity. Equal numbers of male, female, pigeon pea and cotton raised moths were placed in the tank (a total of 172 moths, 43 moths of each type). The moths were on reverse day and night, and were set up just after their dark. The tank was checked every 15 mins for mating moths for a total of 3 hours. This was repeated for 3 nights. Moths were removed from the tank during the day. Mating pairs found in the tank were removed and allowed to continue to mate. Crop origin of partners, time to mate, length of time mating, body length, and mass were recorded. Female moths were then placed in a Chinese Tupperware container for 6 days, which contained a small container of honey water and was covered in nappy liner as a substrate for oviposition. Eggs harvested from the females were incubated for 2 days and the viable eggs and larvae were countered.

Experiment 1b: Wild eggs from sorghum plants

Same procedure as above, using 800 eggs collected from sorghum plants in the field. 140 *Helicoverpa armigera* were raised through to maturity and tested in the Perspex tank. 35 moths of each type were placed in the tank (140 moths total) and tested for four nights.

Experiment 1c: wild caught eggs from pigeon pea and cotton plants

In March 2009 428 eggs were collected from Bt cotton, and 356 eggs were collected from pigeon pea refuges. These were raised as above, and tested in the Perspex tank. 40 moths of each type were placed in the tank (160 moths total) and tested for two nights.

Experiment 2a: Exposing emerging laboratory moths to pigeon pea or cotton.

In October 2009, 400 laboratory colony eggs were set up on diet and raised through until pupae, at which point the pupae were sexed, and set up in same sex groups of about 20 at the base of either cotton or pigeon pea plants in individual cages in the glasshouse. Both *H. armigera* and *H. punctigera* were set up, but too few *H. punctigera* emerged for that experiment to continue. Plants were checked daily for emerged moths, which were subsequently removed. A total of: cotton: 74 females, 64 males; pigeon pea: 79 females, and 66 males were harvested.

One hundred males (50 exposed to each plant type) were set up in individual plastic mating cages (diameter 12cm, depth 6 cm, containing a small container of sugar water) in the evening (temperature 25^0C, 75% humidity, the day before full moon). At 12 mid-night a female was added to each male (50 females exposed to each plant type). Cages were checked every 15 mins and the experiment finished at 12,midday. The same data was collected as before.

Experiment 2b: Exposing emerging wild moths to pigeon pea or cotton.

In November 2011, 800 neonates from four females collected using light traps were set up on diet. Once they had pupated, they were sexed and placed in same sex groups of about 30 at the base of either cotton or pigeon pea plants in individual cages in the glasshouse. The plants were checked daily for emerged moths. When emerged moths were found, the remaining pupae were moved to fresh pot and the emerged moths left for 24 hours, after which time they were collected. This meant that the newly emerged moths had been first exposed to either cotton or pigeon pea plants for between 24 and 48 hours. Any moths exposed to moths of the opposite sex were discarded. The same data was collected as before.

Ninety mating cages were set up as before, except the mating cages were larger (diameter 12cm, depth12 cm). The same data was collected as before.

G. Baker & C. Tann

4. Conduct a landscape scale evaluation of refuge crop efficacy

During the previous project (CRC 1.01.52), we conducted a pilot study in 2008-09 of the spatial and temporal variability of refuge crop (pigeon pea only) performance in attractiveness to and productivity of *Helicoverpa*. In that year we assessed the attractiveness of each refuge crop growing in the St George region (n = 25) according to the Monsanto audit / evaluation index), and the numbers of eggs, larvae and pupae on the plants or in the soil on 4 occasions during the season. That work, presented in the Final Report for CRC 1.01.52, high-lighted the spatial & temporal variability in refuge performance. We have continued such studies throughout the 3 seasons (2009-12) in the current project to build a data-base of the variability of such performance at land-scape scale, with a view to modelling how well the refuges collectively “service” the Bt cotton crops they are dedicated to.

Surveys of Bt cotton and refuge crops in the St George region were fraught with difficulties of access during 2009-2012 because of the repeated flooding experienced there. Nevertheless, we achieved several assessments in each season. During the 2009-10 season, we visited 22 pigeon pea refuge crops and several of their associated Bt cotton crops (note not all, but to check synchronies between crops) on 5 occasions, during the 2010-11 season, we visited 27 pigeon pea refuge crops and their associated Bt cotton crops on 4 occasions, and during the 2011-12 season, we visited 26 pigeon pea refuge crops and their associated Bt cotton crops on 3 occasions.

On each occasion, we used Monsanto's mid-season audit ranking system to measure the attractiveness of the refuges to *Helicoverpa*. Bt cotton crops were assessed in term of their growth stage. The numbers of eggs present in relation to the nearby refuge crop was a measure of attractiveness. The numbers of *Helicoverpa* eggs and larvae were counted on 6 m of row in each crop on each sampling occasion. *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 most sampling occasions for *Helicoverpa* pupae (the first sampling occasion was often not surveyed for pupae, because it was perceived numbers would then be low). Live pupae were also returned to the laboratory for rearing through to moths.

Similarly, we assessed the performances at a set of refuge crops and their associated Bt cotton crops within the Boggabilla group of growers near Goondiwindi in each season from 2009 to 2012. The numbers of crops surveyed were necessarily smaller than at St George – the Goondiwindi surveys were established somewhat as a comparative "anchor" to check for likely parallels / differences occurring in another cotton production region. Whilst the refuges at St George were consistently pigeon pea, those near Goondiwindi were either pigeon pea or unsprayed, conventional cotton. There were 12 refuge crops sampled near Goondiwindi in 2009-10 (visited 5 times; all were pigeon pea), 15 in 2010-11 (visited 5 times; 9 were pigeon pea, 6 unsprayed cotton) and 15 in 2011-12 (visited 4 times; 11 were pigeon pea, 4 unsprayed cotton).

5. Provide a holistic appraisal of costs/ benefits of key refuge crop options

G. Baker & C. Tann

As well as surveying the abundance of eggs, larvae and pupae of *Helicoverpa* spp. in Bt cotton and pigeon pea and unsprayed conventional cotton refuge crops (see above), the presence of selected beneficial species (predatory beetles, bugs and spiders), selected pests (mirids and green vegetable bug) and the incidence of insect disease was also scored (visually), by assessing whether or not they were encountered whilst checking for eggs and larvae (i.e. 6 M of crop row / field). Note these major taxa were divided into different sub-groups (e.g. spiders included orb webbers, lynx and jumping spriders etc), which were separately assessed and the presence data for these different sub-groups were summed.

The incidence of pupal parasitism was assessed by rearing the pupae collected in the field through to emergence in the lab, as was the incidence of parasitism of eggs and larvae.

M. Whitehouse

Over the course of the project we have been trialing ratoon cotton as a potential dryland refuge. We were interesting in whether ratooning cotton offered any benefits to soil nutrient content and structure, so in collaboration with Dr. Michael Braunack, we have been taking soil samples from the ratoon cotton and associated Bt cotton for analysis.
In June 2009 soil samples were taken from non-bt cotton (which was to be ratooned) and associated Bt cotton. Six cores were taken at each site, and a sample from each core was taken at 4 depths: 0-15cm, 15-30 cm, 30-60 cm, and 60-90 cm. The samples were analysed at ACRI. The process was repeated in 2011 when the samples were sent away to Nutrient advantage for analysis. We plan to take a further sample in June 2012.

Janine Powell (CRC economist) provided us with a questionnaire to assess grower's costings for refuges. David Harris's CRC Summer Scholarship involved collaborating with 16 growers, who we asked to fill out a questionnaire supplied by Ms Powell to identify key costs to growing a refuge. Unfortunately we have had little response from the growers, with four completed questionaires, and three partial responses. With this low level of response we have not completed the analysis.

6. Extend research results to industry and scientific colleagues.

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

RESULTS

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

1. Identify new refuge crop options

M. Whitehouse

Are there specific types of refuges that are more attractive or productive than (non-Bt) cotton refuges?

Irrigated cotton

Attractiveness: Egglays

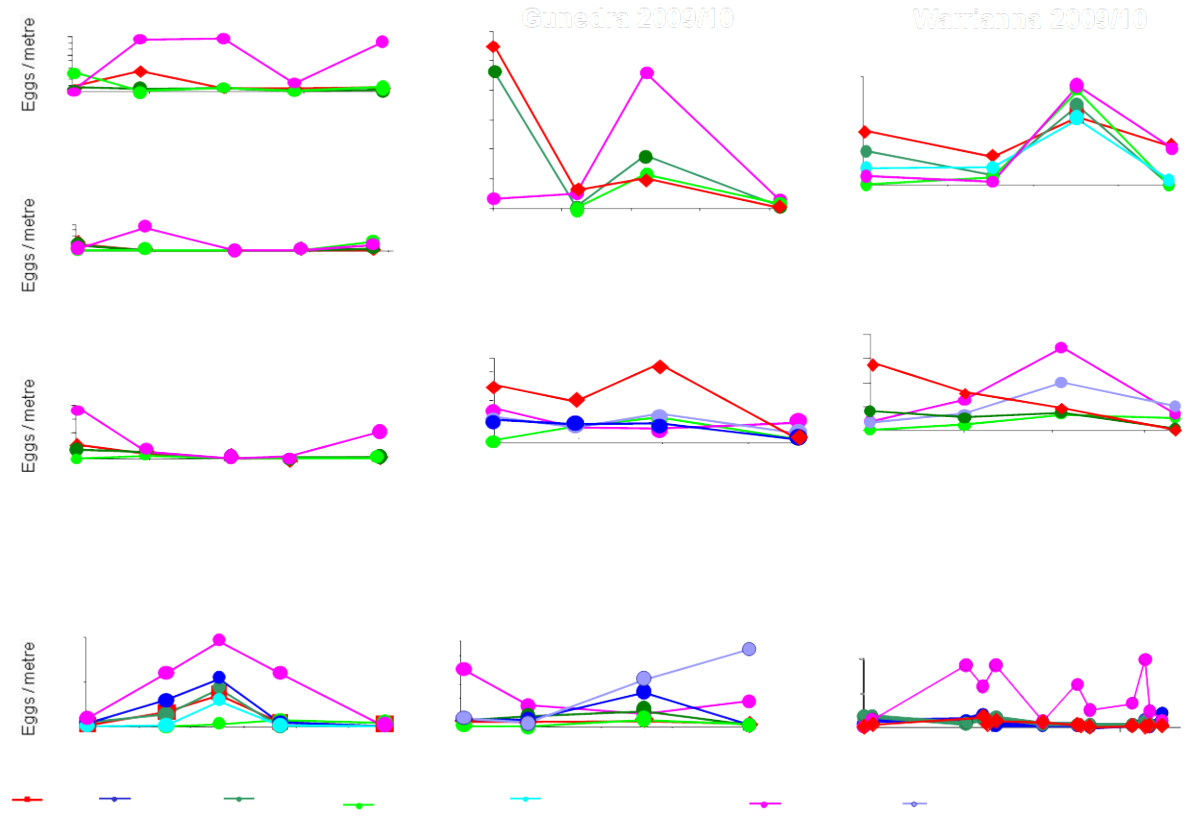


Fig. 6. The number of eggs laid on different crops at the different sites over the seasons.

There was a large range both between and within farms in the egglays attracted to different refuges on different farms (Fig. 6). For example, Gunedra in 2009/10 season had nearly 30 eggs/m on the Bt crop at the beginning of the season, while the highest number of eggs recorded at Tandawanna in the 2009/10 season was only 3 eggs/m on a pigeon pea crop early in the season. At most sites pigeon pea attracted the most eggs, although the attractiveness was not consistent, consisting of one or more peaks.

Smoothing splines were developed from information on egg lays from all crops at all sites, and were used to identify if there were consistent patterns in the attractiveness of the different crops. The smoothing splines revealed that despite the dips in attractiveness over the season within sites, pigeon pea refuges, overall, were attractive for oviposition throughout the season (Fig. 7). In comparison, Bt crops at the beginning of the season were as attractive as the pigeon pea, but their attractiveness decreased significantly over the season, so that by the end of the season, pigeon was significantly more attractive. The attractiveness of other cotton refuges, Sicot 71 and Sicot 80, also dropped off over the season, although there was a trend for these crops to be less attractive than the Bt crop at the beginning of the season. Sicot 80 tended to be more attractive during peak flowering than Sicot 71, but the difference is not significant. Late planted cotton, and cotton with 10% mungbean are significantly less attractive than Bt cotton throughout most of the season. Interestingly, cotton with 10% pigeon pea started the season significantly less attractive than Bt cotton, but by peak flowering it was significantly more attractive.

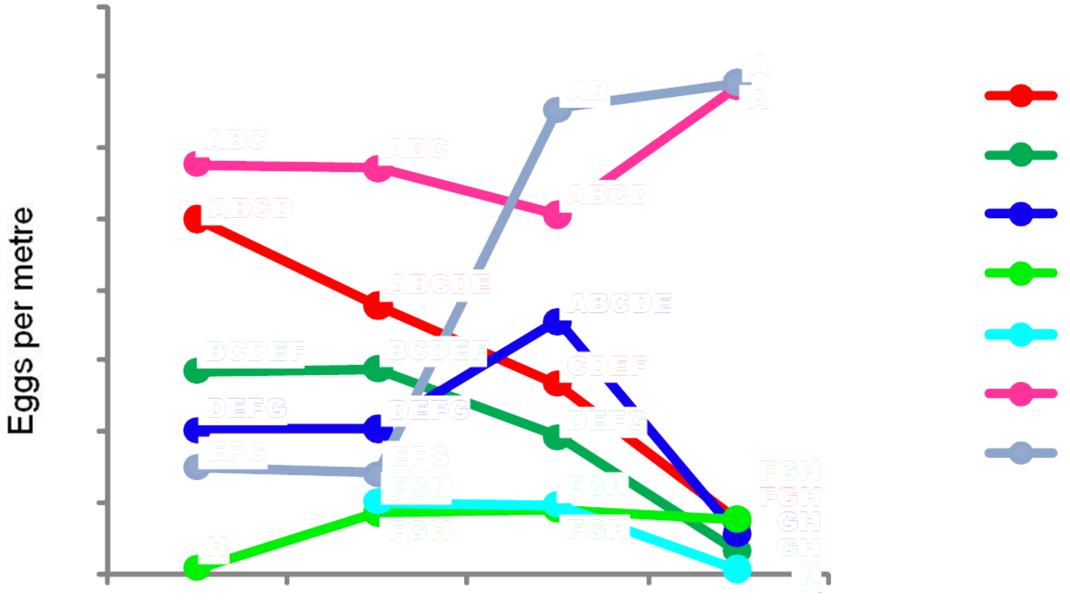


Fig. 7. Summary of smoothing splines of eggs laid over the season on different crops. The number next to the crop indicates the number of sites upon which that crop was grown; the letters on the graph indicate significant differences between eggs per metre.

Productivity: Pupae and Moths

Because the season is only just finishing, we do not have the pupae or moth numbers for this year’s work, so we will be reporting on just the previous year’s work. Fig. E shows all the pupae collected at the different sites over the seasons. Again there is a large amount of variability both between and within farms. For example, Warrianna had the highest local maximum of 12 pupae per metre in 2009/10, and the lowest local maximum a year later of 1.6 pupae per metre (Fig. 8).

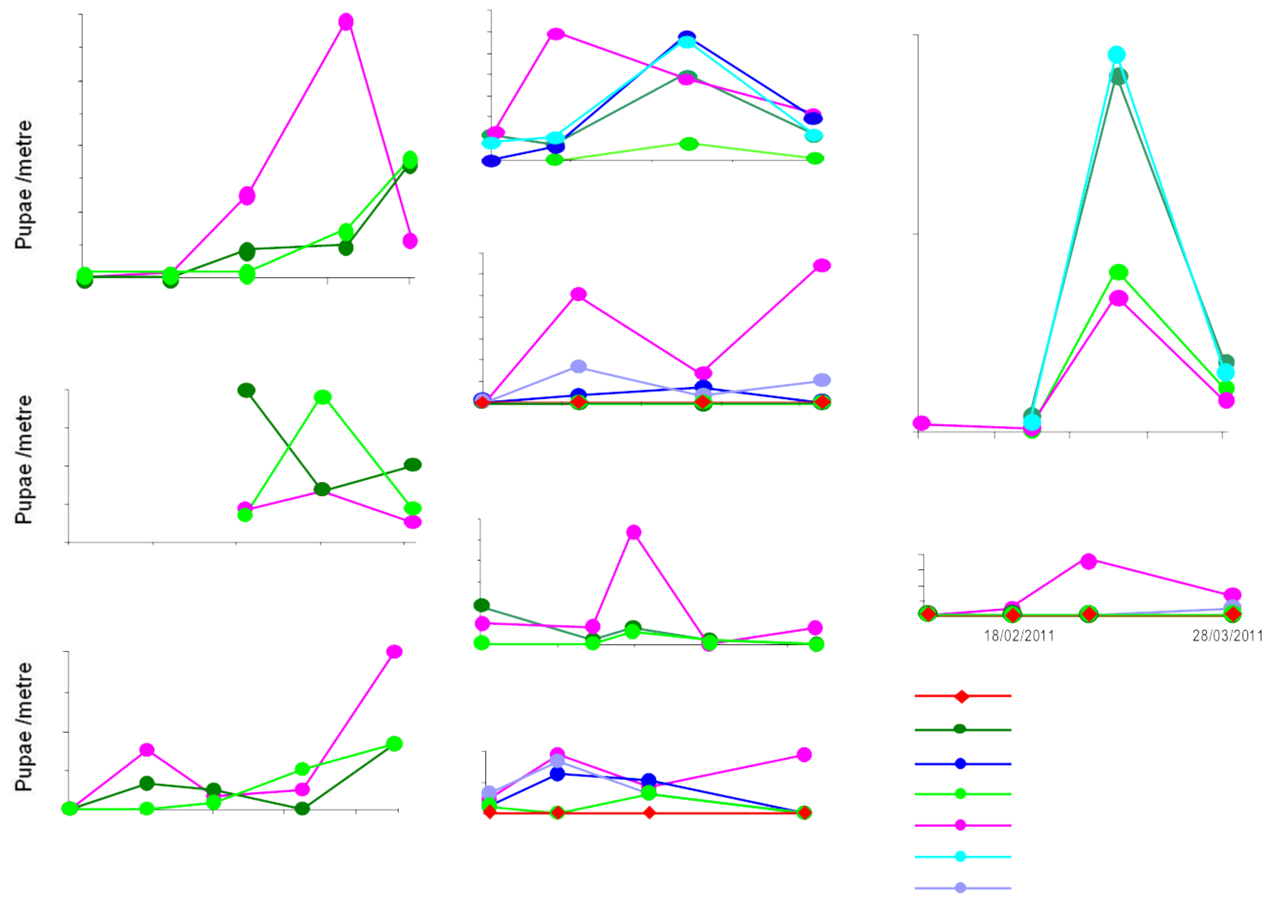
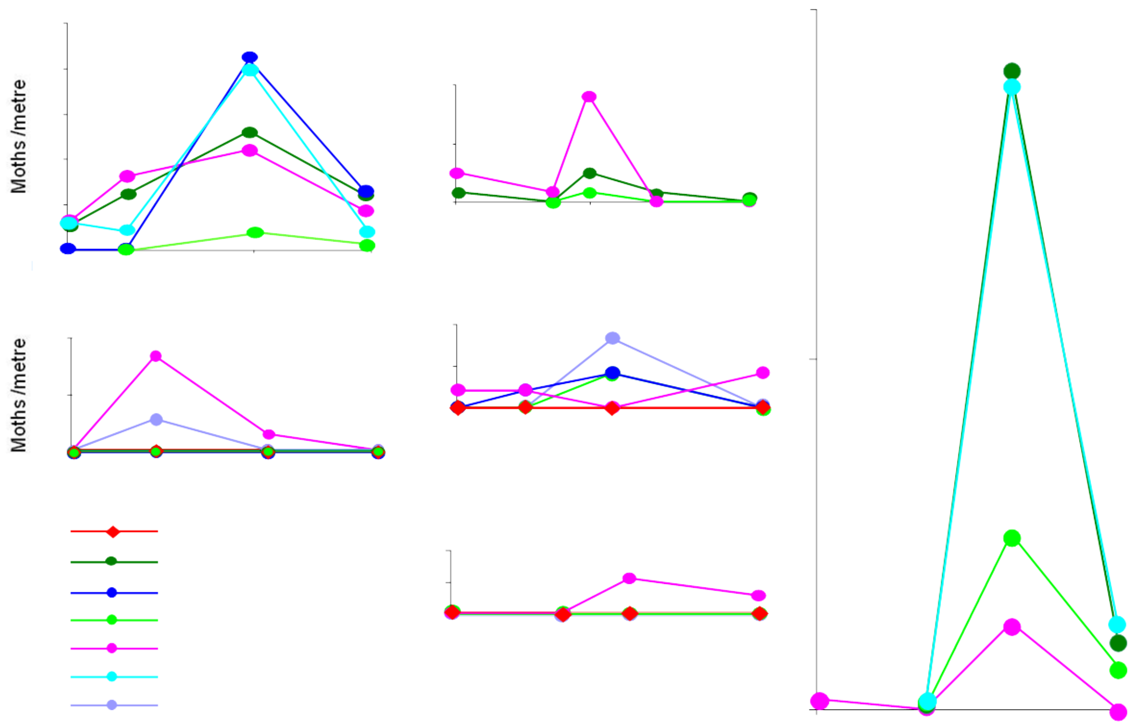


Fig. 8. The number of pupae collected from different crops at different sites over the seasons. Samples from Bt crops were taken only in the 2010/11 season.

The high number of pupae collected from Warrianna mid February from the two conventional cotton crops (Sicot 71 and Sicot 71 + 10% mungbean) in the 2009/10 season (Fig. 8) is reflected in the very high numbers of moths emerging from those same crops (Fig. 9). In the 2010/11 season, the numbers of moths collected were extremely low at all sites, with only a total of three moths retrieved from pupae at Warrianna. More moths were captured using cages from these sites (Fig. 10) but the numbers are still low.



.

Fig. 9. Moths emerging from pupae collected using pupae digs. Numbers in brackets indicate the total number of moths harvested from that site.

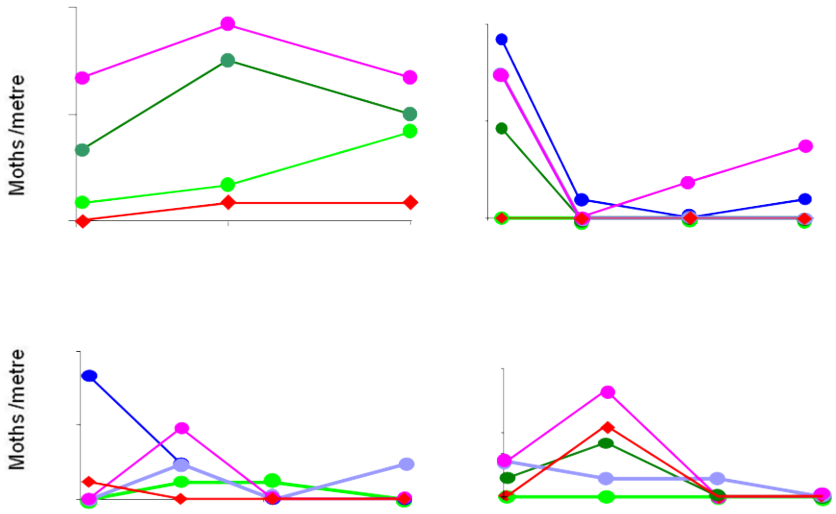


Fig. 10. Moths collected using cages. Key is the same as Fig. F.

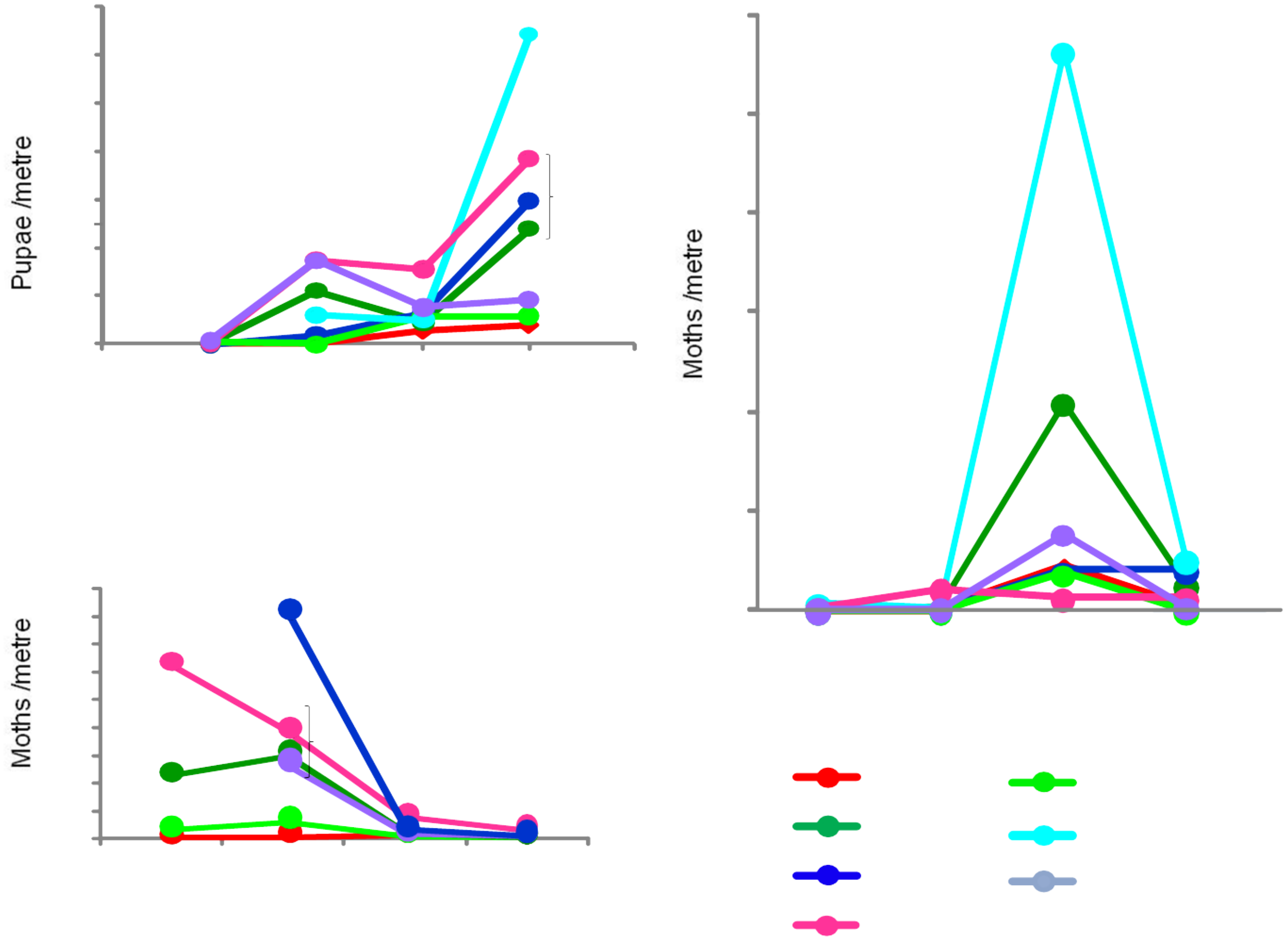


Fig. 11. Summary, using smoothing splines, of pupae and moths collected at all sites (except ACRI 2011/12 season). Crops are colour-coded as before. Samples were grouped by degree days, the highest degree day for each grouping is indicated under the graphs. The coloured numbers indicate the number of sites where that crop was tested. The double asterisks indicate a crop that had significantly higher egg lays than all the other crops on that sample date, single asterisks indicate a lower level of significance.

To combine results for all farms for pupae digs and moths emerging, all samples were standardized to degree days, which were then grouped for analysis (Fig. 11). To allow a comparison between moths emerging from pupae digs, and moths captured in cages, 14 days were added to the pupae dig date for moths emerging from pupae. Results were then combined using smoothing splines (Fig.11).

Because of the low numbers of pupae and moths collected, caution must be taken in analysing these results. For example, the mungbean mix did very well in both the number of pupae collected, and moths emerging from those pupae. Particularly given the low numbers of eggs found on this crop (Fig. 7). At the two sites where mungbean mix was tested (Warrianna 09/10, and ACRI 09/10) cotton refuges also did well. Therefore, it is not clear whether the success of 10% mungbeans reflects the cotton or the presence of mungbeans. Mungbeans were not sampled using cages.

All three graphs show quite different patterns of Helicovpera production. For example, as the season progressed, the overall number of moths collected decreased, while the number of pupae collected increased. The increase in pupae probably reflects an accumulation of pupae in the soil over the season, particularly towards the end of the season as pupae in the Namoi region enter diapause. The increase in pupae at the end of the season was not matched by an increase in moth numbers from pupae after 2100 Degree days.

Diapause may also explain the overall low numbers of moths collected from cages towards the end of the season. The only site where this didn't happen was Laikipia, which is in Emerald where Helicoverpa does not diapause (Fig 10).

Because Helicoverpa pupae are expected to be in very low numbers under Bt cotton, Bt crops are not normally sampled for pupae. We sampled three farms in the 20010/11 season for pupae under Bt crops, and found none (Fig. 8). However, of the four farms sampled using cages, only one did not collect at least one moth from a Bt crop (Fig. 10). Thus moths can be collected from Bt crops using cages.

The combined data indicates that in this study pigeon pea refuges are not always twice as productive as cotton refuges, and that cotton refuges can equal or out perform pigeon pea refuges. This reflects findings from a survey of refuge productivity on commercial farms, carried out by the summer scholarship student, David Harris (see Whitehouse et al, appendix 1). While there are differences between the cotton varieties, these are not consistent. Likewise, while adding pigeon pea to cotton strongly increased egglays at the end of the season (Fig. 7), there was no similar large bounce in terms of moth production, which tended to be similar to cotton refuges without pigeon pea. While mungbean numbers in the combined graph are impressive, they were no more productive than the cotton refuges at the sites they were tested, indicating that there is no clear advantage of mungbeans.

Concluding remarks on irrigated refuges.

Of all the refuges tested, pigeon was usually the most attractive. Currently, pigeon pea refuges only require half the land of non-Bt cotton refuges because they are regarded as twice as attractive as cotton crops. This assumption is not supported by this work. Pigeon pea only became twice as attractive as Bt cotton on these farms at the end of the season if the pigeon pea re-flowered. Thus pigeon pea on these farms was not performing at the level assumed by the models upon which refuges are based.

Pigeon peas' attractiveness at the end of the season could be used by growers to increase the attractiveness of cotton refuges at the end of the season by planting a mixed refuge of cotton with10 % pigeon pea. However this would attract additional costs in terms of planting, weed management and harvesting.

The strong attractiveness of pigeon pea at the end of the season also supports its role as an effective trap crop in the north.

In terms of productivity, there was no clear superior refuge. At individual sites, particularly ACRI, pigeon pea could be the most productive, whereas at other sites it was over-shadowed by cotton. The addition of mungbeans to the cotton appeared to enhance productivity, particularly at the end of the season, but this result was driven by one site (Warrianna 2009/10) where cotton mixed with 10% mungbean was no different to cotton only refuges.

There are no significant differences between the cotton varieties Sicot 71 and 80 in terms of attractiveness and productivity. Sicot 71 may be slightly more attractive at the beginning of the season, and Sicot 80 may be slightly more attractive towards the end of the season. Sicot 80 seems to be more productive than Sicot 71 in terms of moths produced in the mid to late season, using both pupae dig and cage sampling techniques. Which variety will be more productive on any given farm will depend on local conditions. In very low pressure seasons where the refuges are well maintained and watered, Sicot 71 could do better; in higher pressure seasons, particularly with some water stress, the ability of Sicot 80 to set additional fruit after losing the initial crop would be very advantageous. Given that refuges tend to be on poorer soils and may receive less attention than Bt crops, Sicot 80 is probably the best variety for most growers to use.

Late planted cotton was a poor refuge, both in terms of attractiveness and productivity. The one farm where it was particularly attractive at the end of the season (Warrianna 2009/10) resulted in very few moths, presumably because the larvae ate out the crop and were unable to finish development.

These results and others (see Whitehouse et al, appendix 1) indicate that improving refuge management will have a greater effect on refuge efficacy than favouring a particular type of refuge.

Dryland cotton

Attractiveness: Egglays

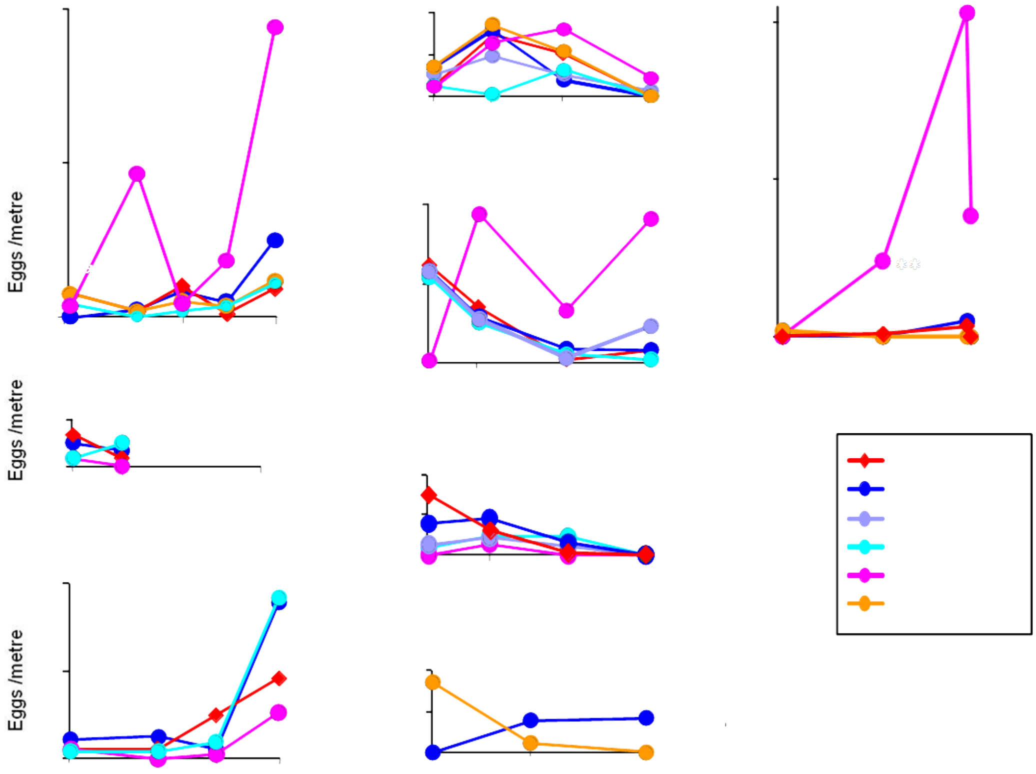


Fig. 12. Attractiveness of dryland refuges in samples taken over a season, as indicated by the eggs per metre laid on each crop. The significant differences between the crops are reported in Table D. The double asterisks indicate a crop that had significantly higher egg lays than the other crops on that sample date, single asterisks indicate a lower level of significance.

In Dryland cotton there was a lot of variability in the attractiveness of crops to moths as oviposition sites, both between seasons and between farms (Fig. 12). Of the eight sites tested, the Dobikin 2009/10 site was ploughed in half way through the season due to poor plant stand, and the Gunedra/Redcamp 09/10 site was opportunistic, and only included two crops. Neither of these two sites showed a significant difference in attractiveness between the refuge crops (Table 4). Of the remaining 6 sites, pigeon pea attracted the most eggs throughout the season at four sites (Table 4) and was particularly attractive at ACRI in the 09/10 and 11/12 seasons; and at Dobikin in the 2010/11 season. At Blue Hills, conventional cotton and Bt cotton were more attractive than pigeon pea over the course of the season.

Within the season, pigeon pea was particularly attractive at the end of the season (Fig. 12: ACRI dryland 90/10, ACRI dryland 11/12, Dobikin 10/11). Bt crops and cotton tended to be more attractive earlier in the season (Fig. 12: Blue Hills 10/11, Dobikin 10/11). Ratoon cotton did well at the beginning of the season at ACRI in the 2010/11 season (Fig 12: ACRI dryland 10/11).

Site	ANOVA	crops					
		Bt	Conv. cot.	10% ppea	10% mungb.	Pigeon pea	Ratoon
ACRI 09/10	P<0.001, F=57, df=4,125 (sqrt trans.)	BC	B		C	A	BC
ACRI 10/11	P<0.001, F=26, df=5,264	AB	AB	B	C	A	
ACRI 11/12	P<0.001, F=105, df=3,80 (sqrt trans.)	B	B			A	B
Dobikin 09/10	N/S	-	-		-	-	
Dobikin 10/11	P<0.001, F=21, df=4,220	B	B	B	B	A	
Blue Hills 09/10	P<0.001, F=8, df=3,80 (sqrt trans.)	A	A		A	B	
Blue Hills 10/11	P<0.001, F=12, df=4,220	A	A	BC	B	C	
Gunedra/ Redcamp 09/10	N/S		-				-

Table 4. Differences between crops in attracting egg lays. The pink shading indicates the crop(s) for that site that were the most attractive; letters or a dash indicate crops tested at that site.

Productivity: Pupae and Moths

At this stage data from the 2011/12 season has not been analysed, so only the 2009/10 and 2010/11 season will be addressed. In addition, the number of pupae and moths retrieved from dryland crops was very low, particularly in the 2010/11 season, and so only limited comparisons can be made between crops.

The only site where there was a significant difference in the number of pupae collected under different refuges was at ACRI in the 2009/10 season (Fig. 13). There was no significant difference between refuges in the number of pupae at any of the other sites.

Productivity was difficult to measure because of the low numbers of moths emerging from the refuges. In the 2010/11 season, only 22 moths were collected throughout the whole season from pupae digs and cages (a total of 372 metres) at all three sites (Fig. 14). There was no significant difference in the number of moths collected from pupae digs or cages or between crop types although there was an effect of sample date, with most moths collected earlier in the season (ANOVA: P=0.018,F=4, df=3,23).

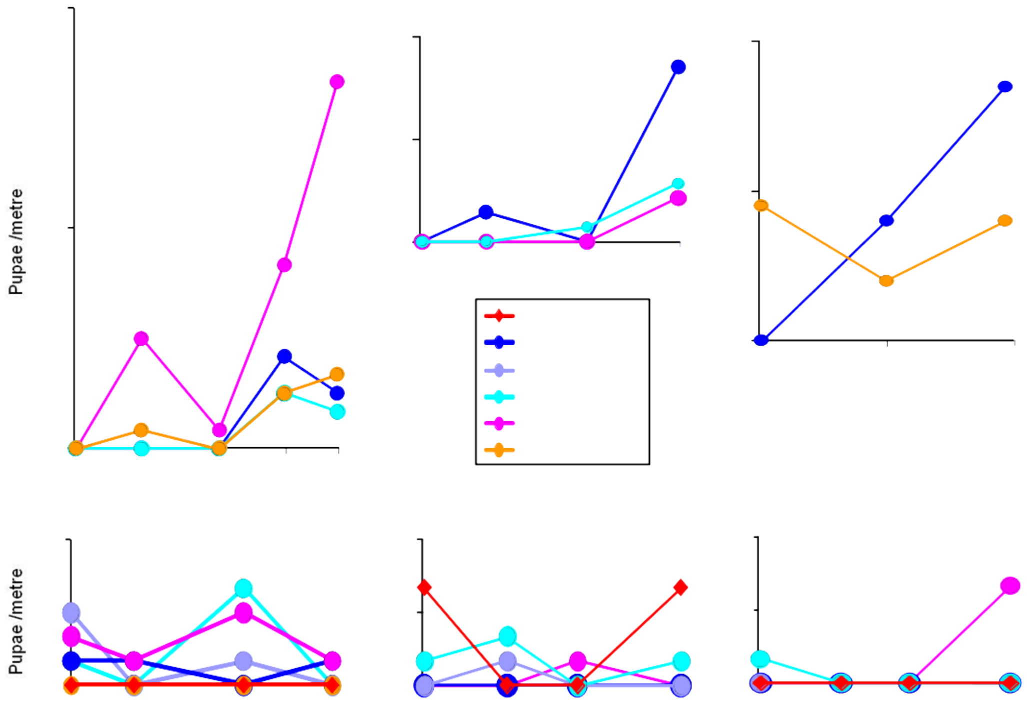


Fig.13. pupae collected from dryland refuges. An asterisk indicates a crop that had significantly higher egg lays than the other crops on that sample date, n= the number of pupae found at that site over the season, The P value indicates if there was an effect of crop type on the number of pupae collected.

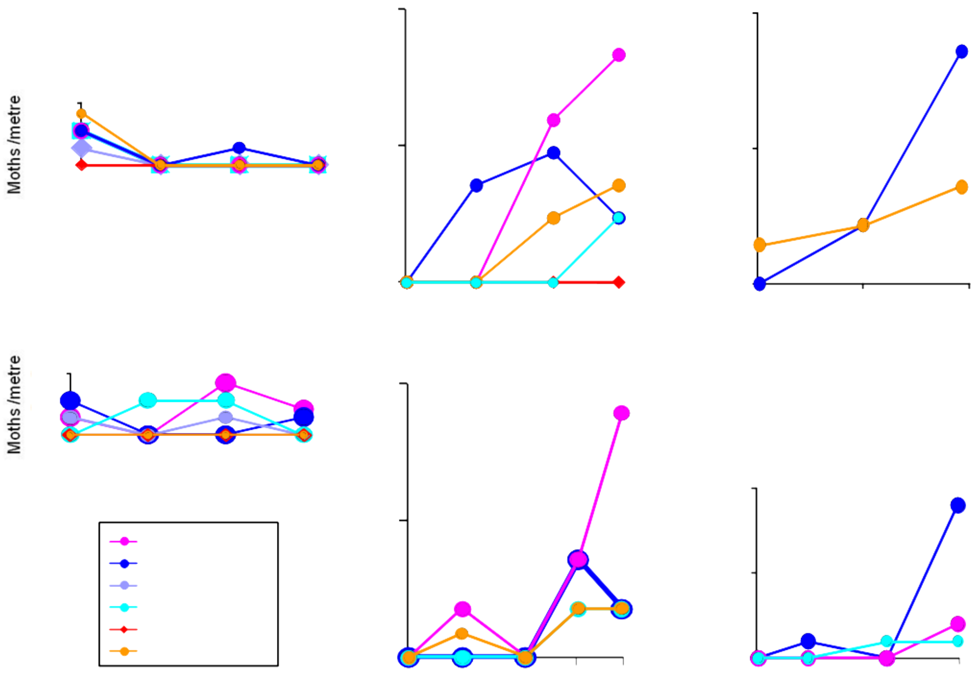


Fig. 14. Productivity as estimated by the number of moths that have emerged from refuges. (Symbols are the same as in Fig. J.).

As with the pupae dig data, the only site where there was a significant difference between crop types in moth production was ACRI in 2009/10 (Fig. 14: CAGES ACRI 09/10, PUPAE DIGS ACRI 09/10). Samples collected using both cages and pupae digs indicate that the pigeon pea crop produced the most moths, particularly in mid to late March. The cage samples indicated that the cotton refuge produced more moths in mid February. Ratoon cotton, or cotton with either pigeon pea or mungbean imbedded in the crop, never produced significantly more moths than other refuge types.

Concluding remarks on dryland refuges

Pigeon pea refuges in dryland cotton have the potential to be very attractive, particularly in high rainfall years, but they are not consistent. They may be farm specific, as they seemed to do particularly well at ACRI, and particularly poorly at Blue Hills. In terms of productivity, again they can be very productive, particularly at the end of the season, but not consistently so. These results suggest that dryland pigeon pea could be used as a refuge in dryland cotton, but not with a reduction in refuge area, because it is unreliable.

At the three sites where it was tested, ratoon cotton was as attractive, or significantly more attractive (at the beginning of the season) than cotton refuges. There was no significant difference in productivity overall between ratoon and cotton refuges, although at ACRI 90/10 cotton refuges produced significantly more moths in mid February than ratoon refuges. As there is little difference between ratoon and cotton refuges, ratoon cotton could be used as a 10% refuge to attract the egglays of early emerging moths. This could be of benefit to the grower as it would avoid the cost of re-planting refuges, and may benefit soil nutrients (see later section). However, this conclusion is largely based on one location (ACRI) and more studies are needed as any benefits would have to be balanced with the potential cost of being a nursery for cotton diseases (the ACRI crop became affected with bungy top in 2010/11) and the cost of weed maintenance, particularly at the end of winter.

The addition of mungbean and pigeon pea to cotton refuges did not provide any advantages or disadvantages in terms of attractiveness or productivity in dryland cotton.

Comparing pupae digs to cages as a sampling unit

All sampling methods have their pros and cons, and pupae digs and cages are no exception. Pupae digs have the advantage that they no only provide moths, but pupae as a unit to measure productivity. This is particularly useful when Helicoverpa pressure is very low. However, it can be a biased measurement, dependant on the ease at which pupae can be removed from the soil (soil cannot be too wet or too dry) and may be affected by the effect of different crop types on soil malleability.

In addition, the period of Helicoverpa activity sampled by pupae digs varies. Helicoverpa stay as pupae for two weeks, unless they are parasitized by wasps, at which point they remain in the ground for three weeks. This means that parasitized pupae have a higher chance of being sampled by pupae digs than non-parasitized pupae. As the season progressed pupae seemed to accumulate in the soil (Fig. 11) as inactive pupae did not emerge, and pupae went into diapause for winter. This gives an inflated view of refuges' productivity at the end of the season.

Removing pupae through pupae digs effectively increases their survival by removing the threat of predation and parasitism. Removing pupae may also protect parasitoids from hyper parasitism. Examination of the by-catch from cages (a full analysis of this is not yet completed) revealed lower than expected levels of parasitoids, and higher than expected levels of hyperparasitism, suggesting the hyperparasitism may be more common than indicated from pupae digs.

Cages have the advantage of being all weather, uniform samplers, but they are time consuming, and need to be checked regularly (at least once a week). Cages are dependant on being set up correctly.

Cages sampled similar numbers of moths if not more moths than pupae digs, indicating that they are as effective as pupae digs in sampling moths. One large advantage was that they were able to sample moths emerging from Bt cotton. This was not achieved using pupae digs. Cages also showed higher moth counts at the beginning of the season than pupae digs and indicated that emergences from cotton refuges were higher than those indicated by pupae digs. Cages have demonstrated that they are a useful tool in measuring refuge productivity.

Do refuges match Bt cotton in attractiveness throughout the season

The aim of Objective one is to establish if different refuge crops can improve refuge efficacy and therefore be more effective at countering any resistant moths emerging from Bt crops. According to the RMP, refuge crops must be planted within 2 km of Bt crops, even though Helicoverpa moths can travel large distances, to ensure maximum likelihood that moths emerging from both crops will mate, and that gravid females have a reasonable chance of encountering attractive refuges. Therefore a conservative approach to refuge management is to ensure that refuges are at least as attractive as Bt cotton within 2 km. As resistant moths could emerge at any stage during the season, refuge efficacy needs to be consistent throughout the season. As Pigeon pea is expected to be twice as attractive as Bt cotton it needs to maintain this level throughout the season. Thus the first step is to establish if refuge attractiveness is correlated to, or greater than, the attractiveness of Bt cotton.

In Irrigated cotton, the attractiveness of Bt cotton was only correlated with that of non-Bt cotton (Fig. 15: se graphs “ Bt vs non-Bt cotton).). In the 2009/10 season, this correlation was ideal, whereas in the 2010/11 season, the non-Bt cotton was attracting about half the egglays as the Bt crop, and therefore was performing well below expectation.

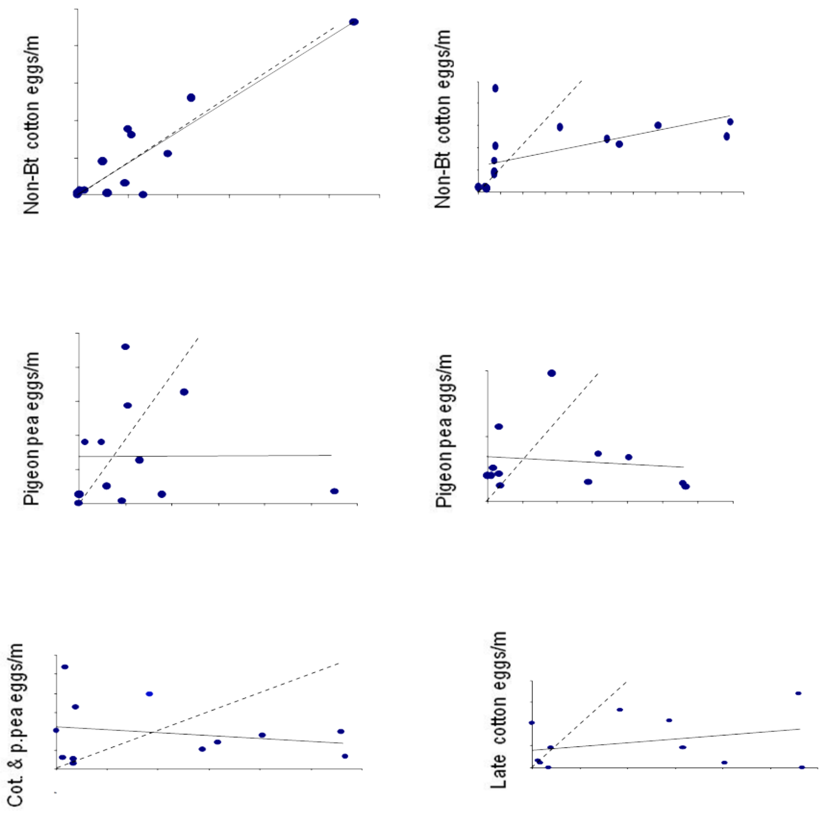


Fig. 15. Correlations between egg lays in Bt cotton and associated refuges in Irrigated cotton. The dashed line indicates the expected relationship, while the solid line indicates the actual relationship.

There was no correlation between Pigeon pea and Bt cotton in irrigated crops, which is not surprising given that pigeon pea's ontogeny is quite different from that of cotton. Nevertheless, pigeon pea is expected to be twice as attractive as cotton (as indicated by the expected relationship line in Fig. 15 and 16 in the graphs "Bt vs Pigeon pea"). Therefore, for pigeon pea to be consistently performing as expected, most data points in Fig. 15 should be above the expected relationship line or close to it. In the 2010/11 season in particular, a large number of points were well below the expected line, indicating that coverage by this refuge could have been compromised on those sampling days. Both cotton with 10% pigeon pea (Fig. 15 :Graph "Bt vs Cotton & Pigeon pea") and late planted cotton (Fig. 15. Graph "Bt vs Late cotton") have many data points below the expected relationship line, indicating that they are not as attractive as Bt cotton.

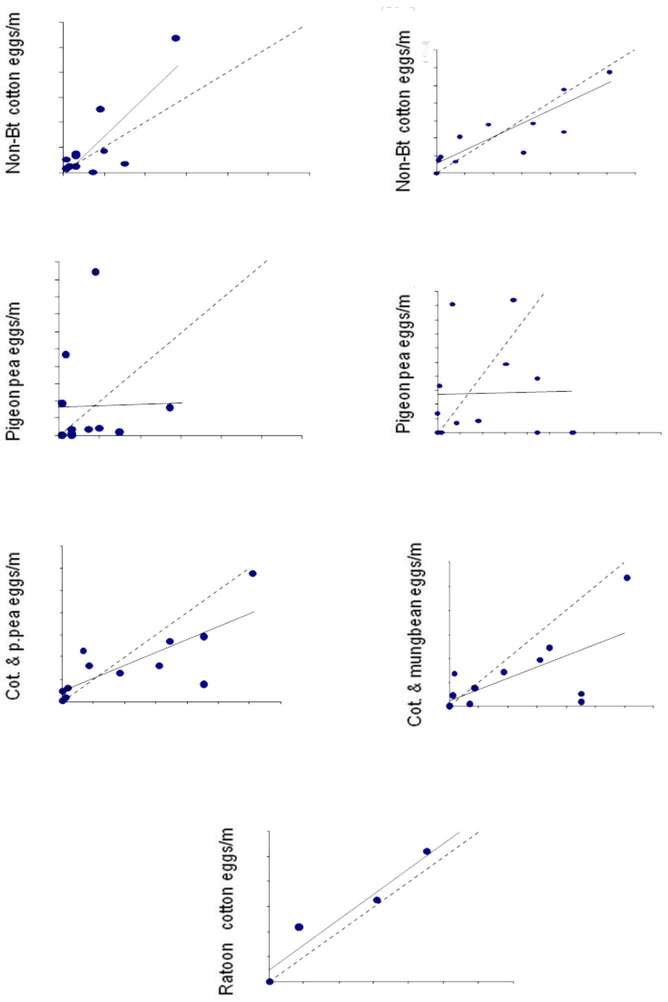


Fig.16. Correlations between egg lays in Bt cotton and associated refuges in Dryland cotton. The dashed line indicates the expected relationship, while the solid line indicates the actual relationship.

In dryland cotton, only pigeon pea was not correlated with Bt cotton (Fig.M. Graphs "Bt vs pigeon pea"). In pigeon pea during both seasons there were a number of samples where the data points were well below the relationship line (following the irrigated assumption that pigeon pea is twice as attractive as cotton). In contrast, in the 2009/10 season, the actual relationship line for non-Bt cotton was above the expected line (although not significantly so; Fig. 16: Graph "Bt vs non-Bt cotton (2009/10)"). In the 2010/11 season non-Bt cotton again matched Bt cotton in its attractiveness to Helicoverpa moths (Fig. 16:

Graph Bt vs non-Bt cotton (2010/11)). Both cotton with 10% mungbean, and cotton with 10% pigeon pea correlated with Bt cotton for attractiveness, but overall were slightly less attractive. Ratoon cotton was as attractive as Bt cotton (Fig. 16: Graph: “Bt vs Ratoon cotton”) but this correlation is based on only three data points..

Concluding remarks on refuge attractiveness

Correlating the eggs lays of Bt cotton and its refuge reveals whether the refuge is performing as expected by the models. Where the curve of the actual relationship falls below that of the expected relationship, the refuge is underperforming and, from a conservative perspective, its efficacy may be compromised. Such refuges, including cotton + 10% pigeon pea, cotton + 10% mungbean, and late cotton may be underperforming in terms of attractiveness and their usefulness as refuges queried. In some cases non-Bt cotton underperformed. This is a concern as it is assumed to be as attractive as Bt cotton. Management factors need to be addressed to ensure that it matches the attractiveness of Bt cotton.

Pigeon pea attractiveness does not correlate well with that of Bt cotton. This is not a problem if its attractiveness is not too much less than its predicted value. However, in this study often it was much less attractive than Bt cotton, particularly in dryland cotton. When pigeon pea has these low levels of attractiveness, its ability to counter resistant moths emerging from the Bt crop could be compromised. To overcome this problem the cotton industry needs to decide how much pigeon pea can deviate below its expected attractiveness (at any time throughout the season) before it compromises its role as a refuge; and how pigeon pea refuges can be improved so that their attractiveness does not drop so low.

Can attractiveness be correlated with productivity?

Refuge productivity is assumed to match that of Bt cotton, (if the Bt cotton did not have Bt toxin). This assumption is extremely difficult to test directly. If productivity could be correlated with attractiveness, then this could be a way of estimating potential productivity of Bt crops. Therefore we tested whether it was possible to correlate attractiveness with productivity and whether there were periods during the season when this correlation was stronger. In presenting the results we will focus on the relationship between egg lays and subsequent pupae or moth counts in irrigated cotton.

Emerald 2009/10.

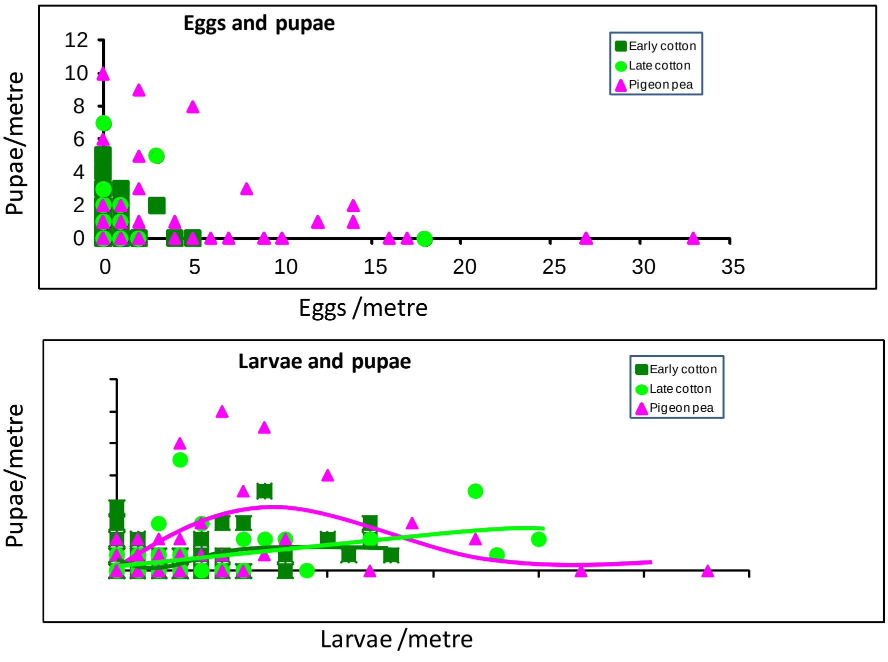


Fig. 17. Relationship between eggs and pupae, and larvae and pupae at Emerald. There was no correlation between eggs laid in a sample and pupae collected from that sample, but there was a correlation between larvae and pupae.

While there was no correlation between eggs and pupae numbers (Regression analysis: P=0.86, df=232, Fig 17: Graph “Eggs and pupae”) there was a correlation between larvae and pupae numbers at Emerald (Regression analysis: P<0.001, df=232, accounting for 10.4% of the variance). Further analysis revealed that a non-linear regression, fitting gausian curves to the different crop types, improved the fit -particularly on transformed data (nonlinear regression: P<0.001, df=3,230; 22.7% of variance explained). In addition the relationship between larvae and pupae numbers differed between the three crops (Fig. 17: Graph “Larvae and pupae”) with pigeon pea pupae numbers peaking at about 8 larvae/m, while the cotton pupae numbers were not as high put had not reached a peak. These differences reflect that cotton and pigeon pea are different crops, and so Helicoverpa population dynamics will differ between the two crops.

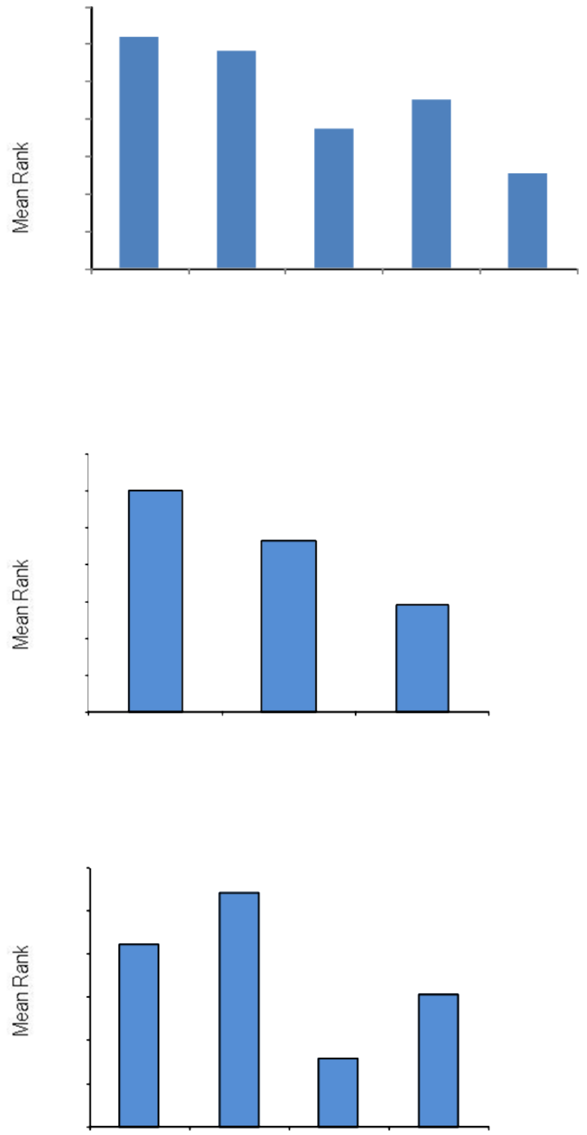


Fig. 18. The relationship between egg and pupae numbers. The higher the rank, the more eggs to pupae, the lower the rank, the more pupae to eggs. At all locations the ranks are higher at the beginning of the season.

As Helicoverpa populations responded differently to pigeon pea and cotton, only early planted cotton data was analysed for an effect of season on the relative number of eggs to pupae. To compare between sample dates, the number of pupae found in a metre was subtracted from the number of eggs, resulting in a positive or negative number. These numbers were compared in a Kruskall Wallis non- parametric analysis of variance which indicated that as the season progressed, number of eggs to pupae dropped (Fig.18; H=24, df=4, P<0.001, n=90).

Namoi Valley 2009/10.

In the Namoi valley, correlations between egg and pupae numbers were undertaken at ACRI and Warrianna in the 2009/10 season. Although there was a strong correlation between larvae and pupae at ACRI, (regression analysis; P,0.001; df=1,91; % explained =31.3) there was no correlation between the number of eggs produced and the number of pupae collected (regression analysis; P=0.46; df=1,91; % explained=3.2). As is clear from Figs. 19& 20, samples in cotton crops either had only eggs, or only pupae. Pigeon pea samples were a distinctive group.

Alternatively, at Warrianna, there was a significant effect of egg number on the number of pupae, and this relationship was influenced by crop type (Fig 19; regression analysis; P<0.001; df=4,72; % explained=43.8). Mungbean mix is not significantly different from early cotton, late planted cotton shows a trend to be significantly different (P=0.053) while the relationship between eggs and pupae in early cotton is significantly different from that in Pigeon pea (P<0.001). Thus there was a stronger positive ratio between the number of eggs laid and the resulting pupae in early cotton and Mungbean than in pigeon pea and late cotton.

The effect of seasonal variation on the correlation between eggs and pupae in cotton is clear at both ACRI and Warrianna, although only at Warrianna is there a correlation between eggs and pupae in the third sample, taken at 1494 Degree days (Fig. 20). As at Emerald, samples in cotton taken early in the season have more eggs to pupae, while those at the end of the season have relatively more pupae to eggs (Fig. 18.).

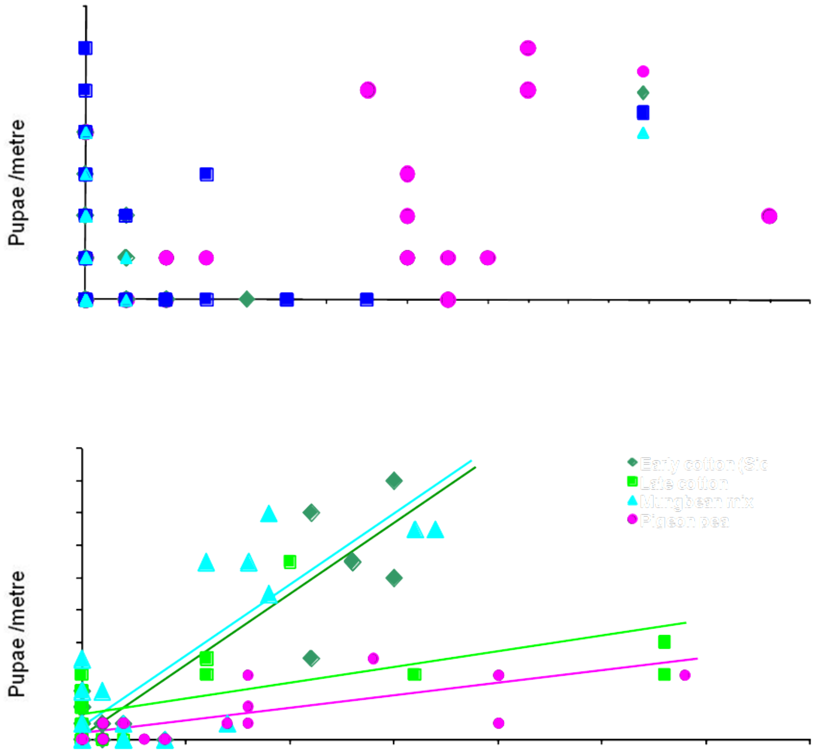


Fig. 19. The relationship between eggs and pupae numbers at ACRI and Warrianna empathizing crop type. There was no correlation between eggs and pupae at ACRI while there was a correlation at Warrianna

Namoi Valley 2010/11.

In the 2010/11 season, Helicoverpa counts were low. Again there was no correlation between egg laid and moths, but there was an effect of sample, with samples on the 18th of Jan (which was equivalent to about 1050 Degree Days) showing a positive relationship between egg lays and moths captured in cages, but not from moth captured using pupae digs (Fig. 21: Graph “Moths from cages”).

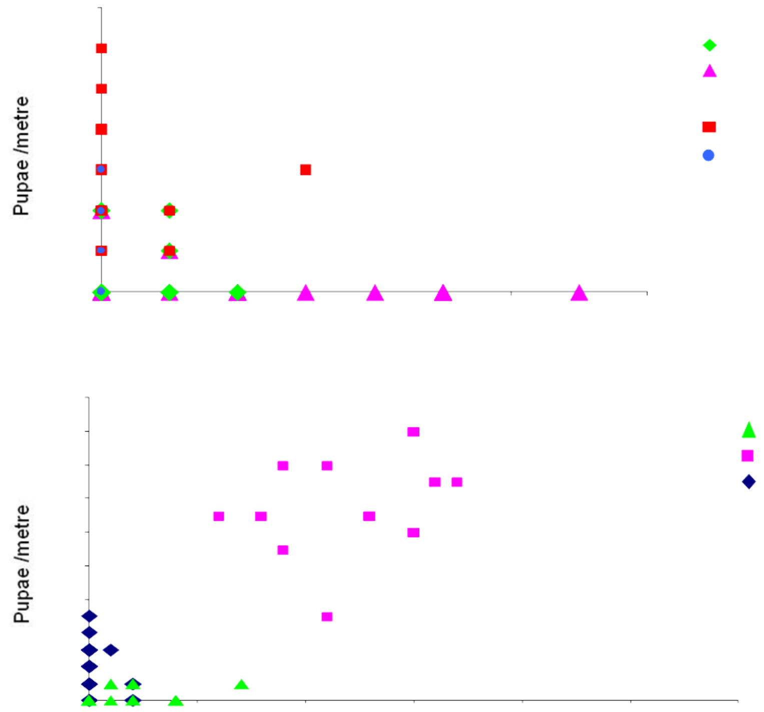


Fig. 20. The effect of sample date on the correlation between eggs and pupae at ACRI and Warrianna in cotton refuges only during the 2009/10 season.. The ANOVA indicates differences in the relationship between eggs and pupae at the different sample dates. At Warrianna only Sample 3 (taken at about 1500 Degree days) is driving the correlation between pupae and eggs.

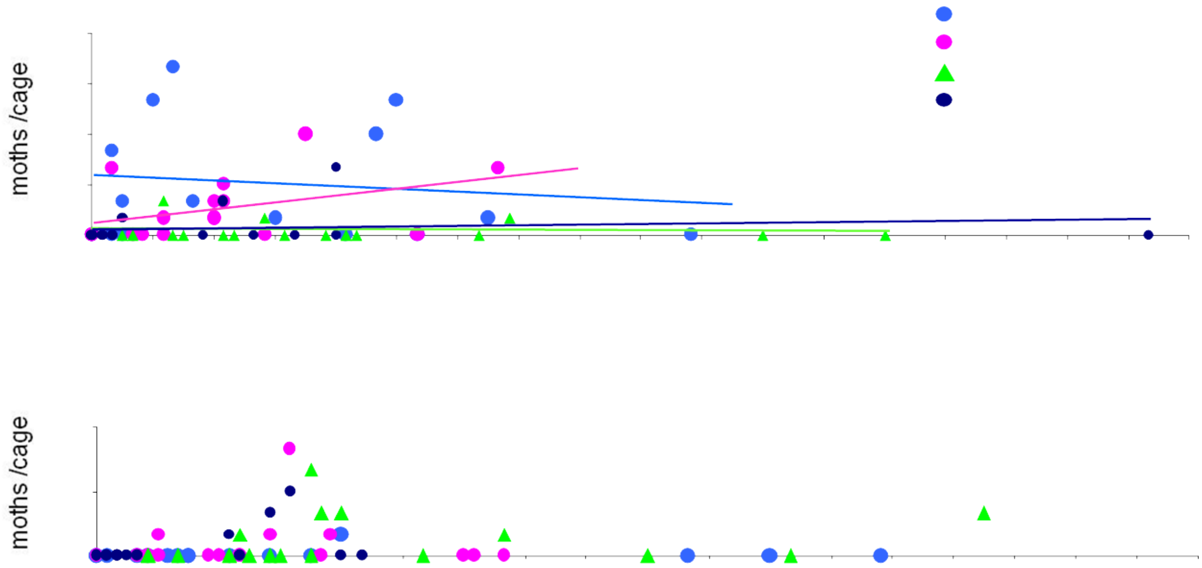


Fig. 21. The relationship between egg and moth numbers at irrigated sites (ACRI, Warrianna and Gunedra) in the 2010/11 season. Only “sample date” influenced the number of moths emerging from cages, and only Sample 2 (18th January) showed a positive correlation between egg lays and moths collected using cages. There was no significant effect of crop, farm or sample date on the number of moths collected using pupae digs.

Concluding remarks on correlating attractiveness with productivity

There is a consistent pattern of egg and pupae numbers throughout the season. Early in the season eggs but not their pupae are found, while late in the season pupae are found that do not correspond to any egg lays. The only time when eggs can be matched to pupae is when the eggs are laid in late January (in the Namoi Valley). Only at this time may it be possible to directly correlate attractiveness to productivity. This possible window of opportunity does not seem to be driven by Degree days, and requires further testing for its reliability.

Most of this analysis has been undertaken in low pressure years. It is likely that the low egg counts in the study were not able to overcome the noise from sampling and survivorship differences between samples. The effect of noise on the correlation between attractiveness and productivity will be further explored once the results of the 2011/12 season have been analysed.

These results demonstrate that at this stage it is not possible to estimate the Helicoverpa productivity of a Bt crop based on its egg lays. Instead, the comparative Helicoverpa productivity of refuges and their Bt crops will need to be assessed using agronomic factors known to affect Helicoverpa survival, such as levels of nitrogen and water content in the soil of each crop.

G. Baker & C. Tann

2009/10 : Split-planting Trials at Gunedra and Neilo

Figs 22 & 23 illustrate the egg, larval and pupal densities on and below the various crops at Gunedra and Neilo. Unlike at Gunedra in 2008-09, there was no clear evidence at these two sites in 2009-10 that split-planting of unsprayed, conventional cotton as a refuge enabled an extension of availability of moth emergences. As noted earlier, the 2nd plantings of the cotton at both sites were damaged by tip worm which would have reduced any advantages these later crops might have otherwise provided. Most notably, the pigeon pea crops at Gunedra and Neilo preformed as well, if not better than, the cotton refuges in terms of production of *Helicoverpa*, especially late in the season.

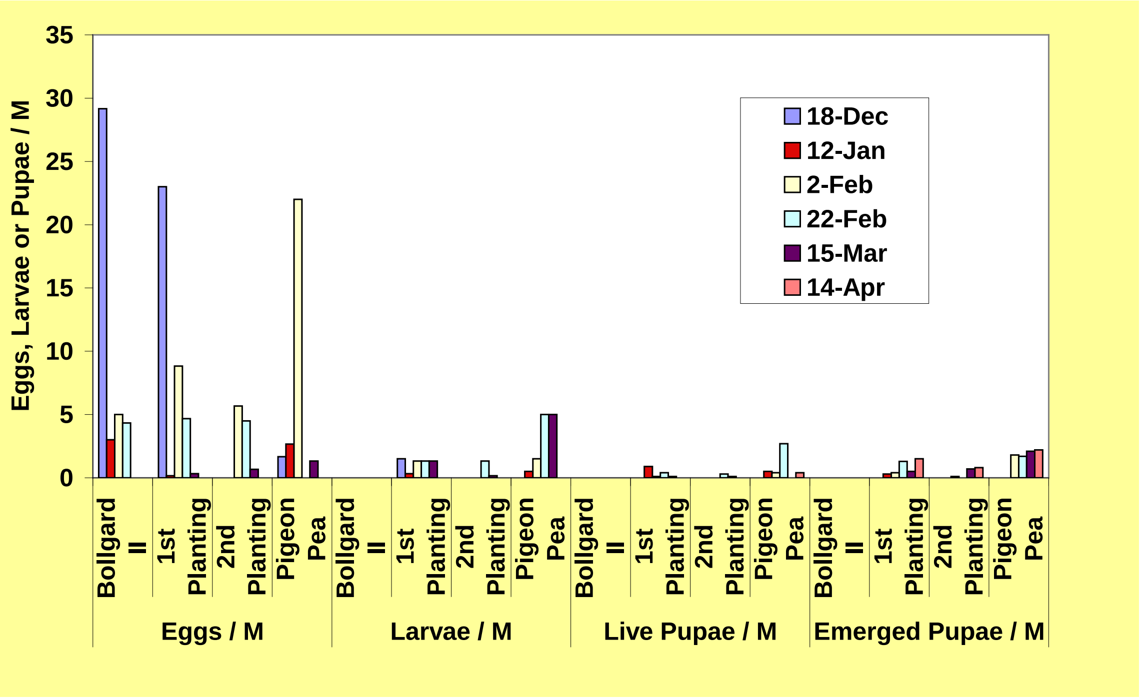


Fig. 22. Abundance of eggs, larvae, live pupae and emerged pupae of *Helicoverpa* on various sampling occasions of Bollgard II cotton, an early planting of unsprayed, conventional cotton, a later planting of unsprayed, conventional cotton, and pigeon pea, at Gunedra (Namoi Valley, 2009-10 season).

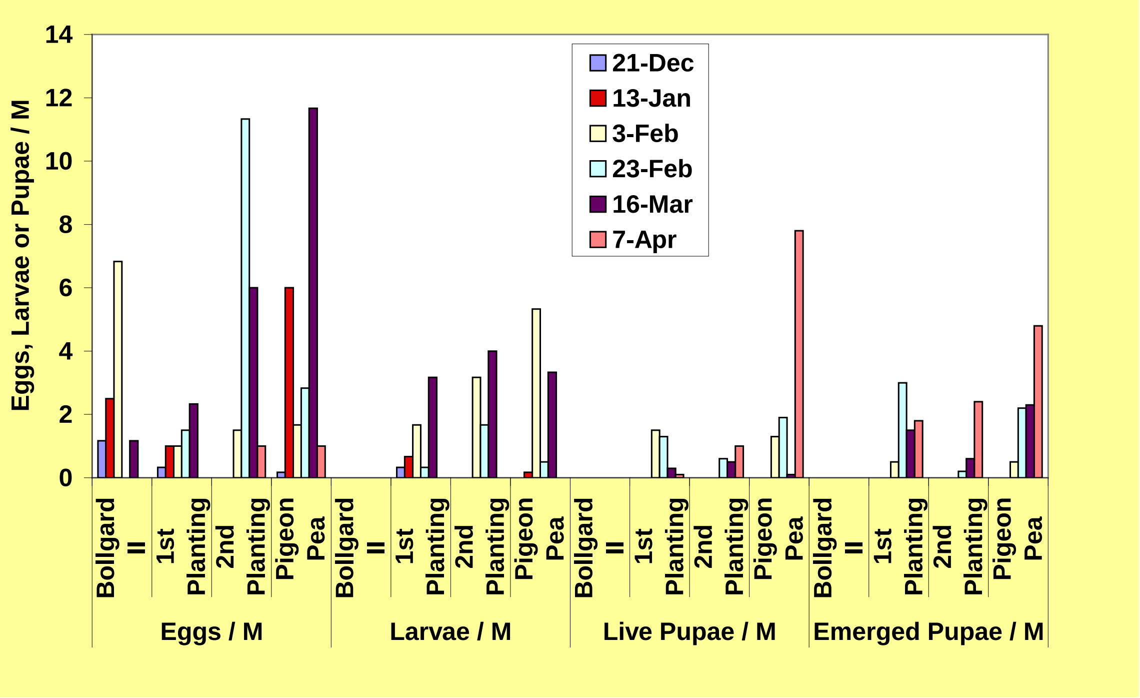


Fig. 23. Abundance of eggs, larvae, live pupae and emerged pupae of *Helicoverpa* on various sampling occasions of Bollgard II cotton, an early planting of unsprayed, conventional cotton, a later planting of unsprayed, conventional cotton, and pigeon pea, at Neilo (Macintyre Valley, 2009-10 season).

2010/11 : Taraba, Gunedra, and CSD (Little Mollee)

There appeared to be little advantage re split plantings of cotton refuge at Taraba in terms of extending productivity of *Helicoverpa* (Figs 24-26). Egg and larval counts were erratic between the two plantings of cotton, and pupae numbers were generally lower in the later plantings compared with the earlier plantings at the same sampling times.

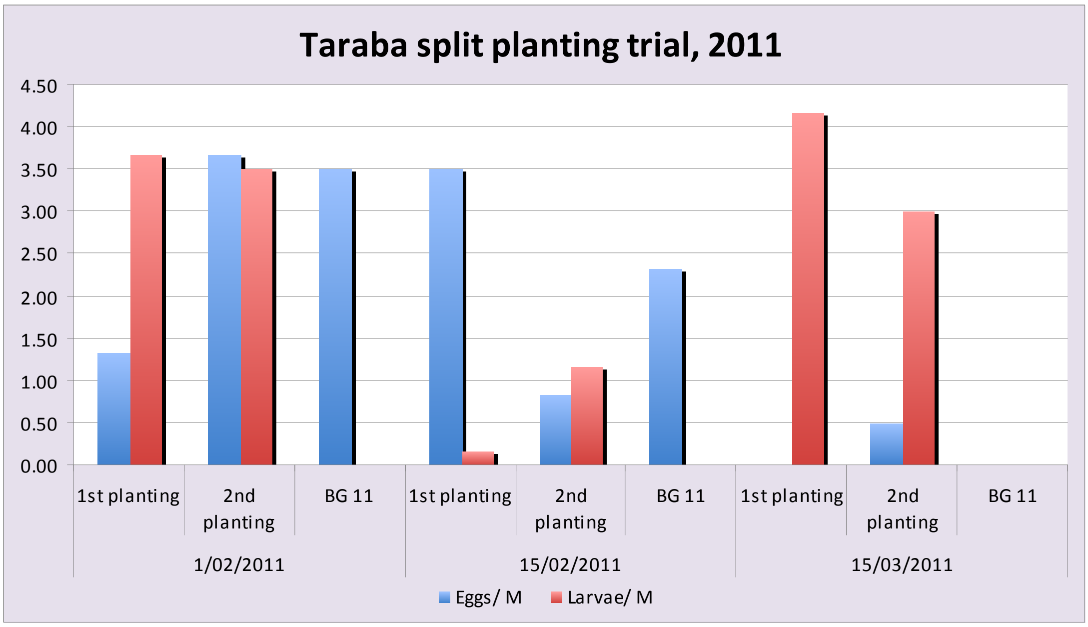


Fig. 24. Eggs and larvae of *Helicoverpa* spp. / m of cotton refuge crop row at Taraba, at different times during early 2011.

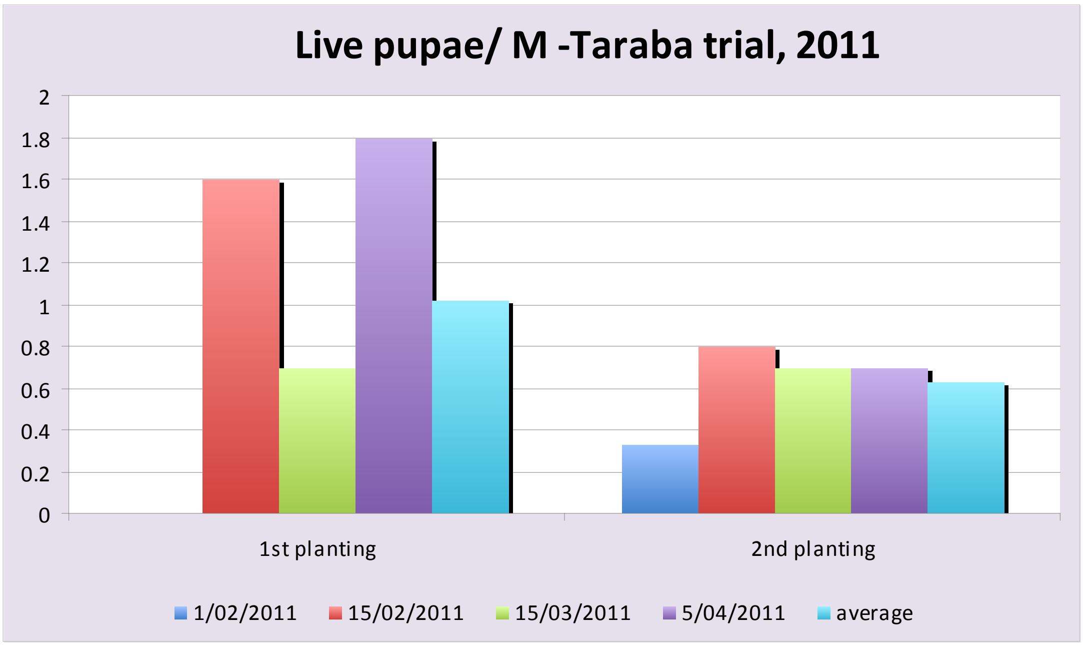


Fig. 25. Live pupae of *Helicoverpa* spp. / sq. m beneath cotton refuge crop at Taraba, at different times during early 2011.

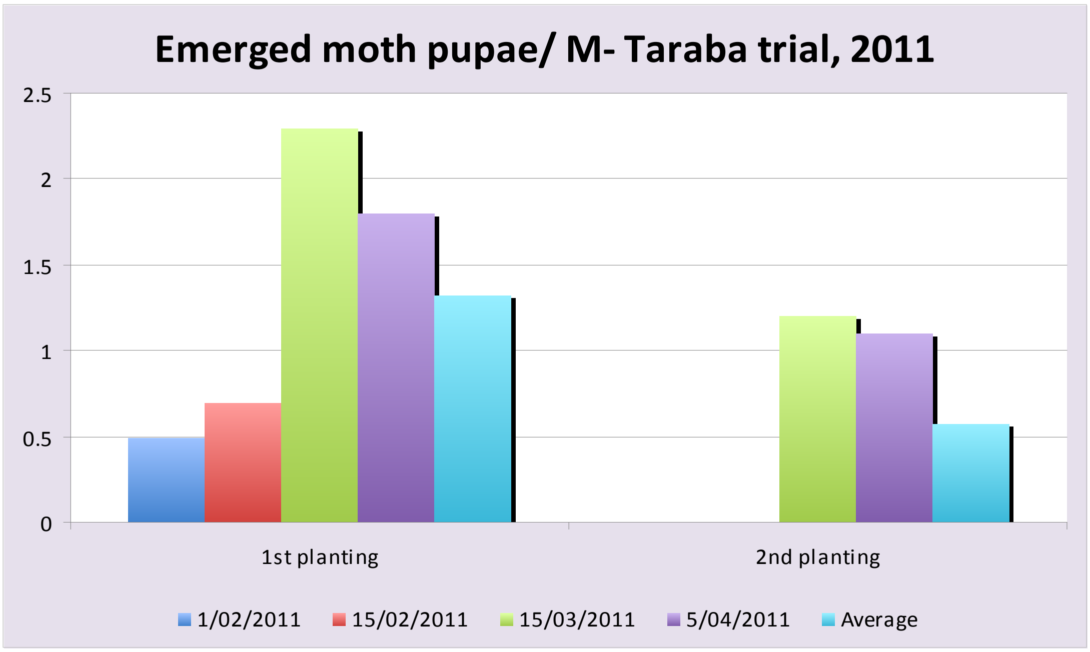


Fig. 26. Emerged pupae of *Helicoverpa* spp. / sq. m beneath cotton refuge crop at Taraba, at different times during early 2011.

Early tipping out of the 2nd planting of conventional cotton at Gunedra again impeded growth and attractiveness at key times. The plants within this trial were well watered but appeared to lack adequate nutrition. Plant height and lushness were poor compared with surrounding Bollgard II crops. Pigeon pea performed well compared with the cotton crops, both in terms of egg lays and pupae production, but there was no evidence that the later planting of cotton added any value to *Helicoverpa* production (Figs 27-29).

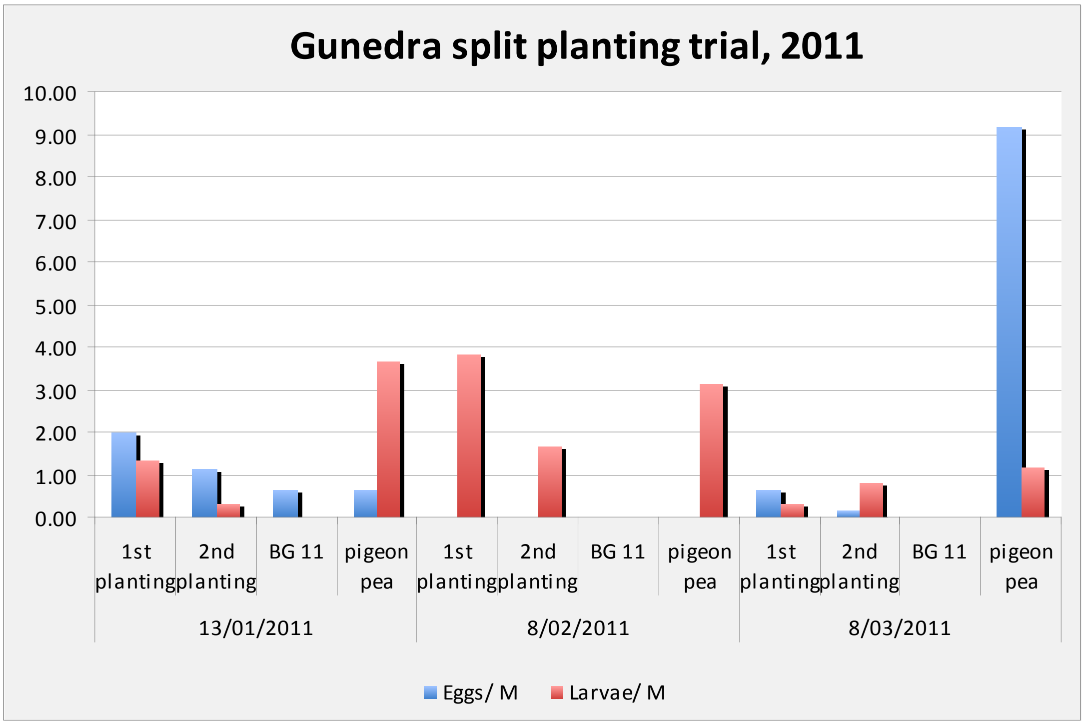


Fig. 27 Eggs and larvae of *Helicoverpa* spp. / m of refuge crop row at Gunedra, at different times during early 2011.

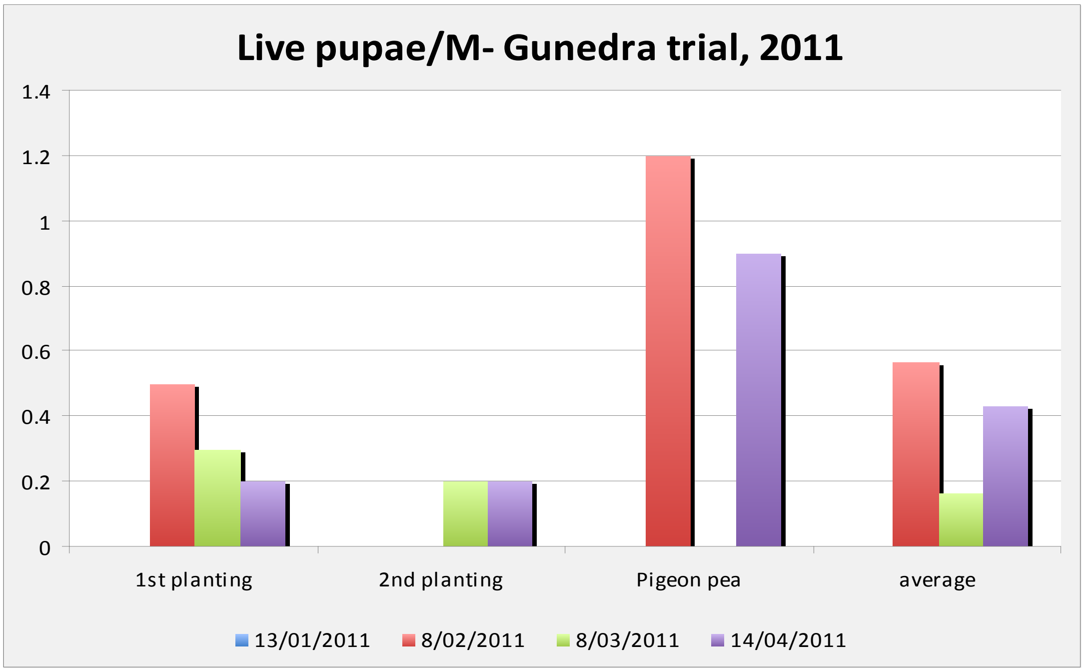


Fig. 28. Live pupae of *Helicoverpa* spp. / sq. m beneath refuge crops at Gunedra, at different times during early 2011.

This work, together with earlier trials (this and the previous project), convinced us that split planting to enhance cotton refuges is not reliable enough to be persuasive as a refuge crop option. Significant damage from pests such as stem borers and cotton loopers frequently depressed any benefit that might otherwise have accrued from 2nd plantings of unsprayed conventional cotton.

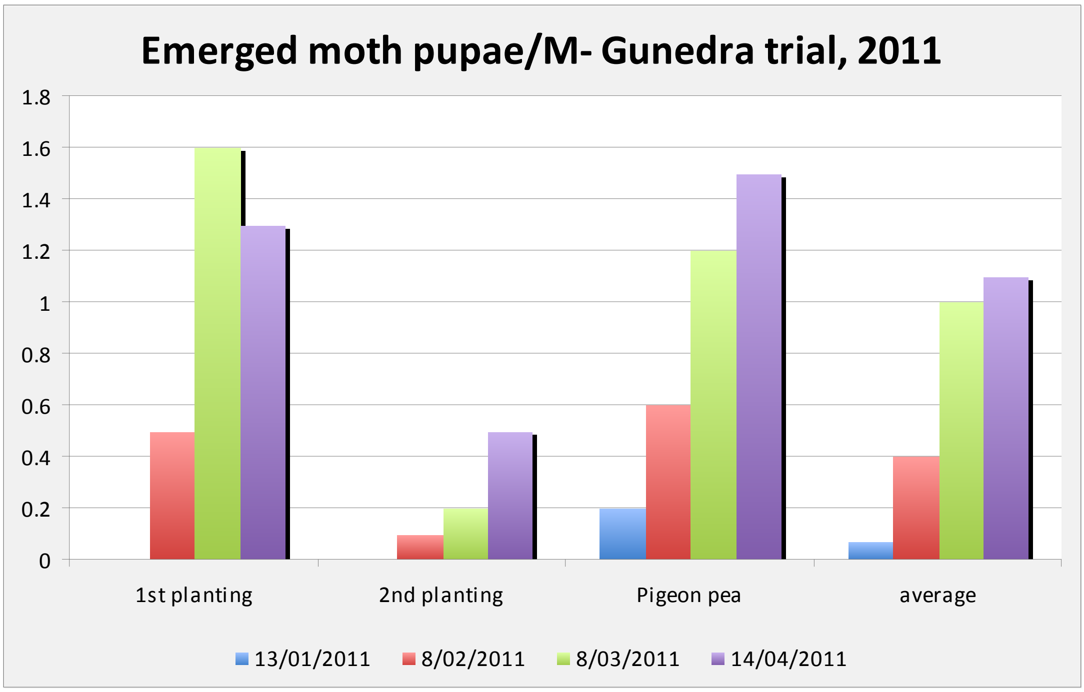


Fig. 29. Emerged pupae of *Helicoverpa* spp. / sq. m beneath refuge crops at Gunedra, at different times during early 2011.

The Sicot80 matured slightly earlier than Sicot 71 at Little Mollee. This may have been simply related to its earlier planting date. But we found no significant difference in attractiveness of the two varieties of conventional cotton to oviposition by *Helicoverpa*, nor did we demonstrate a difference in pupal abundance (a surrogate for moth recruitment) (Figs 30-32). The mix of Sicot 71 and Sicot 43 showed a slight advantage in attractiveness late in the season, but pupal production beneath it was relatively poor.

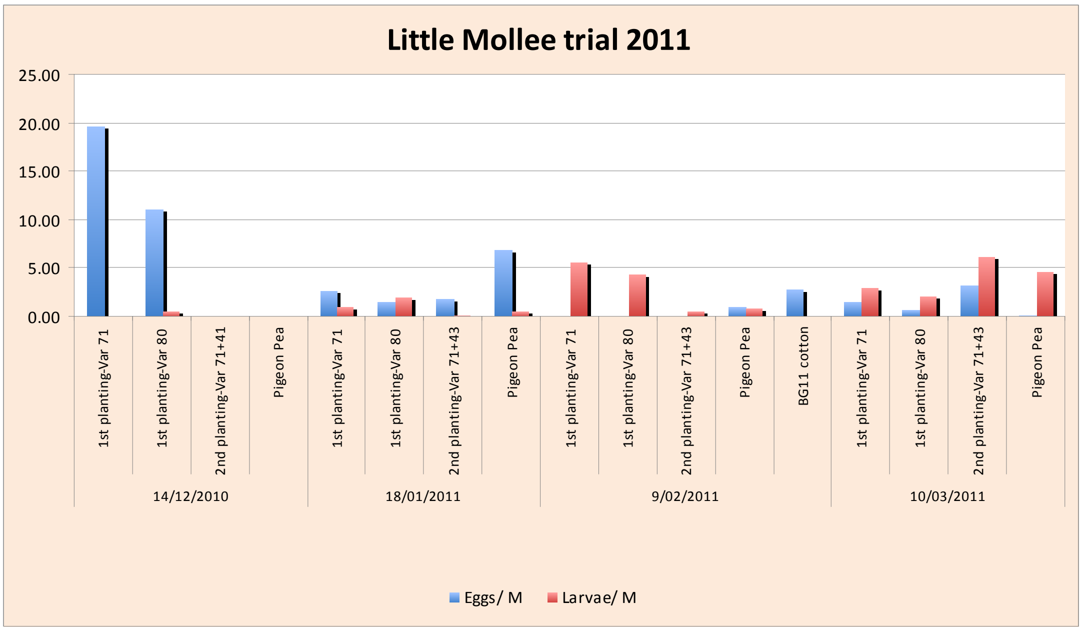


Fig. 30. Eggs and larvae of *Helicoverpa* spp. / m of refuge crop row at Little Mollee, at different times during late 2010 & early 2011.

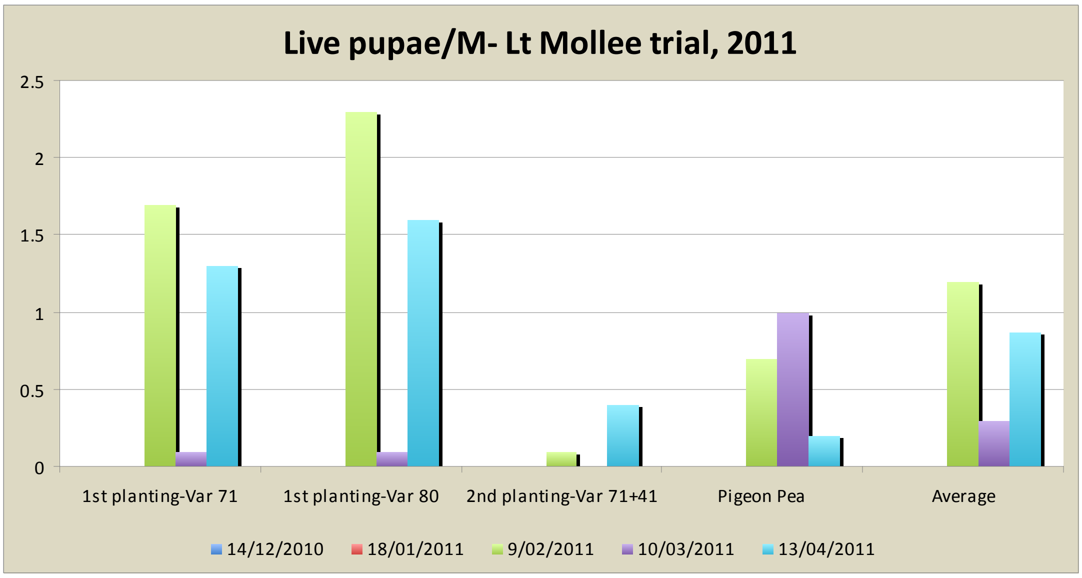


Fig. 31. Live pupae of *Helicoverpa* spp. / sq. m beneath refuge crops at Little Mollee, at different times during late 2010 & early 2011.

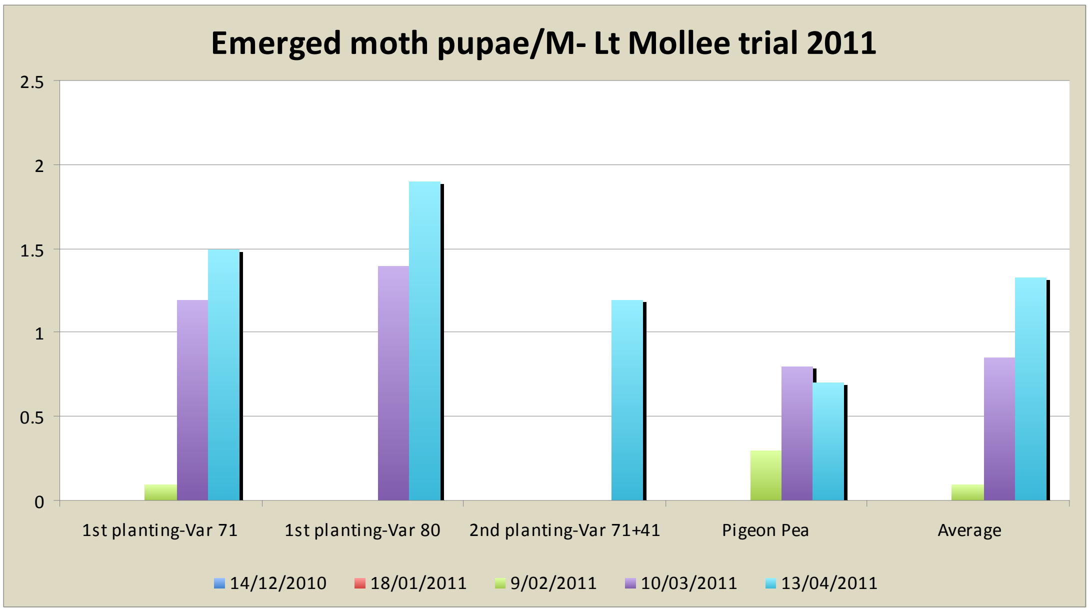


Fig. 32. Emerged pupae of *Helicoverpa* spp. / sq. m beneath refuge crops at Little Mollee, at different times during late 2010 & early 2011.

In comparison with the conventional cotton at this trial, pigeon pea performed moderately throughout the season in terms of *Helicoverpa* pupae production. The lushness of the plants was never good, and this probably reflected in the data obtained. The pigeon pea tended to take longer to re-flower than normally would be the case with more water than the crop received. We suspect the crop suffered by being at the edge of a field where water was not abundant.

2011/12 : Brookstead, Darling Downs

Overall, *Helicoverpa* pressure at Brookstead in 2011-12 was light. NPV (virus) incidence was high and limited *Helicoverpa* survival in early season, as did other natural enemies. Plants were tipped out early by tip worm and *Helicoverpa* and growth was thus impeded.

This damage tended to diminish expected differences between the cotton varieties. The trial site was generally well managed by the farmer, but notably the Bollgard II cotton crop adjacent to the experimental plots received more irrigations.

Unfortunately, there was no marked difference in the development of the mixed cotton crop compared with the individual varieties (Fig. 33). Sicot 43 was shorter than Sicot 80, as was to be expected. There was also no obvious difference in the abundance of eggs and larvae of *Helicoverpa* across the different cotton treatments, but egg and larval loads were higher on the pigeon pea crop, albeit that this crop was at a distance from the trial site (Fig. 34). Similarly, pupae numbers (and associated parasites) as measured in the field showed no treatment effects (Fig. 35). [Note : laboratory rearing of parasites from live and recognisably parasitised pupae is still in progress at time of writing]. Pupae were more common under the pigeon pea crop, in particular late in the season (Fig. 36).

This study thus provided no evidence that mixed varieties of unsprayed conventional cotton might improve the status of cotton as a refuge, but we acknowledge that the 2011-12 season was fraught with low insect pressure and additional issues such as tipping out early in the season limited the utility of the trial.

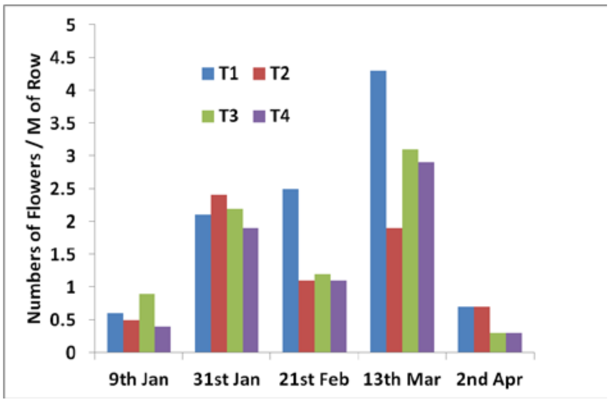


Fig. 33a. Average numbers of flowers per M of crop row of unsprayed conventional cotton within the trial near Brookstead, Darling Downs in 2011-12. T1 = treatment with Sicot 71, T2 = Sicot 80, T3 = Sicot 43, and T4 = equal mix of Sicot 80 & 43.

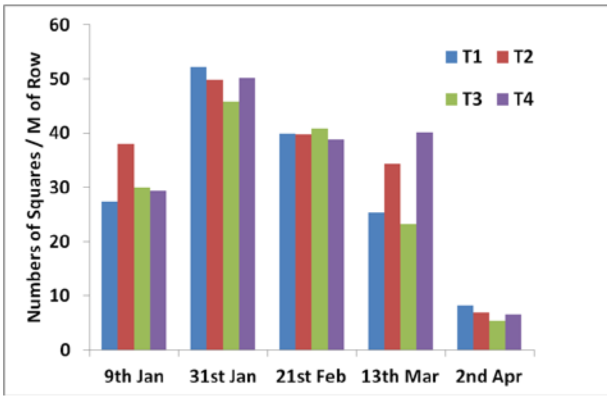


Fig.33b. Average numbers of squares per M of crop row of unsprayed conventional cotton within the trial near Brookstead, Darling Downs in 2011-12. T1 = treatment with Sicot 71, T2 = Sicot 80, T3 = Sicot 43, and T4 = equal mix of Sicot 80 & 43.

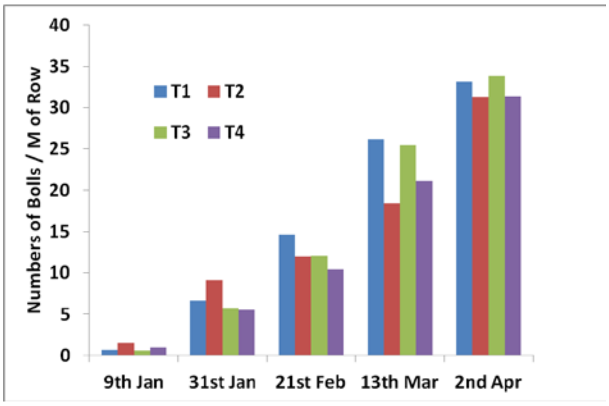


Fig. 33c. Average numbers of bolls per M of crop row of unsprayed conventional cotton within the trial near Brookstead, Darling Downs in 2011-12. T1 = treatment with Sicot 71, T2 = Sicot 80, T3 = Sicot 43, and T4 = equal mix of Sicot 80 & 43.

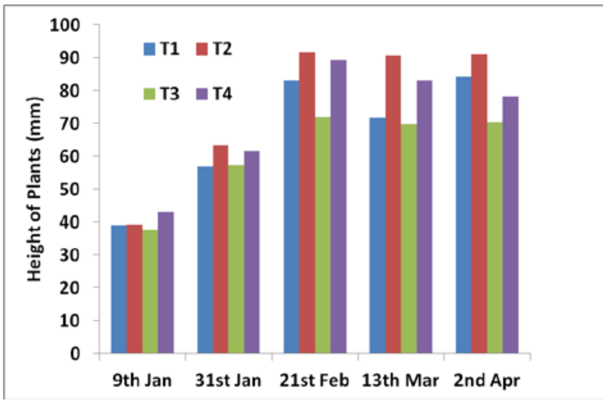


Fig. 33d. Average height of plants within the trial near Brookstead, Darling Downs in 2011-12. T1 = treatment with Sicot 71, T2 = Sicot 80, T3 = Sicot 43, and T4 = equal mix of Sicot 80 & 43.

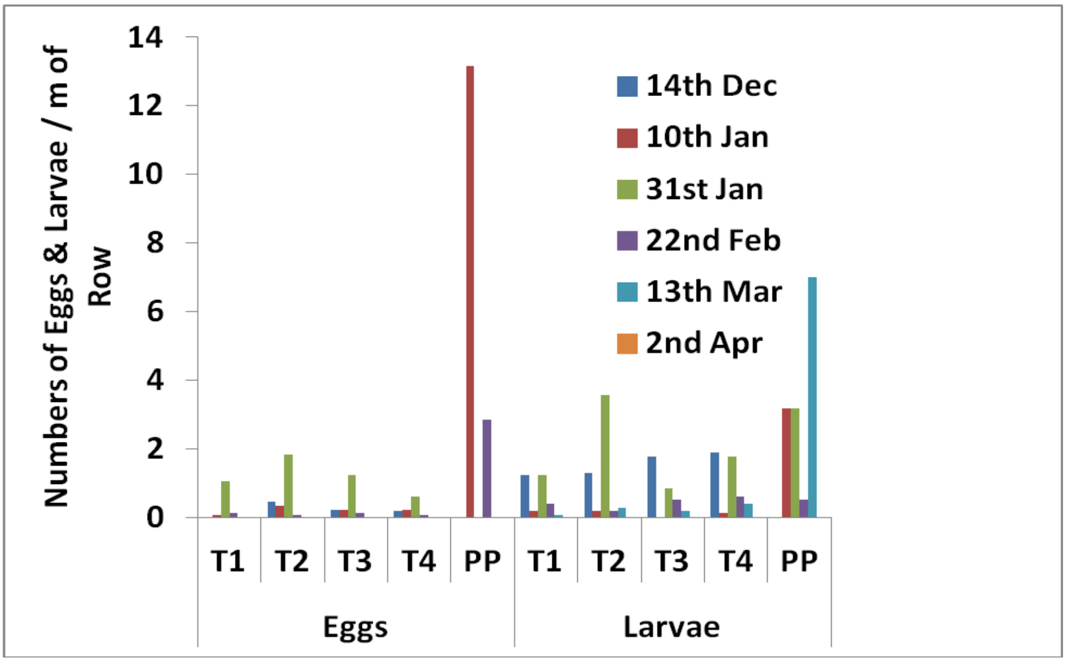


Fig. 34. Average numbers of eggs and larvae of *Helicoverpa* collected per M of crop row of unsprayed conventional cotton and pigeon pea within the trial near Brookstead, Darling Downs and nearby in 2011-12. T1 = treatment with Sicot 71, T2 = Sicot 80, T3 = Sicot 43, T4 = equal mix of Sicot 80 & 43, and PP = pigeon pea.

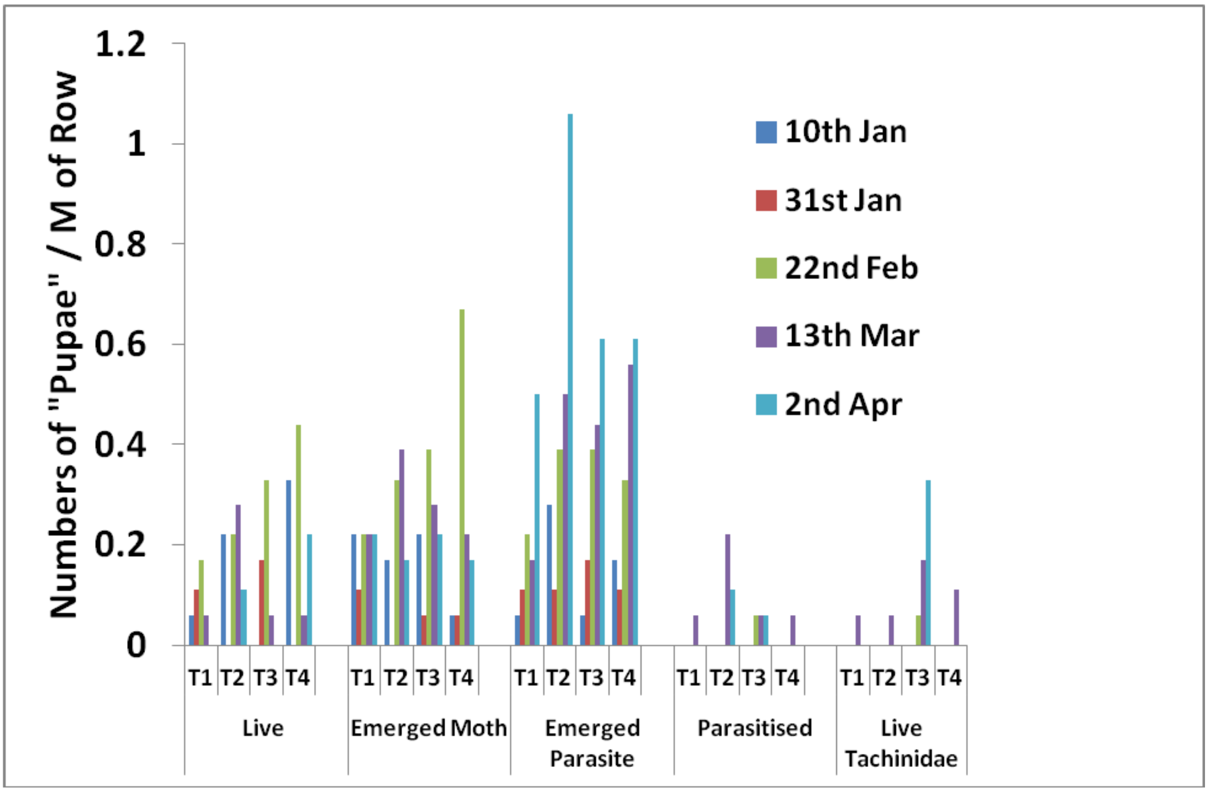


Fig. 35. Average numbers of live *Helicoverpa* pupae, pupal cases indicating emerged moths or parasites, parasitised pupae and pupal cases containing live tachinidae collected per M of crop row of unsprayed conventional cotton within the trial near Brookstead, Darling Downs and nearby in 2011-12. T1 = treatment with Sicot 71, T2 = Sicot 80, T3 = Sicot 43, and T4 = equal mix of Sicot 80 & 43.

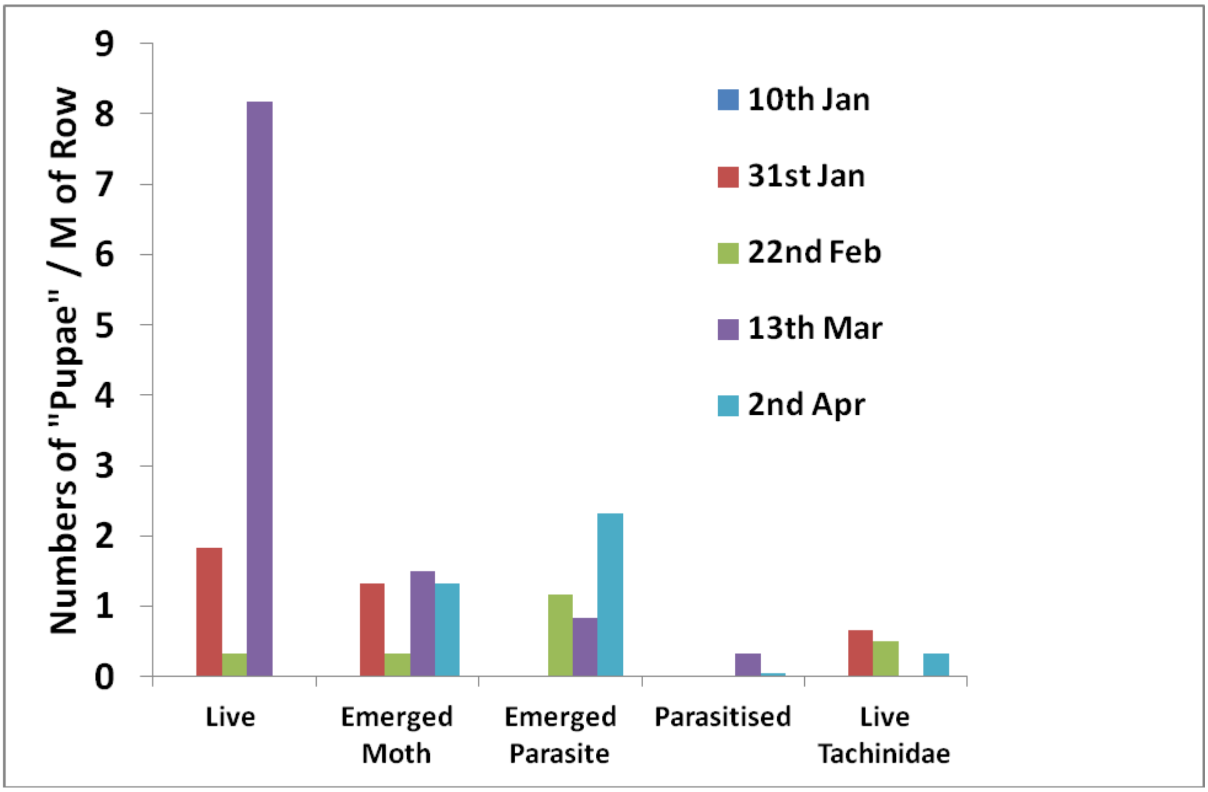


Fig. 36. Average numbers of live *Helicoverpa* pupae, pupal cases indicating emerged moths or parasites, parasitised pupae and pupal cases containing live tachinidae collected per M of crop row of pigeon pea near the trial near Brookstead, Darling Downs and nearby in 2011-12.

No eggs and larvae were recorded towards the end of the trial as plants senesced.

G. Baker & C. Tann

2. Monitor long-term changes in *Helicoverpa* abundance

Pheromone Trapping in the Namoi Valley & St George

The abundance of both *H. armigera* and *H. punctigera* within the pheromone traps set at ACRI, Narrabri during this project was generally lower than previous years (Figs 37-38). *H. punctigera* numbers were particularly low, and the results suggest that very few of these moths have migrated into the cropping region from inland breeding grounds in recent years, in contrast to what has been considered to commonly occur in the past. We suspect the repercussions of a long-term drought and associated issues from grazing pressure may have something to do with the change.

This lack of (or numerically limited) migration, at least on a large scale, may have significant implications for the development of resistance to Bt in *H. punctigera* within the cropping region. But lack of influx of large numbers of moths says little about the actual level of movement displayed within the actual populations caught. There remains a definite need for population genetics' studies (e.g. through the use of modern molecular marker tools) to determine the degree of genetic variability within geographically distant populations and the level of mixing of individuals from such sources to properly assess risks of resistance evolution.

Somewhat similar to the case in 2005-06, when there was an apparent temporal advancement of the 3rd generation of *H. armigera* (probably related to warmer than average temperatures), such that it "encroached" within the period (weeks 21-30) attributed to the 2nd generation (thus seemingly inflating the abundance of that generation in Fig. 41), there was drift in the timings of 1st and 2nd generations of *H. armigera* in 2010-11. In this latter case, there appeared to be a slight delay in the 1st and 2nd generations (Fig. 38). Such variability in the data sets, which ultimately may well prove useful in refining our understanding of the environmental drivers of generation times (and perhaps the efficacy of Bt cotton planting windows as part of the Bt resistance management plan), will need to be controlled when assessing overall, long-term temporal patterns in moth abundance at the end of the survey.

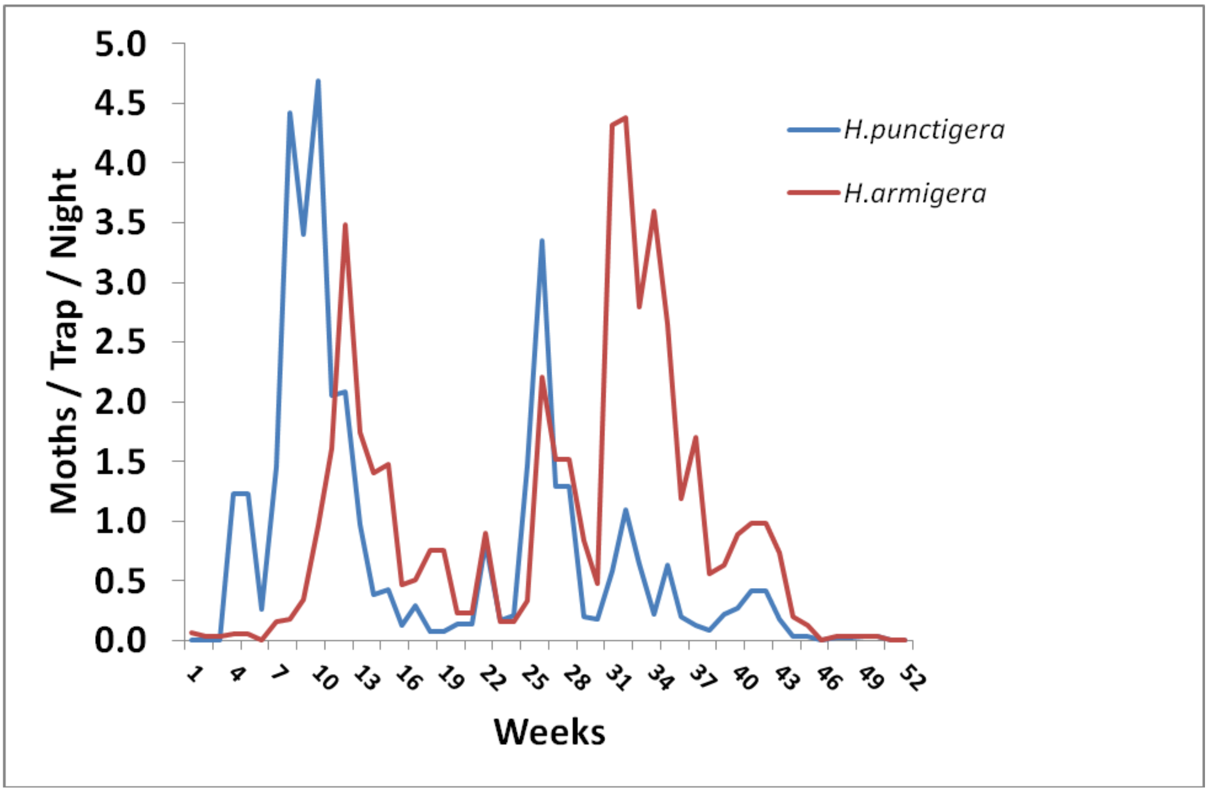


Fig. 37. Pheromone trap catches from the trapping grid near Narrabri for 2009-10. Weeks are from July 1.

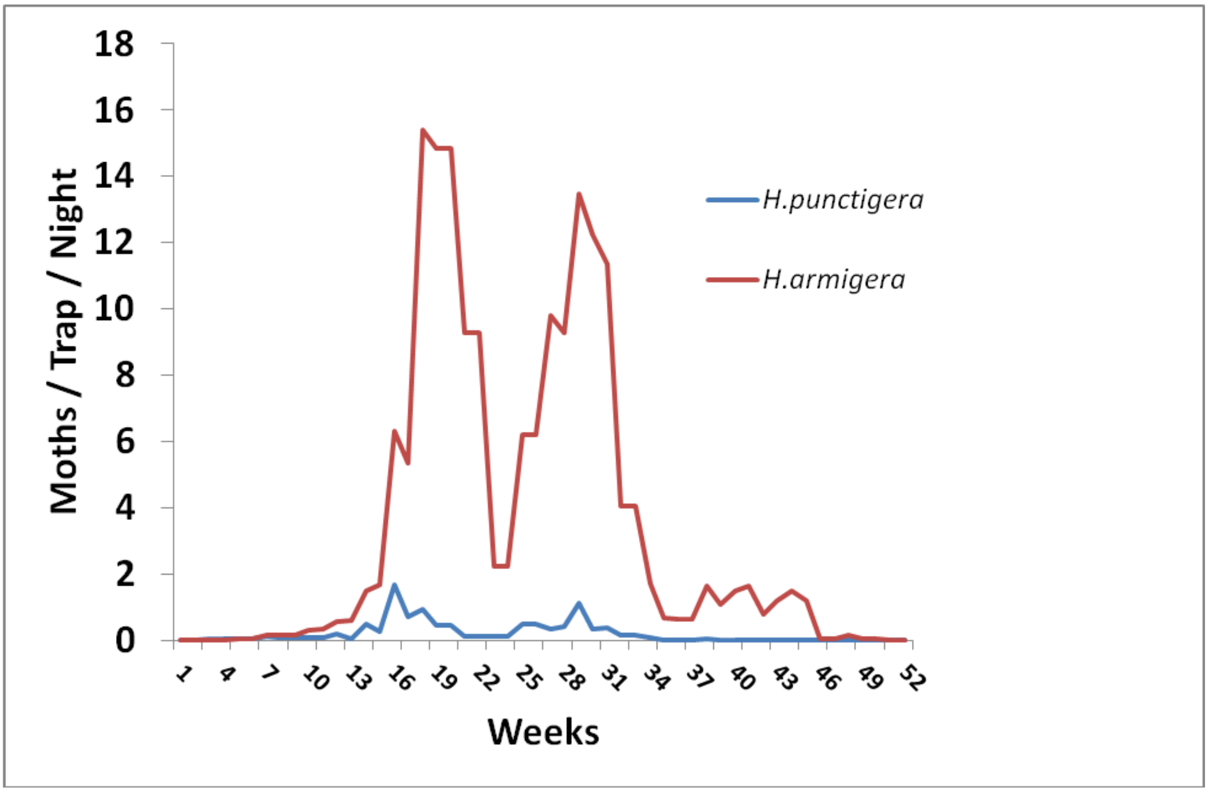


Fig. 38. Pheromone trap catches from the trapping grid near Narrabri for 2010-11. Weeks are from July 1.

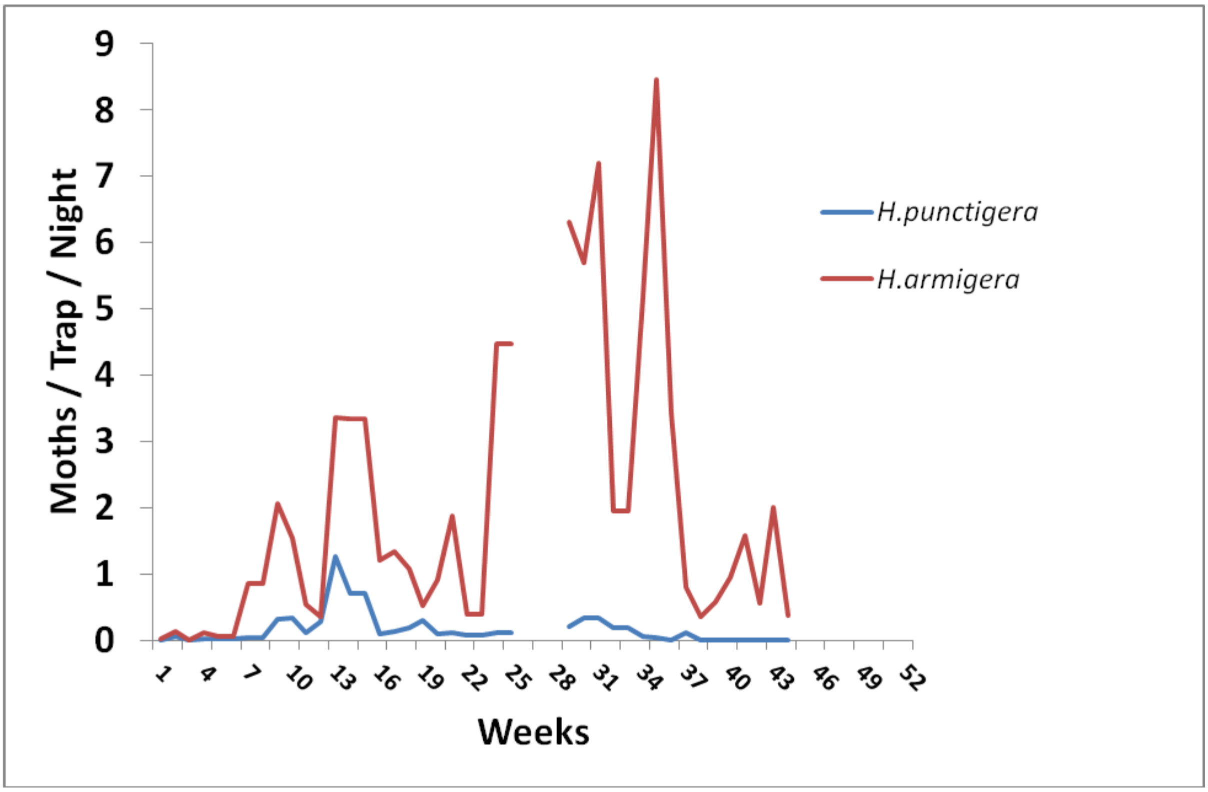


Fig. 39. Pheromone trap catches from the trapping grid near Narrabri for 2011-12. Weeks are from July 1.

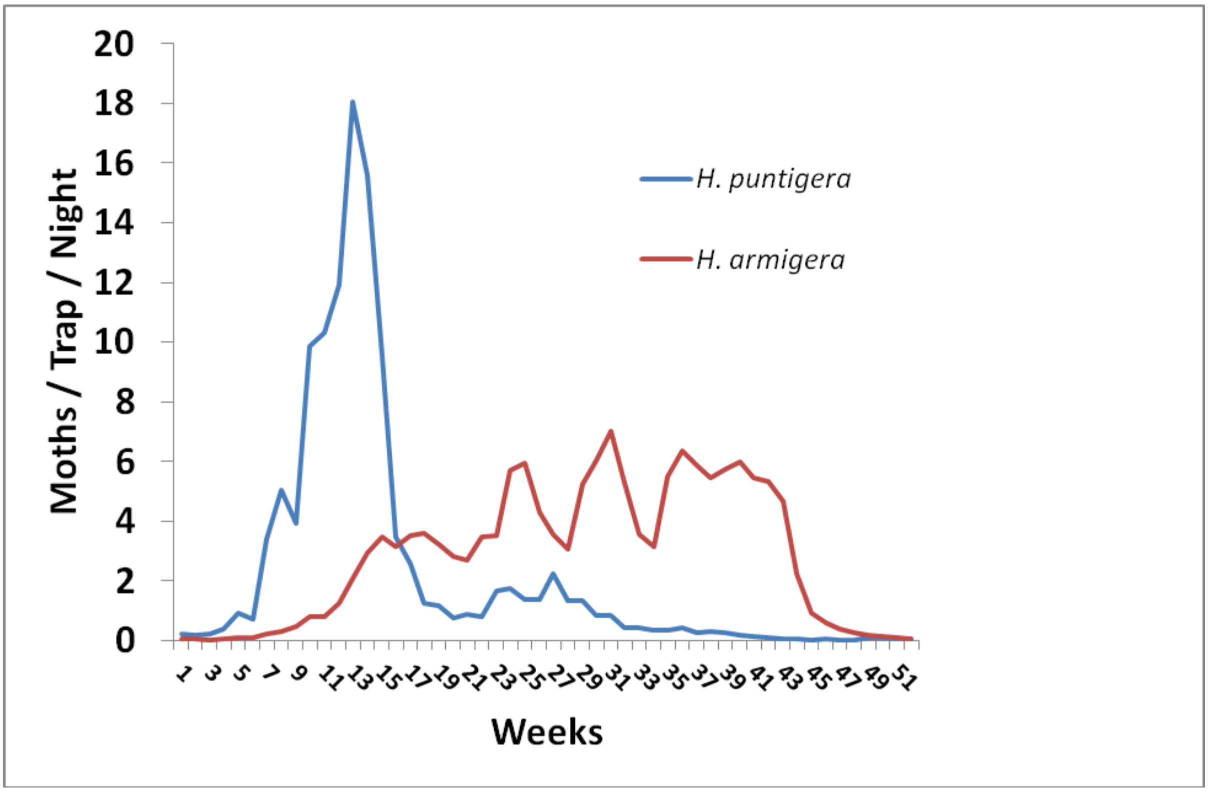


Fig. 40. Long-term average pheromone trap catches from the trapping grid near Narrabri for 1992- 2012. Weeks are from July 1.

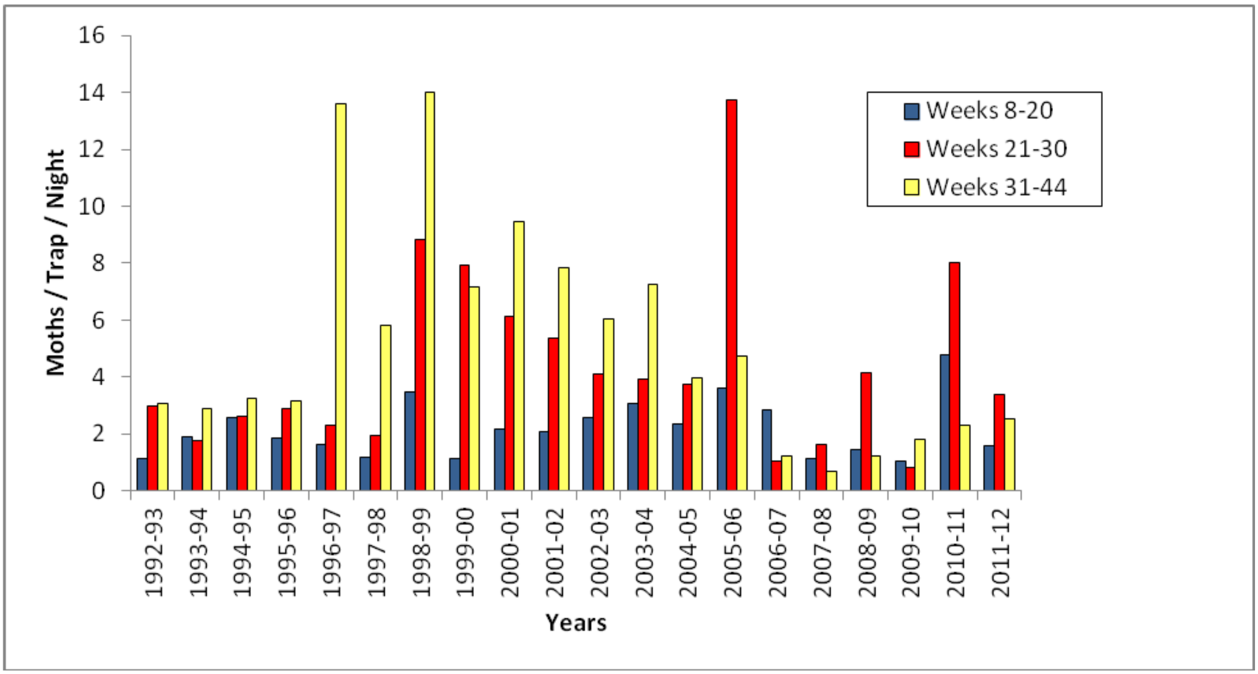


Fig. 41. Abundance of *H. armigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley – for the years 1992-2012. Data are apportioned within each season into 3 groupings : weeks 8-20, 21-30, and 31-44, which approximate the timings of generations of *H. armigera*. Weeks are calculated from July 1.

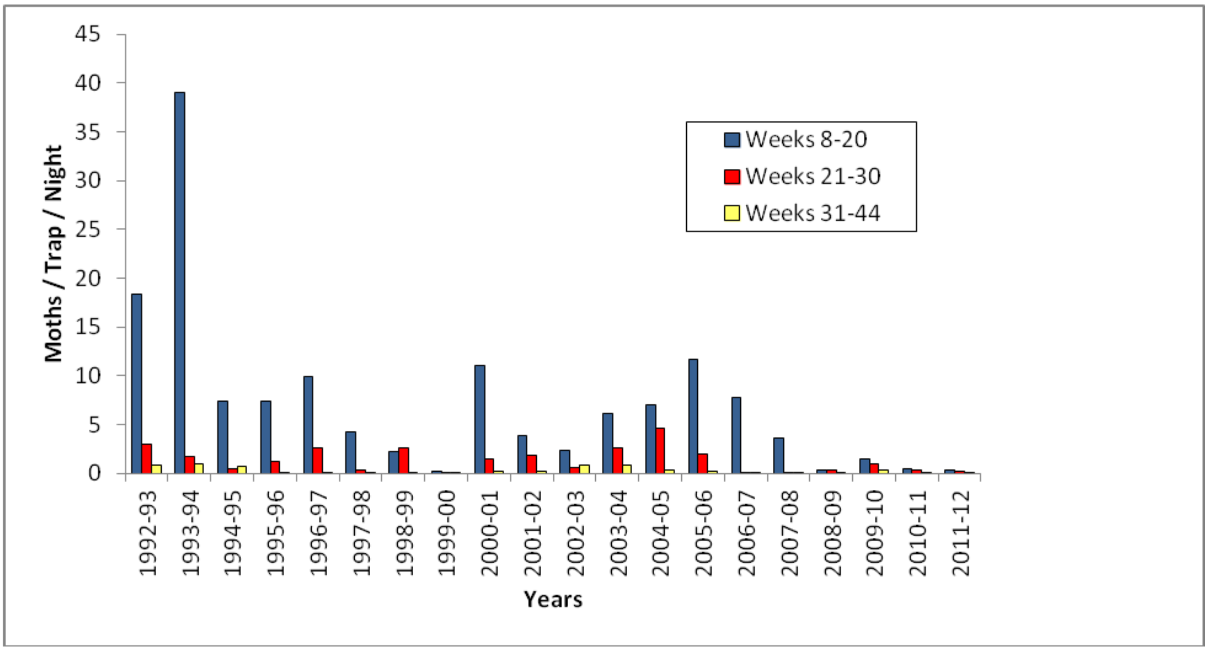


Fig. 42. Abundance of *H. punctigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley – for the years 1992-2012. Data are apportioned within each season into 3 groupings : weeks 8-20, 21-30, and 31-44, which approximate the timings of generations of *H. armigera*. Weeks are calculated from July 1.

The observed trends in pheromone trap catches of *Helicoverpa* moths were correlated with some other landscape scale traits. For example, the hectares sown to cotton (Bt and conventional) in the Namoi Valley (both Upper & Lower) (data provided by Cotton Australia) from 1992/93 to 2010/11 were significantly correlated with the catches of 2nd and 3rd (+) generations of *H. armigera* ($r = 0.520$, $p < 0.05$ and $r = 0.650$, $p < 0.005$ respectively), but not with the 1st generation ($r = 0.298$, $p > 0.05$). Similar relationships were observed between trap catches and yield in bales. However, no significant relationships were observed for *H. punctigera* (e.g. for hectares; r = 0.037, 0.196 and -0.348 for 1st, 2nd and 3rd (+) generations respectively, p for all > 0.05).

It is perhaps not surprising that no relationship was shown between the abundance of the 1st generation of *H. armigera* and cotton production (nor with *H. punctigera*), given the lack of temporal synchrony between these. On the other hand, Fig. 43 depicts the relationship for 3rd generation *H. armigera* and hectares of cotton. At the time of reporting, the hectares of cotton were not available for the 2011/12 season, but expectations are that it will be similar to that recorded for 2010/11. If so, it is becoming apparent that unlike in the 1990's, with the advent of Ingard Bt cotton (in 1996), when the abundance of *H. armigera* seemed to track increased cotton production, and then fell away as the drought reduced cotton production (and Bollgard II replaced Ingard) in the early 2000's, the abundance of *H. armigera* is not increasing again in the most recent years as cotton production is recovering post-drought. It is very tempting to suspect that Bollgard II (now grown at approximately 90% of the cotton crop) might be having a suppressive effect on *H. armigera* abundance cf in comparison with Ingard (with its cap of 30% of the cotton crop, and a more limited toxicity (only single gene, and that being poorly expressed during the 2nd half of the growing season). It is perhaps worth noting here that e.g. where Carriere et al (2003; *PNAS* **100**, 1519-1523) demonstrated suppression of pink bollworm (*Pectinophora gossypiella*) (essentially confined in its feeding to cotton) in the USA in relation to the deployment of Bt cotton (single toxin gene), such was only observed when the % of Bt cotton in the total cotton crop needed to be > 65%. It will be intriguing to see if the abundance of *H. armigera* continues at low levels in the next few years whilst (hopefully) cotton production remains high.

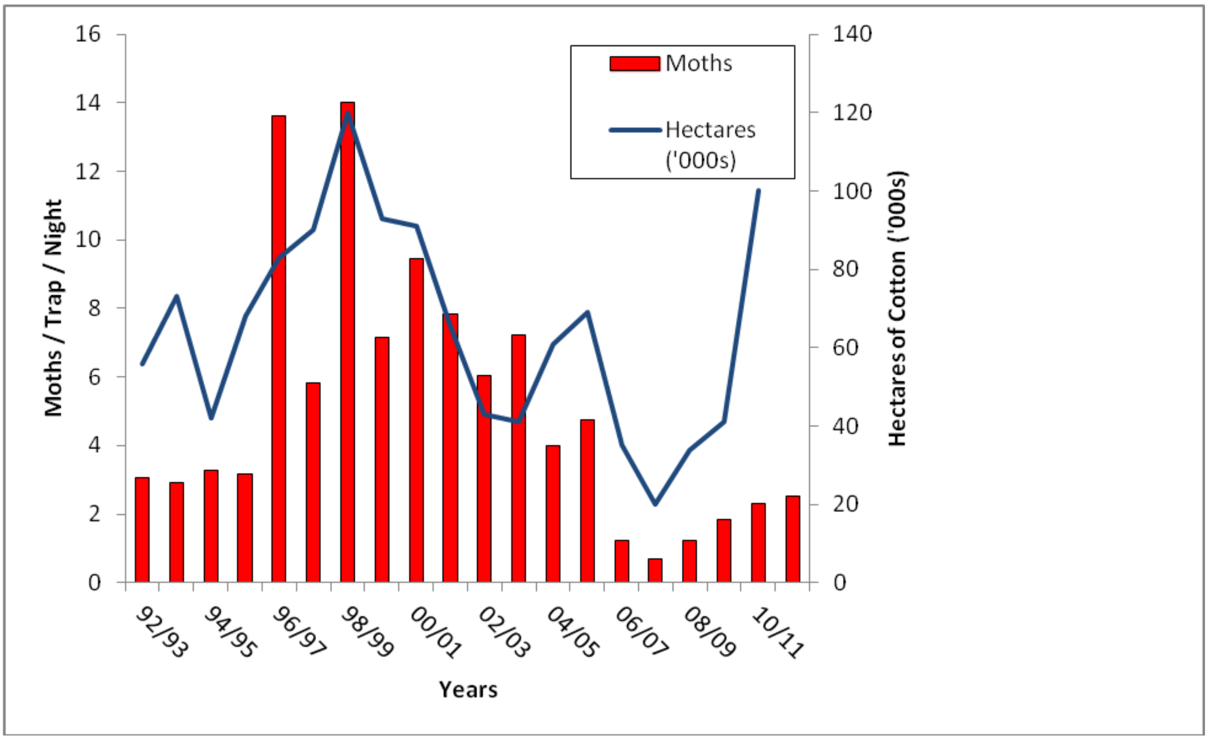


Fig. 43. Abundance of 3rd (+) generation (Weeks 31-44) *H. punctigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley and hectares of cotton sown in the Namoi Valley (data supplied by Cotton Australia) – for the years 1992-2012. Hectare data for 2011-12 not available at time of reporting, but believed to be comparable with 2010-11. Weeks are calculated from July 1.

In addition, we attempted to relate the observed abundances of *H. armigera* moths with weather (rainfall, temperature) recorded at Narrabri, and with hectares of the major crops grown in the Narrabri SLA (data provided by Neil Clark & Associates – via GRDC) that are suitable as plant hosts for these pests. Such cropping data are only available at present up to the 2009/10 season). A strong correlation was again found between 3rd generation moth abundance and hectares of cotton grown ($r = 0.701$, $p < 0.05$), whilst there was no significant correlation with cotton for either the 1st or 2nd generation ($r = 0.113$ and $r = 0.437$ respectively, both with $p > 0.05$). In addition, there was a significant correlation between the abundance of the 2nd generation and the hectares of sunflower ($r = 0.823$, $p < 0.001$), maize ($r = 0.661$, $p < 0.01$), sorghum ($r = 0.636$, $p < 0.05$), and the total crop grown (= sum of hectares of cotton, sorghum, maize, soybean, sunflower, canola, chickpeas, and faba and mungbeans – but note : wheat not included here) ($r = 0.717$, $p < 0.001$). There were however no such correlations demonstrated for these other crops and the 3rd generation of *H. armigera*. For the 1st generation of moths, only one significant correlation with crops (sunflower) was demonstrated ($r = 0.656$, $p < 0.01$). For both the 2nd and 3rd generations, there was a significant correlation between abundance and rainfall during the preceding winter (e.g. May to August) ($r = 0.545$ and 0.571 respectively, for both $p < 0.05$), but this was curiously not the case for the 1st generation (0.306, $p > 0.05$). No significant relationships were detected re temperature. Overall, it seems quite likely that a variety of factors could differentially influence separate cohorts of *H. armigera*, but of course we need to be mindful that some of the observed relationships could simply be auto-correlations.

As for the previous project, we present the relationship between pheromone catches of *H. punctigera* near Narrabri for the last generation in one season and the first generation in the subsequent season (Fig. 44), updated with information collected in recent years. There remains a significant correlation between these data. This could, in absence of population genetic studies of species', reflect local over-wintering of *H. punctigera* (not previously thought to occur, but casual surveys during winter in the Namoi Valley have found *H. punctigera* pupae present), or reflect that in early years spring influxes from inland areas were more consistent between years, thus generating higher 3rd generation numbers.

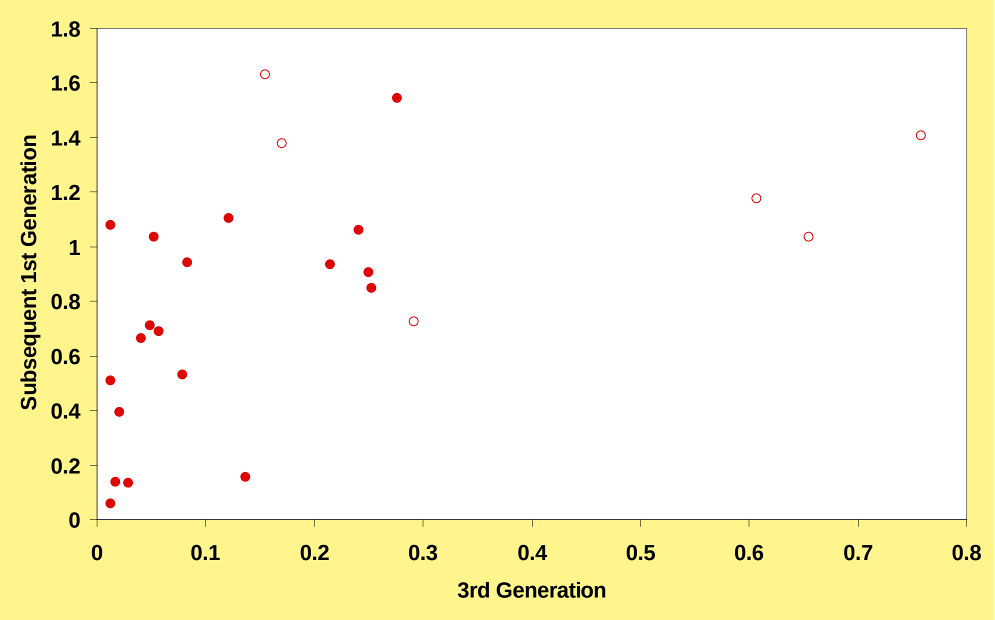


Fig. 44. Relationship between average pheromone trap catches of *H. punctigera* near ACRI, Narrabri (moths / trap / night; expressed as Log [x + 1]) for the 3rd generation in each season and the 1st generation in the subsequent season, from 1987-2011. Open data points are for 1987-1992, when locations of trap sites varied from those used in later years, but were still within the same general area. For 1993-2011, $r = 0.583$, $p < 0.01$. [Note for the *H. armigera* in the same period, $r = 0.079$, $p > 0.05$]

Figures 45 & 46 illustrate long-term trends in inland rainfall. In recent years, annual rainfall has been relatively high in the inland, but autumn-winter rainfall has been moderate. As in earlier analyses, only two significant correlations were obtained between trap catches of 1st generation *H. punctigera* moths (near Narrabri) and preceding (autumn-winter) inland rainfalls (including individual inland regions [see Fig. 4] treated separately, totals for all regions combined, individual months [i.e. April, May, June & July treated separately] and all 4 months summed) : District 17, May ($r = 0.691$, $p < 0.001$) and District 36 July (0.484, $p < 0.05$). The other 58 possible correlations involving rainfall and trap records were not significant. As before, it is reasonable to conclude that the significant correlations we did obtain could easily have occurred by chance. When a modified Bonferroni correction was made to p, to take into account the large numbers of potential correlations and chance significance, only one of the correlations listed above (with District 17, May) was sufficiently strong to remain acceptable (p needed to be < 0.0009). Thus there continues to be little support for the concept that *H. punctigera* abundance in early season in the cropping region (as indicated by results for near Narrabri) reflects preceding autumn-winter rainfalls in relevant parts of the inland, with the implication that such rainfall stimulates population development there, from which moths migrate east. One level of uncertainty that remains is the influence that flood-waters, generated elsewhere but flowing into the inland, have on the population dynamics of *H. punctigera* – such of course aren't represented in direct rainfall there, but are highly likely to influence the abundance (spatial and temporal) of host plants for *H. punctigera*. Also unaccounted for is the degree with which suitable vegetation continues to exist in the inland when moisture levels remain high, when normally it might be expected to diminish as spring approaches. Such may hinder migration away from the inland and thus decrease populations observed further east. Perhaps, the exceptionally low abundance of *H. punctigera* in the vicinity of Narrabri in recent years is, at least in part, explained by such recent moisture effects in the inland. What is very much needed is population genetics studies (using molecular / genomic tools that are now available and enable rapid throughput analyses) to identify genetic structure in populations across relevant geographical regions, and determine the levels of population mixing / movement that occur under varying climatic scenarios. Such would have major implications for the reliability of *H. punctigera* migrations as facets within the Bt resistance management plan for this species.

Oddly, when the same statistical treatment was applied to the trapping data for 1st generation *H. armigera* near Narrabri, more significant correlations ($n = 9$) were obtained than for *H. punctigera*. Significant correlations between inland rainfall and *H. armigera* catches were obtained for : District 15, July ($r = 0.729$, $p < 0.001$); District, 17, April ($r = 0.480$, $p < 0.05$) and July ($r = 0.494$, $p < 0.05$); District 44, July ($r = 0.536$, $p < 0.05$) and All 4 Months Collectively ($r = 0.457$, $p < 0.05$); District 45 July ($r = 0.504$, $p < 0.05$); District 46, July ($r = 0.465$, $p < 0.05$); and All Districts Combined, July ($r = 0.506$, $p < 0.05$) and All 4 Months Collectively ($r = 0.457$, $p < 0.05$). However, only one of these correlations (with District 15, July) was sufficiently strong to remain acceptable with modified Bonferroni correction.

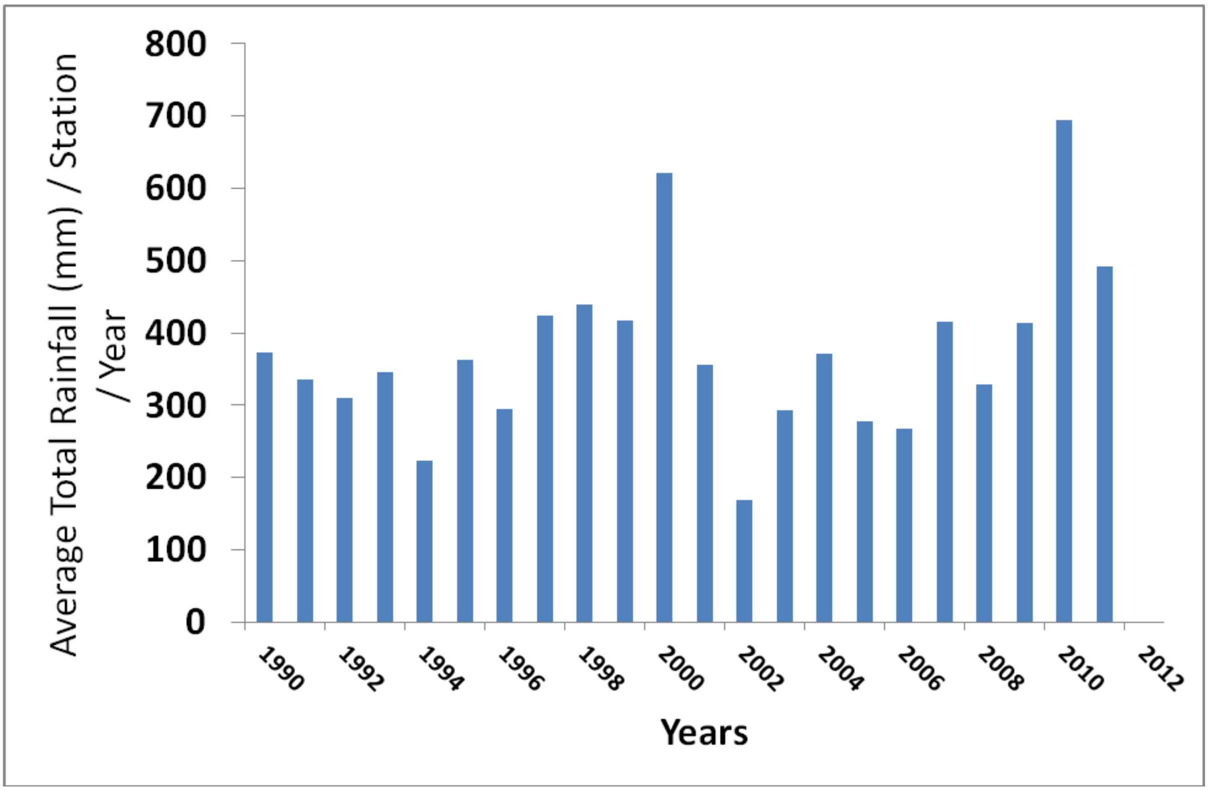


Fig. 45. Average total rainfall (mm) per year across 36 meteorological stations in northern S.A., southern N.T., south-western Qld, and northern N.S.W. Such data (and individual months within these periods) were used to derive correlations with pheromone trap records for *H. punctigera* from 1992-2012.

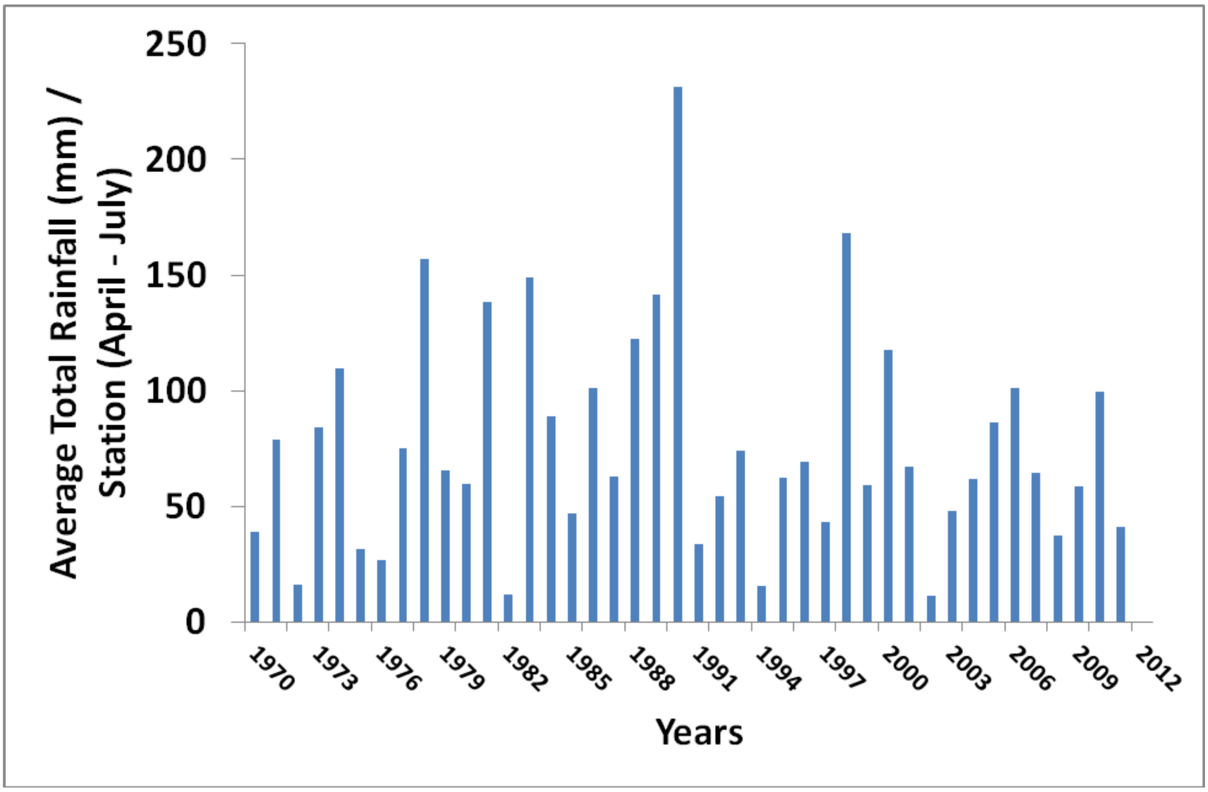


Fig. 46. Average total rainfall (mm) between April to July each year across 36 meteorological stations in northern S.A., southern N.T., south-western Qld, and northern N.S.W. Such data (and individual months within these periods) were used to derive correlations with pheromone trap records for *H. punctigera* from 1992-2012.

For brevity, we do not present here the data (in detail) from the pheromone trapping that occurred at St George during the project. Rather, we compare data recorded at St George with the equivalent near Narrabri, from 1997 when first records were kept at St George to the present (Figs 47 & 48). Because the traps at St George are only checked for part of the season, we can only make such comparisons with surety for the 2nd generation moths (i.e. weeks 21-30). The patterns were quite similar between the two regions, and indeed there were significant correlations in trap catches for both *H. armigera* and *H. punctigera* ($r = 0.695$, $P < 0.01$ and $r = 0.768$, $P < 0.001$ respectively). If we simply treat the average catch each year as the basic data, then more *H. armigera* were generally caught at Narrabri over the 1997-2012 period cf St George (Paired t test $t = 3.26$, $P < 0.01$), but whilst there was a tendency towards more *H. punctigera* being caught at St George (especially in the more recent years), such was not significant, although very nearly so ($t = 2.11$, $P = 0.053$). Interestingly, there was a significant correlation across years between the 2nd generation catches of *H. armigera* and *H. punctigera* at St George ($r = 0.605$, $P < 0.05$), but the same was not so for the traps near Narrabri, either within the same year span as St George ($r = 0.181$, $P > 0.05$), nor within our longer data set for 1992-2012 ($r = 0.135$, $P > 0.05$). Nor was there anything significant for the 1st or 3rd generations ($r = -0.073$, $P > 0.05$ & $r = -0.131$, $P > 0.05$ respectively). At this time we are not sure how to interpret the significant result for St George. But the similar patterns in catches between St George and Narrabri do suggest that our findings from long-term trapping near Narrabri has applicability more broadly than just locally.

The abundance of *Helicoverpa* eggs recorded on cotton in the St George region continued to be relatively high in recent years compared with the 1990's, at least in some months (Fig. 49). Reasons for this trend are not immediately apparent. There have been some particularly large acreages of chick pea grown in the region in recent years and build up of moths on these in spring may be part of the answer.

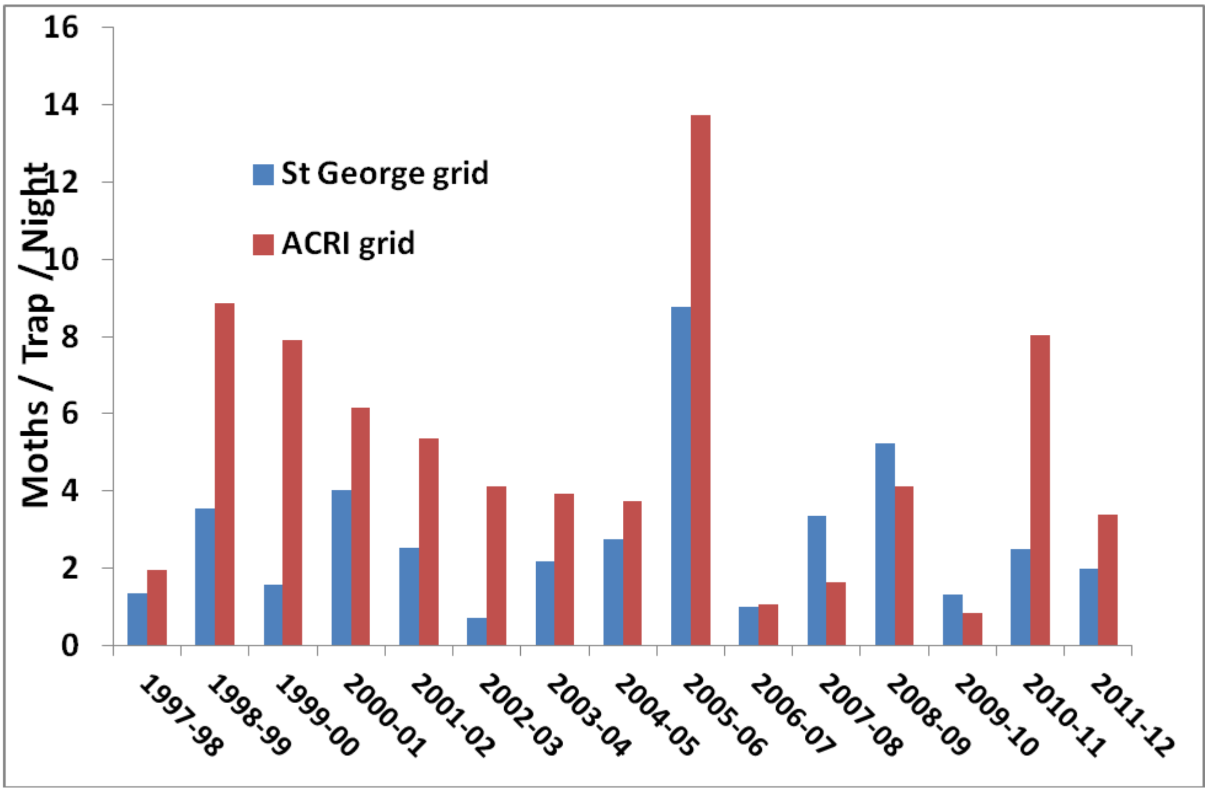


Fig. 47. Mean numbers of 2nd generation (weeks 21-30) *H. armigera* moths caught in grids of pheromone traps near Narrabri and in the St George region from the 1997-98 "season" to the 2011-12 "season".

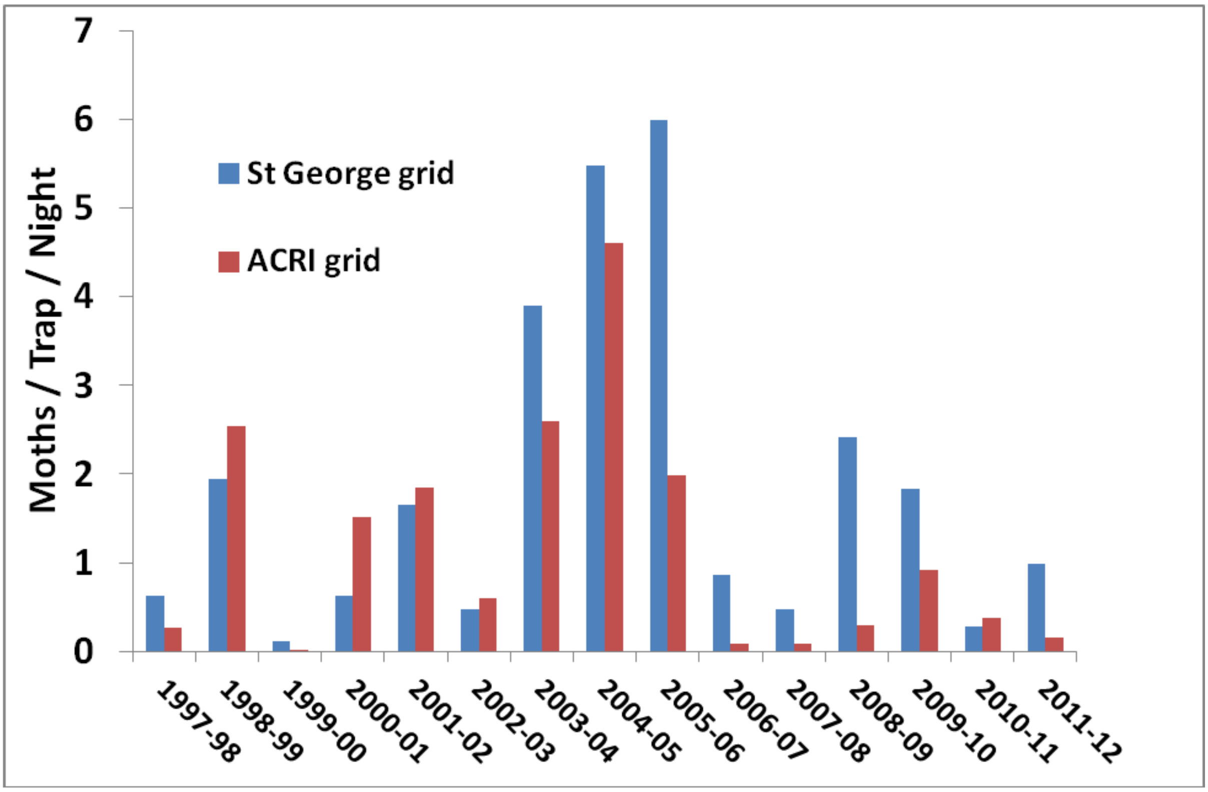


Fig. 48. Mean numbers of 2nd generation (weeks 21-30) *H. punctigera* moths caught in grids of pheromone traps near Narrabri and in the St George region from the 1997-98 "season" to the 2011-12 "season".

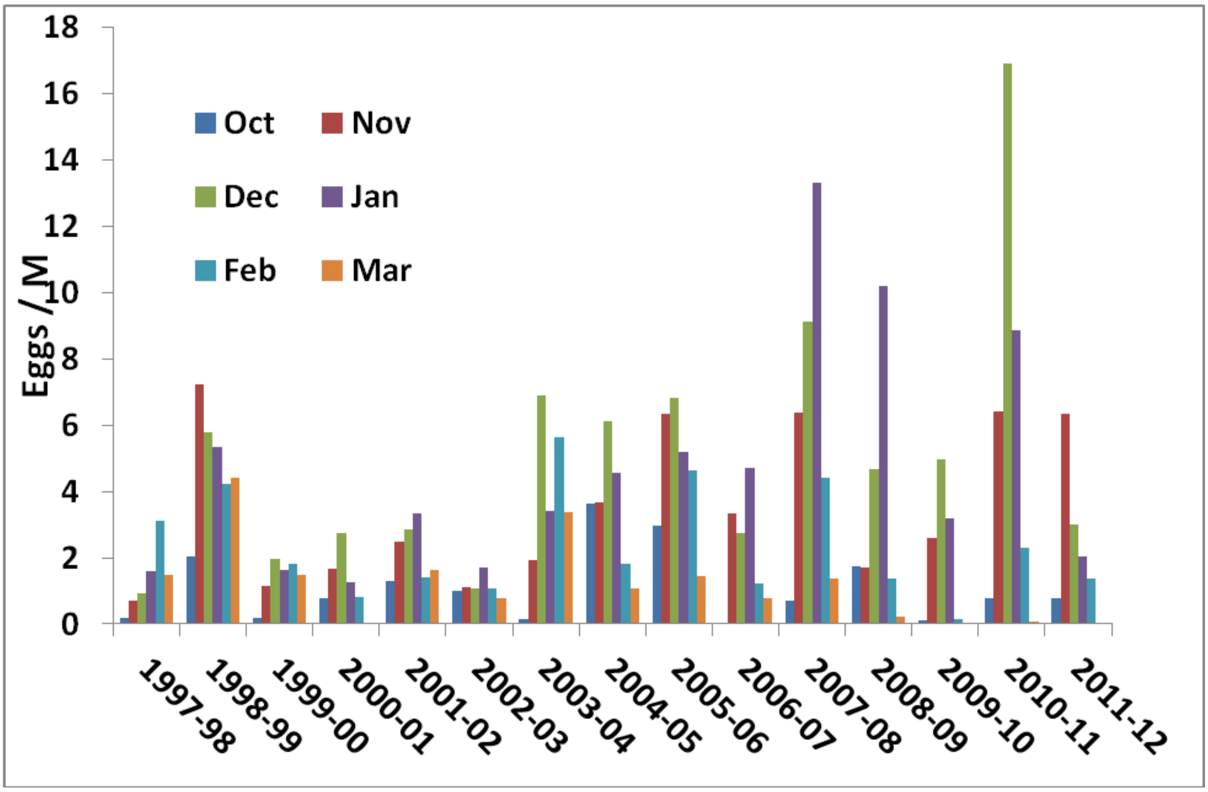


Fig. 49. Average abundance of *Helicoverpa* spp. eggs on cotton (Bt and conventional cotton not separated here) within the St George region, between 1997-2012.

Surveys of weed & non-cotton crop plants conducted each spring in the Namoi region for Helicoverpa *eggs and larvae.*

The abundance of *Helicoverpa* eggs and larvae in late winter-spring in 2009-11 in northern N.S.W. and southern Qld was generally low compared with previous years, probably reflecting, at least in part, the paucity of vegetation following the recent drought. The majority of eggs and larvae were collected from chickpea. *H. punctigera*, as we would expect, was the most dominant species. Parasitoids were mostly Hymenoptera (e.g. *Heteropelma*, *Netelia*, *Microplitis*, *Chelonus* and *Trichogramma*) and Diptera (e.g. Tachindae). Percentage parasitism fluctuated below approx 15% in crops and approx 39% in weeds and native vegetation (means of 4.76 ± 0.84% and 13.09 ± 2.11% respectively) (Figs 50 & 51). The % parasitism was significantly higher in the native vegetation / weeds cf the crops (Wilcoxon Rank Sum Test [Normal approximation with Continuity Correction] = 3.76, $P < 0.001$), but there was no correlation across years between the two habitats ($r = 0.422$, $P > 0.05$). Disease incidence varied below approx 15% in crops and approx 19% in weeds and native vegetation (means of 3.44 ± 0.91% and 5.49 ± 1.25% respectively), with no difference between habitats (WRS = 1.19, $P > 0.05$), nor correlation between habitats ($r = 0.466$, $P > 0.05$). Correlations between % parasitism, % disease and seemingly relevant environmental factors are as yet not apparent (e.g. for % parasitism with late winter-spring rainfall, $r = 0.246$ and $r = 0.059$, both with $P > 0.05$ for crops and weeds / native vegetation respectively; likewise for mean maximum temperature, $r = -0.165$ and $r = 0.044$, both with $P > 0.05$; correlations also N.S. for % disease, but stats outcomes not presented here). This work represents in total, over all years, the rearing of 11,023 eggs / larvae from early season crops and 6,811 from native vegetation and weeds.

Whilst *H. punctigera* was usually the dominant species collected in winter-spring, especially on weeds and native vegetation, it is worth noting that in some years (e.g. 1998, 1999 and 2005), *H. armigera* was the dominant species, at least on early season crops.

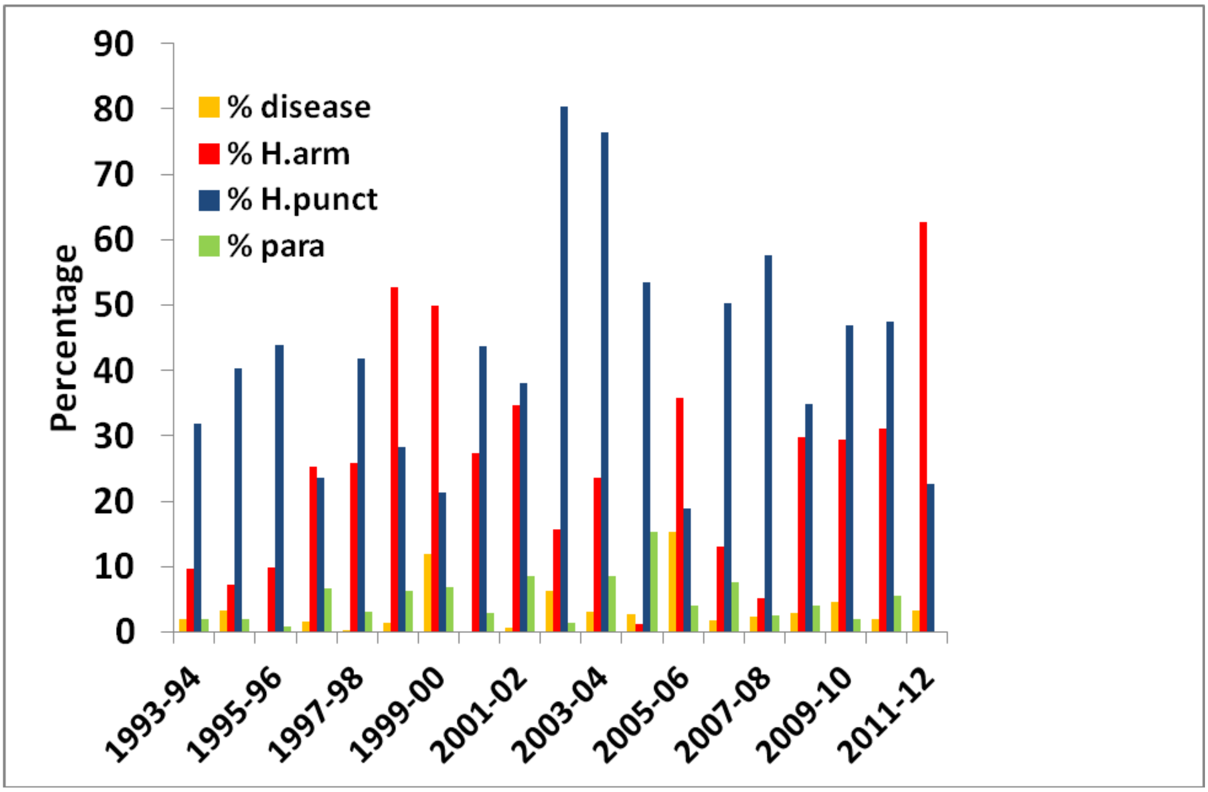


Fig. 50. Proportions of *Helicoverpa* spp. eggs and larvae collected from crops in late winter-spring that when reared in the laboratory were *H.armigera* or *H. punctigera* moths, diseased or parasitised.

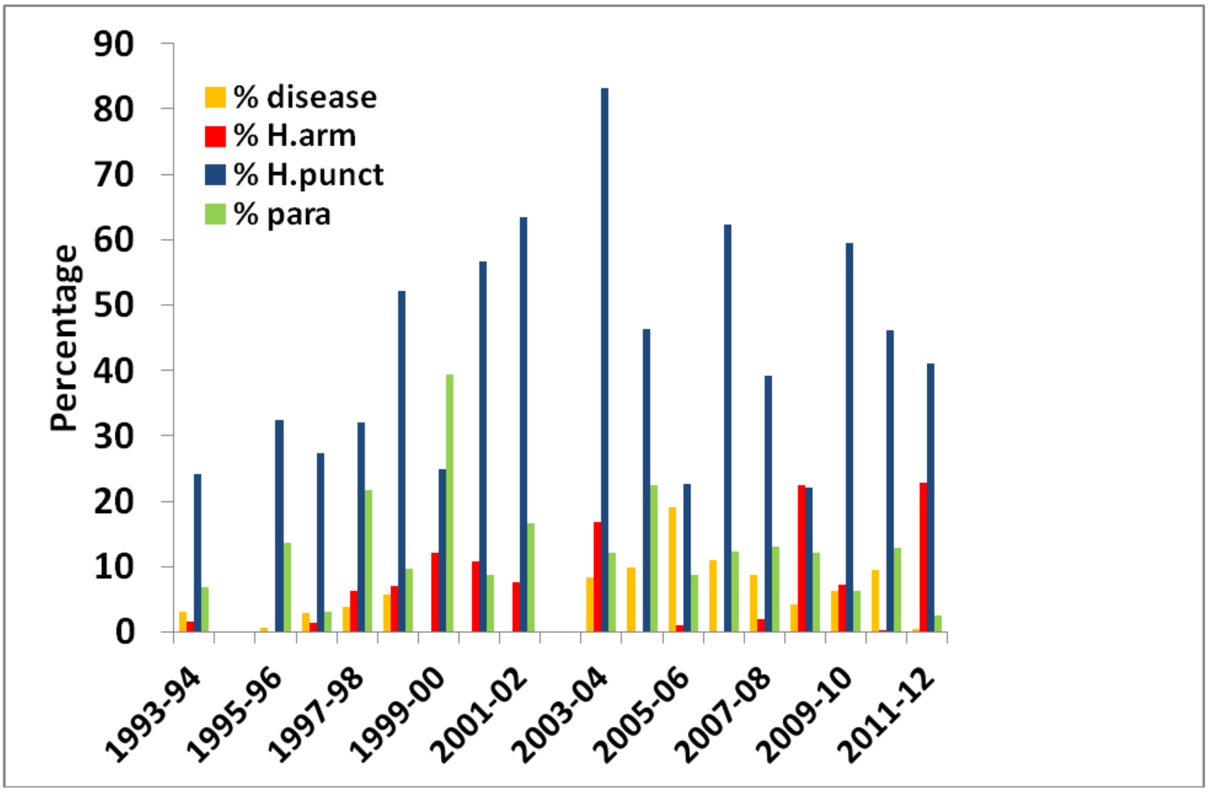


Fig. 51. Proportions of *Helicoverpa* spp. eggs and larvae collected from weeds and native vegetation in late winter-spring that when reared in the laboratory were *H.armigera* or *H. punctigera* moths, diseased or parasitised.

It is convenient to include here information on long-term trends of the rearings of *Helicoverpa* eggs and larvae (all sources) from not only spring but also later in the *Helicoverpa* "season" in the cropping region (Figs 52 & 53) (Note : incidences of parasitism, disease and deaths from unknown causes are not included here. Such are dealt with later in terms of refuge crop performance). The proportion of *H. punctigera* in the rearings diminished as the season progressed, as would be expected. But a couple of points are worth noting. Firstly, there continues to be a small proportion of *H. punctigera* late in the season (March-April), at the time we would expect such individuals to pupate and over-winter. Whilst small, this proportion has become reasonably consistent from year to year. We also found over-wintering pupae of *H. punctigera* during this study (as we did during its predecessor). This (previously under-emphasised) occurrence probably has relevance to the potential emergence of Bt resistance in this species, especially if seasonal (spring) invasions of this species into the cropping region from elsewhere (inland) are slight.

Secondly, it is curious that there has been a clear trend from a dominance by *H. armigera* during summer in the 1990's to more equal proportions of the two *Helicoverpa* species in the 2000's (Fig. 52). Reasons for this are not as yet apparent. i.e. does it reflect a relative shift downwards in the abundance of *H. armigera*, or the opposite for *H. punctigera* ? Trends in catches of the two species in pheromone traps (see earlier) don't seem to provide adequate explanations.

Thirdly, the incidence of *H. armigera* early in the season, sometimes as common as *H. punctigera* in the rearings, needs to be recognised by land-holders / consultants / agronomists who are selecting sprays to control *Helicoverpa* in early season crops such as chickpea and faba bean. Use of (the cheaper option) pyrethroids, where resistance to *H. armigera* is well recognised, is seemingly fraught with risk, without recognition of the dominant *Helicoverpa* sp. present.

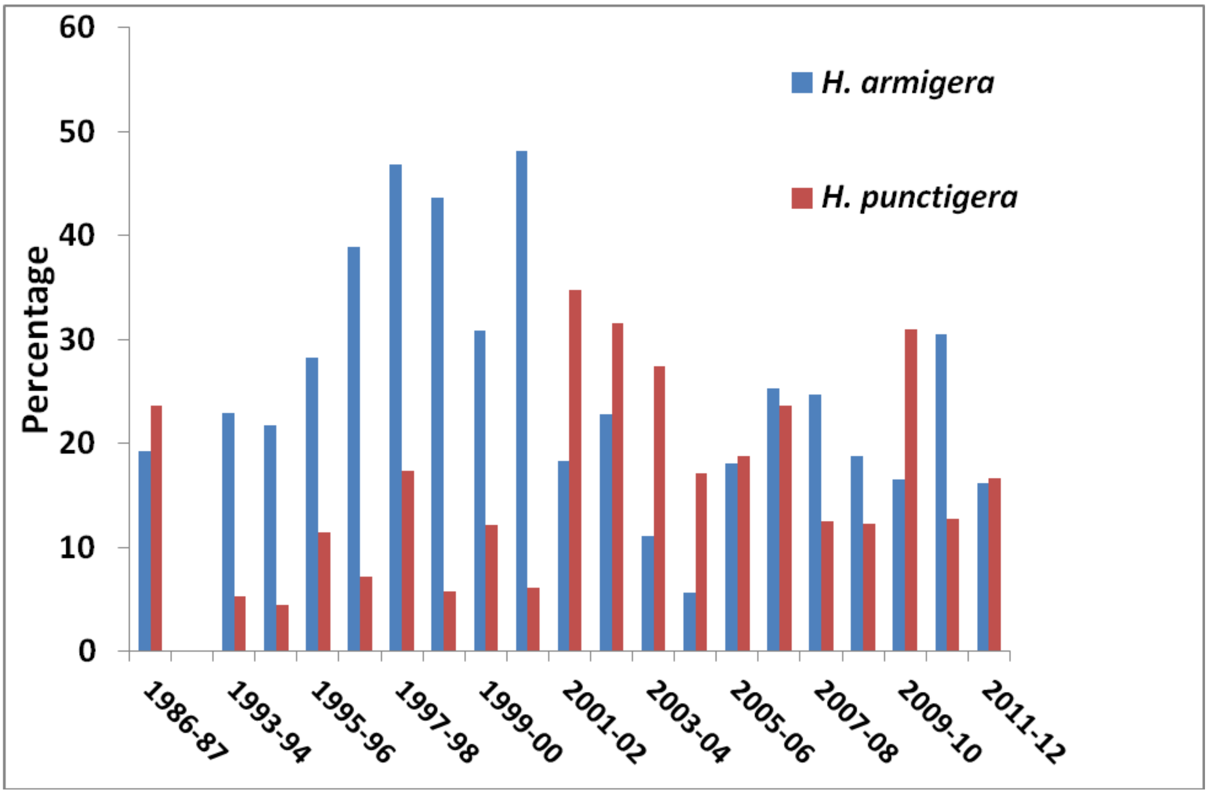


Fig. 52. Proportions of eggs and larvae of *Helicoverpa* spp. that when collected during summer (December to February) and reared in the laboratory yielded *H. armigera* and *H. punctigera* moths.

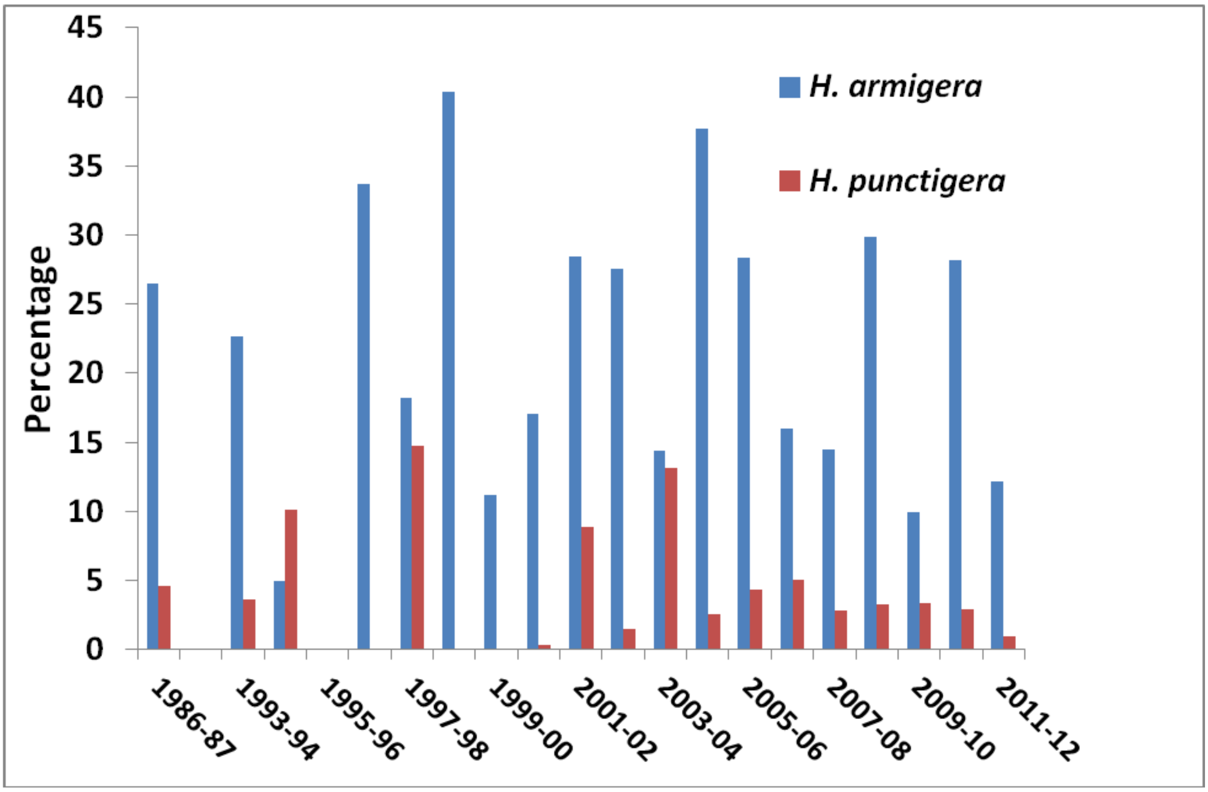


Fig. 53. Proportions of eggs and larvae of *Helicoverpa* spp. that when collected during autumn (March to April) and reared in the laboratory yielded *H. armigera* and *H. punctigera* moths.

3. Identify degree of cross-mating of *Helicoverpa* from different plant host origins

G. Baker & C. Tann

Sites monitored in northern NSW / southern Qld, emergences predicted, night collections made of mating moths & chemical analyses completed to identify both origins.

In the majority of cases, bi-plots of the stable C and N isotope signatures of *H. armigera* moths separated well for C (delta C < -20‰ being moths of C3 plant host origins; delta C > -20‰ being moths of C4 plant host origins) but showed no sign of separation for N (e.g. see Fig. 54). This result thus replicated the preliminary results we reported for CRC Project 1.01.52. We had originally expected that we would be able to discriminate pigeon pea (as a legume) from cotton (non-legume) plant host origins (within the C3 origin cluster), the legume exhibiting a lower delta N than the non-legume, related to differences in N fixation. But analyses of moths reared only on pigeon pea or cotton suggested separation based on delta N differences would be a challenge, and our field data now seem to confirm that. Perhaps pigeon pea's reputation as a poor N fixer is the cause of the difficulty here. An alternative explanation may lie in the N status of (and / or origin in) the soils used for cotton production.

We therefore remain in need of a method to discriminate pigeon pea and cotton, to enable fuller assessment of random mating of moths across the refuge (structured & unstructured) crop strategy. Such would also have broader ramifications as a tool to assess landscape-scale measurement of movements of *Helicoverpa*. In this regard, we are currently exploring (with CSIRO PI, using financial support external to this project) the possibility of using plant cuticular wax signatures that are taken up by *Helicoverpa* larvae when feeding. Early results from this work are encouraging.

However, at one site (Tucka Tucka, Macintyre Valley), results (Fig. 55) varied from those found at the other sites. The moths at Tucka Tucka, in particular those with a delta C (δ^{13}C) typical of C4 plants, were quite obviously differentiated into two distinct δ^{15}N sub-groups (separated at approximately δ^{15}N = 20‰). When the C4 origin moths from Tucka Tucka were split into high (H) and low (L) δ^{15}N traits (at > and < 20‰ respectively), there were 70 individuals classed as the former and 121 individuals classed as the latter. Of the 42 pairs of moths included amongst these individuals, there were 10 HxH matings, 12 LxL matings, and 20 HxL matings. This was not significantly different from what would be expected at random (Multinomial Test : = 4.78, $P > 0.05$; where expected numbers were 5.64, 16.86 and 19.50 for HxH, LxL and HxL matings respectively, based on all moths collected – i.e. pairs and singletons). Given that no significant differences were detected in δ^{15}N between the males and females of *H. armigera*, nor singleton and mating moths, at Tucka Tucka (nor at almost any other site – with one exception – data not included here, but are available from G.B. if needs be), the groupings depicted in Fig. 55 cannot be explained by sex or mating status differences amongst the moths.

We can offer no clear reason for the different result at Tucka Tucka, and whilst our original aspiration in being able to further separate designated C3 and C4 plant host origins using stable N isotopes was realised, it occurred within the C4 moths rather than within the C3 moths (the latter where we most needed discrimination). Perhaps the two groupings within the C4 moths reflected separate host plant origins, but they could also have represented different field origins (where N availability differed).

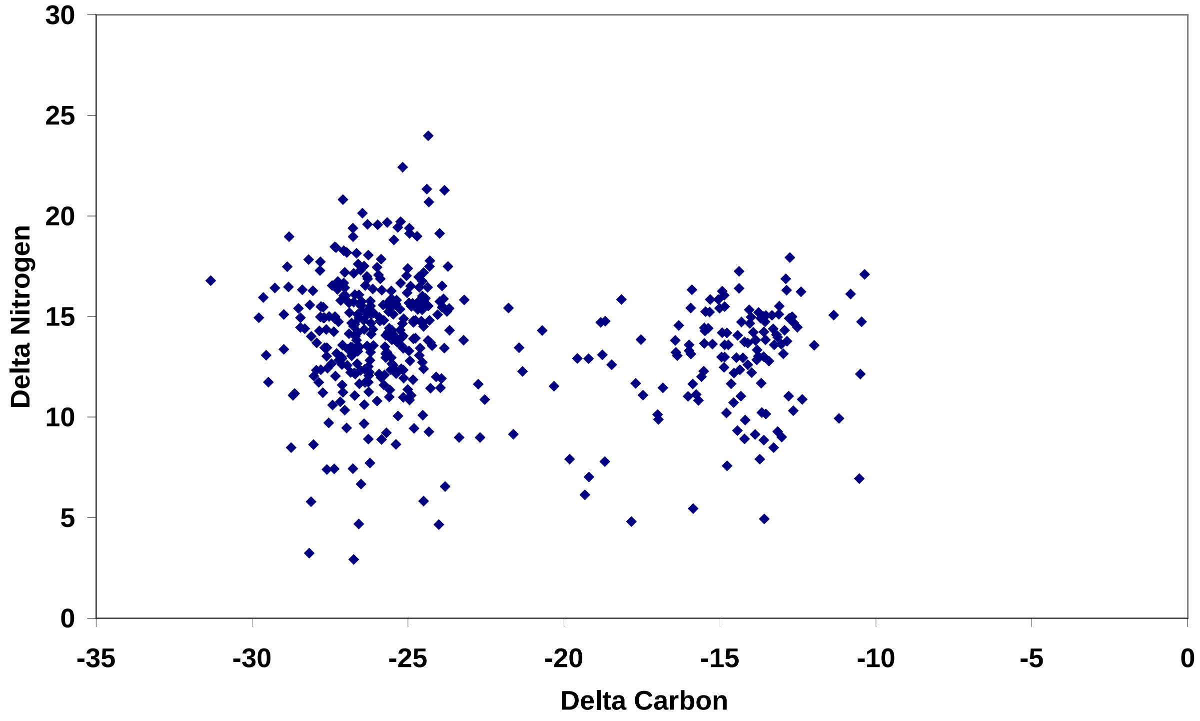


Fig. 54. Bi-plot of stable carbon and nitrogen isotope analyses (δ^{13}C & δ^{15}N (‰)) of *H. armigera* moths collected within a Bt cotton crop at Redcamp, in summer 2006. Mating pairs and singletons are pooled in this case.

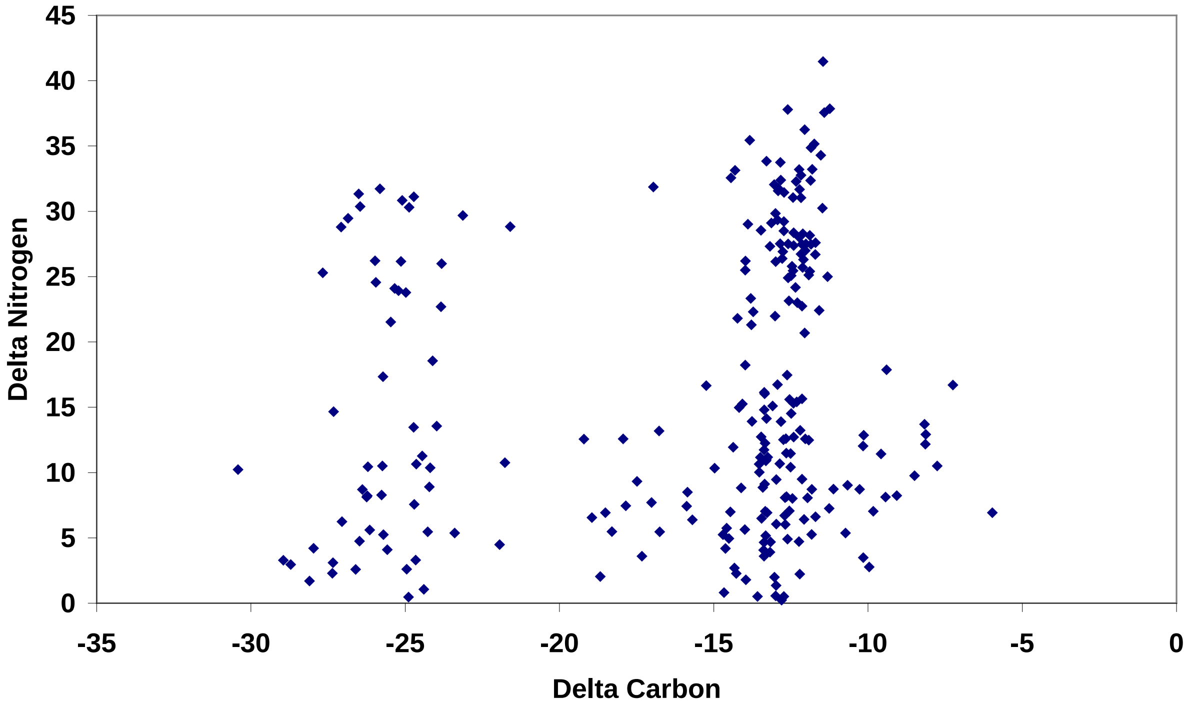


Fig. 55. Bi-plot of stable carbon and nitrogen isotope analyses (δ^{13}C & δ^{15}N (‰)) of *H. armigera* moths collected within a Bt cotton crop at Tucka Tucka, in summer 2009. Mating pairs and singletons are pooled in this case.

The precise locations of the mating moths we collected in the Bt crops were not recorded. However, we did record the time of collection for each moth, and thus had a crude surrogate for its distance from the dedicated refuge crop. On most occasions, there was no convincing pattern in the δ^{13}C and δ^{15}N values related to time of collection (and hence distance into refuge). However, at Redcamp, all the moths collected between 00.37 hr and 01.15 hr (and nearest to the edge of the Bt cotton crop) had C3 signatures, but those collected earlier, between 10.25 hr and 00.21hr (and further into the Bt crop) had a mix of C3 and C4 signatures (53 and 47% respectively). At least for the collections at Redcamp, δ^{13}C values of moths varied spatially (but no similar variation was seen for δ^{15}N). It seems most likely that the observed predominance of C3 origin moths near the edge of the Bt cotton crop at Redcamp simply reflected a high output from a well-functioning conventional cotton refuge crop at the time of our collection. Approximately 10 days prior to this moth collection, the refuge crop was very heavily infested with *Helicoverpa* larvae. Further into the Bt cotton crop we presumably moved into the range of other (C4) sources of moths. Note also, we collected mating moths near the edge of the field at approximately 3.5 times the rate (132 pairs in 38 mins) compared with further away (116 pairs in 119 mins). In future surveys, it would be useful to gather more spatially explicit data on the abundance of moths with different stable isotope signatures within Bt cotton fields. This should better indicate the efficacy of coverage of cotton crops by moths from different host sources.

Overall (all our assessments of moths for stable isotope signatures), 53% of the moths collected within cotton crops had δ^{13}C signatures suggestive of C4 plant origins (i.e. $\delta^{13}\text{C} > -20‰$) (Fig. 37). This perhaps suggests that unstructured refuges can be very influential in producing Bt susceptible moths on cotton landscapes. However, on some occasions when moth collections were made, C4 plants were being grown as the dedicated refuge associated with the Bt cotton crop in focus (e.g. sorghum, prior to it being removed as a recognised refuge option). Care therefore needs to be taken when referring to what was the unstructured refuge at the time in question, although C4 plant refuges were only ever a minority at best (< 7%) of the choices made by farmers. However, we recorded several instances where a large percentage of the moth population captured over the cotton crop was of C4 origin(s), yet the dedicated refuge crop was pigeon pea or unsprayed, conventional cotton. For example, one of these was at Tucka Tucka in the Macintyre Valley, where 77 % of the moths captured in the Bt cotton crop were C4. Such moths must have come from elsewhere in the landscape besides the dedicated (pigeon pea) refuge. On that occasion, there was a sorghum crop nearby. Indeed, in the year of that collection much more sorghum than cotton was grown in the local Namoi Valley (88,449 ha cf 22,621 ha; Australian Bureau Statistics, 2011) i.e. as a crop in its own right. Another example was Taratan (also in the Namoi Valley), where 73% of the moths captured in the Bt cotton crop were C4. In this case, the dedicated refuge was again pigeon pea; but a maize crop (non-refuge), 1 km from the Bt crop, had large numbers of *Helicoverpa* larvae on it in the weeks prior to collecting the moths in the Bt cotton. That maize crop may well have been a source of the captured moths. At the time of this collection, the areas used for sorghum, maize and cotton production in the Namoi Valley were 136,491, 5,896 and 65,327 ha respectively (Australian Bureau Statistics, 2011).

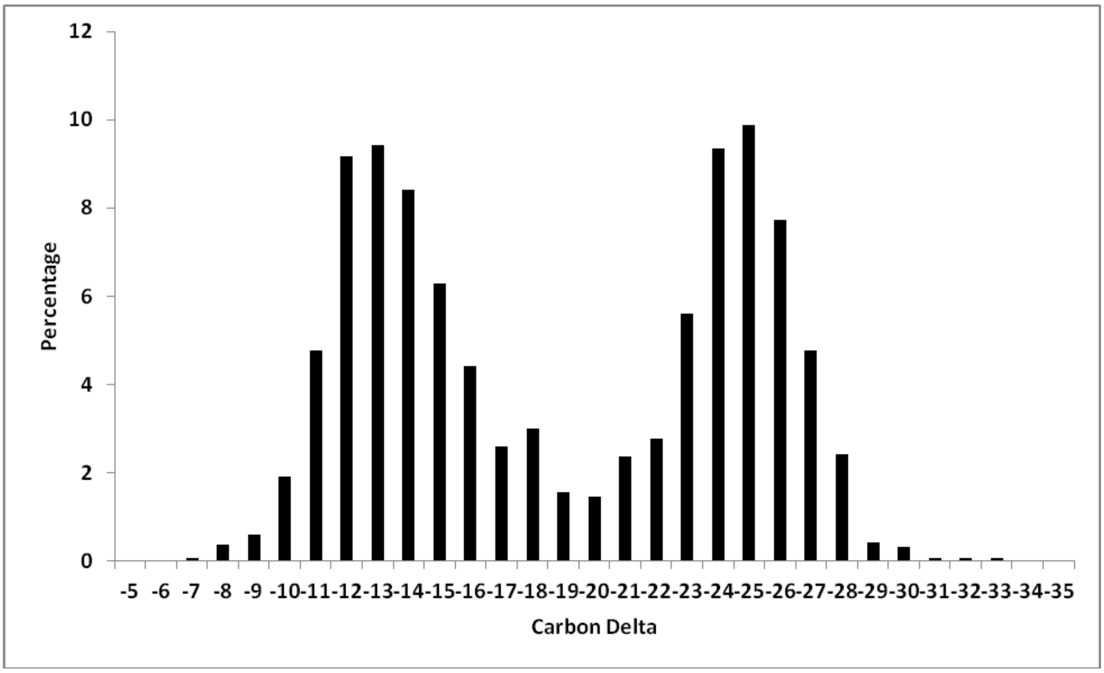


Fig. 56. Percentage of total collection (n = 2,887) of *H. armigera* moths at 20 sites (19 in Bt cotton, 1 in conventional cotton) between 2002-2009, across several cotton production valleys with varying carbon delta signatures.

On the other hand, 47% of the moths captured over cotton had $\delta^{13}C$ signatures suggestive of C3 plant origins. All but one of these cotton crops used for collections was Bt cotton (the exception being a conventional cotton field used at Drayton, Namoi Valley). *Helicoverpa* larvae and pupae are usually rare in Bt cotton crops. It seems therefore highly unlikely, that many of the moths caught at these sites with C3 signatures originated from the Bt crops themselves. Redcamp (Namoi Valley), for example, had crops of sorghum, maize, sunflowers and unsprayed conventional cotton nearby (the latter being the dedicated refuge) which did have large numbers of *Helicoverpa* larvae on them in advance of the moth collections. These crops could well have provided the moths of both C3 and C4 origin caught within the Bollgard II® cotton crop at Redcamp.

A core assumption of the RMP for Bt cotton is that mating between *H. armigera* individuals is random, i.e. irrespective of their host plant origin. In particular, moths generated from refuge crops and Bt cotton should mate with each other. The work conducted in this project supports the RMP assumption by suggesting that mating between moths from separate plant sources (and within Bt cotton crops) is indeed common, and likely to occur at random, at least for *H. armigera*. Whether or not the production and / or fitness of *H. armigera* offspring might vary according to the host plant origins of their parent moths, in particular where mixed origin matings occur, is however unknown.

Our findings with *H. punctigera* are necessarily limited, but results from C stable isotope analyses that have been completed are as follows. At Shangri-la, where most *H. punctigera* were collected (n = 110), moths were predominantly of C3 origins (92%). Of the 42 mating pairs collected, 39 were C3 x C3 crosses, 0 were C4 x C4 crosses, and 8 were C3 x C4 crosses. This compares with the expected 35.50, 0.27 and 6.18 crosses respectively, and the result was not significantly different from what would be expected at random (Multinomial Test = 0.48, *P* > 0.05). Similarly for the small sample at Battery Hill where C3 and C4 moths were collected in equal numbers and there were 9 mating pairs, with 3 in each category of mating pairs, again there was no evidence for other than random mating (Multinomial Test = 1.00, *P* > 0.05). At South Callandoon, all moths were of C4 origin (with 15 mating pairs). This work suggests overall (cautiously) that *H. punctigera*, like *H. armigera*, is mating at random according to plant host origin.

The N stable isotope analyses showed no overt separation into sub-groups at any of the 3 sites. δ^{15}N means ± S,E.s were 8.23 ± 0.27, 6.82 ± 0.61, and 11.64 ± 0.58 at Shangri-la, South Callandoon and Battery Hill respectively. Whilst these means varied significantly (One –Way ANOVA, F = 15.66, $p < 0.001$, Tukey's test separated each mean from the others), this didn't of course help identify different sources of the moths, e.g. within C3 plant host origins, at any one site.

M. Whitehouse

Laboratory experiments conducted to identify cross-mating of moths from plant hosts proving intractable to discern in field (e.g. pigeon pea & cotton).

Experiment 1.

Mating success in the communal mating chamber was low (Exper. 1a: 21 pairs, 24%; Exper. 1b: 6 pairs, 8%; Exper. 1c: 5 pairs, 5%). Therefore the results of all three experiments were pooled for analysis.

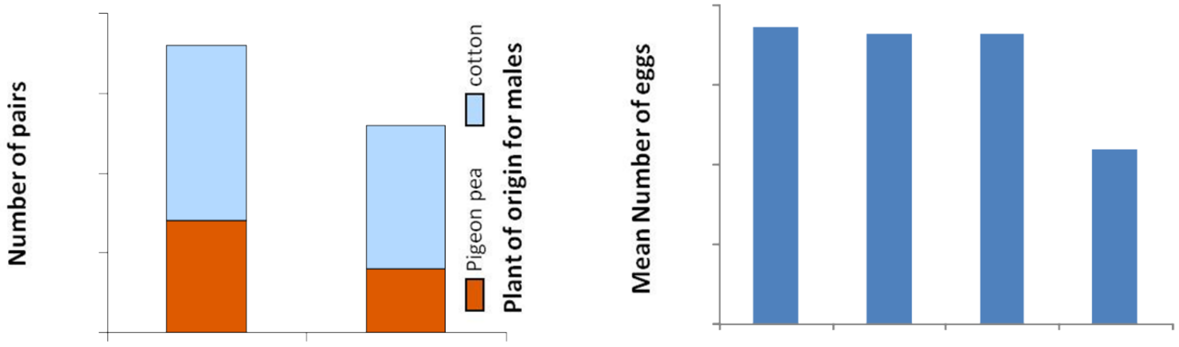


Fig. 57. The results of Experiment 1. The first graph shows the crop origins of mating couples. There was no effect of crop origin on mating preferences. The second graph shows the average number of eggs laid by mated females. The x axis label indicates to origin of the mated couple. For example, "cotpp" indicates that the male of the couple originated from cotton and the female originated from pigeon pea. The origins of the mated couple did not affect fecundity.

Moths which had been raised as larvae on either cotton or pigeon pea and given a choice showed no preference when choosing mating partners from adults raised on either crop type (Chi square =0.22, df=1; NS). The crop of origin of mated pairs did not influence the amount of fertile eggs produced by the mated females (Kruskal Wallis one way ANOVA F=0.51, df=3,27; NS; Fig. 57).Therefore it seems that the crop that a *Helicoverpa* fed on as a larvae has no effect on choice of mate as an adult.

Experiment 2a

In these experiments moths were set up in pairs, and so not given a chance to choose mates. There was concern that 12 females had been exposed to males before the experiment, so their results were removed from the analysis. Other pairs were disrupted during the experiment, and so removed from analysis. Results from Experiment 2a showed that the time to mate was not influenced by crop origin (ANOVA; male crop: F=1.6, P=0.21; female crop: F=2, P=0.17; interaction: F=0.13, P=0.72; df=1,64) and neither was the time spent mating (ANOVA; male crop: F=0.37, NS; female crop: F=17, NS; interaction: F=1.1, NS; df=1,24).

Although mated pairs produced more fertile eggs (ANOVA; F=8, P=0.006, df=1,61) the amount of fertile eggs or larvae produced by the mated females was not influenced by the crop to which she or her partner had been exposed (ANOVA; male crop:.F=2, NS; female crop: F=2.4, NS, interaction: F=0.4 NS; df=1,43). Crop type did not affect the likelihood of mating (Chi sq value = 1.72, P=0.19, n=57).

Experiment 2b
Of the 90 pairs set up, 44 mated. Of these, there was no significant effect of crop origin on their likelihood to mate (Fig. 58; Chi Square value=0.11, NS, df =1, n=44).

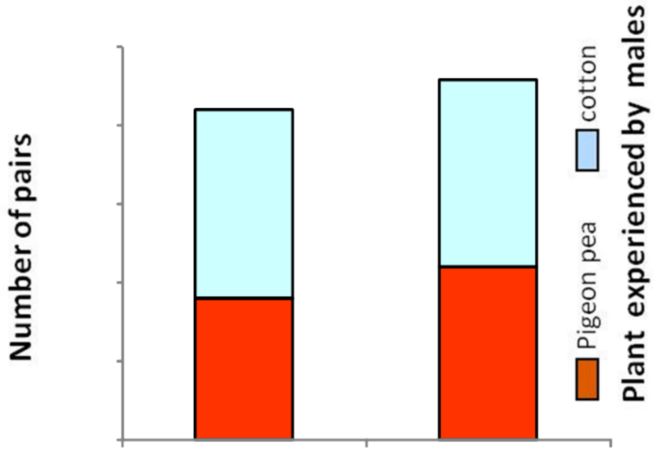


Fig. 58. The crops experienced by mated couples. There was no effect of crop type on likelihood to mate.

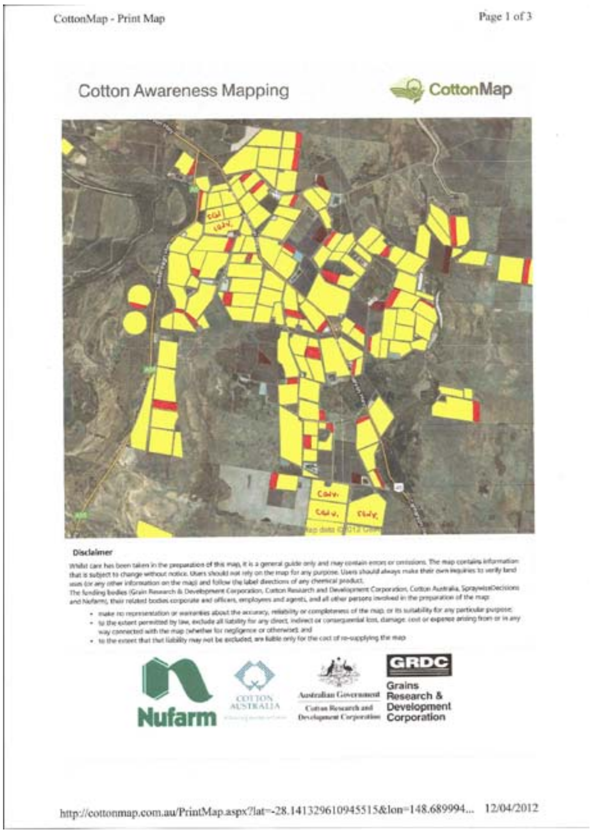
These results suggest that crop exposure as emerged adults does not influence mate choice.

Conclusions on Mate choice

There is no evidence that *Helicoverpa armigera* discriminate between mating partners on the basis of either the crop upon which they fed as juveniles, or the crop they first experience as adults. This pattern is consistant whether Helicoverpa are laboratory raised or wild types. Thus moths from pigeon pea are just as likely to mate with moths from cotton as other moths from cotton. These results support pigeon pea as a viable refuge for cotton.

4. Conduct a landscape scale evaluation of refuge crop efficacy

Distributions of refuges surveyed, 2009-12.
Fig. 59 gives an example of the distribution of cotton crops in the St George region (according to the "CottonMap" website during 2011-12, with our understanding of the distributions of refuge crops superimposed upon it (acknowledgements to Dallas King for much assistance with this information). We used this annual information to further construct the maps detailed below, and for inputs into studies of potential moth dispersal away from individual refuges.



CottonMap - Print Map

Page 1 of 3

Cotton Awareness Mapping

CottonMap

Disclaimer

Whilst care has been taken in the preparation of this map, it is a general guide only and may contain errors or omissions. The map contains information that is subject to change without notice. Users should not rely on the map for any purpose. Users should always make their own inquiries to verify land uses (or any other information on the map) and follow the label directions of any chemical product.

The funding bodies (Grain Research & Development Corporation, Cotton Research and Development Corporation, Cotton Australia, SpraywiseDecisions and Nufarm), their related bodies corporate and officers, employees and agents, and all other persons involved in the preparation of the map:

- make no representation or warranties about the accuracy, reliability or completeness of the map, or its suitability for any particular purpose;
- to the extent permitted by law, exclude all liability for any direct, indirect or consequential loss, damage, cost or expense arising from or in any way connected with the map (whether for negligence or otherwise); and
- to the extent that that liability may not be excluded, are liable only for the cost of re-supplying the map.

Nufarm

COTTON AUSTRALIA

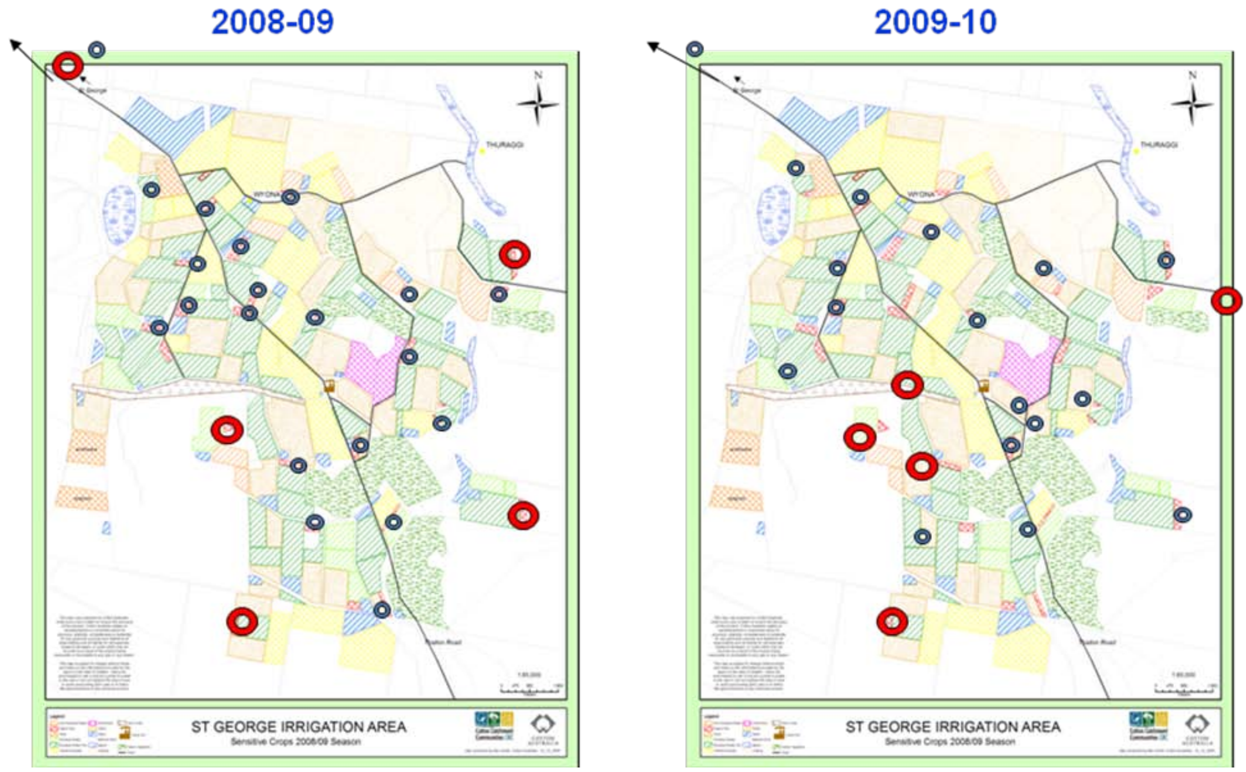
Australian Government
Cotton Research and Development Corporation

GRDC
Grains Research & Development Corporation

http://cottonmap.com.au/PrintMap.aspx?lat=-28.141329610945515&lon=148.689994... 12/04/2012

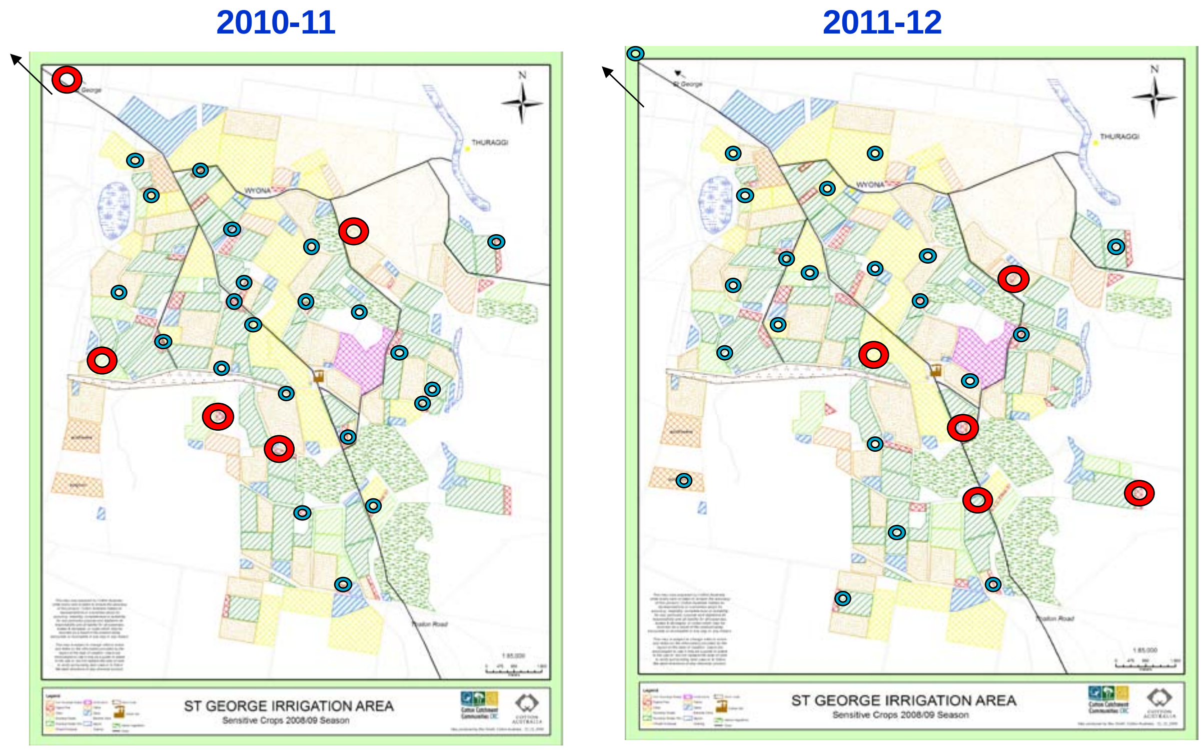
Fig. 59. Extract from the "CottonMap" website, indicating where cotton crops were grown near St George, Qld in 2011.12 (in yellow), with refuge crops added in (in red).

St George
Figs 39 & 40 illustrate the locations of the refuge crops that were sampled each year of the project. The sites used in 2008-09 are included here for reference, given data from this year will be part of the overall study on spatial efficacy of the refuges. Note, for simplicity, the background in each map is the cropping use that occurred in 2008-09, but the locations of fields per se was of course essentially the same across years. A reasonable spatial coverage of the refuge crops within the St George region was achieved in each year. Some refuges lay outside the dimensions of the map (e.g. Wagaby).


2008-09

2009-10

Fig. 60. Locations of refuges surveyed near St George in 2008-09 and 2009-10. Refuges marked in red were the most productive in that season.


2010-11

2011-12

Fig. 61. Locations of refuges surveyed near St George in 2010-11 and 2011-12. Refuges marked in red were the most productive in that season.

<u>Goondiwindi</u>

Maps for the locations of the survey sites near Goondiwindi are still in preparation and will be included in a later report to CRDC.

2009-10 St George & Goondiwindi

Overall, the attractiveness of the pigeon pea refuge crops was slightly higher near St George (mean attractiveness index throughout the season = 3.5), compared with near Goondiwindi (3.1) (Figs 62 & 65). It was thus no surprise that egg lays were higher at St George (overall mean of 5.8 eggs / M) compared with Goondiwindi (3.6 / M) (Figs 63 &66), and there were more live pupae in the soil at St George (2.3 / M) compared with Goondiwindi (0.8 / M) (Figs 64 &67). During 2008-09, the average abundance of live pupae / M in surveys of pigeon pea refuge crops in the St George region was 0.8 / M. We commented in the Final Report for Project 1.01.52 that this was at the bottom end of the range (0.8-2.3 live pupae / M [mean = 1.4 / M]) recorded during more extensive surveys between 1996-2003. At the time, it seemed problematic if the abundance of *Helicoverpa* live pupae had reduced through time under pigeon pea. This result for 2009-10 provided no support for such a decline. In fact, the 2009-10 result was at the top end of the long term range in densities. The 2009-10 result for the Goondiwindi sites is at the low end of the data range. This highlights how variable these abundances are (see later in this report also for generic comments made about long term trends in refuge performance). The observed variations in the data very probably also reflected prevailing weather, and its impacts on refuge crop performance. During 2009-10, summer rainfall was good in the St George region and the quality of the pigeon pea refuge crops, and their performance re *Helicoverpa*, reflected that. Goondiwindi was in rather a "rain-shadow" for the early part of the season. The performances of the pigeon pea crops there were generally poor until late in the season, when rainfall increased. In the absence of prevailing rainfall, a concerted effort to maintain healthy refuge crops (irrigation) seems highly likely to reap benefits for *Helicoverpa* production.

Yet again (cf data for 2008-09), it was obvious how variable the abundances of eggs and live pupae of *Helicoverpa* are amongst pigeon pea refuge crops within regions like St George and Goondiwindi.

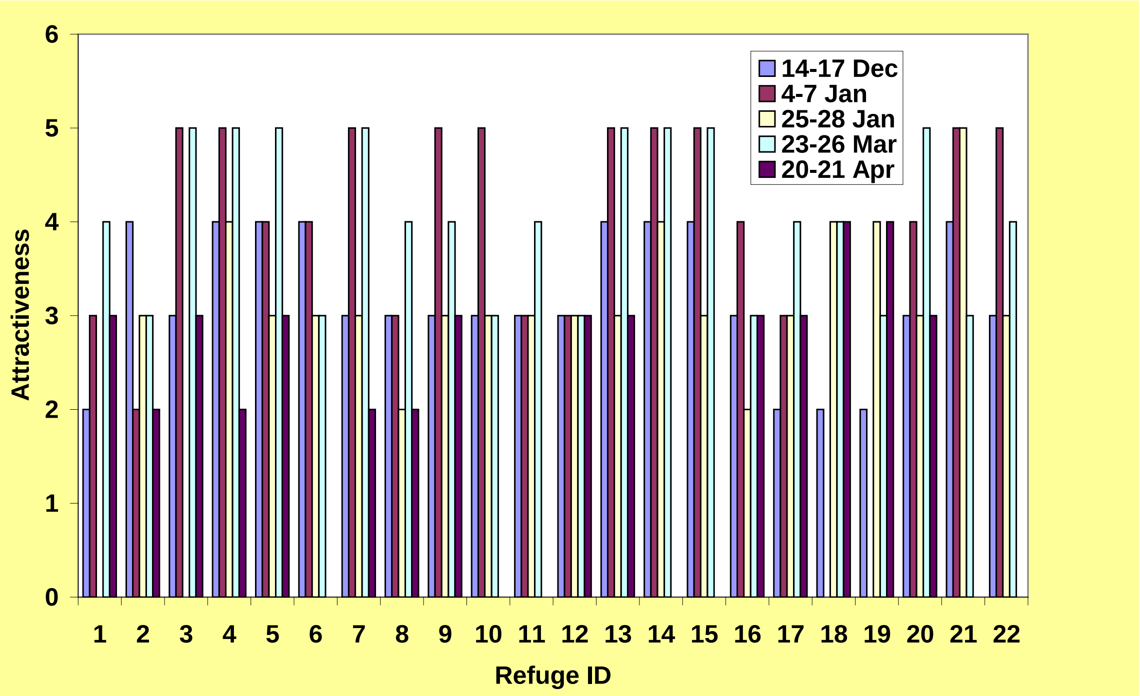


Fig. 62. Attractiveness estimates (Monsanto rank) for *Helicoverpa* oviposition at 22 refuge crops near St George (2009-10), on 5 survey occasions.

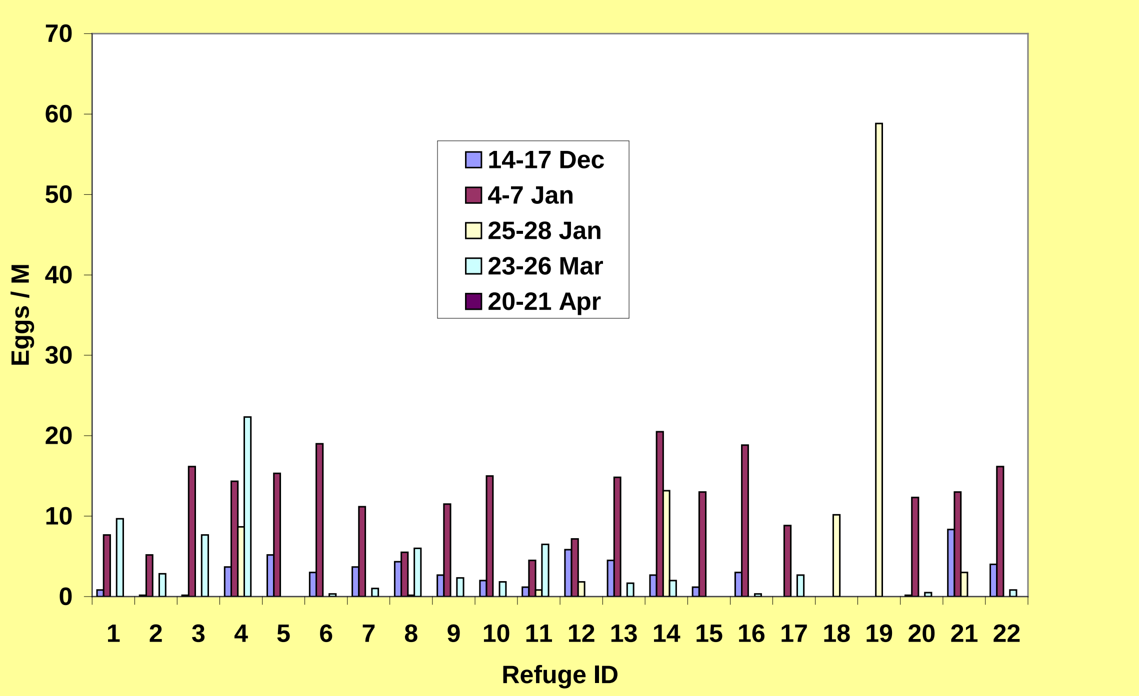


Fig. 63. Numbers of *Helicoverpa* eggs on 22 pigeon pea refuge crops near St George (2009-10), on 4 survey occasions (note no samples taken 20-21 Apr).

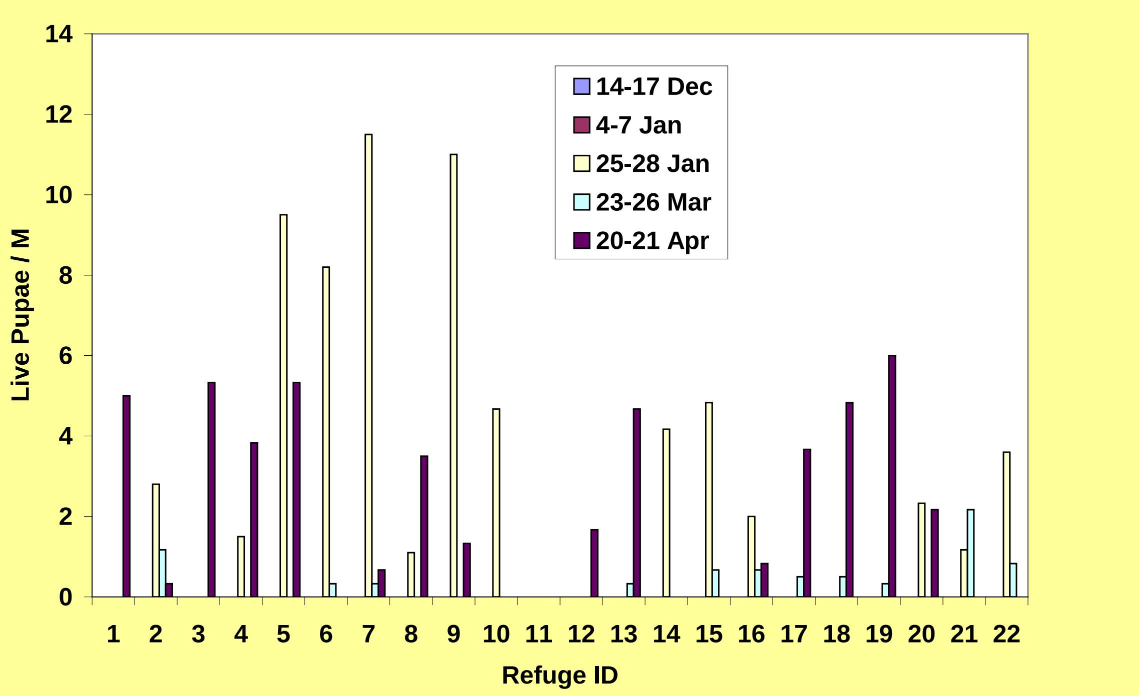


Fig. 64. Numbers of *Helicoverpa* pupae beneath 22 pigeon pea refuge crops near St George (2009-10), on 3 survey occasions (note no samples taken 14-17 Dec & 4-7 Jan).

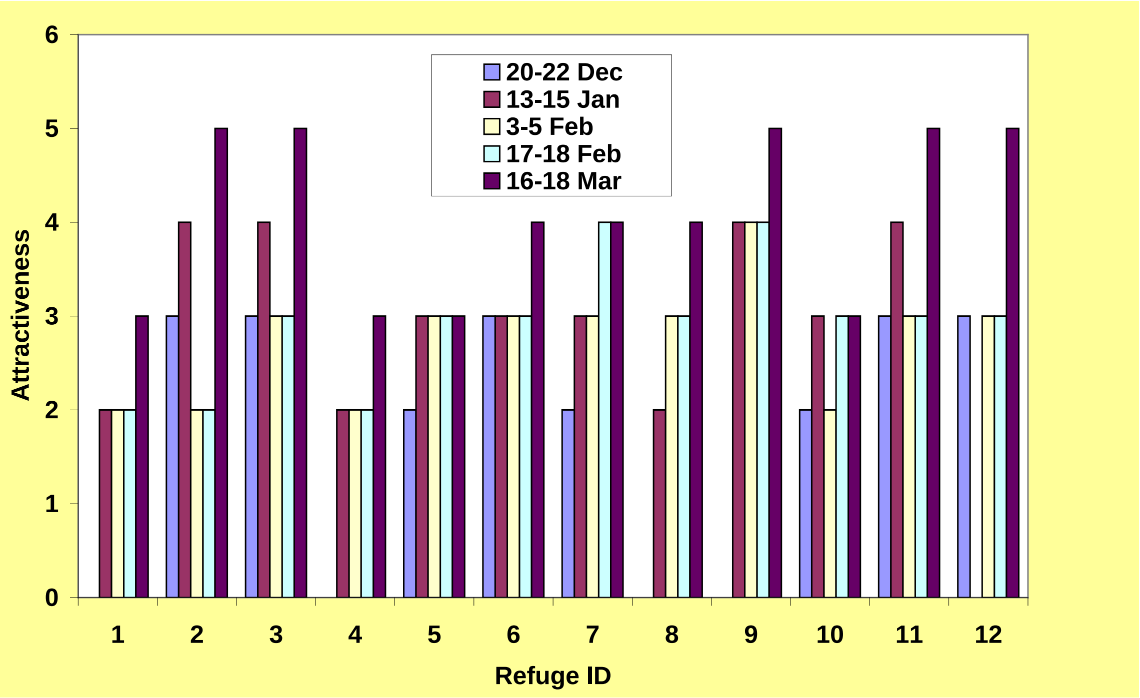


Fig. 65. Attractiveness estimates (Monsanto rank) for *Helicoverpa* oviposition at 12 refuge crops near Goondiwindi (2009-10), on 5 survey occasions.

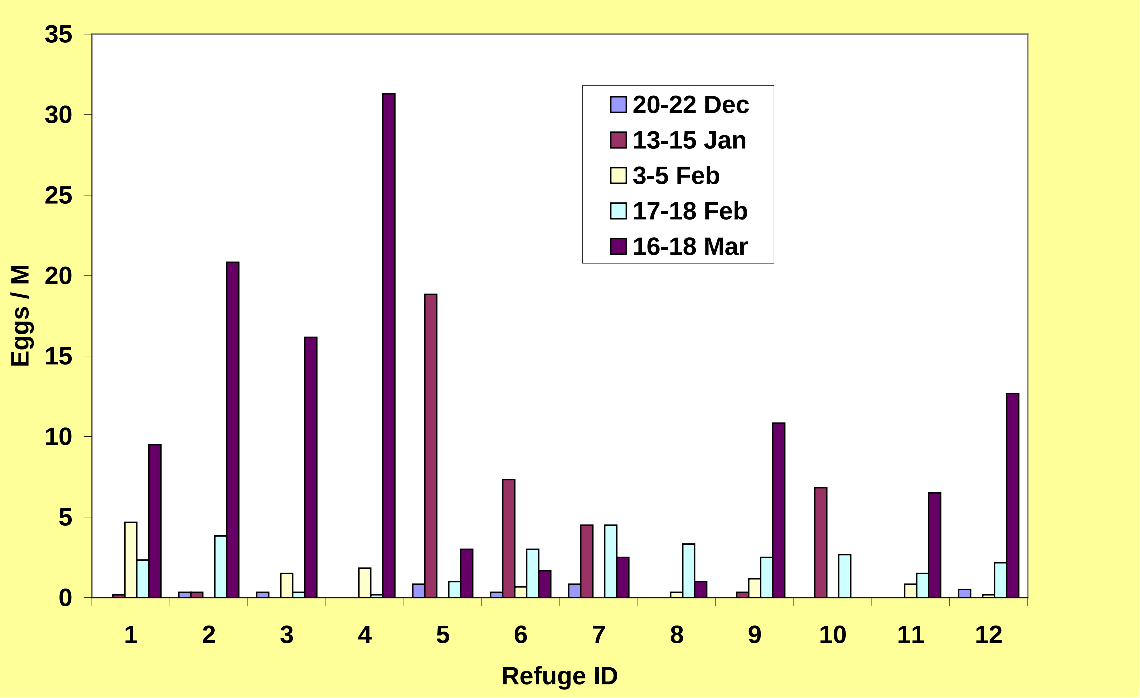


Fig. 66. Numbers of *Helicoverpa* eggs on 12 pigeon pea refuge crops near Goondiwindi (2009-10), on 5 survey occasions.

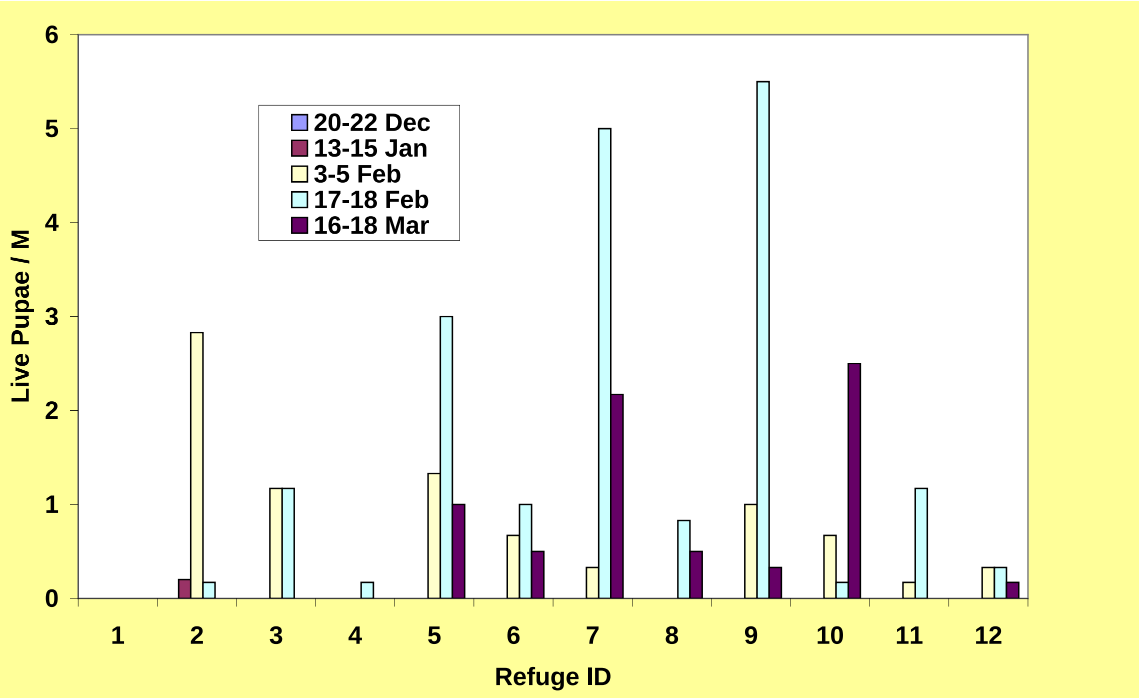


Fig. 67. Numbers of *Helicoverpa* pupae beneath 12 pigeon pea refuge crops near Goondiwindi (2009-10), on 4 survey occasions (note no samples taken 20-22 Dec.).

2010-11 St George & Goondiwindi

We continued to survey refuges for *Helicoverpa* etc at both St George and near Goondiwindi in 2010-11, in a similar way to that conducted in previous years.

At St George all refuges were pigeon pea, and the numbers of such crops increased on previous years, reflecting increased plantings of Bt cotton. We couldn't sample all refuges, but instead sampled most (n = 26 + one site at nearby Wagaby) (three sites, 7, 12 & 19, were dropped out as the season progressed). Ideally, we tried to visit all sites every three weeks to ensure we had accurately documented the activity within the refuges and to compare confidently on a spatial level. However, floods prevented access to the town over January, which unfortunately is a significant period, so there are some gaps in this year's data. In total, we made four visits to each refuge crop, but could only sample pupae on 3 occasions (the soil was too wet for effective pupae sampling on the other).

The quality of refuges was again quite variable (as in previous years), as was the destruction caused by grasshoppers (the main risk to successful moth production this year). In some instances, the grasshoppers were significantly defoliating the refuges, and some growers elected to spray their refuge with insecticide because of this. Many refuges became highly attractive at the end of March in response to late season rain, and in some cases good *Helicoverpa* spp. populations were generated in response. Generally, the majority of refuges were looked after reasonably well with regular watering and good weed management.

The mean overall attractiveness (rank) of the pigeon pea crops to *Helicoverpa* spp. (using the Monsanto system) was 3.73 (Fig. 68). Attractiveness varied between visits (Kruskal-Wallis H = 8.57, $P < 0.05$), being greatest in March and least in late February. The abundance of eggs on the pigeon pea plants varied markedly between sites, and in time (being more common early in the season) (Fig. 69). Likewise, the abundance of live and emerged pupae varied greatly between sites and in time (Figs 70 &71), as has been noted in previous years. The mean number of live pupae over the whole season was 0.82 / m. This result was much less than that obtained in the 2009-10 season (2.3 / m), and comparable with that reported for

2008-09 (0.75 / m). The mean number of pupae found in 2010-11 was again at the lowest end of the range found during 1996-2003 for pigeon pea (overall mean then = 1.4 live pupae / m [range = 0.8-2.3]), when relativities in likely moth productivities for different refuge crop options were assessed (Baker et al 2008, *Aust J. Agric. Res.*).

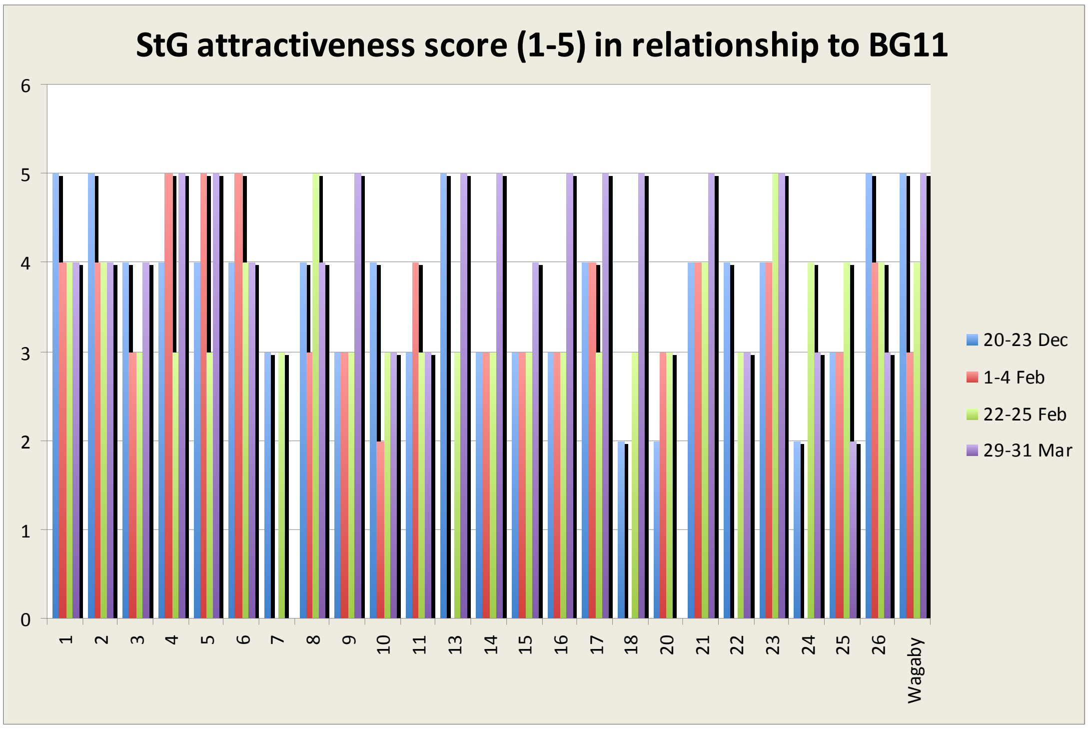


Fig. 68. Attractiveness estimates (Monsanto rank) for *Helicoverpa* oviposition within pigeon pea refuge crops near St George (2010-11), on 4 survey occasions.

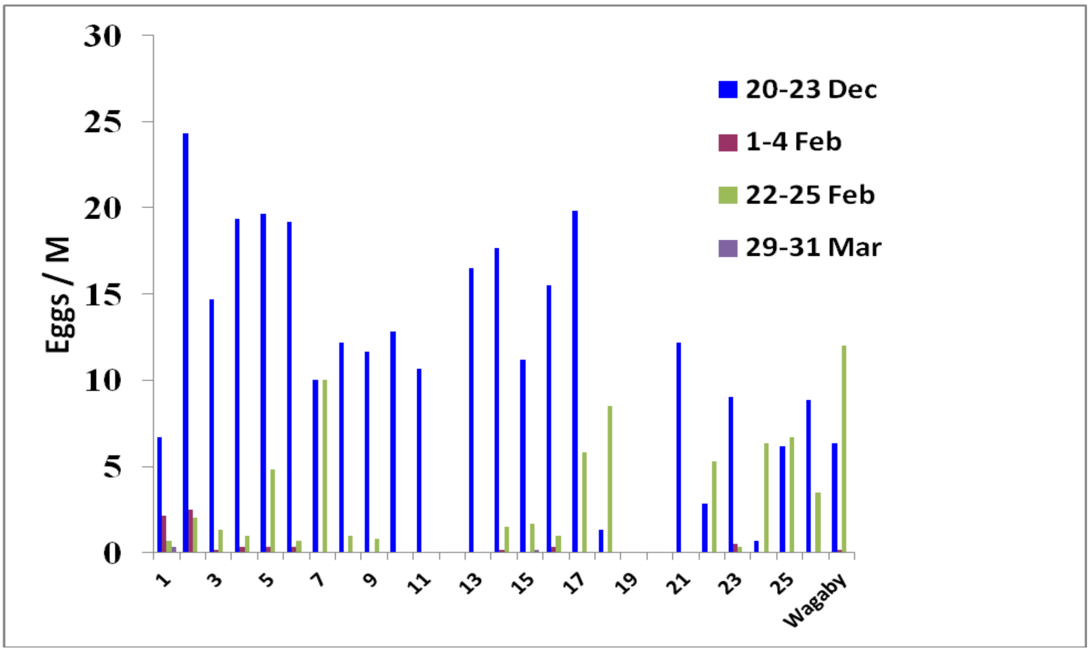


Fig. 69. Numbers of *Helicoverpa* eggs on pigeon pea refuge crops near St George (2010-11), on 4 survey occasions.

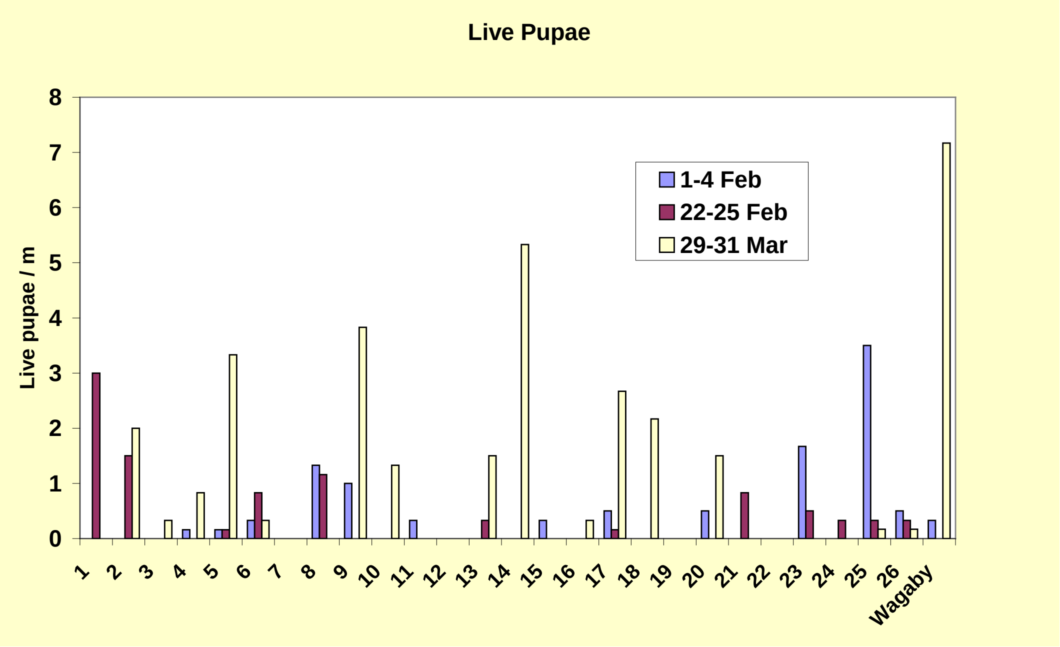


Fig. 49. Numbers of live *Helicoverpa* pupae beneath pigeon pea refuge crops near St George (2010-11), on 3 survey occasions.

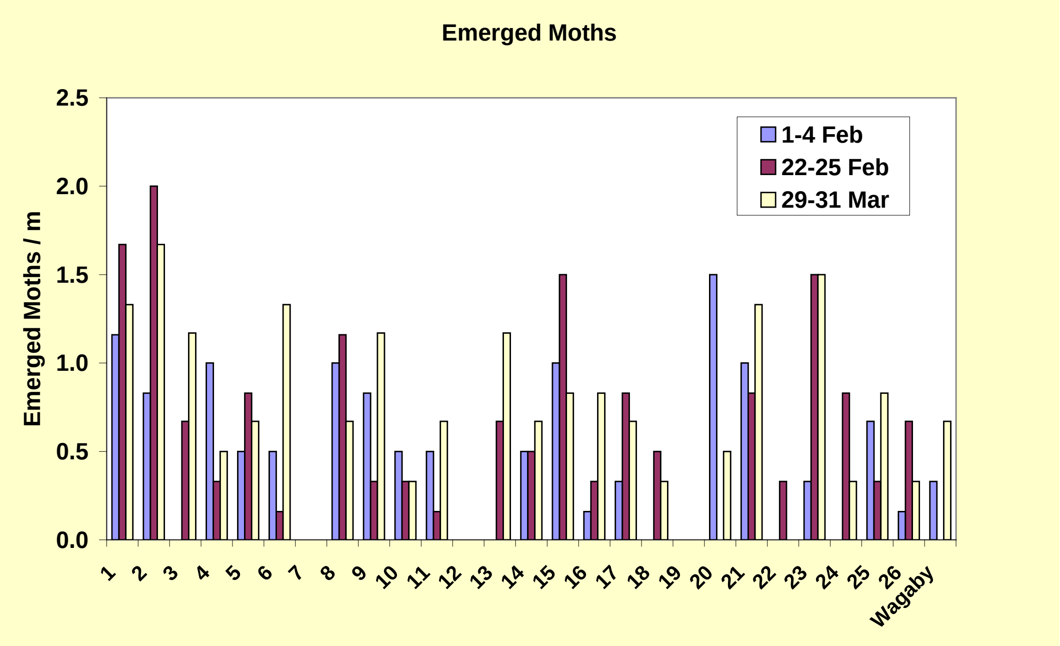


Fig. 71. Numbers of already emerged *Helicoverpa* pupae (as moths) beneath pigeon pea refuge crops near St George (2010-11), on 3 survey occasions.

The numbers of pupal case remains which provided evidence of recently emerged moths, whilst variable between visits, were perhaps more consistent across individual refuges – which would be expected, given they effectively "sample" the pupal population over a longer time period.

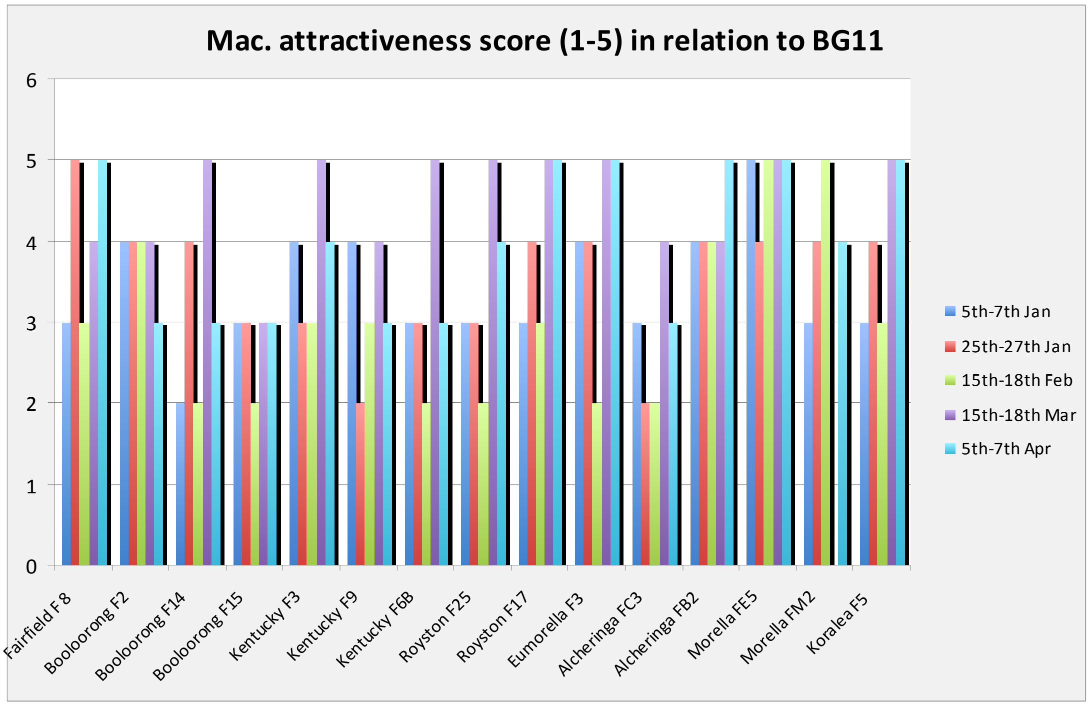


Fig. 72. Attractiveness estimates (Monsanto rank) for *Helicoverpa* oviposition at 15 refuge crops (9 pigeon pea & 6 unsprayed cotton) near Goondiwindi (2010-11), on 5 survey occasions. The refuges at Booloorong F2, Booloorong F15, Kentucky F3, Kentucky F9, Kentucky F6B & Alcheringa FC3 were unsprayed cotton. The others were pigeon pea.

Near Goondiwindi, 9 pigeon pea and 6 unsprayed, conventional cotton refuges were surveyed, giving an opportunity to compare the current performances of the two refuge options, as well as measure inherent variability and spatial pattern amongst refuges within the region. Cotton loopers infested all the unsprayed cotton refuges and by the end of the season in some cases spectacularly defoliated the plants. Flooding was an issue within some of the refuges (and Bt crops) located close to the river where plants were killed by slowly receding water. Our sampling of these refuges resulted in reduced areas available but enough to suffice our needs. In fact there was substantial regrowth occurring with the plants that did survive the flood waters, so they were generally attractive for longer. Refuge management within the study area was generally good. However, as is often the case in most regions in our experience, pigeon pea refuges often missed an irrigation. This is, we believe, unacceptable : continuous lushness and attractiveness are paramount. Rain cannot be relied upon.

There was a tendency for the pigeon pea refuges to be ranked more attractive to *Helicoverpa* spp. (using the Monsanto system) than the unsprayed cotton refuges (mean ranks 3.98 cf 3.42 respectively; Wilcoxon Rank Sum Test (Normal approximation, with Continuity Correction) = 2.53, $P < 0.05$; where variability in time ignored) (Fig. 72). Attractiveness varied between visits, both for pigeon pea (Kruskal-Wallis $H = 16.06$, $P < 0.005$) and unsprayed cotton ($H = 12.63$, $P < 0.05$). In both cases, attractiveness was greatest in March and least in February.

Looking at pigeon pea and unsprayed cotton refuges separately, egg numbers were perhaps more stable across individual sites within cotton (Fig. 73), but the data were too variable to otherwise discern any difference between the two refuge types. However, it was clear that the abundance of live pupae was greater within pigeon pea cf cotton refuges (Fig. 74), as has been shown before (overall means of 0.75 & 0.26 live pupae / m respectively). A similar trend was apparent with pupal cases representing previously emerged moths (0.64 & 0.45 emerged moth pupae / m respectively; data not plotted here for brevity). The abundance of live pupae under pigeon pea refuges was reasonably similar this year at St George and Boggabilla (0.82 cf 0.75 pupae / m respectively).

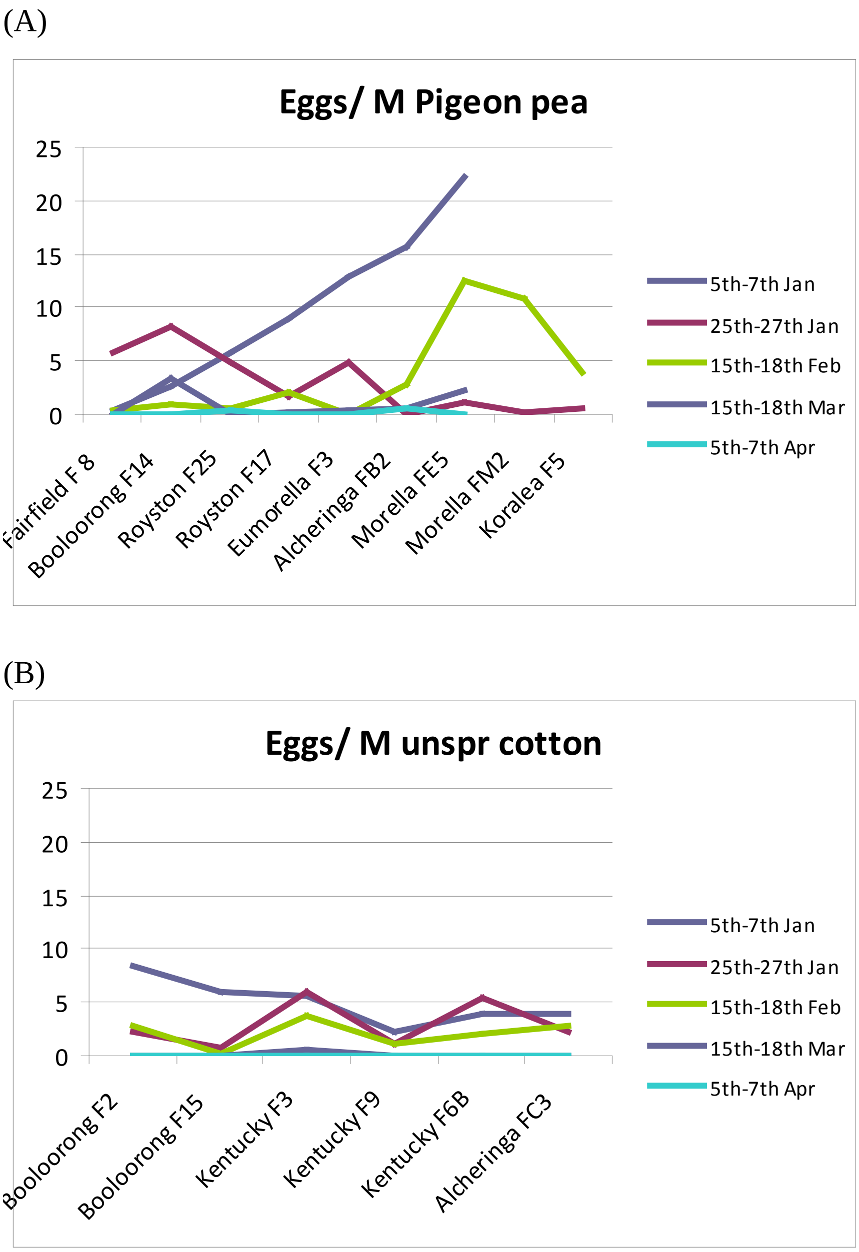
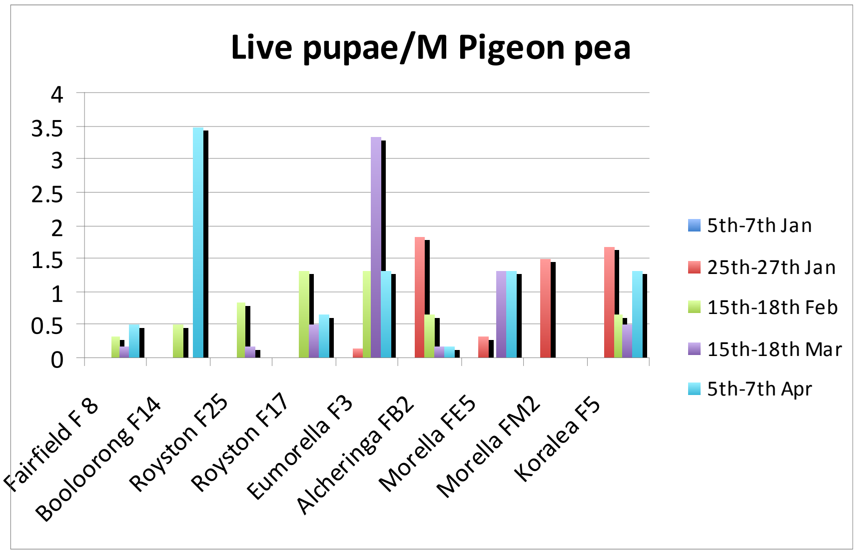


Fig. 73. Numbers of *Helicoverpa* eggs on pigeon pea (A) & unsprayed cotton (B) refuge crops near Goondiwindi (2010-11), on 5 survey occasions.

(A)



(B)

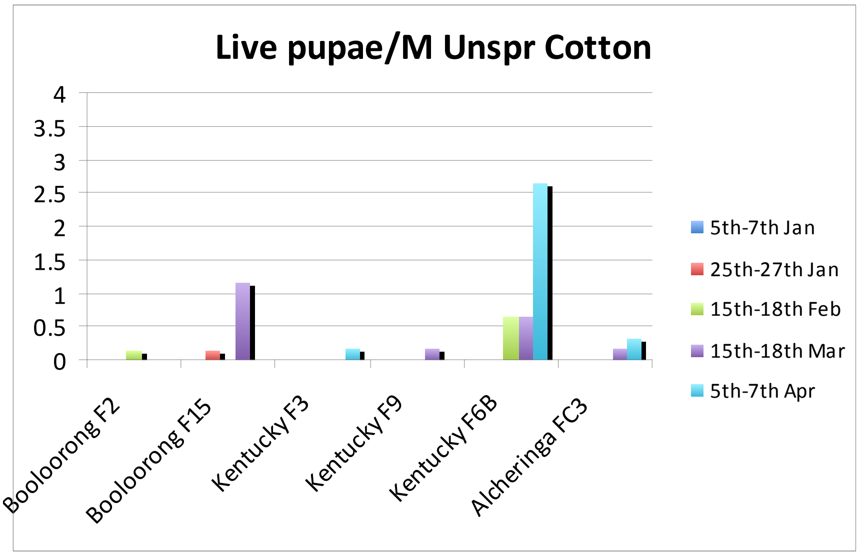


Fig. 74. Numbers of live *Helicoverpa* pupae beneath pigeon pea (A) & unsprayed cotton (B) refuge crops near Goondiwindi (2010-11), on 5 survey occasions.

2011-12 St George & Goondiwindi

Surveys at St George proved more difficult in 2011-12 because of yet another flood. We managed to survey on 3 occasions (one less for pupae), but not all fields were accessible on each occasion (and one site was dropped out during the work). Surveys near Goondiwindi proved more practical (4 visits).

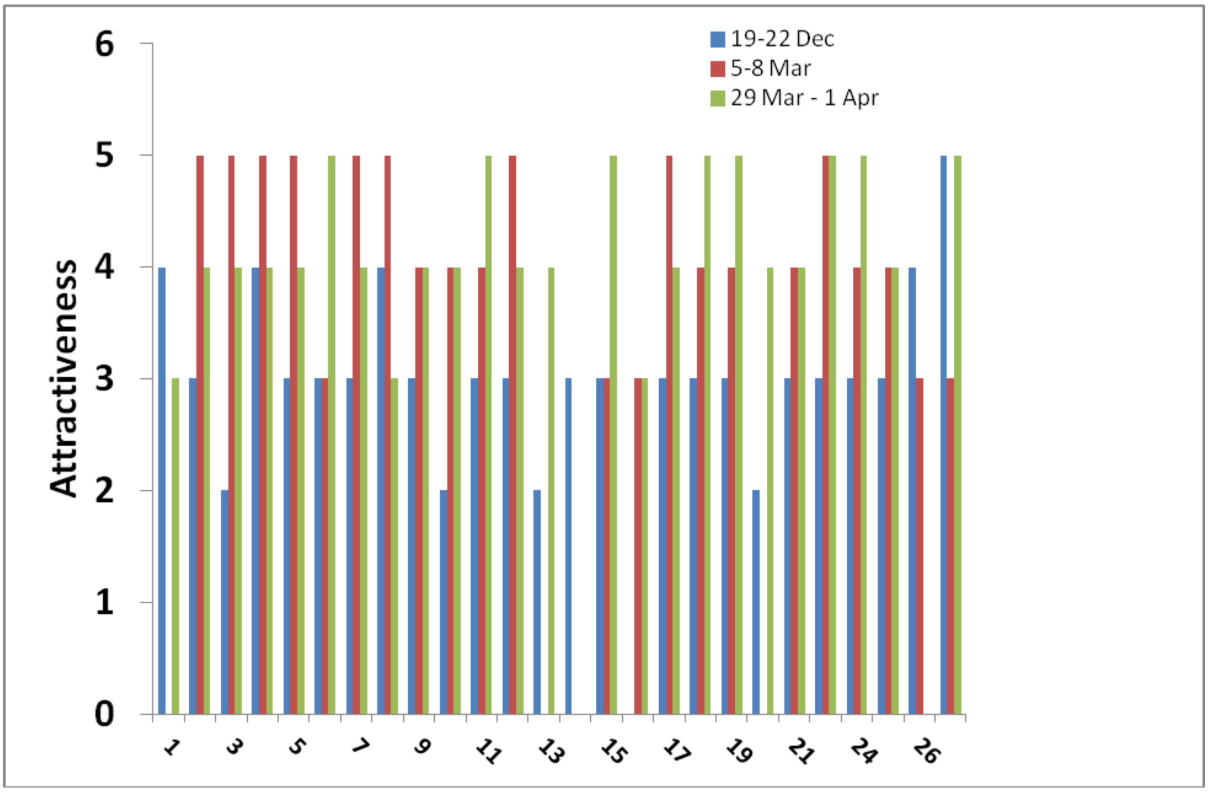


Fig. 75. Attractiveness estimates (Monsanto rank) for *Helicoverpa* oviposition within pigeon pea refuge crops near St George (2011-12), on 3 survey occasions.

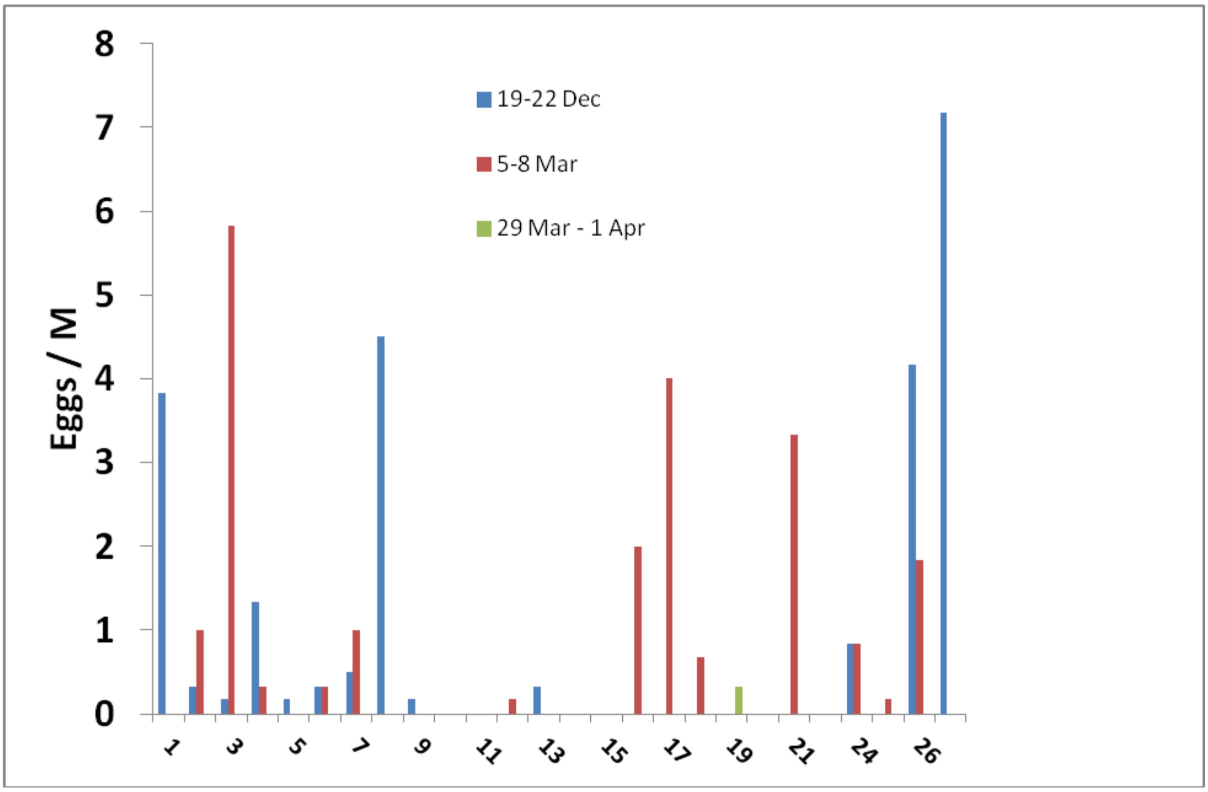


Fig. 76. Numbers of *Helicoverpa* eggs on pigeon pea refuge crops near St George (2011-12), on 3 survey occasions

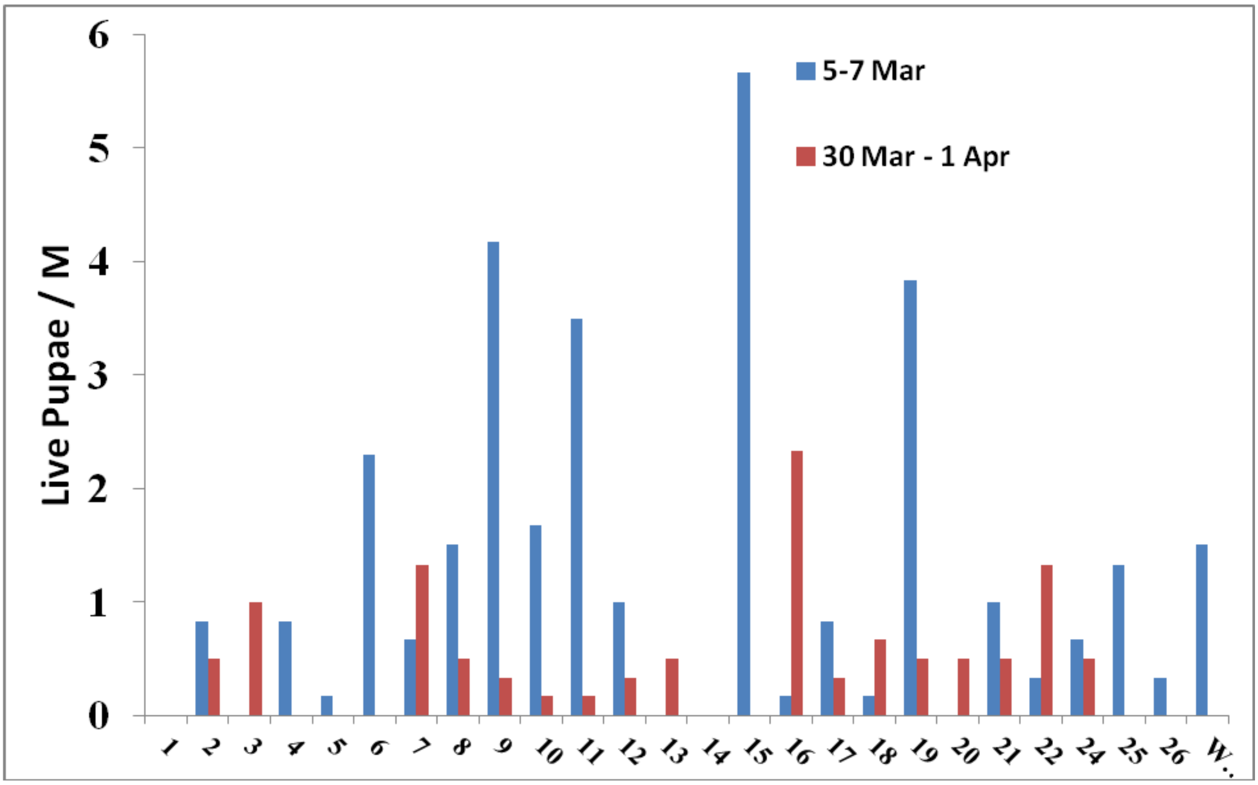


Fig. 77. Numbers of live *Helicoverpa* pupae beneath pigeon pea refuge crops near St George (2011-12), on 2 survey occasions.

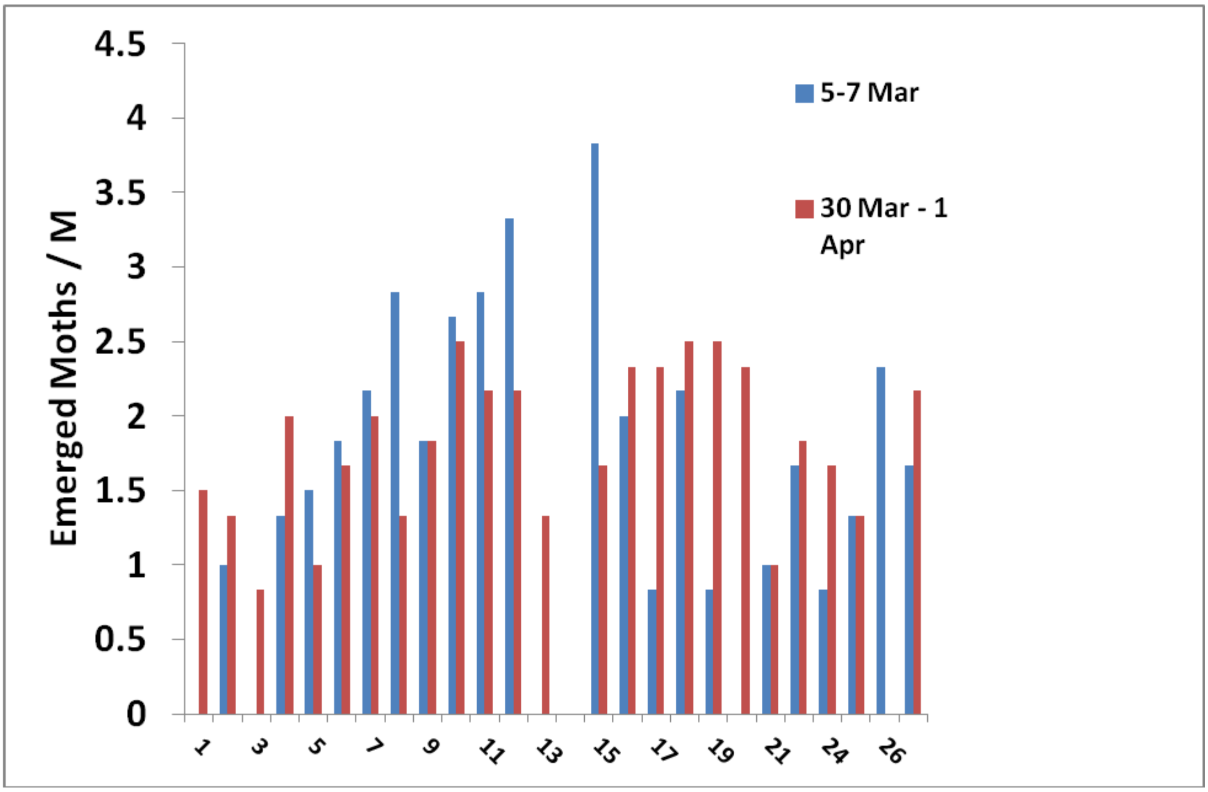


Fig. 78. Numbers of already emerged pupae of *Helicoverpa* (as moths) beneath pigeon pea refuge crops near St George (2011-12), on 2 survey occasions.

The mean overall attractiveness (rank) of the pigeon pea crops to *Helicoverpa* spp. (using the Monsanto system) was 3.80 (Fig. 75). Attractiveness varied between visits (Kruskal-Wallis H = 25.68, P < 0.01), being greatest in March-April and least in December. The abundance of eggs on the pigeon pea plants varied markedly between sites, and in time (being very rare in late March – early April (Fig. 76). Likewise, the abundance of live (and emerged) pupae varied greatly between sites and in time (Figs 77 &78), as has been noted in previous years. The mean number of live pupae over the whole season was 0.98 / m. This result was again lower than the long-term average reported for pigeon pea (1.4 / M) during 1996-2003.

At Goondiwindi in 2011-12, 11 pigeon pea and 3 unsprayed, conventional cotton refuges were surveyed. Generally, these refuges were attractive throughout the season, being assisted by frequent rains early to mid-season. The addition of a timely irrigation later in the season at many sites maintained that attractiveness. But *Helicoverpa* numbers were light this year. There were too few cotton refuges to allow meaningful comparisons of their performance with that of the pigeon pea refuges (Figs 79-82), but there seemed to be a general tendency for pigeon pea to carry more *Helicoverpa*.

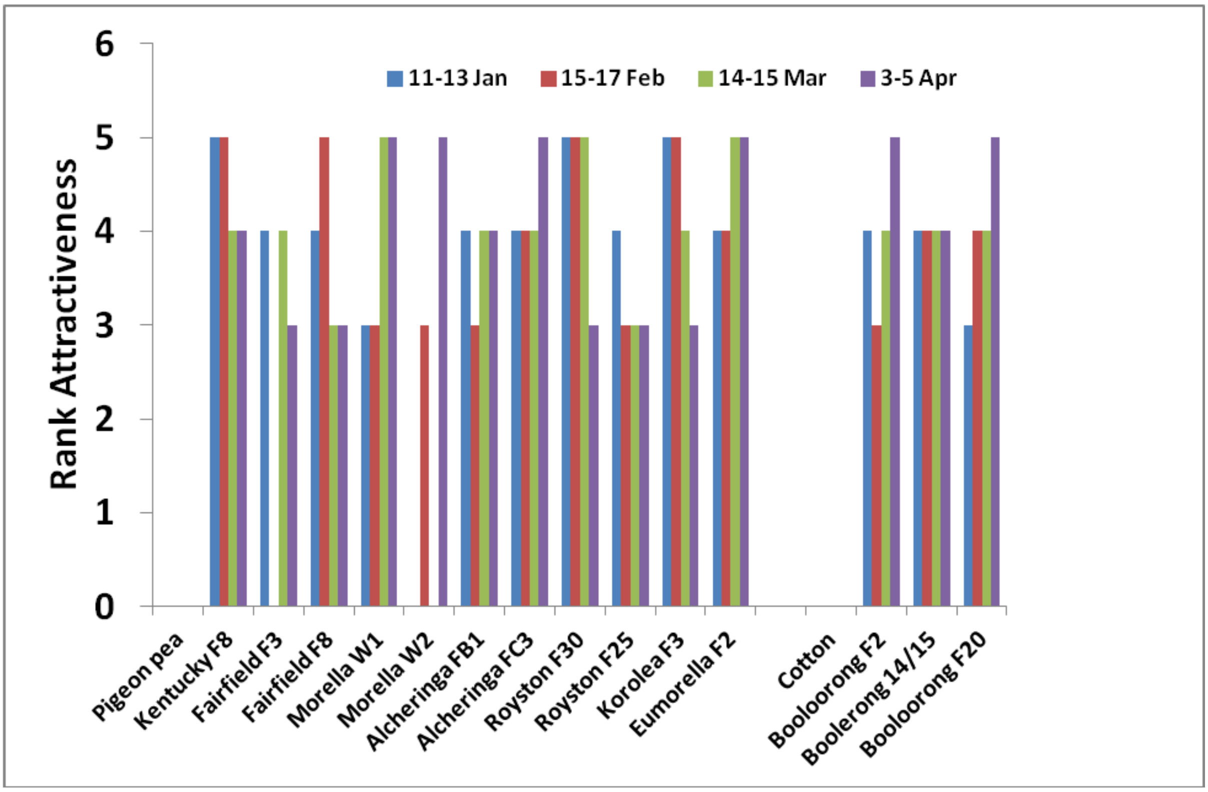


Fig. 79. Attractiveness estimates (Monsanto rank) for *Helicoverpa* oviposition at 14 refuge crops (9 pigeon pea & 3 unsprayed cotton) near Goondiwindi (2011-12), on 4 survey occasions.

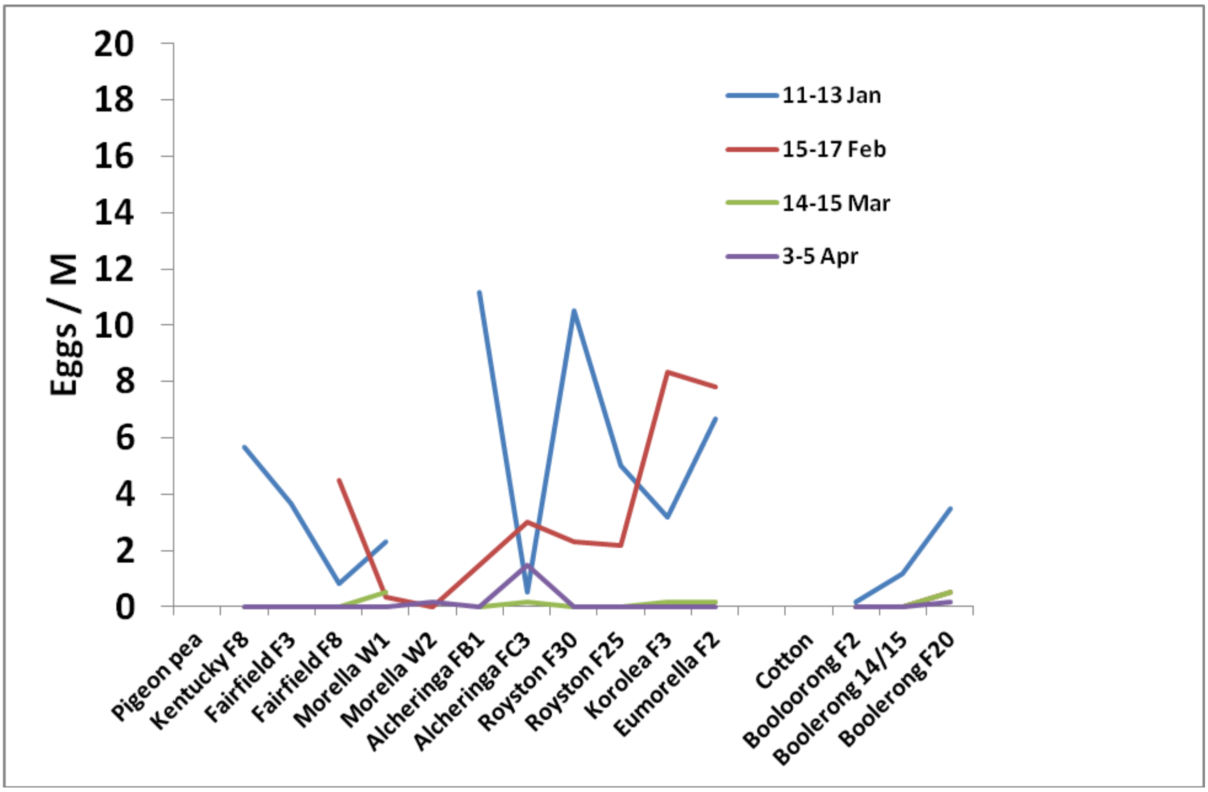


Fig. 80. Numbers of *Helicoverpa* eggs on 14 refuge crops (9 pigeon pea & 3 unsprayed cotton) near Goondiwindi (2011-12), on 4 survey occasions.

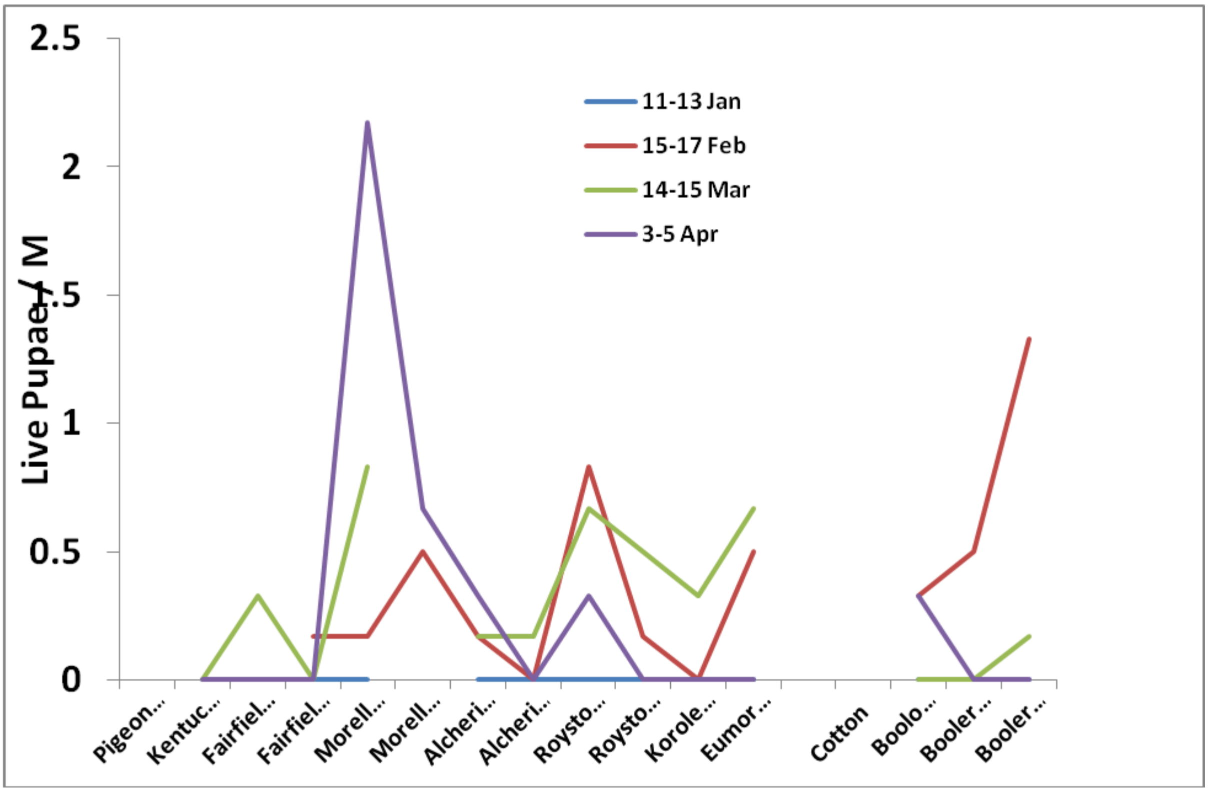


Fig. 81. Numbers of live *Helicoverpa* pupae beneath 14 refuge crops (9 pigeon pea & 3 unsprayed cotton) near Goondiwindi (2011-12), on 4 survey occasions.

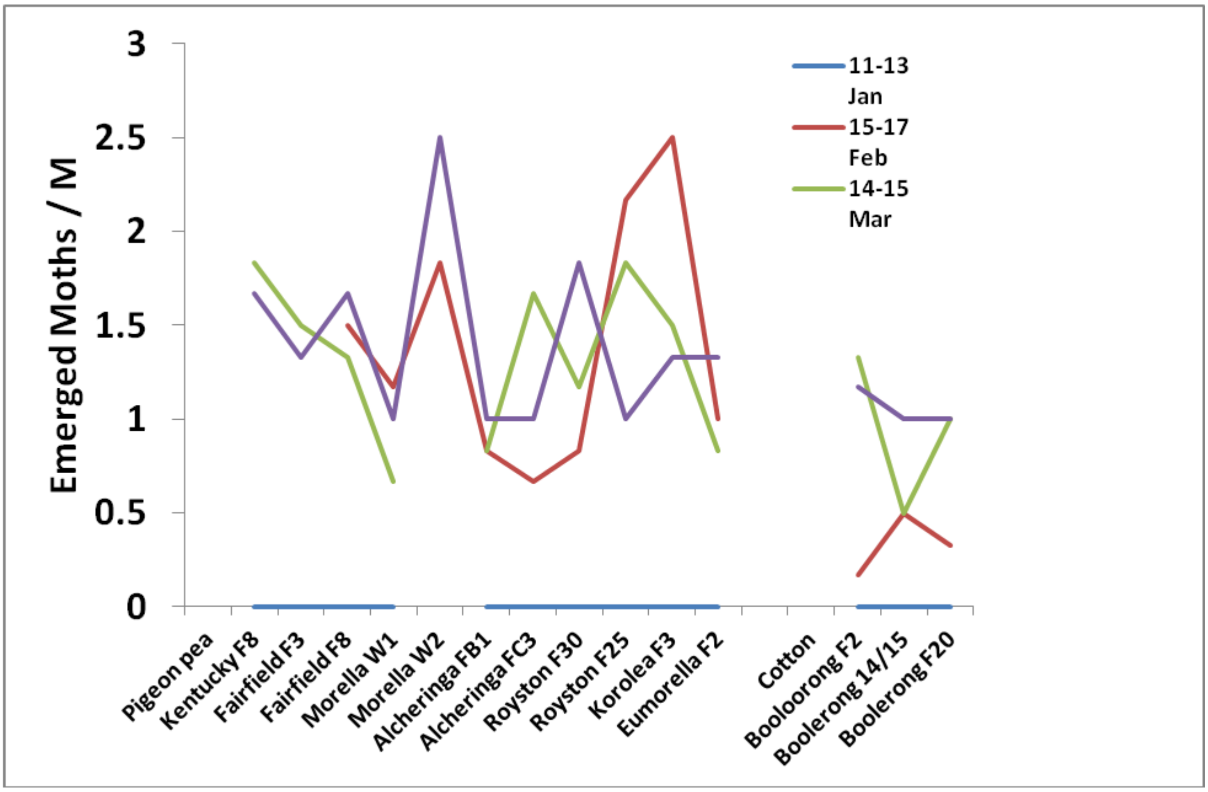


Fig. 82. Numbers of already emerged *Helicoverpa* pupae (as moths) beneath 14 refuge crops (9 pigeon pea & 3 unsprayed cotton) near Goondiwindi (2011-12), on 4 survey occasions.

Capacity for refuge crops to service Helicoverpa *outputs from Bt cotton crops.*
The moth production (using pupae numbers as surrogates) from refuges was highly variable (see earlier Figs, 60-61). In particular at St George, where more data were obtained, the most productive refuges tended to occur near the periphery of the cropping area. An explanation for this apparent pattern, if real, is not obvious. One possible explanation is that moths that are produced within the cropping region (presumably more so than in surrounding regions) disperse and turn back at the cropping boundaries, thereby accumulating near the regional edges. Alternatively, moths may benefit from feeding on nectar from trees and shrubs growing more abundantly in these peripheral areas. Whatever, we are currently modelling the degree of potential coverage of the crop production regions of St George and Goondiwindi (Boggabilla) from the refuges we have sampled, using our pupae abundance data as surrogates for moth production and dispersal kernels according to wind direction and speed (dispersal initially being assumed to be random below a threshold speed, and directional [down wind] above the threshold – as has been used by previous authors). Outcomes will be provided in the Full Report, due late in 2012 to CRDC.

Wind data (direction and speed) have been sourced from Bureau of Meteorology records (e.g. see Fig. 83 for St George). We are using data recorded nightly at 21.00 hr, because it best matches the nocturnal flight behaviour of *Helicoverpa*, and the period December through to April each year, to match the timings of our sampling events in the field. Wind direction was predominantly from the north and east at this time.

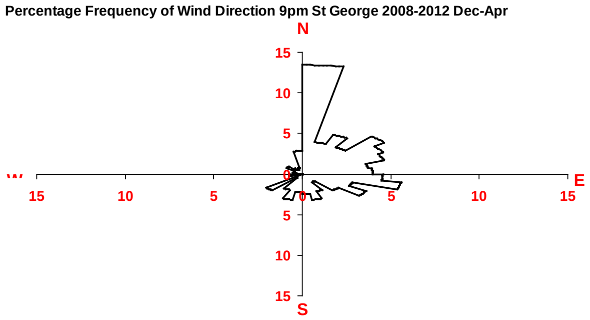


Fig. 83. Frequency of wind direction recorded at St George Airport, Qld between December and April (inclusive) from 2008 - 2012

5. <u>Provide a holistic appraisal of costs/ benefits of key refuge crop options</u>

G. Baker & C. Tann

Comparisons of refuge (pigeon pea & cotton) performance – pests & beneficial species
We have now surveyed a large number of pigeon pea (n = 474) and unsprayed conventional cotton (n = 246) refuge crops for *Helicoverpa* pupae (surrogates of moth production) over many years (1996-2012) and in several valleys, in particular during the deployment of both Ingard and Bollgard II Bt cotton (Fig. 84). These individual fields were surveyed for pupae up to 6 times per season, such that overall 7,220 M of crop row were sampled beneath pigeon pea, and 4,701 M beneath unsprayed conventional cotton. In almost all years, pigeon pea surpassed unsprayed conventional cotton in the total number of pupae recorded across all sites, and indeed the overall average numbers of pupae / m^2 (1.26 for p pea, 0.47 for conv. cotton; where data for individual years averaged) reasonably approximates the expectation set during development of the RMP (i.e. 2 : 1). Whilst the abundance of pupae varied greatly between years, there is no indication in the data of a temporal change in abundance.

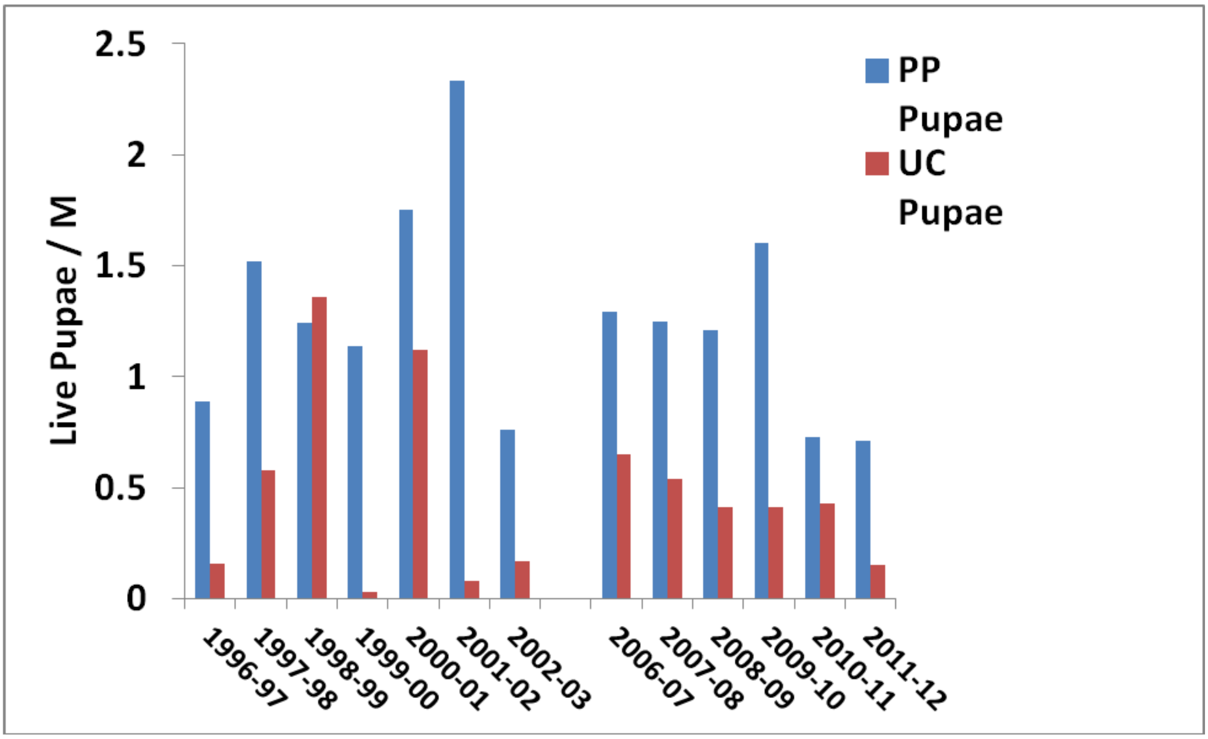


Fig. 84. Mean numbers of live *Helicoverpa* pupae collected during surveys of pigeon pea and unsprayed conventional cotton refuge crops, 1996-2012.

The pupae collected during these surveys were reared to determine levels of parasitism (overall, 7,711 were reared from beneath pigeon pea and 1,505 from beneath cotton) (Fig. 85). Most notably, the levels of parasitism of pupase within pigeon pea refuge crops have increased during the deployment of Bollgard II compared with what occurred at the time of Ingard (for comparisons of 2006-2011 with 1996-2003; means of 49.7 ± 7.0% and 21.4 ± 5.2% respectively, Wilcoxon Rank Sum Test [Normal approximation, with Continuity Correction] = 2.21, $P < 0.05$). On the other hand, although there seems to be a similar trend in the data for cotton refuges this was not significant (means of 40.8 ± 5.2 % and 22.2 ± 9.2 %, WRS test = 1.50, $P > 0.05$, for the same periods respectively). The outcome from the cotton data seems highly likely to have be influenced by the high level of parasitism observed in 1999-2000. During some years, (199-00, 2001-02, 2002-03 & 2006-07), few pupae (< 50 each year) were reared from collections beneath cotton refuges. If these years with low sample size were omitted from the analysis, then there was a significant difference between % pupal parasitism between the Ingard and Bollgard II cotton era (WRS test = 2.33, $P < 0.05$). Perhaps this temporal difference in parasitism, at least in pigeon pea refuges, reflects various changes in landscape management over that time, in particular the well-recognised reduction in pesticide use. As in previous years the dominant parasites were *Heteroplema* and Tachinidae, the former being generally dominant but the latter becoming very common in recent years.

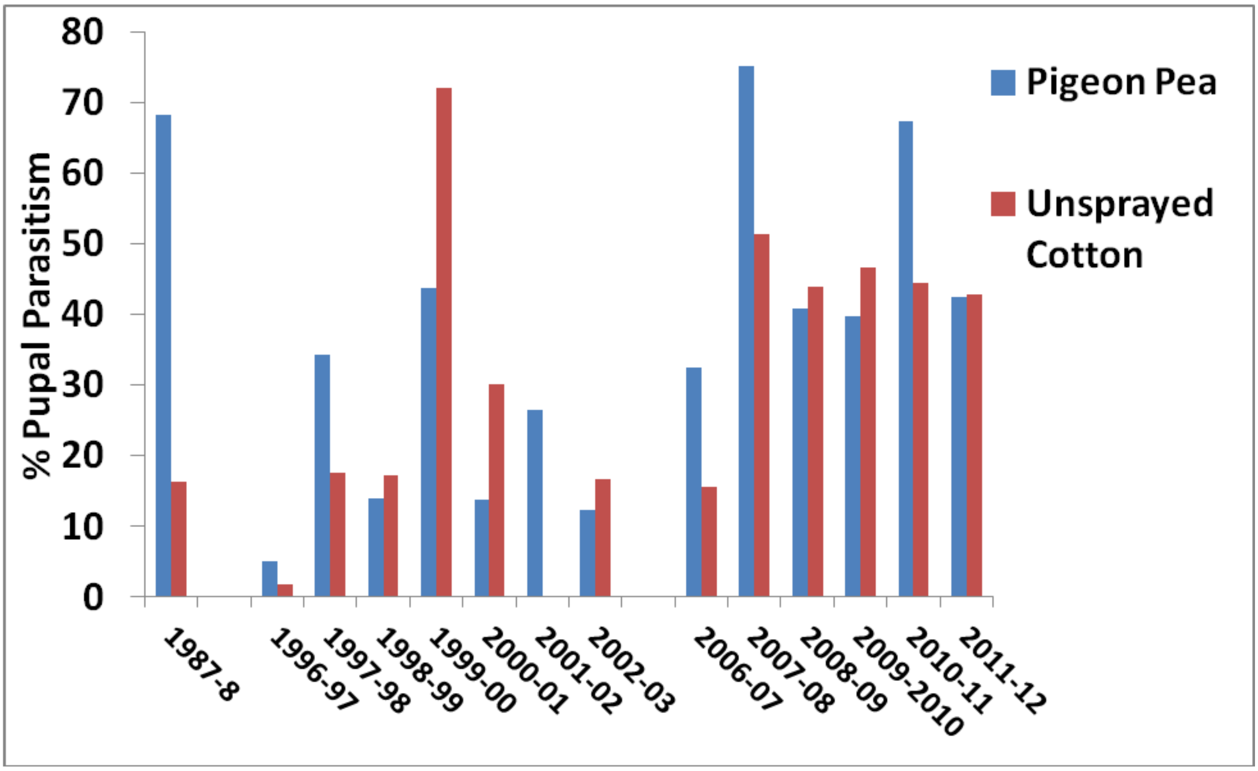


Fig. 85. Mean levels of parasitism of *Helicoverpa* pupae collected during surveys of pigeon pea and unsprayed conventional cotton, 1996-2012, and reared in the laboratory. Data for 2011-12 are only approximate at the time of writing – rearing is continuing. Data on record for 1987-88 at ACRI for surveys of crops are included.

The proportions of moths (*H. armigera* vs *H. punctigera*) that have emerged from the collections made in pigeon pea and unsprayed, conventional cotton refuge crops over the years (several valleys) that we have been surveying have varied markedly (Fig. 86), but there appears to be no obvious temporal trend in such data, nor any difference in the relative proportions of the two species emanating from the two refuge crops. The data do not seem to relate to temporal trends noted in the abundance of moths in pheromone traps (see earlier).

Eggs and larvae of *Helicoverpa* were also collected and reared from various crops (see below) and refuges during summer and autumn in this project (and years before it). There was no consistent evidence of differences in the proportions of *Helicoverpa* spp., parasitism and disease incidence between the most common refuges (pigeon pea and unsprayed conventional cotton) (e.g. Fig. 87 for 2010-11 data). Therefore the different refuge types are not separated for presentation here. Likewise, the data provided here for the “crops” include samples taken in several broad acre crops (e.g. sorghum, maize, sunflowers) (especially in the early 1990’s) as well as Bt cotton (predominant in the records in recent years). In time, we will tease apart the data to plot trends specific to particular crops, but for the moment there are some observations based on the current clustering of the data that are worth note.

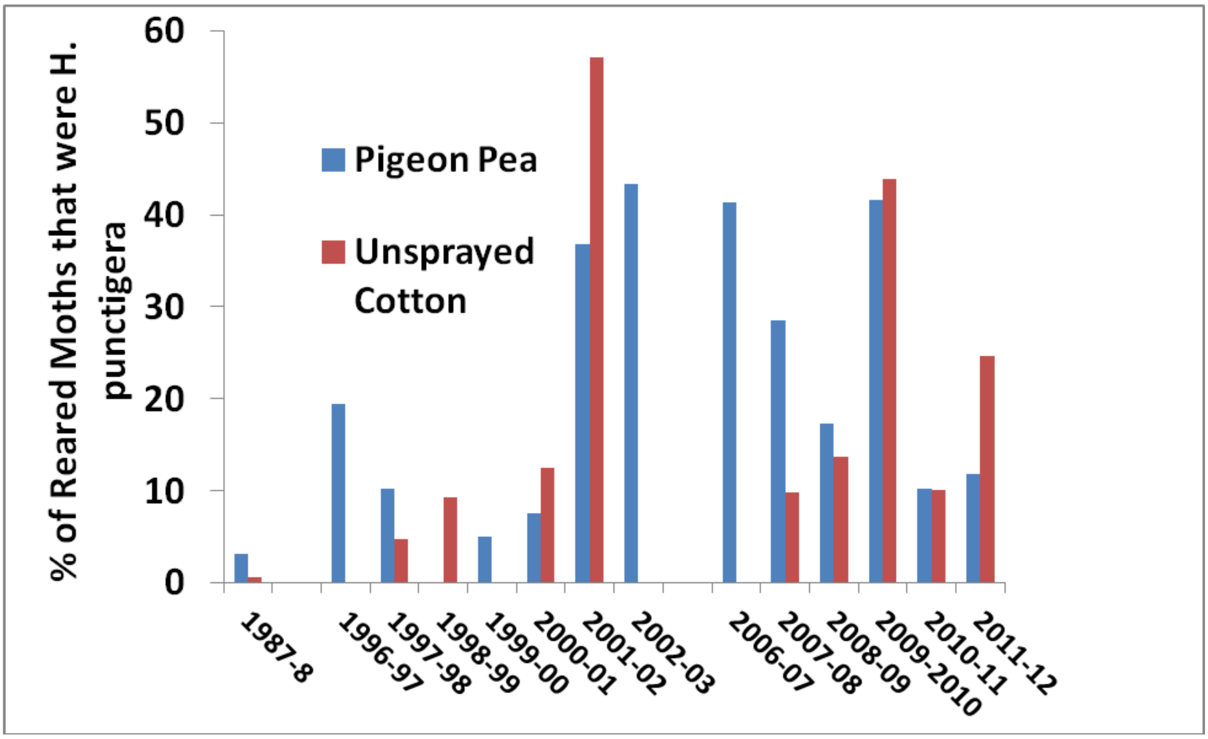


Fig. 86. Proportions of moths that were reared from field collected pupae in refuge crops that were *H. punctigera* (the remainder up to 100% = *H. armigera*).

Percentage parasitism fluctuated below approx 11% in crops and approx 41% in refuges (means of 6.06 ± 0.69% and 11.88 ± 2.52% respectively) (Figs 88 &89). The % parasitism was significantly higher in the refuges cf the crops (WRS Test = 2.13, $P < 0.05$), but there was no correlation across years between the two habitats ($r = 0.206$, $P > 0.05$). Disease incidence varied below approx 17% in crops and approx 33% in refuges (means of 5.13 ± 1.37% and 16.13 ± 3.06% respectively), with a significant difference between habitats (WRS = 2.59, $P < 0.01$), and a correlation between habitats ($r = 0.818$, $P < 0.001$). This work represents in total, over all years, the rearing of 10,526 eggs / larvae from crops and 24,541 from refuges. These natural enemies (diseases and parasitoids) cause substantial losses to the potential refuge production of high moth populations (an estimated 28%). Possible reasons for the higher parasitism and disease incidence in refuges compared with crops include both differential pesticide use and density dependent factors (more insects, means more interactions & greater chance of disease transmission).

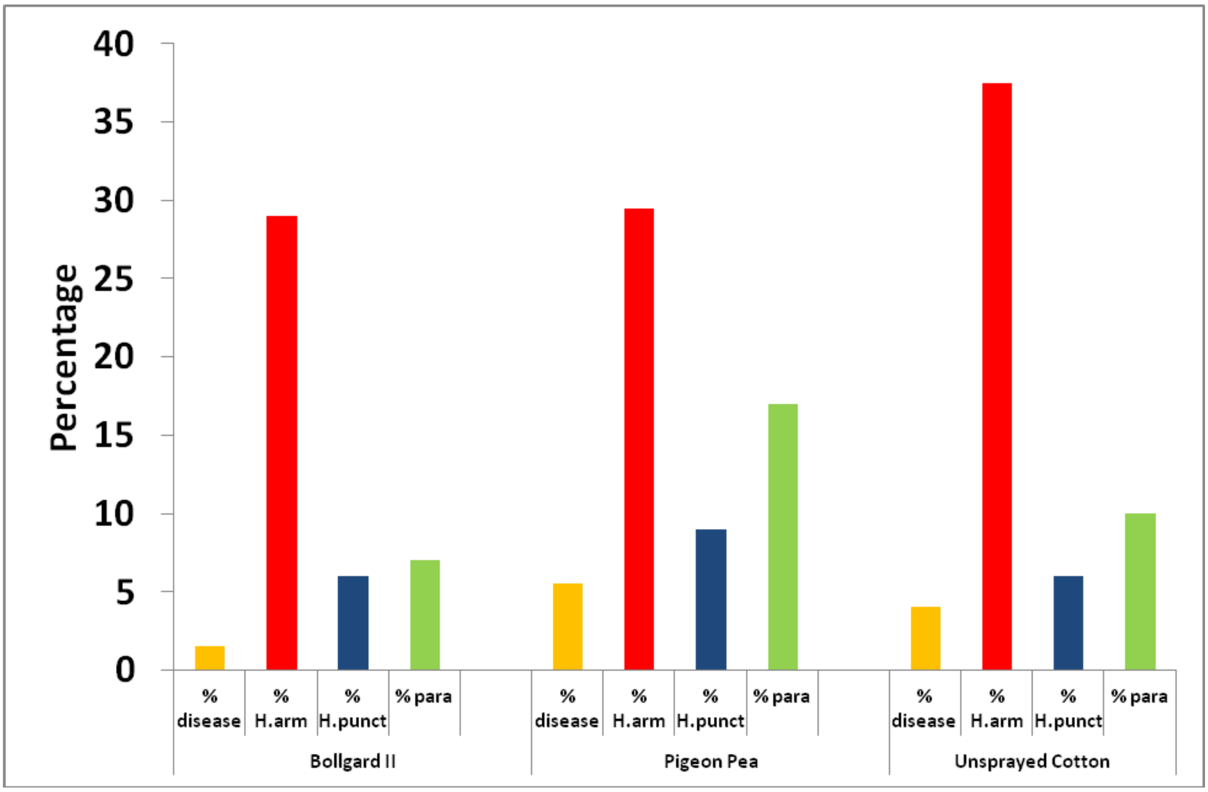


Fig. 87. Proportions of *Helicoverpa* spp. eggs and larvae collected from Bollgard II crops and pigeon pea and unsprayed conventional cotton refuge crops in summer–autumn 2010-11 that, when reared in the laboratory, were *H. armigera* or *H. punctigera* moths, diseased or parasitised.

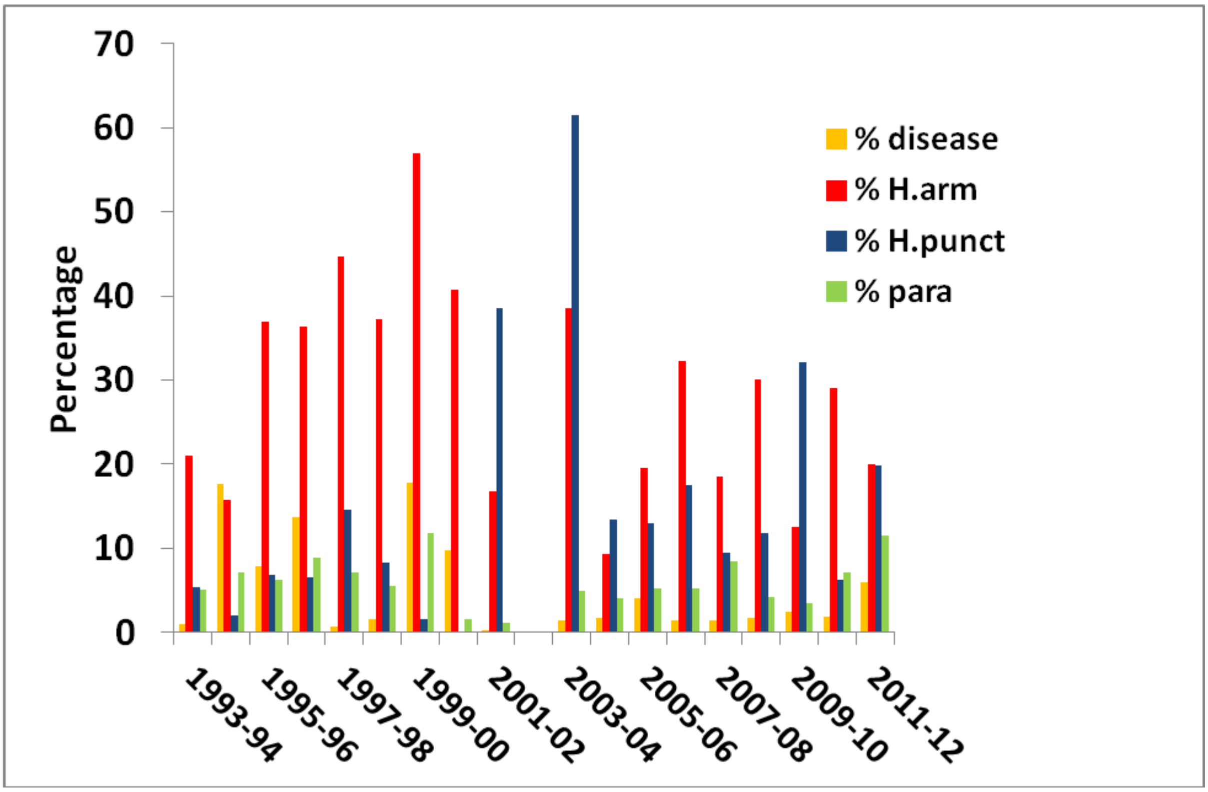


Fig. 88. Proportions of *Helicoverpa* spp. eggs and larvae collected from various crops in summer –autumn that, when reared in the laboratory, were *H. armigera* or *H. punctigera* moths, diseased or parasitised.

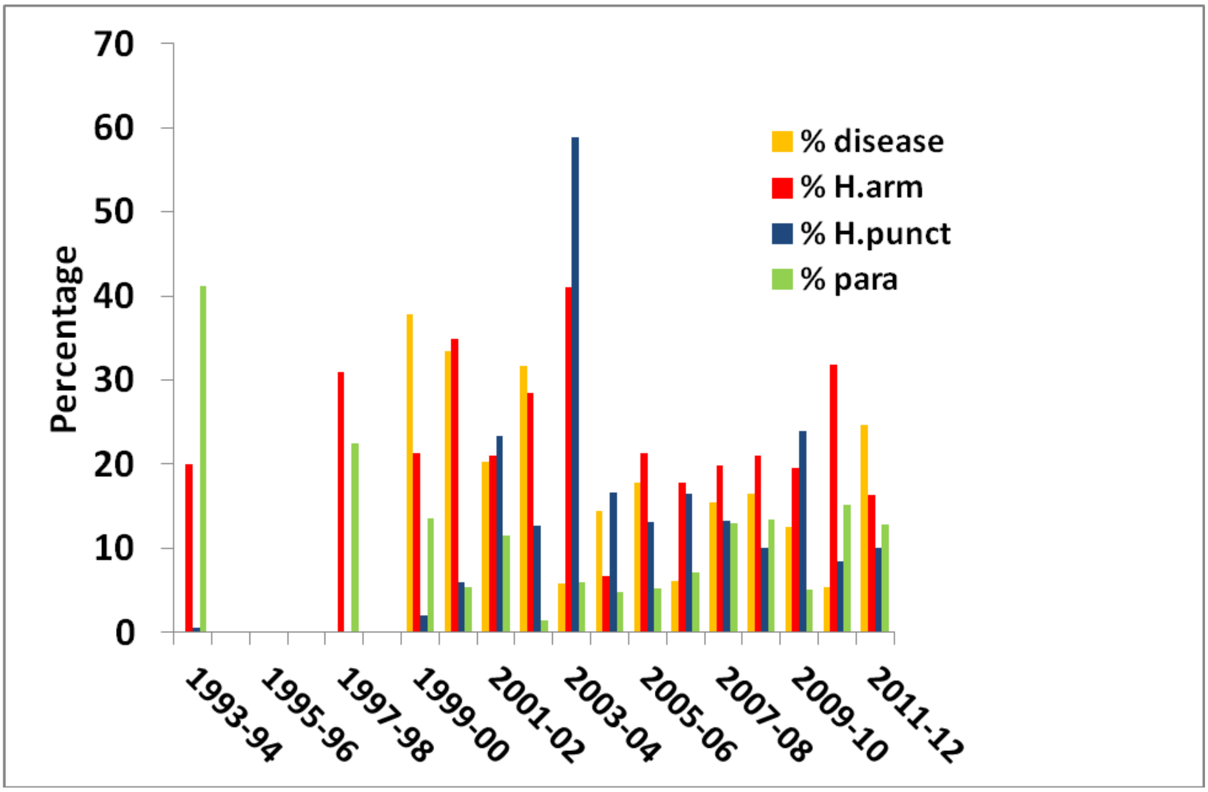


Fig. 89. Proportions of *Helicoverpa* spp. eggs and larvae collected from refuge crops (various types combined) in summer –autumn that, when reared in the laboratory, were *H. armigera* or *H. punctigera* moths, diseased or parasitised.

Figure 90 illustrates the average presence data for predatory beetles, predatory bugs and spiders recorded in different months during the cotton growing season for 2009-10. The data can of course be broken down to individual taxa within these predatory groups (but such data would be far too detailed to provide in this report). Clearly, there were no marked differences between the occurrences of the three key predatory groups within the different habitats studied. Generally, predatory beetle frequencies decreased across the cotton season, whilst predatory bugs and spiders increased. Such data were repeated in the other two years of the project (and in the three years of the previous project). We thus do not repeat all the data for other years here.

Data on patterns in abundance of key secondary pests (e.g. green vegetable bug and mirids) will be presented in a later report to CRDC, as will the community of ground dwelling invertebrates collected by cages.

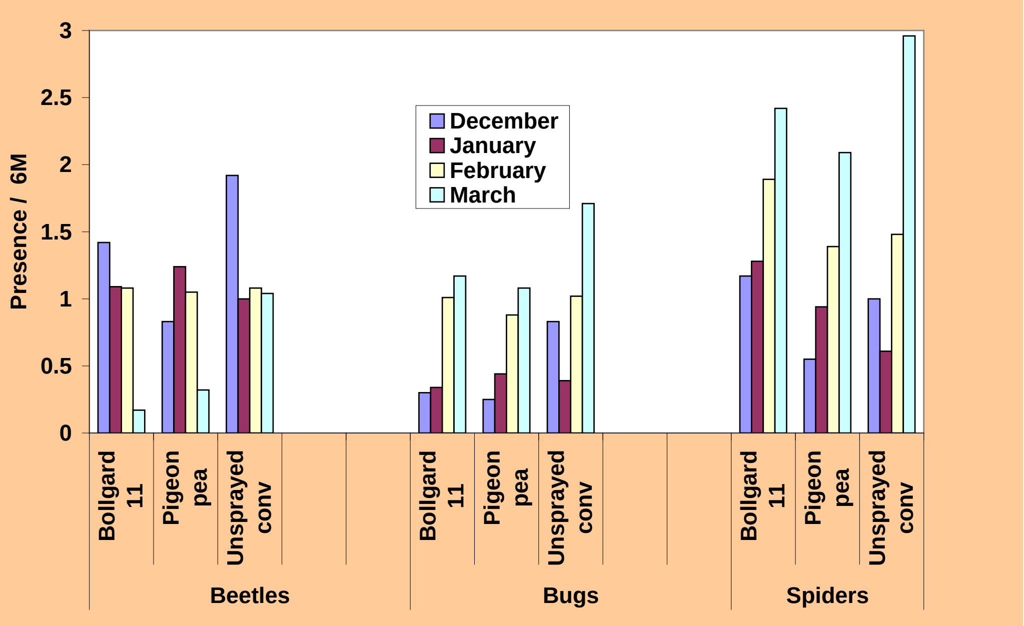


Fig. 90. Incidence of selected beneficial invertebrate taxa, expressed as the average number of 1 M crop rows where their presence was noted, within the 6 M of row checked visually per field (in 2009-10).

M. Whitehouse

Other local variables (e.g. soils, water & nutrients applied, organic C etc).

As we still plan to take the final soil sample in June 2012, a full analysis of the results cannot be completed at this stage.

Fig 91 shows the nitrogen and carbon results of samples taken in 2009 and 2011. The numbers along the y axis indicate the depth of the sample in cm. The 2009 “to be ratoon” samples were taken after the first season of the non-Bt cotton crop that was going to be ratooned. The 2011 ratoon samples are from the same site after 2 years of ratooning, while the 2011 Bt samples were taken from the associated annual Bt crop.

The results suggest that after 2 years, mean carbon concentrations have increased at the top of the soil profile, especially in ratoon cotton. Mean nitrogen levels have dropped at both sites. A likely cause for the drop in nitrogen ppm is a change in field management. The site usually grows irrigated cotton, and the high nitrogen readings at the beginning of the study may reflect previous nitrogen applications to irrigated crops. No nitrogen has been applied to the dryland crops. Nevertheless, the ratoon crop seems to be holding its soil nitrogen content better than the Bt cotton crop, particularly at 15cm below the surface.

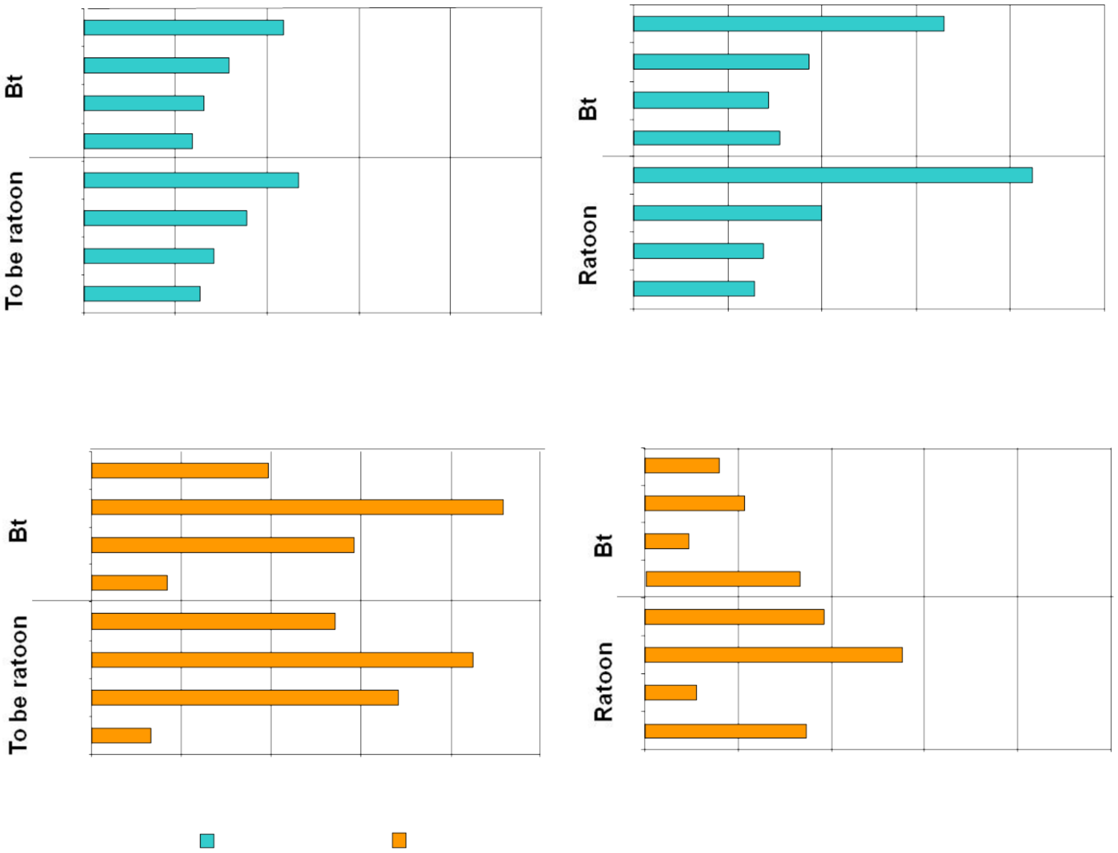


Fig. 91. Mean nitrogen and carbon readings for the Bt and ratoon dryland crops at ACRI in 2009 and 2011.

Concluding remarks on ratoon cotton

Ratooning cotton refuges may offer a slight advantage in terms of carbon soil content. The final sample in 2012 will clarify whether ratooning cotton refuges over 3 years has a significant effect on soil carbon content.

Protocols developed with an economist for collaboration on broad cost / benefit analyses of refuge crop options

Not achieved – see Methods section

G. Baker, C. Tann & M. Whitehouse

6. Extend research results to industry

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

7. Additional Deliverables

M. Whitehouse

The potential role of Magnet to increase refuge attractiveness

METHODS

Magnet ™ is known to attract moths to a crop to feed. Previous work (Addison 2010) found that egg lays increased close to Magnet applications in conventional cotton. In the 2009/10 season, a study was done to test if Magnet increased egg lays in a conventional cotton refuge. In this experiment, the central row of a conventional cotton refuge was sprayed with Magnet three times during the season (once in December, January and February respectively). To confirm that moths had been attracted to the Magnet, flash counts of Helicoverpa moths were taken before the visual samples. To flash count the sampler walks down a cotton row, tossing soil onto the cotton to flush out any Helicoverpa. Flash counts were undertaken on the row sprayed with magnet, and both 15m and 90m rows which were sampled for eggs (Fig. 92). To check for eggs, three visual samples (north, middle and south) were taken at 1m, 15m and 90m on each side of the sprayed row (Fig. 92) 1 day and 4 days after spraying (36 visuals per spray event). The results were analyzed using a repeated measure ANOVA.

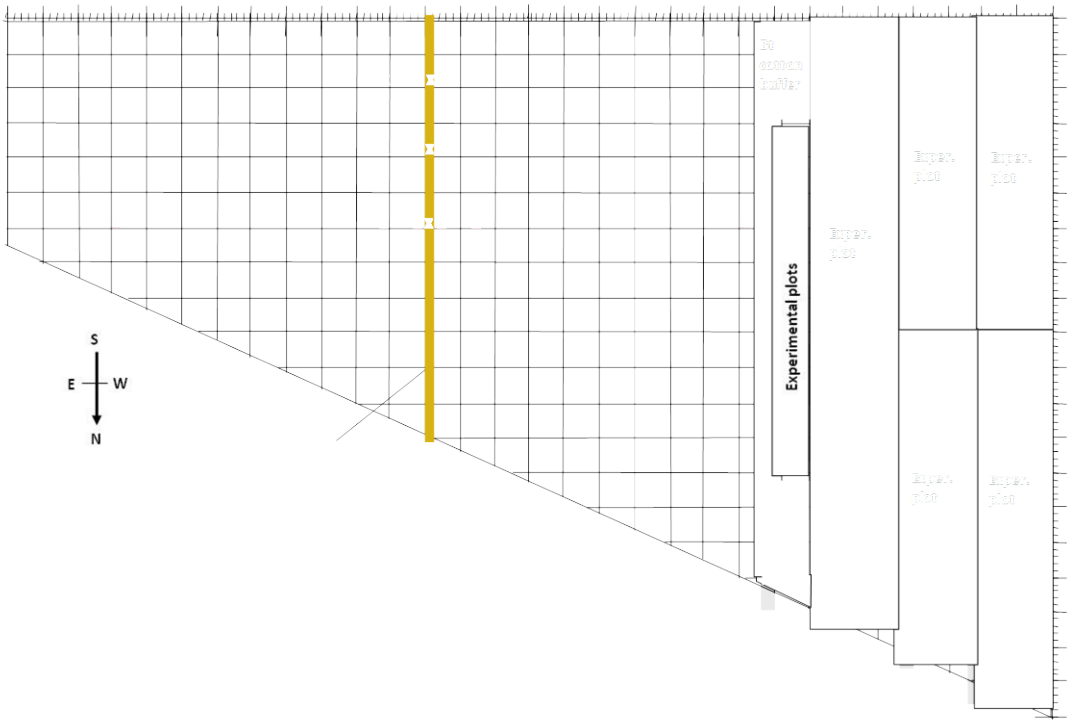


Fig. 92. Basic plan of the field site at ACRI for Magnet™, indicating the sampling locations (red X) with respect to the Magnet application (mustard coloured line)

RESULTS

The biggest problem we encountered with this work was that it was a very low pressure season with low numbers of moths, as indicated in our flush samples (Fig. 93). There was no evidence that moth numbers were higher closer to the Magnet spray, either 1 or 4 days after application.

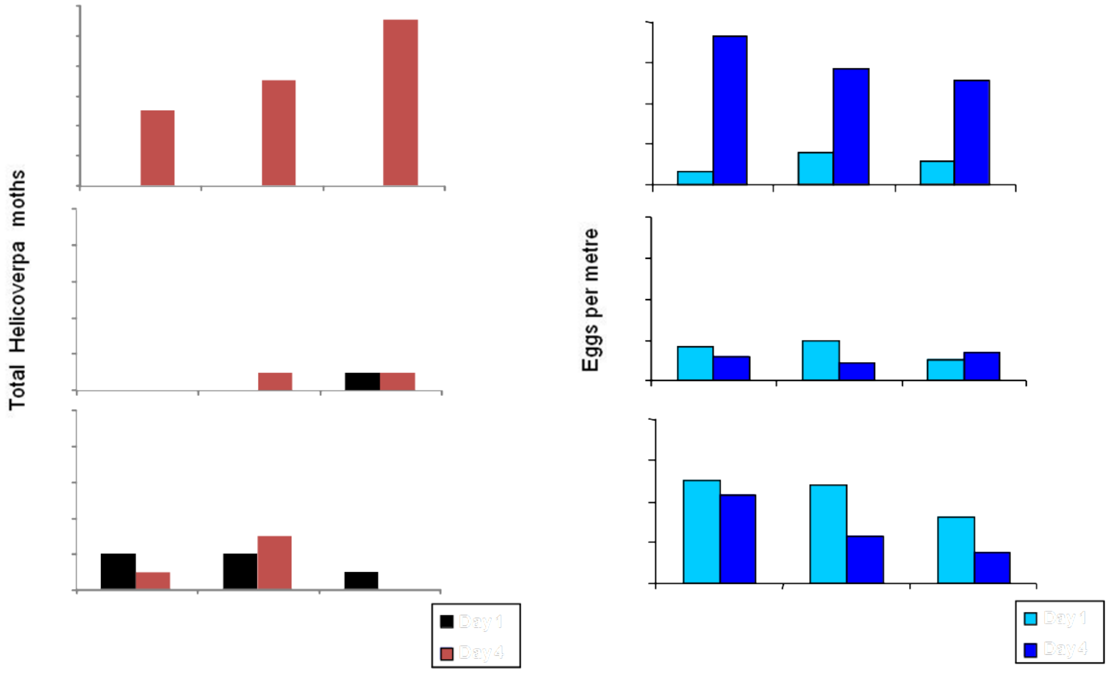


Fig. 93. The total number of moths exposed by flushing, and the number of eggs per metre following an application of Magnet.

These low numbers are also reflected in the low egg counts (Fig. 93) which indicated that there was no significant effect of the Magnet spray on egg numbers in that the distance from the spray did not influence the number of eggs laid (repeated measure ANOVA, distance from spray: F=2, df=2,30, NS). There was a significant difference in the number of eggs laid between the three applications (ANOVA, F=15, df=2, 50, P<0.001) and between Day 1 and 4 (F=1, df=1,30, P=0.049) but there was a strong interaction between the application dates and sample days (F=27, df=2,50, P<0.001) indicating that for different application dates, there were opposing effects on the egg numbers of the Day sampled. This is demonstrated in Fig Cb, where in the December application Day 4 counts are higher, whereas in the February application Day 4 counts are lower. Thus these differences are not likely to be due to the Magnet sprays.

The results presented here do not support the findings of Addison (2010) that egg lays increased close to Magnet applications in conventional cotton. However, because of the low moth and egg counts, this study is not a strong test of the hypothesis. Magnet is a well known attractant of moths, and the lack of moths attracted to Magnet in this study suggests it was undertaken in adverse conditions for its application. The only conclusion that can be drawn from this work is that an effect of Magnet on egg lays may be slight or dependant on favourable local conditions. From this work there is no evidence that Magnet could be used to increase the efficacy of refuges by increasing their attractiveness as indicated by egg lays.

OUTCOMES

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

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

1). How effective are current refuge crop options ?

2). Are novel crop options available ?

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

Through tackling these questions, the project aimed to deliver the following declared outputs, and thus make progress towards the listed science and industry outcomes :

Output A : Field and laboratory trials on the degree of cross mating of moths (including *H. punctigera*) from different crop origins

Output B : A landscape scale assessment of temporal and spatial patterns in *Helicoverpa* production and their overall mating efficacy.

Output C : Field trials assessing *Helicoverpa* production from novel refuge crop options e.g. staggered cotton plantings, mixed species crops, pigeon pea for dryland systems. These to be supplemented by evaluation of current options (see B above). Economic assessment of novel crop options.

Output D : Data sets that indicate long-term temporal shifts in the abundance of *Helicoverpa* at landscape level, and to a lesser degree that of some natural enemies.

How effective are current refuge crop options ?

The project's results demonstrate that the two current refuge crop options, pigeon pea and cotton, are viable refuges. There is, for example, no evidence to suggest that mating between moths from different crop origins is other than random. Both refuge crop types can produce large numbers of (Bt susceptible) moths but such performance can be spatially and temporally patchy. The key issue facing the refuge strategy is if the current level of patchiness is sufficient to sustain an adequate control of the emergence of Bt resistance. Do the moths emerging from the patchiness adequately cover the risk? Are we able to increase the performance of poorly productive refuges, in particular through enhanced agronomic management? [As an aside, the demonstration of patchiness in moth production will help decision-making re the deployment of refuges and where to focus Bt resistance monitoring efforts].

Over many years, pigeon pea has performed better than unsprayed, conventional cotton in terms of producing moths, using pupae production as a surrogate for this. In general, pigeon pea is a more attractive refuge for ovipositing moths than cotton, but it lacks synchrony with Bt cotton in this regard – as would be expected for a different plant species. There can of course be situations where cotton refuges prove superior. Many environmental factors can contribute to the vagaries of refuge production, including farm management of them. Ideally, it would be best to be able to assess refuge productivity in a direct way with that of Bt cotton itself. The project sought to find alternative ways to do this, in particular from initial attractiveness and egg loads, but this proved elusive.

We conclude that it is paramount for refuges to be well managed, akin to that provided for Bt cotton, to optimise attractiveness for *Helicoverpa* (nutrients, adequate water, and if at all possible, strategic control of pests such as tip worm, aphids, whitefly etc which can drastically reduce refuge performance). Enabling this may require some revision of how refuges are incentivised. Currently, most emphasis is on choice of refuge crop and the hectares required. More emphasis could be placed on refuge management per se by farmers. Poorly managed refuges can become resistance threats themselves (e.g. Bt cotton regrowth within them).

Are novel crop options available ?

We recognised the desire of farmers to use conventional cotton as a refuge cf pigeon pea because of the additional benefits it could bring (e.g. yield in a low insect pressure year, less issues with herbicide drift, agronomic simplicity) and the frustration that it is currently set at a higher % area demand than pigeon pea. We experimented with a variety of novel refuge crop options, mostly involving cotton as the basis of a refuge, in both irrigated and dryland situations. In particular, we considered split plantings of cotton and mixing cotton varieties and other crop species (pigeon pea, mungbean) with cotton - to enhance broader attractiveness.

However, results were inconclusive. Unfortunately, the low *Helicoverpa* pressure that was experienced during the project and the repetitive damage incurred from other insect pests (esp. tip worm) made the trials we conducted all the more difficult to interpret. Certainly, any improvements that were noted were not sufficient to merit recommendation as alternative refuge strategies. There was some evidence that optimising the variety of cotton refuges (on a case by case basis, considering local conditions) could be better recognised than it is. Opportunities such as access to more Okra leaf and RRF varieties for use as potential refuges are perhaps worth considering. With regard to dryland cotton, such generally finishes up earlier than irrigated cotton. As a result, the risk of pupae late in the season seems minimal (but very difficult to assess, unless the use of emergence cages indicates differently to the problematic soil sampling that has been hitherto used at these times). The need for pupae busting might well be relaxed in dryland cropping more so than in irrigated cropping ? Given its capacity to grow well in general in dryland situations, we have no issue with pigeon pea being used as a refuge there.

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

The answer to this question seems clear. Pheromone trapping has detected long term trends in the abundance of both *Helicoverpa* spp., *H. armigera* tracking cropping patterns and the frequency of large spring (immigrant) peaks of *H. punctigera* becoming rare in recent years. The intriguing question awaiting resolution for *H. armigera* is if Bollgard II (and Bollgard III in future) will suppress its abundance at landscape scale, where Ingard failed to do so. A couple more years should tell the tale on this. We don’t properly understand why the incidence of spring spikes in abundance of *H. punctigera* has abated lately. There are many possible explanations, related to inland plant host dynamics. But implications for Bt resistance development in *H. punctigera* are of concern. Substantial population mixing within this species could still be happening, although at low densities. Population genetics studies, using modern molecular tools, are called for to unravel these matters. At the same time, we have also demonstrated that there are temporal shifts occurring in the balance of *H. armigera* : *H. punctigera* in late season populations. Again, mechanisms are not clear. In addition, we now know that shifts in *Helicoverpa*’s natural enemies are happening (e.g. parasitism increasing). This can probably be attributed to general reductions in pesticide use. The impact of such growth in natural enemy performance has to be a negative influence on refuge performance, although the increased presence of natural enemies in the environment more generally is no doubt also a positive development in other ways.

8. **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**

M. Whitehouse

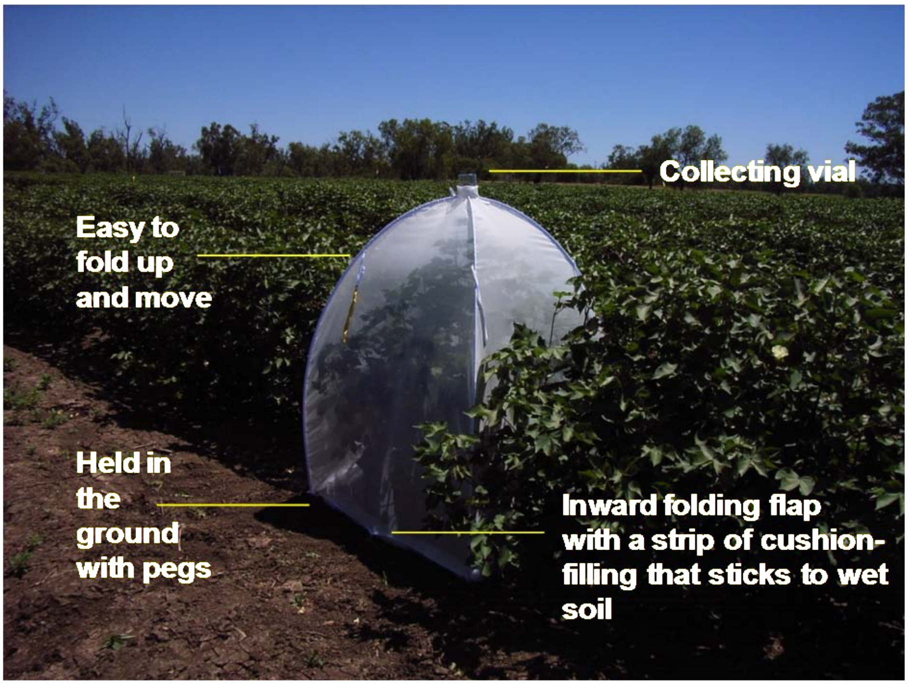


Fig. 94. Emergence cages developed to sample moths in cotton fields

One significant technical development related to this project was the development of 1 metre cages to sample moths (Fig. 94). We needed a cage that: would sample only one metre of cotton, was easy to put up and take down, was reliable, and whose reliability was not compromised by environmental conditions such as very dry or wet soil. These cages were highly successful during the project, catching as many or more moths than pupae digs (for more detail on the cages see Objective 1). The cages will be used more extensively in the new project (Managing Bt resistance and induced tolerance with effective refuge crops) but we will be making some changes, such as modifying the collecting vial so that it is easier to remove, and using shade cloth so that the cages last longer. At the moment they only last 2 years under field conditions.

Another notable technical advance has been the maturing of methods to identify plant host origins of moths. The use of the stable C isotope method to separate C3 and C4 plant origins was developed in this project's predecessor (CRC 1.01.52) and carried forward. As well as that, we had hoped in this project that stable N isotopes could be used to finesse plant host origins within a C3 / C4 framework, based upon the well-recognised differences in stable N signatures that exist between legumes and non-legumes. However, this did not prove possible. Our main focus was of course on separating cotton and pigeon pea origins and this did not therefore happen. We can only conclude that pigeon pea is a poor legume in terms of N fixation (somewhat supported by pers comms from plant / soil nutrient experts), or that the soils themselves and the N applications to them in cotton production systems obscure fixation differences that are transmitted via plants to the insects. It is always possible that stable N signatures might be used to separate plant origins other than the pairing of cotton and pigeon pea, but the rarity with which we noted groupings within the stable N data we obtained for *Helicoverpa* from a variety of sites tends to suggest this is unlikely. At present other quite different chemical markers are being sought by two different organisations (CSIRO & UNE) and such approaches are viewed (cautiously) as offering some potential. Certainly, if such tools for identifying plant hosts (and hence origins) can be refined, then they will offer very broad advantages to the study of *Helicoverpa*'s (and other pests') landscape ecology.

In addition, models of the collective dispersal of moths from refuges within landscapes are being developed at the end of the current project. We anticipate demonstrating the advantages of these in the Final Report that will be provided to CRDC later in 2012.

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

9. 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. Substantial temporal changes are happening in the abundance of *H. armigera* and *H. punctigera* in cotton production systems. This is evidenced by data from pheromone trapping grids in both the Namoi Valley and the St George region and increasing % of *H. punctigera* in field collections of eggs and larvae in summer and pupae entering winter. There are continuing doubts over the reliability of migrations of *H. punctigera* from inland sources, as once accepted in the RMP. Such appear not to have occurred to any major extent in recent years. Population genetics studies (e.g. based on rapid throughput molecular tools) are needed to properly confirm this shift from the perspective of population mixing. There is good reason to suspect that whilst single gene Ingard Bt cotton did not suppress *Helicoverpa* abundance at landscape level, Bollgard II might.

2. Long-term monitoring of pigeon pea and unsprayed conventional cotton refuges (1996-2012) that looks at averages across sites, suggests that the status quo of approx. 2 : 1 performance (in terms of *Helicoverpa* moth production) of these two key refuges has been maintained in the field. Levels of pupal parasitism have increased in recent years in pigeon pea refuges and most likely also in cotton refuges (conclusions limited here because of limited collections in some years).

3. Separate surveys of pigeon pea and cotton refuges during the tenure of this project which look at variation within and among sites, suggest that no consistent difference occurred between pigeon pea and cotton refuge productivity, in either irrigated or dryland situations. We have yet to attempt merging these two data sets to determine overall outcomes for recent years. This work did however find that pigeon pea was generally more attractive to ovipositing *Helicoverpa* than cotton.

4. Many refuges "under-performed" in terms of moth production. Whilst this may in some cases be simply attributable to chance and the vagaries of insect dispersal / establishment and to heavy pressure from natural enemies (some aspects of our work suggested that natural enemies were more abundant in refuges than crops), **we believe strongly that the agronomic management (especially adequate watering) needs to be much better implemented across the industry for refuges to perform to potential.**

Perhaps additional / alternative incentives to promote refuge management might work as well / better than simply required hectares.

5. Various novel refuge crop options were explored, most notably split-planting of unsprayed conventional cotton (to spread the attractiveness of cotton refuges more broadly throughout the season) **and mixing different varieties of cotton, adding other crops to cotton** (e.g. pigeon pea, mungbeans) **and rattooning cotton** (again to spread attractiveness in time)**, but none of these options produced convincing performance that would underpin recommendations for adoption.** Unfortunately, pest pressure was generally low during the tenure of the project, thus making assessments difficult.

6. **The production of *Helicoverpa* from refuges (as for other crops) is highly variable in space and time.** The in depth monitoring of refuges throughout the St George region and a part of the Macintyre Valley highlighted this variability and the spatial dispersion of high and low performances raises concerns for how well refuges perform collectively. It is expected that modelling the potential spread of moths from actual refuges in these real landscapes using field collected data will demonstrate that some parts of landscapes are likely to be more at risk of Bt resistance development than others. Such findings may help direct resistance monitoring to “risky” elements in the landscape and / or suggest how the spatial deployment of refuges might be improved.

7. The key assumption in the Bt RMP of random mating between *Helicoverpa* moths from different plant host origins was upheld. Many field collections of singleton and mating *H. armigera* moths (over two projects) have shown that at least insects from C3 and C4 plant host origins mate at random. Field demonstration of similar random mating of moths of cotton and pigeon pea origins has proven intractable, but laboratory studies provide support for it being likely. Field collections also suggest that unstructured refuges can provide substantial (unheralded) support for the suppression of Bt resistance. Much less data is available for *H. punctigera*, but what there is suggests this species behaves similarly to *H. armigera* with regard to random mating.

8. An additional deliverable suggested it was unlikely that application of Magnet® in refuges could lift refuge performance in terms of *Helicoverpa* production (oviposition as a surrogate). This finding was in agreement with 3 previous years’ study, reported for CRC 1.10.52.

9. Emergence cages were demonstrated to be an effective means to measure *Helicoverpa* abundance in refuge crops, especially in dryland situations wherein pupae digging can be fraught with inefficiencies.

10. Attractiveness (as evidenced by egg lays) in Bollgard cotton was more often correlated with attractiveness in cotton refuges than in pigeon pea refuges. This finding is in agreement with previous studies throughout 1996-2003 (see Final Report for CRC 1.10.52) which showed a close temporal relationship between pupae numbers under Ingard cotton and cotton refuges, but not between pigeon pea refuges.

11. The relative abundance of eggs on refuge crops and Bollgard II cotton were highly variable – in some cases refuges attracted far fewer eggs than Bollgard II, in other cases (especially for pigeon pea) more eggs were laid than on Bollgard II, but only late in the season.

12. The project explored the possibility that egg lays might be correlated with moth productivity, and thus be used as indicators of such (e.g. evaluation of optimum refuges to use). However, association was difficult to demonstrate (links between larvae and subsequent moth productivity were understandably easier to demonstrate). One suggestion is that the relative productivity of Bt cotton and its refuges could be assessed using agronomic factors known to influence *Helicoverpa* survival.

EXTENSION OPPORTUNITIES

10. Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.

One metre emergence cages are being further refined to improve their efficiency.

Alternative methods to trace the plant host origins of moths (chemical signatures) are being researched by CSIRO ES & PI Divisions.

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

Improving refuge management is a difficult task, in part because there are no immediate benefits to the grower. The benefits are more general and longer term for the industry. To improve refuge attractiveness and productivity, growers need incentives that benefit them directly, thus enhancing uptake. We will be discussing these issues with grower groups to appreciate their views better.

To further disseminate our research results, we will be providing talks to grower and consultant groups, publishing articles in industry magazines such as the Australian Cottongrower and CSD Variety Trials annual booklets, giving talks to / providing representation at key industry for a such as REFCOM, TIMS, Cotton Conference, IPM Research Forum etc., and publishing in peer reviewed science journals.

(c) for future research.

In a new project funded by the CRDC ("Managing Bt resistance and induced tolerance with effective refuge crops in preparation for Bollgard III") we will be building on many of the findings from this project. In particular, we will:

1. Undertake experiments on commercial farms to quantify the effect of different agronomic factors on refuge attractiveness and productivity.
2. Promote refuge governance through meetings which promote best refuge management techniques.
3. Work with the industry to develop possible improvements to refuge compliance that better reflects the attractiveness and productivity of refuges.
4. Work with the myBMP group to develop a simple "refuge effectiveness calculator" as a level four practice in IRM for Bollgard cotton. The calculator will enable growers to compare the efficacy of their refuges under different conditions so that they can find the way to grow refuges that is best suited to their farms.

An additional new project is also under negotiation with CRDC for funding through 2012-15 entitled : "Managing Bt resistance through improved knowledge of *Helicoverpa punctigera* ecology and cotton planting window efficacy". Whilst the emphasis on *H. punctigera* will be reduced in what goes forward (in particular research on the population genetics of this species using molecular tools – to better understand the degree of seasonal migration / population mixing that is occurring, even when population abundance is low), core topics such as the long-term monitoring of *Helicoverpa* populations will still proceed. There will also be work (collaboratively with e.g. Monsanto) to assess the effectiveness of the current use of planting windows for Bt cotton to limit Bt resistance development. We anticipate there will also be an element of the research that addresses landscape movements of *Helicoverpa*, as it pertains to the notion of "pest suppressive landscapes".

We remain strongly convinced that it is essential to understand the degree of mixing of populations of *H. punctigera* at large scales, to underpin the RMP. We will continue discussions with CRDC on this.

PUBLICATIONS

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

Papers published during the project (and related to the work) :

Torres J.B., Ruberson J.R., Whitehouse M.E.A. IN PRESS Transgenic cottons for sustainable pest management. *In* **Agric Develop and Sust**. (Book Chapter).

Whitehouse M.E.A., Barnett M., Mansfield S. & Broughton K. 2011 Intraguild interactions and predator effects the potential role of Lynx spiders and damsel bugs in the control of green mirid damage in cotton. **Austral Ecology 36: 687-697** no. doi: 10.1111/j.1442-9993.2010.02204.x

Whitehouse M.E.A. 2011. The IPM of mirids in Australian cotton: understanding why and when pest managers spray for mirids. **Agricultural Systems, 104:** 30-41.

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. **Proceedings of the 3rd International Symposium on Biological Control of Arthropods**, Christchurch, New Zealand (Feb 2009). pp 161-172

Baker, G.H., Tann, C.R. & Fitt, G.P. (2011). A tale of two trapping methods : Helicoverpa spp. (Lepidoptera, Noctuidae) in pheromone and light traps in Australian cotton production systems. **Bulletin Entomological Research 101**, 9-23.

Downes, S., Mahon, R.J., Rossiter, L., Kauter, G., Leven, T., Fitt, G. & Baker, G. (2010). Adaptive management of pest resistance by *Helicoverpa* species (Noctuidae) in Australia to the Cry2Ab Bt toxin in Bollgard II® cotton. **Evolutionary Applications 3**, 574-584.

Tann, C., Baker, G. & Lawrence, L. (2011). Is pigeon pea living up to expectations as a refuge for Bollgard II ? **The Australian Cottongrower** Feb-Mar 2011, 13-16.

Tann, C., Baker, G. & Lawrence, L. (2011). Pigeon pea : living up to expectations as a refuge with Bollgard II cotton in Australia ? **Outlooks on Pest Management** Feb 2011, 41-43.

Tann, C., Baker, G., Downes, S., Whitehouse, M., Mahon, R., & Whitburn, G. (2009). Bt resistance, refuges and Helicoverpa ecology. In : "**2008/09 Variety Trial Results**". Cotton Seed Distributors, Wee Waa, pp. 83-86.

Tann, C., Baker, G., & Downes, S. (2010). Ecology and resistance in Helicoverpa. In : "**2009/10 Variety Trial Results**". Cotton Seed Distributors, Wee Waa, pp. 79-83.

Baker, G., Downes, S. & Tann, C. (2011). Ecology and resistance in Helicoverpa. In : "**2010/11 Variety Trial Results**". Cotton Seed Distributors, Wee Waa, pp. 104-108.

Tann, C & Baker, G. (2010). Pigeon pea as a suitable refuge crop for use in association with Bollgard II® cotton – is it living up to expectations ? **Proceedings Australian Cotton Conference**, Gold Coast, Qld. 6 pp.

Baker, G.H. & Tann, C.R. Mating of *Helicoverpa armigera* (Lepidoptera : Noctuidae) moths and their host plant origins as larvae within Australian cotton farming systems. **Bulletin Entomological Research** Resubmitted after minor revision.

Lu, Z-Z. & Baker, G.H. Spatial and temporal dynamics of *Helicoverpa armigera* (Lepidoptera, Noctuidae) in contrasting agricultural landscapes in north-western China. **International Journal of Pest Management** Submitted.

Next manuscripts (in prep) :

Baker, G.H. & Tann, C.R. Long-term variations in the abundance of *Helicoverpa* spp (Noctuidae), particularly in response to the use of Bt cotton. Intended either for *Science, PNAS* or *J. Anim. Ecol.*

Baker, G.H. & Tann, C.R. Random mating amongst *Helicoverpa punctigera* (Noctuidae), with relevance to Bt cotton and associated refuge crop strategies. Intended for *Aust J. Entomol.*

Baker, G.H. & Tann, C.R. Parasitism spp. in field populations of *Helicoverpa* spp. (Noctuidae) : temporal and habitat comparisons using long-term data sets. Intended for either *Aust. J. Entomol.* or *Austral Ecol.*

Whitehouse M., Mansfield S., Harrris D., Cross D. (submitted to CSIRO for publication in the Australian Cottongrower). Do pigeon pea refuges on commercial farms produce twice many moths as cotton refuges?

Whitehouse M.E.A., Mass S. Irrigated refuges of cotton: are they performing as expected and can they be improved? (to be submitted to Crop Protection).

Whitehouse M.E.A. Comparing refuge crops to increase efficacy against Helicoverpa resistance in dryland cotton. (to be submitted to Crop Protection).

Whitehouse M.E.A., Mansfield S., Nobilo J., Hagler J.R. The effect of predator size and prey type and size on predation in cotton fields using ELISA techniques. (to be submitted to: Entomologia Experimentalis et Applicata).

Whitehouse M.E.A., Wilson L.J., Downes S., Cave L., Norman P. The effect of Bt cotton on spider mite densities. Possible tritrophic effects or differences in plant structure? (To be submitted to Environmental Entomology).

Reports to Farmers

Whitehouse M.E.A. **2011** Report for Dobikin

Whitehouse M.E.A. **2011** Report for Gunedra

Whitehouse M.E.A. **2011** Report for Warrianna

Whitehouse M.E.A. **2011** Report for Blue Hills

Whitehouse M.E.A. **2010** Report for Ian Gourlay at Blue Hills

Whitehouse M.E.A. **2010** Report for Phil Firth at Warrianna

Whitehouse M.E.A. **2010** Report for Richard Cathcart at Redcamp-Gunedra

Talks given :

Whitehouse, M. Researching refuge performance. (REFCOM, Toowoomba, July 2011), including facilitated discussion.

Whitehouse, M. The role of refuges : where we are and where we are going. (Northern Farming Systems IPM Forum, Toowoomba, July 2011.

Whitehouse, M. Talks on spiders and insect communities in cotton to school students (Scientist in Schools program), Narrabri & Wee Waa, 2010 & 2011.

Whitehouse, M. The role of refuges in the resistance management of Helicoverpa moths and the implications of this to IPM in cotton. 3rd Combined Australian & N.Z. Entom. Soc. Conf., New Zealand, Aug 2011.

Whitehouse, M. (Organiser) Australasian Society for the Study of Animal Behaviour, Annual Conference, Narrabri, 2010. Promoted Narrabri & cotton industry.

Whitehouse, M. The IPM of mirids in Australian cotton : understanding why and when pest managers spray for mirids. Aust. Soc. Stud Anim Behav., New Zealand, 2009.

Whitehouse, M et al Integrated pest management and the effects of transgenic cotton on insect communities in Australia : lessons from the past and future directions. 3rd Int. Symp. Biol. Cont. Arthropods, New Zealand, 2009.

Whitehouse, M.et al The use of ELISA techniques to clarify complex predator / prey interactions in cotton . 3rd Int. Symp. Biol. Cont. Arthropods, New Zealand, 2009.

Whitehouse, M. New refuge management options. Cotton CRC Annual Science Review, Narrabri 2010.

Whitehouse, M. Recent developments in cotton refuges. (Northern Farming Systems IPM Forum, Toowoomba, 2010.

Whitehouse, M. The role of refuges in resistance management – are refuges working as expected ? CSIRO, 2011.

Whitehouse, M. Spider diversity in Australian cotton : patterns and significance. Univ. Sydney, 2009, 2010, 2011, 2012.

Baker, G. & Tann C. Efficacy of refuge networks in the landscape. REFCOM (at Aust. Cotton Conf), Gold Coast, August 2010.

Baker, G. & Tann, C. Refuges for resistance management in Bollgard cotton, & related aspects of *Helicoverpa* ecology. Cotton CRC Science Forum ???

Baker, G & Tann, C. Cotton CRC Science Forum, Narrabri, Oct 2010.

Baker, G.H. & Tann, C.R. The landscape ecology of *Helicoverpa* and the efficacy of Bt refuges. Cotton CRC Science Forum, Narrabri, March 2012.

Baker, G., Tann, C. & Whitehouse, M. Refuge crops and the ecology of *Helicoverpa*. REFCOM, Brisbane, February, 2009.

Baker, G., Tann, C. & Whitehouse, M. Refuge crops and the ecology of *Helicoverpa*. Cotton CRC Science Forum, Narrabri, August 2009.

Baker, G & Tann, C. Long-term variations in the abundance of *Helicoverpa* spp. (Noctuidae). What’s driving them ? Ecol. Soc. Aust. 50th Anniv. Conf., Canberra, Dec. 2010.

Tann, C. Northern Farming Systems IPM Forum (Toowoomba, August 2010).

Various talks to grower / industry groups re refuge research etc :

e.g. C. Tann (Lower Namoi CGA, June 2010; CSD field day at Korolea, Macintyre, Apr, 2011; CSD field day at Little Mollee, Namoi, Feb 2011)

e.g. M. Whitehouse (CSD field day at Little Mollee, Namoi, Feb 2011; Emerald Growers Group, 2009 & 2010)

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

No

Part 4 – Final Report Executive Summary

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

Transgenic (Bt) cotton has provided major benefits for the Australia cotton industry (e.g. control of *Helicoverpa* and reduced pesticide use), but the possibility of Bt resistance in these insects is a major threat. Until recently, Bt resistance research focused only on *H. armigera*. However, recognition that Bt resistance alleles becoming increasingly common in *H. punctigera* has moved focus to both species.

As part of a resistance management plan (RMP) for *Helicoverpa*, refuge crops (no exposure to Bt toxins) are required to be grown in association with Bt cotton, at prescribed amounts (ha, as a % of Bt cotton grown), according to historic views of their abilities to produce large numbers of moths that will then disperse, mate with potentially resistant moths emerging from Bt cotton, and thereby reduce the likelihood of Bt resistance emerging to a damaging scale. Initially, several refuge crops were available for use, but this has recently been reduced to only pigeon pea and conventional cotton, because these crops harbour both *Helicoverpa* species. Pigeon pea is the most commonly used refuge because less (5%) is required cf cotton (10% when unsprayed). However, there is significant desire in industry to move to a less demanding requirement of cotton refuge, because of side benefits it could provide (e.g. cotton yield). Refuge crop options are also less available for dryland than irrigated cotton. In tandem with all this, the amount of Bt cotton on the landscape has drastically changed from a 30% cap of all cotton in the 1990s (Ingard) to 80-90% now (Bollgard II). This background led to this project which sought to answer :

1. How effective are current refuge crop options ?,
2. Are there novel refuge crop options ?,
3. Is the abundance of the target pests, *Helicoverpa* spp., changing in time at landscape scale ?

The work has shown that there have been substantial changes in the abundance of both *Helicoverpa* species at landscape scale in recent years. Whilst *H. armigera* has tracked the abundance of host crops, the numbers of spring migrant (susceptible) *H. punctigera* have seemingly diminished. The latter has implications for resistance management in this species. Ingard cotton was unable to suppress the abundance of *H. armigera*, but it is looking increasingly likely that Bollgard II will.

Long-term monitoring of pigeon pea and cotton refuges suggests that the 2 : 1 differential assumed in *Helicoverpa* productivity has been maintained, but separate work within the project has highlighted the large variability in these refuges' performance. At times, cotton refuges can be as productive, or more so, than pigeon pea. Many refuges "under-perform". Modelling is in progress using intensive data sets obtained from surveying refuge crops within regions (St George, Macintyre) to assess the collective performance of refuges in servicing landscapes. Initial results suggest this will demonstrate there are weak links in coverage, where Bt resistance risk will be heightened. Such work will help focus monitoring efforts, and indicate where greater care in placement / management of refuges is needed. This project stresses strongly that high standards of farmer management of refuges (e.g. adequate nutrients, water) are imperative in delivering optimal refuge performance. Perhaps incentives for such management need to be put in place by industry.

Various novel refuge options were explored, especially focusing on improving cotton performance through increased seasonal breadth of attractiveness (e.g. split plantings, mixing varieties and host crop species). However, none of these approaches proved persuasive enough to merit changing options. Pest pressure during the project was generally low, making demonstration of novel refuge options difficult.

A key assumption, implicit in the Bt resistance RMP is that mating occurs at random between moths from different plant host origins. This project supports the assumption. The project also explored the possibility that egg lays (attractiveness) might be correlated with refuge productivity, thereby enabling simpler evaluation of likely refuge performance, in particular relative to Bt cotton. However, such a relationship was not demonstrated clearly. Research in dry-land cotton has been difficult in the past, in part because of the difficulty of traditional methods of assessing refuge productivity (pupae digs, efficiency is compromised in harder soils). This project developed a modified emergence cage which can assist with assessing refuge productivity without the labour of digging.

Contact : Dr Geoff Baker; email Geoff.Baker@csiro.au; ph. 02 6246 4406