Project Title: Ecology of *Helicoverpa* in relation to transgenic cotton and the efficiency of refuge crops

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CRDC Program:
OR CRC Program: **The Farm**

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Part 3 – Final Report Guide (due 31 October 2006)

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

Background

The introduction of transgenic (Bt) cotton to control *Helicoverpa* spp., has significantly influenced pest management practices, most notably through reduced and softer pesticide use, and a greater interest in the management of beneficial invertebrates (predators, parasitoids). What influence, if any, the introduction of Bt cotton (& other, concurrent changes in management practices) have had on the overall abundance of *Helicoverpa* in cotton growing regions is however poorly understood. Will the replacement of Ingard (1 Bt gene) cotton with Bollgard II (2 gene) be influential in this regard ? Through our previous monitoring of early season incidence of *Helicoverpa* spp on non-cotton host plants in northern NSW, and our networks of pheromone trapping & bug-checking in the Namoi and St George regions (the latter a relatively isolated production system) (see projects CSE64C & CSE90C), we established a long-term data set to enable assessment of temporal shifts in *Helicoverpa* abundance (seasonal and across years). We proposed to continue such monitoring to provide on-going assessments for industry and researchers (e.g. via CRC web site).

The major challenge to sustainable use of Bt cotton is the risk that the target pests, *Helicoverpa* spp, may evolve resistance to the engineered toxins. Resistance to conventional Bt sprays has evolved in field populations of other moths (e.g. *Plutella xylostella*), *H. armigera* has consistently developed resistance to synthetic pesticides in the field, and cultures of Bt resistant strains of *H. armigera* have been generated in the lab. Bt resistance concerns are thus well-founded. Much effort has therefore been devoted to developing and implementing pre-emptive resistance management strategies, most notably based on the use of refuges to maintain sources of susceptible moths in the population which will mate with potentially resistant individuals produced in Bt crops - thus dampening the development of resistance. Our previous research has helped identify refuge options. The major options are sprayed (non Bt) conventional cotton, unsprayed conventional cotton, pigeon pea, sorghum and maize - all of which however provide issues to grapple with (e.g. sprayed cotton continues reliance on pesticides, unsprayed cotton is costly for potentially little yield). The desired outcome is clearly for the smallest, most efficient refuge to be identified (probably pigeon pea). With the advent of Bollgard II and the expectation that there will be fewer moths arising from such cotton, there is added pressure on reducing required refuge sizes.

One of the major criteria defining effective refuges is that they will generate enough susceptible moths to ensure that matings between resistant survivors from Bt crops are extremely unlikely. But our knowledge underpinning the optimal placement of refuges within a landscape and how effective their coverage is of moths generated from Bt crops is very limited. Dillon et al (1998) simulated movements of *H. armigera* from refuges to transgenic crops using HEAPS and argued that dispersal from refuges can be patchy according to wind speed and direction and spatial distribution of crops. The qualities of plant hosts at source and sink, aggregative / synchronous movement behaviours of the moths and limits of simple diffusion are also considered likely to be important. But empirical data from the field on all this are scarce. We gathered some preliminary data on the topic in CSE90C, using strontium to mark moths in refuges and recapture them elsewhere. We proposed to expand this work to conduct field experiments and surveys using commercial crops to demonstrate movements of moths from refuges to nearby Bt crops.

Helicoverpa moths are known to mate several times. *H. armigera* moths mate first at about 3 days old & peak in oviposition at 7 days, but the timing of the extra matings, their occurrence relative to crop origin of the moths and their relevance to egg production is poorly understood [Unpublished CSIRO data suggests repeated matings do not influence total fecundity, but the

effect on the genetics of offspring is unknown]. Sperm precedence is known to occur in Lepidoptera, but which mating (first, last, neither) takes precedence is variable between species. No information seems to be available for *H. armigera* on this. Against the backdrop of managing Bt resistance and ensuring that matings between moths from refuges and Bt crops are effective, it is desirable to understand how frequently moths are mating in particular habitats and if sperm precedence is occurring. We proposed experiments to clarify these issues for *H. armigera*.

Whilst refuges are primarily grown to produce Bt susceptible *Helicoverpa*, they also have the potential to produce significant numbers of secondary pests (e.g. green vegetable bug, mirids) as well as beneficial invertebrates of relevance to the cotton industry. Because we hoped to mark-recapture *Helicoverpa* and would be surveying *Helicoverpa* in situ in both refuges and Bt crops, the opportunity should arise to also collect information on the abundance and apparent movement of these other organisms – thus supplementing other projects concentrating on their abundance (within CSIRO & other organisations – most notably CSE103C, M. Dillon).

Plant chemicals such as jasmonic acid & terpenoids are responsible for the induction of many changes in plant resistance that occur following herbivore attack. Several authors (e.g. Thaler 1999, 2002) have suggested these chemicals can affect the abundance and performance of natural enemies, for example by attracting such animals to sites where herbivores are in large numbers. To our knowledge, the possibility that such chemicals might be exploited in the Australian cotton industry, either through direct application to cotton fields or through plant breeding programs with focus on HPR has not been suggested before. We proposed a pilot study to determine if there is a discernible aggregation of beneficial invertebrates within cotton fields when jasmonic acid is applied.

Objectives

1. To monitor temporal changes in the abundance of *Helicoverpa* spp. in the Namoi and St George regions through continued pheromone trapping and egg and larval sampling on early season weeds and crops.

2. To determine the potential for effective mating between moths generated in variously placed refuges and nearby transgenic crops.

3. To establish an accurate predictive model for the emergence of *Helicoverpa* in southern cotton regions.

   [NB. Objective 3 was dropped out, with CRDC agreement, due to funding shortfalls at the start of the project]

4. To measure the movements of sucking pests (mirids, GVB) and beneficial invertebrates between refuges and Bt crops, as opportunity allows, and to test the impact of volatile plant chemicals induced by herbivore damage (jasmonic acid) on the movements of beneficial species.

All the main objectives, with the exception of number three, were successfully achieved within the budgetary and seasonal* restraints of this project. Further research is planned within our present project (CSE 115 : "Maximising the efficiency of Bt refuge crops") that will build on studies of the ecologically important aspects of *Helicoverpa*, especially the efficacy of refuge crops. Such studies are essentially long term but of vital importance to the cotton industry, if we are going to manage Bt resistance in the future .

*[Planned movement experiments are very opportunistic by nature, and need to capitalise on situations where, in particular, abundant *H. armigera* eggs and larvae occur within suitably located refuges. There is therefore a need to be flexible with planning to ensure that we have suitable natural populations to work with. Not every season gives us that opportunity, and 2003-06 seasons were troublesome in this regard. Methodologies are continually being improved upon with regard to moth movements / plant host origins (e.g. carbon isotope analysis techniques and related diagnostic tools) and these will be explored more fully in coming years.]

Methods

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

1. Temporal Changes in the Abundance of *Helicoverpa* spp. on Early Season Weeds and Crops.

As in years prior to this project, the incidences of *Helicoverpa* eggs and larvae on weeds, natural vegetation, and winter-spring crops were monitored through periodic visual and sweep net collections. Collections amongst weeds and natural vegetation were virtually all based on sweep netting (usually 100 sweeps / site). Collections within crops were usually based on visual checks of crop rows (usually 6 m replicates). Crops (during and before this project) included mainly faba bean and chickpea (but also canola, lupins, field peas, linseed, safflower, coriander, wheat and vetch were sampled). The numbers of eggs and larvae counted were expressed against the relevant sampling effort. The collected material was reduced to a sub set when prolific and reared through to maturity or death / parasitoid emergence in the laboratory. The numbers of individuals reared / collection varied, but in most cases was from a few to approx. 50. The primary emphasis in this work was to gather early information on the annual build up in *Helicoverpa*, particularly *H. armigera*, abundance prior to the coming cotton season (i.e. an educated "feel" for what was likely to occur at the start of the season). However, in addition, the surveys enabled broad ranging comparisons across years and between vegetation types of the incidence of diseases and parasitoids affecting *Helicoverpa*, and the relative abundance of the two key *Helicoverpa* species. The work also broadened our understanding of *Helicoverpa* beyond cotton crops.

Collection efforts necessarily varied between years (during this project and its predecessors) according to the availability of crops and weeds etc to sample from (e.g. because of drought [see Fig. 1 for rainfall data collected at Narrabri during the study and earlier], weeds and natural vegetation, and to a lesser extent crops, were not sought in all years). Over the years, data were collected from the major cotton growing valleys in NSW and southern Qld (Namoi, Macquarie, Macintyre, Gwydir, St George), with some data also included from elsewhere, e.g. near Bourke – but sites in the Namoi Valley predominated throughout.

2. Temporal Changes in the Abundance of *Helicoverpa* spp. in Pheromone Traps in the Namoi and St George / Dirranbandi regions

a) Vicinity of ACRI, Narrabri (Lower Namoi Valley)

For the duration of this project, and following on from previous years, a grid of 11 pairs of Agrisense canister pheromone traps (one each for *H. armigera* and *H. punctigera*) was maintained within an approx 10 km radius of ACRI, Narrabri. The traps were emptied weekly, weather permitting. Lures were changed monthly and pesticide strips were changed bi-monthly. Two CSIRO-designed cone light traps were also run during the 2004-05 season at sites on ACRI (Field R5 and Chico A1 block) to assist in interpreting the pheromone

catches (see earlier report of research under CSE90C which indicated key differences in catches from the two types of traps). The light traps were run for a single night, usually at weekly intervals. White eggs were also recorded on cotton adjacent to these traps, using visual assessments (six replicate 1 m rows of crop), done the day after each light trapping.

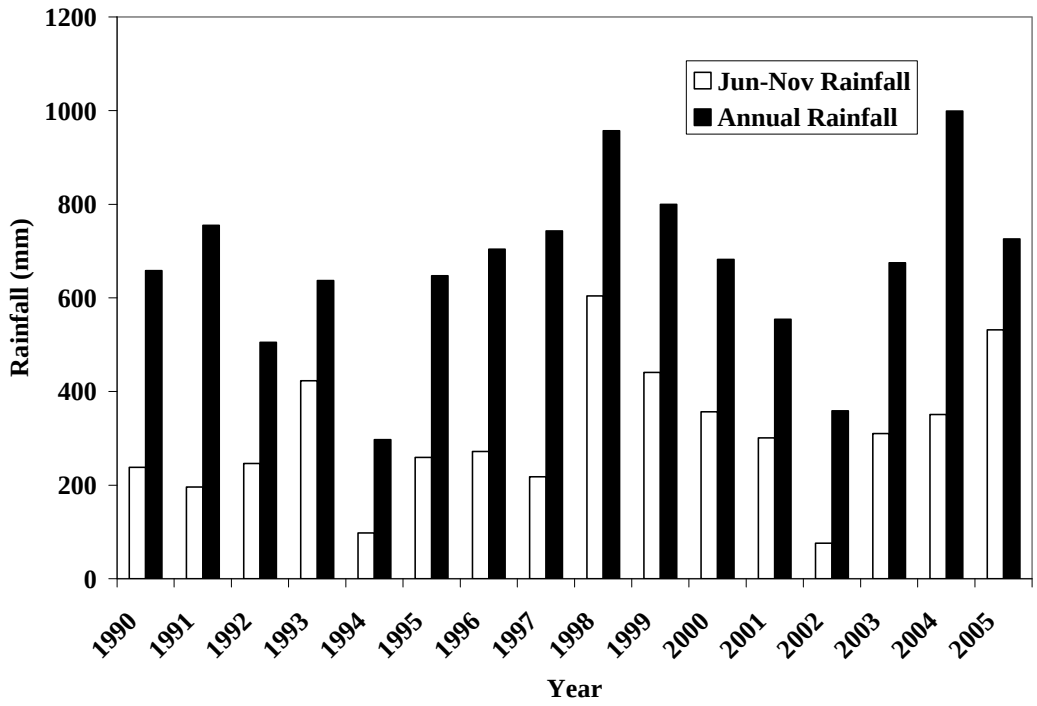

Fig. 1 Rainfalls recorded in Narrabri (BOM) from 1993 to 2005, including winter-spring data.

b) St George and Dirranbandi

Pheromone trapping equipment was supplied to cotton consultants in the cotton growing regions of St George and Dirranbandi who maintained pairs of traps and provided weekly counts of the numbers of moths collected throughout the growing season (note not all year round, as near ACRI), as they had in years prior to this project. Numbers of traps varied between years (e.g. for St George : 8 pairs in 2003-04, 10 pairs in 2004-2005, and 9 pairs in 2005-2006). Cotton consultants in the St George region also provided data on the abundance of white eggs of *Helicoverpa* spp. that they collected in the vicinities of the pheromone traps.

3. Abundance of *Helicoverpa* and Other Invertebrates on Bt Cotton and Associated Refuge Crops.

Several sampling sites were established in each season during the project with the notion of setting up intensive movement studies of moths in and around unsprayed refuges (e.g. into Bt cotton crops) if a suitable population of *H. armigera* developed. The concept was to spray strontium chloride on to the refuge crop at a critical time, such that feeding larvae would take it up through their feeding and become marked through to adult moths (cf successful use of this mark-recapture technique described in the final report for the project's predecessor, CSE90C). At subsequent dates, when modelling suggested such moths would be emerging we would trap them at prescribed distances from the refuge to determine their extents of movements, and ideally what they might be mating with. [We also anticipated that such

work might lead to marking of other invertebrates produced in the refuge crops and enable opportunistic studies of their movements too]. Unfortunately, such populations of *H. armigera* did not develop at the selected sites during the tenure of the project. One site at Getta Getta, North Star was sprayed with strontium during the 2003-04 season (a system of pigeon pea as the refuge crop, associated with Ingard cotton), but too few moths were recovered subsequently within the associated Bt cotton to yield useful results (very high predation and parasitism of larvae, and predominance of *H. punctigera* rather than the desired *H. armigera* hindered progress here). Nonetheless, the allied objective of determining the performances of refuges in supporting *Helicoverpa* spp. populations (and those of other refuge crop canopy invertebrates) in comparison to the associated Bt cotton crops was achieved. Those results are presented here.

From approximately mid December to April each season, we regularly visited farms within the Namoi, Macintyre, and Gwydir Valleys, plus farms in the St George / Dirranbandi regions (19 farms in 2003-04, 24 farms in 2004-05, and 37 farms in 2005-06). Each farm had well-managed unsprayed refuges in close proximity to Ingard and / or Bollgard II cotton crops. Most of the refuges were pigeon pea, in varying stages of development, but we also monitored sorghum crops with multiple staggered plantings, maize and unsprayed cotton crops. [All sites were initially sampled each year, but some sites were discarded during the season as it became obvious they were not likely to be suitable as mark-recapture sites, including lack of ongoing availability of irrigation]. At each site that was monitored throughout each season, suction sampling (D-vac) and visual inspections were usually conducted both within the refuge and within the Bt cotton crop. Occasionally, sweep netting was also conducted within refuges to enhance collections of *Helicoverpa* for subsequent laboratory rearing. At a subset of sites, observations were made within the Bt cotton crop both near its edge (50-200m from edge, adjacent to the refuge) and further into the crop (minimum of 300m from edge). Suction sampling comprised six replicate samples, each covering 10m of crop, taken with each crop at each site. Visual sampling involved checking 6 replicate 1m rows of crop within each field. Sweep netting comprised 100 standard sweeps per field. Whilst +/- data were only collected for most invertebrates in the visual sampling, the abundance of *Helicoverpa* eggs and larvae / m were recorded. A sub-set of *Helicoverpa* eggs and larvae from the visuals (and occasional larvae from sweep netting in the refuge crops) were reared in the laboratory to determine species, parasitism and disease.

4. Incidence of Mating Between *Helicoverpa armigera* Moths From Different Plant Host Origins Within Bt Cotton Crops.

C3 and C4 plants are so designated because of their different photosynthetic pathways. Such plant groups differ in the relative abundance of naturally occurring carbon isotopes, and they pass such differences on to insect herbivores which feed on them. In general, C4 plants are tropical plants, or summer growing annuals in temperate regions. The main group of C4 plants are grasses, including sorghum and corn crops. Both of these latter plants are accepted as refuge options for use in concert with Bt cotton (and only support *H. armigera*). There is evidence that some Chenopodeaceae and many herbaceous weed species may also be C4. C3 plants include legumes such as pigeon pea, as well as cotton, sunflower, canola, and safflower. Pigeon pea is a particularly popular choice as a refuge option for use within the RMP for Bt cotton.

Firstly, to verify the carbon isotope designation of different plant types, we collected pupae of *H. armigera* from beneath C3 (unsprayed cotton and pigeon pea) and C4 (sorghum and corn) crops in the vicinity of ACRI, Narrabri, northern N.S.W, during the 2005-06 cotton season. We reared these pupae to moths in the laboratory, dried them before they fed, and analysed them individually (4-5 mg of head and a small part of the thorax) for their carbon isotope signatures using specialised mass spectrometry at UNE.

Secondly, at the optimum emergence time as indicated by regular surveys of large populations of *Helicoverpa* larvae in several refuge crops in northern NSW during the tenure of this project, we manually collected mating *H. armigera* moths in Bt cotton crops (Bollgard II®) associated with such refuges, at night using head torches.. We also collected various numbers of single moths on these occasions (somewhat driven by our success in finding mating moths !), and we occasionally collected moths over conventional cotton crops grown in the vicinity of the refuges as well. These moths were analysed for carbon isotope signatures, as above. For brevity, we present here data compiled for moths collected near Drayton during the 2004-05 season (sorghum as refuge) and near Wee Waa during the 2005-06 season (corn and sorghum grown as refuges).

5. Influence of Jasmonic Acid Applications on the Localised Abundance of Beneficial Invertebrate Populations on Cotton Plants.

We explored the possibility that jasmonic acid (JA) might attract natural enemies of cotton pests in a series of experiments (varying in design) that ran in all three cotton seasons of the project. All work was conducted at ACRI in fields of unsprayed conventional cotton.

In 2003-04, we conducted two experiments. In the first experiment, ten plots for each of two treatments (JA and Controls) were established (random block design). Each plot was comprised of five cotton plants within one row, isolated by removing four plants each side of the experimental plants in the same row. The plots were placed centrally within an area of cotton that was 12 rows wide and 15 m long. Each such replicate area was separated from its neighbours by buffers of 4 rows of sunflowers. JA (synthetic, commercially available) was applied to relevant plants using a small pump action aerosol applicator at weekly intervals on 3 occasions in February 2004 (13, 20 and 27 Feb). Plots were shielded by plastic sheeting to prevent drift. JA solution was made from distilled water and a small quantity of methanol to facilitate mixing of the JA with the water (1 ml methanol / 50 mg JA active ingredient). 50 ml solution was applied to each plant, such that each plant received 0.2 mg active JA ingredient (similar in quantity to that used by other overseas workers). Control plants received equivalent amounts of water and methanol solution only. Visual inspections of canopy invertebrates were conducted immediately prior to applying the JA.

In the second experiment in 2003-04, the whole experiment was conducted along a single row of cotton. Five plants were selected within replicate 1 m sections of the row of cotton, with 1 m of buffer plants separating such experimental sections of the row. JA and Control treatments were alternated along the row, with ten replicates for each treatment. Application rates etc for JA and Control solutions were the same as per the first experiment. JA was applied on 26 February 2004, and visual inspections of the plants for the abundance of canopy invertebrates were conducted on 1, 3, 5, 8 and 12 March, prior to subsequent applications of JA. JA solution was thus sprayed more frequently on to cotton plants in this second experiment. A visual inspection was also conducted on 26 February, prior to applying the initial JA.

Sentinel *Helicoverpa* larvae and egg cards were placed within the experiments described above to observe predation and parasitism, but such yielded no data of note, and are therefore not reported on further here.

During the 2004-05 season, sixteen plots were established, each four rows wide and 5 m long. There were 2 m buffer sections of unsprayed cotton between the plots. Six cotton plants (two adjacent rows of three plants) were left within each plot, after removing all the other plants within each plot. The plots were arranged in a random block design, with eight

plots for each treatment (JA and Control) initially. Replication was reduced to six plots per treatment as the experiment progressed, simply because of time taken to service the experiment. JA solution (and Control solution) was applied at the same rates as described above. Thirteen weekly applications and associated visual checks for canopy invertebrates (just prior to JA application) were made, starting on 24 November 2004.

In addition, sticky traps were established on 20 December 2004 at ACRI near the JA experiment. These traps consisted of 30 cm diam. white plastic bucket lids secured to wooden pegs, such that the lids were suspended 30 cm above the soil surface. A 4 cm diam. cotton wool pad was secured at the centre of each lid, upon which to apply a Control (2 ml of distilled water) or JA solution (2 ml , such that 2 mg of active ingredient was applied per pad; i.e. a much more concentrated JA solution than used previously on plants). Tanglefoot was applied to the rest of the upper surface of each lid. These sticky traps were placed at 5 m intervals, with 10 replicates per treatment. Inspections were conducted weekly (for 12 weeks) for the invertebrate catch, the catch was removed from the tanglefoot for identification, and Control or JA solutions were re-applied as appropriate.

During the 2005-06 season, further plots were established at ACRI in an area of unsprayed conventional cotton. Each plot consisted of three adjacent 1 m rows of cotton which were sprayed with either JA solution or Control. No surrounding plants were removed on this occasion. Whilst all three rows were sprayed, only the central row was checked visually for invertebrates. There were five replicate plots for each treatment. Plots were arranged at random within the field, at least 20 m distant from their nearest neighbour, and never in the same row of cotton. JA was applied at a higher rate than that used in the previous 2 years on plants (0.5 mg active ingredient per plant). Sprays were applied weekly for 8 weeks, from early January to late February 2006. Inspections were made prior to spraying.

6. Sperm Precedence in *Helicoverpa armigera*.

The use of refuges as part of the Bt resistance management strategy assumes that moths produced there (and Bt susceptible) will mate with moths arising from transgenic cotton crops (and possibly resistant), thus reducing the likelihood of resistance developing in field populations. But if the female moths from refuges mate first in the refuges before leaving them (most likely with other locally produced moths) and if the first mating carries precedence in the nature of the offspring later produced by those female moths, irrespective of subsequent matings (as is known to occur in some species), then the efficacy of the refuge strategy is called into question.

In a collaborative research project, led by Dr Sharon Downes (CSIRO Entomology, ACRI), to determine whether or not sperm precedence exists in *H. armigera*, we conducted a preliminary experiment within which we mated female moths sequentially with male moths from two separate cultures. The females and the two groups of males were all collected from different locations to facilitate the use of DNA finger-printing (micro-satellite profiles) to distinguish amongst offspring sired by the different males. Dr Kirsten Scott (University of Queensland) conducted the micro-satellite analyses for us, using five standard loci, as reported in Scott et al (2004) *Molecular Ecology Notes* 4 : 204-205.

Briefly, from this study, we believed we had established that :

1. Females can mate with more than one male (this was already known).
2. 2. When females mate with more than one male, their offspring can be sired by more than one male (i.e. multiple paternity can occur), but this is not always the case.
3. Some mechanism exists whereby the sperm from some males in a mating series is "stored" for use in insemination up to several days after mating has occurred.

4. There does not appear to be obligatory sperm precedence, at least when two males are involved.
5. The micro-satellite technology has enough power to determine relative proportions of paternity.

However, we were made aware that two research groups were using micro-satellite analyses with *H.armigera* in Australia (Univ. Queensland and Univ. Melbourne) and obtaining very different results. The Univ. Queensland scientists were suggesting genetically structured populations of *H. armigera* existed whilst the Univ. Melbourne scientists were finding much more homogeneous populations. Other scientists, Prof D. Heckle and Prof P. Batterham, also at Univ. Melbourne advised us that micro-satellite analyses are unreliable for molecular markers because they don't originate from a single locus; rather they are mostly buried within repeated sequences which limits their use for the work we intended. P. Batterham's group have been investigating alternative methodologies for paternity testing, based on EPIC (exon primed intron crossing) markers for use with *H. armigera*. We decided to link with Batterham's group in Melbourne to analyse the outcomes from a further sperm precedence experiment.

In this subsequent experiment (conducted by S. Downes), we reared three bulk colonies of *H. armigera* : our Bt susceptible laboratory strain (ANGR) and each of two field derived strains from different locations within the Namoi Valley (Namoi 1 and Namoi 2). The ANGR colony was used to provide the female moths in the study. Mating arenas were constructed from clear perspex containers, with air vents and a covered arm hole to enable easy capture of moths. We ran two concurrent sub-experiments, one with Namoi 1 males as the first mates for the females and the other with Namoi 2 males as first mates.

We reversed the night and day cycle of the moths to enable observations to be conducted during working hours. We placed approximately 100 female ANGR pupae and 100 male Namoi 1 pupae into the first mating arena. Previous work had shown that successful matings take place for at least 30 minutes. Thus, once moths emerged, we observed the arenas for matings at least every 20 minutes for the following 10 days. Any copulating pairs were relocated to an individual small container until mating had finished. Males were then killed and preserved in alcohol. Females were marked with a unique number before being relocated to a second mating arena that contained males from the Namoi 2 colony. Any copulating pairs from this arena would thus be comprised of females mating for a second time. Mating pairs were again relocated to an individual small container until mating had finished. Males were then killed and preserved in alcohol, and eggs were collected from the females 1, 3, 5 and 7 days after their second mating. The female moths and a subset of neonate larvae resulting from the eggs were preserved in alcohol. All preserved material was then submitted to the Univ. Melbourne for paternity analyses. A similar sub-experiment was run, reversing the order of the exposure of the female moths to males from the two Namoi colonies. The work described above was repeated in both 2005 and 2006.

Results

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

1. Temporal Changes in the Abundance of *Helicoverpa* spp. on Early Season Weeds and Crops.

The incidence of *Helicoverpa* spp. on winter-spring crops varied quite markedly between years since records began in 1993-94. For example, in some years (1996-2001), the proportions of eggs and larvae reared from these crops that yielded *H. armigera* were

relatively high (Fig. 2). In other years (e.g. 2002-2004), *H. punctigera* strongly predominated. There is no evidence to suggest these trends can be explained by variations in the crops sampled (most were faba bean and chick pea throughout the years), but it is noteworthy that in the three years in which *H. armigera* particularly predominated in rearings from winter-spring crops (1998, 1999 & 2005), rainfall was highest (Figs 1 & 2). The occurrence of *H. armigera* in spring will also almost certainly reflect to some extent its abundance in the previous summer and how many pupae overwinter from that season. Notably, the 1998-99 season was notorious as an exceptional time for *H. armigera* (see data presented in the Final Report for CSE90C). The frequency of *H. armigera* in spring collections in 1999 may well reflect in part the 1998-99 season. In contrast, the eggs and larvae reared from weeds and native vegetation were consistently dominated by *H. punctigera*. Parasitoids of *Helicoverpa* spp. also varied quite markedly between years. Parasitoids included Tachinidae (Diptera) and various Hymenoptera, including *Microplitis* sp, *Telenomus* sp., *Chelonus* sp., *Heteroplema scaposum*, *Netelia producta* and *Lissiopimpla excelsa*. Data on the incidence of individual parasitoids through time and locality are available on request. Deaths resulting from diseases such as virus, bacteria and fungi were common in the rearings (data not shown here).

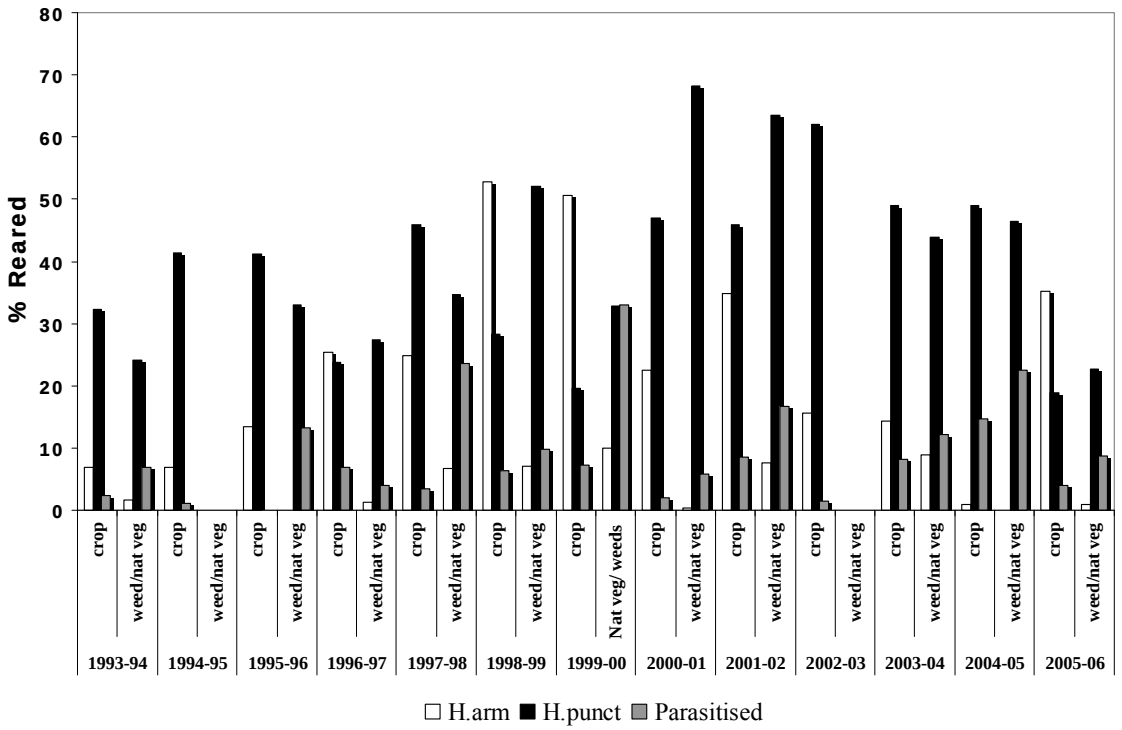

Fig. 2. Incidence of *H. armigera* and *H. punctigera* in eggs and larvae of *Helicoverpa* spp. collected from crops and weeds and native vegetation during July to November and reared through successfully to moths each year from 1993 to 2005. The incidence of parasitism of such eggs and larvae (i.e. emergence of parasitoids from collected material) is also illustrated.

Previous studies have suggested that *H. punctigera* is more mobile than *H. armigera*, the former breeding in large numbers on a variety of hosts in inland Australia and migrating across to the eastern cropping regions as conditions in inland Australia become less favourable for them. *H. punctigera* are argued to be more reliant upon weeds and native vegetation than *H. armigera*, which is thought to be more dependent upon established crops.

The data set presented here supports this perceived difference in plant host preference for the two *Helicoverpa* spp. Monitoring of different suites of plant communities is likely to be a key factor in determining early season predictions of high populations for each of the two *Helicoverpa* spp. in subsequent summer cropping seasons. Certainly, it seems likely that flushes of weeds and native vegetation in spring are likely to stimulate *H. punctigera* abundance more so than *H. armigera*. The lack of weeds and suitable native vegetation to sample in the springs of 1994 and 2002 reflected the dry prevailing weather at the time (Figs 1 & 2).

As a result of all the spring sampling we have conducted over the years from 1993 to the present, we have accumulated a substantial data base on the actual abundance (cf proportions that are *H. armigera* and *H. punctigera* as displayed here) of *Helicoverpa* spp. eggs and larvae on a variety of plant hosts. We are still in the process of analysing these data sets to determine their utility as predictors of forthcoming seasonal pest abundance. Nevertheless, we do indicate here that the three years in which *H. armigera* were particularly predominant amongst collections of *Helicoverpa* eggs and larvae on winter-spring crops (1998, 1999 and 2005) corresponded with those years in which mid-season abundances of *H. armigera* were highest in pheromone traps in the Namoi Valley (see Fig. 7). Such associations are not evident later in the seasons, but many more conflicting environmental drivers may well have had opportunity to be influential by then.

2. Temporal Changes in the Abundance of *Helicoverpa* spp. in Pheromone Traps in the Namoi and St George / Dirranbandi regions.

a) Vicinity of ACRI, Narrabri (Lower Namoi Valley)

The abundance of *H. armigera* and *H. punctigera* in the pheromone traps in the vicinity of ACRI in each year of the project are presented in Figs 3-5. In all 3 years, there was evidence of a major peak in the abundance of *H. punctigera* during spring – thought to be the result of movement of this species to the region from inland Australia, and typical of data collected within the same grid of traps in previous years (Fig. 6). However, in the 2003-04 and the 2004-05 seasons, there was clear evidence of a further / later peak in *H. punctigera* abundance, to the point that it exceeded the catch of *H. armigera* at this time. Such has not been the norm in earlier years (Fig. 6). The abundance of *H. punctigera* in 2005-06 in the pheromone traps near ACRI was more "normal". The abundance patterns for *H. armigera* were rather erratic, but in general displayed the usual increase in numbers as the cotton season progressed.

The abundance of *H. punctigera* in the pheromone traps later in the season than usual, at least in 2003-04 and 2004-05, is all the more noteworthy given that pheromone traps have tended not to be particularly efficient (cf light traps) in catching this species beyond week 20 (see Final Report for CSE90C). We speculate that the apparent increase that we have observed in *H. punctigera* abundance later in the season compared with previous years [and that other observations have also supported, e.g. our own monitoring and rearing of *Helicoverpa* spp. eggs and larvae from crops and independent observations made through surveys for *Helicoverpa* eggs to estimate Bt resistance in field populations] is related to a landscape scale reduction in pesticide use associated with the advent of Bt cottons. Such may have allowed greater survival and reproduction of *H. punctigera* populations than hitherto was possible under heavy pesticide use. Other explanations may of course be tenable (e.g. later movements into the region of additional generations from elsewhere).

We have been monitoring long term changes in the catches of *Helicoverpa* spp. within the pheromone trap grid near ACRI since 1992. Fig. 7 illustrates data that have now accumulated in this regard for *H. armigera*. The data have been disaggregated into 3 periods

during the cotton season (weeks 8-20, 21-30, and 31-44) which more or less define the timings of the three generations that are common for this species (more clearly delineated through light trap catches cf pheromone traps catches – see Final Report for CSE90C – but also see Fig. 3 above). Since the 1996-97 season (when Bt cottons were first introduced in Australia, including the Namoi Valley), the abundance of *H. armigera* has generally been disproportionately high in late season compared with years prior to 1996-97. The 2004-05 season appears to be an exception to the rule, and at first glance the 2005-06 season seems to be also (Fig. 7). However, we believe the 2005-06 result is misleading (and related to the rather crude way we chose to divide the season into the three periods). The 2005-06 season was particularly hot and the third generation of *H. armigera* seems to have arrived much earlier than normal (Fig. 5) – in fact it clearly has fitted into the mid-season period normally assigned to the second generation (weeks 21-30). If such is taken into consideration, then the pattern in abundance of *H. armigera* in 2005-06 probably is still quite similar to most other years since 1996-97, i.e. with a bias to particularly high numbers in late season. We are unsure quite what is driving these landscape scale, temporal patterns in the abundance of *H. armigera* but speculate that it is a response to reduced use of pesticide brought about by the use of Bt cottons, exacerbated in the early years of transgenic crop deployment when Ingard cotton was known to be less effective late in the season (poor Bt expression). It is also possible that changes in crop types grown in the region (and with varying suitabilities for *H. armigera*) have contributed to the observed moth dynamics. We are currently gathering data to investigate this possibility. Quite why 2004-05 should deviate from the trend is a puzzle, but notably this season had a very dry finish (e.g. 4.5 mm of rain fell in Narrabri in March April 2005, cf 21-215 mm (mean = 110 mm) in the same two months in other years since 1996). Perhaps such weather had an over-riding impact on the abundance of *H. armigera* on the landscape.

The catches of moths (males only shown) in light traps at ACRI during the 2004-05 season are illustrated in Figs 8 & 9, as well as concurrent, nearby catches in pheromone traps and white egg counts. [Data for Chico block only at ACRI are shown for brevity]. The catches of moths using the two different methods were clearly quite different, although they did show some similarity in pattern, more convincingly for *H. armigera* than for *H. punctigera* (see also the Final Report for CSE90C). The catches of moths (note they were males, because of male pheromones being used) showed little similarity with the patterns observed in egg counts (the latter, a surrogate for female moth activity ?).

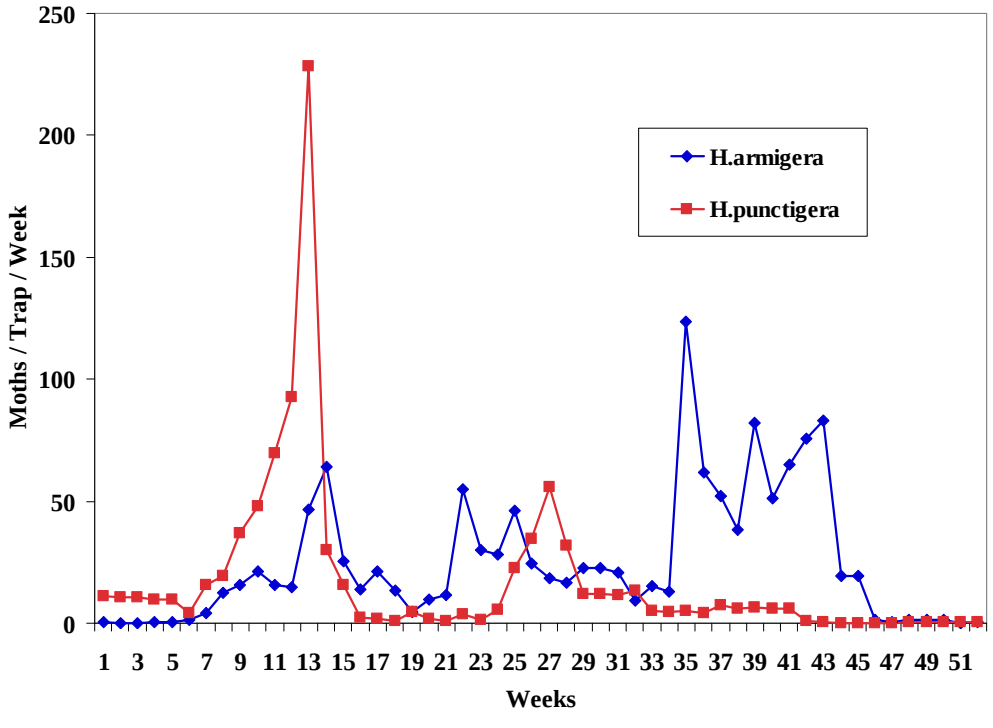

Fig. 3. Abundance of *H. armigera* and *H. punctigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley during 2003-04. Weeks are from 1 July to 30 June.

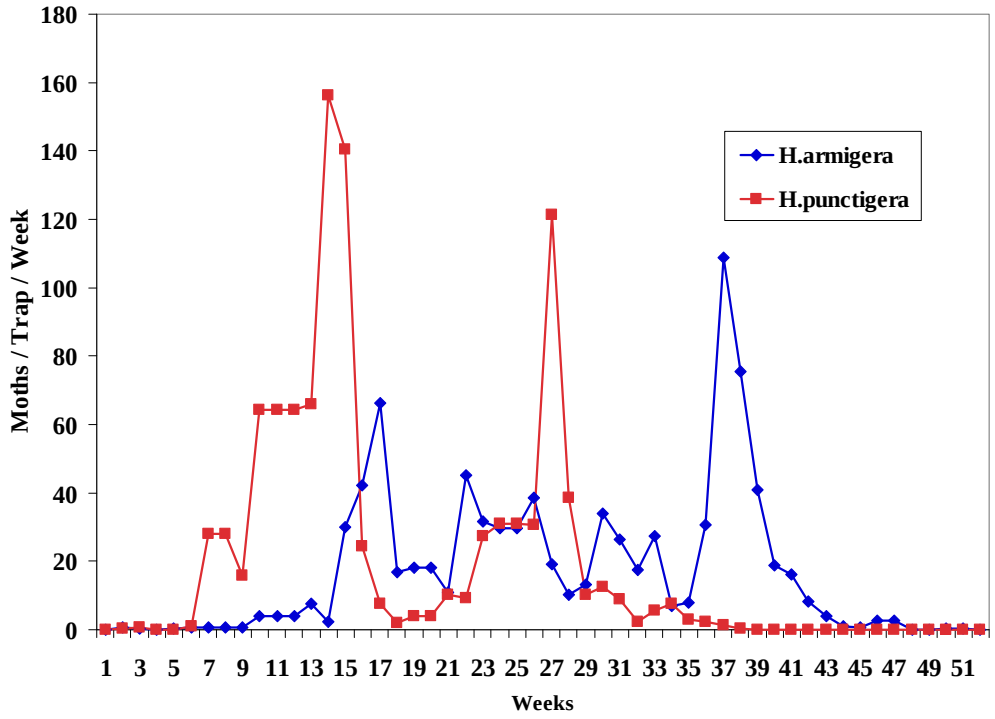

Fig. 4. Abundance of *H. armigera* and *H. punctigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley during 2004-05. Weeks are from 1 July to 30 June.

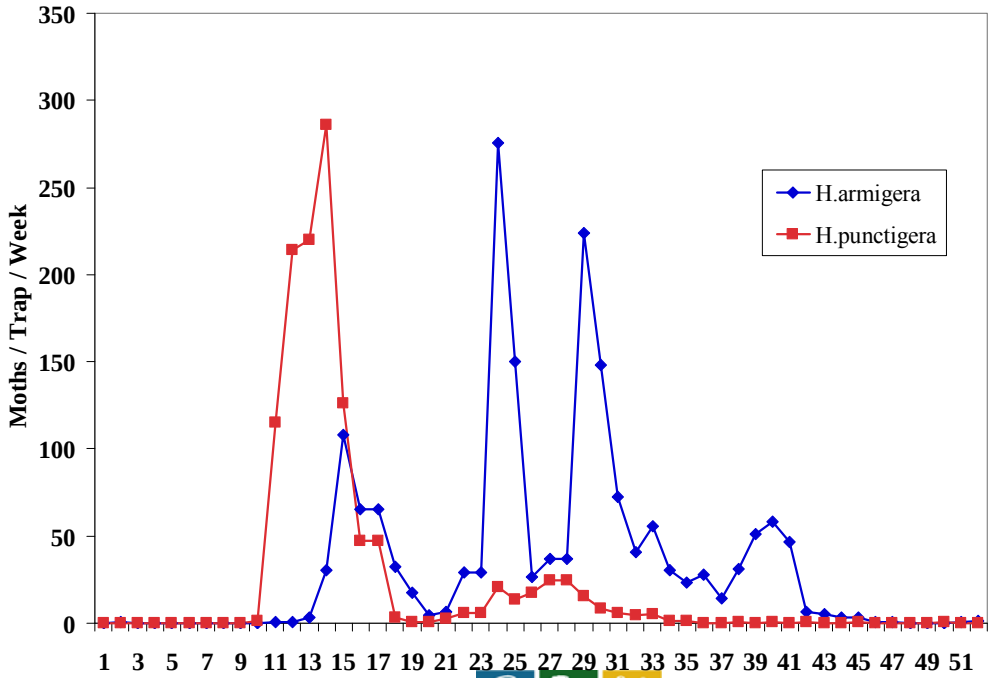

Fig. 5. Abundance of *H. armigera* and *H. punctigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley during 2005-06. Weeks are from 1 July to 30 June.

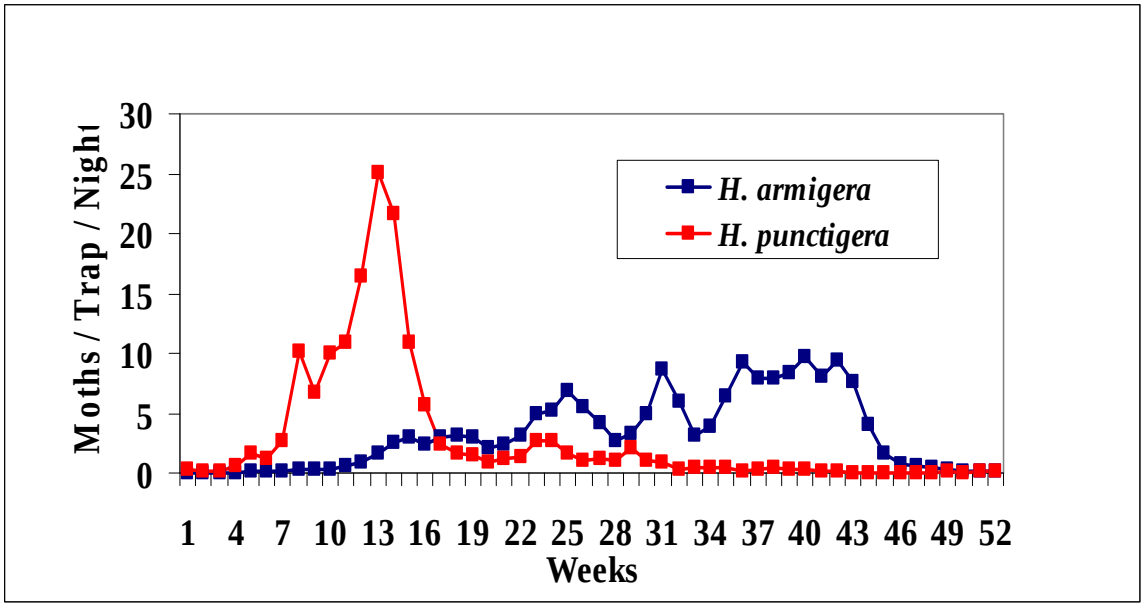

Fig. 6. Abundance of *H. armigera* and *H. punctigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley – average data for the years 1992-2002. Weeks are from 1 July to 30 June.

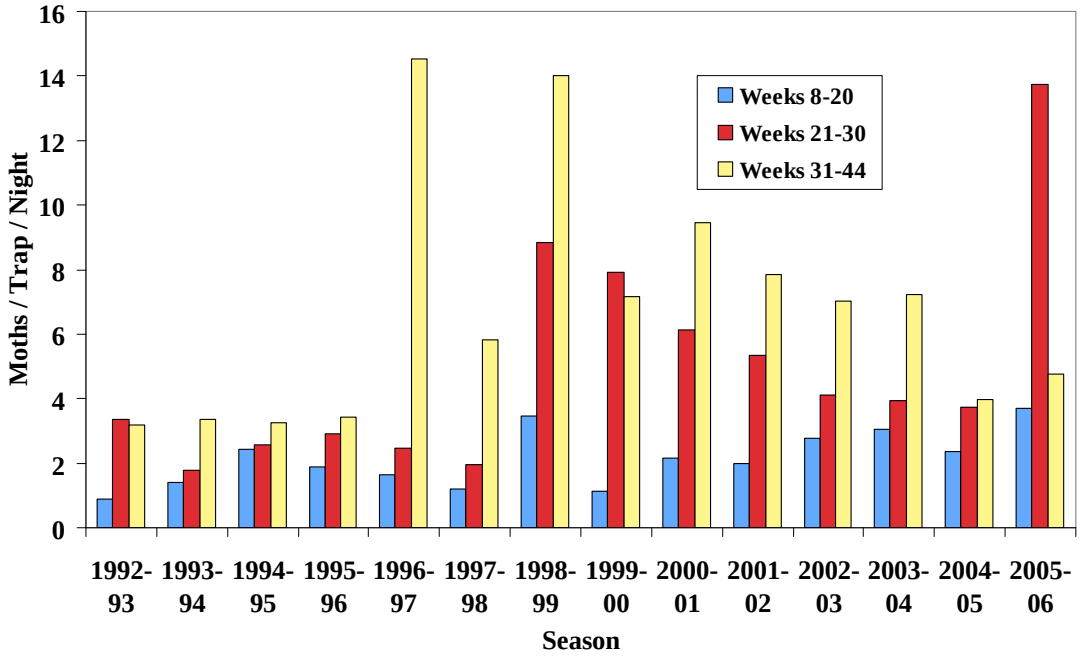

Fig. 7. Abundance of *H. armigera* male moths in pheromone traps set within a 10 km radius of ACRI, Narrabri in the Namoi Valley – for the years 1992-2006. Data are

apportioned within each season into three groupings : weeks 8-20, 21-30, and 31-44, which approximate the timings of generations of *H. armigera*. Weeks are calculated from July 1.

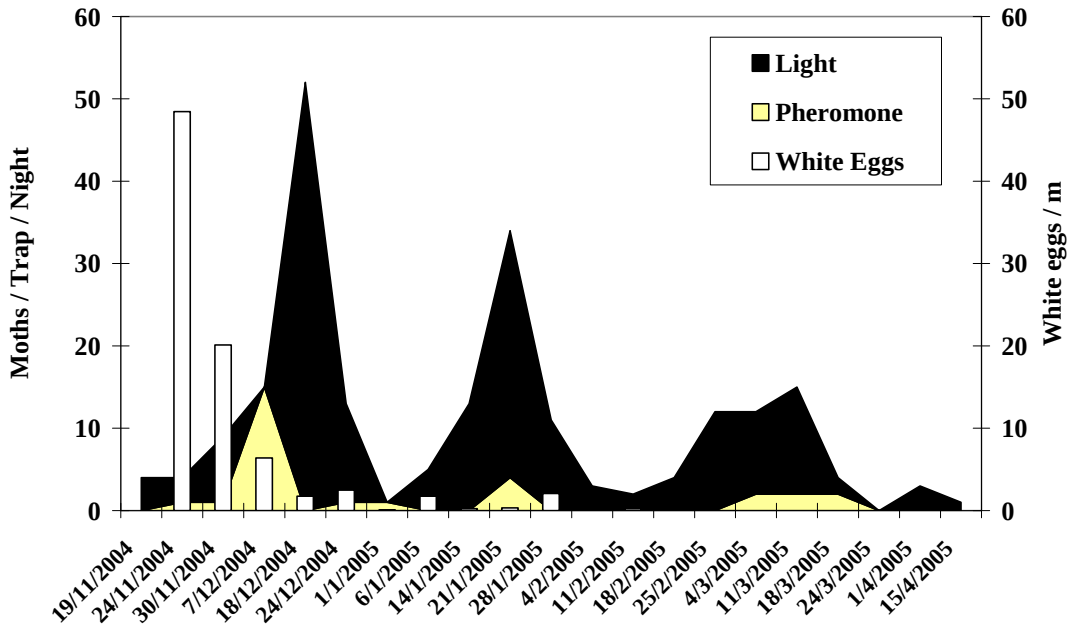

Fig. 8. Abundance of male *H. armigera* in pheromone and light traps and white eggs on cotton plants in Chico Block, ACRI during the 2004-05 season.

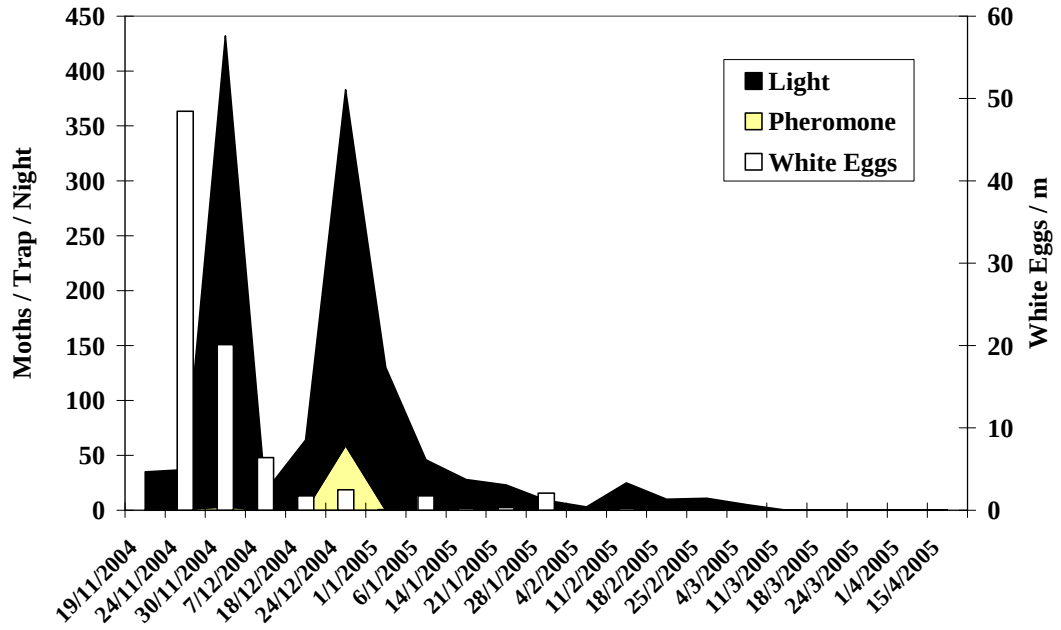

Fig. 9. Abundance of male *H. punctigera* in pheromone and light traps and white eggs on cotton plants in Chico Block, ACRI during the 2004-05 season.

b) St George and Dirranbandi

The abundance of *H. armigera* and *H. punct*igera in the pheromone traps set at St George are presented in Figs 10-12. [Dirranbandi data are not presented here, for brevity]. Note : the restricted operation of the traps (within season, rather than all year round) meant that the early migration of *H. punctigera* into the region prior to the trapping period is not evident in the graphs. What is clear however is the much greater abundance of *H. arm*igera in the 2005-06 season, compared with 2003-04 and 2004-05 (i.e. similar to data collected near ACRI – see above). In addition, the abundance of *H. punctigera* frequently exceeded that of *H. armigera*, in particular during December – January in 2003-04 and 2004-05 (again similar to the ACRI data set). Figs 13 & 14 illustrate the abundance of *H. armigera* and *H. punctigera* over the 9 years that pheromone traps have operated near St George (segregated into data for individual months). These data show a very erratic "pattern" in the abundance of *H. armigera*, but a tendency for larger catches of *H.punctigera* in recent years, particularly later in the growing season. Egg lays (Fig 15) seem to have been erratic between years near St George, but particularly high in the last three years, as well as in 1998-99. Unfortunately, we don't have data prior to the advent of Bt cotton in the St George / Dirranbandi region, but there doesn't seem to have been the same marked abundance of *H. armigera* at the end of the cotton season there since 1996, as observed in the Namoi (perhaps a difference in regional cropping strategies helps explain this ?).

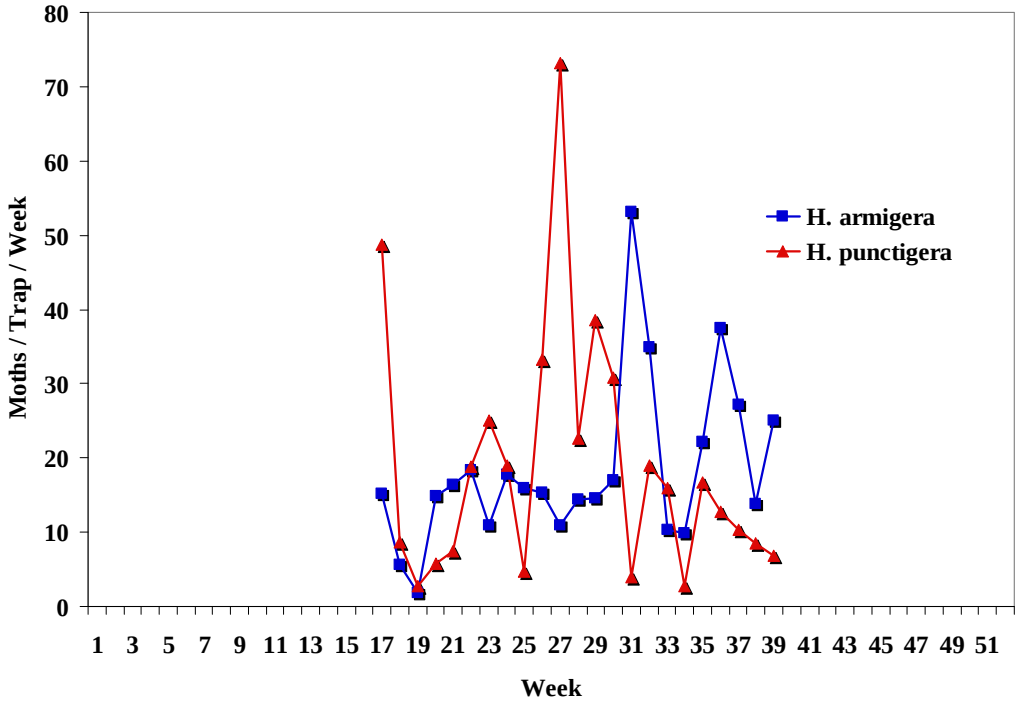

Fig. 10. Abundance of *H. armigera* and *H. punctigera* male moths in pheromone traps set near St George during 2003-04. Weeks are from 1 July to 30 June.

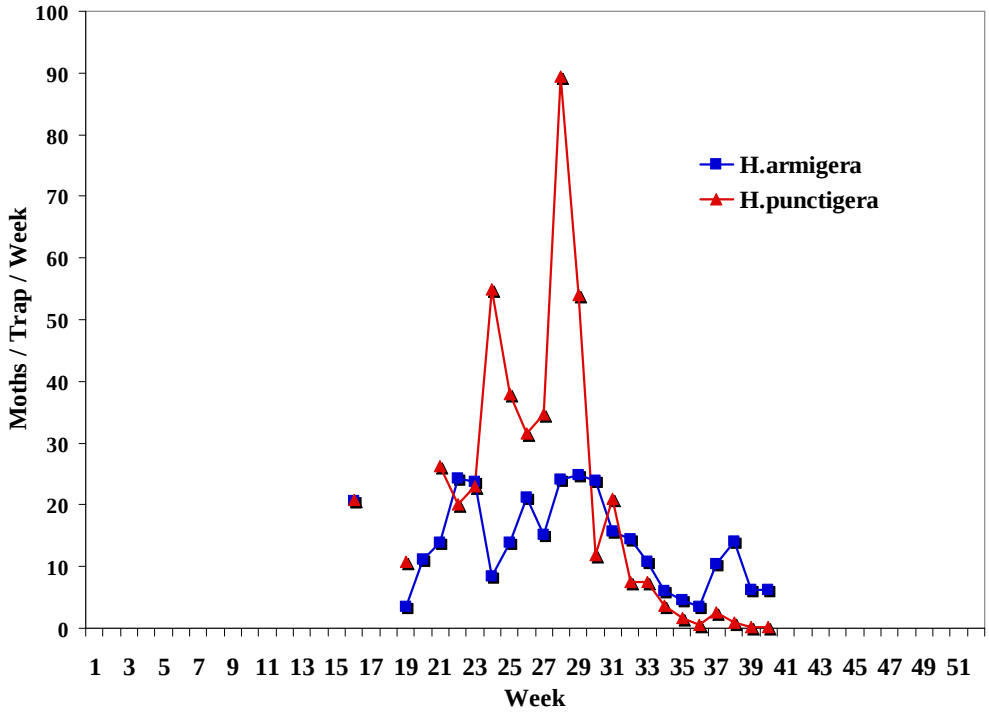

Fig. 11. Abundance of *H. armigera* and *H. punctigera* male moths in pheromone traps set near St George during 2004-05. Weeks are from 1 July to 30 June.

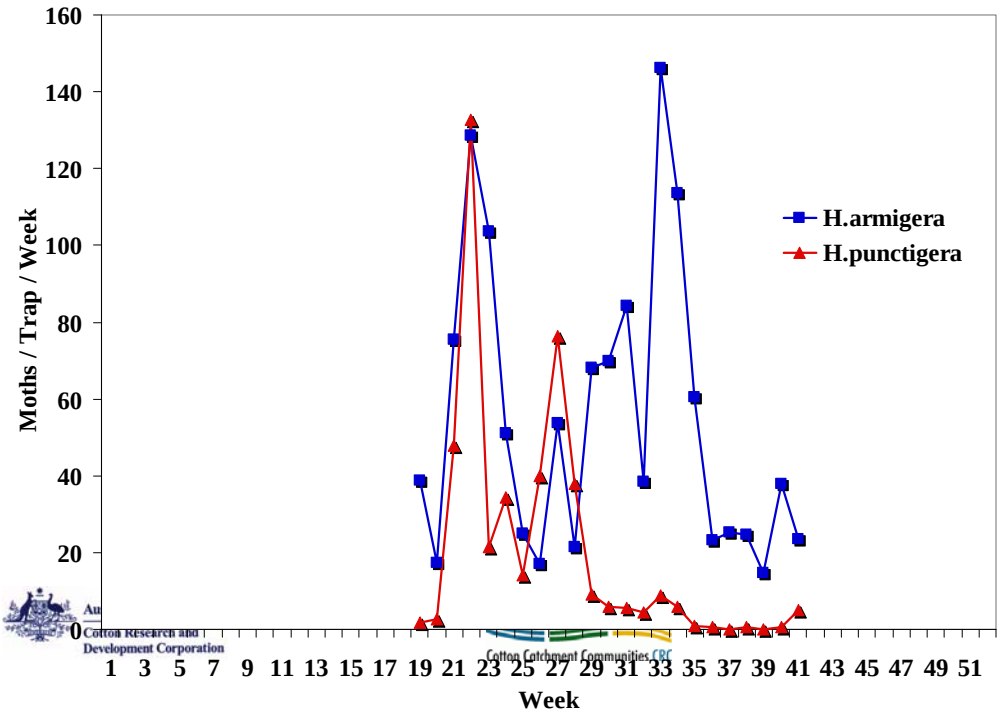

Fig. 12. Abundance of *H. armigera* and *H. punctigera* male moths in pheromone traps set near St George during 2005-06. Weeks are from 1 July to 30 June.

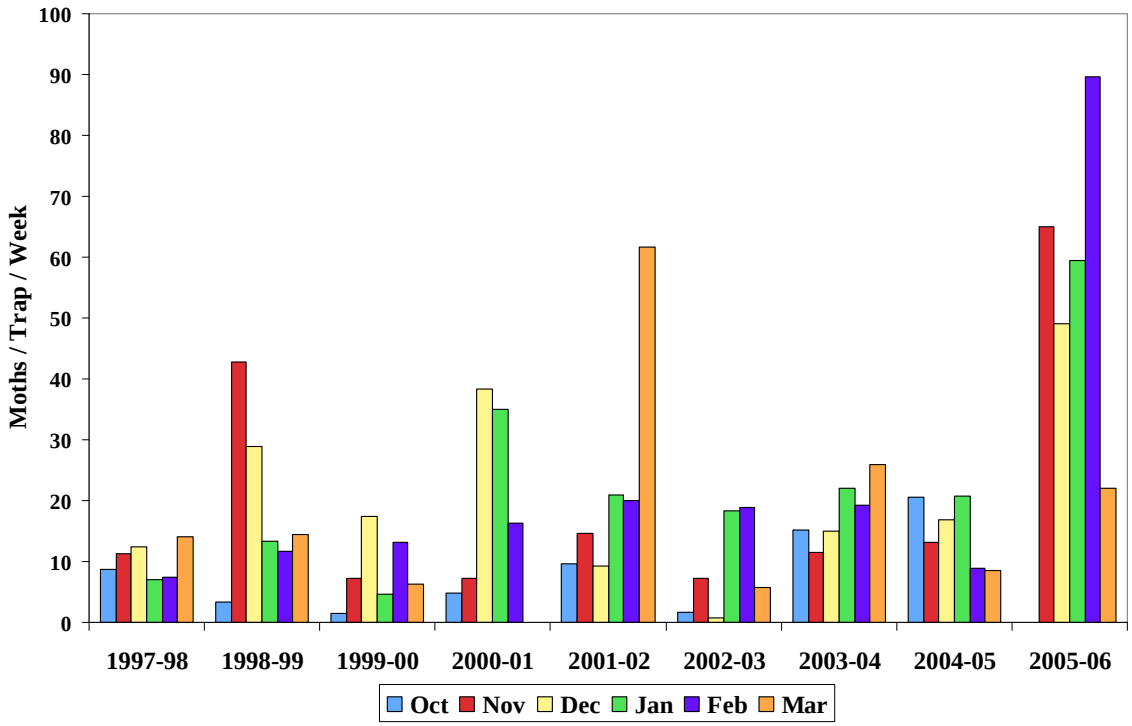

Fig. 13. Abundance of *H. armigera* male moths in pheromone traps set near St George during 1997-2006. Data are segregated into months within each season.

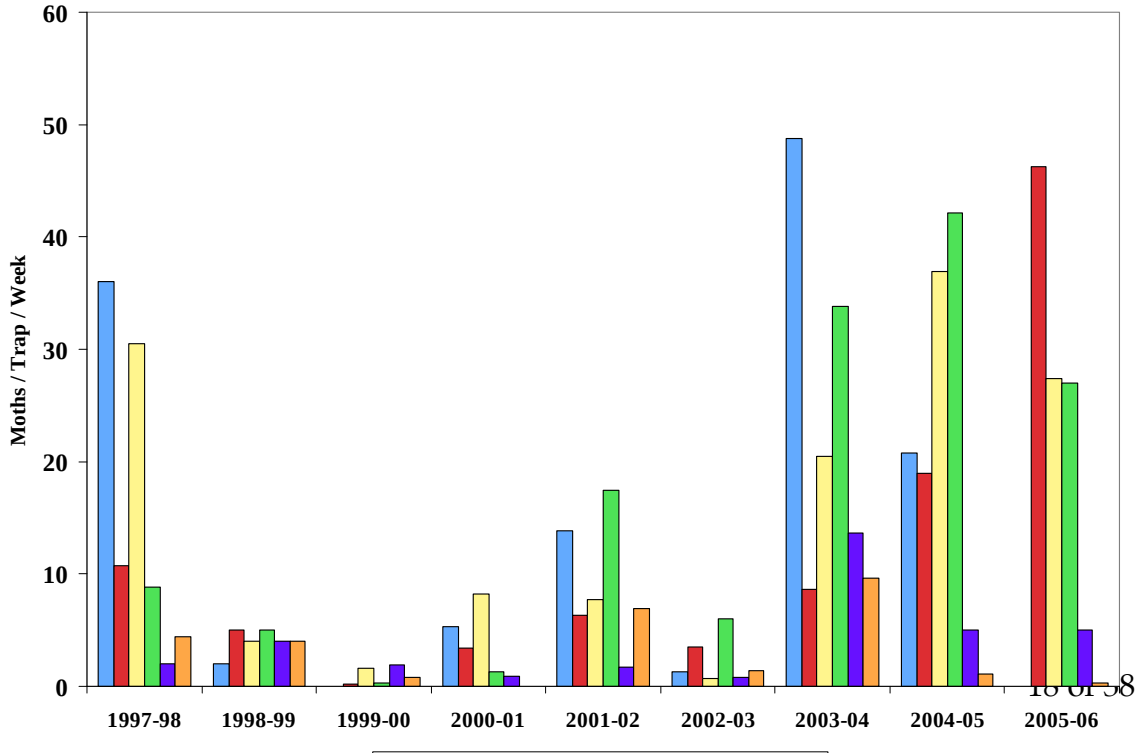

Fig. 14. Abundance of *H. punctigera* male moths in pheromone traps set near St George during 1997-2006. Data are segregated into months within each season.

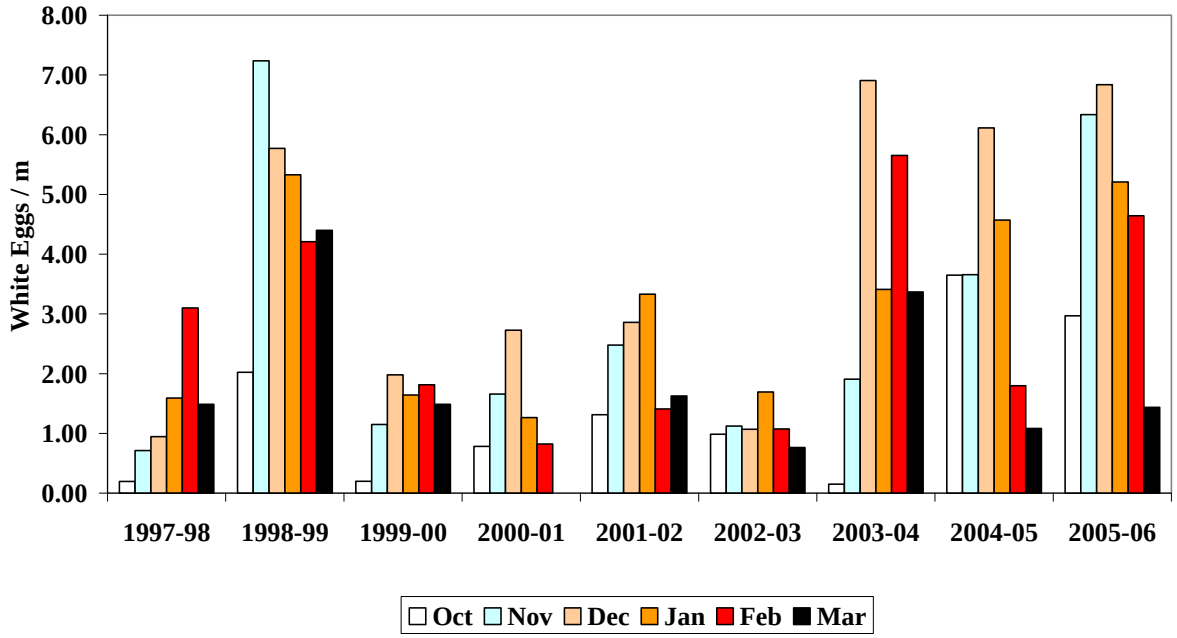

Fig. 15. Abundance of *Helicoverpa* spp. white eggs on cotton crops in the vicinity of pheromone traps near St George during 1997-2006. Data are segregated into months within each season. Data kindly provided by consultants in the St George region.

3. Abundance of *Helicoverpa* and Other Invertebrates on Bt Cotton and Associated Refuge Crops.

Figs 16-18 illustrate the abundance of selected invertebrate taxa collected within Bollgard II crops (and occasional Ingards crop during 2003-04) , both near the edge and further into the field, and within their associated refuges (always pigeon pea) using suction (D-vac) sampling during the three years of the project. Whilst there was no evidence of any difference in the abundance of spiders across these three "habitats", there was clear consistency in predatory beetles being more abundant in the refuge crops compared with the Bollgard II crops, and more abundant near the edges of Bollgard II crops compared with further into such crops. Predatory bugs and mirids were more abundant within the refuge crops compared with Bollgard II crops, but there was no evidence of them varying in abundance within the Bollgard II crops in a similar way to the predatory beetles.

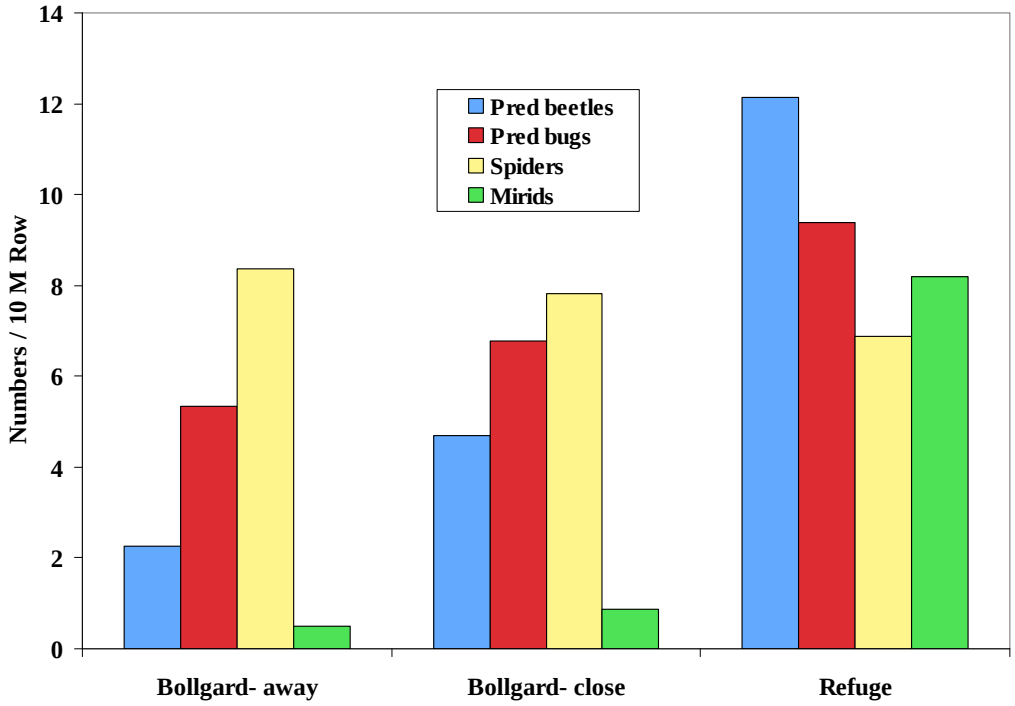

Fig. 16. Abundance of selected canopy invertebrates collected by suction (D-vac) sampling in Bollgard II cotton crops and associated refuge crops in northern NSW and southern Qld during 2003-04.

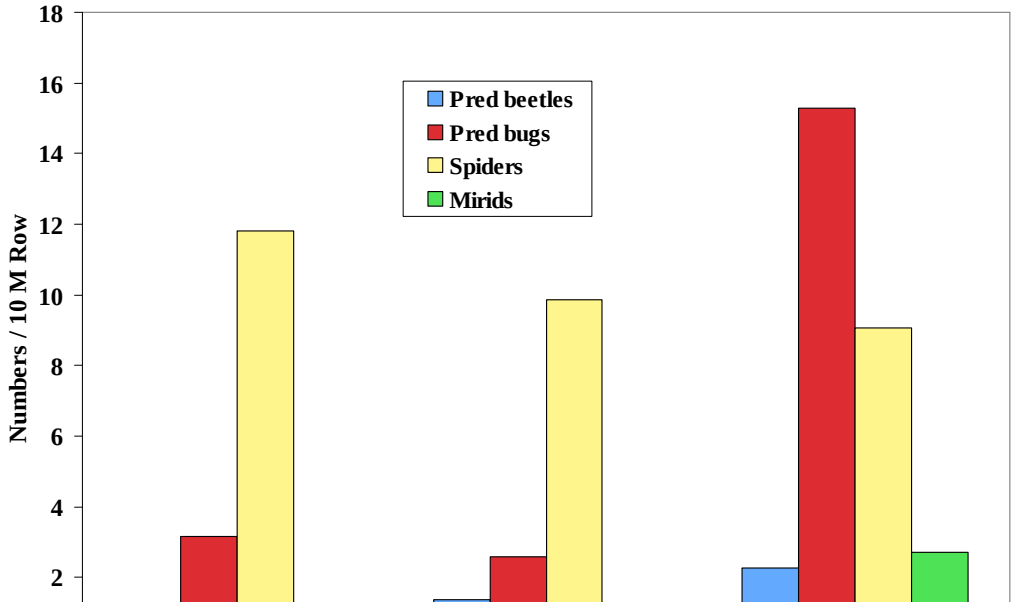

Fig. 17. Abundance of selected canopy invertebrates collected by suction (D-vac) sampling in Bollgard II cotton crops and associated refuge crops in northern NSW and southern Qld during 2004-05.

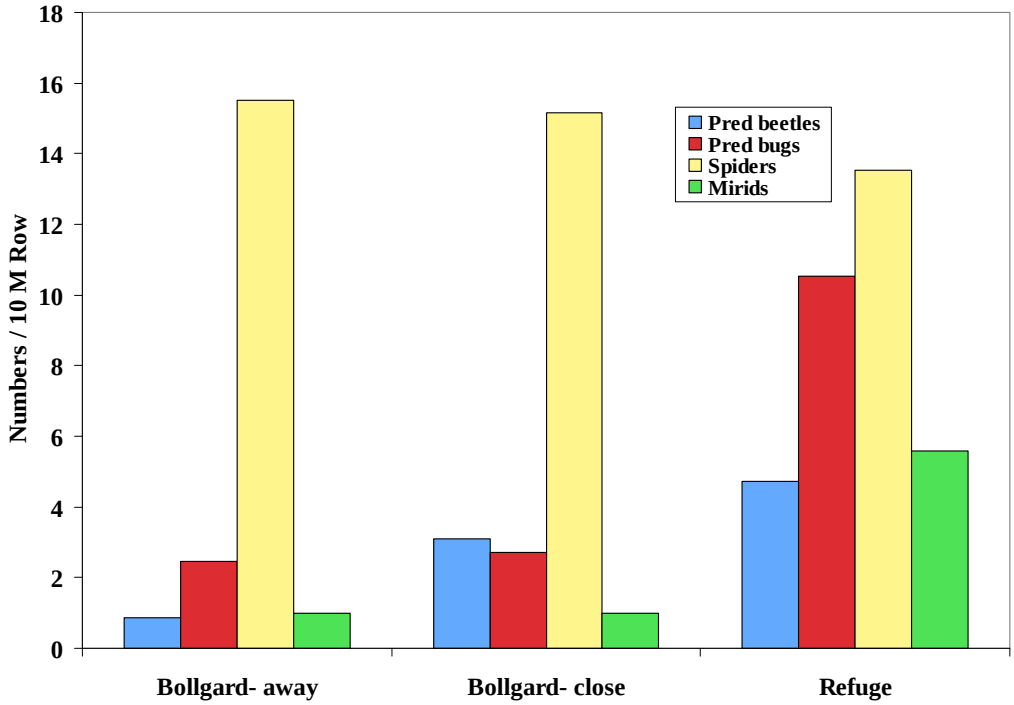

Fig. 18. Abundance of selected canopy invertebrates collected by suction (D-vac) sampling in Bollgard II cotton crops and associated refuge crops in northern NSW and southern Qld during 2005-06.

Visual sampling at these same sites mostly yielded *H. punctigera* from pigeon pea refuge crops, when eggs and larvae were reared through to moths in the laboratory (and frustratingly so – since we were hoping for *H. armige*ra to build up at these sites to enable mark-recapture studies !) (Figs 19-21). There was no consistent *Helicoverpa* species bias within eggs and larvae, when reared through to moths, from collections made near the edge or further within Bollgard II crops, nor between Bollgard and pigeon pea refuge crops. There was no obvious difference between the low numbers of *Helicoverpa* eggs laid within the three "habitats" (refuge, inner and outer of Bt cotton crops). Larval abundance was also overall generally low, but higher within the refuge crops than within the Bollgard II crops, as would be expected. Parasitism was erratic.

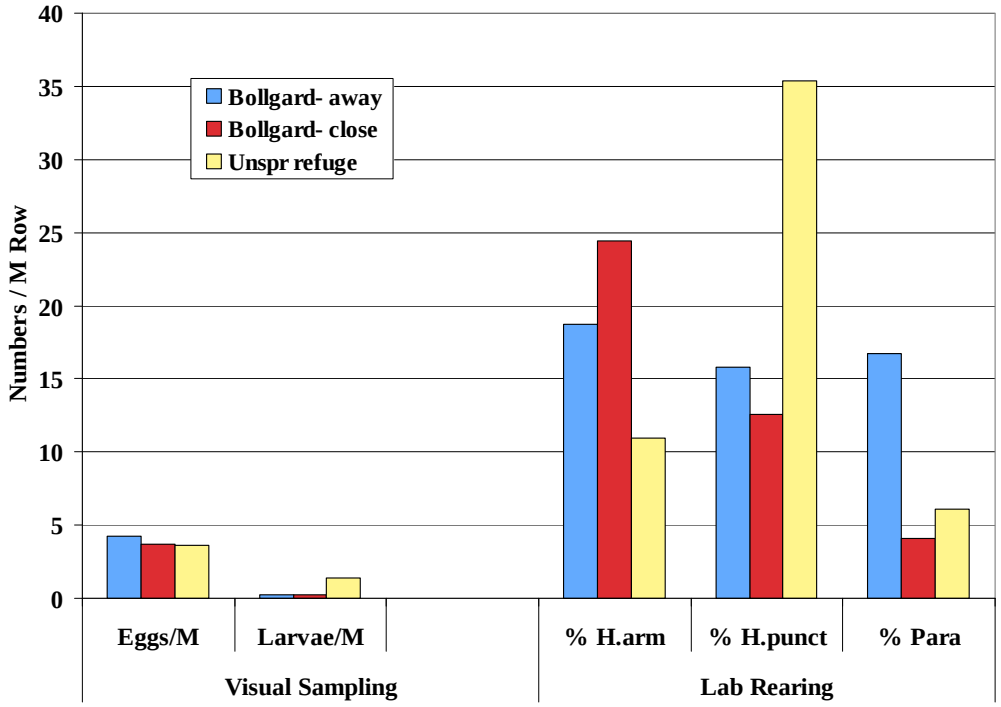

Fig. 19. Abundance of *Helicoverpa* spp. eggs and larvae collected during visual surveys of Bollgard II cotton crops and associated pigeon pea refuge crops in northern NSW and southern Qld during 2003-04, and incidence of *H. armigera, H. punctigera* and parasitism when such were reared in the laboratory.

Figs 22-24 present data from the visual surveys for *Helicoverpa* eggs and larvae, and the outcomes from laboratory rearings of these eggs and larvae from the Bollgard II (and occasional Ingard) crops and their associated pigeon pea refuge crops at all the sites surveyed (i.e. not just those wherein Bollgard II crops were sampled near their edges and further into the fields). The data are reasonably similar to those presented in Figs 19-21, although a preponderance of *H. punctigera* in the rearings from pigeon pea refuge crops was not found in all three years. Data for the relatively small number of other Bt cotton crops and their associated unsprayed conventional cotton, sorghum or maize refuge crops that were also sampled are not provided here, but are available on request.

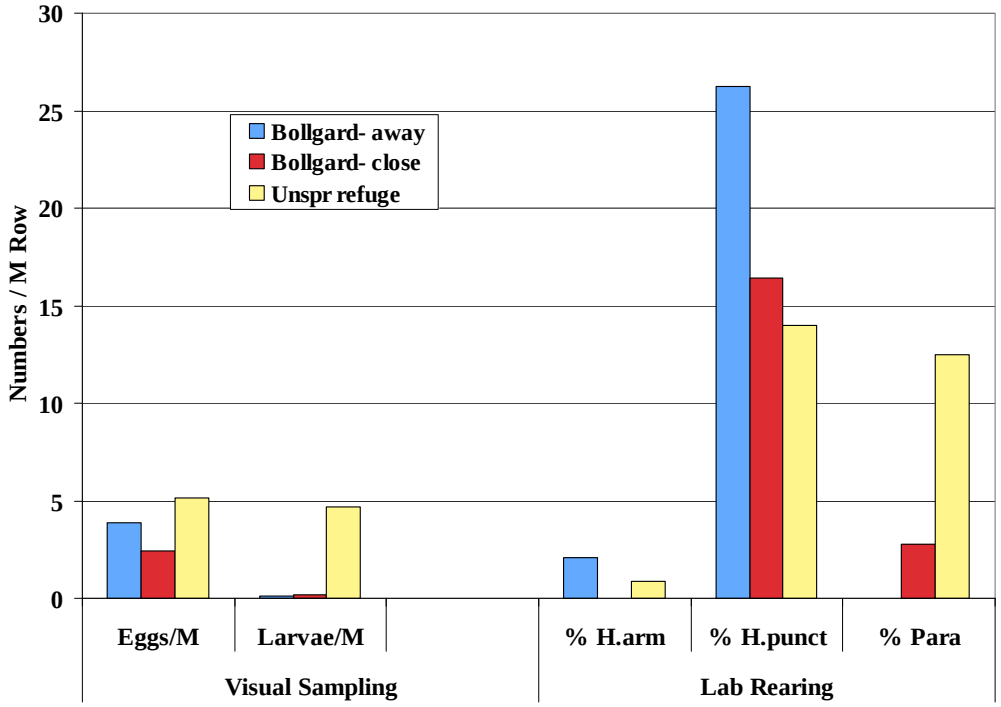

Fig. 20. Abundance of *Helicoverpa* spp. eggs and larvae collected during visual surveys of Bollgard II cotton crops and associated pigeon pea refuge crops in northern NSW and southern Qld during 2004-05, and incidence of *H. armigera*, *H. punctigera* and parasitism when such were reared in the laboratory.

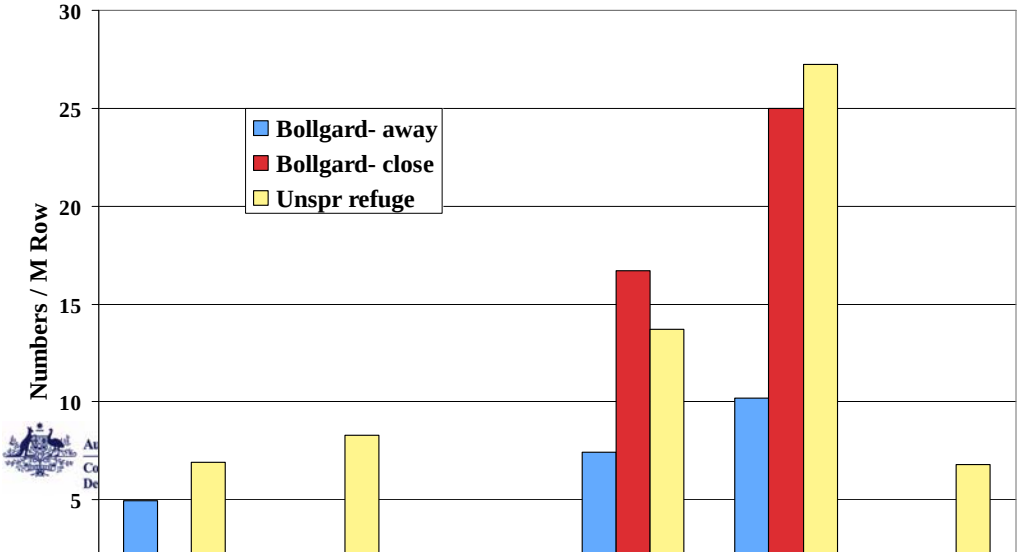

Fig. 21. Abundance of *Helicoverpa* spp. eggs and larvae collected during visual surveys of Bollgard II cotton crops and associated pigeon pea refuge crops in northern NSW and southern Qld during 2005-06, and incidence of *H. armigera, H. punctigera* and parasitism when such were reared in the laboratory.

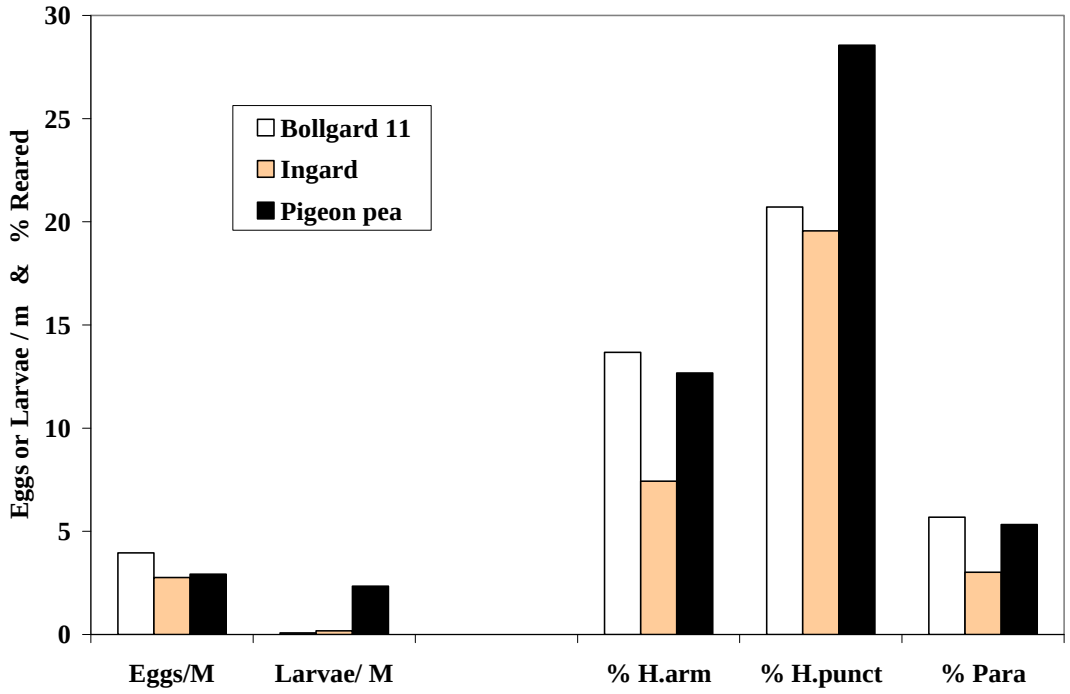

Fig. 22. Abundance of *Helicoverpa* spp. eggs and larvae collected during visual surveys of Bt cotton crops (mostly Bollgard II) and associated pigeon pea refuge crops in northern NSW and southern Qld during 2003-04, and incidence of *H. armigera, H. punctigera* and parasitism when such were reared in the laboratory.

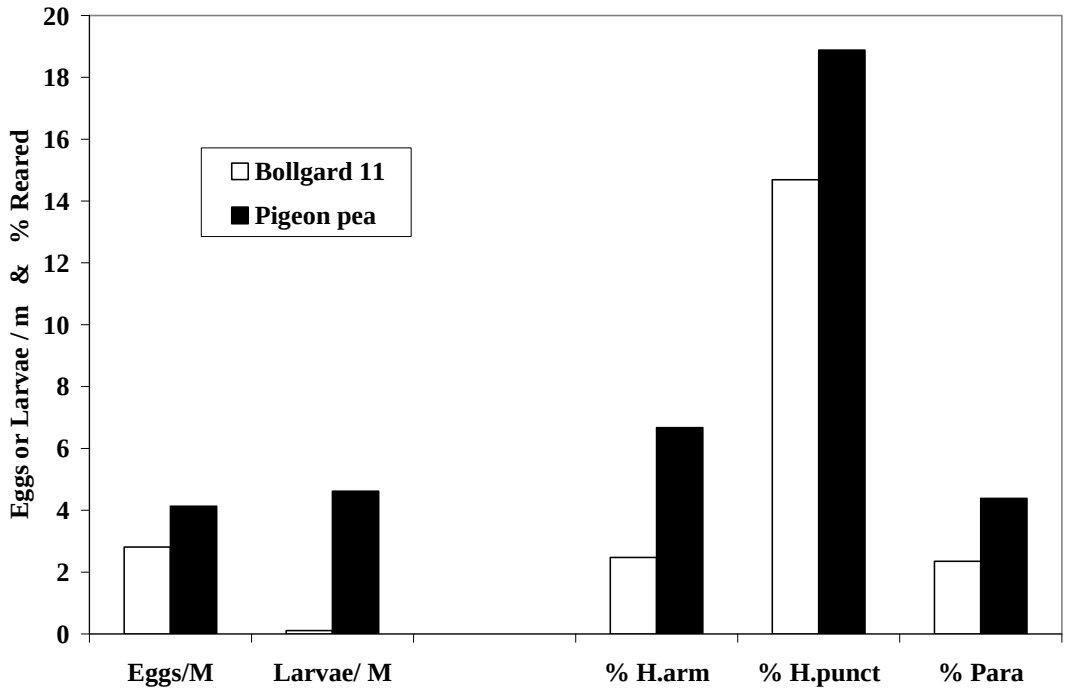

Fig. 23. Abundance of *Helicoverpa* spp. eggs and larvae collected during visual surveys of Bollgard II cotton crops and associated pigeon pea refuge crops in northern NSW and southern Qld during 2004-05, and incidence of *H. armigera, H. punctigera* and parasitism when such were reared in the laboratory.

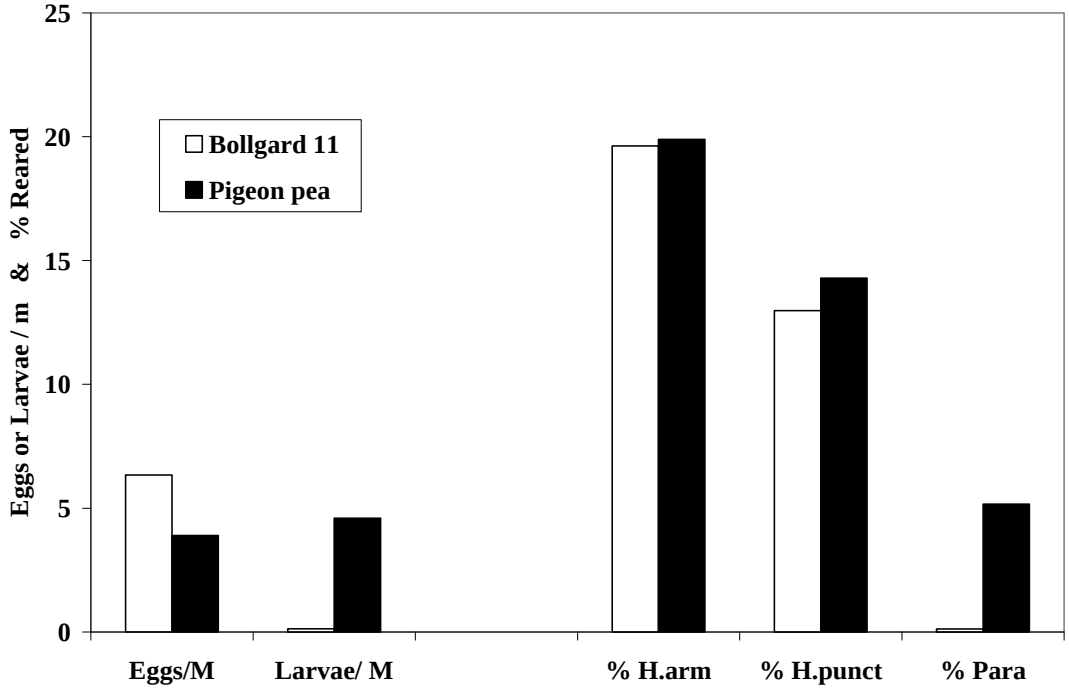

Fig. 24. Abundance of *Helicoverpa* spp. eggs and larvae collected during visual surveys of Bollgard II cotton crops and associated pigeon pea refuge crops in northern NSW and southern Qld during 2005-06, and incidence of *H. armigera, H. punctigera* and parasitism when such were reared in the laboratory.

Table 1 lists presence only data for various taxa collected during these surveys of Bt cotton crops and their associated refuge crops. Whilst the data are necessarily crude (and only data for the 2003-04 season are presented here for brevity), they do indicate the most frequently encountered species. Amongst these, red and blue beetles, damsel, big-eyed and apple dimpling bugs, lynx and flower / crab spiders, mirids, thrips, jassids and whiteflies were the most commonly encountered invertebrates in Bt cotton crops (not including *Helicoverpa* spp.). The same taxa predominated in pigeon pea refuge crops nearby. In other years (2004-05 and 2005-06; data not shown), more or less the same taxa also predominated. Exceptions were : a relative scarcity of damsel, big-eyed and apple dimpling bugs and mirids and white fly in subsequent years (compared with 2003-04). The most common predatory taxa scored during similar work noted in the Final Report for CSE90C were much the same as those identified here.

4. Incidence of Mating Between *Helicoverpa armigera* Moths From Different Plant Host Origins Within Bt Cotton Crops

Carbon delta results (mean ± S.E.) varied significantly amongst moths reared from the pupae collected in soil beneath the four crops (unsprayed cotton = -28.79 ± 0.33, pigeon pea = -26.38 ± 0.34, sorghum = -13.24 ± 0.20, corn = -11.45 ± 0.09; One way ANOVA [based on first 10 moths analysed from each treatment], $F = 1153.1$, $p < 0.001$) (Fig. 25). The carbon delta means differed between all field sources (LSD test).

Table 1. Recorded presences of various predatory invertebrates (and occasional secondary pests) noted in suction sampling (D-Vac), visual surveys and occasional sweep netting of Bt cotton crops and associated refuge crops in northern NSW and southern Qld during 2003-04. Numbers following refuge types indicate frequency of sampling occasions (some crops could not always be accessed – e.g. due to recent flood irrigation. Bt cotton and refuge visits thus do not necessarily equate). Note data are aggregated together for all sampling methods.

Taxa	Bt Cotton (41)	Pigeon Pea (34)	Unsprayed Cotton (5)	Sorghum (6)	Maize (0) (sampled in other years)
Beetles					
Anthicus sp.	19	18	3	6	
Red & Blue	37	32	4	6	
Green soldier	2	4	0	0	
Carabidae sp.	0	0	0	0	
2 spotted L/B	13	8	2	4	
Transverse L/B	5	4	2	3	
Striped L/B	4	3	1	0	
3 banded L/B	7	4	2	4	
Variable L/B	2	2	0	1	
Spotted L/B	4	3	1	2	
Hippo L/B	14	12	2	3	
L/B larvae	1	1	0	3	
Stethorus sp.	6	2	0	2	
Bugs					
Brown smudge	6	4	0	4	
Glossy shield	2	5	1	0	
Pred Shield	8	11	1	3	
Damsel	33	34	5	2	
Pirate	20	12	1	5	
Assassin	1	0	0	0	
Big-eyed	36	15	3	5	
Apple Dimpling	36	32	3	3	
Lacewings					
Green	16	19	2	2	
Brown	11	6	1	4	

L/W larvae	21	12	3	1
Spiders				
Lynx	40	33	5	6
Night stalkers	22	16	4	3
Tangle web	15	2	3	3
Jumping	27	7	2	5
Flower / crab	33	28	4	3
Orb weaver	12	6	4	1
Others				
Hoverfly larvae	6	2	1	0
Ants	10	13	1	2
Mirids	28	30	3	3
GVB	4	4	1	0
Thrips	37	34	5	6
Aphids	13	1	1	5
Jassids	40	33	5	6
Mites	1	1	0	0
Whitefly	31	15	4	0

Very few *H. armigera* moths were collected at Drayton in the Bollgard II and sorghum crops (Fig. 26). But large numbers of *H. armigera* were collected in the conventional cotton crop (Fig. 26), 64 of which were mating (i.e. 32 pairs). If we accept that a carbon delta value of < - 20 is indicative of a C3 plant host and a carbon delta value of > - 20 is indicative of a C4 plant host (see results given above for moths reared from pupae collected beneath such plant hosts), then overall we observed the following matings amongst the 32 pairs : 7 pairs representing matings between C3 derived individuals, 10 pairs representing matings between C4 derived individuals, 13 pairs representing mixed pairings (C3 x C4) and 2 pairs which were inconclusive. Given the proportions of C3 and C4 moths present in total, these pairings are not significantly different from what would be expected by chance ($\chi^2 = 0.48$, $p > 0.05$), although there is a slight tendency for mixed matings to be less than expected. That is it seems likely from these results that mating is happening at random over cotton between moths from different host plant sources. This is a very encouraging finding : such random mating is a basic assumption of the current Bt resistance management strategy.

However, the results from Wee Waa were different. Fig. 27. illustrates carbon delta data for the first 140 mating pairs of *H. armigera* collected and analysed from Bollgard II® cotton crops near Wee Waa (a subset of the total collection of 265 mating pairs). Data are arranged with the two members of each mating pair randomly assigned as 'A' or 'B' and vertically aligned. Clearly, the moths varied in their carbon isotope signatures. Within some mating pairs, carbon delta data were similar for individual moths; within other mating pairs, carbon delta data differed markedly. On the same basis of accepting a carbon delta value of < -20 as the discriminator between C3 and C4 host plant origin, 63 matings were observed between moths from C3 sources only, 96 matings between moths from C4 sources only, and 106 matings where moths came from different plant hosts (i.e. C3 x C4 matings), across the 265 mating pairs collected. If we assume that the mating moths reflect a representative sample of those present in the Bt cotton crops, then we can also calculate from the results if the observed matings between moths of C3 and C4 plant origins differ from what we might expect to occur at random (given the relative abundance of such moth types in our catches). From our catches we would expect by chance (i.e. random mating) to have found 50.8 C3 x C3 matings, 130.4 C3 x C4 matings and 83.8 C4 x C4 matings. Chi square analysis suggests that our findings differ significantly from what we would expect through random mating ($\chi^2 = 9.28$, $p < 0.05$). That is, we observed more matings between moths from the same plant origins, and fewer matings between moths from different plant origins than we would have expected by chance. Thus these results bring into question the assumption of random mating that underpins the Bt resistance management strategy.

The precise locations of the mating moths we collected in the Bt crops near Wee Waa were not recorded. The mating moths were collected during random walks throughout the cotton

fields, up to 1 Km from the dedicated refuge we had earlier been monitoring. In future studies we intend to gather more spatially explicit data (perhaps via GPS) on the incidence of inter-mating of moths from different plant hosts within Bt cotton fields. This should better indicate the efficacy of such mating.

The mating moths we collected near Wee Waa mostly had carbon delta values of either -25 to -28 or -11 to -17. Whilst the former match quite closely with carbon delta data observed for moths emerging from pupae collected under cotton and pigeon pea crops, the latter data was not as closely matched with the carbon delta values collected from moths emerging from pupae collected under sorghum and corn. Whether or not this variation reflects that these field collected moths originated from other C4 plant hosts on the landscape, or some "contamination" through feeding since emergence is unknown.

One of the intriguing results from our work near Wee Waa was the abundance of the mating moths that we collected in the Bollgard II crops that bore C3 carbon isotope signatures (i.e. 44 % of the mating moths). It seems highly unlikely that these moths originated from the Bollgard II cotton crops (larvae were rarely seen on these crops). They may have come from other cotton crops in the region, from pigeon pea, or some other unknown source. We intend to analyse the remains of the C3 – signatured moths we have collected (e.g. for gossypol which is a characteristic for cotton), to determine if their origins were cotton or another plant.

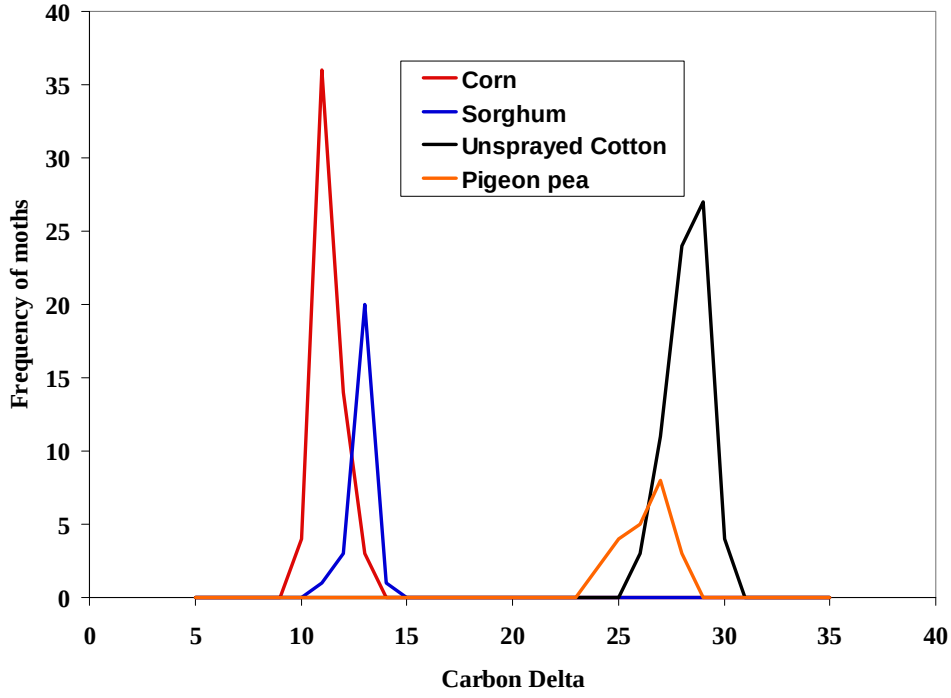

Fig. 25. Carbon isotope analyses for *H. armigera* moths reared from pupae collected beneath crops (maize and sorghum – C4 plants and cotton and pigeon pea – C3 plants).

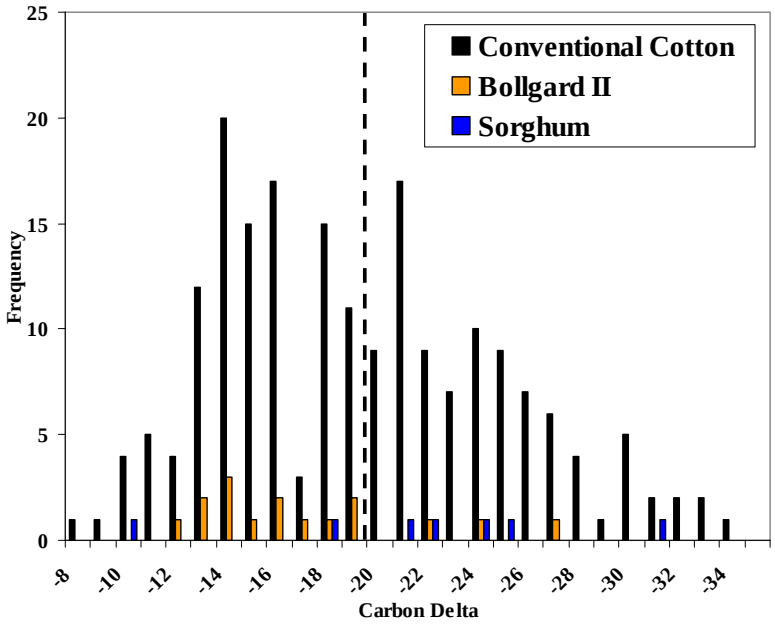

Fig. 26. Carbon isotope analyses of *H. armigera* moths caught over conventional unsprayed cotton, Bollgard II cotton and sorghum crops near Drayton, NSW.

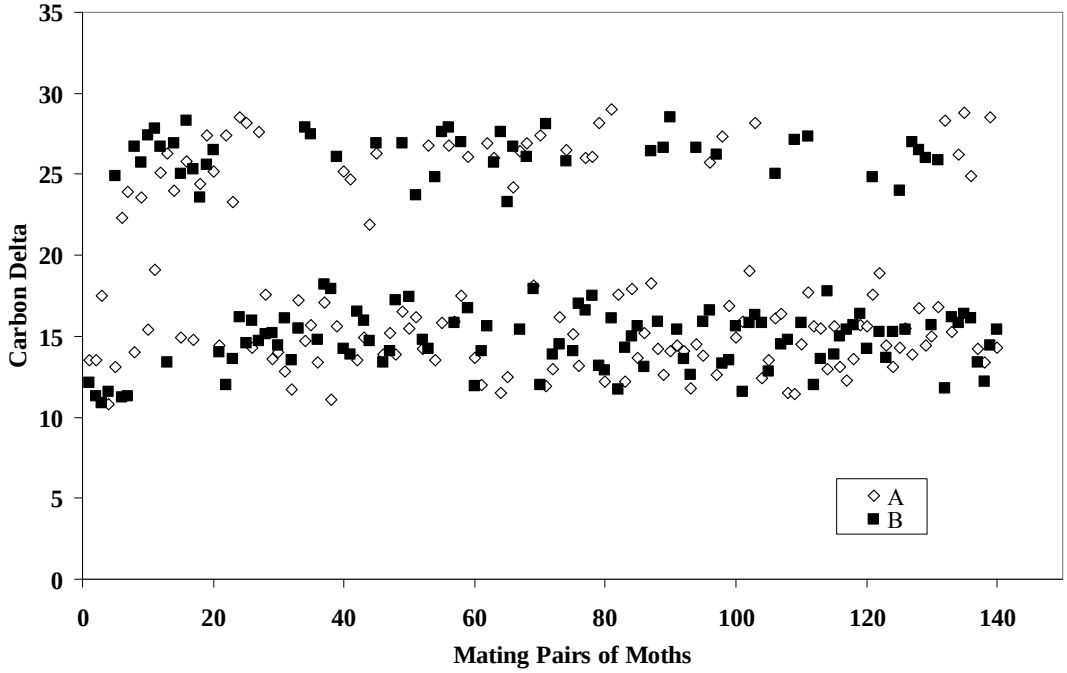

Fig. 27. Carbon isotope analyses of *H. armigera* moths caught over Bollgard II cotton crops near Wee Waa, NSW. Associated refuge crops were C4 plants (sorghum and maize). A and B represent different individuals (males and females) within each of a subset (n = 140) of the mating pairs of moths that were collected.

5. Influence of Jasmonic Acid Applications on the Localised Abundance of Beneficial Invertebrate Populations on Cotton Plants.

Whilst there were trends in both experiments in 2003-04 towards greater numbers of predatory invertebrates (analysed by key groups of taxa) on cotton plants sprayed with jasmonic acid compared with control plants, such trends were not significant, and in subsequent years no significant differences between treatments were observed either (Figs 29-31). No significant differences were observed between catches in sticky traps surrounding cotton wool impregnated with jasmonic acid and water (Fig. 32). There was a consistent tendency for *Helicoverpa* eggs to be laid in greater numbers on cotton plants sprayed with jasmonic acid, but this trend was never significant on a particular occasion. The conclusion has to be that applications of jasmonic acid are unlikely, at least at the rates we applied [which were similar to those used by other, overseas authors], to provide a means of attracting beneficial species into cotton crops. There could of course be a different response if much greater areas of cotton plants were to be sprayed, and this issue of scale somehow influenced attraction of beneficial species in our studies. Another possibility is that the nature of the beneficial community we worked with did not include, by chance, the most responsive beneficial species. But this possibility was offset by conducting our studies in three consecutive years.

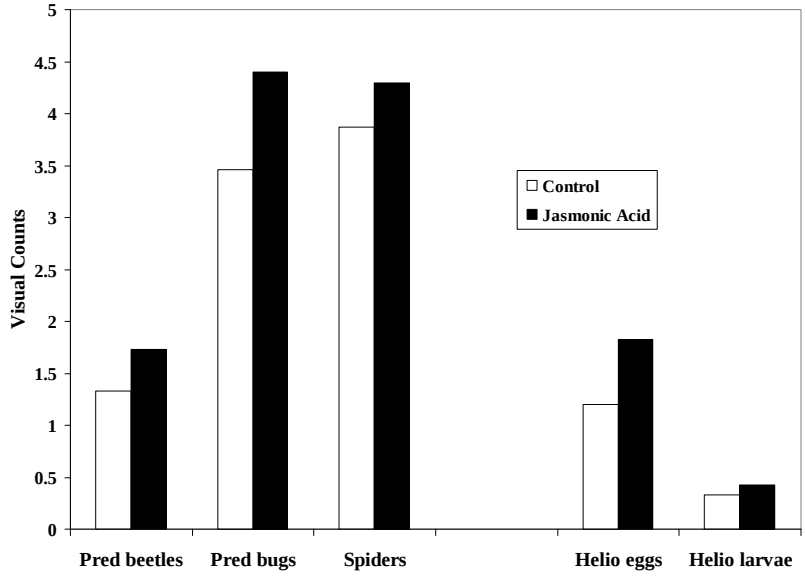

Fig. 28. Abundance of *Helicoverpa* spp. eggs and larvae and various predatory invertebrates collected during visual surveys of conventional cotton plants sprayed with

either jasmonic acid solution or water (Controls) at ACRI, during 2003-04. (Experiment 1).

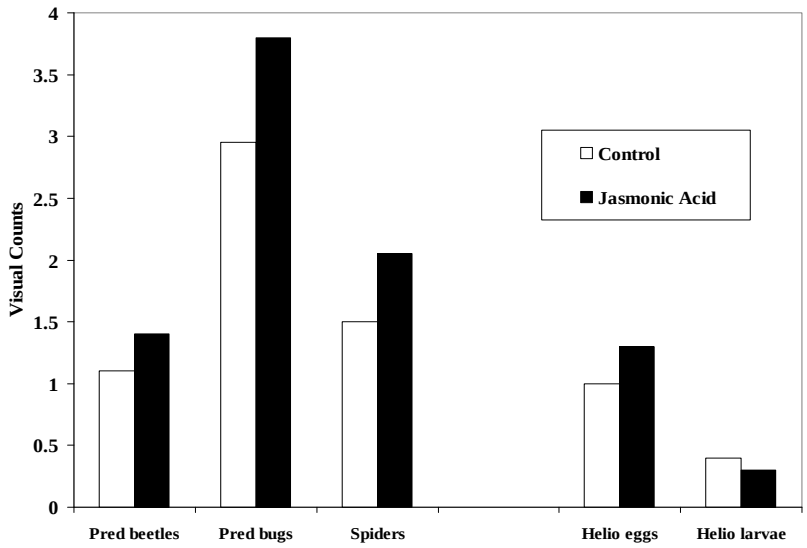

Fig. 29. Abundance of *Helicoverpa* spp. eggs and larvae and various predatory invertebrates collected during visual surveys of conventional cotton plants sprayed with either jasmonic acid solution or water (Controls) at ACRI, during 2003-04. (Experiment 2).

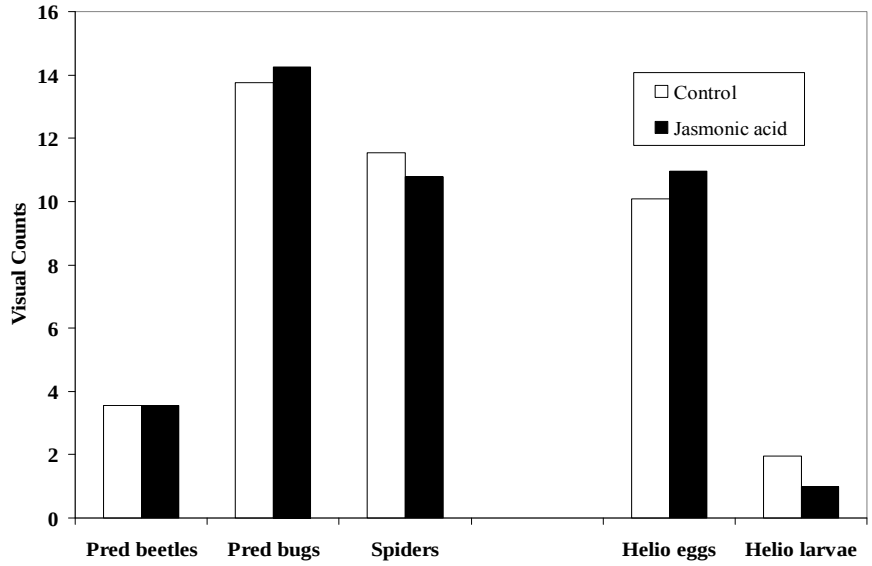

Fig. 30. Abundance of *Helicoverpa* spp. eggs and larvae and various predatory invertebrates collected during visual surveys of conventional cotton plants sprayed with either jasmonic acid solution or water (Controls) at ACRI, during 2004-05.

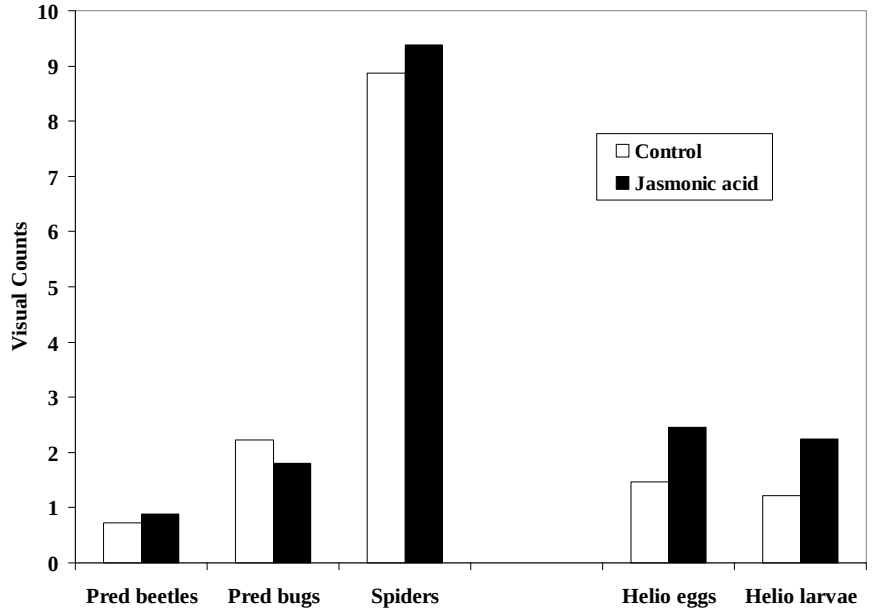

Fig. 31. Abundance of *Helicoverpa* spp. eggs and larvae and various predatory invertebrates collected during visual surveys of conventional cotton plants sprayed with either jasmonic acid solution or water (Controls) at ACRI, during 2005-06.

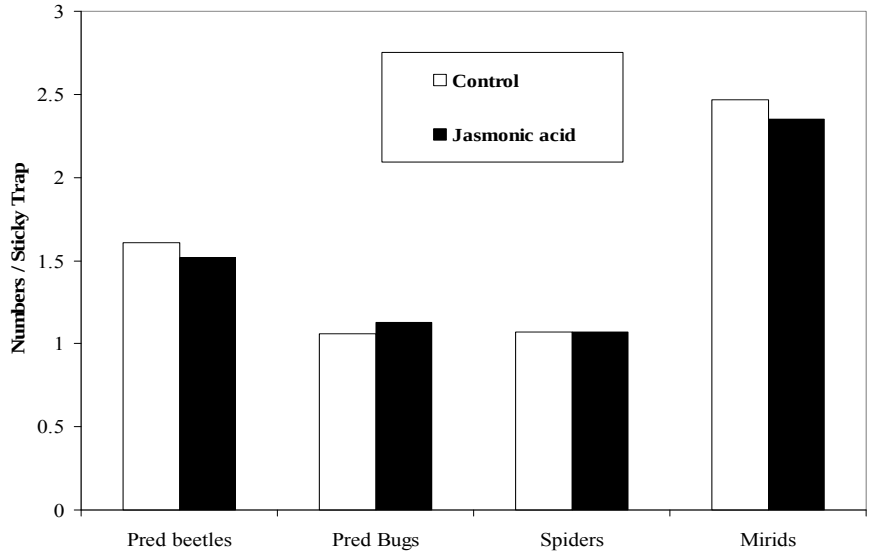

Fig. 32. Abundance of *Helicoverpa* spp. eggs and larvae and various predatory invertebrates collected in sticky traps treated with either jasmonic acid solution or water (Controls) at ACRI, during 2004-05.

6. Sperm Precedence in *Helicoverpa armigera*.

We are still awaiting the results of the paternity tests for the progeny from the *H. armigera* female moths that were mated consecutively with males from different locations and are currently being analysed at Univ Melbourne using EPIC markers. The results of this work will be reported to CRDC in a separate communication.

Outcomes

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

Helicoverpa spp. are major pests of Australian cotton. Transgenic (Bt) cotton represents a substantial investment by the cotton industry to control *Helicoverpa* and reduce reliance on insecticides. The long-term success of Bt cotton relies heavily on a sound ecological knowledge of *Helicoverpa* and effective management strategies to counter the development of resistance against Bt by these pests. As stated in the original project application, this project focused particularly on determining changes in the abundance of *Helicoverpa* at landscape scale (e.g. what temporal patterns are evident in the abundance of *Helicoverpa* with the advent of Bt cotton ?) and the likely effectiveness of refuges in limiting Bt resistance development (e.g. are refuge crops producing large enough numbers of *Helicoverpa* and are these resultant moths mating at random on the landscape with moths from other plant hosts (including Bt cotton), as assumed ?). In addition, the research aimed to gather data on the dynamics of secondary pests and beneficial invertebrates at the refuge crop / Bt crop interface. The broad aims of the research were to 1) improve industry confidence in the use of refuge crops as a valid component of the Resistance Management Plan for transgenic cotton, especially at a time of change (Ingard to Bollgard II cotton), and 2) enhance sustainability and environmental health through reduced use of pesticides and greater capture of services from beneficial species. The latter of course is a flow on from the establishment of the transgenic technology, and not a direct target of this project's research.

The project's outputs (directly related to its planned outcomes) included demonstration of temporal changes in the abundances of both *H. armigera* and *H. punctigera* at landscape scale, in part perhaps associated with the deployment of Bt cotton. Production of *Helicoverpa* (in particular *H. armigera*) from refuge crops was low during the project – indeed *H. punctigera* generally dominated the production within refuges. This trend deserves ongoing scrutiny to determine if such is simply transient or more ongoing. The monitoring of moth mating in Bt cotton crops represents a small data set thus far, but the findings suggest that there is substantial cross mating on the landscape of *H. armigera* from different plant host origins. However, the project also generated data which questions the RMS assumption of random mating. Further research is needed to be confident in these regards.

In addition, the project provided data to indicate that refuge crops can support significant populations of secondary pests of importance in cotton production. For example, populations of mirids were substantially more abundant (per m of crop row) in pigeon pea refuge crops compared with their associated Bt cotton crops nearby. Refuge crops may be sources of mirid recruits to cotton crops, but direct movement studies are required to be persuasive on this. Counter-balancing these findings, some beneficial species (e.g. predatory bugs and

beetles, but seemingly not spiders) were consistently more abundant in refuge crops compared with their associated Bt cotton crops. Again, direct movement studies are required to determine if refuge crops provide effective sources of beneficial species, thus enhancing IPM within cotton crops. Whatever, all this data begs the question of whether or not predatory / parasitic beneficial species in refuges represent net benefits on the landscape, or in fact hinder the productivity of refuges – which after all are intended to produce high numbers of *H. armigera*. This issue needs further resolution.

The project also sought, through a minor aim, to evaluate the potential of jasmonic acid as an attractant for beneficial species in cotton crops. Literature from elsewhere in the world suggested such might be the case. If so, a potential tool might be identified to enhance biological control of the *Helicoverpa* that manage to survive within transgenic crops (with potential for resistance) and secondary pests unaffected directly by Bt. The results of our work however provided no indication of an aggregative effect of jasmonic acid on beneficial invertebrates.

Please describe any:-

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

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

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

The most significant technical advance developed through the project is the use of carbon isotopes to identify plant host origins of moths. Whilst this methodology was being developed during previous projects, and has been used successfully overseas, this project has substantially expanded the use of the technique in application to cotton entomology in Australia.

c) required changes to the Intellectual Property register.

None required.

Conclusion

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). The abundance of key cotton pests, *Helicoverpa* spp. (both *H. armigera* and *H. punctigera*) have changed on the landscape since the deployment of transgenic (Bt) cotton. Causative links, if any, are not clear at this stage. Spring surveys of crops, weeds and native vegetation, as well as pheromone trapping, show promise as early warning indicators of the forthcoming seasonal bias in *Helicoverpa* spp. in cotton crops.

2). Mixed matings of *Helicoverpa* moths from different plant host origins (with implications for refuge crop / Bt cotton crop combinations) are common on the landscape, but the assumption of random mating in such instances, which underpins the current Resistance Management Plan for deployment of Bt cotton crops, may not strictly be true. More research is planned to help tighten up conclusions in this regard.

3). Refuge crops (especially pigeon pea crops which were the focus of much of the work done in this project) support substantial populations of key secondary pests of importance to cotton production, and of beneficial invertebrates. The balance of effect of such pests and

beneficial species needs further study with respect to movements to cotton crops and hindrances to effective *Helicoverpa* production within refuges.

4). Identification of attractants for beneficial species could provide tools to enhance IPM in Australian cotton systems. Jasmonic acid, whilst suggested by overseas researchers to attract beneficial invertebrates (through links to damage caused by herbivorous pests), showed no capacity to do so in field experiments conducted in cotton fields at ACRI.

Extension Opportunities

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

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

The primary skill developed through this project, and that will be further developed in the new project that follows it (CSE 115), is the use of naturally occurring isotopes (C and we hope to develop extra capability with N) to trace the plant-host origins of *Helicoverpa* moths within the landscape (e.g. distinguishing C3 and C4 plant origins). We are also endeavouring to develop methods to clearly distinguish within C3 plants, such as cotton and pigeon pea, e.g. gossypol or legume chemical traits).

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

Project results will continue to be communicated through participation in farmer meetings, trial books, seminars, conference presentations, industry magazines and media articles, linkages with key industry committees (e.g. TIMS) and scientific publications, and through existing linkages with IDOs and the Cotton CRC extension teams activities. [see below re intended publications and manuscripts in prep.]

(c) for future research.

A new project (2006-09) has begun (CSE 115 : "Maximising the efficiency of Bt refuge crops"), the objectives of which are :

- To demonstrate the efficiency of coverage of Bt cotton crops by moths from non-cotton sources and the degree of cross-mating of moths from different plant hosts,
- To evaluate methods to enhance the production of *Helicoverpa* within refuge crops (e.g. "Magnet")
- To continue monitoring of landscape-scale changes in the abundance of *Helicoverpa* spp.

There is substantial interest in an ongoing evaluation of the productivity of recognised refuges & the environmental / management factors influencing such (thus temporal shifts in efficacy across the industry), but such is currently out of scope in this new project because of budgetary limitations.

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

Tann, C. & Baker, G. (2004). Research Comments Entomology. In : "Variety Trial Results 2004". Cotton Seed Distributors, Wee Waa, pp. 79-80.

Tann, C., Baker, G. & Downes, S. (2005). Research Comments. Entomology. In : "Variety Trial Results 2005". Cotton Seed Distributors, Wee Waa, pp. 95-96.

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

Lawrence, L. & Tann, C. (2004). An unusual season for *Helicoverpa punctigera* in Australian cotton. *Outlook on Pest Management*, 2004 : 163-4.

Lawrence, L. & Tann, C. (2004). An unusual season for *Helicoverpa punctigera.* *Australian Cottongrower* 25 (3) : 8-10.

Lawrence, L., Tann, C. & Baker, G. (2004). Changes in *Helicoverpa* abundance in the Namoi. The *Australian Cottongrower. Cotton Yearbook 2004*, 98-100.

Tann, C.R. (2004). Changes in *Helicoverpa* abundance in cotton growing areas over the past few years. Lower Namoi Valley Trial & Year Book, 2004.

Baker, G.H. & Tann, C.R. (2006). Mating of *Helicoverpa armigera* moths in Bollgard II cotton. Aust. Cotton Conf, Gold Coast, Qld. In press.

Other Articles :
Occasional articles have been published by other authors which refer in part to our work e.g.

Lawrence, L & Clayton, S. (2005). CSIRO divisions combine for grower benefits. The *Australian Cottongrower. Cotton Yearbook 2005*, 96-100.

Rossiter, L, Downes, S & Mahon, R. (2006). *Helicoverpa* : species mix, parasitism and resistance monitoring. *Australian Cottongrower* 26 (7) : 66-69.

Manuscripts nearing completion :

Baker, G.H., Tann, C.R & Fitt, G.P. Production of *Helicoverpa* spp. (Noctuidae) within different refuge crops to accompany transgenic cotton plantings in eastern Australia. Intended for *Aust. J. Agric. Res.*

Baker, G.H., Fitt, G.P. & Tann, C.R. Long term temporal changes in the abundance of *Helicoverpa* spp. (Noctuidae) in pheromone and light traps in northern New South Wales, Australia. Intended for *Aust. J. Agric. Res.*

Baker, G.H. & Tann C.R. Comparison of pheromone and light trap efficiencies for *Helicoverpa* spp. (Noctuidae). Intended for *Aust. J. Agric. Res.*

Planned Manuscripts :

Baker, G.H. & Tann, C.R. Incidence of mating of *Helicoverpa* spp. (Noctuidae) moths from different plant origins. Intended for *J. Aust. Ent. Soc*.

Baker, G.H. & Tann, C.R. Refuge crops as sources of secondary cotton pests and beneficial invertebrate species. Intended for *Australian Cottongrower*.

Presentations to Industry Groups

Research results from the project were disseminated to industry through a variety of ways :

C. Tann presented several talks to cotton grower groups (e.g. through AWM meetings, farm walks etc) in the St George / Dirranbandi, Namoi, Gwydir and Macintyre regions. Talks

were also given by C. Tann and G. Baker at e.g. IPM Forum (Brisbane), CRDC Bt Resistance / Refuge Workshops (Narrabri), and various media articles and interviews were published / recorded (e.g. "The Courier" (Narrabri), CSD interviews). Overviews of the research were provided in CSIRO news letters (e.g. "CSIRO Times" circulated at the Aust Cotton Conference). Ongoing pheromone trap data were provided to "The Courier" (Narrabri) and published as the newspaper determined.

The research was presented at various scientific venues by G. Baker and C. Tann, e.g. CSIRO Reviews and seminars, ANU (Canberra) undergraduate lectures, British Ecological Society Annual Conference (UK), University of Darmstadt (Germany), DEH Symposium on GMOs (Canberra), XXI International Congress of Entomology (Brisbane), Aust. Entomol. Soc. Ann. Conf. (Hobart), Aust. Ecolog. Soc. Ann. Conf. (Armidale).

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

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

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 varieties provide a substantial basis for economically and environmentally sustainable insect pest management within the Australian cotton industry. The introduction of Bt cotton has significantly reduced pesticide use for the control of key pests such as *Helicoverpa* spp. and encouraged a greater emphasis on the management of beneficial invertebrates in pest control. A major risk now facing the cotton industry, from a pest management perspective, is the development within *H. armigera* of resistance to Bt. As a result, mandatory requirements are placed on growers of Bt cotton to provide refuge crops (no Bt exposure) as sources of susceptible moths that will mate with any potentially resistant moths arising from the Bt crops – thus swamping resistance development. Various refuge crops are available as options (pigeon pea, maize, sorghum, conventional cotton). To properly evaluate the utility of such refuges, we need improved knowledge of the degree to which moths generated by them and those emerging from Bt crops effectively mate. A core assumption of the current Bt Resistance Management Plan for Bt cotton is that moths from different crop origins mate at random. Using carbon isotope signatures characteristic of C3 and C4 plants (e.g. cotton and pigeon pea cf maize and sorghum), this project demonstrated that mixed matings of *Helicoverpa* moths from such different plant host origins can be common on the landscape, but the assumption of random mating in such instances may not strictly be true. More research is planned to further confirm these conclusions.

This project also continued monitoring of long-term (and seasonal) changes in *Helicoverpa* abundance in cotton growing regions through networks of pheromone trapping in the Namoi Valley and St George / Dirranbandi regions that began prior to the introduction of Bt cotton.

The pheromone trap catches for *H. armigera* in the Namoi Valley suggested this species has increased in abundance (at landscape scale), especially late in the cotton growing season, since the advent of Bt cotton. The mechanisms driving such change are not understood, but could be related to reductions in pesticide use and / or concurrent variations in the use of other crops on the landscape that are attractive to the moth. The abundance of *H. punctigera* has also increased in the last few years. Spring surveys of crops, weeds and native vegetation showed promise as early warning indicators of the forthcoming seasonal bias in *Helicoverpa* spp. in cotton crops.

Concurrent with our work on *Helicoverpa*, we opportunistically gathered data on the abundance of secondary cotton pests and beneficial invertebrates within refuge crops and their associated Bt cotton crops. Refuge crops (especially pigeon pea crops which were the focus of much of the work done in this project) supported substantial populations of mirids and predatory beetles and bugs. The balance of effect of such pests and beneficial species for cotton production needs further study with respect to movements to cotton crops and the hindrances beneficial species may provide to effective *Helicoverpa* production within refuges.

In addition, we explored the potential of jasmonic acid, a chemical involved in plant response to herbivore damage, to act as an attractant for beneficial invertebrates in cotton crops. Such responses have been suggested by overseas research. Identification of attractants for beneficial species could provide useful tools to enhance IPM in Australian cotton systems. However, jasmonic acid failed to influence aggregative behaviour of beneficial invertebrates in trials we conducted in conventional cotton fields.

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