Cotton
RESEARCH & DEVELOPMENT

Annual, Progress and Final Reports

REPORTS

Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: **CSE90C**

Annual Report: ☐ Due 30-September

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Part 3 - Final Report

1. 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) has had on the overall abundance of *Helicoverpa* in cotton growing regions is however poorly understood. Our previous monitoring of the early season incidence of *Helicoverpa* spp. on various host plants in northern NSW and our networks of pheromone trapping & bug-checking surveys in the Namoi and St George regions (the latter a relatively isolated production system) (see project CSE64C), have enabled us to establish long-term data sets to assess temporal shifts in *Helicoverpa* abundance (both seasonal and across years). This project sought to continue such monitoring to provide on-going assessments (e.g. via CRC web site) and an improved understanding of the mechanisms behind the population dynamics of *Helicoverpa* spp. for industry and researchers.

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. This project aimed to help identify the most productive refuge options. Such information is essential to allow robust estimates of refuge sizes. Crops considered in CSE90C included sprayed (non Bt) conventional cotton, unsprayed conventional cotton, pigeon pea, sorghum, maize and soy bean.

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 well the moths generated there disperse to Bt crops is very limited. Some studies have simulated movements of *H. armigera* from refuges to transgenic crops using the model 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. In CSE90C, we sought to use strontium to mark moths in refuges and set traps to recapture them in nearby Ingard cotton crops.

Many questions about the seasonal abundance and resistance dynamics of *Helicoverpa* require knowledge of which crops contribute to local populations. While we can infer something about the relative importance of different crops from pupal numbers, the definitive answers require that we can assign moths to probable crop origin. We intended to do this, and thus infer local movements of moths, using the ratios of carbon isotopes that vary between C3 (cotton, legumes) and C4 (maize, sorghum) plants and thus are likely to be transferred to moths reared on them.

Despite some previous research on cultivation and its usefulness in reducing the abundance of over-wintering pupae of *Helicoverpa* ("pupae busting"), questions often arise as to the best tillage methods to use to achieve this. We intended in CSE90C to establish field trials, in collaboration with soil scientists, to determine optimum methods amongst commonly available machinery to destroy pupae, whilst taking into consideration the impact of such methods on soil structure and fertility.

A substantial mass of data on the ecology of *Helicoverpa* spp. has accumulated at ACRI through previous projects, in particular in relation to moth trapping programs, egg and larval incidence in field surveys, management of over-wintering pupae and performance of Ingard refuge crops. It is important that such data be analysed and published in order to inform and enable input to future research and management decisions in the broadest sense. This project sought to facilitate this delivery.

2. Project Objectives

The main objectives of this study were to :

(i) Quantify the value and effectiveness of unsprayed refuges for management of Ingard cotton.
Substantially Achieved : The relative production of various refuge options was widely surveyed throughout northern N.S.W and southern Qld. Pigeon pea was identified as the most productive and reliable refuge. An initial study of movement of moths between a refuge and a Bt crop was completed. Further studies were thwarted by low insect pressure.

(ii) Monitor the seasonal phenology of *Helicoverpa* species in the Namoi and St George regions as representative systems for the cotton industry.
Achieved : Studies were completed using trapping methods (pheromone and light traps), egg and larval surveys of a broad range of host plants, pupal surveys in Bt cotton, conventional sprayed cotton and unsprayed refuge crops, and rearings of eggs, larvae and pupae to determine moth species identification and level of parasitism and disease.

(iii) Quantify the crop origin of *Helicoverpa* moths as an aid in interpreting seasonal patterns of abundance and resistance frequency.
Partially Achieved : Our original intention was to utilise carbon isotope analyses to discriminate the crop origin of moths broadly on landscapes including cotton. As indicated in Progress Reports, we reduced emphasis on this due to the lack of sensitivity of the technique in complex cropping systems. We did however apply the technique to two systems, wherein we surveyed with the intent to monitor movements between refuges and Bt cotton crops. Analyses are still pending (due to analytical equipment failure for several months).

(iv) Finalise research on the management of over-wintering pupae.
Partially Achieved : We conducted a trial, in collaboration with soil scientists measuring soil physical properties, to determine optimal tillage equipment for destroying *Helicoverpa* pupae in winter. Whilst the results indicated a trend towards most mortality with greatest soil disturbance, differences in the data were not significant. Low pupal numbers and particularly patchy distributions at the trial site inhibited the study.

(v) Finalise data analysis and publication of information collected over the past 8 years.
Partially Achieved : This report is evidence of a concerted effort to organise, analyse and document a substantial amount of data that has accumulated at ACRI through preceding projects as well as this particular one. Our intent is to now prepare manuscripts for scientific publication based on this data.

3. Methodology

1. Light and Pheromone Trapping

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 or more often, weather permitting. Lures were changed monthly and pesticide strips were changed bi-monthly. Two CSIRO-designed cone light traps were also run (for the first two years of the project) at sites on ACRI (Leitch’s and Chico block), as they had been in previous years. The light traps were emptied at weekly intervals (or more frequently), the moths were sexed and identified to species and spermatophore counts were made for each female to determine mated status. Whereas the pheromone traps were run all year round, the light traps were only run for 36 weeks, encompassing the cotton growing season (often the case in previous years as well).

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. Numbers of traps varied between years (13 pairs in 2000-01, 14 pairs in 2001-2002, 11 pairs in 2002-2003).

c) Trapping and Egg Counts

Egg pressure (eggs / m cotton row) are measured at ACRI in the vicinity of the pheromone and light traps reported above and across the St George and Dirranbandi cotton growing regions, by ACRI staff and consultants respectively. This provides an opportunity to measure how closely the trapping and egg abundance data match. It also enables a measure of annual variability in pest abundance (albeit crude because of varying availabilities of suitable plant hosts on the landscape which are not "controlled").

2. General Field Monitoring

As in years prior to this project, the incidence of *Helicoverpa* eggs and larvae on weeds, natural vegetation, crops other than cotton (including designated Bt cotton refuges) and cotton crops (occasionally) was 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 based on visual checks of crop rows (usually 6 m replicates) and sweep netting. 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* 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 and concurrently with our focus in the latter.

Collection efforts necessarily varied between years according to the availability of crops and weeds etc to sample from (e.g. because of drought, weeds and natural vegetation were not sought or were unavailable in 2002-03). Most 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.

3. Ingard Cotton and Refuge Crop

a) Namoi, Gwydir, Macintyre & Macquarie Valleys

Regular three weekly visits to sites in the Namoi, Gwydir, Macintyre and Macquarie valleys were conducted in the three cotton seasons in this project (147, 120 and 103 visits in total in each of the three years) to survey for pupal abundance in Ingard cotton and associated refuge crops. This work thus continued similar work in the earlier project. Pupae were collected from December to March / April from replicated (n = at least 14) 1 m rows of cotton (essentially 1 m^2 quadrats) within which the soil was dug to 10 cm depth. Samples were at least 20 m apart and were taken within sets of fields at each site, one field being Ingard cotton and the other its dedicated refuge, either an unsprayed crop (usually non-cotton) and / or conventional sprayed cotton. Dryland crops were included along with irrigated crops (the latter in the vast majority). Live and emerged pupae were counted. Live pupae (a sub-sample) were reared in the laboratory to confirm species identification and level of parasitism. Note was taken of the sowing times of the refuges (sometimes seemingly sub-optimal because of the vagaries of weather) and their maturity and likely attractiveness to *Helicoverpa*.

Monsanto provided extra finance to survey an additional 19 field sites in 2000-01 to evaluate the efficacy of late planted refuges (mostly pigeon pea; planted late November-early December 2000) –

due to a late realisation of an over-planting of Ingard cotton in this season. Data was collected from five regions : Darling Downs (n = 6 sites), Byee (2), Namoi (8), Gwydir (2), Mungindi (1). Sites were visited during March – April 2001. Most sites were visited twice. Qualitative crop information (crop development stage, plant height etc) was recorded. *Helicoverpa* pupae were sampled in the standard way, recording live and emerged pupae. Sampling was conducted both in the refuge crops and the Ingard cotton associated with them (where it occurred in the immediate area, 14 of the 19 sites). Live pupae were reared in the laboratory to identify *Helicoverpa* spp and parasitoid incidence within them. The abundance of pupae of *Netelia producta* and Tachinidae, species which parasitise *Helicoverpa* pupae, was also recorded in the field. In addition, the refuge crops were sampled to measure the abundance of *Helicoverpa* larvae using 100 sweeps on each sampling occasion.

During 2000-01, a replicated trial was established at J. Warnock's farm at Maules Ck, Lower Namoi, in response to a request by TIMS, to evaluate the merit in using soybean as a refuge. Comparisons were made with the performance of pigeon pea. This followed grower interest in the potential of other legume refuge options, besides the highly favoured pigeon pea, which may offer advantages of greater residual nitrogen in the soil, marketability of produce and ease of management. CSIRO had previously conducted research on the value of soybean as a refuge in 1996-97 and 1997-98 when soybean was included as one of several potential crops in replicated studies in several regions. Soybean was grown at only one location however. Those studies showed that soybean was not a highly effective refuge, producing lower densities in both seasons than all other crops other than lucerne.

The trial at Maules Ck in 2000-01 involved three replicated strips of soybean and pigeon pea. Plots were 48 or 96 rows wide and 750 m long. Crops were sown in late November and were regularly monitored throughout the growing season from early January until April. Data was collected on larval and pupal densities in each "refuge" crop and in nearby Ingard and conventional cotton as well. Stand density, stage of development and height of each crop were also recorded at each sampling date.

This work was followed up with further comparisons of soybean and pigeon pea as appropriate refuges during the following season at Goondiwindi in the Macintyre Valley and Boggabri in the Upper Namoi Valley. Unfortunately, the initial trial site at Maules Ck was unavailable for a repeat study because of water shortages. At Boggabri, pigeon pea and soy bean were planted in late November 2001, with four replicate plots of each. Unfortunately, poor crop establishment caused from significant regrowth of the previous year's cotton and large infestations of summer weeds made clear comparisons between the pigeon pea and soy bean well nigh impossible. However one pupal sampling (in mid March) did demonstrate the pigeon pea was generating *Helicoverpa* (0.3 – 0.65 pupae / m^2) whilst no pupae were found below the soy bean (which appeared of better quality). In contrast a successful trial was conducted at Goodiwindi. Again pigeon pea and soy bean were sown in late November, with four replicate plots of each planted alternatively (each plot with 14 rows and approx 900 m long). The eight plots were separated from Ingard cotton by additional pigeon pea (grown as a trap crop). Eggs and larvae were sampled in the soy bean and pigeon pea on three occasions during the growing season, by checking 3 m of crop within each replicate plot. Collected individuals were reared through in the laboratory for identification. In addition, the soil within three 1m rows of crop (essentially 3 m^2) within each pigeon pea and soy bean plot was hand sorted for pupae on four occasions, along with samples also taken in the Ingard cotton crop and a nearby conventional cotton crop. Stand density, stage of development and height of each crop were also recorded at each sampling date.

b) St George & Dirranbandi

Regular, three-weekly visits to sites in the St George and Dirranbandi regions were also conducted in the three cotton seasons in this project (except Dirranbandi in 2002-03 when no cotton was grown due to drought) to survey for *Helicoverpa* pupal abundance (103, 132 and 54 visits in total in each of the three years). This again followed on from similar work in the previous project. Pupae were collected from replicated $1m^2$ quadrats, in the same way as described above for other valleys, within paired fields at each site, one field being Ingard cotton and the other its refuge. Initially, virtually all the refuges were conventional sprayed cotton. In later years, unsprayed non-cotton refuges (e.g. pigeon pea) and unsprayed cotton were also included in the sampling. Pupae were reared in the laboratory to confirm species identification and level of parasitism.

Egg counts were provided by consultants in St George and Dirranbandi (for Ingard and conventional cotton – no distinction made between these), which provided a concurrent measure of egg pressure.

c) Further Observations on Natural Enemies

In 2001-02 (Dec – Mar), we surveyed the abundance of predatory invertebrates in a set (n = 9 farms) of unsprayed refuges and their related Ingard cotton crops with a view to identifying the common invertebrate predators in the two types of habitat and clarifying any contributory role the refuges might make to such beneficial populations in the Ingard cotton. Samples were taken within pigeon pea and unsprayed cotton refuges, in Ingard cotton adjacent to the refuges (within 50-200m of the edge of the Ingard crop), and further into the Ingard cotton crop (>> 200m from the crop edge abutting the refuge). The first 50 m of all crops that was adjacent to tail and head ditches was avoided. Firstly, replicate 1 m rows of crop (n = 6 / field) were visually checked for predators (same plants as used for egg and larvae checks for *Helicoverpa*) and their abundance was recorded. Secondly, 100 sweeps were taken in the same region of each crop and the presence (only) of predators was also noted in the catch. Some fields were surveyed only once during the season, whilst others were surveyed up to four times (n = 51 field visits in total). Farms were within the Namoi, Macintyre & St George regions. In addition, visual checks for predatory invertebrate abundance and sweep net surveys to record the presence of predators were made in refuge crops (only) (n = 40 field visits) in the same regions, plus Dirranbandi and Gwydir. The refuges were pigeon pea (25), unsprayed cotton (11) and soybean (4).

In 2003 (Jan – Mar), we also surveyed the presence of predatory invertebrates in a set of unsprayed refuges (n = 21 farms) in the Namoi, Macintyre, Gwydir, Croppa Creek, North Star & St George regions using 100 sweeps near where egg and larval checks on crops were made. Most fields were visited twice, others only once (n = 39 field visits in total). The refuges included pigeon pea (n= 30 visits), sorghum (n = 6) and unsprayed cotton (n = 3).

4. Tillage / Emergence Experiment

During 2002, we conducted a trial in Field A2 – Chico Block (grey clay soil), ACRI to determine the influence of various tillage methods, applied during the winter months, on the survival of *Helicoverpa* pupae, as expressed via their emergence as moths during the following spring. The work was done in collaboration with Dr N. Hulugalle (NSW Agric.) who monitored various soil properties concurrently. We only report the entomological aspects of the work here.

There was six tillage treatments, with different tillage intensities :

1) Centre Busting / Discs : Large central tyne to break up the centre of the bed, followed by a set of discs to totally destroy the beds in a cross way action. This may often leave large clods of soil that are not adequately broken up.
2) Aerway : Rotary action cultivator that disturbs the bed and rebuilds it. It will disturb to 10 cm but fails to address the furrow regions. Offers good disturbance within the main target area and performs best for the moist conditions present in the experiment.
3) Centre Busting / Go-Devils : Large central tyne to break up the centre of the bed, followed by a set of rolling cultivators to disturb the shoulder of the hill and furrow. The Go-Devils do not cut very deep and are mainly used to tidy up the bed.
4) Eliminator / Wheat Planter : Stalk cutter operating just below the surface and mulching the stalks, followed by a planter designed to plant wheat into trash using single disc opener – 7 inch spacing.
5) Wheat Planter : Wheat planter drilled into standing cotton stubble with little disturbance. Cotton stubble slashed afterwards.
6) Untreated Control (No Tillage) : No soil disturbance of any kind. Standing cotton stubble slashed afterwards.

These treatments are arranged in decreasing order of degree of soil disturbance . The field had been used for an unsprayed, conventional cotton crop during the 2001-02 season. Preliminary sampling of the field in autumn, after the cotton was harvested, suggested an average number of *Helicoverpa* spp / m^2 = 4.3 were likely to over-winter. The field was tilled on 1 July.

Average soil moisture (0-30 cm depth) at the time of tillage (in controls) was 18.8% (g/g) (n = 12). There was 3 replicates for each tillage treatment, each replicate = 12 rows of cotton x 186m long (= 0.22 ha) in size, arranged in a random block design.

Emergence of *Helicoverpa* moths (and other moth species and pupal parasites of Lepidoptera) was monitored from mid August 2002 until mid-January 2003, using emergence tents and cages which were set within each tillage replicate during July 2002. Each replicate had two 6 x 1m emergence tents and one $1m^2$ cage, thus each effectively surveying moth emergence from $13m^2$. Collection vials, located at the tops of the tents and cages, were checked every few days. The tents and cages were arranged regularly along lines within each tillage replicate.

5. Movements of Moths from Refuges to Ingard Crops

During the project we monitored several refuge crops with the intent of establishing mark- recapture experiments (using strontium chloride as the marker) to help determine if moths produced in refuges are likely to mate with moths in the nearby Ingard cotton crops. In most cases we were unable to conduct such intense work, because of the low insect pressure prevailing during the study and the need to be assured of high numbers of marked moths emerging from the selected refuges. However, in the 2000-01 season, we identified an opportunity using a pigeon pea refuge near Warren in the Macquarie Valley. After spraying the refuge with strontium (10 kg / ha; 2.5 ha pigeon pea sprayed, 0.5 ha pigeon pea left unsprayed) to mark the developing *Helicoverpa* larvae (on 8 February), an intensive collecting period followed when modelling suggested emergence was most likely. We ran a set of 6 light traps for 5 nights (7 – 11 March) and collected mating pairs and single moths as observed within the refuge and adjacent Ingard cotton crop. Two light traps were set within the refuge, and single traps were set within Ingard cotton, at each of 380, 560, 670 and 1100 m distance from the central (trapping) area of the refuge. We also collected pigeon pea plant samples within sprayed and unsprayed areas of the crop (3 plants from each area) and set 8 emergence cages (4 within each of the unsprayed and sprayed areas) to collect moths and test for expected marking. These emergence catches also acted as a check on our predicted emergence time. The emergence cages ran concurrently with the light traps. Collections were analysed for strontium using CIROS Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES) at CSIRO PI in Adelaide.

6. Crop Origins of Moths

To test the potential of using carbon isotope ratios to discriminate C3 and C4 plants as original host plants for captured moths, seven *H. armigera* larvae were collected from each of the following crops growing in the Lower Namoi Valley : unsprayed cotton and pigeon pea (C3) and maize and sorghum (C4). Carbon isotope ratios were measured by specialised mass spectrometry at U.N.E. in collaboration with Dr P. Gregg. Having found that moths could be separated in this way (see Results), we have work in progress to analyse carbon isotopes from moths collected within Ingard crops to see if we can shed light on their probable origin – in particular if they may have come from designated refuges or elsewhere. At Ketah in the Gwydir Valley during early 2003, we located a system of clusters of irrigated plantings of Ingard cotton with sorghum used as refuge, and little else planted in the vicinity because of the prevailing drought. We collected 50 mating pairs of *H. armigera* and 59 single moths from within Ingard cotton on 10 February 2003. We added to this collection, 4 mating pairs and 8 singles that were collected on 11 March. In addition, we located a 1,000 acre Ingard cotton crop under centre pivot irrigation, surrounding a 100 acre pigeon pea crop (the designated refuge). Surrounding the Ingard crop in turn was a 700 acre dryland crop of sorghum. Both the pigeon pea and sorghum were in good condition and likely to be attractive to *Helicoverpa* (e.g. we measured an average of 2 *Helicoverpa* larvae / m row in the sorghum). On 25 February 2003, we collected 16 mating pairs of *H. armigera* and 89 single moths. All these moths are awaiting carbon isotope analyses at U.N.E. Unfortunately, there have been mechanical failures with the equipment at U.N.E. for several months and this has delayed results.

4. Results

1. Light and Pheromone Trapping

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

Figs 1-3 indicate the observed pheromone trapping data for *H. armigera* and *H. punctigera* during the three years of the study. Fig. 4 indicates the averaged data for years since the 1992-93 season (when the current grid of pheromone trapping sites became settled into a routine, thus facilitating long term monitoring). The typical early season peak of *H. punctigera* catches was not apparent in 2002-03.

Similarly, Figs 5-8 indicate the observed light trap catches of *H. armigera* and *H. punctigera* (both sexes presented separately) for the two seasons, 200-01 and 2001-02 [Light traps were not run in 2002-03]. Figs 9-10 provide the averaged data for years 1992-2002. Notably males predominated in both species. Curiously, male *H. punctigera* proved particularly abundant in the first generation (approx. weeks 8-20, i.e. spring), a period traditionally attributed to *H. punctigera* invading from elsewhere. Reasons for this strong sexual bias, particularly in *H. punctigera* in spring, are not clear. The catches of H. punctigera were markedly different between 2000-01 and 2001-02, with the first generation predominating in the first year, and the second generation predominating in the second year.

The long term averaged data from pheromone and light trapping are compared directly in Figs 11-12 for *H. armigera* and *H. punctigera* (using only data for males). Very similar graphs appear for *H. armigera*, but gross differences are apparent for *H. punctigera*. For the latter species, the initial generation (weeks 8-20) is predominant in pheromone traps, but the second generation (weeks 21-30) is predominant in light traps. Reasons for these distinct differences in trapping success of the early generations of *H. punctigera* are not understood. The pheromone traps that were run in close proximity to the light traps at ACRI yielded catches (data not shown here) similar to those amongst the rest of the pheromone traps further away from ACRI. The data differences were therefore not a result of varying regions being trapped by the different methods.

Seemingly, one or both trapping methods vary in efficiency to the extent that these differences in pattern of catches are generated. We are currently seeking explanations for the differences.

In addition, the long term changes in trap catches (annual variations) have been plotted in Figs 13-16. In each figure, the catches have been divided into periods of 8-20, 21-30 and 31-44 weeks, thus corresponding approximately with generations 1, 2 and later (as has been done by previous authors). Most notably, the numbers of *H. armigera* have increased markedly in the pheromone trap catches since 1996, especially in later generations. In contrast, no similar pattern is evident in the light trap catches for *H. armigera*, nor in either the pheromone or light trap catches for *H. punctigera*. Reasons for the clear increase in pheromone trap catches of *H. armigera* are not known. One possibility might have been a change in the design / operation of the pheromone traps for *H. armigera* during the course of data collection (e.g. an unnoticed subtle change in the commercial lure used). But no such variation in technique is known to have occurred – and if such had been the case, then surely a similar increase would have been evident in the first as well as the later generations – but it was not.

The pheromone traps located near the light traps at ACRI yielded similar variations in the abundance of the separate generations of *H. armigera* across the years to the traps placed further afield (data not shown here). Thus, again, the greater regional catchment of moths in the pheromone traps cannot explain the observed discrepancy between catches using the two trapping techniques.

Most likely, the change in *H. armigera* numbers in pheromone traps reflects a change in efficacy of the traps / behaviour of the moths, rather than a change in moth abundance per se. But why should male moths, especially later generations, be suddenly caught more efficiently from 1996 onwards ?

One possibility is that the abundance of *H. armigera* has in fact declined, particularly late in the season, to the point that pheromone traps are more efficient in trapping males because there are fewer "calling" females in the region – thus less competition for the traps.

This all begs the question as to what might have happened on the landscape to possibly cause a shift in moth catches. The obvious change was of course the release of Ingard cotton in 1996, which does indeed correspond with the observed change in *H. armigera* catches. Could the introduction of Ingard with its kill of larvae hatching on it (especially early to mid-season) be reducing the abundance of moths enough to generate change such as depicted in Fig. 13 ? The introduction of Ingard was gradual and the quantities grown in 1996 and in the few years prior to that in and around ACRI would seem unlikely to drive the sudden changes in trap catches. Also, why don't we see changes in light trap catches if this were true ?

We are currently exploring concurrent changes that have occurred over the last decade or so in the Lower Namoi that might help explain the changes we have observed in our traps (e.g. pesticide use, crop acreages). This includes finer analysis of the data set we have gathered through the general monitoring of eggs and larvae of *Helicoverpa* on crops, weeds and natural vegetation (see below). We are also seeking available long-term data sets from CCA on egg pressure variations. Some changes that have occurred on the local landscape are illustrated in Figs 17-18 (data kindly provided by T. Farrell, ACRI). Mung bean and soy bean acreages have for example increased markedly since 1996. Both crops are hosts for *H. armigera*, but the recent increases in their acreages seem not to match the *H. armigera* trap catch changes adequately. Plantings of other crops (e.g. faba bean and chick pea) are also likely to have changed significantly in recent years, but data for them are yet to be obtained. Rainfall patterns do not match with observed changes in trap catches.

Analyses of the spermatophore counts for female moths caught in the light traps at ACRI are still proceeding.

The data collected from our pheromone trapping in the Lower Namoi have been regularly posted on the Australian Cotton CRC web site for broad readership of current trends in catches.

b) St George and Dirranbandi

Figs 19-24 indicate the observed pheromone trapping data for *H. armigera* and *H. punctigera* for St George for the last 3 years. Fig. 25 indicates the averaged data for traps near St George for the 6 years since records began. Data were similar for the traps at Dirranbandi and are not presented here. Note data up to week 14 and after week 40 are sparse and have not been plotted for this reason in Fig. 25. There is little evidence of an early season peak in *H. punctigera* abundance, as found in the Namoi region, but as noted above, early season trapping was limited in most years.

The longer term changes in abundance of *H. armigera* and *H. punctigera* in traps near St George are plotted in Figs 26-27, as was done earlier for the vicinity of ACRI. No trends in abundance across years are evident, but it is notable that the "seasonal" changes as seen near ACRI are not apparent in St George. Instead of a tendency towards the last generations of *H. armigera* and the first generation of *H. punctigera* being most common, the pattern for *H. armigera* was more variable and the first two generations of *H. punctigera* tended to dominate at St George.

c) Trapping and Egg Counts

Very many examples could be drawn from the data to indicate the match (or lack thereof) between the abundance of moths in traps and egg counts, but by way of example, cases are presented here for traps and egg counts at ACRI in 1998-99 (Figs 28-29). Neither pheromone nor light traps tracked egg abundance closely, but overall light traps gave a better fit. Unfortunately, as has been pointed out by previous authors, the tedium, cost and inherent time lag for analysing light trap catches makes them an unlikely tool for indicating egg lays in practice.

Recent annual changes in *Helicoverpa* egg abundance on cotton at ACRI (Chico Block) and in the St George and Dirranbandi regions are indicated in Fig. 30. In both St George and Dirranbandi, egg pressure was particularly high in 1998-99 and has remained relatively low since then. At ACRI, this trend has not been the case. We are currently seeking access to earlier data to relate these egg pressure data to observed changes in trap catches (see above).

2. General Field Monitoring

This is a huge data set and only some general themes evident in it are documented here. Data from the last three years are included along with older data (back to the 1993-94 season), given that all have been worked through recently.

Firstly, 1,937 sites were surveyed, including those sites at which no *Helicoverpa* were found (a minority, since sites e.g. in natural vegetation were deliberately selected where *Helicoverpa* might be expected to occur). Fig 31 illustrates the outcomes of the rearings of the many thousands of eggs and larvae that were collected. Only 43% of the reared material reached adulthood and could be identified. A further 16% died from either disease (virus) or successful parasitism. The remainder of the eggs and larvae died from unknown causes. Note parasitism was recorded only if a parasitoid emerged from the *Helicoverpa* (i.e. not if only some passing evidence was recorded, e.g. a parasitoid egg was noted on a larva).

Next, Figs 32-37 illustrate the outcomes of the rearings from the main habitats surveyed in winter-spring and summer-autumn respectively (rarely surveyed habitats, such as volunteer cotton in winter, have been ignored). In winter-spring, *H. punctigera* was generally more common in the rearings than *H. armigera*, especially from weed and natural vegetation sources. However, intriguingly, the proportion of *H. armigera* appears to have increased post 1996 (see earlier observations on *H. armigera* pheromone traps in the vicinity of ACRI, Narrabri, but note there the abundance of *H. armigera* moths increased later in the season). We are currently checking the accumulated data to determine if there were subtle changes in the habitat types that were surveyed which might help explain the temporal changes observed in the proportion of *H. armigera*. Levels of parasitism were erratic between years, but were generally higher in rearings from weeds and natural vegetation. Likewise, viral disease incidence was erratic, but there was no obvious tendency for it to predominate in one habitat more than another.

During summer-autumn, *H. armigera* generally predominated over *H. punctigera* in the rearings from the crops (other than cotton) and the dedicated refuges. There was no evidence of such a consistent predominance of *H. armigera* in the rearings from the weeds and natural vegetation. Parasitism rates were generally low (< 20%) in all habitats. Notably, the incidence of viral disease appeared consistently higher in the dedicated refuges than in other habitats, probably reflecting denser populations of hosts.

Table 1. Parasitoids reared from field collections of eggs and larvae of *Helicoverpa* spp. (1993-94 to 2002-03).

Parasitoids	**Winter-Spring**		**Summer-Autumn**			
& Year	**Weeds/ Nat. Veg.**	**Crops**	**Crops**	**Unsprayed refuge**	**Weeds/ Nat. Veg.**	**Ingard**
Egg parasitoids						
1993-94	0	0	0	0	0	
1994-95	0	5	0		0	
1995-96	0	0	0		0	
1996-97	8	2	82		5	
1997-98	0	6	0	0	0	
1998-99	12	0	1		3	0
1999-00	7	1	0	0	0	
2000-01	0	1	0	0	0	
2001-02	0	0	0	1		0
2002-03	12	3	0			
Larval parasitoids						
1993-94	132	9	45	18	4	

1994-95	0	15	79		39	
1995-96	25	1	46		2	
1996-97	28	11	37		0	
1997-98	12	41	27	16	6	
1998-99	143	14	6		3	0
1999-00	20	44	20	99	1	
2000-01	8	21	1	54	0	
2001-02	4	41	0	112		1
2002-03		4		107		

Overall, these data serve to indicate the levels of parasitism, viral disease incidence and balance of *Helicoverpa* species composition in habitats other than cotton. No attempt has yet been made to fine tune the data by teasing out differences in time more precisely (e.g. separate winter and spring), variations in the plant type surveyed (e.g. separate the individual weed species and native vegetation plants), or the relative frequencies of the various parasitoids that were reared. However, Table 1 does provide an indication of the frequency of egg and larval parasitoids recorded from the rearings with reference to the broad timings and habitat types they came from (see Figs 32-37 for number of sites surveyed). Weeds and natural vegetation in winter – spring and unsprayed refuges in summer – autumn provided significant sources of parasitois. Egg parasitoids were generally rare and included *Chelonus* (most common, actually emerges from larva, 145 records) and occasional *Trichogramma* spp. The most common larval parasitoids were tachinid flies (531 records). *Microplitis* and *Heteropelma* spp. wasps were next (366 and 337 records respectively), followed by *Netelia* spp. (33) and others (unidentified, 31).

3. Ingard Cotton and Refuge Crops

a) Namoi, Gwydir, Macintyre & Macquarie Valleys

The numbers of live and total pupae collected throughout 1996-97 (when surveys began) to 2002-03 in unsprayed refuges and conventional and Ingard cotton crops in these regions are given in Tables 2-5. In most cases, Ingard cotton had fewer *Helicoverpa* pupae than conventional cotton (overall 2:3). Unsprayed refuges produced 6-10 x the number of pupae in Ingard cotton. Data for other regions that were less frequently surveyed (e.g. Bourke, Walgett, Darling Downs, Mungindi, Kingaroy) are available from G. Baker and C. Tann.

Table 2. Average numbers of *Helicoverpa* pupae / m^2 recorded in Ingard and conventional cotton crops and unsprayed refuges in the Namoi Valley.

Season	Ingard Cotton			Conventional Cotton			Unsprayed Refuges		
	Total Pupae	Live Pupae	Site Visits	Total Pupae	Live Pupae	Site Visits	Total Pupae	Live Pupae	Site Visits
96-97	0.79	0.23	54	1.30	0.38	56	0.46	0.17	21
97-98	0.36	0.07	70	0.64	0.16	60	0.63	0.44	84
98-99	0.87	0.27	68	1.63	0.69	65	-	-	-
99-00	0.17	0.06	16	1.08	0.45	13	2.35	0.77	15
00-01	0.25	0.05	26	1.02	0.32	7	2.48	1.03	33
01-02	0.04	0.01	19	0.28	0.09	15	5.47	3.79	16
02-03	0.17	0.09	19	0.34	0.19	13	1.60	0.89	18
Average	**0.38**	**0.11**		**0.90**	**0.33**		**2.17**	**1.18**	

Table 3. Average numbers of *Helicoverpa* pupae / m^2 recorded in Ingard and conventional cotton crops and unsprayed refuges in the Gwydir Valley.

Season	Ingard Cotton			Conventional Cotton			Unsprayed Refuges		
	Total Pupae	Live Pupae	Site Visits	Total Pupae	Live Pupae	Site Visits	Total Pupae	Live Pupae	Site Visits
96-97	0.20	0.08	34	0.50	0.14	38	0.33	0.14	26
97-98	0.36	0.10	31	0.44	0.13	30	2.14	1.18	57
98-99	1.35	0.36	21	1.84	0.63	15	6.04	1.99	5
99-00	0.14	0.09	10	0.21	0.05	3	1.18	0.29	10
00-01	0.42	0.05	14	0.26	0.03	5	2.94	0.54	17
01-02	0	0	6	0.39	0.21	5	0.33	0.07	5
02-03	0.66	0.29	10	0.84	0.27	4	1.06	0.32	15
Average	**0.45**	**0.14**		**0.64**	**0.21**		**2.00**	**0.65**	

Table 4. Average numbers of *Helicoverpa* pupae / m^2 recorded in Ingard and conventional cotton crops and unsprayed refuges in the Macintyre Valley.

Season	Ingard Cotton			Conventional Cotton			Unsprayed Refuges		
	Total Pupae	Live Pupae	Site Visits	Total Pupae	Live Pupae	Site Visits	Total Pupae	Live Pupae	Site Visits
96-97	0.77	0.13	9	1.18	0.35	9	1.63	0.90	39
97-98	0.59	0.01	10	1.15	0.20	8	6.03	1.42	8
98-99	2.22	0.46	15	1.49	0.33	11	15.71	12.73	7
99-00	0.01	0	17	0.22	0.03	7	0.10	0.03	15
00-01	0.41	0.03	13	0.64	0.08	6	4.70	1.29	13
01-02	0.07	0.01	18	0.19	0.13	13	2.29	0.82	23
02-03	0.47	0.07	9	0.63	0.23	5	2.34	1.05	10
Average	**0.65**	**0.10**		**0.79**	**0.19**		**4.69**	**2.61**	

Table 6 provides data on the average densities of *Helicoverpa* pupae found within the "additional" refuges and their associated Ingard cotton crops, as funded by Monsanto in 2000-2001. Whilst the abundance of *Helicoverpa* pupae (in particular the emerged pupae) appears to have been much greater under the sorghum and maize crops compared with the pigeon pea crops, such a conclusion needs to be cautious, given the limited replication of the former. The maize and sorghum refuge crops also were already in place when the need for additional refuge crops was identified. They "became" refuges by avoiding pesticide applications. In contrast, the pigeon pea refuges were deliberately planted (late).

The abundance of *Helicoverpa* pupae under the "late" pigeon pea refuges was higher compared with the Ingard cotton. Far more (as a proportion) of the pupae had emerged under the maize and sorghum refuges and the cotton than under the pigeon pea. The pigeon pea refuges showed a clear increase in pupal density over the sampling period (peaking in April, data not shown here) and this matched high densities of larvae on these crops in March. It seems likely that many of the larvae observed in March would have entered a pupal diapause, induced by declining temperatures and day lengths. They would probably have been destroyed once the refuge crops were removed and thus provided little benefit. Pupal parasitism varied from 3% in pigeon pea to 18% in the sorghum.

Table 5. Average numbers of *Helicoverpa* pupae / m^2 recorded in Ingard and conventional cotton crops and unsprayed refuges in the Macquarie Valley.

Season	Ingard Cotton			Conventional Cotton			Unsprayed Refuges		
	Total Pupae	Live Pupae	Site Visits	Total Pupae	Live Pupae	Site Visits	Total Pupae	Live Pupae	Site Visits
96-97	0.46	0.17	29	0.58	0.21	39	0.97	0.39	70
97-98	0.27	0.10	35	0.44	0.06	36	0.56	0.27	32
98-99	0.34	0.09	29	1.16	0.37	27	6.80	0.90	1
99-00	0.06	0.01	23	0.11	0	2	4.31	1.19	26
00-01	1.07	0.38	5	1.43	0	1	6.75	1.66	7
01-02	-	-	-	-	-	-	-	-	-
02-03	-	-	-	-	-	-	-	-	-
Average	**0.44**	**0.15**		**0.74**	**0.13**		**3.88**	**0.88**	

Table 6. Average densities of *Helicoverpa* pupae recorded within "additional" refuges and associated Ingard cotton crops at 19 sites in March – April 2001.

Crop	Treatment	Number of samplings	Live pupae / m^2	Emerged pupae / m^2	Total pupae / m^2
Ingard cotton	Sprayed	24	0.12	0.18	0.29
Conv. Cotton	Sprayed	1	0	0.71	0.71
Pigeon pea	Unsprayed	32	0.73	0.12	0.85
Sorghum	Unsprayed	3	0.21	6.64	6.86
Maize	Unsprayed	4	0.11	3.02	3.18

Table 7 provides a comparison with the previous Table by listing the average pupal densities under "standard" (i.e. earlier) refuges planted across nine cotton regions (see sampling methods elsewhere in this report). The "standard" pigeon pea refuges produced many more pupae during March – April compared with the "additional" refuges.

Table 7. Average densities of *Helicoverpa* pupae recorded within "standard" refuges and associated Ingard cotton crops at 36 sites in March – April 2001.

Crop	Treatment	Number of samplings	Live pupae / m^2	Emerged pupae / m^2	Total pupae / m^2
Ingard cotton	Sprayed	35	0.11	0.62	0.74
Conv. Cotton	Sprayed	21	0.22	1.12	1.34
Pigeon pea	Unsprayed	17	3.56	4.79	8.34
Sorghum	Unsprayed	5	0.49	2.01	2.50

In addition, it is worthy of note that poor establishment and management of the "additional" refuges may well have contributed to their lower productivity of *Helicoverpa.* The stand density of the pigeon pea was very low at several sites, weed infestations were common and two sites had never been irrigated.

Overall, our conclusion was that, although the "additional" pigeon pea refuges did produce *Helicoverpa* pupae and moths, their value was limited, in particular in comparison to refuges planted earlier. The "additional" maize and sorghum crops, which in reality were planted early, were probably quite productive.

In the soybean refuge evaluation at Maules Ck, soybean established at a much higher plant density than pigeon pea (12.1 plants / m of row cf 2.2). This may have influenced the relative attractiveness of the crops and subsequent productivity of moths. Both plant types appeared to be well managed and attractive throughout most of the season. Soybean and pigeon pea have very different growth habits : the former establishes more quickly and produces a dense canopy of vegetation with flowers along the stem, whilst the latter has more open, spindly growth, with flowering at the terminals. *Helicoverpa* spp larvae appear to prefer to feed on the pods of pigeon pea and the foliage of soybean. Possibly this difference reflects the plant forms noted above, and perhaps the very hairy nature of soybean pods. We also noted that the soil dried out more rapidly beneath pigeon pea.

Fig. 38 shows the average density of *Helicoverpa* pupae (data lumped over the 5 sampling dates). Pupae were equally more abundant under pigeon pea and soybean, compared with conventional and Ingard cotton (for total pupae, $F = 0.72$, $p > 0.05$ when pigeon pea and soybean were compared). Pigeon pea and soybean produced pupae at a similar rate throughout the season (Fig. 39) (for total pupae, $F = 2.59$, $p > 0.05$ when pigeon pea and soybean compared through time). The proportions of pupae that were parasitised were similar between pigeon pea and soybean (up to 10-20%) – data not shown here. 90% of successfully reared pupae were *H. armigera*. By April, most of the pupae that were found under both pigeon pea and soybean had emerged (Fig. 40), but no marked differences were apparent between crops.

Larval densities were similar in soybean and pigeon pea crops during January – February, but were much higher on pigeon pea (20 very small & small larvae / m) compared with soybean (3 larvae / m) in April (similar difference ratio for medium and large larvae on the two crops). By April, the soybean had senesced and was unattractive to *Helicoverpa*, whilst the pigeon pea was flowering still and hence attractive.

Although this trial supported the view that soybean can be a productive refuge, we considered further trials were needed at other sites to give such a claim more substance. We were also mindful that pigeon pea, because of its multiple cycles of flowering, has broad seasonal coverage as a refuge (especially earlier than the Maules Ck trial evaluated). Could multiple planting time for soybean also achieve longer attraction ?

In the 2001-02 trial at Goondiwindi, plant establishment of the pigeon pea and soy bean was more comparable (14.4 cf 12.8 plants per m of row respectively) than observed at Maules Ck. The trial site received four irrigations during the season. Whilst both crop types were lush, we failed to find any *Helicoverpa* spp on the soy bean. The only lepidopteran present there was *Chrysodiexus eriosoma* (Noctuidae). In contrast, we collected eggs and larvae of *Helicoverpa* on the pigeon pea on all three sampling occasions (e.g. up to 9 larvae / m of row). Approximately equal numbers of *H. armigera* and *H. punctigera* were reared from the eggs and larvae collected. Parasitism was 13% or less; death due to virus was 10% or less.

The 2001-02 season was generally regarded as a low activity year for *Helicoverpa* in the region and the pupal abundance data for the pigeon pea crops reflected this (average of 1.1 – 1.6 live pupae found / m^2, with 0.25 – 0.33 of these parasitised). The most common parasitoid was *Heteroplema scaposum*. Again, approximately equal numbers of *H. armigera* and *H. punctigera* were reared from the pupae collected. No pupae were found beneath the soy bean and Ingard cotton. Negligible numbers of pupae were found beneath conventional cotton.

Whilst the failure of the soy bean to attract *Helicoverpa* at Goondiwindi was somewhat a puzzle, the results serve to indicate a greater unreliability of soy bean compared with pigeon pea as a refuge crop.

b) St George & Dirranbandi

The abundance of live and total pupae collected in Ingard and conventional cotton crops from 1996-97 to 2002-03 in the St George and Dirranbandi regions are shown in Tables 8-9. The numbers of pupae found in crops other than cotton that were used as refuges in the more recent years are also provided.

Helicoverpa abundance was clearly higher in 1998-99 compared with other years (see also other valleys above – but note Macquarie valley exception for 2000-01). In the 3 years of this project, abundance was quite low. The abundance of *Helicoverpa* pupae was generally greater in the conventional crops compared with the Ingard crops, and much greater under the refuges (similar proportions to the other valleys noted above). The low abundance under refuges in 2002-03 can be attributed, at least in part, to reduced water availability.

Table 8. Average numbers of *Helicoverpa* pupae / m^2 recorded in Ingard and conventional cotton crops and unsprayed refuges near St George.

Season	Ingard Cotton			Conventional Cotton			Unsprayed Refuges		
	Total Pupae	Live Pupae	Site Visits	Total Pupae	Live Pupae	Site Visits	Total Pupae	Live Pupae	Site Visits
96-97	0.13	0.05	5	0.44	0.20	5	-	-	-
97-98	0.33	0.08	20	0.36	0.08	19	-	-	-
98-99	0.48	0.18	28	0.76	0.34	25	-	-	-
99-00	0.27	0.11	27	0.35	0.12	25	1.07	0.14	1
00-01	0.40	0.12	33	0.46	0.12	28	10.32	6.20	6
01-02	0.01	0.01	30	0.10	0.03	21	4.48	1.49	24
02-03	0.20	0.08	25	0.25	0.08	22	0.48	0.04	7
Average	**0.26**	**0.09**		**0.39**	**0.14**		**4.09**	**1.97**	

Table 9. Average numbers of *Helicoverpa* pupae / m^2 recorded in Ingard and conventional cotton crops and unsprayed refuges near Dirranbandi.

Season	Ingard Cotton			Conventional Cotton			Unsprayed Refuges		
	Total Pupae	Live Pupae	Site Visits	Total Pupae	Live Pupae	Site Visits	Total Pupae	Live Pupae	Site Visits
96-97	-	-	-	-	-	-	-	-	-
97-98	0.57	0.05	8	0.34	0.04	8	-	-	-
98-99	0.77	0.31	18	1.30	0.58	15	-	-	-
99-00	0.14	0.03	26	0.29	0.07	23	-	-	-
00-01	0.30	0.07	17	0.85	0.23	19	-	-	-
01-02	0.10	0.03	26	0.21	0.10	27	0.36	0.05	4
02-03	-	-	-	-	-	-	-	-	-
Average	**0.38**	**0.10**		**0.60**	**0.20**				

c) All Valleys Combined

The numbers of live and total pupae collected throughout 1996-97 (when surveys began) to 2002-03 in the various refuges and Ingard cotton crops across all surveyed regions are given in Figs 41-42. Pigeon pea produced high numbers of *Helicoverpa* pupae most consistently of all the refuges. Sorghum also produced very large numbers of *Helicoverpa* pupae, but only in one year during the study.

More pupae (as a %), when reared in the laboratory, produced *Helicoverpa* moths from the conventional cotton refuges and the Ingard cotton (Fig. 43). Moth emergence was particularly poor in the sorghum refuges, where parasitism of pupae was highest. The vast majority of emerging moths were *H. armigera*. *H. punctigera* was particularly rare in sorghum and maize.

Data indicating variability in % rearing from live pupae between years are available from G. Baker and C. Tann. Extremes are indicated in Table 10. Clearly, the success in rearing *H. armigera* (the object of using refuges) was very variable between years – in all crops. Conservatively, taking the average % emergence data from Fig. 43, and thus "correcting" the live pupae numbers recorded in Fig. 41 for each refuge type, suggests that pigeon pea is still the most productive refuge.

Table 10. Annual extremes in average % rearings from live *Helicoverpa* pupae collected from various refuges and Ingard cotton crops during 1996 – 2003.

Crop	% *H. armigera*	% *H. punctigera*	% Parasitised
Pigeon pea	21.6 / 79.9	0 / 17.5	5.1 / 43.7
Sorghum	0 / 52.9	0 / 0	1.1 / 66.7
Maize	35.4 / 53.2	0 / 0.2	2.6 / 4.3
Unsprayed cotton	0 / 60.5	0 / 9.1	0 / 30.0
Conventional cotton	30.9 / 78.8	0 / 12.9	0.8 / 24.5
Ingard cotton	7.6 / 89.9	0 / 22.5	2.0 / 25.8

The most common parasitoids reared from the *Helicoverpa* pupae were *Heteropelma scaposum*, *Pterocormus* spp., *Lissopimpla excelsa*, *Netelia producta* and various Tachinidae. Table 11 indicates the relative frequency of these groups in pupae reared from collections made in Ingard and conventional cotton crops during this study. Similar data for rearings from unsprayed refuges are still being collated, but a similar suite of parasitoids was encountered there.

Table 11. Numbers of parasitoids reared from live pupae collected during surveys of conventional and Ingard cotton crops, 1996-97 to 2002-03.

Cotton Crop &	Cotton Season						
Parasitoid	96-97	97-98	98-99	99-00	00-01	01-02	02-03
Conventional							
Pupae reared	193	202	180	73	66	81	44
Tachinidae	5	6	24	0	2	2	0
Heteroplema	1	25	135	17	10	8	1
Pterocormus	7	4	7	2	0	1	0
Netelia	0	0	2	0	1	0	0
Lissopimpla	0	3	0	0	0	0	0
Total parasitoids	13	38	168	19	13	11	1
Ingard							
Pupae reared	175	218	223	119	108	99	63
Tachinidae	4	4	16	2	0	1	2
Heteroplema	0	14	52	8	2	3	2
Pterocormus	2	1	12	0	0	0	3
Netelia	0	0	2	0	0	0	0
Lissopimpla	0	1	3	0	0	0	0
Total parasitoids	6	20	85	10	2	4	7

d) Further Observations on Natural Enemies

The abundance of predatory invertebrates was much higher in unsprayed cotton and pigeon pea refuges compared with sites located within Ingard cotton crops, well away from the edges of fields (Fig. 44). These data combine the 9 farms where all the cropping variables listed in Fig. 44 were represented with the additional observations made on refuge crops only. Data for the 9 farms, treated separately, are available from G. Baker and C. Tann. Trends are similar to those depicted in Fig. 44. Nearer to the edges of Ingard crops, the abundance of predators more closely resembled the nearby refuges. These results suggest that there may be some net movement of predators into Ingard crops, but further studies using marked individuals are needed to confirm this. The results also beg the question of how to design and evaluate experiments on the influence of transgenic cotton on non-target invertebrates. If the surveyed "plots" of transgenic cotton are small and prone to edge effects (i.e. invasion from nearby, different habitat types), then the influence(s) of the transgenic crop might be masked. Care should thus be taken to ensure that plots are indeed as large as needed to enable rigorous evaluations. The plot sizes used previously for non-target evaluations of Ingard and Bollgard II should perhaps be reviewed in light of the data presented here.

Table 12 lists the presence data for the predators collected in Ingard cotton and their associated refuge crops during the 2001-02 season. The data provide further support to the likelihood of predatory groups being less abundant well within Ingard cotton crops, compared with their edges, and that perhaps invasion of the edges of Ingard crops might explain this pattern. Table 13 lists the presence data for the predators collected in the additional refuges surveyed during the 2001-02 season. The data are dominated by pigeon pea crops. However, it is clear, that in this particular season, red & blue beetles (*Dicranolaius bellulus*) were widepsread, as were transverse ladybeetles (*Coccinella transversalis*), damsel bugs (*Nabis kinbergii*) and jumping spiders (Salticidae). Assassin bugs (*Prithesancus* spp.) were commonly collected in the pigeon pea, but not so frequently in the other refuges.

Table 12. Recorded presences of various predatory invertebrates through sweep netting of Ingard and associated refuge crops in Dec 2001 – Mar 2002. Numbers following refuge types indicate numbers of sampling occasions. Note other spiders in this and later tables could include small specimens of the families listed separately.

Predators	**Ingard – close to refuge (17)**	**Ingard – far from refuge (17)**	**Pigeon Pea (12)**	**Unspr'd Cotton (5)**
Beetles (Total)	**40**	**18**	**34**	**12**
Dicranolaius bellulus	17	10	11	5
Carabidae	0	0	1	0
Diomus notescens	2	0	0	0
Coccinella transversalis	11	5	9	4
Micraspis frenata	0	1	4	0
Harmonia octomaculata	8	1	8	3
Coelophora inaequalis	1	0	0	0
Harmonia conformis	1	1	1	0
Bugs (Total)	**14**	**9**	**23**	**8**
Cermatulus nasalis	1	1	0	0
Ochelia schellenbergii	2	1	4	0
Nabis kinbergii	10	4	10	4
Geocoris lubra	1	3	1	2
Prithesancus spp.	0	0	8	2
Lacewings (Total)	**3**	**3**	**9**	**2**
Mallada spp.	2	0	7	1
Lacewing larvae	1	3	2	1
Spiders (Total)	**45**	**35**	**35**	**16**
Oxyopidae	6	2	8	4
Clubionidae	3	0	2	1
Theridiidae	0	0	2	0
Salticidae	11	10	6	3
Thomisidae	8	6	5	4
Araneidae	3	1	1	0
Other spiders	14	16	11	4
Ants	**7**	**1**	**2**	**0**

Table 14 lists the presence data for the predators collected in the refuges during the 2002-03 season. The data are again dominated by pigeon pea crops. Even so, it is clear, that in this particular season too red & blue beetles were widespread, as were flower / crab spiders (Thomisidae), in pigeon pea refuges, and probably also in sorghum and unsprayed cotton refuges. The most commonly seen lady bird beetles were *Hippodamia variegata* (note not see the previous year) and the transverse ladybeetles, although neither of these was observed in the few unsprayed cotton crops that were surveyed. Amongst predatory bugs, apple dimpling bugs (*Campylomma liebknechti*) were widespread in pigeon pea refuges (and probably also unsprayed cotton) whilst damsel, predatory shield (*Ochelia schellenbergii*) and big-eyed (*Geocoris lubra*) bugs were also moderately common. Green lace wings (*Mallada* spp.) were common in pigeon pea as were mirids (not identified further) and lynx (Oxyopidae) and jumping spiders, the latter occurring in all three unsprayed cotton crops surveyed.

Table 13. Recorded presences of various predatory invertebrates through sweep netting of additional refuge crops in Dec 2001 – Mar 2002. Numbers following refuge types indicate numbers of sampling occasions.

Predators	**Pigeon Pea (25)**	**Unspr'd Cotton (11)**	**Soybean (4)**
Beetles (Total)	**70**	**33**	**19**
Dicranolaius bellulus	23	10	4
Chauliognathus pulchellus	3	0	1
Carabidae	1	2	1
Diomus notescens	1	3	0
Coccinella transversalis	19	8	4
Micraspis frenata	8	5	2
Harmonia octomaculata	14	5	6
Harmonia conformis	1	0	1
Bugs (Total)	**57**	**28**	**13**
Cermatulus nasalis	2	1	2
Ochelia schellenbergii	14	4	1
Nabis kinbergii	21	10	4
Geocoris lubra	2	7	3
Prithesancus spp..	17	2	2
Campylomma liebknechti	1	3	0
Orius spp.	0	1	1
Lacewings (Total)	**15**	**9**	**2**
Mallada spp.	13	7	2
Lacewing larvae	2	2	0
Spiders (Total)	**74**	**41**	**12**
Oxyopidae	12	8	2
Clubionidae	5	3	1
Theridiidae	2	0	1
Salticidae	16	10	2
Thomisidae	15	6	1
Araneidae	3	3	1
Other spiders	21	11	4
Ants	**10**	**5**	**0**

Table 14. Recorded presences of various predatory invertebrates through sweep netting of refuge crops in Jan – Mar 2003. Numbers following refuge types indicate numbers of sampling occasions.

Predators	Pigeon Pea (30)	Sorghum (6)	Unspr'd Cotton (3)
Beetles (Total)	**60**	**14**	**3**
Dicranolaius bellulus	25	3	3
Coccinella transversalis	10	3	0
Micraspis frenata	2	0	0
Harmonia octomaculata	7	3	0
Coelophora inaequalis	1	0	0
Harmonia conformis	3	1	0
Hippodamia variegata	12	4	0
Bugs (Total)	**73**	**9**	**7**
Cermatulus nasalis	4	0	0
Ochelia schellenbergii	12	1	1
Nabis kinbergii	15	2	0
Geocoris lubra	11	2	3
Prithesancus spp.	4	0	0
Campylomma liebknechti	20	1	3
Orius spp.	7	3	0
Lacewings (Totals)	**22**	**3**	**2**
Mallada spp.	16	2	1
Micromus tasmaniae	3	0	0
Lacewing larvae	3	1	1
Spiders (Totals)	**71**	**15**	**9**
Oxyopidae	16	4	2
Clubionidae	2	2	0
Therididae	0	1	0
Salticidae	16	3	3
Thomisidae	26	2	2
Araneidae	4	1	1
Other spiders	7	2	1
Mirids	**25**	**3**	**2**
Ants	**12**	**0**	**0**

4. Tillage / Emergence Experiment

Table 15 gives the total numbers of *Helicoverpa* (all but two were *H. armigera*) that emerged in each tillage treatment, along with the total numbers of pupal parasitoids and other moth species. The parasitoids included *Heteropelma scaposu*m, *Ichneumon promissorius* & unidentified Tachinidae. The other moths included *Leucana* sp., *Agrotis* sp., *Earias huegeliana* & *Chrysidiexis punctifera*. Captures of emerging *Helicoverpa* were low, and highly variable between replicates (e.g. 10 of the 13 *H. armigera* emerging from the Eliminator / Wheat Planter treatment were in one replicate). Consequently, although there appears to be a tendency in the data for the Aerway and Centre Busting / Disc treatments to have been more destructive of lepidopteran pupae and their parasitoids than the other treatments, this was not statistically significant (e.g. One Way ANOVA for *Helicoverpa* emergence, $F = 1.31$, $p > 0.05$). Transformation of the data did not change the stats outcomes. The experiment was inhibited by low insect pressure, yielding few pupae to work with. A future experiment should also consider greater replication (plots &/or emergence cages) to overcome the high degree of patchiness in insect abundance observed.

Table 15. Total numbers of *Helicoverpa* spp.(*H.a. = H.armigera; H.p. = H. punctigera*), pupal parasitoids and other moths emerging per tillage treatment at ACRI in 2002-03.

Tillage treatment	***H. a.***	***H.p.***	**Parasitoids**	**Other Moths**	**Total**
Centre Busting / Discs	2	0	3	8	11
Aerway	2	0	0	2	4
Centre Busting / Go-Devils	3	0	22	3	28
Eliminator / Wheat planter	13	0	7	16	36
Wheat Planter	9	1	21	3	34
Untreated Control	10	1	28	3	42

5. Movements of Moths from Refuges to Ingard Crops

The mark – recapture experiment near Warren using strontium chloride as a marker of larvae developing on a pigeon pea refuge yielded 503 *H. armigera* (353 males, 150 females) and 35 *H. punctigera* (29 males, 6 females) in the light traps. There was no obvious pattern in trap catches between crops (80 and 91 total *Helicoverpa* moths in the two light traps in the pigeon pea, and 52, 179, 89 and 47 total *Helicoverpa* moths in the four light traps in the Ingard cotton, in order of increasing distance from the pigeon pea). The collected numbers of mated pairs of *H.armigera* (n = 22) was surprisingly and disappointingly small, in part caused by unexpected irrigation and insecticidal spraying of the site which curtailed our ability to make further collections.

The levels of strontium in the sprayed and unsprayed pigeon pea plant samples were quite distinct (mean = 713 mg / kg, range = 560 – 830 mg / kg for the sprayed samples; mean = 107 mg / kg, range = 105 – 110 mg / kg for the unsprayed samples). The levels of strontium in moths caught emerging from sprayed and unsprayed pigeon pea were markedly different (Fig. 45), but there was a great deal of overlap between the data (range for sprayed moths = 1.79 – 50.28 mg / kg; range for unsprayed moths = 0.51 – 6.55 mg / kg). Clearly, most moths emerging from the sprayed pigeon pea were not “marked” strongly with strontium, but several were. No moths that emerged from the unsprayed pigeon pea had strontium levels in excess of 7 mg / kg.

The level of strontium in the mated moths collected in the Ingard cotton crop (n = 34) and the pigeon pea refuge (n= 8) (data pooled) was comparable with that recorded in the unsprayed refuge (mean ± S.E. = 2.50 ± 0.20 mg / kg, range = 0.82 – 5.59) (note 2 mated moths escaped prior to strontium analyses !). None of these moths could be considered as definitively originating from the refuge. Strontium analyses of the trapped moths showed no differences between traps (Fig. 46). Fig. 46 refers only to *H. armigera*. *H. punctigera* were also trapped, but their numbers were low (n = 35). All but 2 had strontium levels less than 7 mg / kg. The 2 exceptions had 7.8 and 10.0 mg strontium / kg.

Whilst it was disappointing that we trapped few moths with high levels of strontium, it was encouraging that similar numbers of moths with strontium levels > 7 mg / kg (i.e. more than the greatest level we observed in moths caught emerging from unsprayed pigeon pea) were trapped in both pigeon pea and Ingard cotton. This could be interpreted as evidence that moths produced within pigeon pea refuges are indeed venturing into the Ingard cotton crops as “intended” (note a dilution of marked moths would be expected with distance away from the pigeon pea, but we saw no evidence of this). The lack of any mated moths with a high strontium level however brings into question whether or not refuge-generated moths are mating in Ingard cotton crops. We now need repeat experiments to give greater rigour – e.g. studies at other sites, heavier marking to enable increased recapture data, surveys of emergence in other nearby habitats besides the designated refuge to check natural strontium levels produced there.

6. Crop Origins of Moths

The percentage carbon and the carbon delta data from the preliminary analyses of *H. armigera* larvae collected and reared from known crop hosts are given in Table 16. There was a clear distinction in carbon delta for the C3 and C4 plants ($F = 256.8$, $p < 0.001$), verifying this would be a useful way to discriminate moth origins. As indicated in the methods, analyses of further collections of *H. armigera* moths within Ingard cotton at Ketah in the Gwydir Valley and Myall Downs near North Star are awaiting isotope analyses.

Table 16. Results of carbon isotope ratio analyses on *H. armigera* moths raised from known plant hosts. Data are means ± S.E.

Moth Origin	% Carbon	Carbon Delta
C3 Plants		
Unsprayed Cotton	52.7 ± 3.1	25.8 ± 0.5
Pigeon pea	49.8 ± 1.7	26.4 ± 0.5
C4 Plants		
Sorghum	51.6 ± 2.1	13.6 ± 0.5
Maize	55.4 ± 2.3	13.1 ± 0.4

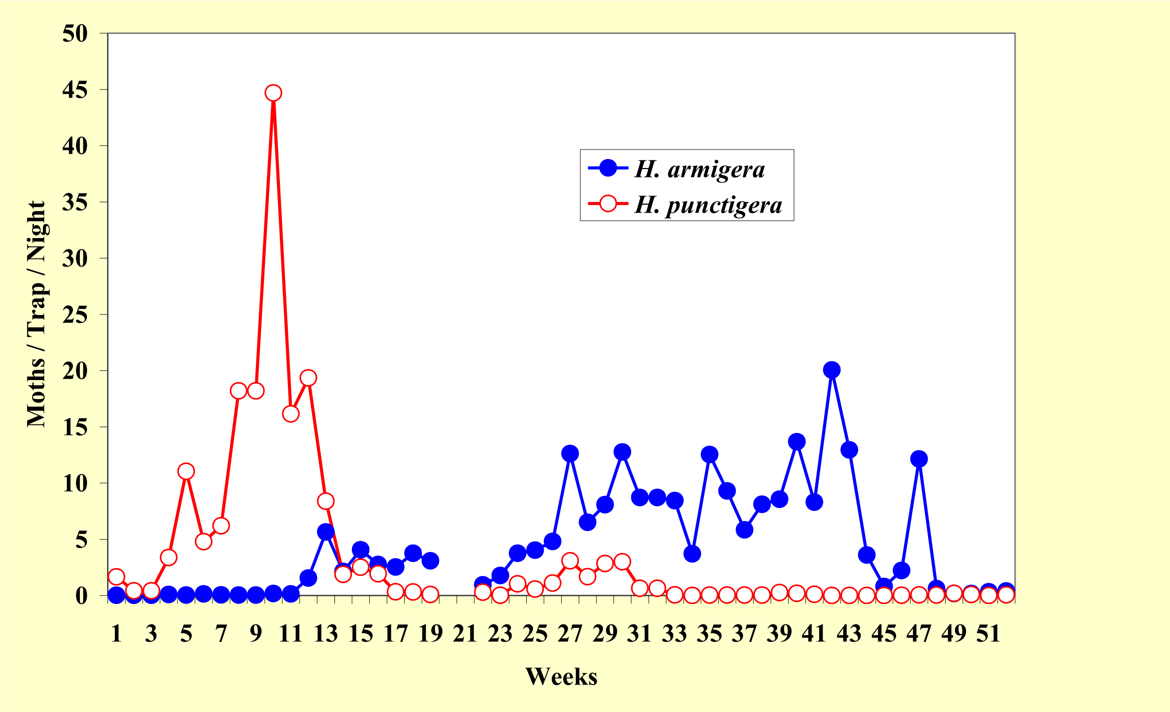

Fig. 1. Pheromone trap catches for *H. armigera* and *H. punctigera* near ACRI, Narrabri, 2000-01.

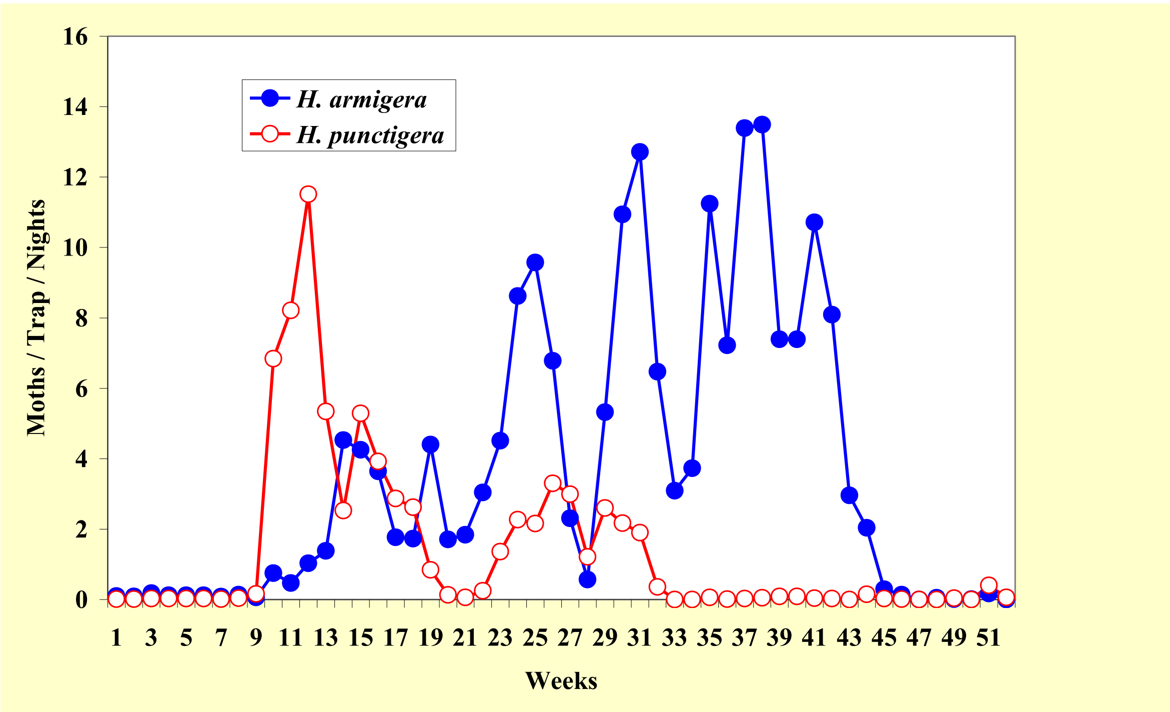

Fig. 2. Pheromone trap catches for *H. armigera* and *H. punctigera* near ACRI, Narrabri, 2001-02.

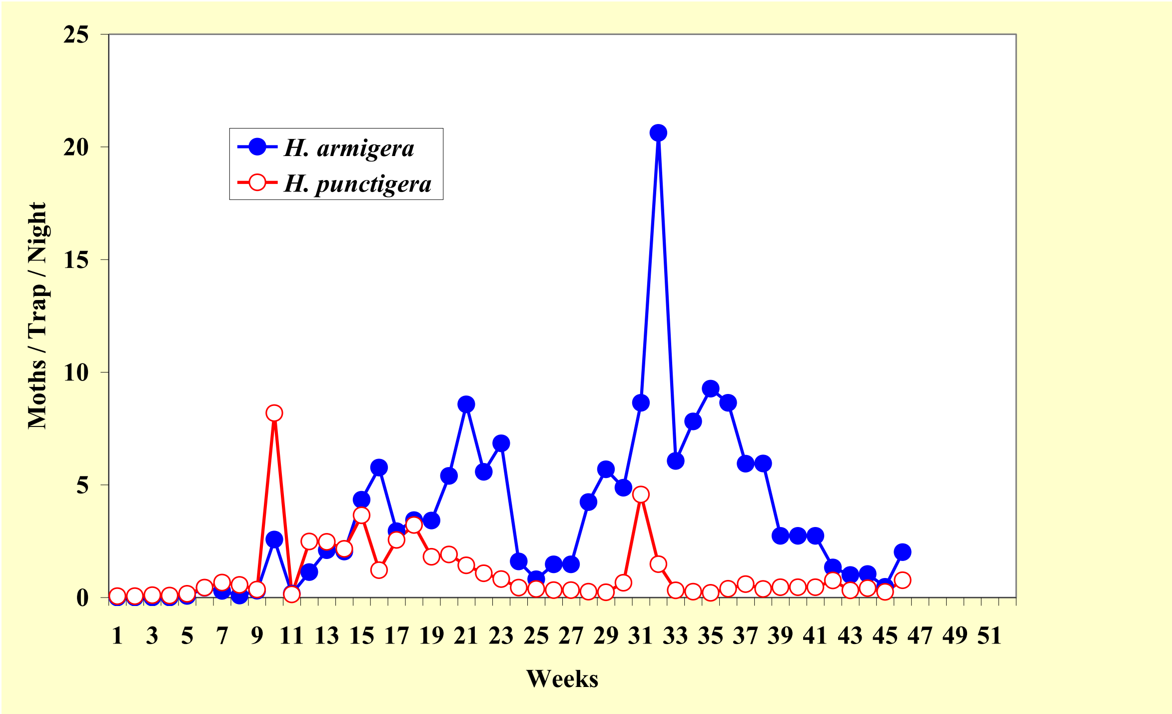

Fig. 3. Pheromone trap catches for *H. armigera* and *H. punctigera* near ACRI, Narrabri, 2002-03.

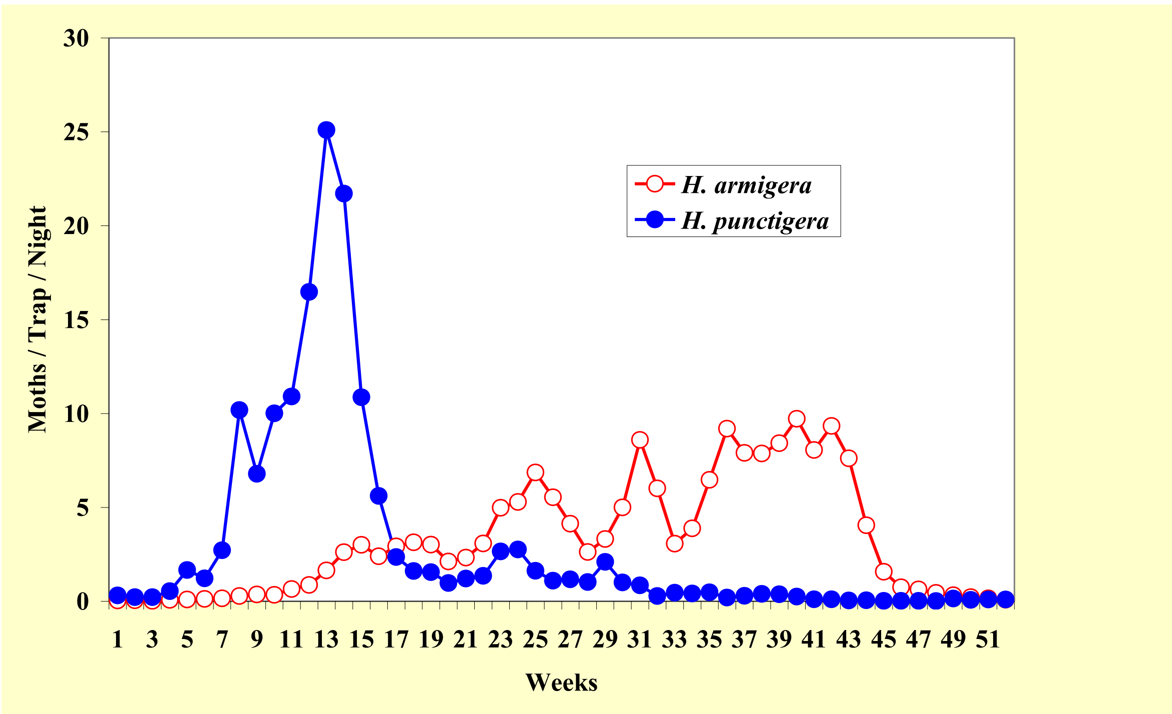

Fig. 4. Average pheromone trap catches for *H. armigera* and *H. punctigera* near ACRI, Narrabri for 1992-93 to 2001-02.

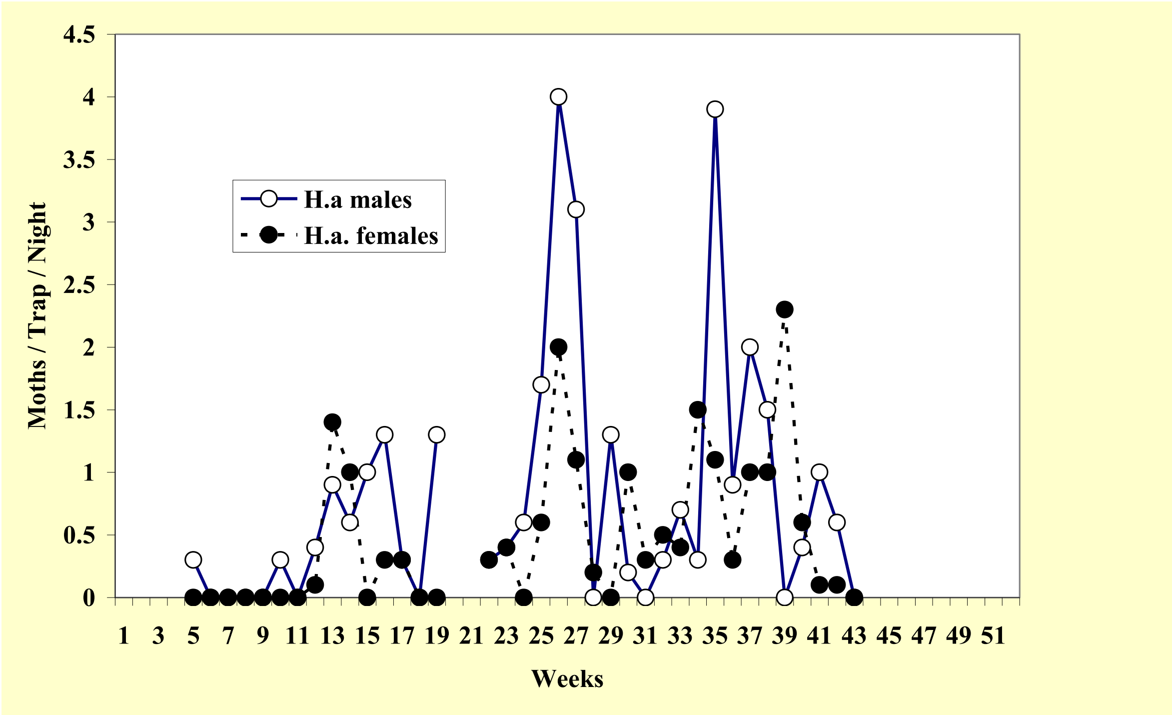

Fig. 5. Light trap catches for *H. armigera* near ACRI, Narrabri, 2000-01 (males and females shown).

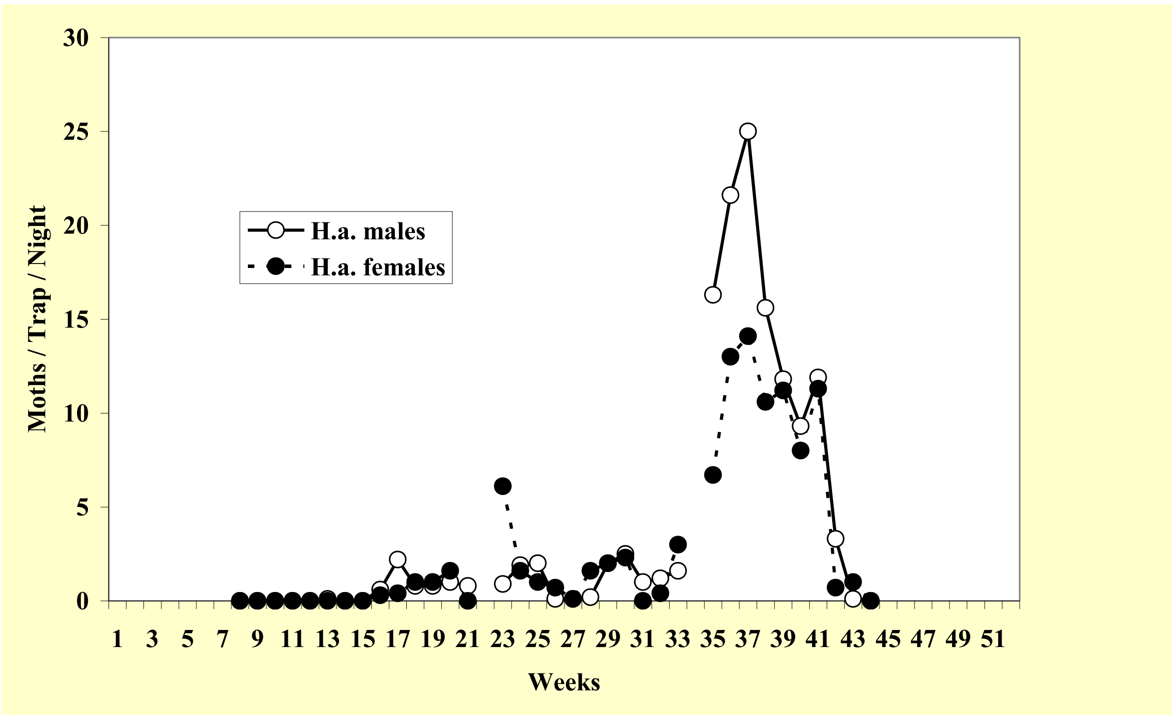

Fig. 6. Light trap catches for *H. armigera* near ACRI, Narrabri, 2001-02 (males and females shown).

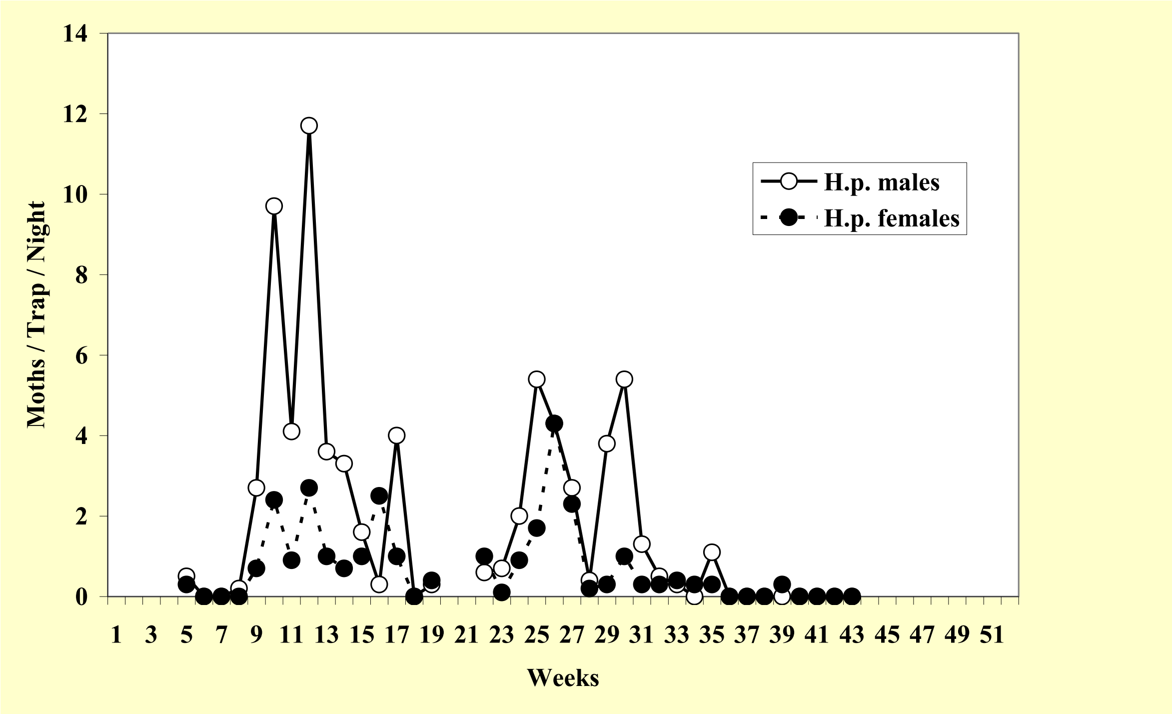

Fig. 7. Light trap catches for *H. punctigera* near ACRI, Narrabri, 2000-01 (males and females shown).

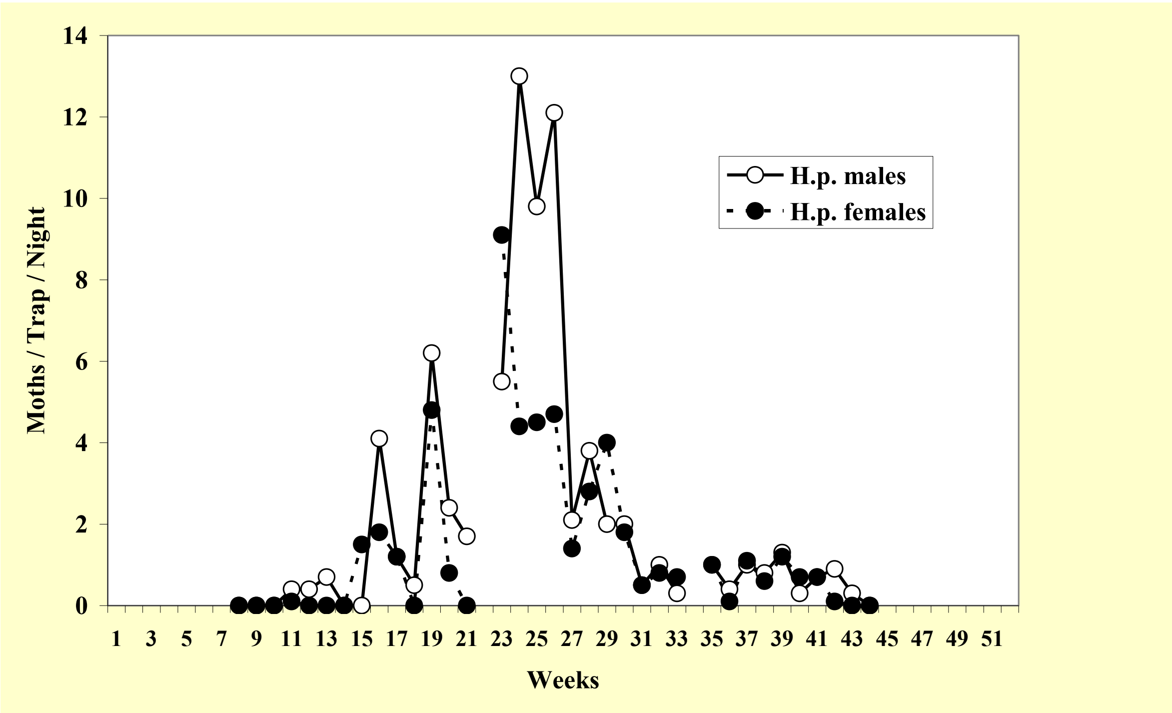

Fig. 8. Light trap catches for *H. punctigera* near ACRI, Narrabri, 2001-02 (males and females shown).

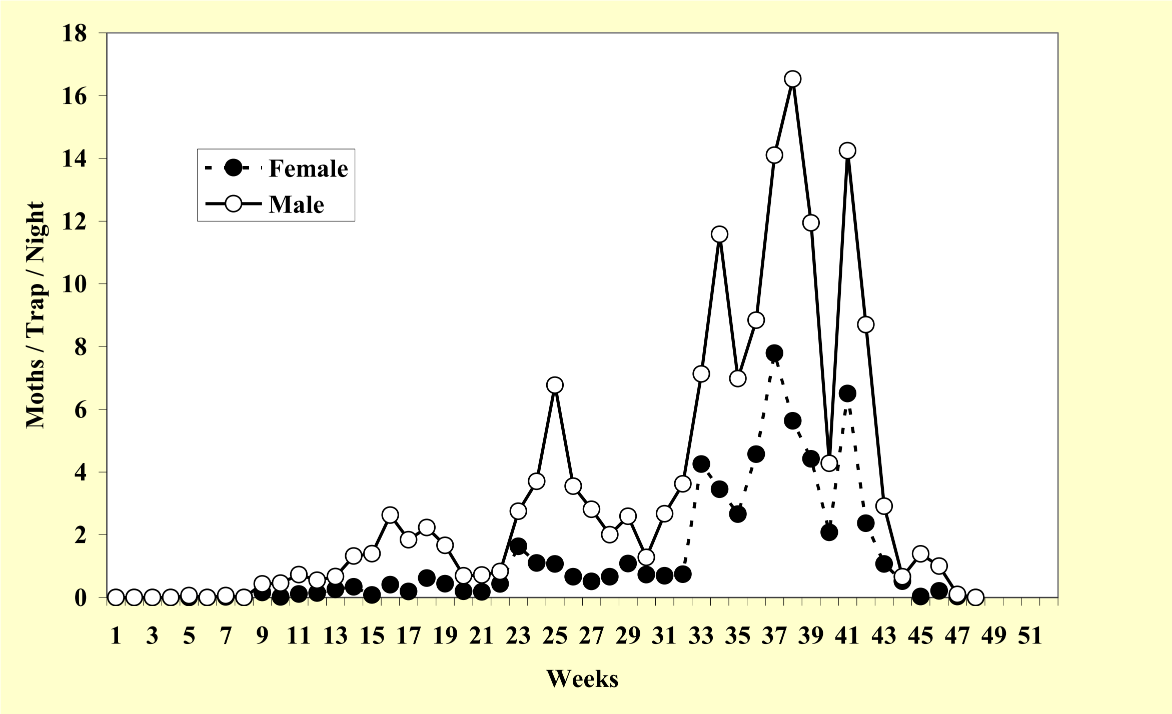

Fig. 9. Average light trap catches for *H. armigera* near ACRI, Narrabri, 19992-93 to 2001-2002 (males and females shown).

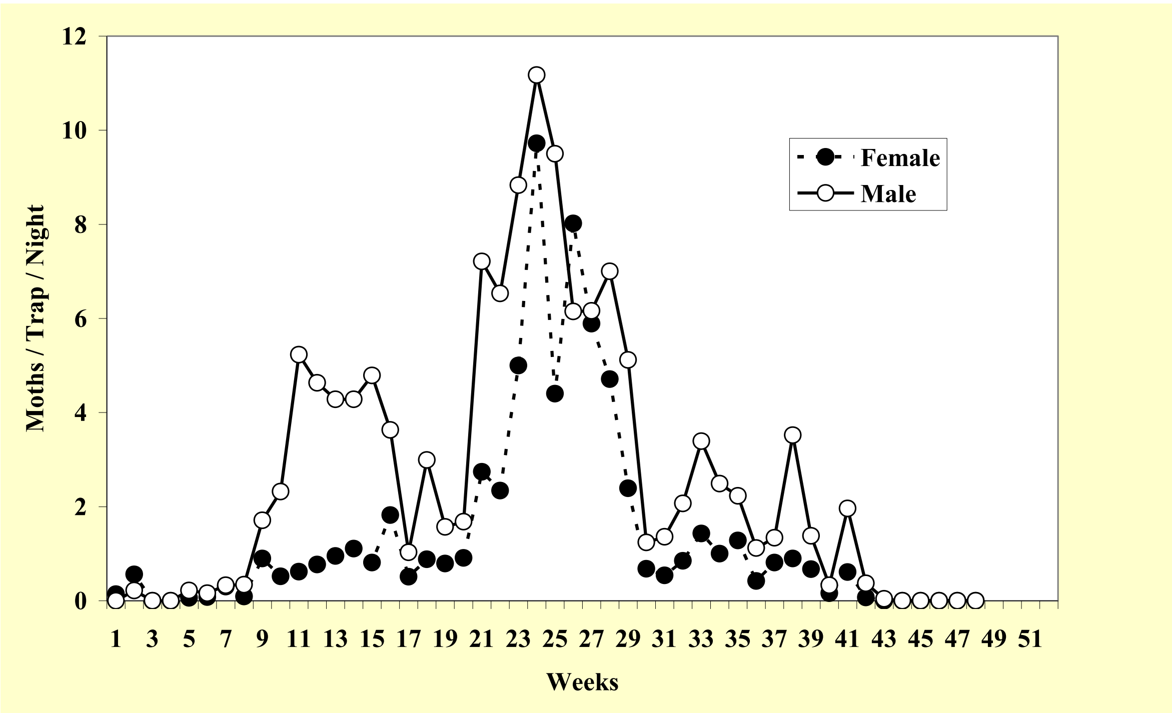

Fig. 10. Average light trap catches for *H. punctigera* near ACRI, Narrabri, 19992-93 to 2001-2002 (males and females shown).

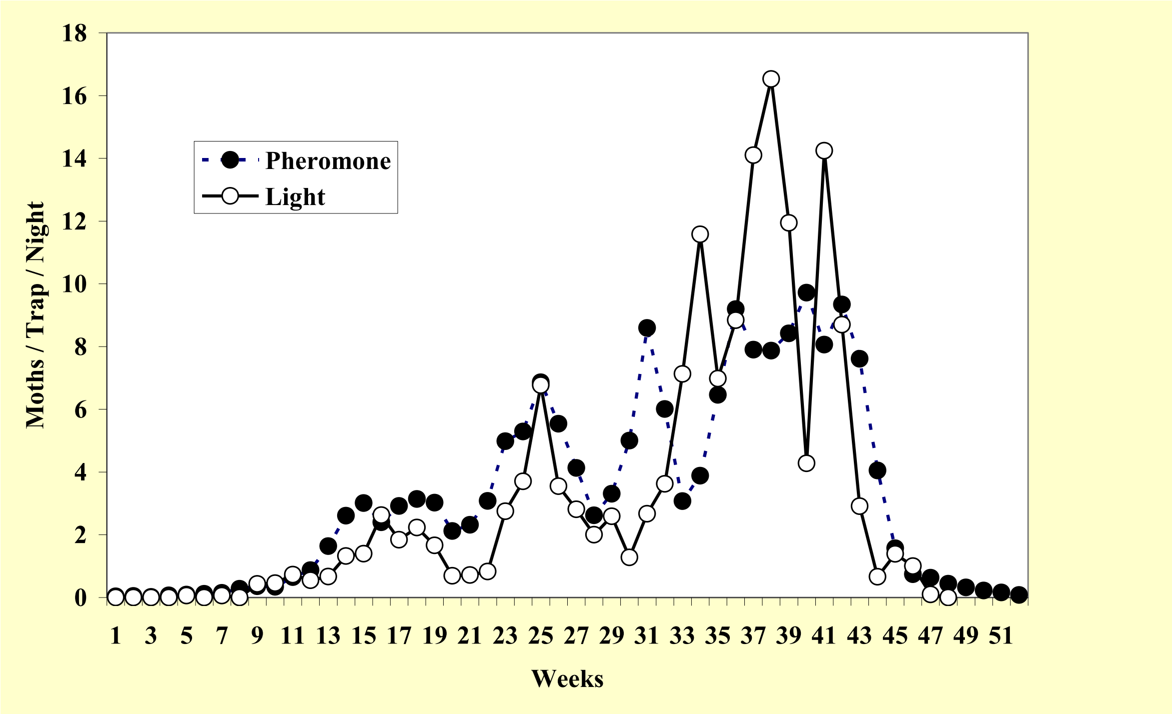

Fig. 11. Comparison of long-term averages (1992-2002) for pheromone trap catches and light trap catches of *H. armigera* (males only) near ACRI, Narrabri.

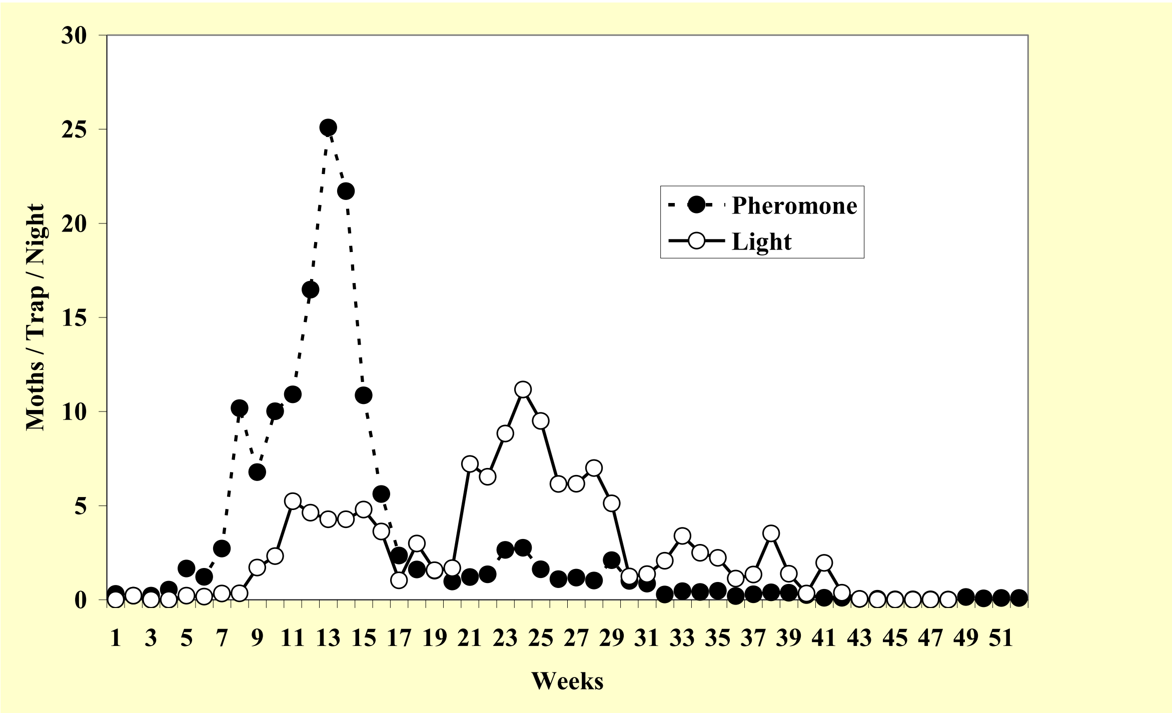

Fig. 12. Comparison of long-term averages (1992-2002) for pheromone trap catches and light trap catches of *H. punctigera* (males only) near ACRI, Narrabri.

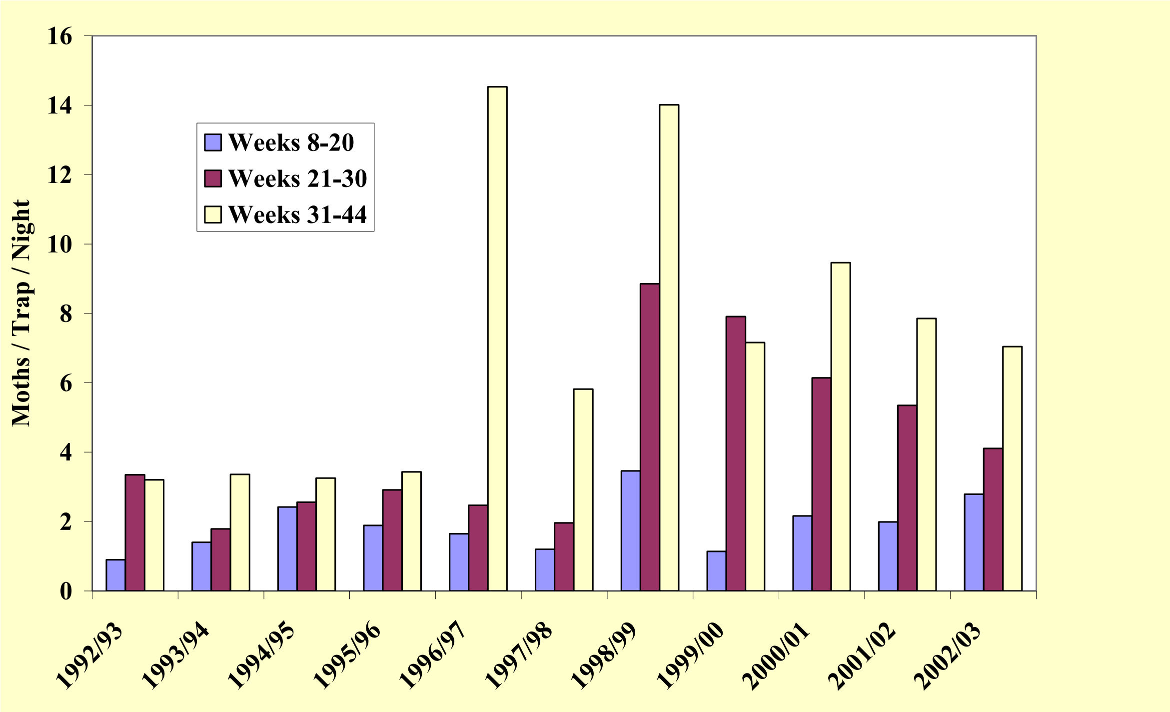

Fig. 13. Average pheromone trap catches for *H. armigera* near ACRI, Narrabri from 1992-93 to 2002-03. Data are separated into clusters of 8-20, 21-30 and 31-44 weeks since July 1 in each year.

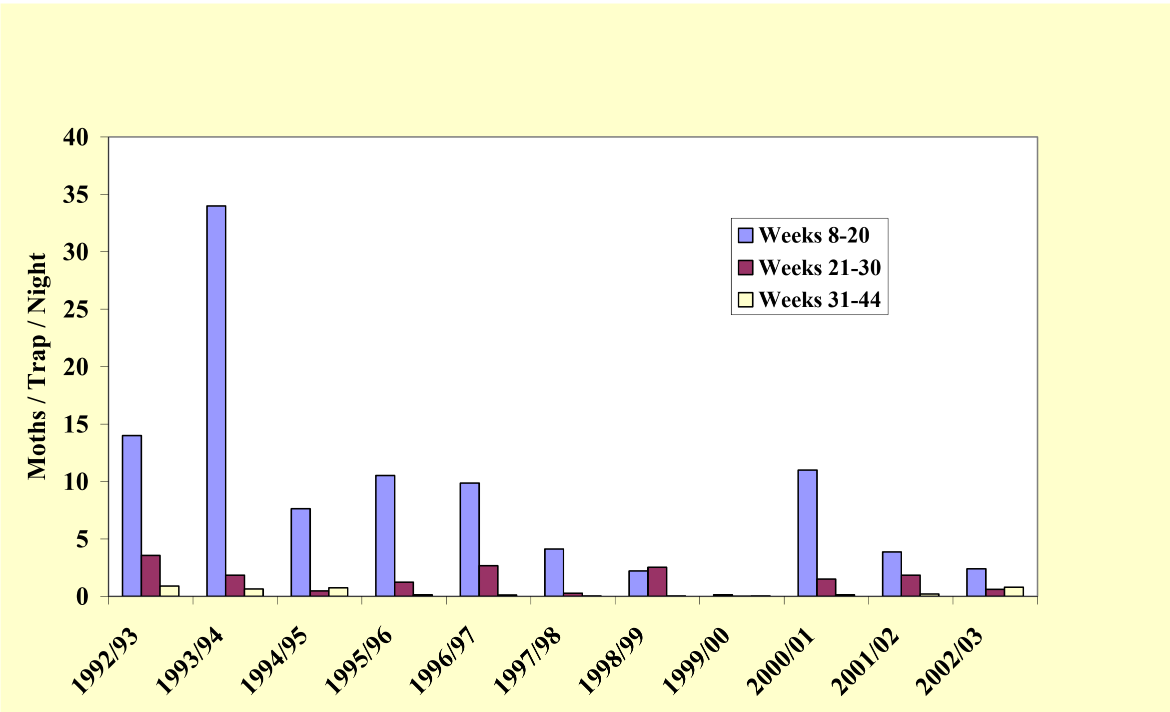

Fig. 14. Average pheromone trap catches for *H. punctigera* near ACRI, Narrabri from 1992-93 to 2002-03. Data are separated into clusters of 8-20, 21-30 and 31-44 weeks since July 1 in each year.

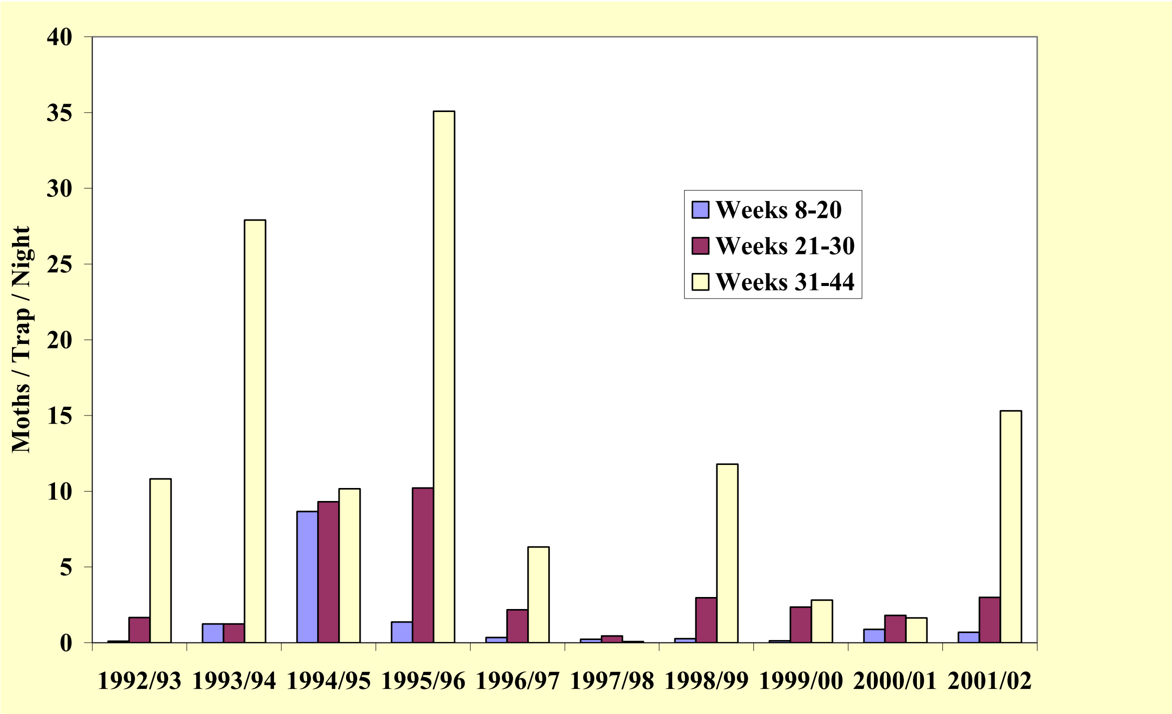

Fig. 15. Average light trap catches for *H. armigera* near ACRI, Narrabri from 1992-93 to 2001-02. Data are separated into clusters of 8-20, 21-30 and 31-44 weeks since July 1 in each year.

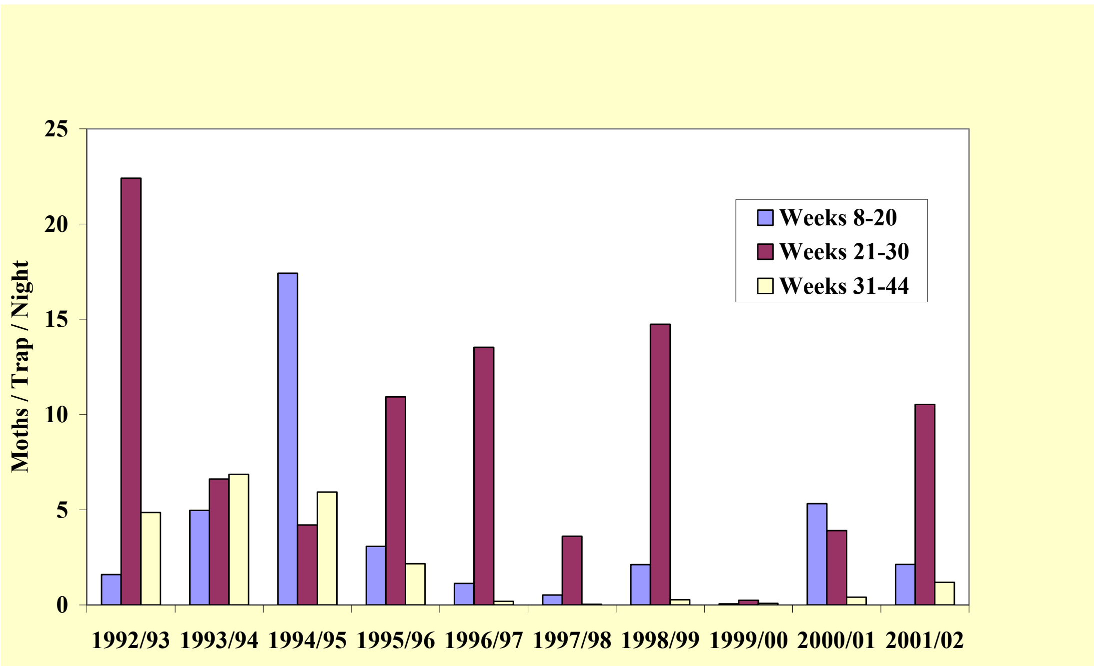

Fig. 16. Average light trap catches for *H. punctigera* near ACRI, Narrabri from 1992-93 to 2001-02. Data are separated into clusters of 8-20, 21-30 and 31-44 weeks since July 1 in each year.

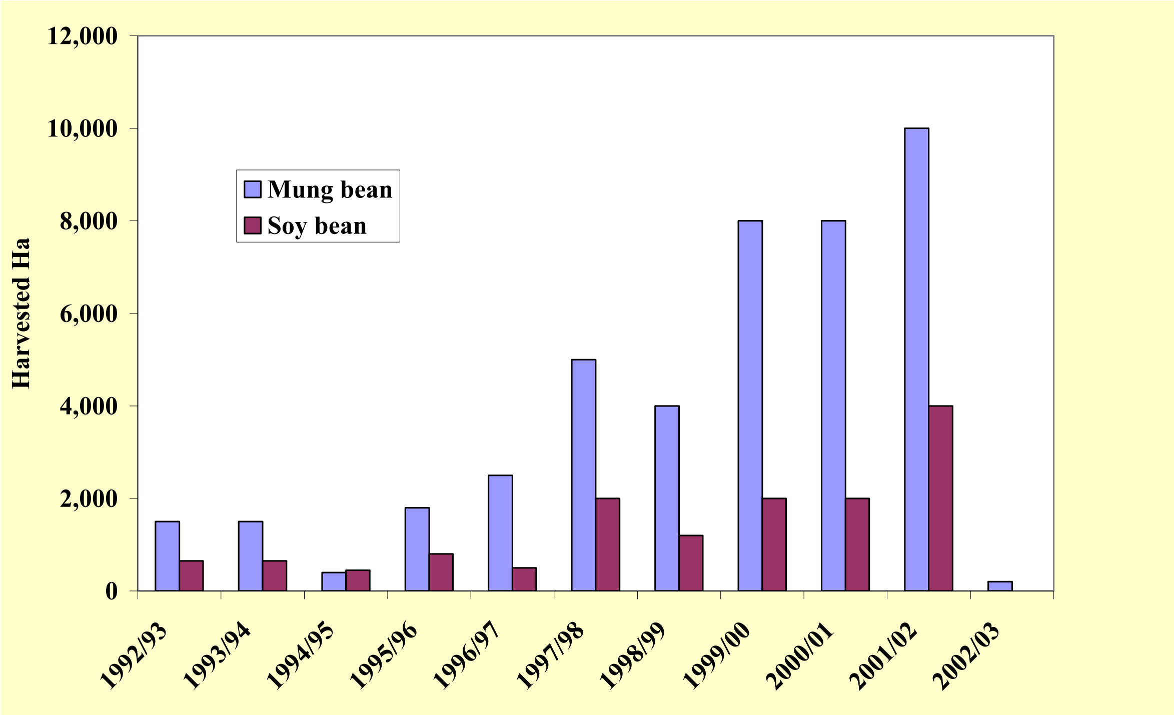

Fig. 17. Approximate areas of selected grain crops harvested in the Narrabri Shire, N.S.W.

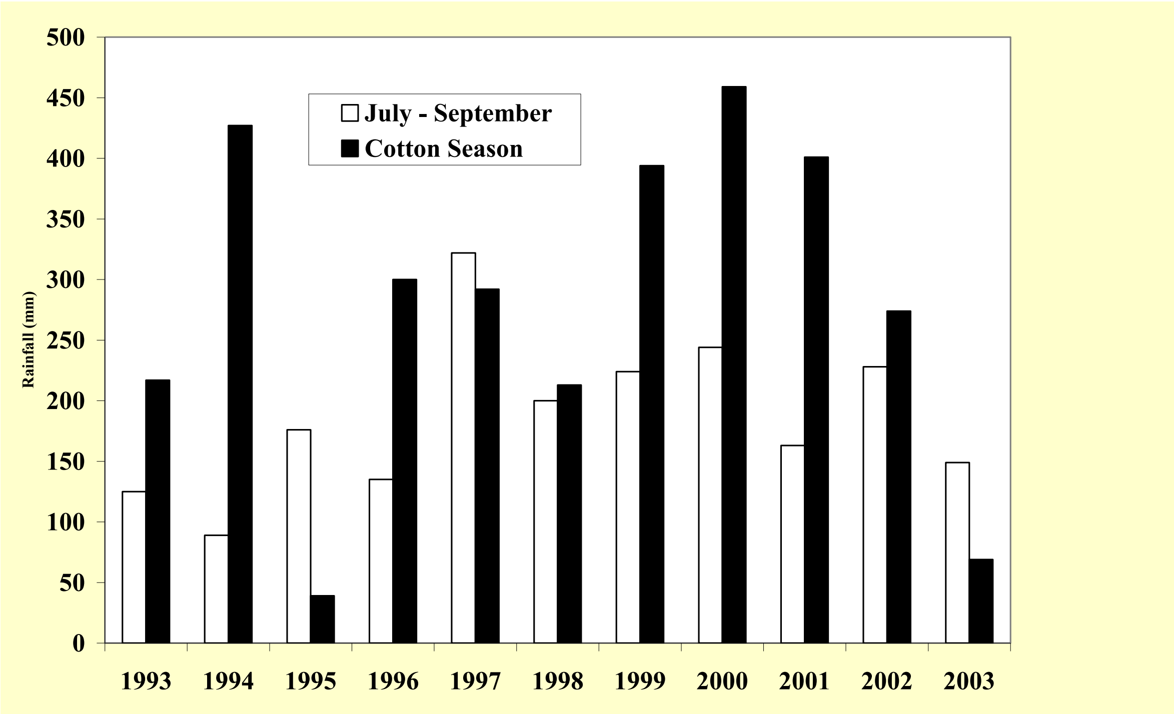

Fig. 18. Rainfalls recorded at ACRI, Narrabri in the winter months preceding each cotton season and during the cotton growing seasons (taken as October – April). Year is that of the harvest.

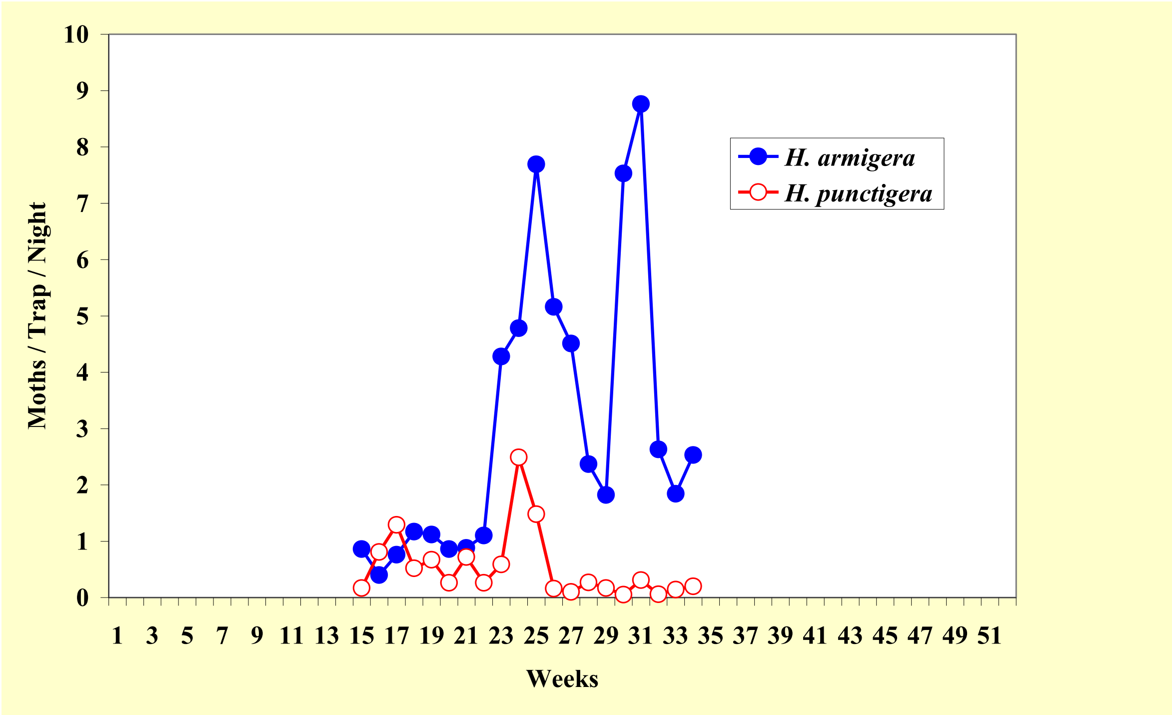

Fig. 19. Pheromone trap catches for *H. armigera* and *H. punctigera* near St George, 2000-01.

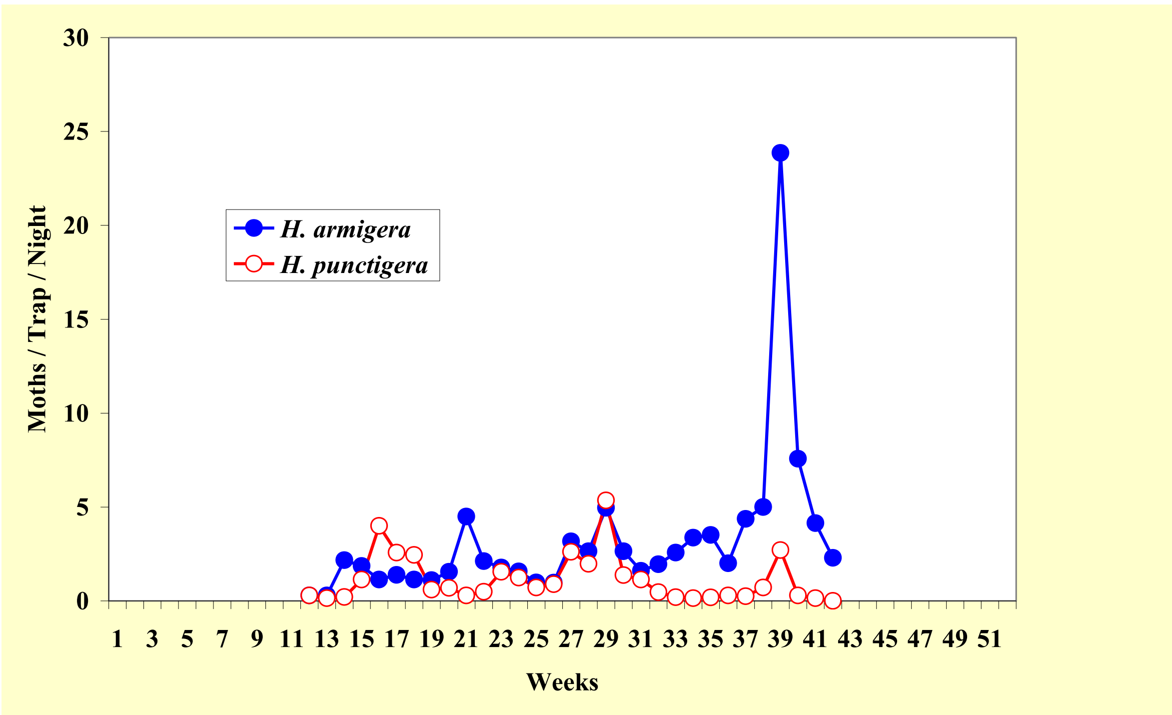

Fig. 20. Pheromone trap catches for *H. armigera* and *H. punctigera* near St George, 2001-02.

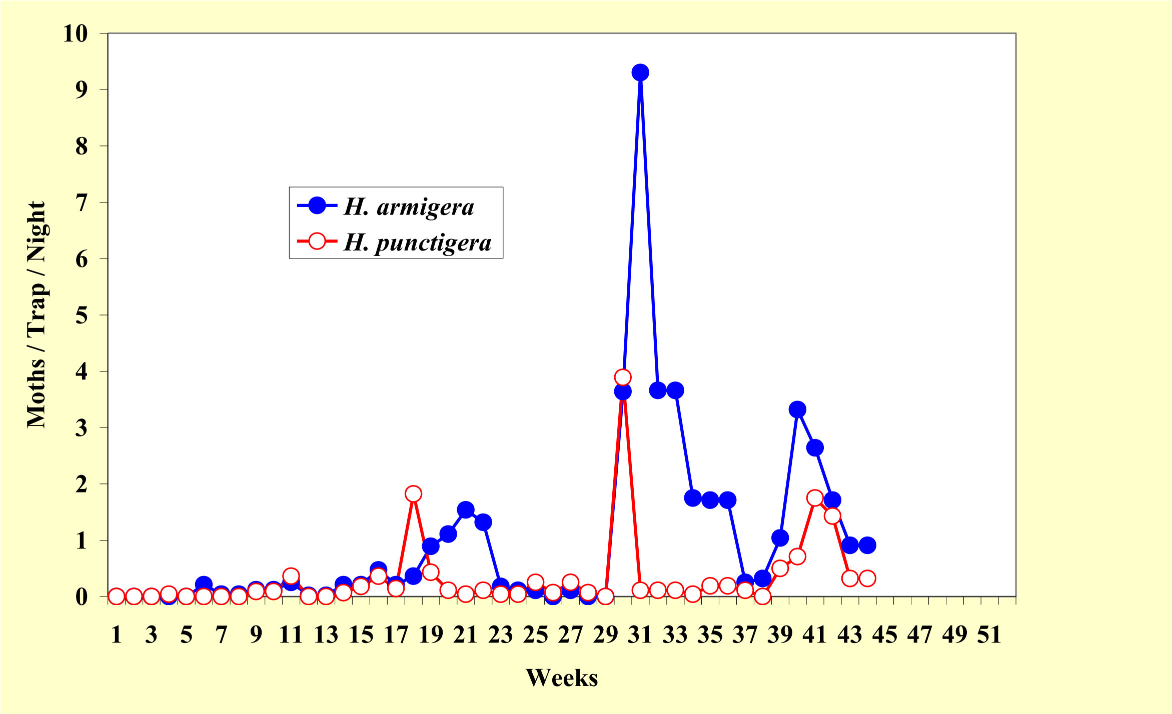

Fig. 21. Pheromone trap catches for *H. armigera* and *H. punctigera* near St George, 2002-03.

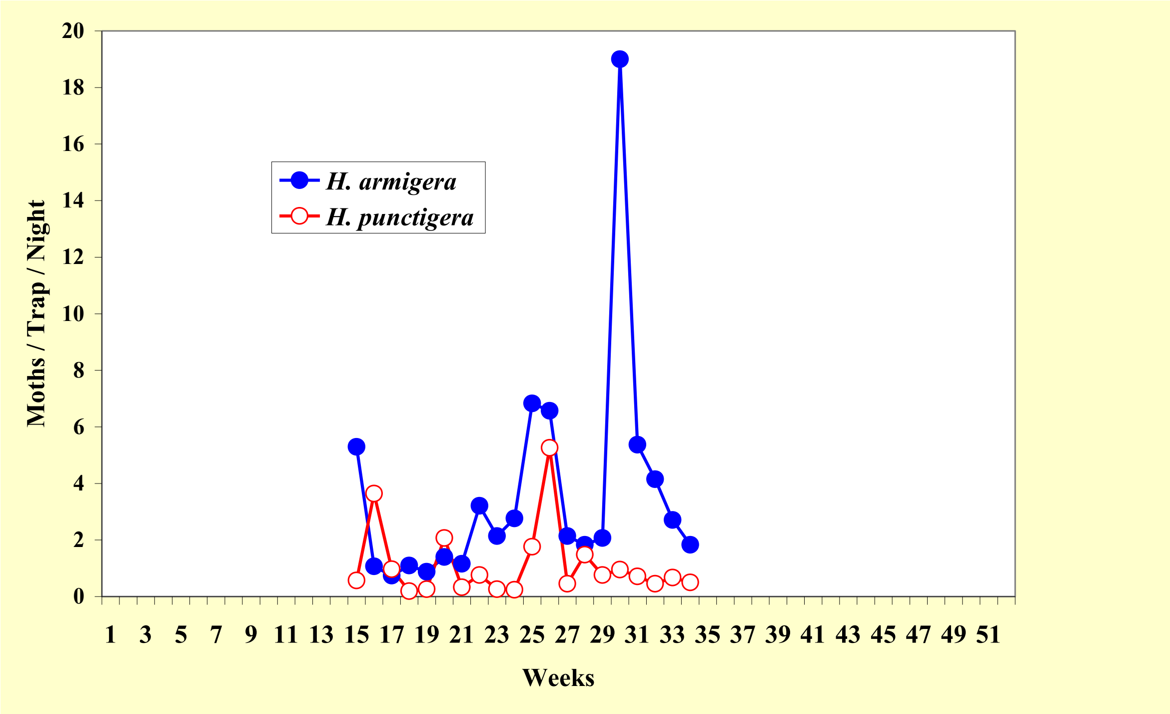

Fig. 22. Pheromone trap catches for *H. armigera* and *H. punctigera* near Dirranbandi, 2000-01.

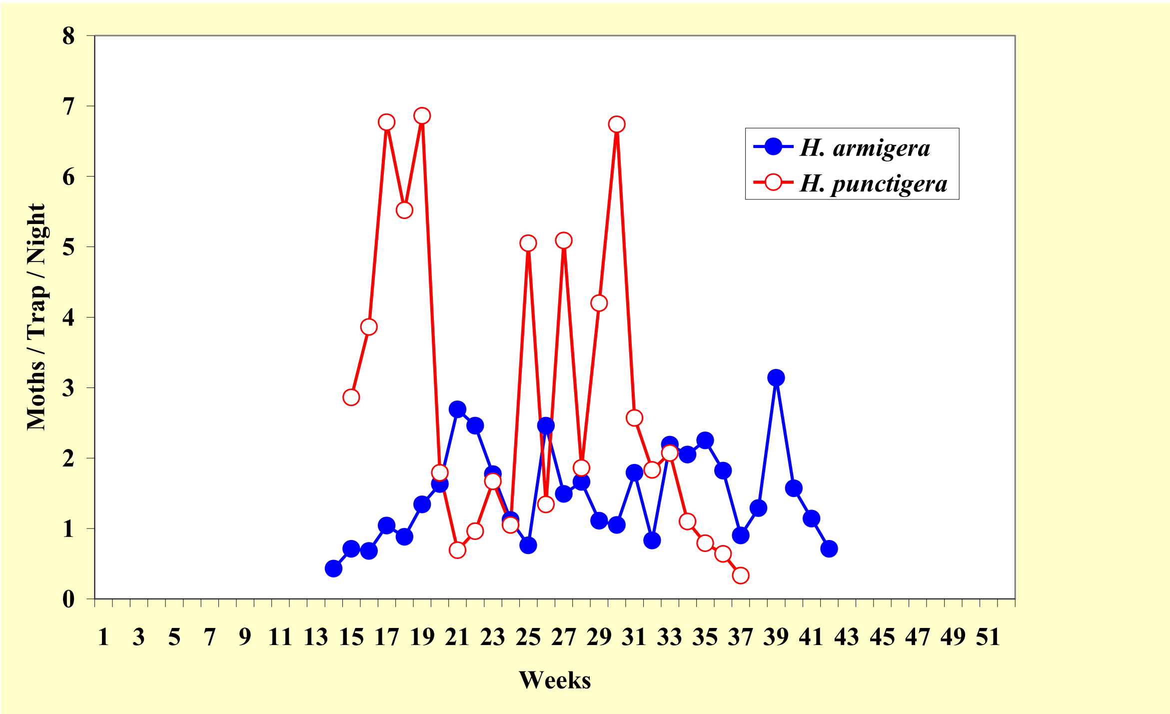

Fig. 23. Pheromone trap catches for *H. armigera* and *H. punctigera* near Dirranbandi, 2001-02.

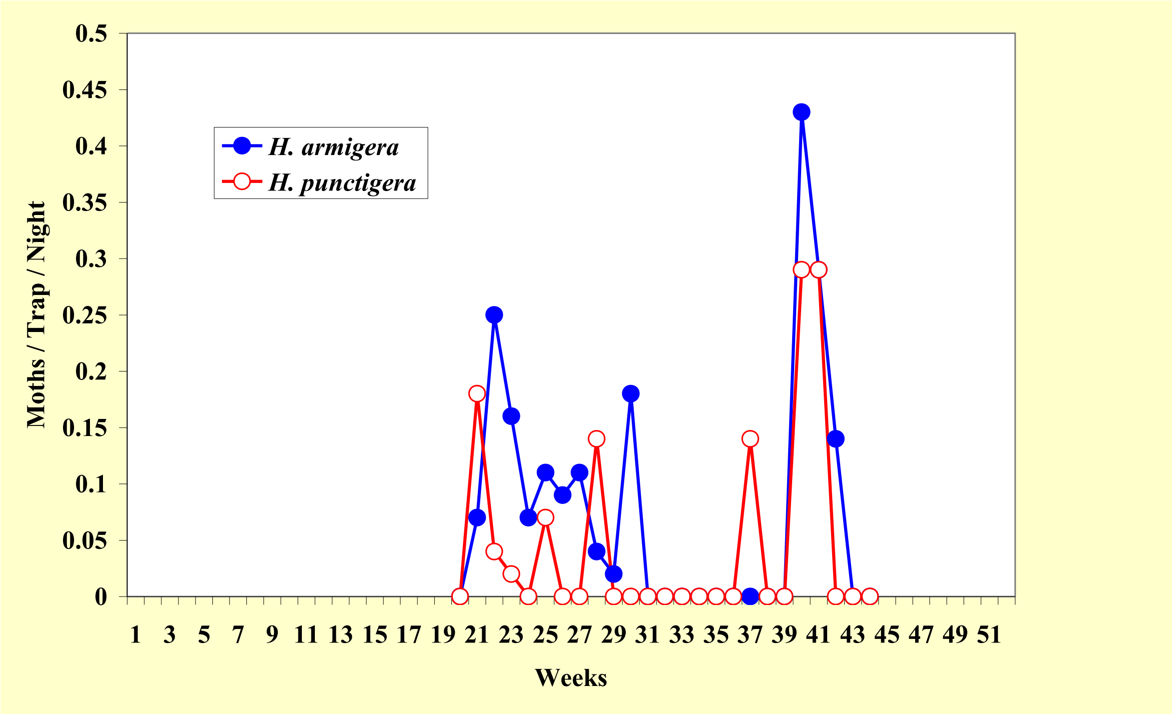

Fig. 24. Pheromone trap catches for *H. armigera* and *H. punctigera* near Dirranbandi, 2002-03.

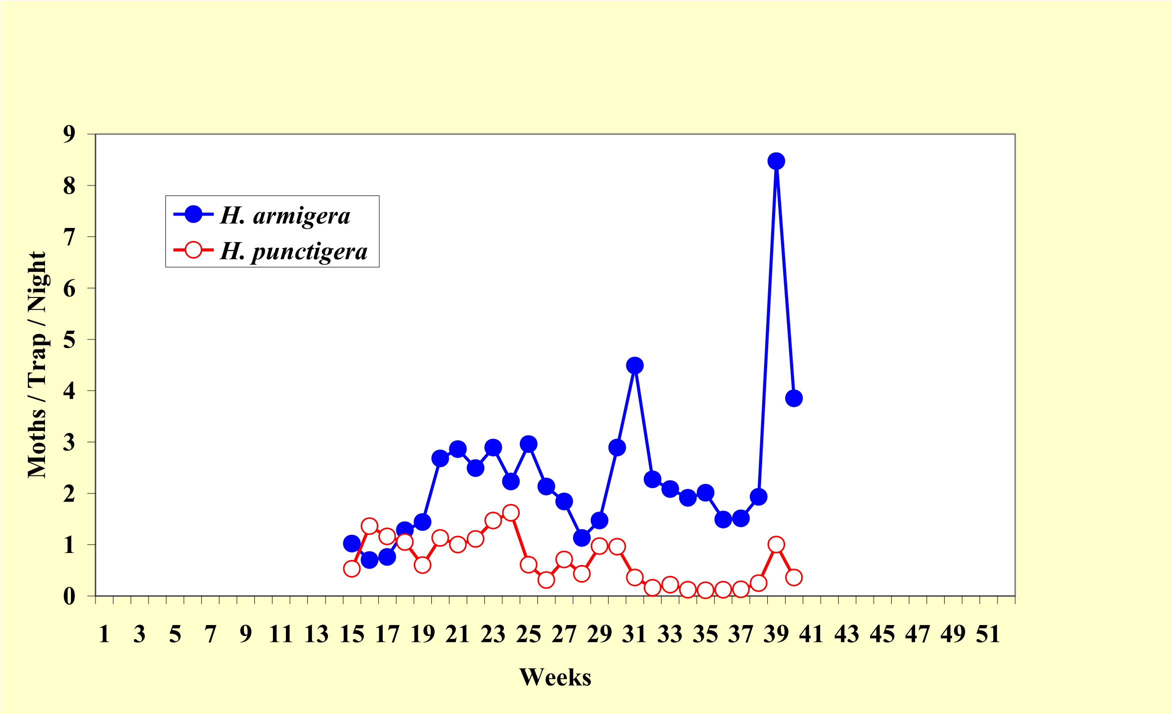

Fig. 25. Average pheromone trap catches for *H. armigera* and *H. punctigera* near St George for 1997-98 to 2002-03.

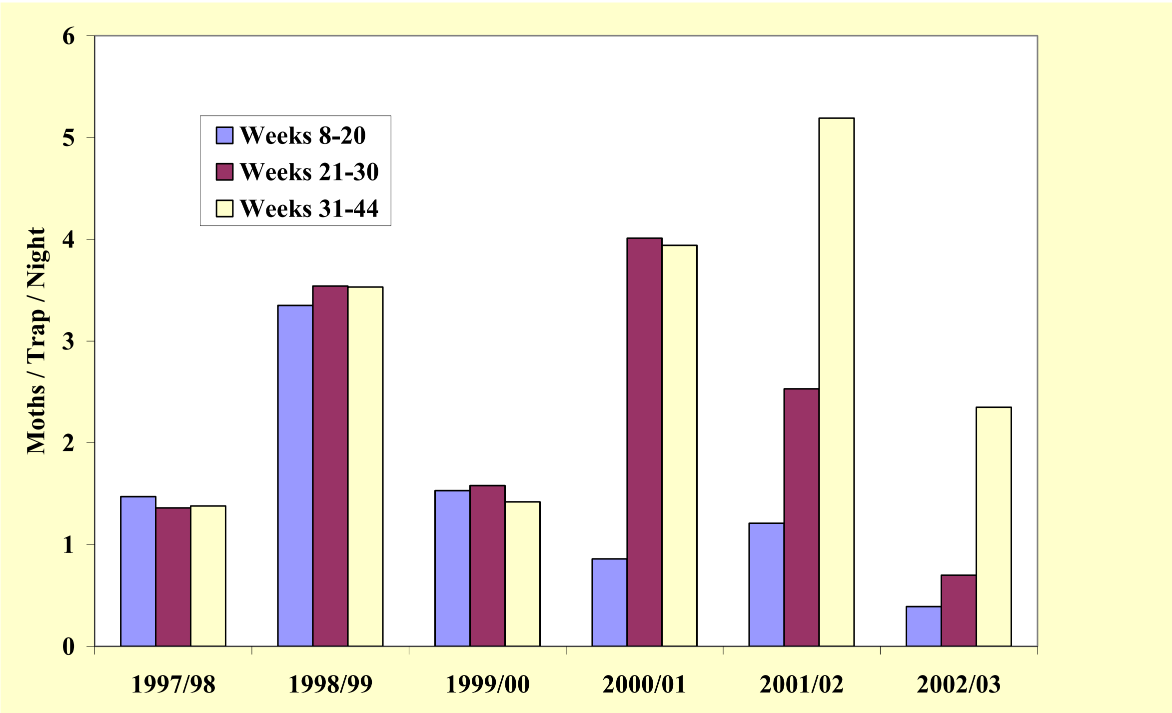

Fig. 26. Average pheromone trap catches for *H. armigera* near St George from 1997-98 to 2002-03. Data separated into clusters of 8-20, 21-30 and 31-44 weeks since July 1 in each year.

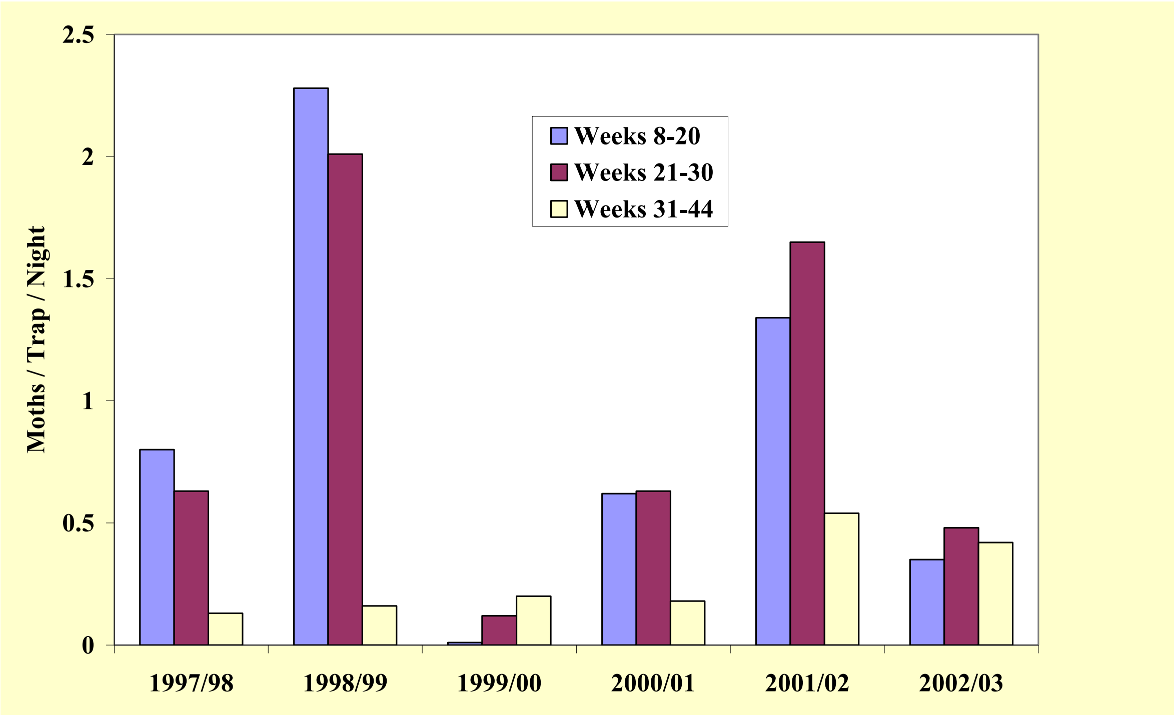

Fig. 27. Average pheromone trap catches for *H. punctigera* near St George from 1997-98 to 2002-03. Data separated into clusters of 8-20, 21-30 and 31-44 weeks since July 1 in each year.

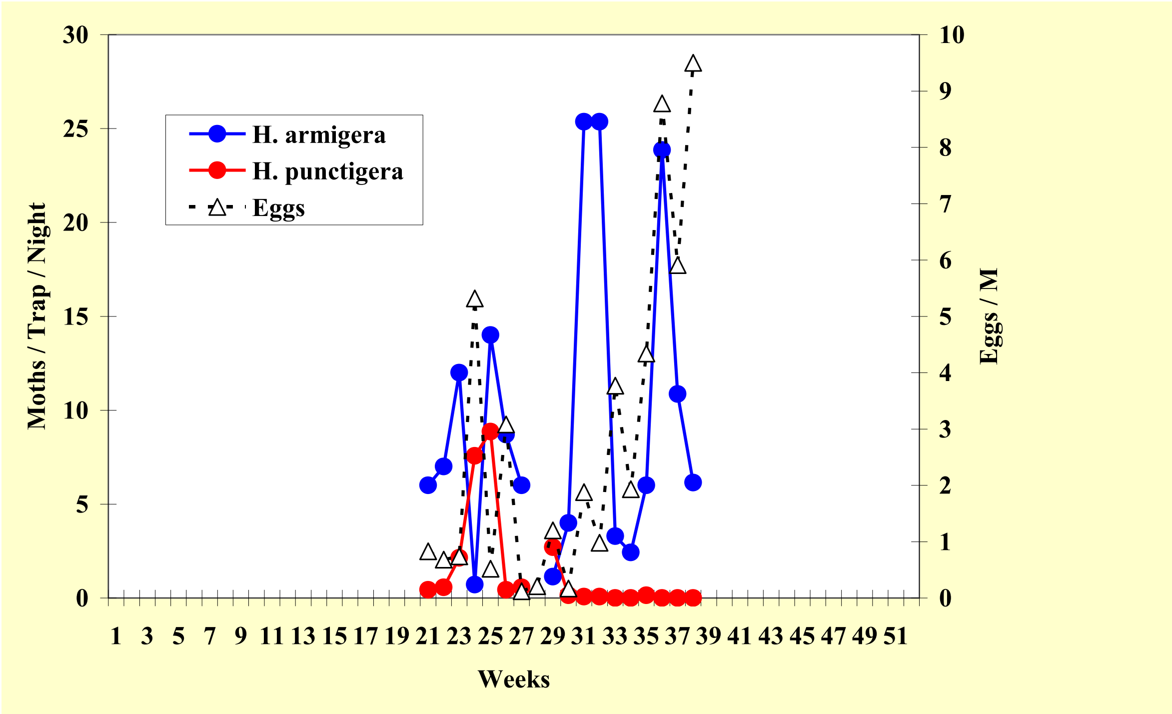

Fig. 28. Average numbers of *H. armigera* and *H. punctigera* caught in pheromone traps and *Helicoverpa* eggs collected from cotton in Chico Block, ACRI, Narrabri during the 1998-99 season.

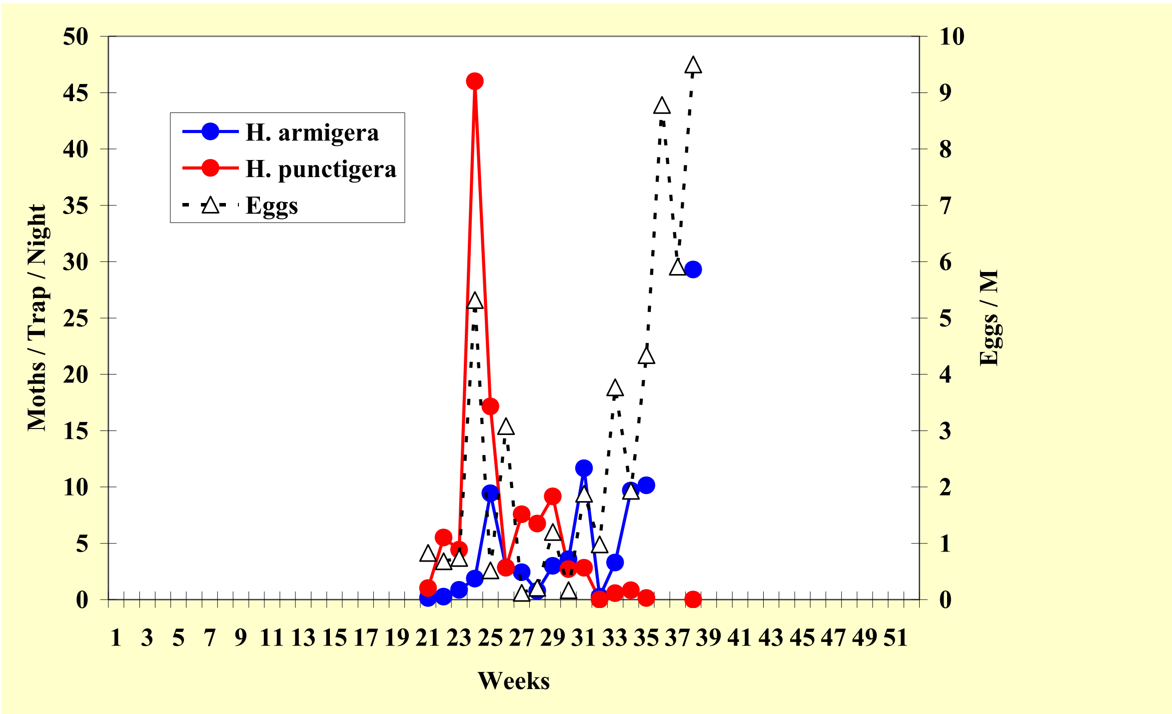

Fig. 29. Average numbers of *H. armigera* and *H. punctigera* caught in light traps and *Helicoverpa* eggs collected from cotton in Chico Block, ACRI, Narrabri during the 1998-99 season.

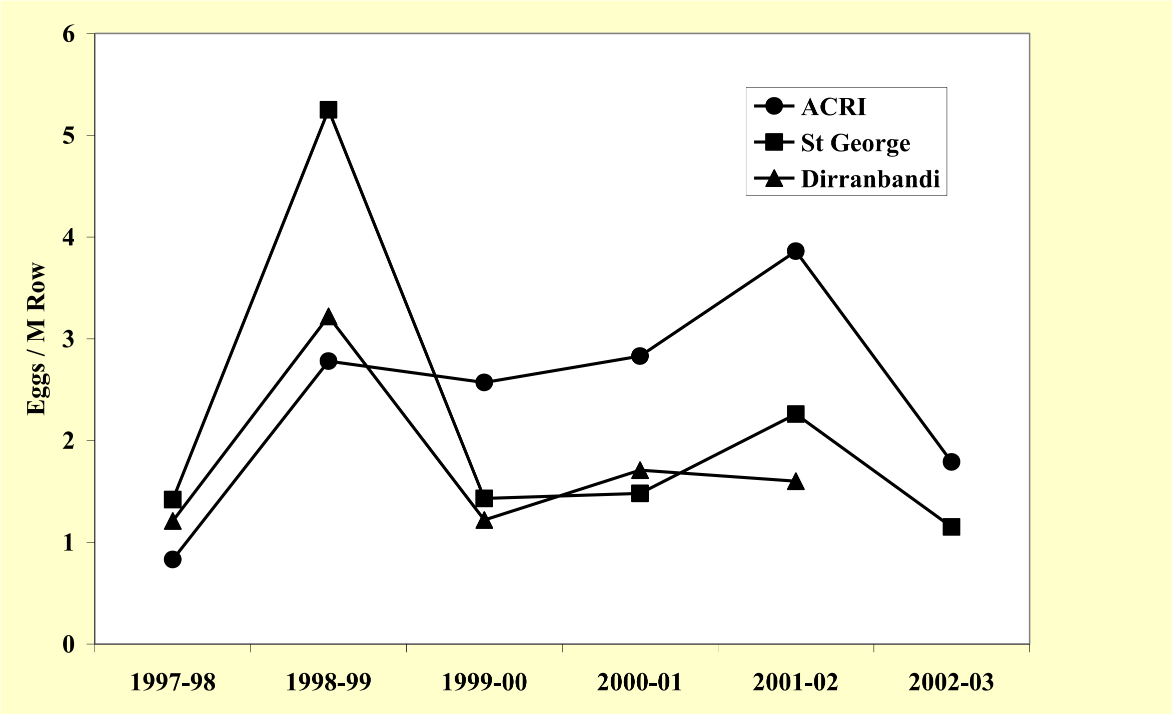

Fig. 30. Abundance of *Helicoverpa* eggs on cotton crops at ACRI (Chico Block), Narrabri and near St George and Dirranbandi from 1997-98 to 2002-03.

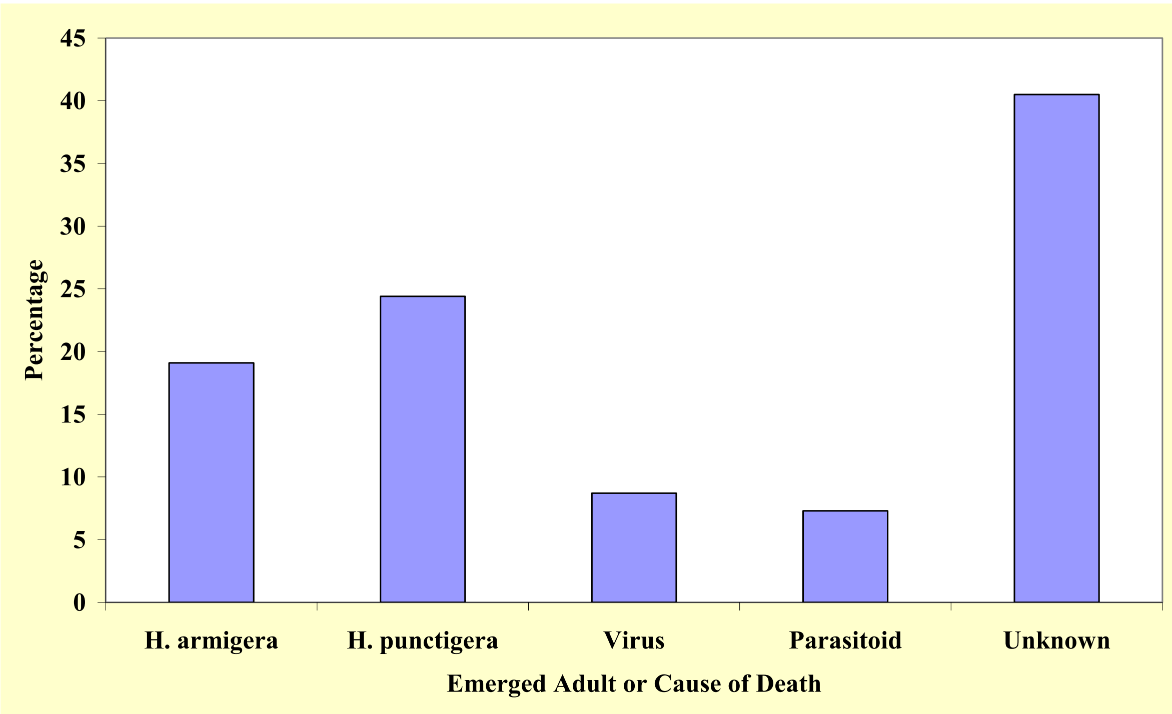

Fig. 31. Rearing outcomes in the laboratory from field collected *Helicoverpa* eggs and larvae (1,937 sites, 1993-94 to 2002-03). Data are expressed as a % of the rearings.

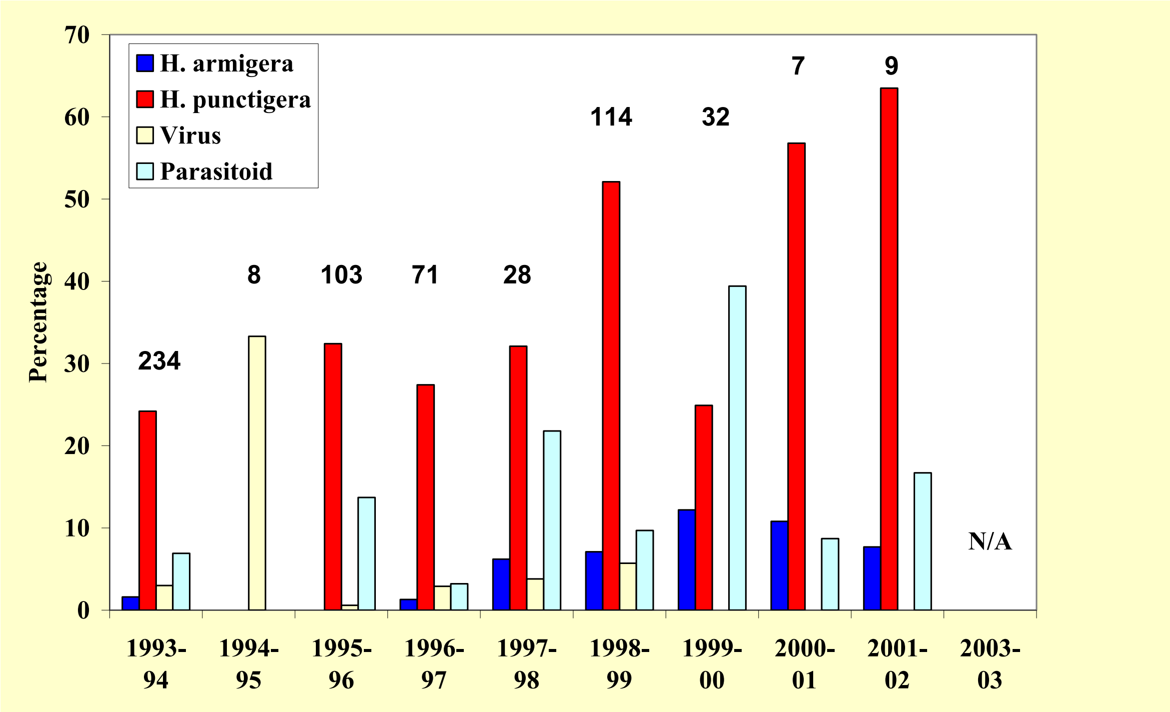

Fig. 32. Rearing outcomes in the laboratory from field collected *Helicoverpa* eggs and larvae (1993-94 to 2002-03) in winter-spring (June – November) on weeds and native vegetation. Numbers above histograms indicate sites surveyed in a particular year. Data are expressed as a % of the rearings. Deaths from unknown causes are not shown.

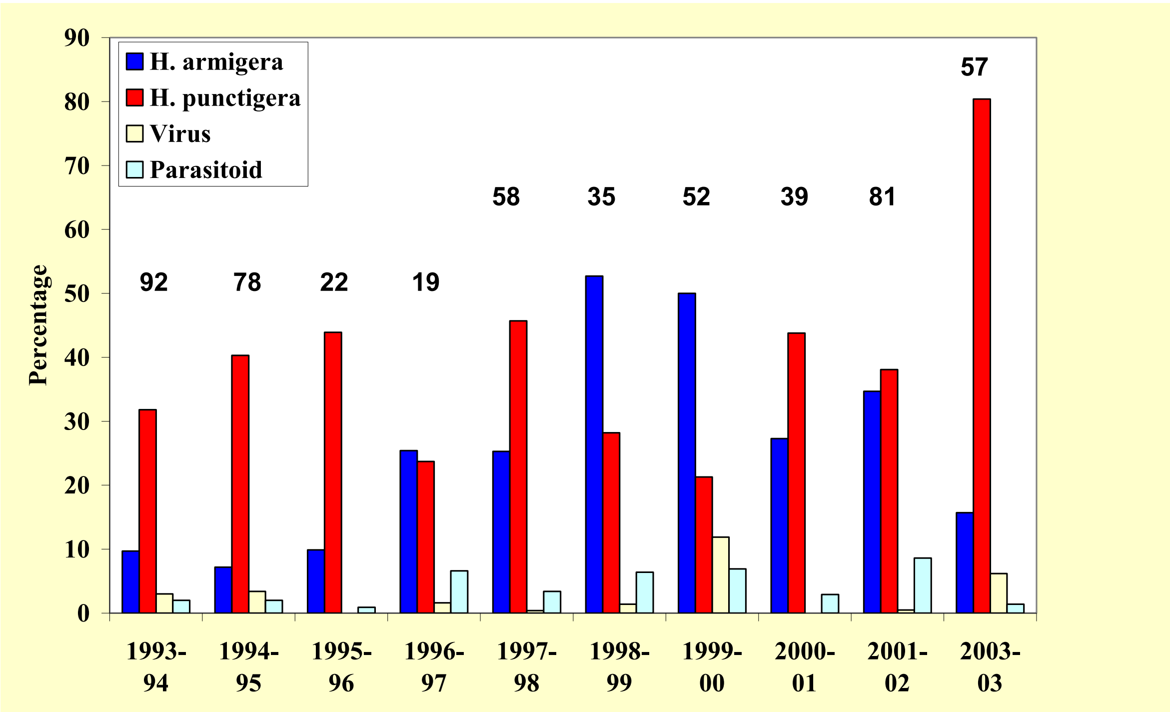

Fig. 33. Rearing outcomes in the laboratory from field collected *Helicoverpa* eggs and larvae (1993-94 to 2002-03) in winter-spring (June - November) on crops other than cotton. Numbers above histograms indicate sites surveyed in a particular year. Data are expressed as a % of the rearings. Deaths from unknown causes are not shown.

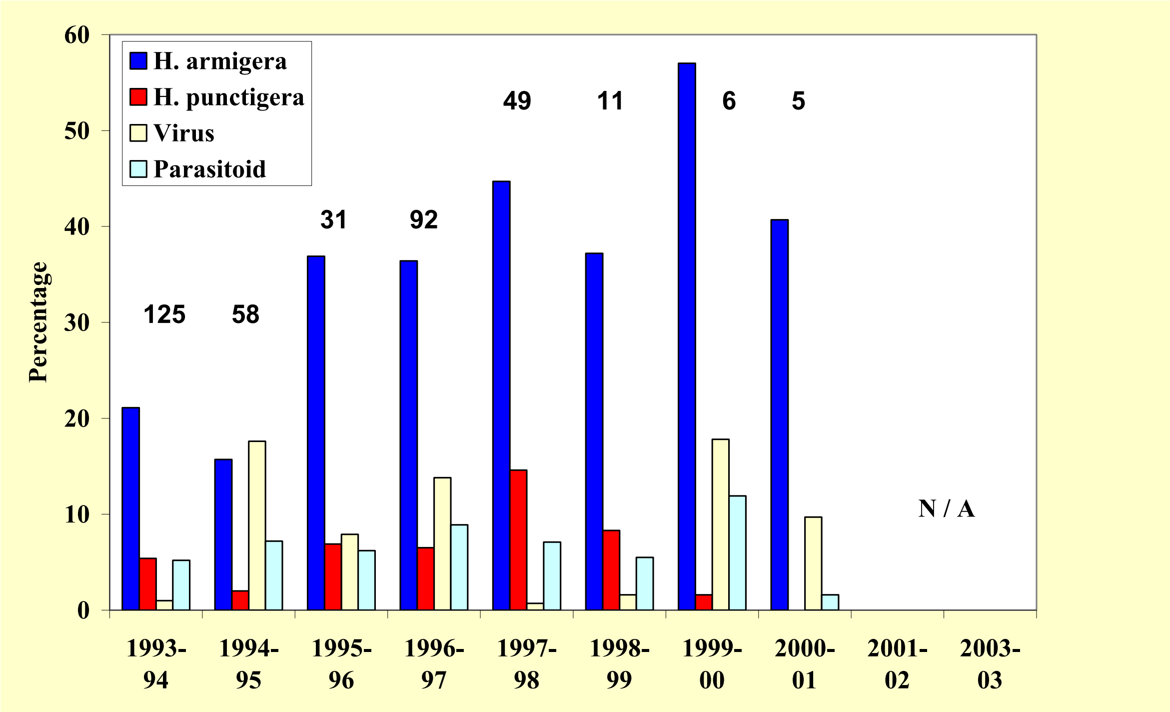

Fig. 34. Rearing outcomes in the laboratory from field collected *Helicoverpa* eggs and larvae (1993-94 to 2002-03) in summer-autumn (December – May) on crops other than cotton or Ingard cotton refuges. Numbers above histograms indicate sites surveyed in a particular year. Data are expressed as a % of the rearings. Deaths from unknown causes are not shown.

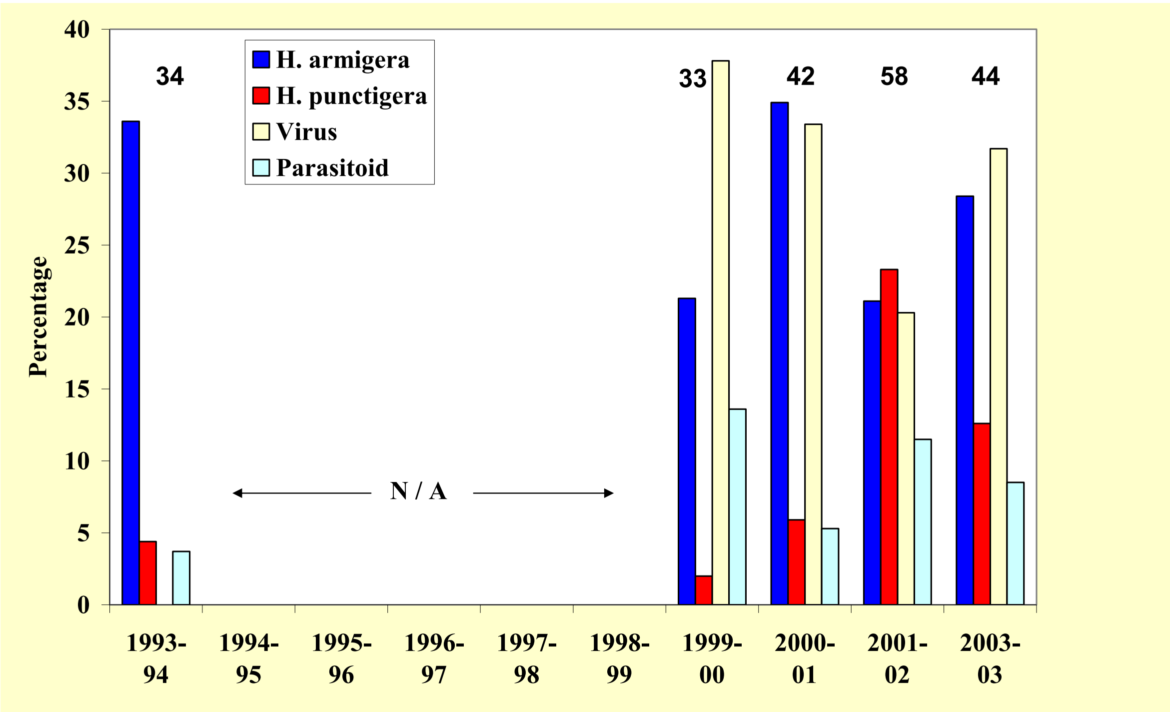

Fig. 35. Rearing outcomes in the laboratory from field collected *Helicoverpa* eggs and larvae (1993-94 to 2002-03) in summer-autumn (December – May) on unsprayed Ingard refuge crops (mostly pigeon pea and sorghum). Numbers above histograms indicate sites surveyed in a particular year. Data are expressed as a % of the rearings. Deaths from unknown causes are not shown.

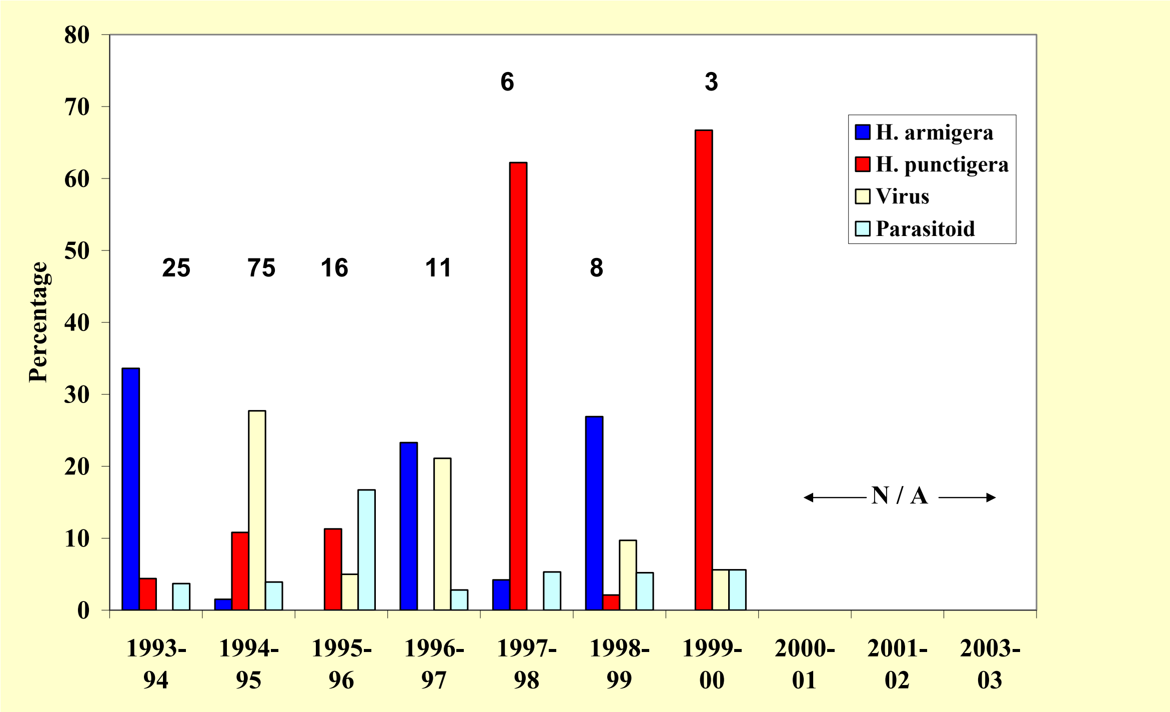

Fig. 36. Rearing outcomes in the laboratory from field collected *Helicoverpa* eggs and larvae (1993-94 to 2002-03) in summer-autumn (December – May) on weeds and native vegetation. Numbers above histograms indicate sites surveyed in a particular year. Data are expressed as a % of the rearings. Deaths from unknown causes are not shown.

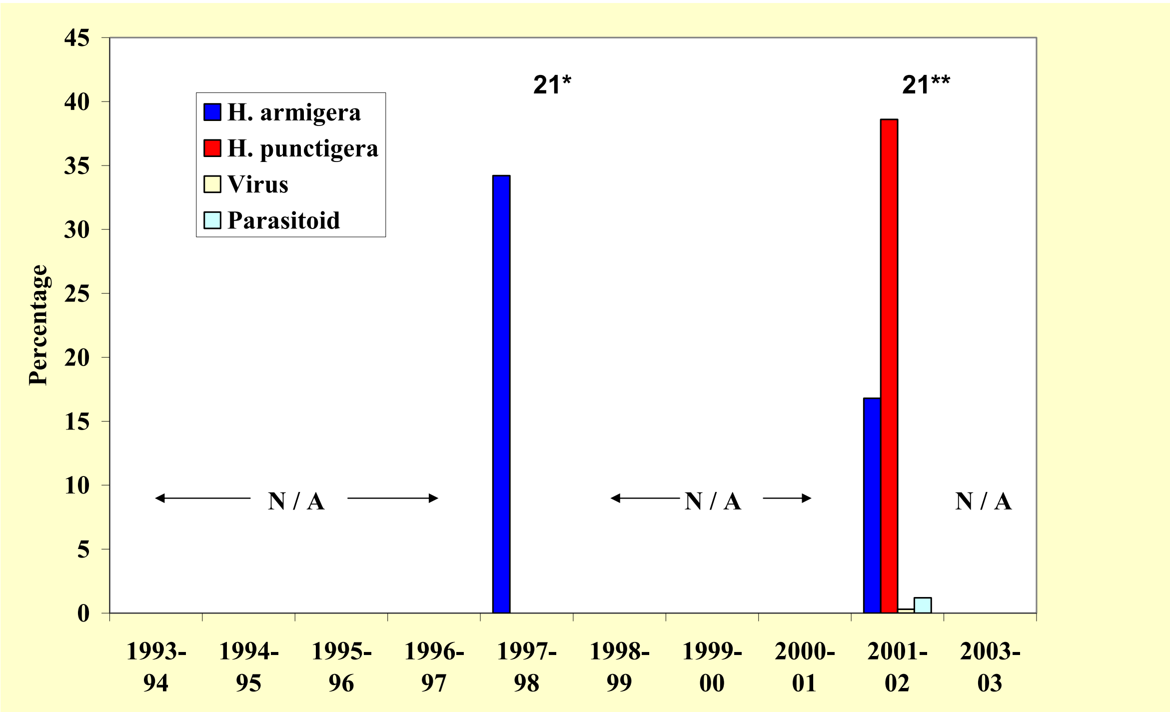

Fig. 37. Rearing outcomes in the laboratory from field collected *Helicoverpa* eggs and larvae (1993-94 to 2002-03) in summer-autumn (December – May) on conventional* and Ingard** cotton crops. Numbers above histograms indicate sites surveyed in a particular year. Data are expressed as a % of the rearings. Deaths from unknown causes are not shown.

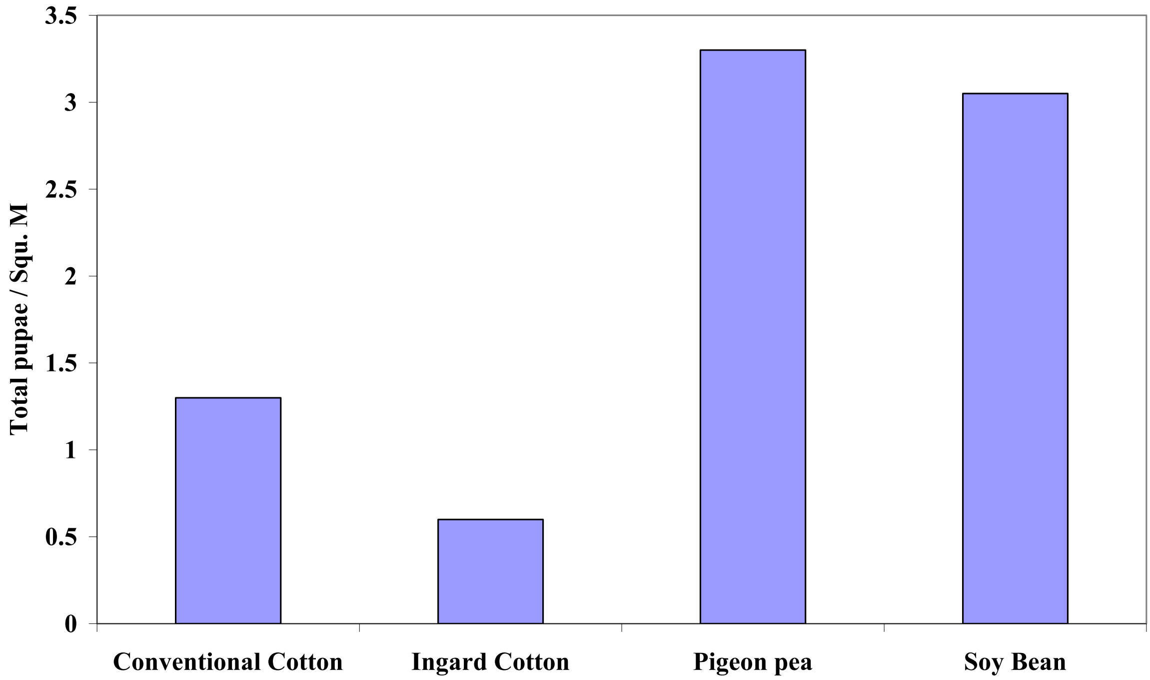

Fig. 38. Abundance of total *Helicoverpa* pupae (averaged across five sampling dates) under various crops at Maules Ck, 2001.

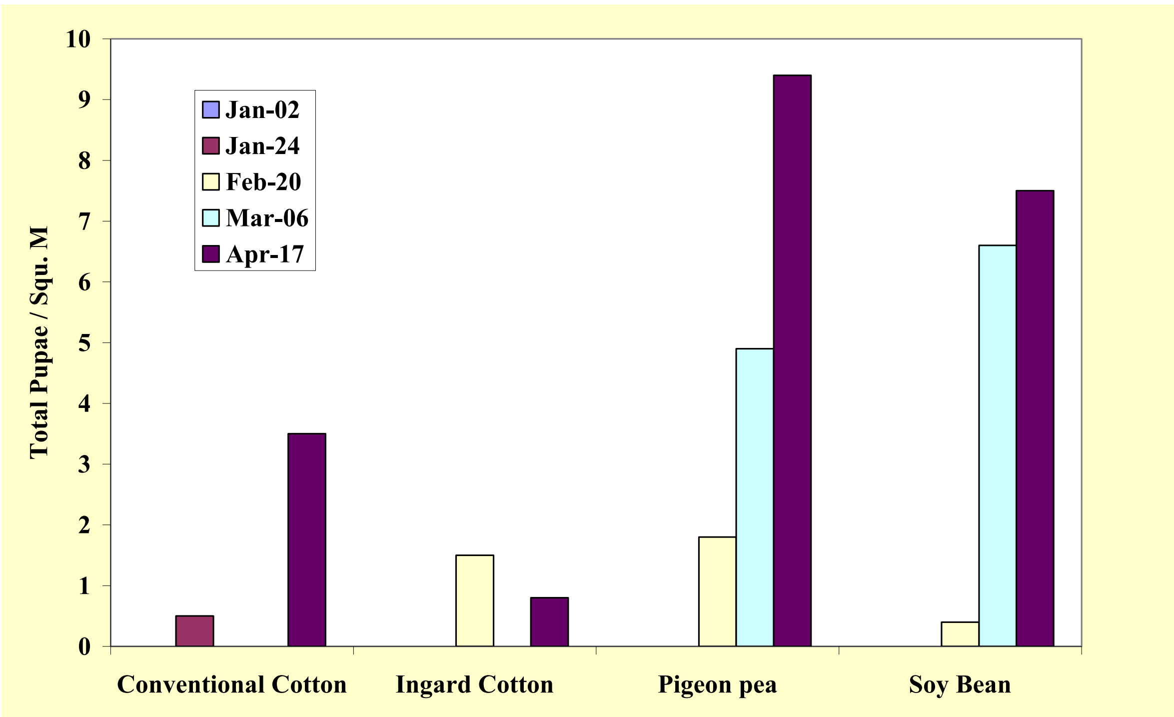

Fig. 39. Abundance of total *Helicoverpa* pupae under various crops at Maules Ck on five sampling dates.

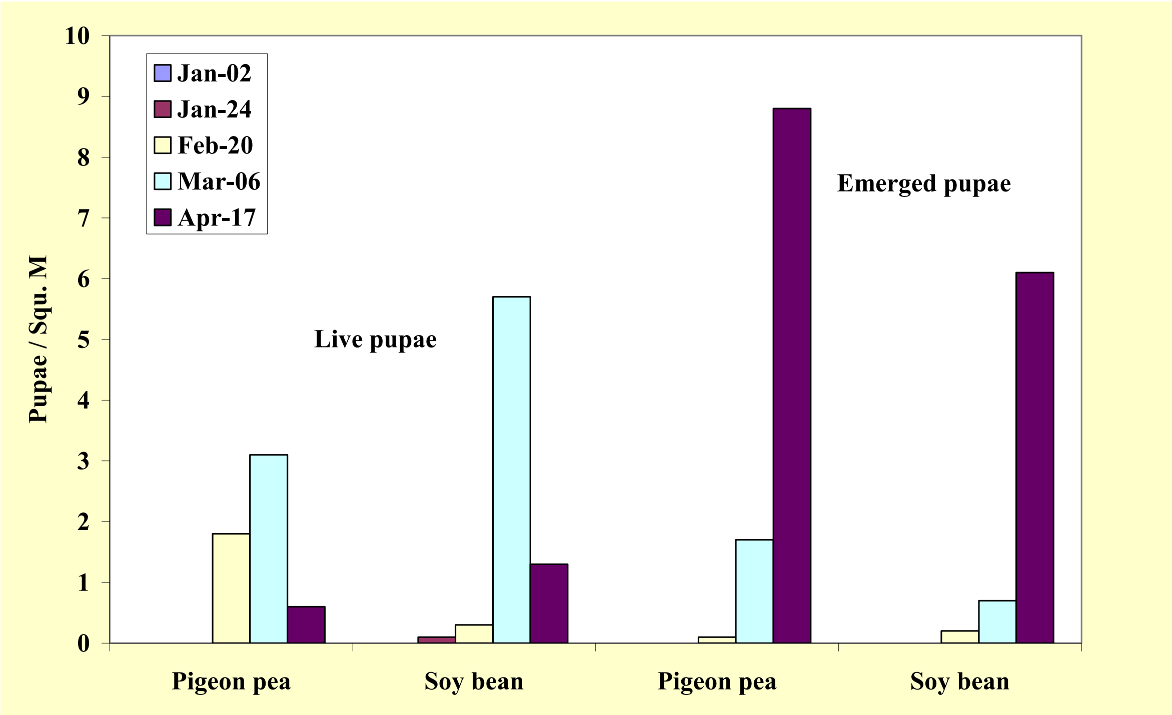

Fig. 40. Abundance of live and emerged *Helicoverpa* pupae under pigeon pea and soy bean at Maules Ck on five sampling dates.

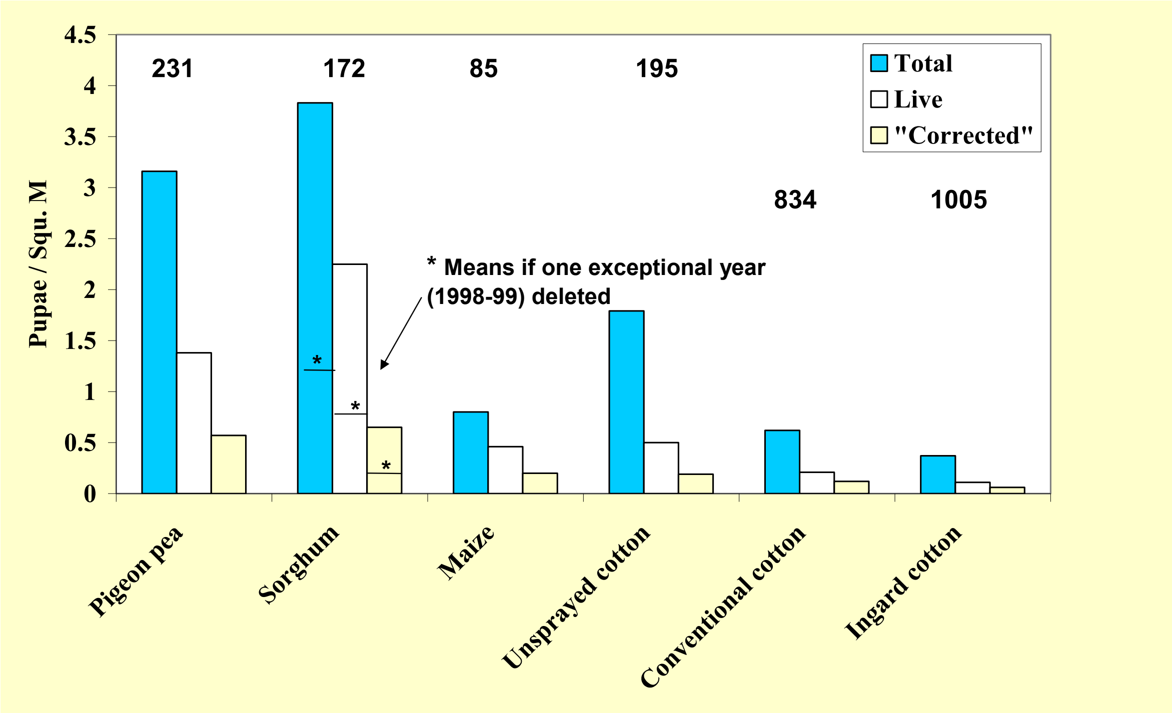

Fig. 41. Average numbers of total, live and "corrected" live (for *H. armigera*) *Helicoverpa* pupae collected from soil under Ingard cotton and various refuge crops across all valleys (1996-2003). Numbers above histogram clusters indicate sampling visits for each crop. For further explanation of "corrected" see text.

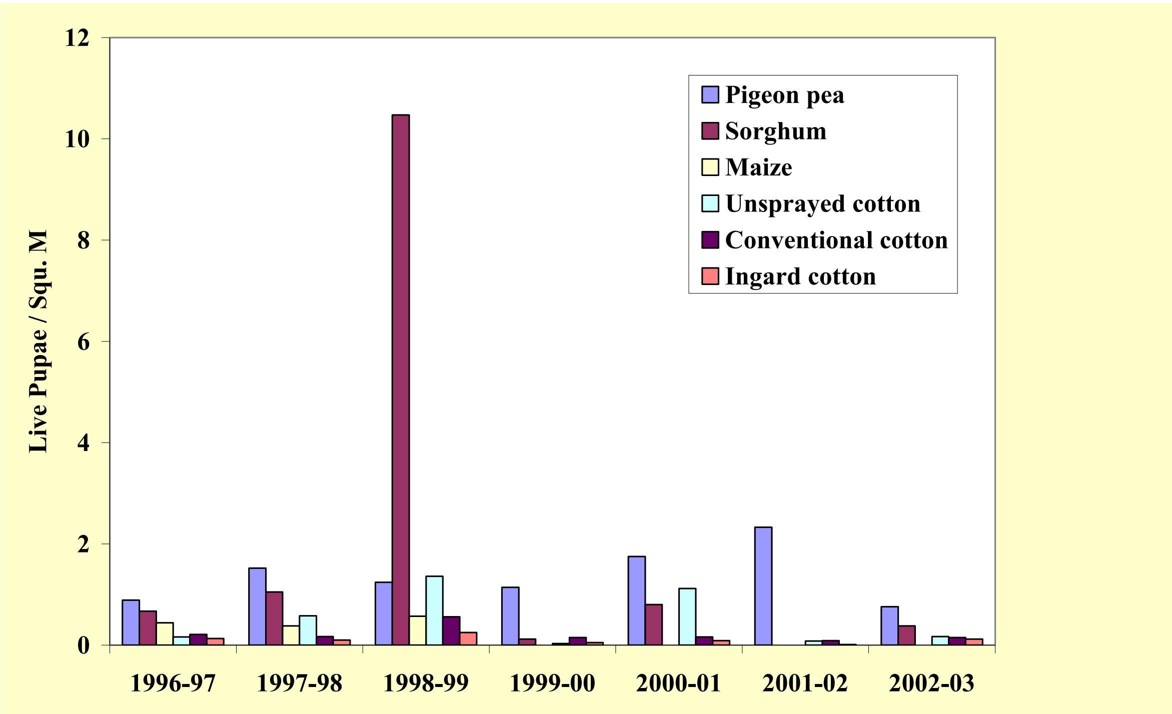

Fig. 42. Average numbers of live *Helicoverpa* pupae collected from soil under Ingard cotton and various refuge crops across all valleys in individual years. N/A = not sampled.

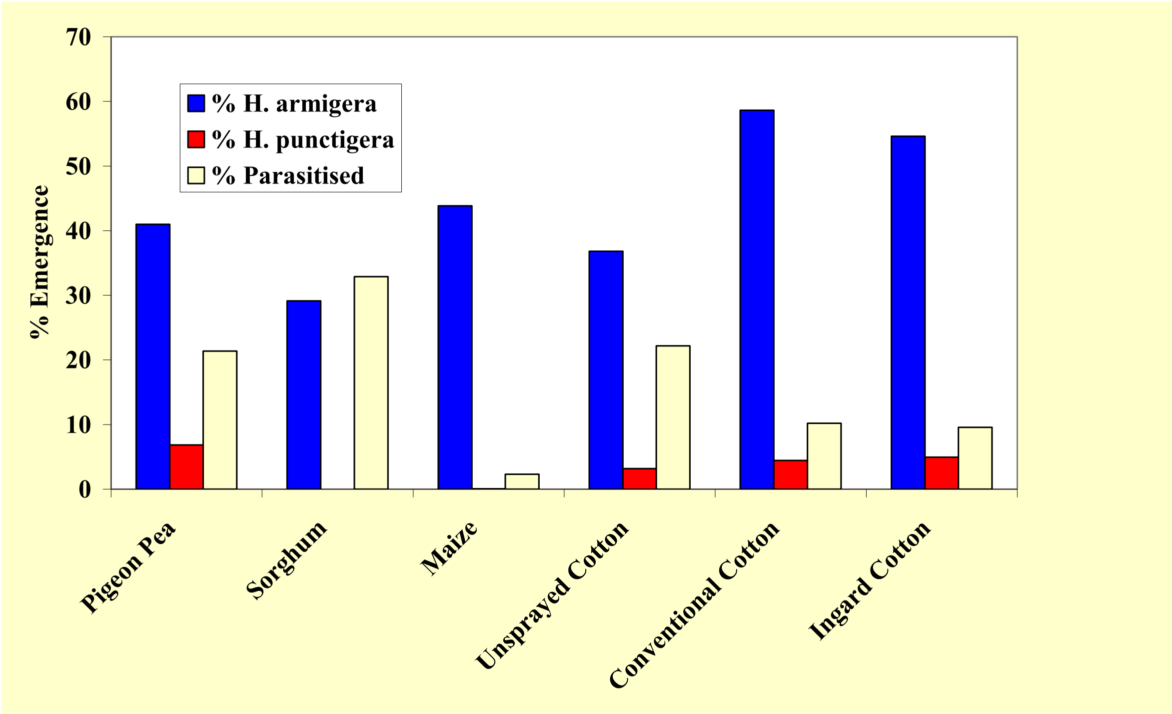

Fig. 43. Rearing outcomes in the laboratory from field collected (live) *Helicoverpa* pupae (1996-2003) from all valleys, according to crop sampled. Deaths from unknown causes are not shown.

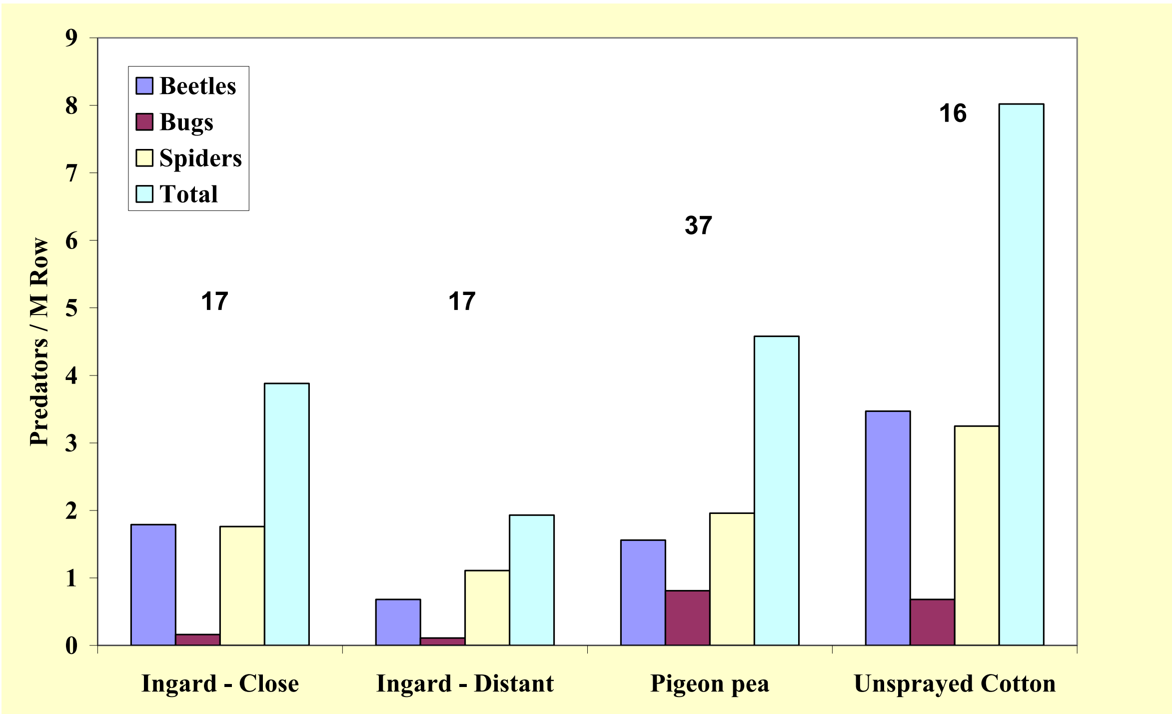

Fig. 44. Average numbers of predatory invertebrates recorded during visual checks in pigeon pea and unsprayed cotton refuges and Ingard cotton crops during the 2001-02 season. Numbers above histograms indicate numbers of field visits.

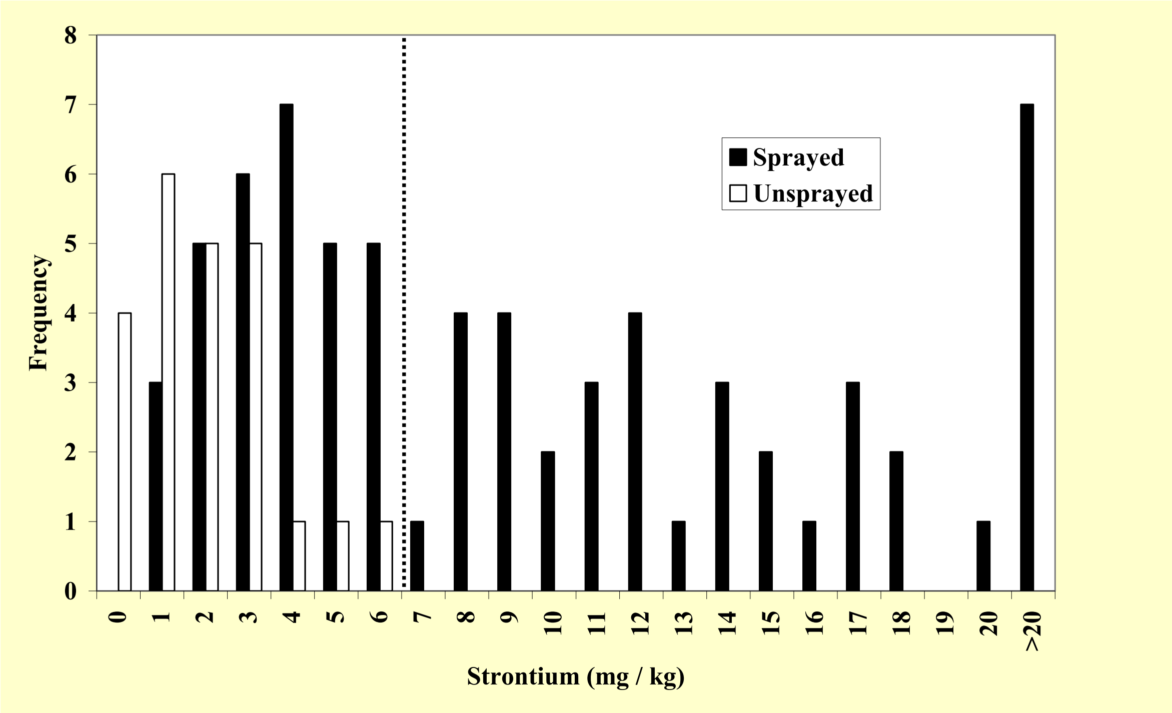

Fig. 45. Frequency of *H. armigera* moths with varying levels of strontium emerging from sprayed and unsprayed sections of the pigeon pea refuge near Warren, March 2001.

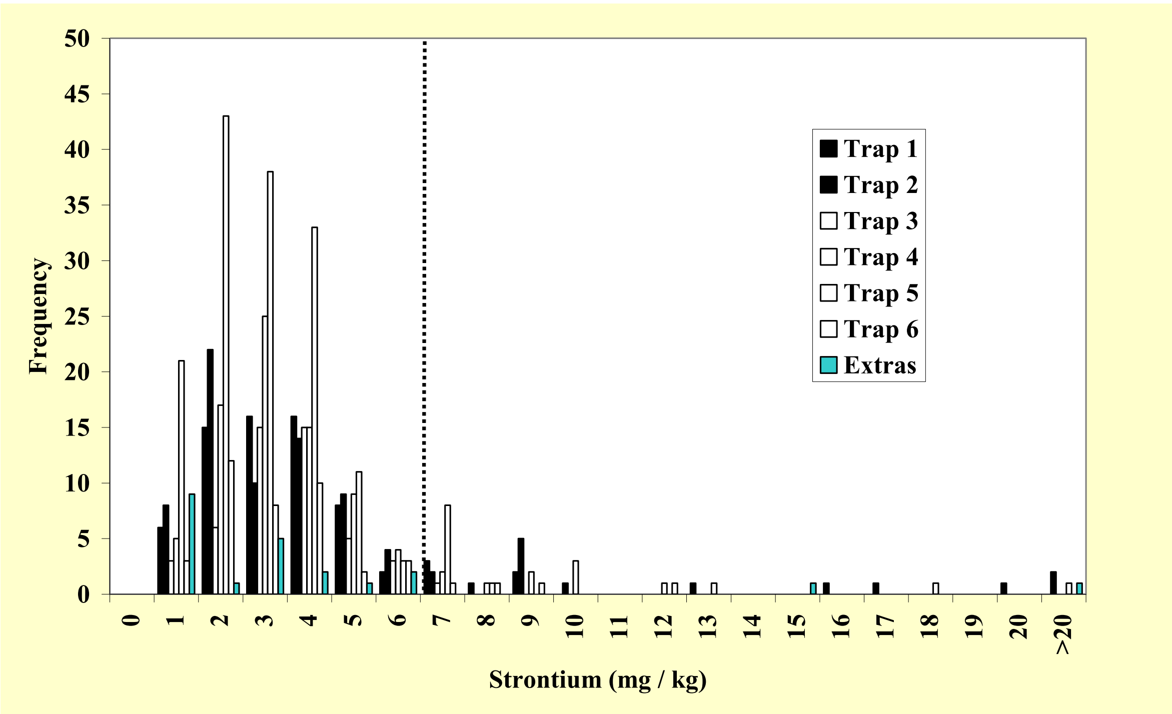

Fig. 46. Frequency of *H. armigera* moths with varying levels of strontium collected from light traps set in pigeon pea refuge (Traps 1 & 2) and Ingard cotton (Traps 3-6) near Warren, March 2001. Extra moths collected by hand in the pigeon pea refuge are also included.

5. Conclusions – Take Home messages

• Long term changes in the abundance of *H. armigera* are apparent, but mechanisms controlling these are poorly understood.

• Commonly used methods for assessing abundance of *Helicoverpa* moths (pheromone and light traps) provide conflicting data.

• Weeds and natural vegetation in winter- spring and unsprayed refuges in summer - autumn can provide significant sources of parasitoids of *Helicoverpa*.

• Unsprayed refuges generally produced many more pupae than conventional sprayed and Ingard cotton (see Results for relative productions). Pigeon pea was clearly the most reliable and productive refuge option. Parasitism can greatly reduce refuge performance, but obviously brings other benefits.

• Predatory invertebrates appear to be rarer well within Ingard cotton crops compared with the edges of such crops and adjacent refuges. This pattern may be a response to invasive movements. Care should be taken in determining the spatial scale of field trials to assess non-target effects of Bt cottons – to allow for significant edge effects.

• Substantial soil disturbance may be required to destroy the majority of over-wintering *Helicoverpa* pupae – but data generated in this study were not significant in this regard.

• Moths from refuges can be found within Ingard cotton crops, but the data supporting this assertion are based on one trial and the jury is still out on whether or not these refuge-derived moths effectively mate there with locally-produced (resistant ?) moths.

6. Research in Relation to CRDC's 3 Outputs : Economic, Environmental & Social

This research has direct relevance to the sustainability and profitability of the Australian cotton industry through monitoring of shifts in the abundance of major insect pests (*Helicoverpa* spp), scrutiny of the effectiveness of refuge strategies in limiting the development of resistance to Bt, and furthering understanding of the influence of refuges in the dynamics of beneficial species. Such research underpins the use of transgenic cotton as a major tool to limit economic damage, reduce pesticide use and utilise beneficial species more in IPM.

7. Summary

a) Technical Advances

The major technical result of a commercial nature to arise from this work has been the identification of the relative merits of various crops as refuges for use with Bt cottons and confirmation of pigeon pea as the most reliable and productive option.

b) Other Information Developed

The research has provided insight into the relative use of common monitoring tools (pheromone and light traps) for *Helicoverpa* and initiated the use of marking methods to follow movements of moths between refuges and Bt cotton crops. Some preliminary observations of the distribution of beneficial species in Bt cotton crops have relevance to the spatial scale of future studies assessing the influence of such crops on non-target fauna. Attempts to identify optimal tillage implements for "pupae busting" in winter were limited by low and patchy pupal abundance at the trial site.

c) Changes to Intellectual Property Register

None required.

8. Future Plans

a) Further Development of Project Technology

In the new project (see below), focus will be on demonstrations of moth movements between variously placed refuges and Bt crops, and effective mating between such populations using strontium marking, carbon isotope analyses, micro-satellite DNA signatures, spermatophore counts and sperm precedence studies (to determine which mating amongst several is most important).

b) Presentation and Dissemination of Project Outcomes

A major focus of this project has been to firstly organise, analyse and document data sets that have accumulated through ongoing research at ACRI for many years. We now intend to begin preparing scientific manuscripts based on the data presented here in the next few months.

Findings from the research will be further extended to industry through participation in field days, talks to farmer groups (e,g, AWM), articles in grower magazines & booklets (e.g. Aust Cotton Grower, CSD Variety Trial Results) and presentations and posters at workshops (e.g. for IDO's) and conferences (e.g. Aust. Cotton Conf). We intend to present research results in novel fora for the cotton industry (e.g. Aust. Ecological Soc. Annual Conf, Dec 2003). Scientific papers will be submitted to internationally recognised journals. We anticipate the project's findings will be used in industry reviews of IPM and resistance management (e.g. TIMS).

c) Future Research

A new project (A004 : Ecology of *Helicoverpa* in relation to transgenic cotton and the efficiency of refuge crops) began in 2003. The primary objectives of this project are to :

(i) monitor temporal changes in the abundance of *Helicoverpa armigera* and *H. punctigera* in the Namoi and St George regions through continued pheromone trapping and egg and larval sampling on various host plants.

(ii) determine the productivity and potential for effective mating between moths generated in variously placed refuges and nearby transgenic crops.

This new project is thus a natural progression from the results generated in CSE90C.

9. Publications Arising and Planned

Trapping data were regularly updated and displayed on the Australian Cotton CRC web site and are well used by cotton consultants and agronomists. Project staff addressed various workshops, field days and research reviews involving cotton consultants, IDOs, AWM groups and other scientists on topics such as early season abundance of *Helicoverpa*, pupal control and Ingard refuges studies (e.g. in the Namoi, Macintyre, St George, Walgett regions). Articles were published in regional cotton year books (e.g. Macintyre, St George). An article was published in the "Australian Cotton Grower" (Tann, C. Fitt, G, & Baker, G. (2002). Selecting the right refuges for Bt cotton. ACG 23 (1) : 8-10) and we overviewed various aspects of our work in CSD's Variety Trial Results booklets (2001, 2002, 2003). The work has been presented at several conferences and overseas talks (.e.g. C. Tann, Aust Ent Soc Annual Conf., Hobart; G. Fitt, invited plenary lecture at 3rd Intern. Conf. Biopesticides, Kuala Lumpur, Malaysia; G. Baker, World Cotton Conference, Cape Town, S, Africa; G. Baker, invited lecture UCD, Dublin, Ireland). The work has also featured in undergraduate lectures (e.g. G. Baker, A.N.U. Canberra) and in the production of industry guidelines (e.g. G. Fitt's contribution to "A guide to the Ingard resistance management plan for 2002/2003", Aust Cotton CRC & Monsanto).

We intend submitting manuscripts on long-term changes in *Helicoverpa* abundance as indicated by pheromone and light trapping and relative refuge productivity to scientific journals within the current financial year.

10. Impact of Project Results and Conclusions for Cotton Industry

The development of effective resistance management strategies represents a major plank in the deployment of Bt cottons in the Australian cotton industry. The optimal use of refuges is an integral part of the resistance management strategy and a significant cost burden to cotton farmers. This work assists in the identification of the most appropriate refuge options.

Part 4 - Final Report Executive Summary

Transgenic (Bt) cotton varieties provide a substantial basis for economically and environmentally sustainable insect pest management within the Australian cotton industry. Whilst the introduction of Bt cotton has significantly reduced pesticide use and encouraged a greater emphasis on the management of beneficial invertebrates in pest control, the impact of Bt cotton and concurrent changes on the landscape on the overall abundance of the target pests, *Helicoverpa* spp., is poorly understood. We have therefore continued long-term studies of the population dynamics of *Helicoverpa* spp. and their natural enemies in northern N.S.W. and southern Qld.

The greatest risk 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. To properly evaluate the utility of refuges, we need improved knowledge of their relative production of moths and the degree to which moths from refuges and Bt crops effectively mate.

Analyses of data, accumulated since the early 90's, from the deployment of pheromone and light traps in the vicinity of ACRI, Narrabri demonstrated long-term changes in the abundance of *H. armigera*. Increases in mid-late season abundance were recorded in recent years in pheromone, but not light traps. Similar changes in the abundance of *H. punctigera* were not found. Mechanisms driving the increase in *H. armigera* are currently unclear. The research also served to highlight fundamental differences between the two commonly used trapping techniques in terms of the seasonal patterns of activity that they depict, in particular for *H. punctigera*. In addition, pheromone traps were maintained in the St George and Dirranbandi regions in more recent years. These catches differed in seasonal pattern from those observed near ACRI. The abundance of *Helicoverpa* eggs was monitored near ACRI, St George and Dirranbandi. Neither pheromone nor light trap catches of moths tracked egg pressure closely, but light traps gave the best fit.

Almost 2,000 surveys were conducted of weeds, natural vegetation and crops other than cotton in northern N.S.W. and southern Qld from 1993 to 2003 to assess the abundance of *Helicoverpa* eggs and larvae. The surveys used visual checks of host plants and sweep netting. Collected material was reared in the laboratory to determine moth species identity and levels of parasitism and viral disease. Increased incidence of *H. armigera* was noted in recent years on winter-spring plants. Weeds and natural vegetation in winter-spring and unsprayed refuges in summer-autumn yielded large numbers of parasitoids of *Helicoverpa*.

Comparisons were made between the abundance of *Helicoverpa* pupae (within the cotton season) in soils used for Ingard cotton, conventional sprayed cotton and unsprayed refuges from 19996 to 2003 in the major cotton producing valleys of northern NSW, plus St George and Dirranbandi in southern Qld. These studies were supplemented with trials evaluating specific issues, notably the value of additional refuges sown late in 2000-01 due to accidental over-planting of Ingard cotton that year and the relative merit of soy bean as a refuge crop. Overall, these data confirmed pigeon pea as the most reliable and productive refuge option. Rearing of live pupae indicated the level of parasitism can be substantial in some crops (especially sorghum).

Surveys of predatory invertebrates on vegetation within Ingard cotton crops and associated refuge crops illustrated spatial patterns in abundance, in particular that abundance was lower well within Ingard crops compared with nearer the edges. This pattern may reflect invasion from adjacent habitats. The result suggests the spatial scale used to establish field evaluations of the non-target effects of Bt cottons need to be carefully chosen.

Although substantial research has been done on the need to control over-wintering pupae of *Helicoverpa* through tillage ("pupae busting") to limit the development of resistance, the optimal tillage equipment to use is not well understood. A trial which sought to evaluate a range of tillage methods in this regard yielded data suggestive of greater mortality of pupae when most soil disturbance occurred – but the data were equivocal due to low and patchy pupal abundance at the trial site.

A mark – recapture study of moth abundance in a pigeon pea refuge and nearby Ingard cotton crop demonstrated movement from the refuge to the cotton. However, none amongst the small number of mating moths captured in the Ingard crop were obviously produced in the refuge. Further research to underpin our knowledge of the movements and matings of moths between refuges and Bt cotton crops are the subject of a new research project (2003-06).