January, August & Final Reports

REPORTS

Part 1 - Summary Details

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

CRDC Project Number: **CSE76C**

January Report:	☐	Due 29-Jan-01
August Report:	☐	Due 03-Aug-01
Final Report:	☒	Due within 3 months of project completion

Project Title: Augmentation and conservation of parasitoids of Helicoverpa spp. in cotton

Project Commencement Date: 7/98 **Project Completion Date:** 6/02

Research Program: Insect Management

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Part 3 – Final Report Format

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

1. Outline the background to the project.

In Australian cotton fields, the eggs, larvae and pupae of *Helicoverpa armigera* and *H. punctigera* are attacked by a diverse range of parasitic wasps and flies. These parasitoids help to reduce the overall abundance of *Helicoverpa* spp in the agroecosystem by removing a proportion of the developing populations (Fitt 1994 *Ann. Rev. Entomol.* 39:543-562). However, despite their importance our knowledge of the biology and ecology of many key parasitoid species is severely lacking. While research on Trichogrammatid wasps and *Microplitis demolitor* has provided detailed information on biology and ecology of these parasitiods (Scholz 1990 *MSc thesis, Uni. Queensland* pp203) other key species have never been studied. For example, although *Heteropelma scaposum,* is often the most abundant *Helicoverpa* parasitoid encountered in the cotton agroecosystem (Fitt and Mares 1992 *Proc.6th Aust. Cotton Conf.* pp 269-276, Fitt and Walker pers. obs.) nothing is known about the biology or ecology of this wasp. Furthermore, we have little information on the habitat requirements of *Ichneumon promissorius*, a true pupal parasitoid of *Helicoverpa* recently exported and successfully established in the USA for control of *H. zea* in corn crops.

New technology may increase the importance of parasitods by enhancing their numbers. The development of transgenic varieties of cotton producing Bt proteins has been a major advancement in the fight against *Helicoverpa* spp. As part of the management plan to reduce the chance of *Helicoverpa* spp developing resistance to Bt, the use of refuge crops has been endorsed. The use of refuge crops, while designed to produce more *Helicoverpa* (Fitt 1996 *Proc.8th Aust. Cotton Conf.* pp 69-76) may inadvertently increase populations of egg, larval and pupal parasitoids. Similarly, the practice of planting pigeon pea as a trap crop for *Helicoverpa* in transgenic cotton (Anon. 1997 *Aust. Cotton Grower* 18:82) may significantly increase egg and larval parasitoid populations. If so, can these parasitoids originating from refuge or trap crops be attracted back into the transgenic cotton crops to parasitise late season *Helicoverpa* larvae or pupae thus further lowering the chances of resistance to Bt protein developing?

The aim of this project is to gain a more comprehensive understanding of the biology and ecology of key *Helicoverpa* parasitoids, and thereby indicate ways of effectively augmenting and conserving parasitoid populations in cotton using the most appropriate management techniques.

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

The original objectives outlined in the proposal by Dr G. Fitt and Dr R. Mensah, with Dr P. Walker as the principal investigator, were to:

1. Study the biology and ecology of key *Helicoverpa* parasitoids to gain a better understanding of their behaviour, host range, habitat requirements and seasonal dynamics.
2. Determine how natural populations of *Helicoverpa* parasitoids can be effectively augmented and conserved in cotton through the use of nursery crops, refuges, trap crops, food sprays or other novel methods.
3. Determine which insecticides have the least disruptive effect on key parasitoid populations in cotton to improve the integration of biological and chemical control agents.

However, Dr Walker resigned from CSIRO in September 1998. Dr N. Schellhorn, took over the role of principal investigator for the project from July 1999 until January 2001. She modified the objectives to:

(1) Quantify species relative abundance and phenology in non-crops and crops. The experimental phase of the research would focus on parasitoid movement, reproduction and longevity.
(2) To determine if parasitoid abundance is increased in cotton because the source population (i.e early season crops and within field strips of non-crop vegetation) is nearby.
(3) To determine the mechanisms for movement from a source population (i.e. early season crop or refuge) to a sink crop (i.e. cotton) either because (a) the source crop is unsuitable or (b) the sink crop is more attractive.
(4) To determine: (a) if parasitoids feed on floral nectar, extra-floral nectar and pollen, (b) if feeding increases adult fecundity and longevity, and (c) if an increase in parasitoid fecundity and longevity increases their abundance via increased attack rates.

Objectives 1 and 4 were completed in total. Marking techniques were developed to monitor insect movement for objectives 2 and 3. The results from objective 2 were inconclusive (see later) and were not repeated as Dr Schellhorn left the project.

The objectives were further modified by Dr M. Whitehouse who was the principal investigator for nine months from October 2001 to June 2002:

1. To examine movement of parasitoids between cotton and adjacent trap/refuge crops through mass release via mass rearing, which would also provide information on the biology of the parasitoids.

2. To demonstrate movement of parasitoids between cotton and adjacent trap/refuge crops by following the movement of individual large parasitoids.

3. To determine parasitism rates of caterpillars in different field crops (cotton, wheat, pigeon pea) and weeds (Paterson's curse, feral sunflower) in the Namoi district from data archived at ACRI and collected during the 2001-02 season.

4. To inoculate a commercial cotton field with the egg parasitoid *Trichogramma*.

Objectives 1, 2, and 4 were completed in their entirety. Analysis for objective 3 is still in progress, as part of a large review paper (in collaboration with C. Tann, N. Schellhorn and P. Walker) examining nearly 20 years of data on parasitism rates.

3. How has your research addressed the Corporations three outputs: Sustainability, profitability and international competitiveness, and/or people and community?

The research has directly contributed to the "sustainability of natural resources" by 1) monitoring populations of native parasitoids in crops and non-crops, 2) determining how we can increase parasitoid abundance on-farm in crops and non-crops, and 3) identifying when and how much mortality parasitoids cause to *Helicoverpa* spp., thus enabling further development of robust IPM strategies which could lead to reduced insecticide use. By doing this, the research has also indirectly addressed the corporation's output of "profitability and competiveness" and "people and communities". The overall intent of the project is to thus reduce insecticide use, increase profit, reduce environmental pollution, and create greater community acceptance of cotton production.

4. Detail the methodology and justify the methodology used.

Schellhorn's work (1999-2000)

Parasitoid abundance in crops and non-crops within* Helicoverpa *larvae and pupae

To identify the spring and summer crops and non-crops that are good sources for parasitoids during the 1999-2000 season, we sampled for larvae and pupae of *Helicoverpa* spp., returned them to the laboratory and reared them to determine the species that emerged. Due to their minute size, direct sampling for egg parasitoids was treated separately and is explained below. A sweep net was used to collect *Helicoverpa* larvae from vegetation. A sample consisted of 25 swings of the net or the equivalent of 20 row metres. Depending on the size of the area to sample, 4 to 10 transects, or groups of 25 swings, were taken on several occasions. The amount swept was dependent upon the size and frequency of a particular type of vegetation. We sampled lucerne (800 m) from the property "Oakville" in Narrabri, "Norwood" in Moree, and Steve Esther's property at Malevu, lupin (800 m) from "Lynbrae" in Wee Waa, pigeon pea (2720 m) at "Iona" at Warren, "Killoween" at Warren, "Lowana" at Pilliga, and "Yarral" at Narrabri, sorghum (360 m pre-tasselling plus 43 tassel spins post-tasselling) at "Oakbank" in Narrabri, and "Unfarm" in Narrabri, feral sunflowers (egg parasitism only) along roadside, unsprayed cotton (625 m) at "Lowana" at Pilliga, wheat (525 m) at ACRI field A1 and field 6, "Lowana" at Pilliga, and "Tinsfield" in Wee Waa, and non-crops along roadsides and fallow fields (within a 100 km radius of ACRI) including native plants and weeds in a variety of plant families, e.g. *Heliptimum apiculata* (100 m), wild radish, *Raphinestrum* spp. (200 m), and *Echium* spp. (2000 m). With the exception of wheat, all crops were sampled throughout their life, from young vegetative to mature reproductive. Wheat was not sampled until late vegetative - pre-reproductive. Pupal digging only represented 5% of our sampling efforts, and was done primarily in un-sprayed cotton. A randomly chosen 1 m x 50 cm section directly below the cotton plants was methodically scraped to reveal tunnels made by *Helicoverpa* spp. When pre-pupae or pupae were located they were returned to the lab to rear out for parasitoids.

Egg parasitism

In addition to collecting naturally oviposited eggs of *Helicoverpa* spp., and checking for parasitism, we also experimentally assessed egg parasitism. Previously egg parasitism was assessed in the Namoi Valley by stapling index cards to cotton plants that contained eggs of *H. armigera*. The results from this work (Walker and Fitt 1999 final report: CRC for Sustainable Cotton)suggested that egg parasitoids were present in the area, but extremely rare. Egg parasitoids are thought to use several cues to locate their host, including scales left by the moth. To better assess populations of rare individuals, we chose to use naturally oviposited eggs. We placed a net bag over the branch or stem of a plant and placed a reproductive female *H. armigera* moth in the net bag at numerous locations (see below) from 12 Nov 99 until early March. She was allowed to lay eggs over night. To vary the number of eggs laid (anywhere from 0 to 300) we varied the age of the moths that were placed in each bag. The next day we removed the bag and the moth, and allowed the eggs to be exposed to predators and parasitoids for 48-72 hours depending on temperature. After this time the plant material with eggs was removed, returned to the laboratory and placed on agar. Placing leaves in agar significantly slowed desiccation, assisting the assessment of parasitism. After 24 hours, if the egg was parasitised it would turn black. However, to determine the species of parasitoid, the eggs had to remain on the agar for approximately 10 days until the parasitoid emerged. Overall, we bagged 327 plants in a variety of crops and non-crops that were found to contain eggs of *Helicoverpa* spp. Two hundred and eighty-five plants were bagged within a 100km radius of Narrabri, and 42 plants were bagged in sorghum and cotton in Warren, NSW. Of the 285 bagged plants near Narrabri, 113 were placed at the Platt Family's property, Lowana, in Pillage, NSW on unsprayed cotton, pigeon pea, and sprayed cotton. We bagged lucerne at "Norwood" in Moree and "Oakville" in Narrabri, feral sunflower along the roadsides in Narrabri near ACRI and Wee Waa, and the road between Wee Waa and Yarrie Lake, *Echium* spp (Patterson's curse) along the roadside between Narrabri and Wee Waa, pigeon pea at "Lowana" at Pilliga, sorghum at "Oakville" in Narrabri, conventional cotton at ACRI in field 6 and at "Lowana" at Pilliga, *Bt* cotton at "Lowana" at Pilliga and "Iona" at Warren, and unsprayed cotton at "Lowana" at Pilliga, throughout spring and summer. We would have included lupin in our samples, however we did not establish our sampling methods until lupin was close to harvesting.

Feeding on floral and extra-floral nectar – with Andrew Manners, University of QLD

We conducted glasshouse experiments to determine if parasitoids feed on floral and extra-floral nectar, if feeding increases adult fecundity (i.e. number of eggs) and longevity (i.e. life-span), and if an increase in parasitoid fecundity and longevity increases their abundance via increased attacks on *Helicoverpa* spp. We established 4 feeding treatments, honey solution (ca. 15% honey), cotton extra-floral nectar, lucerne floral nectar, and water only. Our choice of floral and extra-floral nectar was based on preliminary findings where we determined that the wasp, *Ichneumon promissorius*, a pupal parasitoid, does feed on floral and extra-floral nectar and that these wasps spend most of the time searching on cotton and lucerne plants. Our expectation was that honey should provide the greatest longevity, lifetime fecundity and parasitism rate, water should provide the lowest, and the lucerne and cotton should be in between. A single mated female parasitoid, *I. promissorius*, was placed into a container ca. 22cm x 38cm that included water, a continuous supply of one of the food items mentioned above, and shelters for shade. The wasps were given 6-8 hosts every other day for 17 hours (5 light, 12 dark) throughout their life span. Each feeding treatment was replicated 12 times for a total of 48 individuals. We monitored longevity, lifetime fecundity, average daily parasitism rate, and overall parasitism rate.

Schellhorn's work (2000 - 2001)

Monitoring populations of beneficials

Early Season. In July 2000, we planted 9 Ha of lupin at ACRI, **adjacent** to a field ear-marked for cotton, and **far** (1000 m away) from the same field ear-marked for cotton.

From September until early December, we sampled the lupin and cotton (late October for cotton) using a D-VAC suction device and sweep nets. We monitored populations of *Helicoverpa* spp, parasitoids and predators. In the lupin, we sampled 6 metres with the D-VAC 10-20 times, and 10 metres with the sweep net 10-20 times. Insects were frozen and sorted for the key insect predators, spiders, and parasitic wasps. Although the project was about parasitoids, due to the 3.5 weeks of rain and subsequent flood that occurred in late 2000, we observed that insect densities in early season crops were very low, especially *Helicoverpa* spp., as well as the parasitoids that attack them. Therefore, we counted a variety of beneficials including predatory shield bug, *Oechalia schellenbergii*, bigeyed bug, *Geocoris lubra*, minute pirate bug, *Orius* spp., damsel bug, *Nabis kinbergii*, red and blue beetle, *Dicranolaius bellulus*, transverse ladybird beetle, *Coccinella trasversalis*, three-banded ladybird beetle, *Harmonia octomaculata*, striped ladybird beetle, *Micraspis frenata*, minute two-spot ladybird, *Diomus notescens*, green lacewings, *Mallada* spp., brown lacewings, *Micromus tasmaniae*, hover flies, jumping spiders, family Salticidae, crab spider, family Thomisidae, lynx spider, *Oxyopes* spp., tangle web spider, *Achaearanea* spp., *Microplitis demolitor*, two-toned caterpillar parasite, *Heteropelma scaposum*, orange caterpiller parasite, *Netelia producta*, banded caterpillar parasite, *Ichneumon promissorius*, orchid dupe wasp, *Lissopimpla excelsa*, and vespids.

In addition, as part of a movement study (see below) we experimented with Yellow Sticky Buckets (YSB) to capture and monitor insect populations. We placed nineYSB in the lupin field, 3 within a dyed zone (also see below) and 6 outside the dyed zone, 20 in the adjacent cotton, and 16 in the cotton 1000m from the lupin. Each bucket was placed 1.3 m above ground. In the cotton, four lines of buckets, ca. 80 m apart, were placed from the start of the adjacent cotton field (25 m from the edge of the lupin), every 40 m. In the far cotton field, four lines of buckets were placed 65 m apart, every 40 m. Each YSB was monitored weekly for the key parasitoids and predators. The YSB were especially designed for the emerging cotton because the plants were too small to sample using a D-VAC or sweep net, but we still wanted to know what insects were present and moving into the field.

At Lowana in the Pilliga scrub, 7 Ha of lupin were also planted. The combination of poor germination and rain and flood resulted in samples taken on two occasions only, and low insect densities.

Mid-Season. In late August 2000, 8 Ha of pigeon pea were planted at Lowana in the Pilliga scrub 90 m from a 8 Ha cotton crop. Both the pigeon pea and cotton were sampled from December-February using a D-VAC, sweep net, and YSB. Six and 10 metre sections were D-VACed and swept, respectively, 10-20 times per date.

We placed YSB in the cotton and pigeon pea fields to monitor insect populations. The YSB were not part of the movement study because they could not be deployed and checked at the appropriate time intervals. This was due to irrigation schedules and aerial application of insecticides on adjacent cotton fields.

Dyeing insects in the field to monitor movement

To determine whether insects from early- and mid-season legumes move into cotton, we evaluated and developed a marking technique to dye natural field populations of beneficial insects. We experimented with fluorescent oil and water soluble dyes in the laboratory, and water soluble dyes in the field. In early-season at ACRI, we sprayed a 2 Ha section of the 9 Ha field of lupins (see above) with Rhodamine B mixed with a wetter (ECO-teric, non-toxic surfactant) 3 times at 2 rates, 0.3 gm/L and 0.6 gm/L. Rhodamine B granules (HCA Colours Australia) mixed with water are bright pink and fluoresce orange. After the second application of dye, insects were sampled in the dye-sprayed section and unsprayed section of the lupins 12 hours and 5 days later. All insects were fluoresced under a Labino UV 35 watt lamp to determine whether they were marked with the dye. After the third application of dye, the lupins were slashed and the cotton adjacent and far from the lupin were sampled for marked insects. Insects were sampled from the adjacent cotton by using D-VACS, but were not sampled with D-VAC in the cotton 1000 m from the lupin because the plants were used for physiology experiments and could not be disturbed. Instead we used the YSB to measure insect movement from lupin to cotton.

In mid-season, we wanted to determine if pigeon pea was providing a source of parasitic wasps and predators, and whether insects moved back and forth between the pigeon pea and the cotton. We first monitored insect populations prior to spraying the field with dye. Although the populations were quite low, we decide to treat it as a preliminary experiment and proceeded anyway recognising that the insect density was not high enough to show a pattern. We sprayed a 1.5 Ha centre section of the 8 Ha cotton field at Lowana with panacryl brillant flavine 10GFF (HCA Colours Australia) once at a rate of 0.6 gm / L, and a 1.5 Ha centre section of the adjacent pigeon pea field with Rhodamine B. Twelve hours later we collected insects using a D-VAC from 20, 6 row metre sections in the sprayed section, and the same in the unsprayed section of each crop. We repeated the sampling nine days later. Although the intent was to also sample five days later again, we were unable because of field irrigation. After the insects were collected they were frozen, sorted and scanned under a UV lamp to detect fluorescent dye.

Sentinel larvae

To assess whether parasitism rates were higher in cotton adjacent to the early-season lupin compared to the cotton 1000m away at ACRI, we placed 3 potted cotton plants (pre-flowering) at each YSB in both cotton fields, for a total of 108 plants. We inoculated each with 5-50 neonate *H. armigera* (difficulty in recovering them resulted in trying several densities) on three separate occasions and then recovered larvae one week later to assess parasitism. It was necessary to use potted plants because the cotton in the field was just emerging and very small. We wanted to monitor parasitism from the time that the lupin was mature until it "hayed-off".

Adult parasitoid abundance throughout the season

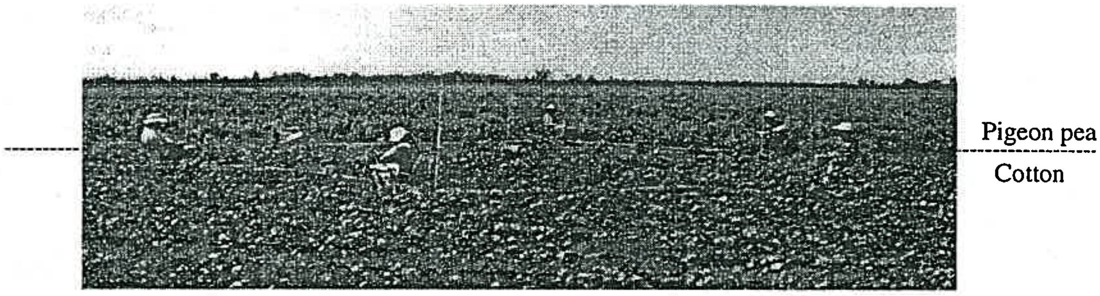
In addition to monitoring parasitism of *Helicoverpa* spp. in crops and non-crops, we also monitored the abundance of adult parasitoids. We used Malaise traps at Lowana during

1999, 2000, and 2001. In 1999-2000, we placed Malaise traps in sprayed and unsprayed cotton, pigeon pea, and the interface row between pigeon pea and unsprayed cotton. In 2000-01, we placed Malaise traps in Bt cotton, unsprayed cotton, pigeon pea, conventional cotton. We placed cubes of dichlorvos in the collection jar because some of the parasitoids have been (and may still be) analysed for the presence of insect-borne pollen. We removed captured insects weekly working around spraying and irrigation schedules and weather. We sorted out the five main parasitoids, *M. demolitor*, *H. scaposum*, *N. producta*, *I. promissorius*, *L. excelsa*, and vespids.

Whitehouse's work (2001-2002)

Whilst Dr. Shelhorn attempted to monitor the movement of parasitoids in general by marking them with dyes in the field, Dr Whitehouse tried two other approaches to see if the large parasitoid wasps *Netelia producta* (Orange caterpillar parasite) and *Heterpelma scaposum* (Two-toned caterpillar parasite) in particular moved between different crop types. These approaches involved mass releases of parasitoids into cotton and adjacent pigeon pea, and following the movements of individual wasps in the field. In both of these approaches, the movements of wasps between cotton and pigeon pea were observed. Pigeon pea was chosen as it is commonly used as a refuge crop in the Namoi Valley.

Mass releases



Workers positioned around the hexagon to observe the wasps leave the release point

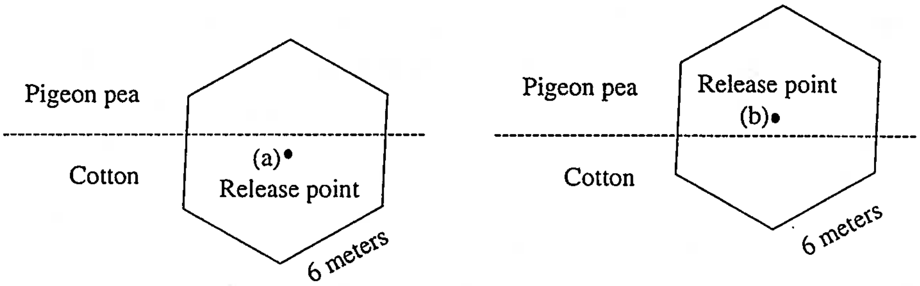


Fig 1. Field characteristics of wasp mass releases showing the position of release point when wasps were accustomed to cotton (a) or pigeon pea (b).

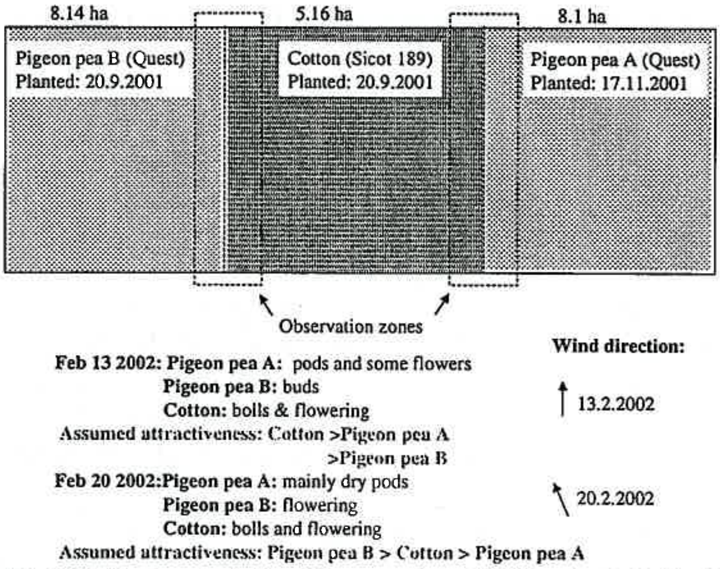
Large numbers of *N. producta* and *H. scaposum* were raised in the laboratory on the cotton bollworm *H. armigera* during the cotton season. Details of the rearing method can be seen in the attached manuscript (**Appendix 1**). Once wasps emerged they were housed with either cotton or pigeon pea plants to see if familiarity affected their plant host preferences in the field. All wasps were marked with a dot of coloured correcting fluid on the dorsal surface of their thorax to identify in the field if they were trained and released on cotton or pigeon pea. Two releases were made at "Lowana" (Owner: Mr. Ken Platt), at Pilliga, Upper Namoi: one on 18 Dec 2001, and the other on 18 Jan 2001. The second release also contained *Ichneumon promissorius* (Banded caterpillar parasite).

To release the wasps we placed the release cage on the border between the cotton (variety: Sicot 189) and pigeon pea (variety: Quest) fields. We then marked out with ribbon a hexagon (with 6 m sides), centred on the release point, and positioned six workers at the points of the hexagon (Fig 1a). Each worker was assigned a side of the hexagon and told to record on counters the number of wasps they saw flying over the ribbon. The hexagon shifted 1 m into the cotton when wasps that had been housed with cotton were released, and 1 m into the pigeon pea when wasps that had been housed with pigeon pea were released (Fig 1b). After the release, 22 yellow sticky traps were placed in a circle 17 m from the release point to see if wasps released in one crop would move to the other crop over time. The sticky traps were checked after 24 hours.

Following individual wasps

To observe the movement of wasps between Cotton and Pigeon pea, we sampled wasps on field 2 at "Lowana". This field contained a strip of cotton sandwiched between two pigeon pea strips at different stages of maturity and therefore presumably differing in attractiveness to the wasps (Fig 2).

Observations were made between 8:30am and 11am. To observe the wasps, a pair of workers walked slowly along the cotton/pigeon pea edge. One was 1 row into the cotton, the other 1 row into the pigeon pea. When the workers reached the end of the field, they swapped crop and walked slowly back. One worker looked for wasps while the other recorded the sightings. When a wasp was found, it was identified to species and if possible sex and observed for up to 5 mins. We recorded when it flew between or along rows, and when it landed on a plant



Feb 13 2002: Pigeon pea A: pods and some flowers
Pigeon pea B: buds
Cotton: bolls & flowering
Assumed attractiveness: Cotton >Pigeon pea A >Pigeon pea B
Feb 20 2002:Pigeon pea A: mainly dry pods
Pigeon pea B: flowering
Cotton: bolls and flowering
Assumed attractiveness: Pigeon pea B > Cotton > Pigeon pea A

Fig 2: Field characteristics of the experiment "Following individual wasps".

Abundance of wasps in different crops

We continued with the work of previous years, including Dr Schellhorn's work on the abundance of parasitoids in crops and non-crops in the Namoi region. This work involved collecting larva from crops throughout the season to establish the level of parasitism in different crops. As in other years, we sampled larvae by taking 4 transects in each field, with each transect consisting of 25 sweeps. C. Tann (CSIRO Entomology) also conducted pupae digs at separate sites in which 14 quadrats ($1m^2$) per field were scraped clear to a depth of 10 cm in search of *Helicoverpa* pupae. Any pupae or larvae collected were taken back to the laboratory and reared through.

Release of Trichogramma *into a commercial cotton field*

This work followed on from Dr Schellhorn's work on egg parasitism (see above) and was conducted in collaboration with Dr S. Mansfield. *Trichogramma pretiosum* is a parasitoid of lepidopteran eggs. It is an aggressive species that has been used to successfully control *Helicoverpa* spp. on sweet corn in the Lockyer Valley. Following this success it was released into crops in the Darling Downs with the aim of establishing the species there for use in IPM programs to control *Helicoverpa* spp in cotton. Recent reports suggest that the establishment has been successful, with egg parasitism rates reaching 100% in INGARD® cotton fields in late January in the last two seasons, despite high *Helicoverpa* spp. pressure (e.g. 90 eggs/metre) (Scholz and Lloyd pers. comm.). The effectiveness of *T. pretiosum* at controlling *Helicoverpa* spp. in cotton on the Darling Downs was so impressive that we decided to see if it could be established further south in the Namoi Valley. Consequently we did a pilot study to see if *T. pretiosum* could be successfully introduced into cotton fields in the Namoi valley in New South Wales.

Three inoculative releases of 177,000 wasps were made, over the course of 23 days, in a 90 Ha field of Ingard cotton (variety Sicot 80). The wasps were released via cards containing 1,000 wasps (supplied by the company Bugs for Bugs; Mundubbera, Qld) by stapling one card to the upper leaf of a cotton plant with plants at 8 metre spacings in a 14x12 grid over an area of $9,152m^2$ (excess cards were placed randomly within the release site). Originally, sentinel cards were used to see if *T. pretiosum* had established itself at the site. Each sentinel card contained about 20 *H. armigera* eggs which had been killed by freezing (to prevent *H. armigera* establishment) the night before release. A sentinel card was stapled to a cotton plant every 15 metres in a 9x10 grid over an area of $16,200m^2$ (90 sentinel cards) which included the release site and extended about 16 meters in all directions from the release site. About 500 metres from the release site within the same field we set up a control site in which we put 9 sentinel cards 15 m apart in a 3x3 grid over an area of $900m^2$. Sentinel cards at both sites were set up 4 times.

We later used *Helicoverpa* spp. eggs which had been laid within the sentinel card and control grid to establish parasitism rates. Eggs were collected and confined individually in multicelled plastic trays. Eggs that were parasitized yielded wasps.

5. Detail results including the statistical analysis of results

Schellhorn's work (1999-2000)

Parasitoid abundance in crops and non-crops

In spring, the majority of larval parasitoids were found in lupin, lucerne, and *Echium* spp. (i.e. Patterson's curse), while in summer the majority were found in pigeon pea and sorghum (Fig. 3). A total of 1,128 larvae were collected and returned to the lab to assess parasitism. Of those, 796 larvae survived long enough to determine whether they were infected with virus, parasitised, or healthy and an adult *Helicoverpa* spp. emerged. *Heteropelma scaposum* was the most abundant parasitoid species followed by *Microplitis demolitor* (both preferentially attack 2nd and 3rd instar larvae, otherwise called 'small' larvae). In total there were 231 *H. scaposum*, 91 *M. demolitor*, 5 *I. promissorius*, and 1 *Netelia producta*. Larvae from lucerne and pigeon pea had the highest incidence of virus (6% and 12%, respectively). However, most of the virus-infected-larvae from pigeon pea was due to the application of Gem Star® late in the season, while virus-infected-larvae from lucerne was due to "natural" levels of virus in the field. Of the spring plants sampled, larvae from lupin had the highest parasitism rate at 74%, and of the summer plants sampled, pigeon pea has the highest parasitism rate at 46% (Fig. 3).

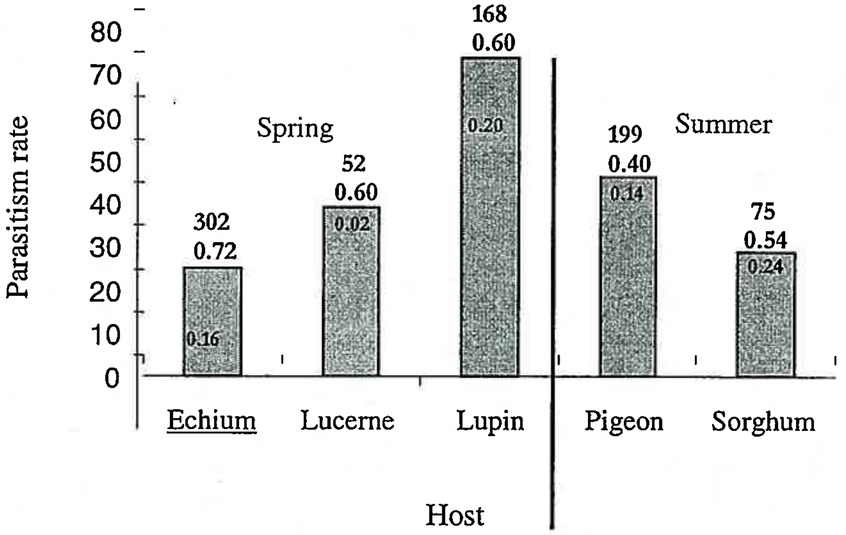


Fig. 3. Larval parasitoid abundance in crops and non-crops represented as percent parasitism of *Helicoverpa* larvae. *Echium* ,Lucerne, and Lupin are spring crops sampled from early-October to mid-December. Pigeon pea and sorghum are summer crops and were sampled from late-December to early-April. The top number above each bar is the number of larvae collected, the middle number is the proportion of the collected larvae that survived to assess parasitism, disease, or healthy individuals, the bottom number is the average density of larvae per metre sampled.

There were also two larvae (one *H. armigera* and one *H. puntigera*) collected from *H. apiculata*, neither was parasitised; one *H. armigera* was collected from *Raphinistrum* spp.; and five larvae from unsprayed cotton, only two that were identified before dying, one *H. armigera* and one *H. punctigera*. Those 5 larvae collected from unsprayed cotton were all that was collected from 400 m of sweeps! Because the objective of the study was to describe the relative parasitism rates among crops and non-crops, the data is presented as percent parasitism, including the proportion of the collected larvae that survived, and the average larval density per metre.

In general, pupae sampling represented a very small proportion of our total sampling effort. In unsprayed cotton, we dug 30 replicates of 1 m x 40 cm and only found one pupa. In pigeon pea, a total of 36 replicates of 1 m x 40 cm were dug on 31 March and 3 April 2000. A total of 592 pupal casings (moth or parasitoid already emerged) and 206 pupae were uncovered after 36 metres of digging. One-hundred and seventy-seven survived, and of those 122 were parasitised (71%). Ninety-five percent of these were parasitised by *H. scaposum*, and 5% by *I. promissorius.*

Egg parasitism

Egg parasitoids exist in the Namoi Valley, however they are rare and parasitism is highly variable among plants within a crop and among crops (Fig. 4). Females laid 22,152 eggs on the 327 plants that were "bagged" to assess egg parasitism,. After 48-72 hours in the field, 5,993 eggs were retrieved and viable. Of the viable eggs, 430 were parasitised by one of two species of egg parasitoids (and possibly three, positive species identification has not been completed), two species of *Trichogramma* and one species of *Telenomus.*

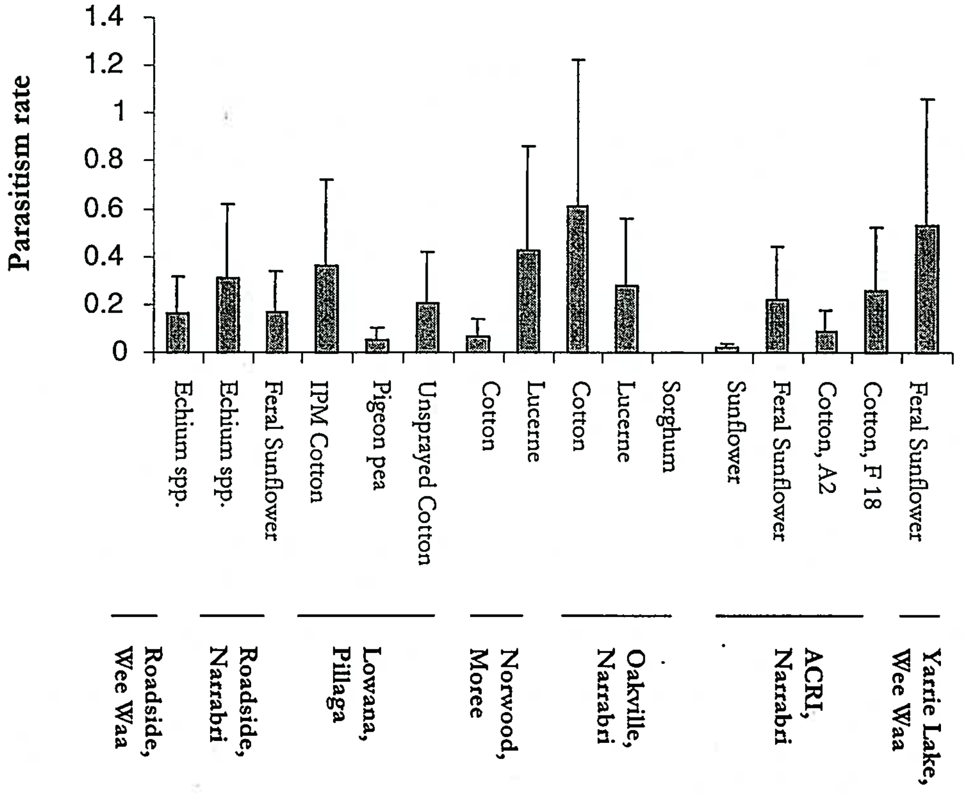


Fig. 4. Mean (+ 1 SD) proportion of *H. armigera* eggs parasitised from different crops and non-crops at several locations. The bars below the plant species name indicates the different crops sampled at a given location

Egg predation (eggs that were missing when we retrieved them, most likely taken by ants, and eggs that were clearly chewed on or the contents sucked out) ranged from 9-95%, but the median value was 76 % predation for the 15 locations listed in Fig. 4. In contrast, of the 168 naturally oviposited eggs that we collected from crops in the same region without bagging, none were parasitised. Furthermore, of the ca. 650 eggs collected at other times by other scientific officers, only one was parasitised. Again, because the objective was to describe the presence and relative abundance of egg parasitism in different crops and non-crops, the data is described using measures of means and standard deviations. A statistical test was not necessary.

Feeding on floral and extra-floral nectar

Our results showed that wasps that fed on the honey solution lived longer and parasitised more *H. armigera* than wasps that only had access to water or lucerne floral nectar (Table 1). Wasps that had access to cotton extra-floral nectar also did not live as long as wasps that fed on honey solution, nor as high a parasitism rate, but there was a tendency for these wasps to be more similar in these regards to wasps that fed on honey than wasps that fed on lucerne floral nectar or water (Table 1). Although, wasps were observed to spend similar amounts of time searching on lucerne (average of 52 seconds per visit) and cotton (average of 67 seconds per visit), they seem not to benefit as much from lucerne in terms of parasitism rate or life-time fecundity as they do from cotton extra-floral nectar.

Table 1. Results from feeding on floral and extra-floral nectar by the pupal parasitoid, *Ichneumon promissorius*.

	$\overline{X}$ Longevity	**$\overline{X}$ DPR[1]**	**$\overline{X}$ Parasitism rate**	**$\overline{X}$ LTF[2]**
Honey solution	19.6 a	0.18 a	0.16 a	12.5 a
Cotton extra-floral nectar	11.0 b	0.18 a	0.11 b	7.9 ab
Lucerne floral nectar	8.5 b	0.08 a	0.05 b	3.77 b
Water only	9.5 b	0.11 a	0.06 b	4.6 b

[1] DPR denotes daily parasitism rate

[2] LTF denotes life-time fecundity.

Letters that differ denote significant difference among treatments @ $P < 0.05$.

Schellhorn's work (2000-2001)

Monitoring populations of beneficials and pests

Early-season. Beneficial insects were abundant in lupin at ACRI, and they peaked at ca. 8.5 per row metre in early October, and decreased to ca. 2 per row metre as the lupin and volunteer sunflower 'hayed-off' (there was a very even stand of volunteer sunflower that germinated once the lupin started to 'hay-off'). Once the cotton plants adjacent to the lupin were large enough to sample, ca. 5 leaf stage, they averaged 1 beneficial per row metre (Fig. 5).

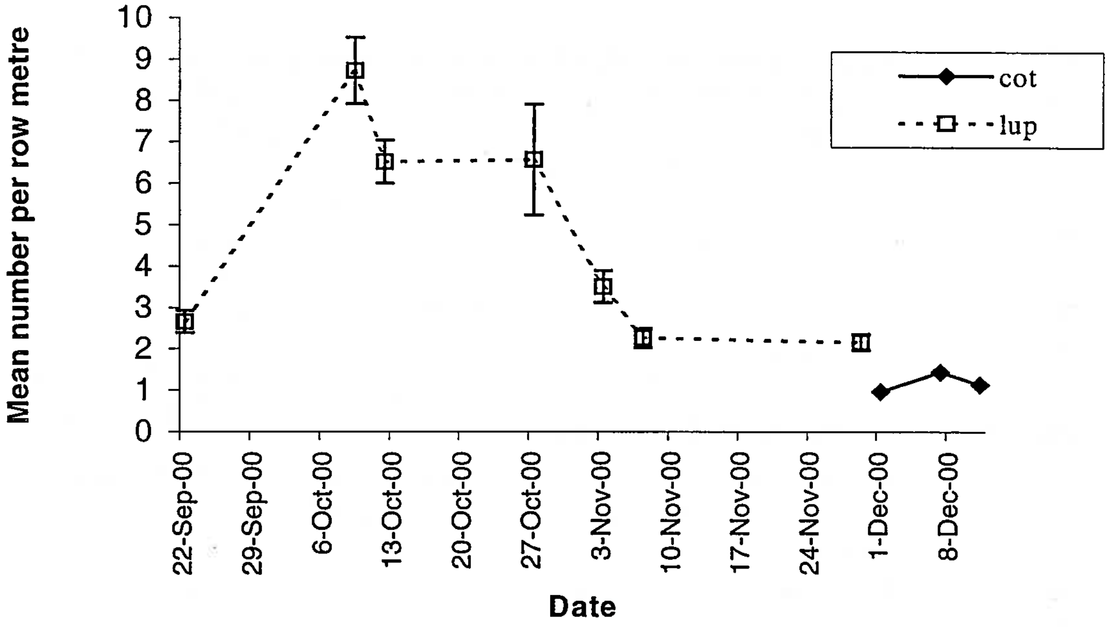
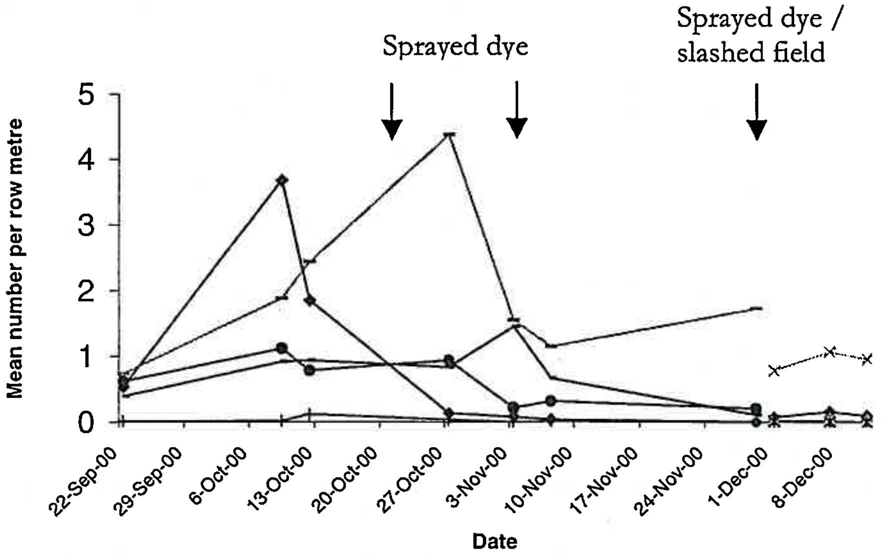


Fig. 5. Mean (± SE) beneficials in lupin and adjacent cotton at ACRI.

A closer look at the groups of beneficial species included in Fig. 5 shows that parasitic hymenoptera were nearly non-existent while spiders were most abundant (Fig. 6). Spiders were much more abundant in lupin than in cotton (Fig. 6). After spiders, nabids were the most abundant of the other beneficial species, early in the season, and then they declined. Coccinellids, and lacewings (*M. tasmaniae* represented the majority) were next most abundant (Fig. 6). Although other insect groups / species were considered, they were rare compared to those shown.


Sprayed dye

Sprayed dye / slashed field

Fig. 6. Mean number of beneficial invertebrates in lupin from 22 Sept – 29 November 2000, and adjacent unsprayed cotton from 1 Dec – 11 Dec 2000. Spiders in lupin (-) and cotton (x),nabids in lupin (diamond), and undetectable in cotton. Coccinellids in lupin (solid circle), lacewings in lupin (-), parasitic hymenopter (+) in lupin, and undetectable in cotton.

The first dye application proved to be too light to mark insects in the field. This was initially surprising, given our laboratory pre-trials, but once applied in the field it most likely broke down under UV (see below). The second application showed great promise, but we received a heavy, unexpected downpour 12 hours after the application. By the third application we were able to assess the proportion of dyed insects in the field (Table 2).

Table 2. Proportion of dyed insects collected from the dyed and un-dyed section of lupin. Number in parenthesis is the number of insects collected.

	Time After Spraying Dye	
	12 hours	**5 days**
Brown lacewing		
Dyed	0.74 (191)	0.40 (98)
Un-dyed	0.11 (149)	0.08 (79)
Spiders		
Dyed	0.26 (129)	0.11(140)
Un-dyed	0.02 (105)	0.01(104)

Spiders and brown lacewings were the most abundant groups / species of beneficial invertebrates that the dye would stain in lupin. Coccinellids were often more abundant than lacewings, but the dye did not stain the glabrous, waxy elytra. The water soluble dye was effective at marking natural field populations of lacewings and spiders. Twelve hours after dying the 1.8 Ha centre section of lupins, 74% of the brown lacewings captured were dyed, and only 11% were dyed in the un-dyed section. For spiders, only 26% of the population was dyed. This was probably due to the different behaviour of the different species. Spiders are less mobile than brown lacewings, they may be covered more by vegetation which may result in fewer individuals being hit by a spray droplet. Five days after spraying the dye, half as many brown lacewings were dyed and collected. A similar phenomenon was seen with the spiders. This could be attributed to emigration from the sampling area, death of insects or degradation of the dye in UV light. Although we can not rule out emigration and death of insects, we now know that the water soluble dye starts to break down when exposed to UV within the first hour (Schellhorn et al. unpublished data). This is not the case with the resin-based fluorescent dyes which remain stable for up to 17 days, the length of the trial.

Parasitic hymenoptera were nearly absent from the lupin field. This was not surprising because *H. punctigera* populations started to increase in late September to 0.35 (± 0.024; $\bar{x}$ ± SE) per row metre, but once the rains came that preceded the flood, the population crashed and did not recover in the lupin. The 2000-01 cotton season proved to be a light *Helicoverpa* year.

After the third application of dye, the lupins were slashed and insect movement was monitored using YSB into the cotton adjacent and far from the lupin. When considering total beneficial populations, there was an increase post-slashing in both the adjacent and far cotton fields (Table 3).

Table 3. Mean number of beneficial insects on the YSB in cotton and lupin pre- and post-slashing of lupin.

	Date*	Adjacent ($\bar{x}$ / day ± SE)	1000 m Away ($\bar{x}$ / day ± SE)	Lupin ($\bar{x}$ / day ± SE)
Pre-slashing	27 Nov 00	0.45 (0.19)	0.52 (0.26)	2.18 (0.66)
Post-slashing	1 Dec 00	1.12(0.67)	0.82 (0.49)	-

* $P < 0.05$, F-statistic, 2-way ANOVA (analysis was conducted on the adjacent and 1000 m away cotton).

When considering only brown lacewings, there was also an effect of date (Table 4). However, this was due to the increase in brown lacewings in the cotton 1000 m away from lupin from pre- to post-slashing.

Table 4. Mean number of brown lacewings on the YSB in cotton and lupin pre- and post-slashing of lupin.

	Date*	Adjacent ($\bar{x}$ / day ± SE)	1000 m Away ($\bar{x}$ / day ± SE)	Lupin ($\bar{x}$ / day ± SE)
Pre-slashing	27 Nov 00	0.02 (0.03)	0	0.14 (0.66)
Post-slashing	1 Dec 00	0.02 (0.08)	0.10 (0.18)	-

* $P < 0.05$, F-statistic, 2-way ANOVA (analysis was conducted on the adjacent and 1000 m away cotton).

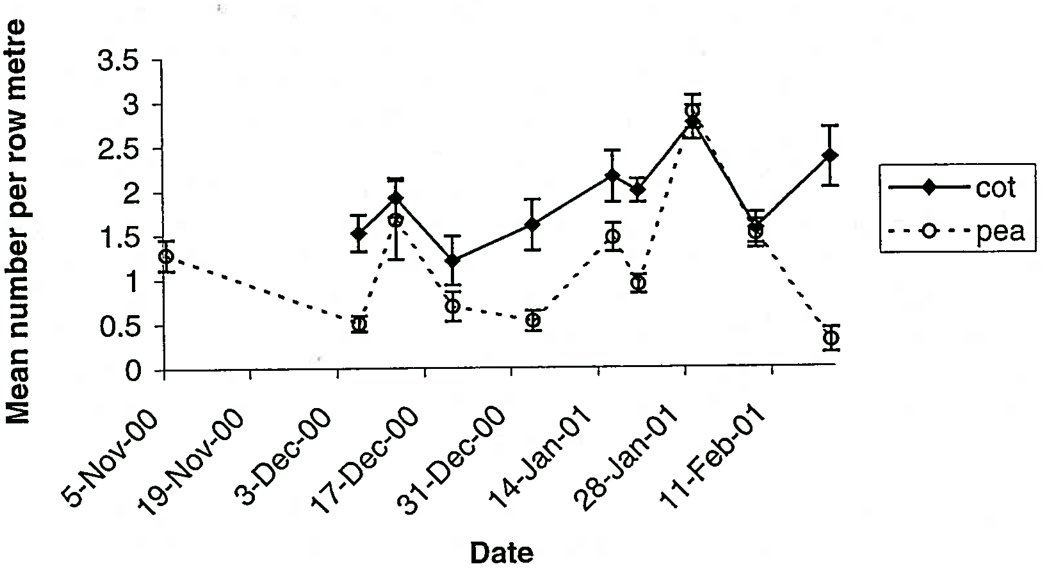


Fig. 7. Mean number ($\bar{x}$ ± SE) of beneficial invertebrates in unsprayed cotton and pigeon pea at Lowana.

A significant correlation with date is not sufficient to imply what, if any, effect the early season beneficial species from lupin had on the adjacent and far cotton. Marked individuals moving from the lupin into cotton need to be monitored. This will require marking a larger population of beneficial insects, using a resin-based fluorescent dye that is stable under UV, and increasing the number of and lowering the YSB to ca. 20 cm from the ground.

Mid-season. Beneficial insects were present in unsprayed cotton and pigeon pea throughout the sampling period (Fig. 7). The seasonal pattern and densities were similar among the two crops, except on occasion cotton supported higher densities than pigeon pea.As seen with early season results in lupin and cotton from 2000-01, parasitic hymenoptera were rarely captured in cotton and pigeon pea later in the season using D-VAC and sweep nets (Fig. 8; however, see results from Malaise traps below). This was most likely a function of low densities (as supported by Malaise trap data) and the fact that D-VAC suction samplers may disturb hymenoptera making it difficult to capture them.

Coccinellids, nabids, and lacewings were the most abundant insect predators, but spiders were the most abundant predator (Figs 9-12). *C. transversalis* was the most abundant coccinellid. The increase in density in early February (Fig. 9) was a direct result of an aphid outbreak in the cotton. Nabids were relatively abundant in unsprayed cotton and pigeon pea, with peak densities in December of ca. 1 per 3 metres (Fig. 10). Lacewings were frequently observed in wheat prior to the cotton season. Although, lacewings are one of the most commonly encountered insect predators in the system, their density is relatively low compared to other predatory insects (Fig. 11). Like coccinellids, their increase in early February was also due to the aphid outbreak. Spiders were the most abundant predator with ca. 1-2 per metre in cotton for several months, ca. 0.5-1.5 in pigeon pea. Although several spiders were identified to family or genera, the majority were not.

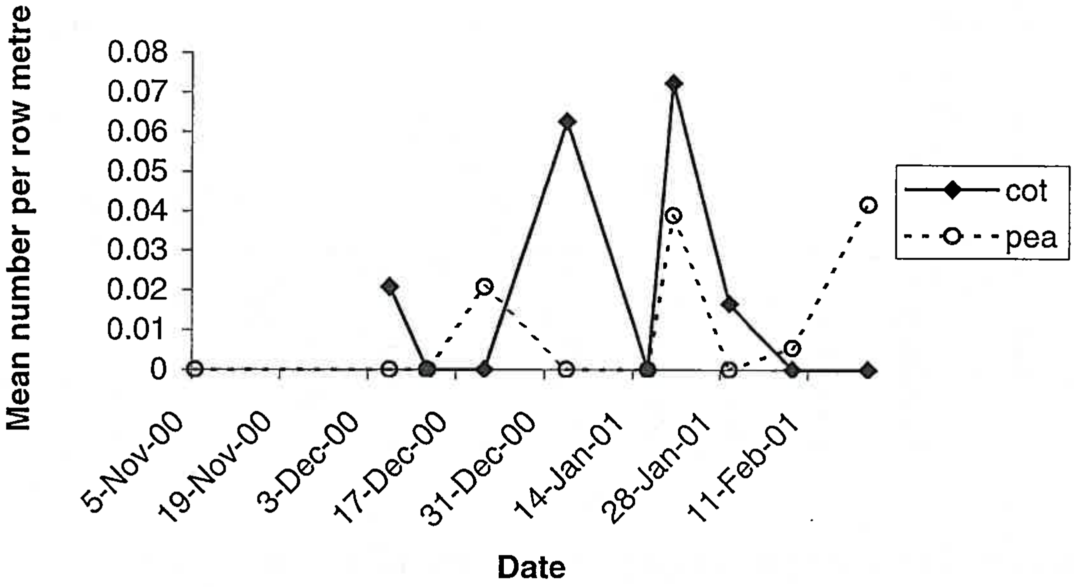


Fig. 8. Mean number of parasitic hymenoptera in unsprayed cotton and pigeon pea.

The results from the YSB at Lowana were somewhat similar to the D-VAC samples. Results from the YSB showed coccinellids to be the most abundant insect collected, representing 78% of all predators. There were three times as many coccinellids captured in unsprayed cotton compared to pigeon pea, and sixty times as many captured there, compared with in Bt cotton. This was largely due to the aphid outbreak that happened over a short period of time in unsprayed cotton combined with the frequent spraying of Wizard in Bt cotton from January onward. Earwigs were the next most abundant, representing 11% of all predators! The high densities of earwigs on YSB compared to D-VAC suction samples suggest that earwigs must be attracted to the YSB or the D-VAC is unable to consistently collect them.

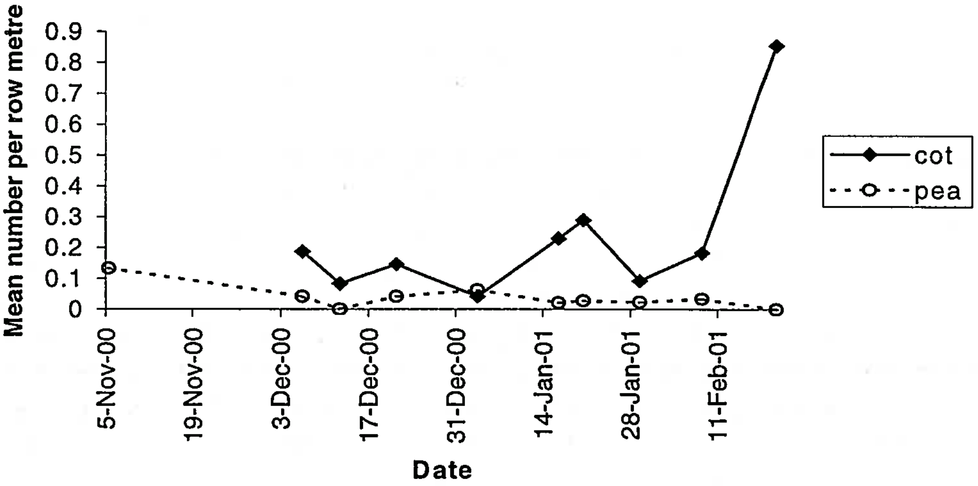


Fig. 9. Mean number of coccinellids in unsprayed cotton and pigeon pea at Lowana.

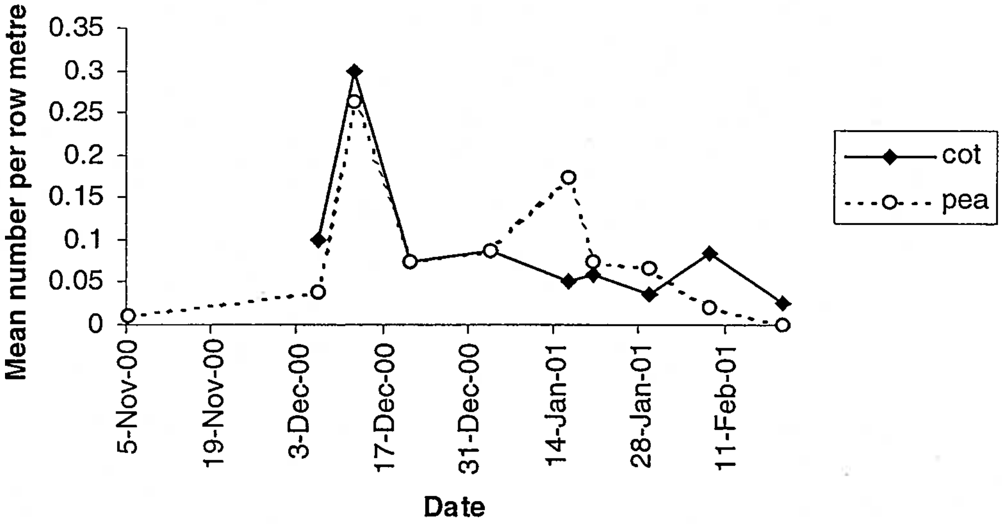


Fig. 10. Mean number of nabids in unsprayed cotton and pigeon pea at Lowana.

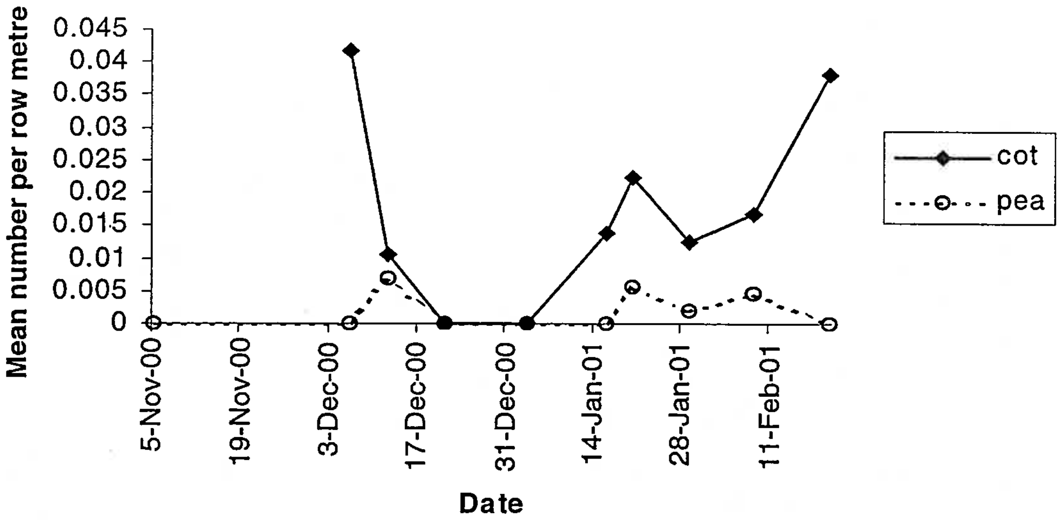


Fig. 11. Mean number of lacewings in unsprayed cotton and pigeon pea at Lowana.

The dye experiments that we conducted to determine if pigeon pea was providing a source of beneficials to cotton, and vice versa resulted in ca. 13% of beneficials marked in the dyed section of cotton and pigeon pea (Table 5). This was considerably lower than the proportion marked during our experiments at ACRI in lupin, and was most likely due to lower densities of beneficials at Lowana or the wind (Table 5). For example, at ACRI we considered only the two most abundant species, brown lacewings and spiders, and collected nearly twice as many compared with when we counted several groups of beneficials at Lowana, including spiders, coccinellid larvae, green and brown lacewings, and nabids. From our independent work marking insects in Adelaide, we require a population density of ca. 1 per metre of each insect species that is being monitored. Less than 1 per metre results in too few individuals being marked. As a group of beneficials there was nearly 1 per metre in the dyed section of the cotton field, but too few considering the different species and different behaviours of each species.

Of those individuals recovered from cotton that were dyed, only one was an immigrant from the pigeon pea (as indicated by the pink dye – the other individuals were marked with greenish-yellow). A single immigrant from pigeon pea was also found in the undyed section of cotton. No emigrants from cotton were recovered in pigeon pea. Another explanation for why the proportion of dyed individuals was lower at Lowana may be the windy conditions. When we sprayed, the wind was between 14-20 knots. In Adelaide, we have observed that the insects don't move when the wind is much above 7 knots, hence possibly reducing the probability of dye making contact with the insect.

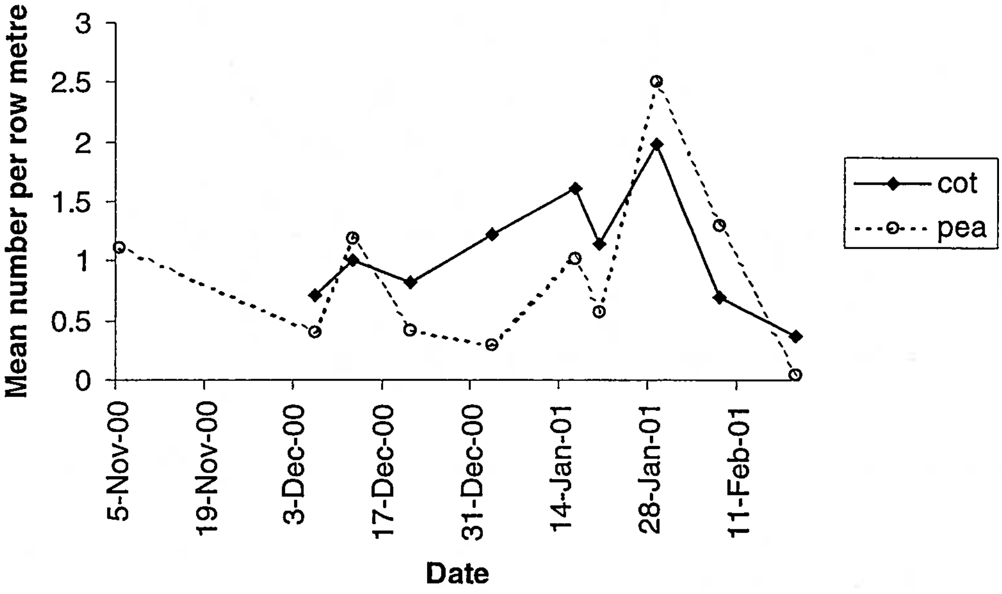


Fig. 12. Mean number of spiders in unsprayed cotton and pigeon pea at Lowana.

Table 5. Proportion of dyed insects collected from the unsprayed cotton and pigeon pea at Lowana. Number in parenthesis is the number of beneficial invertebrates collected.

	Crop	
Field section	***Cotton***	***Pigeon pea***
Dyed	0.13 (108)	0.125 (64)
Un-dyed	0.017 (235)	0.008 (129)

Sentinel larvae

We were unable to accurately assess parasitism rates using sentinel larvae placed on potted cotton plants in the cotton adjacent and far from the lupins. Although numerous neonates were released on the plants (ranging from 5-50), very few were recovered. This phenomenon of "losing" *H. armigera* neonates and older larvae has been reported by others (Gary Fitt, personal communication). On 4 Nov 2000, we recovered 50 third instar larvae, 6 were parasitised, most from a single plant. On 28 Nov 2000, we recovered 20 larvae from the far cotton field, 11 were parasitised, 3 emerged as *H. armigera*, and the rest died. On 5 Dec 2000, we recovered 8 sentinel larvae from the cotton adjacent to the lupin and 1 was parasitised, whilst 1 sentinel larvae was recovered from the cotton far from the lupin, and it was not parasitised. Although using sentinel larvae can provide insight into instantaneous parasitism rates, the number of plants, and hosts (larvae) recovered would have to be significantly higher.

Adult parasitoid abundance throughout the season

Catches from the Malaise traps in 1999-2000 showed adult parasitoid abundance peaks in late February and March (Fig. 13). *M. demolitor* was the most abundant species and represented over 90% of all individuals collected. *N. producta* and *H. scaposum* were the next most abundant, but were still rare compared to *M. demolitor*. Pigeon pea supported the highest densities of parasitoids (Fig. 13).

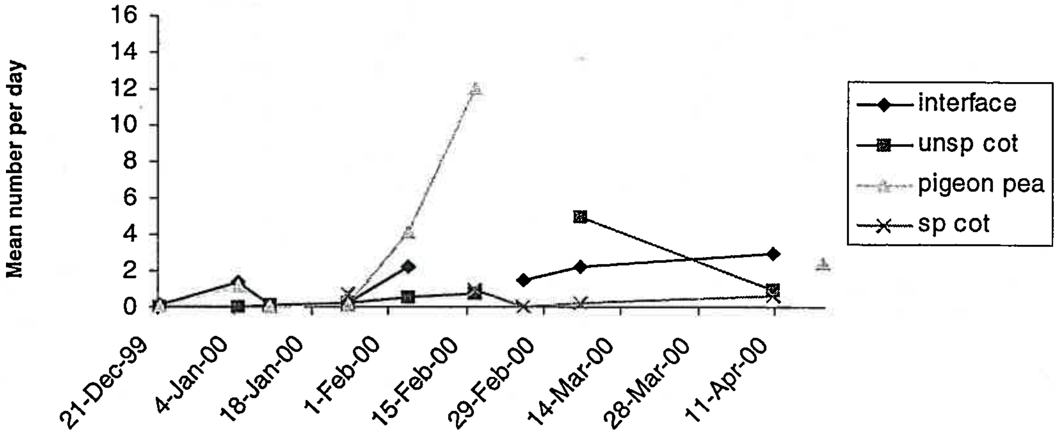


Fig. 13. Malaise trap catches at Lowana 1999-2000. Interface is the boundary between unsprayed cotton and pigeon pea, unsp cot is unsprayed conventional cotton, and sp cot is sprayed conventional cotton. Samples were collected at weekly intervals working around weather, irrigation and spray schedules.

In 2000-01, parasitoid densities of ca. 4-6 per day were reached a month earlier than in 1999-2000 season (Fig. 14). However, the densities were never as high as in the 1999-2000 season. Again, *M. demolitor* was the most abundant parasitoid species collected. With the exception of Bt cotton in early January, the highest densities of parasitoids were in unsprayed cotton and pigeon pea.

Unsprayed cotton and pigeon pea were the crops that supported the highest densities of parasitoids, and *M. demolitor* was the most abundant in each followed by *N. producta*, in cotton and *L. excelsa* in pigeon pea (Fig. 15).

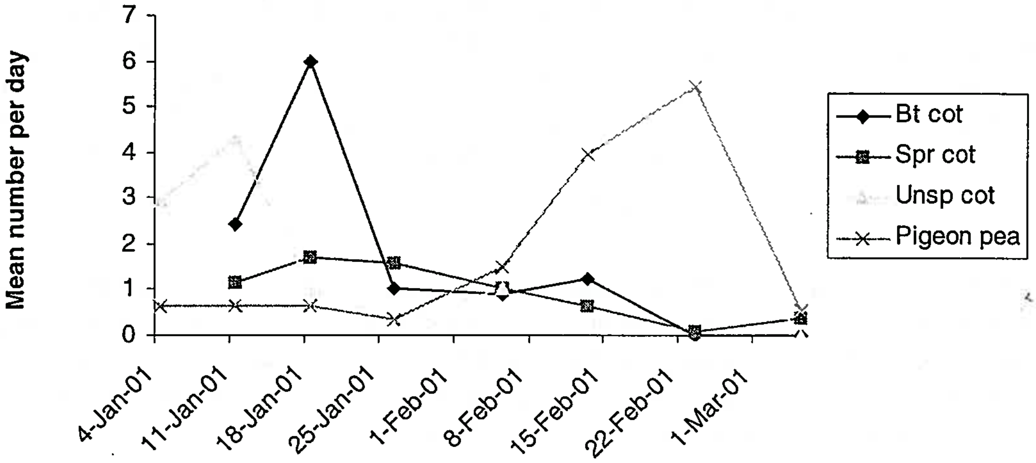


Fig. 14. Malaise trap catches at Lowana 2000-01.

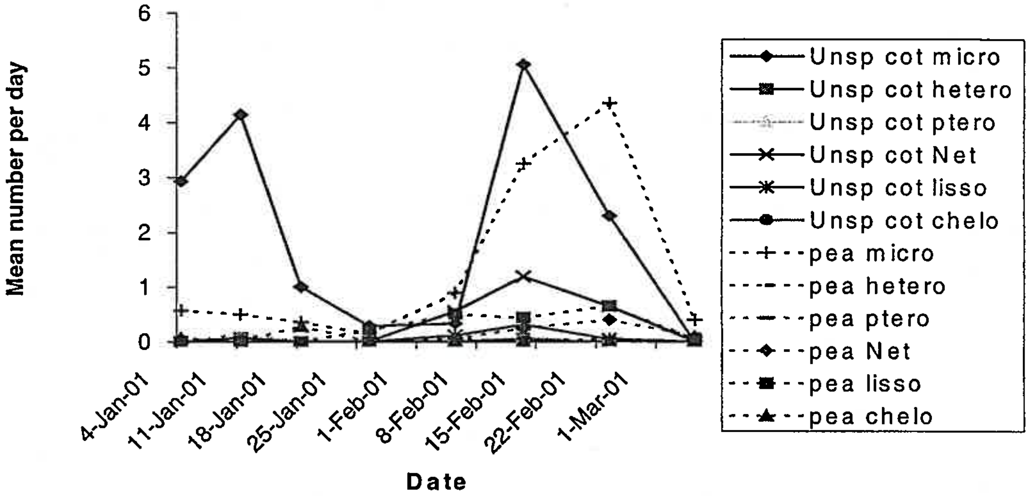


Fig. 15. Mean number of parasitoid species per day in unsprayed cotton and pigeon pea at Lowana, 2000-2001.

From mid-January to early-February, *M. demolitor* populations crashed for reasons that are unclear, and then increased again from mid- to late-Februray. This is a similar pattern to 1999-2000 cotton and pigeon pea results, and a personal observation, (Gary Fitt and Paul Walker) that parasitoid densities do not peak until mid-February and March.

Whitehouse's work (2001-02)

The natural histories of the wasps *Netelia producta* and *Heteropelma scaposum* are described in **Appendix 1**. In summary, we had much greater success at rearing large numbers of *N. producta* than *H. scaposum*, partly because *N. producta* was longer lived in the laboratory (3 weeks compared with 1 week for *H. scaposum*), and because it was easier to confirm when a larva was parasitised by *N. producta.* Our cultures were also dominated by male *H. scaposum* (75%) which made breeding difficult. Our overall parasitism success rate was low (29% of larvae exposed for *N. producta* , and 19% for *H. scaposum*) but this was partly due to the large number of *Helicoverpa* larval deaths. *N. producta* often killed the caterpillars before they could yield wasps by: feeding on the larvae as adults, attacking small caterpillars, and by an excessive parasitoid egg load per caterpillar. An egg load of six or more eggs usually led to the larva's premature death. The parasitism success rate of *N. producta* reached a peak (61% of larvae exposed yielding parasitoids) at five parasitoid eggs per caterpillar.

Mass releases.

Over 6,600 caterpillars were presented to parasitoid wasps in our mass rearing program. From these caterpillars, we successfully raised over 1,600 wasps. However, because of their short lifespan, many wasps died before they could be used in our mass release program. Nevertheless, we did manage to release a total of 614 wasps, of which we observed the flight direction of 396 individuals (Table 6).

Table 6: Number of wasps released and observed.

		Trained on cotton				Trained on pigeon pea			
		male	female	**Total released**	**Total observed**	male	female	**Total released**	**Total observed**
18	*Netelia producta*	10	140	**150**	**89**	6	111	**117**	**50**
Dec	*Heteropelma scaposium*	42	7	**49**	**30**	20	0	**20**	**8**
2001	*Ichneumon promissorius*	0	0	**0**	**0**	0	0	**0**	**0**
18	*Netelia producta*	42	43	**85**	**75**	58	36	**94**	**81**
Jan	*Heteropelma scaposium*	14	10	**24**	**23**	11	4	**15**	**10**
2002	*Ichneumon promissorius*	9	20	**29**	**10**	15	16	**31**	**20**
	TOTAL			**337**	**227**			**277**	**169**

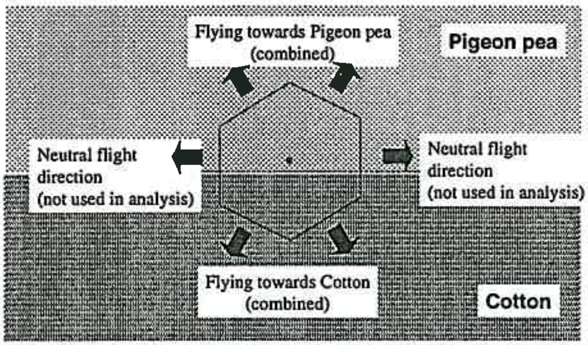


Fig 16. Flight directions used in the log-linear model.

On the 18th of December the pigeon pea was flowering and had a *Helicoverpa* egg count of 47.17eggs/m, and a caterpillar count of 2.83 larvae/m, while the cotton had squares and had an egg count of 1.83 eggs/m (*Helicoverpa* data courtesy of T.Staines and M.Dillon). Because the pigeon pea had large numbers of larvae and was flowering, we assumed that it would be more attractive than the cotton to the parasitoids.

When we released the parasitoids, there was a wind blowing from the cotton to the pigeon pea which confounded results, as many wasps were reluctant to fly in the wind and flew to plants within the hexagon before taking off once the wind eased. To examine factors influencing on flight direction, we used a log-linear model to analyse a multiway contingency table consisting of croptype, training, and species. To analyse crop-type for each release time, we discarded the neutral flights (Fig. 16) and combined the two flight paths towards the cotton, and the two flight paths towards the pigeon pea (Fig. 16). We found that training and release time did not affect the direction that the wasps flew (df = 1, χ^2 =0.1, NS; df = 4, χ^2 =3.4, NS; respectively) whereas crop-type did (df = 1, χ^2 =15.19, P<0.0001). There were no significant interactions between these three factors. Thus we found that more wasps flew towards the pigeon pea than the cotton, irrespective of training (Fig. 17). However, as the wind was also blowing towards the pigeon pea, we cannot rule out that the wasps were passively moved by the wind towards the pigeon pea.

An inspection of the sticky traps on Dec 19th yielded no wasps, and no wasps were caught on the sticky traps after a week. Nevertheless, a casual survey on Dec 19th of the crops revealed 9 *H. scaposum* in the pigeon pea, and 2 in the cotton, suggesting that the wasps did prefer the pigeon pea.

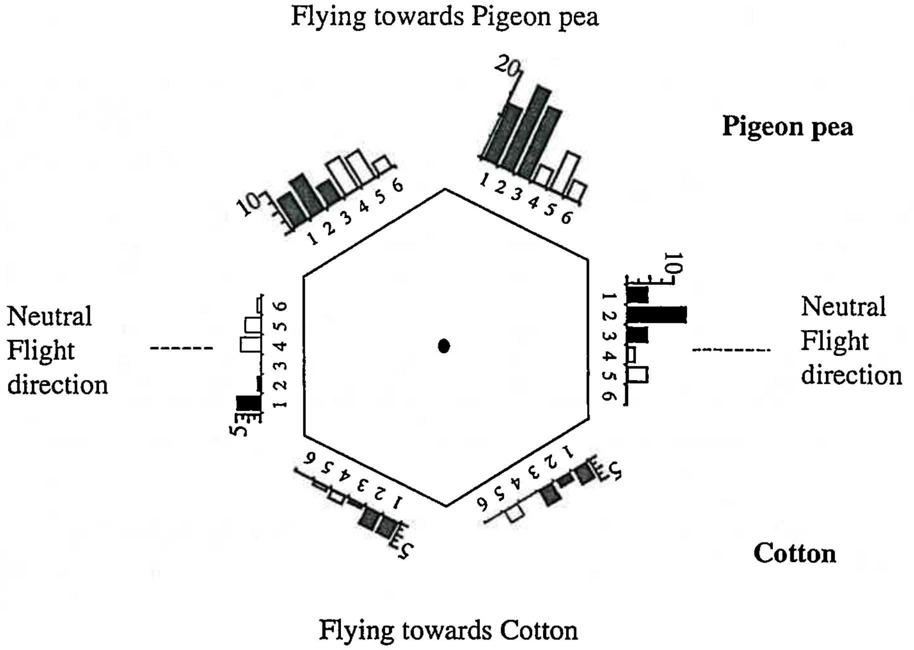


Fig 17. The number of wasps that flew towards cotton or pigeon pea on Dec 18 2001. The numbers under the histogram are the release numbers (there were six releases; *Netelia* releases: 1,2,4,5; *Heteropelma* releases: 3,6) the black histograms represent wasps trained and released in cotton while the white histograms are wasps trained and released in pigeon pea.

On the 18th of January the pigeon pea was forming pods while the cotton was flowering. A *Helicoverpa* check conducted by T. Staines on the 21st of January recorded 2.5 eggs/m and 0.5 larvae/m in the cotton, and 4 eggs/m and 0.5 larvae/m in the pigeon pea. As the larval counts in the two crops were the same, but the cotton was flowering, we assumed that cotton would be slightly more attractive than the pigeon pea.

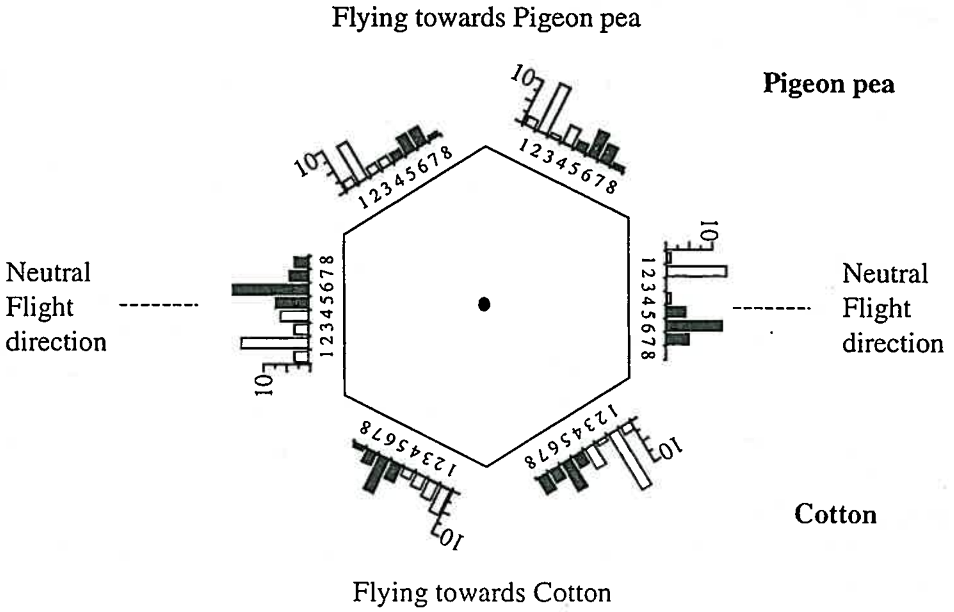


Fig.18. The number of wasps that flew towards cotton or pigeon pea on Jan 18 2002. *Netelia* releases: 1,2,5,6; *Heteropelma* releases:3,7; *Ichneumon* releases: 4,8. See Fig. 17 for the terminology.

This time when we released the parasitoids there was no wind. The wasps seemed to fly randomly into the fields, perhaps with a slight tendency to fly towards the cotton (Fig. 18). Again we used a log-linear model to analyse a multiway contingency table consisting of crop type, training, and time of release. In this analysis we found no effect of flight direction, training, or species.

An inspection of the sticky traps on Jan 19th yielded three wasps, all *N. producta*, and all found in the cotton. Two had been raised and released on pigeon pea, and one had been raised and released on cotton. No wasps were caught on the sticky traps after a week. A casual survey of the crops on Jan 19th revealed 5 wasps in the cotton (4 *N. producta* and 1 *Ichneumon* sp.) and none in the pigeon pea.

These results suggest that wasps readily fly between crops, irrespective of their previous experience as adults with different crops.

Following individual wasps.

The closest *Helicoverpa* egg and caterpillar count to the survey dates was Feb 7th, when no larvae were found. Because of the lack of larvae, we estimated the order of attractiveness by the flowering stage (Fig. 2).

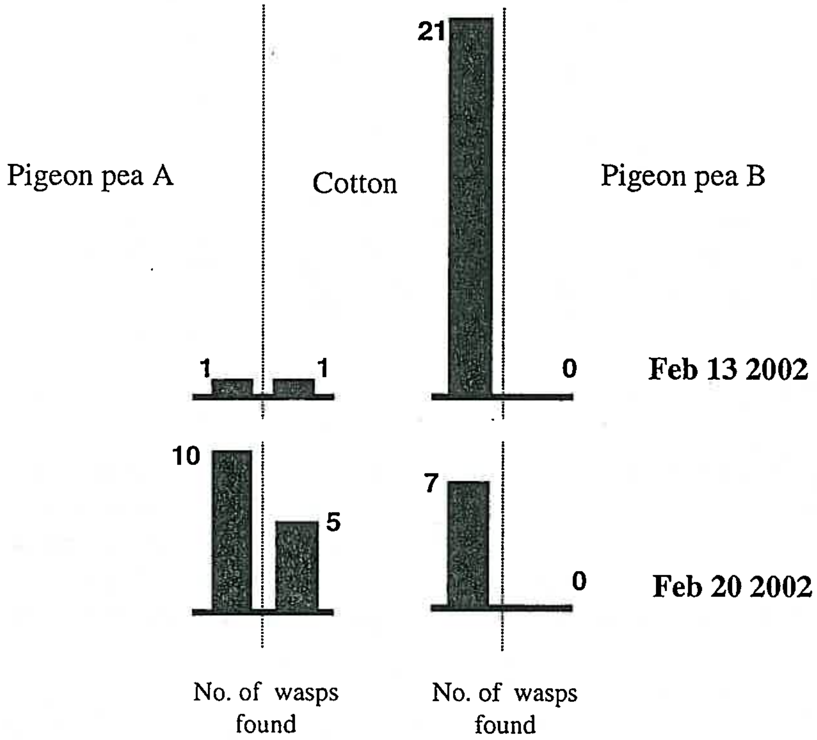


Fig. 19 The number of wasps found at the border between pigeon pea A and B and cotton on Feb 13 and 20.

We were able to locate 45 wasps (23 on Feb 13, 22 on Feb 20) in the field within 5 rows of the border between the pigeon pea and cotton at the two sites. Only one wasp was *N.*

producta, the rest were *H. scaposum.* Of the *H. scaposum,* we were able to sex eight individuals. Five were male and three were female. On Feb 13, the wind direction was along the rows, and so unlikely to affect results. We expected that the cotton would be more attractive than both the older and younger pigeon pea. We found most of the wasps at the border between the older pigeon pea and the cotton, and all of these wasps were in the cotton (Fig. 19) while very few wasps were at the border between the young pigeon pea and cotton.

On Feb 20, the wind direction was again along the rows. We assumed that the younger pigeon pea would be more attractive than the cotton, which in turn would be more attractive than the older pigeon pea. This is what we observed (Fig. 19). All the wasps at the border between the older pigeon pea and the cotton were within the cotton, but at the border between the younger pigeon pea and the cotton we found more in the pigeon pea. The shift from cotton to pigeon pea at the two sites on Feb 20 is significant (Fisher's exact test: P= 0.0053, df = 1, n = 21).

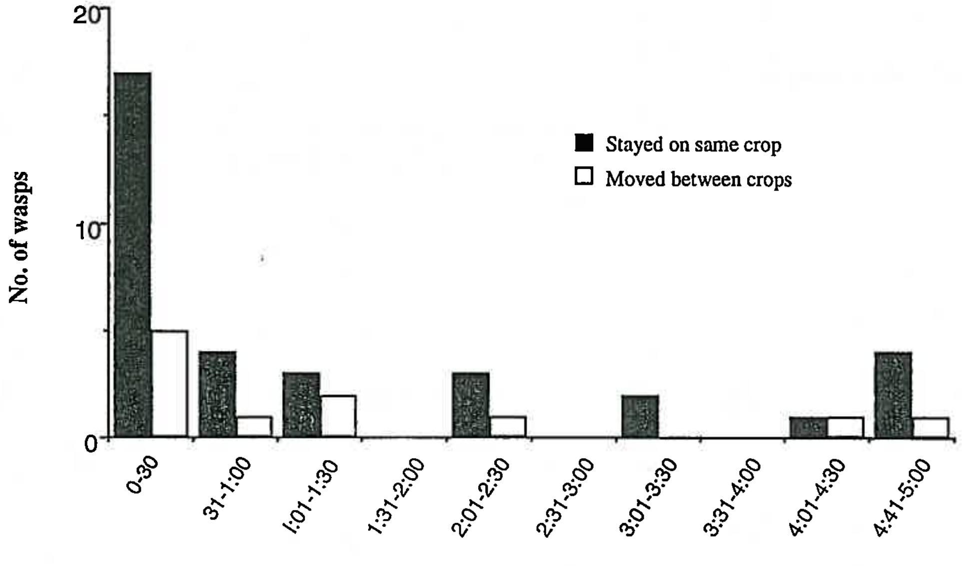


Fig 20. The length of time that individual wasps were observed

Of the 45 wasps we located, 34 stayed on the same crop, while 12 moved between the crops. We were able to follow the movements of the wasps from between 4 seconds to 5 minutes (Fig. 20). There was no difference in the amount of time we observed wasps that flew between crops, and those that stayed in the same crop (Mann Whitney U test: U = 178.5, P = 0.82, n = 45; mean rank of wasps that: (i) stayed in the crop = 23.2, (ii) moved between crops = 22.2).

Of the 23 wasps we found on Feb 13, only 5 moved between the crops (Chi-sq. goodness of fit test: df = 1, $\chi^2 = 6.26$, $P < 0.05$) Consequently, there was a strong tendency not to move between crops, but to stay in the cotton. Of the 22 wasps we found on Feb 20, 14 stayed in the crop in which we found them, and 8 moved between crops (Chi-square goodness of fit test: df = 1, $\chi^2 = 0.4$, NS) indicating there was no difference in the tendency to stay or move between field types. Consequently, we concluded that wasps will move between crops, but the degree to which they will do so seems to be dependant on the relative attractiveness of the different crops.

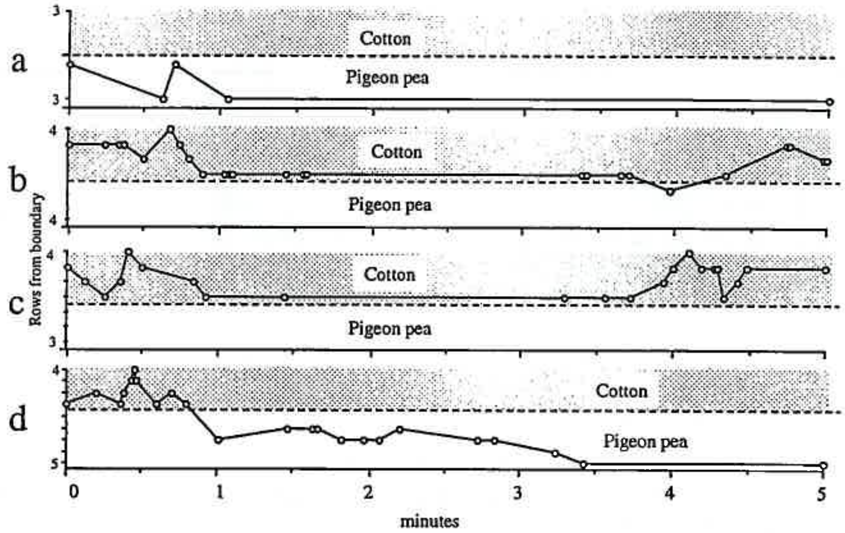


Fig 21. Pattern of movement of individual wasps followed for 5 mins at the border between cotton and pigeon pea. Wasps **b** and **c** show a strong preference for cotton, while wasp **d** moved from cotton to pigeon pea.

The wasps had two main types of flying behaviour. One was moving slowly around the plants ("searching"), and the other was moving quickly above the plants ("travelling"). Our observations were mainly limited to wasps searching. Once the wasps flew above the crop canopy and were travelling, they moved very quickly and were difficult to follow. We found that while searching, the wasps varied in the amount of time they spent moving between plants and landing on the plants and that they readily moved between rows (Fig. 21).

Abundance of wasps in different crops

We collected 6,146 larvae during 2001. Of these, the majority were collected from pigeon pea (2,190) chick pea (2,101) cotton (939) and sorghum (577) (Fig. 22). The parasitism rates are very similar to those reported by Walker (1999) in his final report to CRDC (CSE51C) but are down on those reported by N. Schellhorn for 2000 (see earlier).

A closer look at the data for cotton crops revealed that the parasitism rates differed between larvae collected from Ingard, unsprayed cotton, and conventional cotton (Fig. 23). Although the overall larval numbers were much lower in Ingard cotton, the proportion of larvae brought to the laboratory that matured into moths was higher, and the successful parasitism rate was lower. Therefore Ingard cotton yielded proportionally fewer parasitoids.

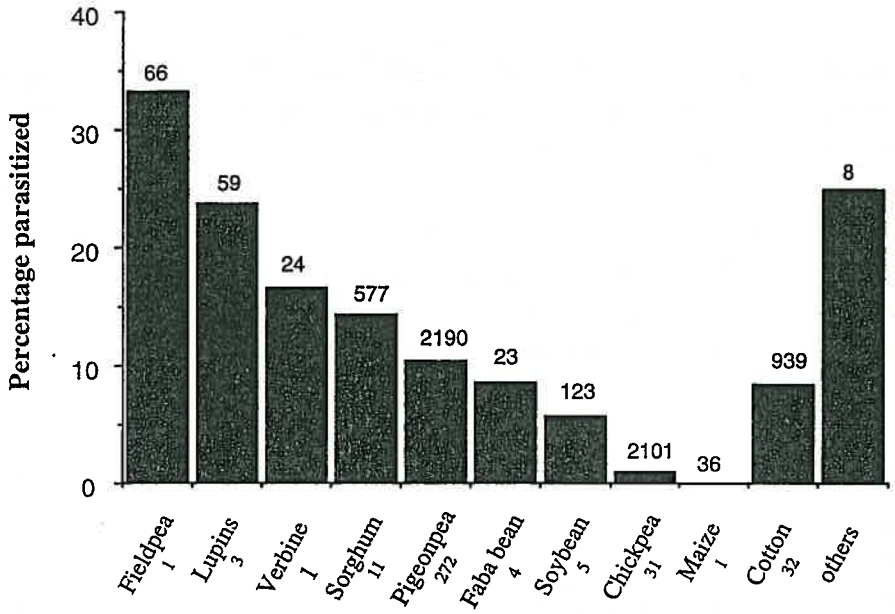


Fig 22. Rates of parasitism of *Helicoverpa* spp in crops in 2001. The number above the histogram is the number of larvae collected. The number below the crop name is the number of samples taken. "Others" consists of Lucerne, Wheat and Paterson's curse.

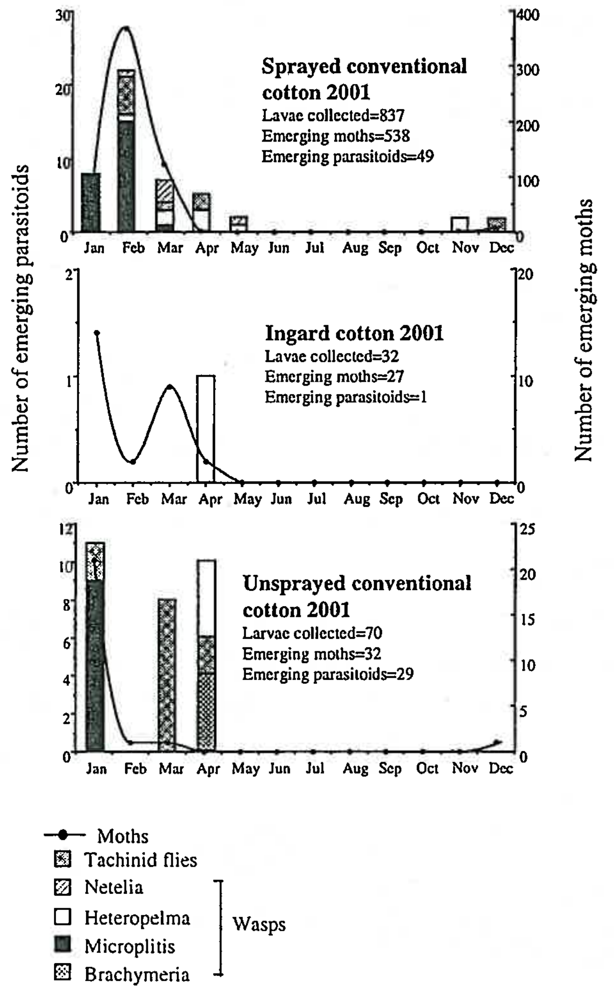


Fig 23. The proportion of parasitoids emerging from larvae collected from different types of cotton throughout the year, in relation to the number of moths emerging. Note scales on y axes vary.

Parasitoid numbers from larvae collected in cotton were highest in February for *Microplitis* sp.

For a crop to be a useful source of parasitoids for cotton, it needs to produce the right parasitoids early in the season. Field peas, Lupins, and verbine, were good sources of parasitoids, but were only sampled once (field peas and verbine) or three times (lupins). The field peas were sampled in October, yielding 22 parasitoids, 3 of which were *N. producta*, 1 was a *Microplitis* sp. and 16 were *H. scaposum*. The verbine was sampled in November, yielding 4 *Heteropelma*. The Lupins were sampled in August and September. The August sample (n = 7 larvae) yielded 1 *Microplitis* sp., while the September samples (n = 52 larvae) yielded 5 *Microplitis* sp., 5 *H. scaposum*, and 3 Tachinids. From these few samples it appears that field pea and verbine could provide a good source of the parasitoids commonly found in cotton early in the season. Our finding that Lupin is a good source of parasitoids confirms N. Schellhorn's findings (see earlier).

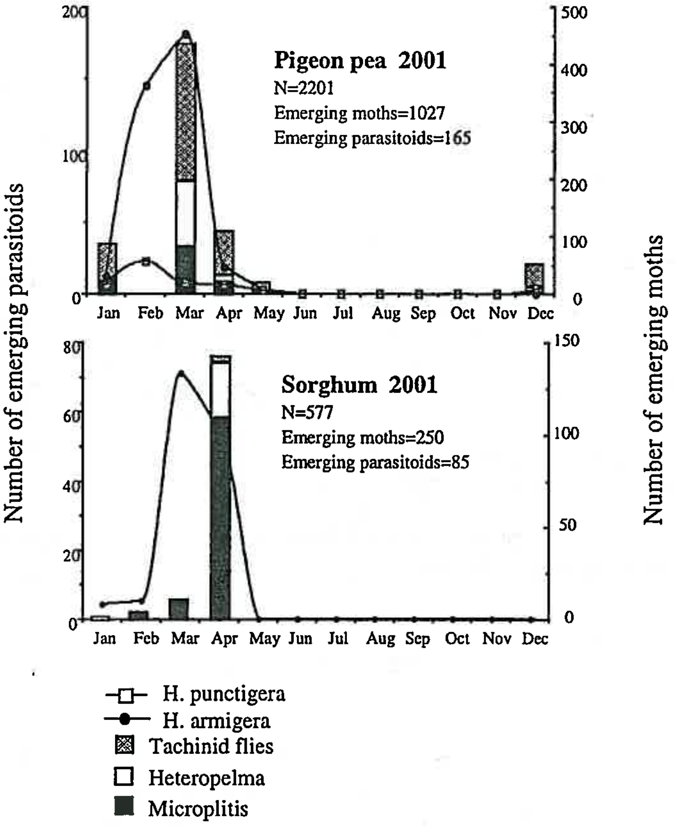


Fig 24. The proportion of parasitoids emerging from larvae collected from different types of cotton throughout the year, in relation to the number of moths emerging. Pigeon pea yielded a large number of *H. punctigera* as well as *H. armigera.*

The most commonly sampled crops that had a reasonable parasitoid load were sorghum and pigeon pea (Fig. 24). Both of these produced parasitoids commonly found in cotton, but they tended to peak later in the season (April for sorghum) although early crops of pigeon pea may produce parasitoids in December/January as well.

1,894 pupae were excavated in 2001. Of these, the majority were removed from fields of pigeon pea and cotton (Fig. 25). Field pea, faba bean and sorghum again yielded a high proportion of pupal parasitoids.

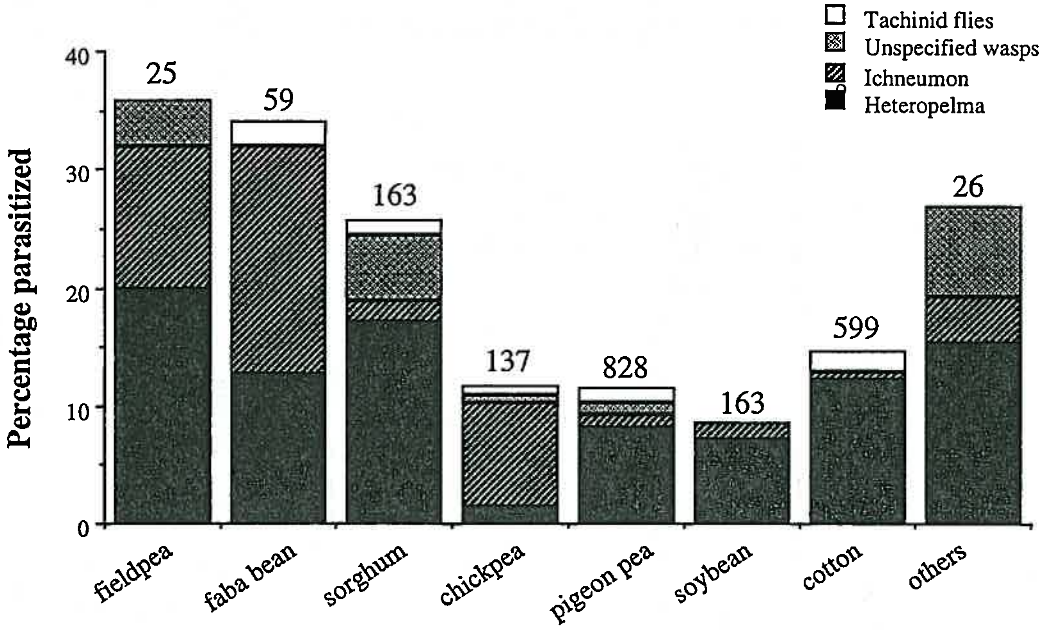


Fig 25. The proportion of pupae parasitized in different crops. The number above the histogram indicates the total number of pupae collected.

Release of Trichogramma *into a commercial cotton field*

Sentinel cards proved to be a very ineffective way of assessing the presence of egg parasitoids in the field. Cards were difficult to relocate, and often no longer carried eggs. Delays in retrieving the cards (caused by unavoidable events such as storms) also reduced their effectiveness. From all the 297 sentinel cards that we placed in the field we recorded only 2 eggs which had been parasitised, both of which had been collected from within the release site.

Field collected eggs proved to be much more effective at monitoring egg parasitism rates. In our first sample we collected 29 eggs from within the release site, and 10 eggs from within the control site. Of the 29 eggs collected, 13 were parasitized, yielding 25 *Trichogramma pretiosum*. Five of the 10 control eggs were also parasitized, but these yielded 5 *Telenomus* wasps. This sample was taken 41 days after the first release of wasps, and 18 days after the final wasp release. This result suggests that the inoculation of *T. pretiosum* into the field was successful.

However, after the first collection of field laid eggs, insecticide sprays such as Tracer 2 and Affirm were applied to the field. Tracer is known to affect small wasps, and in the subsequent 4 collections, only 3 parasitized eggs were found, all within the release site and all producing one *T. pretiosum* each.

6. Discuss the results, and include an analysis of research outcomes compared with objectives.

Schellhorn's work

Parasitoid abundance in crops and non-crops

Our first objective was to identify spring and early summer crops that are good sources for parasitoids. We met our objective and found that larvae of *Helicoverpa* spp. collected from lupin and pigeon pea had the highest parasitism rate on the spring and summer plants sampled, respectively. Lucerne was also shown to have high parasitism, but larval densities were very low and virus incidence was relatively high. However, lucerne has been shown to provide a good habitat for predators of cotton pests (Mensah 1999, *Int. J. Pest Man.* 45:91-100; Walker *et al* 1997, Proc. 1997 Aust. Ent. Soc. Con. pp57). These findings suggests that the most logical rotation crops to use to generate on-farm source populations of parasitoids are lupin in the winter and spring, before cotton is planted, and pigeon pea in the summer while cotton is growing. There are additional rotation crops that may also be suitable to use to generate parasitoid populations. Previous sampling by Walker et al (1997) found that faba beans may also be a good crop to generate spring populations of parasitoids, and adzuki bean, mung beans, sorghum and sunflowers may generate summer populations of parasitoids. However, none of these crops produced parasitism rates as high as lupin and pigeon pea. It is not specifically known at this time what is responsible for the high parasitism rate on lupin and pigeon pea compared to other plants. The higher parasitism rate is most likely due to different plant characters: e.g. morphology of the plants (wasps may have better access to *Helicoverpa* larvae on lupin or pigeon pea than on other plants), the leaf surface (chick peas are known to produce an acid on the leaves which may make searching for hosts difficult) and cues produced by *Helicoverpa* larvae feeding on plants such as the specific frass or waste material produced by the larvae feeding on lupin compared to sunflowers (wasps use the frass to locate hosts; Quicke 1997 *Parasitic wasps*, Chapman and Hall publishers). There may also be seasonal variation.

Egg parasitism

Egg parasitoids were widespread in the Namoi valley from spring to late summer in crops and non-crops that were not sprayed with OP's and SP's. Once OP's and SP's were sprayed on the back section of ACRI, Narrabri, we no longer found parasitised eggs at that location. However, in unsprayed crops or crops that used "soft options" parasitoids still seemed rare and parasitism was highly variable among plants within a crop and among crops.

One of the more puzzling questions was why we did not find parasitised eggs that were naturally oviposited at the same location that we found parasitised eggs from our "baited-eggs"? The most likely explanation is due to our method of baiting. Tricosane (a chemical in moth scales) is a contact kairomone (a chemical produced by *Helicoverpa* that wasps use to locate their hosts, i.e. *Helicoverpa* egg or larvae) and is known to increase searching by wasps for hosts (Lewis et al. 1975, An. Entomol. Soc. Am. 65:1087-1089). When we bag a moth on a plant for up to 18 hours, she probably leaves a considerable amount of scales on the plant, more so than a moth that is laying eggs "naturally". Therefore, when a wasp does land on the plant with the additional moth scales, she probably searches longer, and subsequently has a higher probability of parasitising an egg, compared with when she lands on a plant with a naturally oviposited egg. The difference in egg parasitism rates between the naturally oviposited eggs and "baited-eggs" contributes to our conclusion that egg parasitoids are rare in the Namoi Valley.

Trichogramma spp. are known to be quite common and fairly effective in agricultural systems (including cotton) in the Darling Downs, Queensland (personal communication Brad Scholz and Dave Murry, Queensland, DPI). Based on research to date, we can only speculate on the possible explanations for the rarity of egg parasitoids in the Namoi Valley. There are three possible explanations, none of which are mutually exclusive. The first is that the Namoi Valley may be near the southern end of the geographical distribution of *Trichogramma* spp. This explanation is based on our egg baiting results from Warren, NSW (further west and a little south) where 744 viable eggs were recovered from unsprayed pigeon pea and cotton crops and only seven eggs were parasitised by *Trichogramma.* We purposely chose these spots because they had extremely high densities of larval and pupal parasitoids. A species geographic distribution is most frequently controlled by environmental factors such as temperature and humidity, for example. The second explanation is that compared to the Darling Downs, where a variety of crops are grown throughout the year, the Namoi Valley predominately grows cotton and autumn and winter are often characterized by large expanses of bare ground that has been prepared for the next cotton growing season. The poor availability of habitats during autumn and winter may contribute to the low population density of *Trichogramma* spp. in the spring. The third possible explanation is that the combination of broad scale insecticide use (especially OP's'and SP's') in cotton and the absence of on-farm nursery crops for egg parasitoids to avoid the spray may exacerbate the decline of a population that is already at a low density. Although egg parasitoids are one of many options to increase mortality of *Helicoverpa*, it may be of interest to conduct a few inundative releases on farms where we control for autumn and winter habitats, and frequent placements of on-farm nursery crops. Regardless, all 3 of the explanations are speculative and may be investigated further.

Feeding on floral and extra-floral nectar

Another objective was to determine if parasitoids (a) feed on floral and extra-floral nectar, (b) if feeding increases adult fecundity (i.e. number of eggs) and longevity, and (c) if an increase in parasitoid fecundity and longevity increases their abundance via increased attacks on *Helicoverpa* spp. Our results suggest that nectar sources vary in quality and wasps that have access to higher quality nectar may live longer and kill more pests. Because cotton appears to provide an adequate source of sugar in the extra-floral nectar, our findings stress the need to identify plants that provide a food source early in the season, before cotton is sown.

Our findings have implications for how we design on-farm landscapes in cotton IPM systems. The crops chosen as nursery crops to build-up parasitoid populations (and predators) in the spring and early summer should provide hosts for wasps to parasitise and subsequently increase their populations, and a high quality food source. These systems will have to be monitored carefully to determine the response of both parasitoid and *Helicoverpa* spp. populations in the spring and early summer. On-farm crop diversity will play a role in cotton IPM systems, however, the right type of diversity will be important.

Monitoring populations of beneficial invertebrates and pests and their dispersal

Our results from surveys of cotton and lupin (and *Echium* spp.) suggest that the low densities of parasitoids observed early in the cotton season are a classic parasitoid-host 'lag response' rather than a seasonal or developmental lag. This is supported by the fact that parasitoid densities can be quite high in September / October in spring crops. This finding would further support the idea of creating early season on-farm populations of beneficial insects to minimise the population 'lag' between *Helicoverpa* (or other pests) and parasitoids / predators. Of course, the right type of early season and mid season crop / vegetation will have to be determined. Lupin and pigeon pea (or unsprayed cotton) show great promise because of their agronomic benefits of crop rotation. They may also be part of the Bt cotton refuge strategy.

There are numerous species of predators and parasitoids in cotton and the surrounding landscape. The fact that parasitoids and predators have to re-colonise cotton each season, and often from tremendous distances, limits the real opportunity to integrate them formerly into early-season pest control. In the mid-season, unsprayed cotton and pigeon pea support more beneficial invertebrates than conventional cotton. Either of these crops could be used as a nursery crop for beneficial insect, but the critical question of whether the beneficial species will move from the nursery crop into the conventional or Bt cotton is still unknown. Such information is necessary before nursery crops can be managed on-farm for beneficials.

Unfortunately, the spring rains and flood of 1999 reduced early-season hosts (*H. punctigera*) and subsequent parasitoid populations. This pattern seem to hold for the entire year because we never saw high densities of *Helicoverpa*, and densities of the most abundant parasitoid, *M. demolitor*, were half that of the previous year. This result led us to quantify predator as well as parasitoid populations.

Although we were unable to monitor parasitoid movement with the dye experiment at ACRI, the work did enable development of novel methods for marking field populations of insects. Our later work in Adelaide (over the past year) with resin-based fluorescent dyes (as opposed to the water soluble fluorescent dyes) has shown great promise. We've been able to simultaneously mark pests and beneficials and monitor their movement under different agricultural practices. This information will contribute to the design of the farm that will increase the abundance of beneficial insects while making sure pest populations are under control. Furthermore, we have also found that the height of the YSB is extremely important for the Diamondback moth – brassica vegetable system, and that buckets placed 20 cm from the ground capture significantly more Diamondback moths and parasitoids than YSB placed 1.3 metres above the ground. These marking and capturing techniques could also contribute greatly to information on insect movement in the cotton agro-ecosystem.

Whitehouse's work

Mass rearing and releases

Our mass releases indicated that parasitoids will move between crops, irrespective of past experience. However, mass rearing *H. scaposum* and *N. producta* for field inoculation does not seem to be a viable option. Although *H. scaposum* was common in the samples, it was difficult to rear. Alternatively, whilst *N. producta* was not common in the samples, but was easier to rear than *H. scaposum*, this process was still very labour intensive.

The low numbers of *N. producta* found in the field may be partly due to the sampling techniques used. Firstly, we sampled all *Helicoverpa* larval classes. *N. producta* is restricted to large *Helicoverpa* larvae. Because all sizes of larvae were sampled, the proportion of larvae parasitized by *N. producta* would necessarily be low. Secondly, *N. producta* cannot be collected in *Helicoverpa* pupae because it produces its own pupae rather than uses that of its host. Finally, *N. producta* tend to be nocturnal. Diurnal, visual searches for wasps could not be expected to yield many *N. producta*.

Following individual wasps

The results of these studies showed that wasps will move between crops, that they show preferences for different crops, and that the preferences change as crops change in attractiveness. The high gradient of wasps on February 13th in the cotton field between the side next to the early season pigeon pea and that next to the late season pigeon pea suggests that the presence of early season pigeon pea increased the number of parasitoids in the adjacent cotton. An alternative explanation is that the wasps had been blown to the edge of the cotton by the wind. This is unlikely because there was no cross wind at the time of the sampling. How the presence of early season pigeon pea increased the number of wasps in the adjacent cotton is unclear. The wasps could have been initially attracted to the early season pigeon pea (which had a very high larval count early in the season) and then moved into the neighbouring cotton when the pigeon pea was no longer attractive. Alternatively, the edge of the cotton next to the early season pigeon pea may have produced a large number of wasps, which we then observed. Whatever the reason, this apparent edge effect needs further investigation.

Abundance of wasps in different crops

The observed differences in the parasitism rates of *Helicoverpa* larvae in conventional and Bt cotton may be due to many factors, including the low density of larvae in the Bt cotton, which would attract fewer parasitoids. The relatively high numbers of parasitoids in the unsprayed conventional cotton, and the low survivorship of the *Helicoverpa* larvae, is very encouraging from an IPM standpoint. Unsprayed cotton may not only act as a refuge for *Helicoverpa*, but also a source for parasitoids.

The results indicate that field peas, lupins (also see earlier for conclusions from N. Schellhorn's work), and Verbine could be good sources of larval parasitoids early in the season – but we acknowledge that sampling efforts for these crops in 2001-02 were limited in scope. Pigeon pea was a reasonably good source of parasitoids in 2001-02, but most parasitism in this crop occurred later than that in cotton. A concern may be that pigeon pea can become a sink rather than a source for parasitoids if managed inappropriately in the cotton environs.

Release of **Trichogramma** *into a commercial cotton field.*

T. pretiosum can be successfully inoculated into cotton fields in the Namoi valley. However, they appear to be susceptible to relatively soft sprays, such as Tracer 2, as has been reported in the literature. In the sample in which the parasitism rate by *T. pretiosum* was relatively high within the release site, the parasitism rate by *Telenomus* was high in the control site. Endemic egg parasitism rates are usually regarded as low within the Namoi valley, however this finding suggests that at least at this site natural egg parasitism was important. As no *Telenomus* sp. were found within the release site it is possible that *T.pretiosum* may have displaced or been in direct competition with *Telenomus*. Perhaps *Telenomus* is spatially erratic in its abundance, but the interaction between *T. pretiosum* and other endemic egg parasitoids should nevertheless be assessed.

These findings suggest that *T. pretiosum* can be established in the Namoi valley on cotton, at least in the short term. Whether they can become established regionally to the extent that they are in the Darling Downs is yet to be determined.

Overall discussion

The aim of this project was to gain a more comprehensive understanding of the biology and ecology of key *Helicoverpa* parasitoids, and thereby indicate ways of effectively augmenting and conserving parasitoid populations in cotton using the most appropriate management techniques. Our work with the egg parasitoids, showed that *Trichogramma* sp and *Telenomus* sp are naturally present at low levels. We found that it is possible to successfully inoculate a field with *T. pretiosum*, but that these animals are very susceptible to sprays as even relatively soft sprays such as Tracer will wipe them out. Whether *Trichogramma* can be established in the Namoi to the degree that it has a strong effect on the survivorship of *Helicoverpa* is unclear.

Our work with the larval parasitoids *N. producta* and *H. scaposum* indicates that inoculation of fields using these animals is unrealistic because their rearing is too labour intensive. Nevertheless, we established that *H. scaposum* is more effective against smaller larvae, whilst *N. producta* is more effective with larger larvae. *N. producta* may also be effective at increasing the mortality of larvae by weakening or killing the larvae by host feeding.

We found that lupin is a good source of larval parasitoids in spring. Field peas and verbine are also potentially good sources for spring parasitoids, but these crops need to be more extensively surveyed to confirm this finding. Unsprayed cotton and Pigeon pea were effective sources of parasitoids in summer. Early planted Pigeon pea may be also a good source in late spring.

Our work on parasitoid movement showed that larval parasitoids will readily move between crops, and that the relative attractiveness of adjacent crops is important, as an attractive crop could draw parasitoids away from an adjacent cotton crop. This potential problem has to be balanced against the likelihood that the attractive crop would also be drawing off *Helicoverpa* moths! Nevertheless the close proximity of an attractive early season crop, such as early season pigeon pea, may increase the number of parasitoids in the adjacent cotton crop, once the pigeon pea loses its attractiveness. We found that the presence of nectar increases fecundity and survivorship of parasitoids. This may also contribute to the relative attractiveness and effectiveness of different crops as sources of parasitoids.

7. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. Where possible include a statement of the costs and potential benefits to the Australian cotton industry and future research needs.

The results from the project clearly demonstrate that parasitic hymenoptera can cause significant mortality to *Helicoverpa* spp., that their populations are high in early- and mid-season legumes, and it may be possible to augment their populations on-farm by using legumes as part of a rotation. These results in conjunction with numerous others on insect predators should continue to substantiate the importance of beneficial insects as part of an IPM program. Increasing reliance on beneficial insects, especially early season, can save growers significant amounts of money in sprays. Additional research is needed to know how to manage beneficial populations with early- and mid-season legumes, and the mechanisms responsible for their movement into the cotton crop.

8. Describe the project technology (eg. commercially significant developments, patents applied for or granted licenses etc).

This is not relevant to this project

9. Provide a technical summary of any other information developed as part of the research project. Include discoveries in methodology, equipment design, etc.

This is not relevant to this project

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

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

Our findings suggest that the presence of early season pigeon pea enhances the number of parasitoids in adjacent cotton. This finding needs to be further tested to establish the extent of the influence, and conditions under which the effect is enhanced.

This finding has direct relevance to work by M. Dillon on the AWM of beneficials (CSE 103c). M. Dillon is looking at the influence of the spatial distrubution of crops in the landscape on the abundance and distribution of beneficials. Our findings, including the observations that 1) parasitoids move between crops, 2) there appears to be strong edge affects, and 3) the relative attractiveness of cotton and pigeon pea to parasitoids changes during the season; will have a strong bearing on the patchwork distribution of parasitoids within a region.

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

One of us (MEAW) plans to give a short presentation of the results at the TRAMS AWM group which includes growers upon whose land some of the work was conducted.

In addition, we plan to publish the following papers:

Tann C., Whitehouse M.E.A., Schellhorn, N.A., G.P. Fitt, and Baker, G. Abundance and distribution of hymenopteran parasitoids of *Helicoverpa* spp in crops and non-crops in Australia.

Manners, A.; Schellhorn N.A., and Fitt, G.P. Access to floral and extra-floral nectar: Increasing longevity, and fecundity of a parasitoid, *Ichneumon promissorius,* of *Helicoverpa* in cotton.

Whitehouse, M.E.A; Johns, C. A. and Baker, G. The movement of large parasitoids between adjacent crops: The importance of timing.

Schellhorn, N.A., Schellhorn, N.A., Siekmann, G., Paull, C., and G. Furness. Using dyes and dusts to mark natural populations of insects in the field. Journal of International Pest Management.

11. List the publications arising from the research project.

Schellhorn, N.A. and L.X. Silberbauer. 2003. The role of surrounding vegetation and refuges: Increasing the effectiveness of natural enemies in cotton and broccoli systems. **Proceedings for the 1st International Symposium on the Biological Control of Arthropods**. Honolulu, Hawaii, USA. *In press for release 2003*

Schellhorn, N. A. 2001. Parasitoids in cotton. **Australian Cotton Grower**. 22:44-47.

Schellhorn, N.A., A. Manners, and G.P. Fitt. 2000. Augmentation and conservation of parasitoids of Helicoverpa spp. Findings from the first field season. **Proceedings, 10th Cotton Conference**. Brisbane, Queensland, Australia.pp103-111.

Publications submitted

Johns C. V. and Whitehouse M.E.A. Laboratory rearing of two larval parasitoids of *Helicoverpa* spp. (Lepidoptera: Noctuidae): *Netelia producta* (Brullé) and *Heteropelma scaposum* (Morley) (Hymenoptera: Ichneumonidae). Submitted to: the **Australian Journal of Entomology**.

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12. Are changes to the Intellectual Property register required?

No.

Part 4 – Final Report Plain English Summary

Provide a half to one page Plain English Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web.

The aim of this project was to gain a more comprehensive understanding of the biology and ecology of key *Helicoverpa* parasitoids in cotton ecosystems, and thereby indicate ways of effectively conserving or introducing them there, using the most appropriate management techniques. Our work with the egg parasitoids showed that *Trichogramma* sp and *Telenomus* spp. are naturally present at low levels. We found that it is possible to successfully introduce *Trichogramma pretiosum* into a field of cotton in the Namoi region, but that these animals are very susceptible to sprays as even relatively soft sprays such as Tracer will wipe them out. It is unclear whether *Trichogramma* can be established in the Namoi region to the degree that it has a strong effect on the survivorship of *Helicoverpa.*

Our work with the larval parasitoids *Netelia producta* and *Heterpelma scaposum* indicated that effectively introducing these animals into cotton fields is unrealistic because their rearing is too labour intensive. Nevertheless we established that *H. scaposum* is more effective against smaller larvae, whilst *N. producta* is more effective with larger larvae. *N. producta* may also be effective at increasing the mortality of larvae by weakening or killing them through direct feeding.

We found that lupin was a good source of larval parasitoids in spring. Field peas and verbine are also potentially good sources for spring parasitoids, but these plants need to be more extensively surveyed to confirm this finding. Unsprayed cotton and pigeon pea were effective sources of parasitoids in summer. Early planted pigeon pea may also be a good source of parasitoids in late spring.

Our work on parasitoid movement showed that larval parasitoids will readily move between crops, and that the relative attractiveness of adjacent crops is important, as an attractive crop could draw parasitoids away from an adjacent cotton crop. This potential problem has to be balanced against the likelihood that the attractive crop would also be drawing off *Helicoverpa* moths! Nevertheless the close proximity of an attractive early season crop, such as early season pigeon pea, may increase the number of parasitoids in the adjacent cotton crop, once the pigeon pea loses its attractiveness.

We found that the presence of nectar increases fecundity and survivourship of parasitoids. This may also contribute to the relative attractiveness and effectiveness of different crops as sources of parasitoids.

Appendix 1

Laboratory rearing of two larval parasitoids of *Helicoverpa* spp. (Lepidoptera: Noctuidae): *Netelia producta* (Brullé) and *Heteropelma scaposum* (Morley) (Hymenoptera: Ichneumonidae).

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Running title: Rearing parasitoids *Netelia* & *Heteropelma*

ABSTRACT

Parasitoids are becoming an increasingly important part of our biological arsenal in Integrated Pest Management strategies against major insect pests such as the moth *Helicoverpa armigera*. In order to use them effectively we need to be able to rear large numbers for mass release. In this paper we report our attempts to rear larval parasitoids of *Helicoverpa* spp: *Heteropelma scaposum* and *Netelia producta*. Our results suggest that *N. producta* is the easier species to culture, although our parasitism rate with both wasps was not high (29% for *N. producta* and 19% for *H. scaposum*). We suggest ways in which the parasitism rate could be increased and culturing made easier. We also found that culturing *H. scaposum* resulted in a very male biased sex ratio - not very suitable for mass release. We discuss possible reasons for the bias, including single locus complementary sex determination (CSD) which can result in a high proportion of homozygotic diploid males occurring in inbred populations. Although we suggest that single locus CSD is a plausible explanation for the male biased sex ratio, we caution that more work is needed to confirm that it can occur in *H. scaposum*.

Key words: mass rearing, sex ratio, IPM

INTRODUCTION

Helicoverpa armigera (Hubner), an insect pest of major economic significance in Australian cotton, has proven difficult to manage due to its rapid development of resistance to a range of pesticides, including endosulfan, pyrethroids, carbamates, and organophosphates (Pyke & Brown 1996). Considerable research into alternative control methods, including utilization of natural enemy populations, is occurring in order to combat the growing resistance problem (e.g. Walker *et al.* 1997; Mensah 1999; Johnson *et al.,* 2000).

One group that has been identified as potentially important as biologicial control agents of *Helicoverpa* spp. are parasitoids. The egg, larval, and pupal stages of *Helicoverpa* spp. in field crops in Australia are parasitised by at least forty five different native wasp and tachinid fly species (Johnson *et al.* 2000). Hymenoptera reared from *H. armigera* or *H. punctigera* in Australian cotton include species from the families Ichneumonidae (eg *Heteropelma scaposum* (Morley), *Ichneumon promissorius* Erichson, *Lissopimpla excelsa* (Costa) and *Netelia producta* (Brulle)); Braconidae (eg *Microplitus* spp. and *Cardiochiles* sp.); Trichogrammatidae (eg *Trichogramma* spp., *Trichogrammatoidea* spp. and *Trichogrammatoides* spp.); Scelionidae (eg *Telenomus* spp.); Chalcidae (eg *Brachymeria* spp.) and Pteromalidae (Zalucki *et al.,*1986; Johnson *et al.*, 2000).

Most previous research effort has been focused on the potential usefulness in Integrated Pest Management (IPM) of the trichogrammatid egg parasitoids, and also the braconid larval parasitoids *Microplitis demolitor* (Wilkinson) and *Cotesia kazak* (Telenga) (reviewed by Johnson *et al.*, 2000). Although the ichneumonids *Heteropelma* and *Netelia* are frequently encountered parasitoids of larval *Helicoverpa armigera* (Pyke and Brown, 1996; Johnson *et al.*, 2000), relatively little is known about the biology of these genera and their potential usefulness as biocontrol agents.

To be useful biocontrol agents, parasitoids must be easily cultured to allow for the inoculative, augmentative and/or inundative releases of large numbers. A factor that may limit the culturing of large numbers, and therefore the potential importance of a parasitoid species, is the presence of single locus complementary sex determination

(CSD, Stouthamer *et al.* 1992, Cook 1993). In species with single locus CSD, one locus determines sex. Females result when the animal is diploid (heterozygotic) for that locus, while males normally result when the animal is haploid (hemizygotic). However, when diversity is low, diploid males can also occur when the animal is homozygotic for that locus. Homozygotic males either don't survive or are sterile, so that if a female only mates with these males, she can only produce males. The chance of obtaining homozygotic males increases at a genetic bottleneck, such as that which occurs when a small sample of individuals are used to rear up large populations during culturing. It has been hypothesized that the relatively high rate of biocontrol failures with Ichneumonidae and Braconidae wasps may stem from the reduced genetic variablility at the sex locus caused in part by inbreeding during culturing (Stouthamer *et al.* 1992).

This paper reports on our efforts to mass rear two common ichneumonid larval parasitoids of *Helicoverpa armigera*: *Heteropelma scaposum* (Brullé) an endoparasite (commonly called the two-toned caterpillar parasite) and *Netelia producta* (Morley) an ectoparasite (commonly called the orange caterpillar parasite). Our primary aims in carrying out this task were to determine which species was the easiest to rear in the laboratory, and to rear a large number of these wasps over a short period of time for field release. We discuss life history characteristics of these animals, factors that influenced the relative suitability of each species for mass rearing, and some ways in which our rearing technique could be modified to increase production.

METHODS

Our laboratory colonies of *N. producta* and *H. scaposum* were established in October 2001 using adult wasps collected from wheat, lucerne, lupin and sunflower crops in the Namoi valley (*H. scaposum* specimens are deposited in the CSIRO Entomology collection, Narrabri, *N. producta* specimens are deposited in the CSIRO Entomology collection, Canberra). Further *H. scaposum* individuals were also obtained throughout the rearing period (October 2001 till mid-January 2002) from *H. armigera* pupae collected under cotton, field pea, faba bean, lucerne, pigeon pea and chick pea crops in the Namoi valley.

Wasps were housed in one of 11 perspex cages with cloth sleeves for access. Cage sizes varied: four small (33x33x30cm) six medium (40cm x 40cm x 40cm), and one large (60x61x90cm). These cages contained a maximum of 50, 100 or 400 wasps respectively. Depending on wasp numbers, one to five cages were used to house each species at a time. Water and a 10% honey solution were supplied to the wasps via a cotton wool wick emerging from the lid of a 70ml screw-top jar, half filled with water or honey solution. Bunches of cut flowers and foliage were also put into each cage (cotton, pigeon pea, lucerne or brassica) which provided the wasps with an alternative food source and with surfaces to perch on. Without the foliage the wasps crowded into the corners of the cages, struggling with each other while they attempted to climb the smooth walls. Cage interiors were wiped clean every two days using a weak solution of Cavicide™, and washed in a 5% bleach solution approximately once per fortnight.

We supplied *H. armigera* caterpillars (instars 3-5) reared on a soy-based diet medium (Teakle & Jenson 1985) to the colonies to serve as hosts. These remained in the cages, with food, from half a day to five days depending on wasp density, before replacement. After removal from a wasp colony, each caterpillar was allocated a unique reference number and was reared in a correspondingly numbered container on artificial diet. When caterpillars reached the pre-pupal non-feeding stage they were moved into 30ml containers half-filled with vermiculite within which they could burrow to pupate. After pupation the vermiculite was removed so that emerging wasps would have room to move and could be easily observed.

Sub-samples of the caterpillars removed from the wasp colonies were immediately placed under a dissecting microscope so that the numbers of eggs (*N. producta*) or ovipositor puncture marks (*H. armigera*) per host could be recorded. Towards the end of the rearing program we re-introduced caterpillars that had not been initially attacked to the *N. producta* colony.

We recorded data on the fates of all hosts, including when they were added to and removed from the wasp cages, deaths, whether and when moths emerged, and the development times and sexes of parasitoids that emerged. Newly emerged parasitoids were added to the main breeding colonies. Wasp behaviour was casually observed when caterpillars were added and removed from the cages.

The calculation of parasitoid development time was a problem because caterpillars were exposed to the parasitoid colonies for half a day to 5 days. Development times were calculated for all wasps as the number of days between the oviposition date and the date of adult wasp emergence. When caterpillars were in a cage for more than one day, oviposition date was estimated as the average date (rounded up to the nearest whole day).

RESULTS

N. producta

Determining successful parasitoid oviposition and development

N. producta females oviposited on the host surface, usually in body creases close behind the host's head where they could not be removed by the host's mandibles. The presence or absence of *N. producta* eggs or larvae could be determined with the naked eye. *N. producta* larvae remained feeding on the exterior of the host until after the host burrowed into the substrate and created a pupal chamber. At this point the parasitoid larva would finish feeding from the host, causing it to die, before pupating in a black silken cocoon within the hosts pupal chamber. *N. producta* larvae would only complete development if the host remained healthy enough to burrow into the vermiculite provided and form a pupal cell. Where the host was not sufficiently healthy to burrow into the substrate, regardless of size, the wasp larva did not succeed in spinning a full cocoon or in completing development. Some were observed to reach almost complete development on top of the substrate without a full cocoon (approximately 60-70 individuals, 1180 wasps successfully emerged), but these appeared to become dessicated and did not survive, or they were weak and survived for less than 12 hours in cages with other adults.

Effect of host instar on oviposition success

Our observations indicated that *N. producta* females were more effective with large (5th and 6th instar) caterpillars. *N. producta* females stung the caterpillars one or more times to paralyse them before ovipositing. After ovipositing, *N. producta* would often host feed before the caterpillar recovered from paralysis.

Larger caterpillars were more capable of surviving the combination of *N. producta* stings, oviposition and feeding than smaller (3rd and 4th instar) caterpillars, which often died whilst caged or shortly after removal. Third and 4th instars suffered such high mortality with *N. producta* that we quickly stopped offering them to the wasps. Small caterpillars that survived with high *N. producta* egg loads appeared to be less effective hosts than larger caterpillars. The burden of the parasitoid larvae would often overwhelm small caterpillars before they burrowed into the substrate, resulting in the death of both host and parasitoids.

Parasitism success and sex ratios

In total, 4063 caterpillars were presented to *N. producta*, producing 1180 wasps. The overall parasitism success (measured as % of hosts yielding wasps) was 29%. *N. producta* commonly laid multiple eggs on a single host and we observed numerous individual caterpillars that had multiple *N. producta* larvae feeding and developing on the outside. Despite the high levels of superparasitism, only 2% of the 1152 caterpillars that yielded wasps, produced more than one wasp.

Of the 2%, there were 26 cases of single hosts yielding two *N. producta* adults (10 with 2 males, 11 with 2 females, 5 with 1 male and 1 female) and one case where 3 wasps emerged (2 females and 1 male). There were significantly fewer mixed pairs than expected by chance (Goodness-of-fit test, $df = 2$, $\chi^2 = 9.92$, $P < 0.01$). Of the 26 hosts that yielded two *N. producta*, 5 had been checked for parasitoid eggs. Of these, 2 had 3 eggs, 1 had either 4, 5, or 8 eggs.

Effect of egg numbers on wasp production

Production of *N. producta* increased with the number of eggs laid on the caterpillar host, peaking at 61% for five eggs per host. Host and parasitoid mortality rapidly increased as the egg load rose above five (Fig. 1).

Effect of egg numbers on sex ratio

Fifty-three percent of *N. producta* were female. There was no evidence that the number of eggs on the caterpillar influenced the likelihood of a male or female wasp emerging (Mann-Whitney U test, U=4080, P=0.69, mean female rank=95, mean male rank = 92).

H. scaposum

Determining successful parasitoid oviposition and development

We found it difficult to accurately determine whether a caterpillar had been attacked by *H. scaposum*. *H. scaposum* oviposited through the integument of the host, often leaving little evidence of parasitism on the surface, especially when the host was heavily pigmented. The first reliable indication of successful parasitism occurred after host pupation, when parasitised individuals became darkly spotted, longitudinally distended, and immobile whilst the wasp pupated within.

Effect of host instar on oviposition success

Our observations indicated that *H. scaposum* females were not effective with large (5th and 6th instar) caterpillars as they were likely to be dislodged by the struggling and biting of large caterpillars. *H. scaposum* females were more effective with smaller (3rd and 4th instar) caterpillars.

Parasitism success and sex ratios

In total, 2590 caterpillars were presented to *H. scaposum*, producing 483 wasps. The overall parasitism success was 19%. Although *H. scaposum* commonly laid multiple eggs in a single host (Fig. 2) only one wasp ever emerged from a host.

Effect of scar numbers on wasp production

Puncture marks from *H. scaposum* were difficult to count accurately on heavily pigmented caterpillars, such as those that moulted just prior to puncture counts. Of the 423 caterpillars on which punctures were counted, 90% had ≤ four punctures. Twenty-eight caterpillars that had no puncture marks produced wasps. Mortality increased rapidly as the number of punctures approached four. Above four punctures it was difficult to get a clear picture of the relationship between puncture marks and host fate because so few caterpillars had more than four puncture marks. There was no clear relationship between punctures counted (starting at one puncture) and the success of hosts in yielding wasps compared to hosts dieing or developing into moths (Mann Whitney U test, U=2126, P=0.44, average wasp rank =78, average moth/death rank= , Fig.2).

The percentage yield of *H. scaposum* was highest when 12, 14, 20, or 22 punctures were observed (100% wasp emergence), however only one host occurred in each of these categories. Fifty percent wasp yield was found when there were 5 punctures (n = 2). The next highest yields were of 29%, from one and seven punctures (n = 52 and n = 7 respectively).

Effect of puncture scars on sex ratio

Only 25% of *H. scaposum* were female. There was no evidence that the number of scars on the caterpillar influenced the likelihood of a male or female wasp emerging (Mann-Whitney U test, U=352, P=0.27, mean female rank=36, mean male rank=31).

N. producta and *H. scaposum* development times

Development occurred significantly faster for *N. producta* than for *H. scaposum* individuals (ANOVA, F= 748, P<0.001, *N. producta*: n=995, mean= 27days, *H. scaposum*: n=493, mean= 31 days to develop DF=3, Fig 3). There was no significant effect of sex on development time (ANOVA, F = 2.77, P = 0.096 DF=3).

We did not collect quantitative data on the lifespans of individuals in this study, however *H. scaposum* adults survived in cages for about 2-3 weeks whilst *N. producta* in cages generally survived for longer (approximately 4 weeks in total).

DISCUSSION

Parasitism success.

Over the course of three months we were able to rear 1663 wasps for mass release from 6653 caterpillars. Overall, we found *N. producta* to be more amenable to mass rearing in the laboratory than *H. scaposum*. This was due to several differences between the life history strategies of the species, including ease with which parasitism rates could be determined, host instars used, development times, adult lifespan, and sex ratio.

Multiple wasps from one host

While *H. scaposum*- parasitised caterpillars produced a maximum of only one wasp, some caterpillars parasitised by *N. producta* yielded multiple wasps. This may indicate that different types of larval competition are occurring in the two species. Larvae competing for food in a host may use scramble competition, where all individuals have an equal probability of acquiring food, or by contest competition, where some individuals have a competitive advantage and gain a greater share of the food (Nicholson 1954). As *H. scaposum* larvae feed inside the host, they would use contest competiton, as they would be in direct competition for food, providing the opportunity of one larvae to kill the others, as is common in such parasitoids (Quicke 1997). *N. producta,* alternatively, feed on the outside of the host. They could compete through scramble competition, with the larvae that fed for the longest period surviving, or possibly by indirect contest competition, where a larvae may inhibit the development of others by injecting an inhibitor into the host while it feeds. Whatever the means, dominance by one larvae was not complete in *N. producta* as multiple wasps were able to emerge.

Host size at the time of parasitism may have affected the likelihood of more than one *N. producta* completing development per host, although we have no size data to confirm this. Despite the potential for multiple wasps from one host, only 2% of caterpillars that yielded wasps actually produced more than one *N. producta,* indicating that this phenomenon is unlikely to greatly contribute to *N. producta* wasp production in a mass rearing program.

Although there was no difference in the likelihood of obtaining all male or all female *N. producta* “twins”, same sex twins occurred more often than expected by chance. This suggests that there may be some unevenness in larval competition between the sexes, or that different-sexed larvae on the same host are less compatible.

Parasitism rates.

We obtained higher parasitism rates for *N. producta* (29%) compared to *H. scaposum* (19%) suggesting that the former wasp may be more suitable for mass rearing. However, the differences between *N. producta* and *H. scaposum* may be artificially inflated. *H. scaposum*-attacked caterpillars were difficult to dissern as puncture marks were time-consuming to find and difficult to count accurately (as is indicated by the number of caterpillars that were recorded with no puncture marks, but which nevertheless yielded wasps). However *N. producta* attacked caterpillars were easy to discern as they attached eggs to the outside of the host. Towards the end of the study we used this knowledge to reduce time wastage and improve *N. producta* yield by rearing only hosts that were parasitised. This may have artificially raised the overall parasitism success rate for *N. producta*.

Host age.

The use of younger hosts for *H. scaposum* meant that more time and handling of infected caterpillars was necessary during rearing than was the case for *N. producta*. Older hosts could be placed directly into vermiculite with a small piece of diet on top and left to pupate without further handling. Younger caterpillars required much more food, and so needed to be transferred first on to diet, then sometimes transferred again onto fresh diet before going into the vermiculite. They could not be put straight on to vermiculite with a larger amount of diet as the diet would become mouldy before feeding was complete.

Development time.

Longer development times for *H. scaposum* than for *N. producta* (Fig. 3) contributed to slower population growth. The need for *H. scaposum* to spend a longer period of time feeding on the caterpillar host meant that more effort was spent on host rearing. It may be possible to rear *H. scaposum* hosts from parasitism until pupation without transferral into fresh containers, thereby decreasing handling time. Survivorship may however be reduced as healthy *H. armigera* allowed to pupate in diet suffer higher pupal mortality than those that pupate in vermiculite (pers. com. T. Parker, 2001).

Differences in sex ratio

The proportion of females present was higher for *N. producta* than for *H. scaposum*. This may have contributed to our greater success in building up *N. producta* numbers quickly, and again indicates that *N. producta* may be more amenable to mass rearing.

The fact that we produced such a high number of male *H. scaposum* (75%) is intriguing, and could be due to a number of factors. First, it could indicate that many of the females producing offspring were unmated. This is unlikely given the large numbers of males in the colonies.

Second, it could indicate that *Helicoverpa* are inferior hosts. When parasitoids encounter inferior hosts, they tend to produce males rather than females (Charnov et al 1981, Godfray 1994, Quicke 1997). It is unlikely that the *Helicoverpa* larvae were too small and therefore inferior because *H. scaposum* preferred to attack smaller individuals, and full development was delayed until the caterpillar was large enough to pupate. *Helicoverpa* larvae are unlikely to be an inferior host species because local wild-caught caterpillars raised in the laboratory produced equal proportions of male and female *H. scaposum* during the 2001/2002 season (unpubl. data, C. Tann & T. Parker).

Third, hyperparasitism can result in altered sex ratios, as later females tend to deposit more males (Godfray 1994). In solitary species this would alter the sex ratio if the later (male) arrivals were more effective at killing older competitors. We found no evidence to support this scenario. The number of eggs implanted on the host did not affect the sex of the wasp for either *H. scaposum* or *N. producta*, suggesting that larval competition within the host did not benefit either sex. If hyperparasitizing wasps were more likely to implant males, they did not gain an advantage.

A further possible reason for the male biased sex ratio is that single locus complementary sex determination may occur in *H. scaposum*. CSD is known to occur in a number of Ichneumonoids, including *Diadromus pulchellus* , *Habrobracon hebetor*, *Habrobracon serinopae* and *Microplitis croceipes* (Stouthamer et al 1992) but there have been no reports of skewed sex ratios or of CSD in *M. demolitor*, even though this species has be subject to intensive rearing studies (Murray & Rynne, 1991). In addition, attempts to rear *Ichneumon promissorius*, which is in the same sub-family (Anomaloninae) as *D. pulchellus* (Gauld 1984), have found no evidence of a biased sex ratio (T.Parker, pers. comm.).

Single locus CSD means that both haploid and diploid males can be produced. Diploid males occur when the alleles at the sex determining locus are homozygous (heterozygous alleles result in a female) and are more likely to occur when genetic variance is low, such as during culture rearing. In our rearing program we supplemented our colony with individuals cultured from parasitised wild-caught caterpillars. *H. scaposum* cultured from parasitised wild-caught caterpillars did not have a biased sex ratio, but their addition to the colony did not improve the sex ratio of offspring produced. This indicates that either some other mechanism may be causing the sex ratio bias of our colony, or that the genetic diversity of the additional *H. scaposum* was not enough to offset the production of diploid males.

The male biased sex ratio that we found rearing *H. scaposum* may indicate the presence of single locus complementary sex determination in this species. Further studies, however, are needed to establish if this is correct. If *H. scaposum* does have CSD, then this could further limit its potential as a biological control agent.

Improvements to management techniques

To fully understand the life history of *N. producta* and *H. scaposum* and improve our chances of success with mass rearing, we still need to know about the fecundity of individual females. Further investigations are also needed into adult longevity and the relationships between adult age and reproductive output for both species.

Despite these gaps in our understanding, our experience at rearing these parasitoids has enabled us to draw some conclusions to enhance future mass rearing programs. In the present study we reared all hosts separately in numbered containers in order to collect data on host fate such as individual development times and wasp sex. If the fates of individuals are not tracked in this way, the production process for mass rearing would be much less labour intensive. In addition, if individual wasp emergence dates do not need to be known, pupae from each cohort would not need to be separated into individual containers, again saving time.

We recommend that in order to produce large numbers of larval parasitoids quickly, *N. producta* rather than *H. scaposum* wasps should be used. Caterpillars should be raised individually until 5th instar, and then introduced to parasitoids for 1-2 days.

Uninfected caterpillars can be re-introduced. An optimum of 4 eggs per caterpillar should be aimed for. Once attacked, caterpillars can go straight into vermiculite trays and adult wasps harvested as they emerge.

Although the mass rearing of egg parasitoids, such as *Trichogramma* spp, is widely applied to control lepidopteran pests, little attempt has been made to mass rear larval parasitoids in cotton, although attempts have been made with the small larval parasitoids, *Microphytis demolitor* (Murray and Rynne 1991) and *Cotesta kazak* (Harrington 1983, D. Murray pers. comm.). Our work indicates that it could be possible to mass rear *N. producta*, but currently this process is very labour intensive. More work needs to be done to establish the impact of larval parasitoids on *Helicoverpa* populations and whether the impact of larval parasitoids such as *N. producta* is great enough that a mass release of these beneficials would be economically advantageous.

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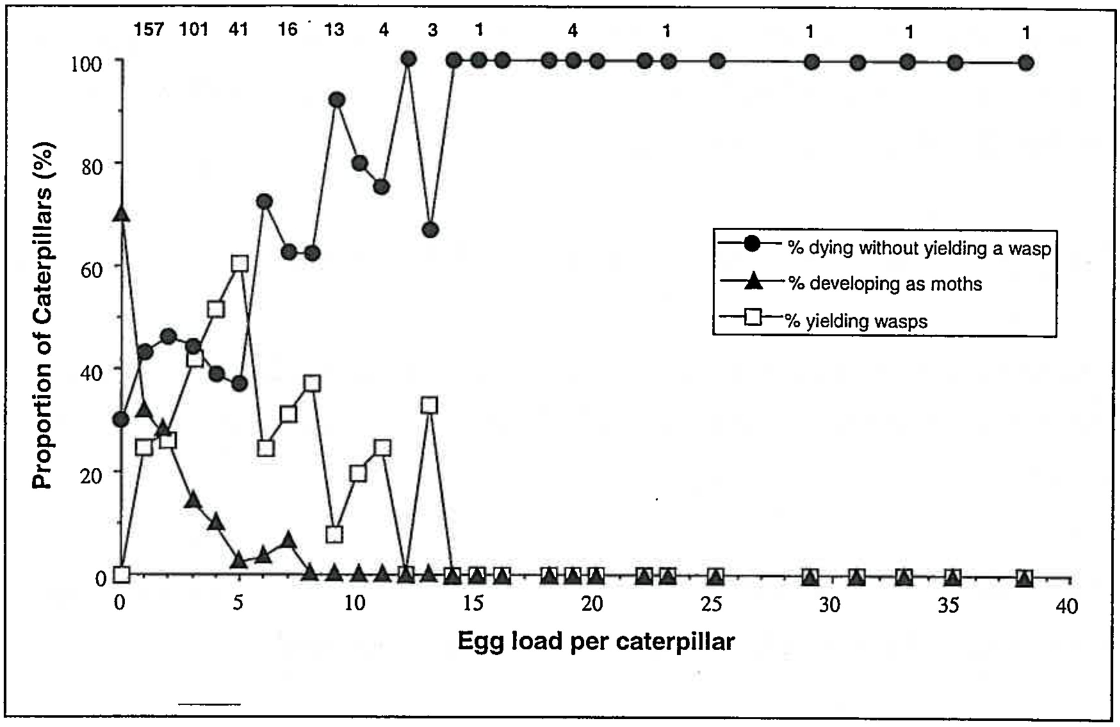

Fig. 1. The fate of caterpillars atacked by *N. producta* (expressed as a percentage). The numbers along the top indicate the total number of caterpillars with approximately every second recorded egg load.

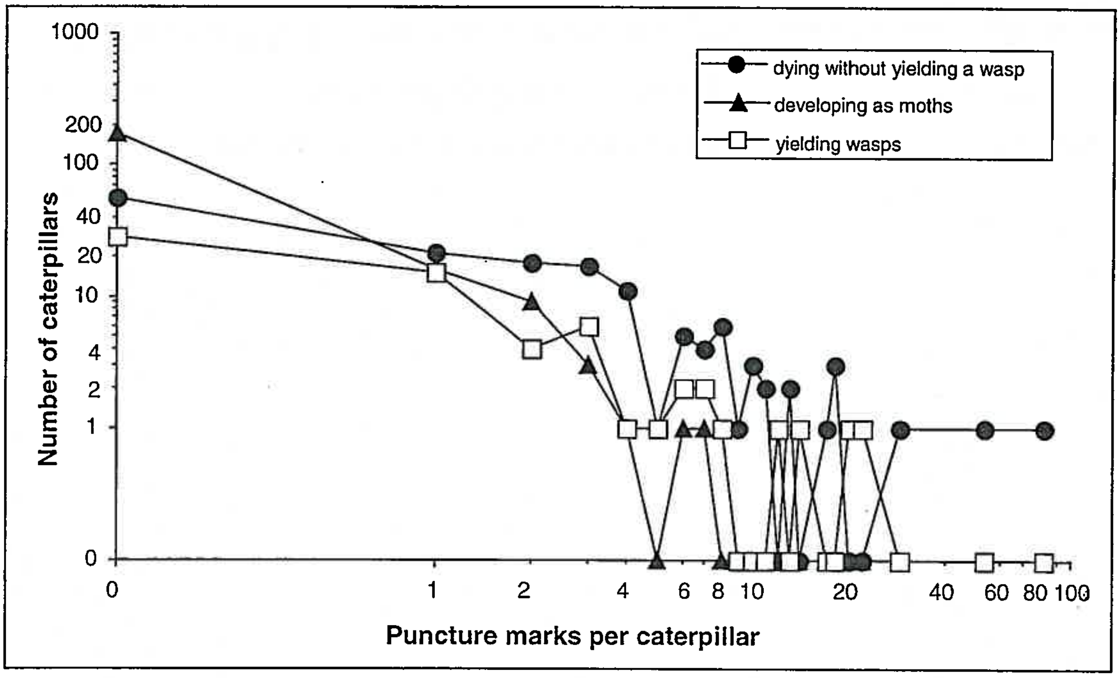

Fig. 2. The fate of caterpillars attacked by *H. scaposum* on a semi log/log scale. Twenty-eight caterpillars that had no visible puncture marks produced wasps.

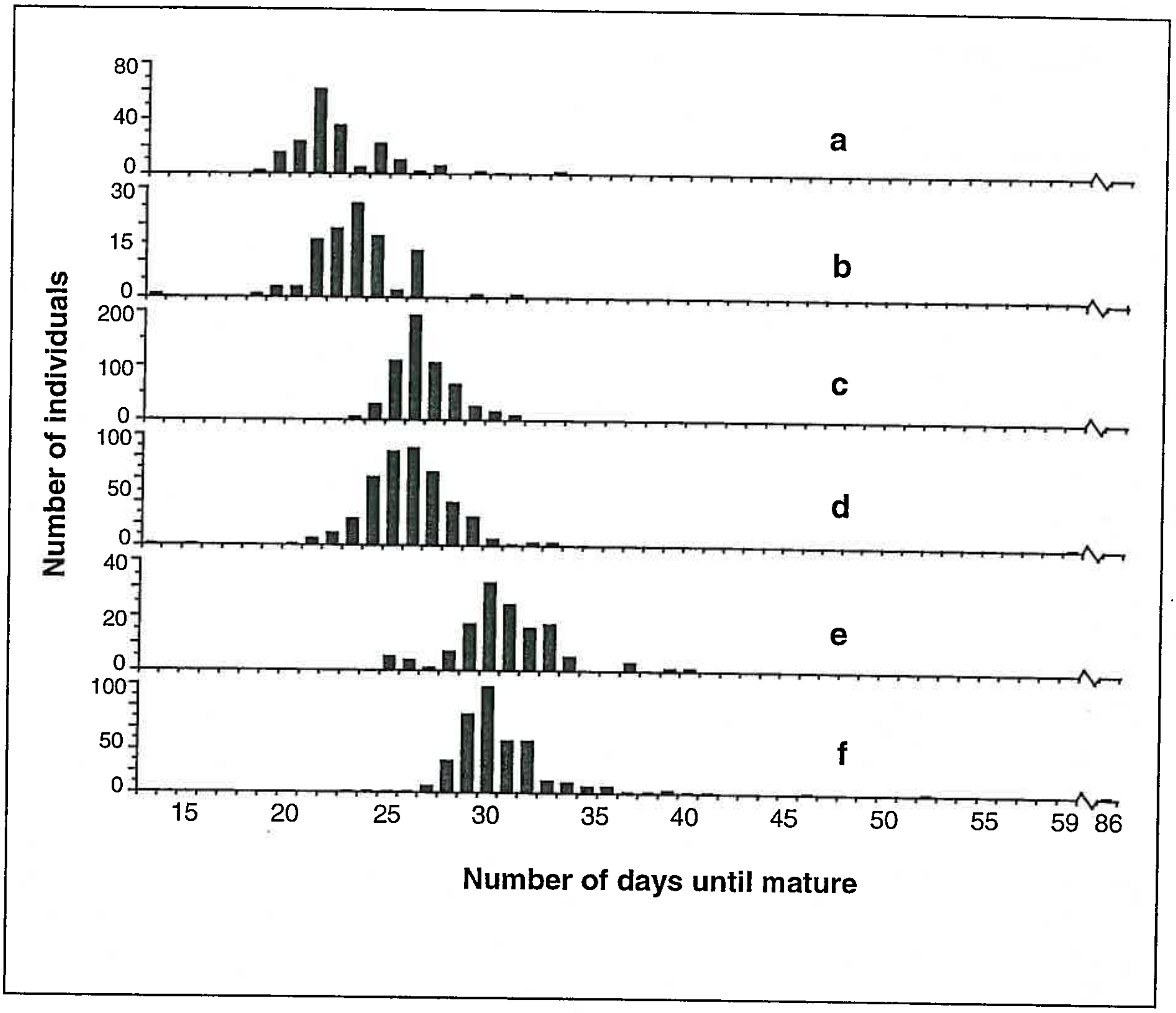

Fig. 3. Developmental times of moths and parasitoids from caterpillars exposed to parasitoid wasps. All parasitoids took longer than moths to develop, and *H. scaposum* wasps took longer than *N. producta* wasps to develop. **a**: moths exposed as caterpillars to *H. scaposum*; **b**: moths exposed as caterpillars to *N. producta*; **c**: *N. producta* females wasps; **d**: *N. producta* male wasps; **e**: *H. scaposum* female wasps; **f**: *H. scaposum* male wasps.

Appendix 2

Publications

THE ROLE OF SURROUNDING VEGETATION AND REFUGES: INCREASING THE EFFECTIVENESS OF PREDATORS AND PARASITOIDS IN COTTON AND BROCCOLI SYSTEMS

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ABSTRACT

Cultural practices such as crop rotation and planting of hedge rows or wind breaks can enhance the abundance of insect predators and parasitoids. However, in assessing the value of such practices, too little attention has been given to the consequences of natural enemy movement. We used novel marking techniques (insect-borne pollen and resin-based fluorescent dyes) in two distinct systems to monitor movement of natural populations of insect predators and parasitoids at the landscape and farm levels. For the cotton system, our results show that the two most abundant insect predators, *Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae) and *Diomus notescens* (Blackburn) (Coleoptera: Coccinellidae), visited several types of vegetation (crop and non-crop) before visiting cotton, and that they moved back and forth between cotton and other types of vegetation. These results show that particular types of vegetation are attractive to predatory insects found in cotton, suggesting the manipulation of vegetation might be used to increase these predators' abundance in the landscape and on-farm. For the broccoli system, we found that the majority of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) dispersed to the adjacent planting of broccoli (within 12 m) before and after cultivation. However for the parasitoid, *Diadegma semiclausum* (Hellén) (Hymenoptera: Ichneumonidae), the majority of marked individuals dispersed further than the adjacent broccoli and 5% of marked individuals dispersed to 60 m in 48 hours before cultivation, and 7% of marked individuals dispersed to 108 m after cultivation. These findings show that *P. xylostella* (L.) and D. *semiclausum* are dispersing differently, and that disturbance from cultivation caused more *D. semiclausum* to disperse further.

Use of these marking techniques allows us to understand links between alternative habitats and crops, enhancing cultural and biological control of pests.

INTRODUCTION

The direct effects of cultural practices on insect pests have been extensively evaluated (Rabb *et al.*, 1984; Herzog and Funderburk, 1986; Dent, 2000). The majority of these practices have been designed to modify crop production to lower pest densities through sanitation, destruction of alternate habitats or hosts used by the pest, tillage, crop rotation or fallowing, manipulation of planting and harvesting dates, trap cropping, and manipulation of vegetational diversity. How cultural practices can be used to increase the effectiveness of natural enemies of insect pests has been less studied (Schellhorn *et al.*, 2000). Cultural practices can affect natural enemy population density and species diversity, and manipulation of these preactices can provide the foundation for conservation biological control.

Many studies demonstrate that cultural practices affect natural enemies. Trap crops (Corbett *et al.*, 1991), rotation crops (Xia, 1994), creation of hedge rows (Coombes and Sotherton, 1986; Wratten and Thomas, 1990; Dennis *et al.*, 2000), and manipulation of non-crop habitat can enhance natural enemy abundance (Banks, 1955; Perrin, 1975; Andow, 1991; Schellhorn and Sork, 1997; Landis *et al*, 2000). However the majority of these studies are descriptive and usually only compare the abundance of natural enemies in one production system or habitat to another. In order to develop predictions about how particular cultural practices change the abundance or effectiveness of predators and parasitoids, it is necessary to understand the underlying population processes, such as movement, reproduction, and longevity (Corbett and Plant, 1993; Prasifka *et al.*, 1999; Schellhorn *et al.*, 2000).

Here we report on the use of novel marking techniques to monitor the movement of natural populations of insect predators and parasitoids at the landscape and whole farm levels. We conducted studies in two distinct systems in Australia, cotton in New South Wales and broccoli in South Australia. The cotton (*Gossypium hirsutum* L.) system was characterised by a summer crop that grows for six months, followed by bare soil for six months, requiring that pests and natural enemies colonize each field anew at the beginning of the cropping season. The broccoli *(Brassica oleracea* L. *var. "marathon")* system was characterised by Mediterranean climate (hot-dry summers, and cool-wet winters), where brassica vegetables are in continuous production year round. This results in resident populations of the major pest and its parasitoids.

MATERIALS AND METHODS

Cotton System

Insect predator abundance in crops and non-crops. Insect samples were taken from vegetation in the Namoi Valley in northern New South Wales, Australia. Sampling focused on three cotton fields, one on each of three farms, which were within a 4 km radius of each other. At each site we employed a standardised sampling technique of running a suction sampler across random 20 m sections of vegetation, repeated five times. The details of this method including the types of vegetation sampled are outlined in Silberbauer and Gregg (2002).

Movement of insect predators. To determine whether insect predators were moving among the different types of vegetation, a sub-sample (n=199) of insects collected were examined for pollen. Insect specimens were prepared for scanning electron microscopy (SEM) by breaking them into four or five pieces, and then adhering them to SEM stubs using double-sided poster tape. SEM stubs were placed in a low-temperature oven (40-60°C) for at least 12 hours prior to sputter coating with gold. Each piece of insect was then examined under at least 500x magnification.

Any pollen found was examined under at least 1000x magnification and identified using Peter Gregg's pollen SEM photographic library (unpublished) and Jones *et al.* (1995). As many pollen species as possible were identified to species or family level. Because the descriptions of Australia's pollen flora is still incomplete, many of the pollen grains could not be identified, and thus were just labelled with numbers. All pollen species found were photographed and given identifying numbers.

Broccoli System

The Adelaide plains is the main vegetable producing area of South Australia. Brassica vegetables are in continuous production year round, which results in a resident population of the major pest, the diamondback moth, *Plutella xylostella* (L.), and its most abundant parasitoid, *Diadegma semiclausum* (Hellén).

Pest and parasitoid abundance in mature broccoli. Monitoring the movement of natural populations of insects in fields involves three steps. First the density of insects of interest must be sufficiently high to allow the use of mark and capture techniques. Second, the mark must be identifiable on the species of insects that are to be evaluated. Third, the capture methods must not cause any cross contamination or removal of the mark, and any biases should be known. To monitor the movements of *P. xylostella* and *D. semiclausum* from mature to young broccoli , we created conditions favouring rapid insect population growth by withholding irrigation for two weeks and insecticides for five weeks. In addition, because the experiment took place on a grower's property, we arranged for the grower to withhold insecticides on all adjacent broccoli bays (long narrow adjacent fields, usually 210 m x 10 m, separated by a 1.5 m ally) for ten days (Fig. 1), even though Dipel® was the only product used on the property over the previous three months and was used infrequently. To determine if the mature bay of broccoli had sufficiently high insect densities to successfully employ spray of a fluorescent dye as a marker, we sampled a bay (210 x 10 m) for insects using a suction sampler at 30 row meters of plants, replicated ten times. From past experience we had determined that the density of a species needed to be ca. one per row metre to have enough individuals in a field to mark and monitor movement.

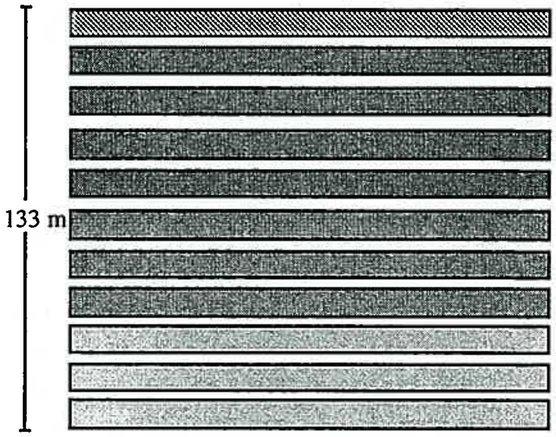

Fig. 1. Layout of experimental field at Newman and Sons property, Virginia, South Australia, Australia. The checked rectangle represents the bay of broccoli sprayed with fluorescent dye. Each additional rectangle represents a bay of broccoli from mature to young differing in age by one week.

Movement of pest and parasitoids. The experiment to assess moth and parasitoid movement was conducted in Virginia, South Australia, on a property that had eleven bays of broccoli in production (each bay measuring 210 x 10 m with a 1.5 m ally), and bare cultivated soil surrounding a cropped field (Fig. 1). We used a novel marking technique by spraying a non-toxic, fluorescent resin-based dye (SARDI Fluorescent Pigment) in the broccoli field to mark natural populations of *P. xylostella* moths and their main larval parasitoid, *D. semiclausum*. In a prior experiment, we established that our dye marked the moth and the parasitoid, and that we were able to capture these species with suction sampling and yellow-sticky-bucket traps (inverted 9 liter buckets, with a 5 cm wide ring made of particle board around the base; all coated with tangle trap) placed 20 cm from the ground. At the time of our experiment, the youngest bay of broccoli was three weeks from harvest and the three most mature bays of broccoli were no longer suitable to harvest so plants in all bays were similar in the amount of vegetative growth.

To determine if *P. xylostella* and *D. semiclausum* move from mature to young broccoli when there is no disturbance from cultivation, we sprayed 120 liters of the dye mixture on the entire 210 x 10 m bay of broccoli. Next, we placed four yellow-sticky-bucket traps 20 cm from the ground per bay in each of five alternating bays, plus the bay that was sprayed with the fluorescent dye. Forty-eight hours after spraying the dye, we used a suction sampler in each of the ten bays not treated with dye (the dyed bay was excluded because suction sampling on plants with the resin picks up fluorescent dye and cross contaminates samples) to sample 30 m sections at three locations per bay. Five days after spraying the dye, we removed the yellow-sticky-bucket traps from all plots and sub-sampled two alternate quadrats of the traps for moths and parasitoids. To determine whether diamondback moth and *D. semiclausum* move from mature to younger broccoli when there is a disturbance from cultivation, we proceeded in the same manner as above. However, after spraying the dye and placing the yellow-sticky-buckets in the bays of broccoli, the dyed bay was cultivated (the usual practice after broccoli is harvested) leaving only bare soil, thus forcing the mobile insects from the broccoli. The yellow-sticky-buckets were removed from all bays three days after setting them up.

Statistical analysis. The Kolmogorov-Smirnov goodness of fit test was used to determine the difference in the distribution of the parasitoids and moths before and after cultivation. A sign test was used to detect the difference in direction of the pattern of dispersal for the parasitoid and moth, before and after cultivation.

RESULTS

Cotton System

Insect predator abundance in crops and non-crops. There were six species of abundant generalist insect predators extracted from the samples: transverse ladybird, *Coccinella transversalis* (Fabricus); minute two-spotted ladybird, *Diomus notescens* (Blackburn); a damsel bug, *Nabis (Tropiconabis) kinbergii* Reuter; red and blue beetle, *Dicranolaius bellulus* (Guerin-Meneville); a green lacewing, *Mallada signatus* (Schneider); and a brown lacewing, *Micromus tasmaniae* (Walker). The average number of individuals of these six species summed that were collected from each type of vegetation through the season varied (Fig. 2). Cotton, lucerne, and wheat had the highest densities of adult insect predators in spring; cotton, sorghum, and sunflower had the highest densities in early summer; and by mid summer the highest populations were in sorghum. By late summer the abundance of insect predators dropped to almost zero, with a few remaining in cotton, sorghum, pasture, or lucerne.

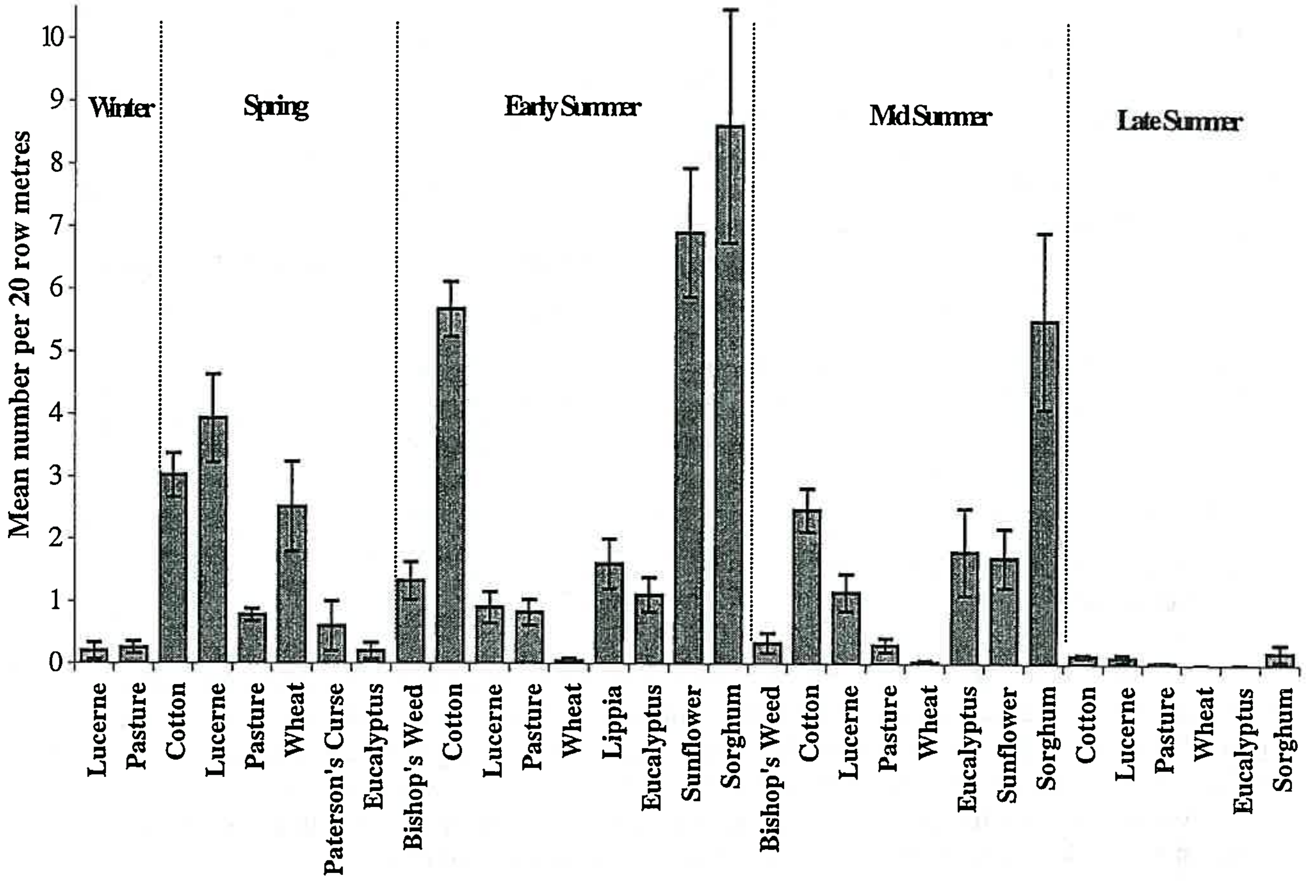

Fig. 2. Mean number (± 1 SE) of the six most abundant insect predators summed in each type of vegetation per seasonal period.

Movement of insect predators around cotton landscape. Of the 199 individuals examined, 170 (85%) carried pollen, and all six predator species had individuals that carried pollen. Of the individuals with pollen, 151 (89%) carried more than one type ("species") of pollen. This pattern was similar for the two most abundant species captured in cotton; 72% of *D. notescens* (n = 47) and 82% of *M. tasmaniae* (n = 35) carried more than one type of pollen. The pollen types carried most frequently by *M. tasmaniae* were Bishop's weed, *Ammi majus*, (L.), cotton, other Malvaceae and *Eucalyptus* spp., and by *D. notescens* were other Malvaceae, Bishop's weed and *Eucalyptus* spp. For those individuals captured outside of cotton, 90% of *M. tasmaniae* (n = 10), carried more than one type of pollen and 75% carried cotton pollen; and for *D. notescens* (n = 14) 57% carried more than one type of pollen and 40% carried cotton pollen.

Broccoli System

Pest and parasitoid abundance and movement from mature to young broccoli. Immediately before spraying fluorescent dye on the mature broccoli bay (and resident insect populations), the adult *P. xylostella* and *D. semiclausum* densities were 0.75 ±0.57 (SD) and 1.01 ± 0.50 (SD) per row meter, respectively. Based on results from suction sampling before cultivation, marked *P. xylostella* did not appear to move far as all marked individuals were captured within 36 m of the dyed bay. However, after cultivation, one marked *P. xylostella* was captured as far as 60 m from the dyed bay, yet there was no difference in their distribution before and after cultivation ($D = 0.10$, df = 9, $P = 0.666$). The result from the yellow-sticky-bucket traps was different than from the suction sampling due to the time and type of capture. The moth *P. xylostella* moved as far as 108 m (the furthest distance sampled in broccoli) from the dyed bay both before and after cultivation, (two and one marked individuals, respectively) but their distribution did not differ ($D = 0.33$, df = 5, $P = 0.400$; Table1). When considering the pattern of dispersal found on yellow-sticky-bucket traps, the direction of the difference of marked *P. xylostella* captured was greater at each distance before cultivation compared to after cultivation ($C_{0.05\,(0)6}$, $P < 0.01$).

Table 1. Proportion of marked *P. xylostella* moths captured on yellow-sticky-bucket traps before and after cultivation at 0 to 108 m from the source of marked insects.

	Distance from dyed broccoli (m)					
	0	12	36	60	84	108
Before cultivation (n=1128)	0.40	0.13	0.03	0.05	0.02	0.01
After cultivation (n=1137)		0.08	0.01	0.03	0.00	0.01

The dispersal pattern of *D. semiclausum* was different from that of *P. xylostella* adults. Based on results from suction sampling before cultivation, 5% of marked *D. semiclausum* were captured 60 m from the dyed bay (Fig. 3). After cultivation, the dispersal of marked *D. semiclausum* was greater ($D = 0.50$, df = 9, $P = 0.037$), and 7% of marked individuals were captured at 108 m from the dyed bay with greater than 50% of marked individuals dispersing further than the closest bay of broccoli, 12 m from the dyed broccoli (Fig. 3).

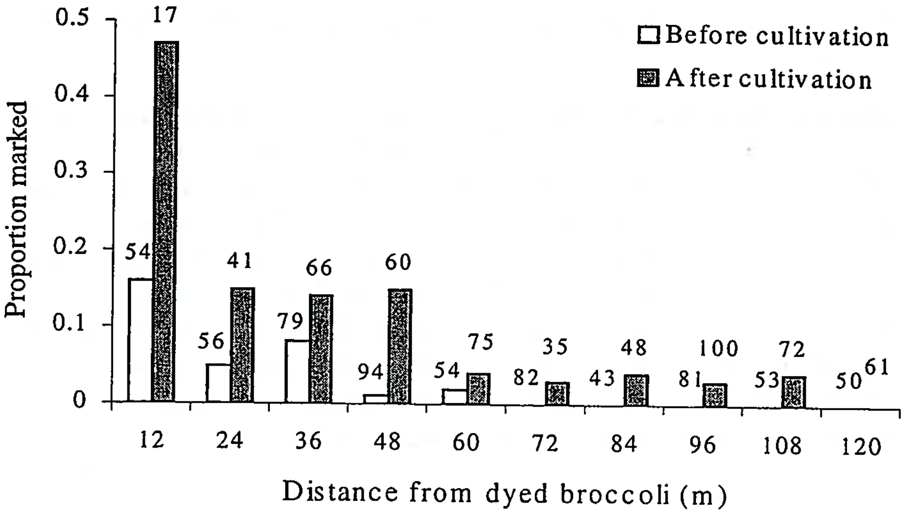

Fig. 3. Proportion of *D. semiclausum* captured with a suction sampler that were marked in relation to distance from source of marked insects (number above the bars are the number of wasps captured).

D. semiclausum was captured on the yellow-sticky-bucket traps as far as 108 m before and after cultivation, and there was no difference in their dispersal ($D = 0.50$, df = 5, $P = 0.208$; Fig. 4). However, the direction of the difference in dispersal of marked *D. semiclausum* was greater after cultivation than before cultivation; the opposite from DBM ($C_{0.05(1)6}$, $P < 0.01$).

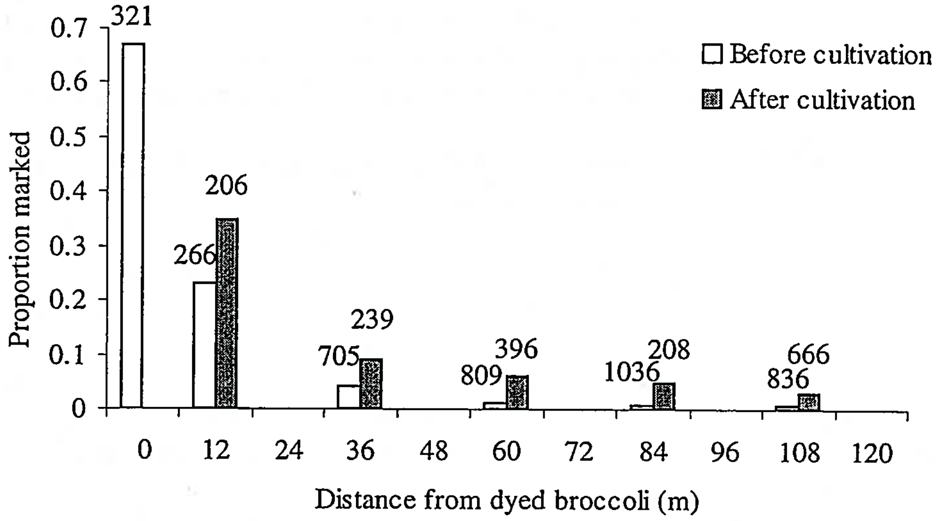

Fig. 4. Proportion of *D. semiclausum* captured on yellow-sticky-bucket traps before and after cultivation that were marked, in relation to distance from source of marked insects (number above the bars are the number of wasps captured).

Although we have focused on dispersal before and after cultivation, it should be noted that dispersal over time maybe confounded with cultivation for the moths. This could have only been avoided if two colors of resin-based dye had been available (which we now have). Results from our laboratory experiments showed that 99.8% of *D. semiclausum* die within 5 days without a sugar source, and the average longevity is 2.9 days, and 99.8% of *P. xylostella* die within 11 days and the average longevity is 5.7 days (Schellhorn, unpublished data). There were five days between the cultivation experiments which suggests that the parasitoids were unlikely to have lived long enough for this issue to be important. However, *P. xylostella* is likely to have lived long enough, yet there was equal or less dispersal after cultivation which suggests that the issue was not important for the moths.

DISCUSSION

Our results from the cotton system show that insect predators of cotton pests are present in several types of vegetation throughout the year. In addition, we found that the two most abundant insect predators, *M. tasmaniae* and *D. notescens*, visit several types of vegetation before moving into cotton, and move back and forth between cotton and other types of vegetation. These findings suggest that particular types of vegetation on-farm or in the larger landscape may conserve and enhance local populations of insect predators.

Our results from the broccoli system show that the patterns of movement for *P. xylostella* adults and *D. semiclausum* before and after cultivation were different. For *P. xylostella* adults, the results from suction sampling suggest limited dispersal before and after cultivation, a finding similar to that of our preliminary experiments (Schellhorn unpublished data). Results from the yellow-sticky-bucket traps showed that *P. xylostella* dispersed to 108 m, but the majority of marked individuals dispersed to the adjacent broccoli before and after cultivation. However, for *D. semiclausum*, the majority of marked individuals dispersed further than the adjacent broccoli. Fewer *D. semiclausum* dispersed before cultivation than after cultivation, suggesting that disturbance increased parasitoid movement, which was not the case for *P. xylostella.* The difference in the degree of dispersal suggested by suction sampling versus yellow-sticky-bucket traps before cultivation was most likely caused by the effect of sampling date: suction sampling being done 48 hours after the broccoli bay was first treated with dye, versus 96 hours for the yellow-sticky-bucket traps.

In large-scale monocultures, such as New South Wales cotton, planting of early-season annuals or early-flowering perennials may improve overwintering conditions, or increase colonisation and subsequent population increase by *M. tasmaniae* or *D. notescens* before the occurrence of populations of summer pests. This appears to be happening in grapes in the western United States of America, where the solitary egg parasitoid *Anagras* spp., overwinters in French prune trees that harbor an alternative host (Doutt and Nakata, 1973; Kido *et al.*, 1984). In the early spring, *Anagras* spp. colonizes adjacent vineyards and plays a critical role in increasing parasitism and controlling populations of western grape leafhopper (Corbett and Rosenheim, 1996; Murphy *et al.*, 1998). The next study in cotton will be to test particular annuals or perennials for improved overwintering and subsequent colonization of cotton.

In the broccoli system, production is continuous so natural enemies have to be maintained, disturbance minimised and population increase encouraged throughout the year without causing an increase in pests. Maintaining bays of harvested, un-cultivated broccoli (a type of refuge) at 70 m intervals may allow parasitoid populations to build-up and move into adjacent, younger plantings. Disturbance, such as harvest or cultivation, can disrupt biological control (Schellhorn *et al.*, 2002; Honěk 1982; Carillo 1985). Maintaining on-farm refuges may reduce the effects of such disturbances on natural enemies and increase re-colonisation (van den Bosch *et al.*, 1966; Mullens *et al.*, 1996).

Pollen and resin-based fluorescent dye are excellent tools to monitor movement of field populations of natural enemies and pests. The data from this project show that information on species-specific behaviour and population processes, particularly movement, are helpful to manage cultural practices intended to increase natural enemy abundance as part of a biological control program. By increasing our knowledges of natural enemies and pests in relation to habitat use, we should be able to make predictions about how to implement effective cultural practices to manage pest insects.

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Augmentation and conservation of parasitoids of *Helicoverpa* spp.: Findings from the first field season

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Introduction

One of the major objectives of the cotton industry is to reduce dependence on insecticides. This may be achieved by incorporating parasitoids into the pest management strategy. Parasitoids are wasps or flies that "attack" other insects. The "attack"means that the parasitic wasp or fly lays an egg inside the eggs, larvae or pupae of a host insect (i.e. *Helicoverpa* spp.). Once the egg of the wasps or fly hatches, it feeds on the internal organs of the host species (i.e. *Helicoverpa* eggs, larvae or pupae) eventually killing it. The eggs, larvae and pupae of *Helicoverpa* are attacked by several native parasitoids that rely on *Helicoverpa* for survival. In order to place greater reliance on parasitoids for *Helicoverpa* spp. control we need to understand how to increase parasitoid abundance in cotton.

Cultural practices can affect parasitoid and predator abundance and species diversity. Historically, the term cultural control is used for insect pests and includes any modification in the way a crop is produced that results in lower pest populations or damage (i.e. trap cropping, tillage, crop rotation and fallow). Cultural control excludes production practices that act directly on insect pests, for example insecticide application, classical biological control (Schellhorn et al. 2000). All of these direct cultural effects on insect pests have been evaluated for many decades and excellent reviews of most of these controls have been published. However, cultural control practices also affect beneficial insects of pests and can influence the ability of the natural enemies to suppress pest populations. Increased density of a particular beneficial species or a greater number of beneficial species can result in greater mortality of the target pest. There are numerous examples in the literature demonstrating that cultural practices can enhance natural enemy abundance, and possibly their efficiency, however, the majority only compare abundance in one production system to another (Schellhorn et al. 2000). In order to place greater reliance on parasitoids for the control of *Helicoverpa* spp. we need to understand how cultural practices can result in higher densities of parasitoids in cotton crops, thus higher mortality to *Helicoverpa* spp.

Our research focuses on the conditions under which parasitoids will become more abundant in cotton and parasitise *Helicoverpa* spp. The research has 4 main objectives:

1. Identify spring and early summer crops that are good sources for parasitoids,
2. Determine whether parasitoids move from a source crop (i.e. early season legumes or summer legumes) into cotton, and if they move into cotton, does the spatial proximity of the source population to cotton matter?,
3. When parasitoids move from source crop into cotton, is it because the source-crop in no longer suitable (i.e. "haying off") or because the cotton crop is more attractive?
4. Determine if parasitoids (a) feed on floral and extra-floral nectar, (b) if feeding increases adult fecundity (i.e. number of eggs) and longevity, and (c) if an increase in parasitoid fecundity and longevity increases their abundance via increased attacks on *Helicoverpa* spp.

Below I present some of the results from the first field season that focus on objectives one and four: parasitoid abundance and parasitism rate in several crops and non-crops. These results prepare us to investigate objectives two and three for the 2000-2001 field season.

Materials and Methods

Parasitoid abundance in crops and non-crops

To identify the spring and summer crops and non-crops that are good sources for parasitoids, during the 1999-2000 season we sampled for larvae and pupae of *Helicoverpa* spp. (due to their minute size, sampling for egg parasitoids was treated separately and is explained below). Direct observation was used to find eggs, a sweep net to find larvae, and digging in the soil to find pupae. We sampled crops including, lucerne, lupin, pigeon pea, sorghum, sunflower, unsprayed cotton and wheat, and non-crops including native plants and weeds in a variety of plant families (i.e. the daisy family - Asteraceae, cabbage family - Brassicaceae). To determine which species of parasitoid was present and parasitism rate, individual eggs, larvae and puapae were returned to the laboratory and reared to see what emerged.

Egg parasitism

In addition to collecting naturally oviposited eggs of *Helicoverpa* spp and checking for parasitism, We also experimentally assessed egg parasitism. We placed a net bag over the branch or stem of a plant and placed a reproductive female *H. armigera* moth in a net bag. She was allowed to lay eggs over night. To vary the number of eggs laid (anywhere from 0 to 300) we varied the age of the moths that were placed in the bags. The next day we removed the bag and the moth, and allowed the eggs to be exposed to predators and parasitoids for 48-72 hours depending on temperature. After this time the eggs were removed, returned to the laboratory and placed on agar. After 24 hours, if the egg was parasitised it would turn black. However, to determine the species of parasitoid, the eggs had to remain on the agar for approximately 10 days until the egg parasitoid emerged. We bagged 327 plants in a variety of crops and non-crops that were found to contain eggs of *Helicoverpa* spp.; lucerne, feral sunflower, *Echium* spp (Patterson's curse), pigeon pea, sorghum, conventional cotton, *Bt* cotton, unsprayed cotton, throughout spring and summer. We would have included lupin in our samples, however we did not establish our sampling methods until lupin was close to harvesting. Two hundred and eighty-five plants were bagged within a 100km radius of Narrabri, and 42 plants were bagged in sorghum and cotton in Warren, NSW. Of the 285 bagged plants, 113 were placed at the Platt Family's property, Lowana, in Pillage, NSW.

Feeding on floral and extra-floral nectar

We conducted glasshouse experiments to determine if parasitoids feed on floral and extra-floral nectar, if feeding increases adult fecundity (i.e. number of eggs) and longevity (i.e. life-span), and if an increase in parasitoid fecundity and longevity increases their abundance via increased attacks on *Helicoverpa* spp. We established 4 feeding treatments, honey solution (ca. 15% honey), cotton extra-floral nectar, lucerne floral nectar, and water only. Our choice of floral and extra-floral nectar was based on preliminary findings where we determined that *I. Promissorius*, a pupal parasitoid, does feed on floral and extra-floral nectar and that wasps spent the most time searching on cotton and lucerne plants. Our expectation was that honey should provide the greatest longevity, lifetime fecundity and parasitism rate, water should provide the lowest, and the lucerne and cotton should be in between. A single mated female parasitoid, *Ichneumon promissorius*, was placed into a container ca. 22cm x 38cm that included water, a continuous supply of one of the food items mentioned above, and shelters for shade. They were given 6-8 hosts every other day for 17 hours (5 light, 12 dark) for their life span. Each feeding treatment was replicated 12 times for a total of 48 individuals. We monitored their longevity, lifetime fecundity, average daily parasitism rate, and overall parasitism rate.

Result

Parasitoid abundance in crops and non-crops

In the spring, the majority of larval and pupal parasitoids were found in lupin, lucerne, and *Echium* spp. (i.e. Patterson's curse), while in the summer the majority were found in pigeon pea and sorghum (Figure 1). A total of 1334 larvae and pupae were collected and returned to the lab to assess parasitism. Of those, 707 survived long enough to determine whether they were infected with virus, parasitised or healthy and an adult *Helicoverpa* spp emerged. *Heteropelma scaposum* was the most abundant species followed by *Microplitis demolitor* (both preferentially attack 2nd and 3rd instar larvae, otherwise called 'small' larvae). Larvae from lucerne and pigeon pea had the highest incidence of virus (6% and 12%, respectively). However, most of the virus-infected-larvae from pigeon pea was due to the application of Gem Star® late in the season, while virus-infected-larvae from lucerne was due to "natural" levels of virus in the field. Of the spring plants sampled, larvae from lupin has the highest parasitism rate at 74%, and of the summer plants sampled, pigeon pea has the highest parasitism rate at 46% (Figure 1).

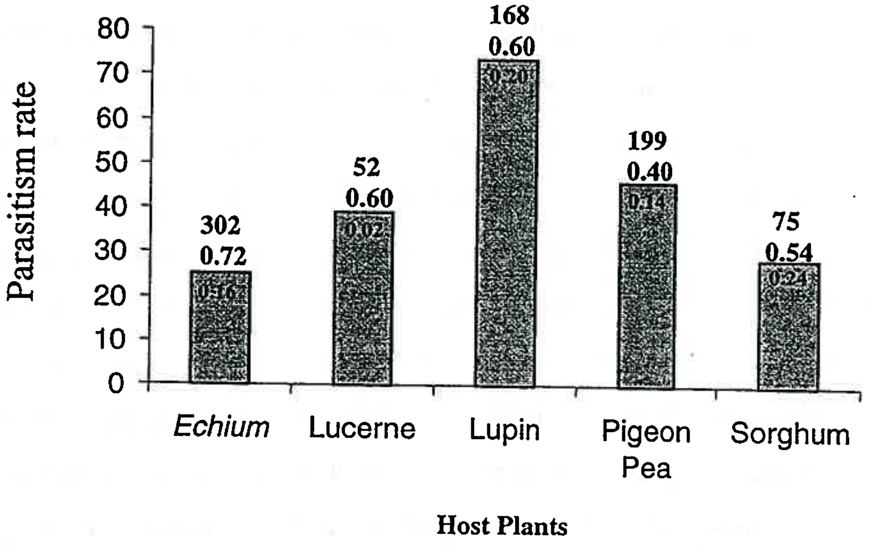

Figure 1. Larval parasitoid abundance in crops and non-crops represented as parasitism rate of *Helicoverpa* larvae. The top number above each bar is the number of larvae collected, the middle number is the proportion of the collected larvae that survived to assess parasitism, disease, or healthy individuals, the bottom number is the average density of larvae sampled.

In general, pupae sampling represented only 5% of our sampling effort and primarily in unsprayed cotton (where only one pupae was found after 30 meters of digging) and pigeon pea. Of the 36 m sampled, 142 live pupae were uncovered and 83% were parasitised by *H. scaposum*, and *I. Promissorius*. We will increase our sampling effort for pupae during the 2000-2001 field season.

Egg parasitism

Egg parasitoids exist in the Namoi Valley, however they are rare and parasitism is highly variable among plants within a crop and among crops (Figure 2). Females laid 22,152 eggs on the 327 plants that were "bagged" to assess egg parasitism,. After 48-72 hours in the field, 5993 eggs were retrieved and viable. Of the viable eggs, 430 were parasitised by at least two species of egg parasitoids (and possibly three, positive species identification has not been completed), two species of *Trichogramma* and one species of *Telenomus*. Egg predation (eggs that were missing when we retrieved them, most likely taken by ants, and eggs that were clearly chewed on or the contents sucked out) ranged from 9-95%, but the median value was 76 % predation for the 15 locations listed in Figure 2. Of the 168 naturally oviposited eggs, none were parasitised. Furthermore, of the ca. 650 eggs collected by other researchers, only one was parasitised.

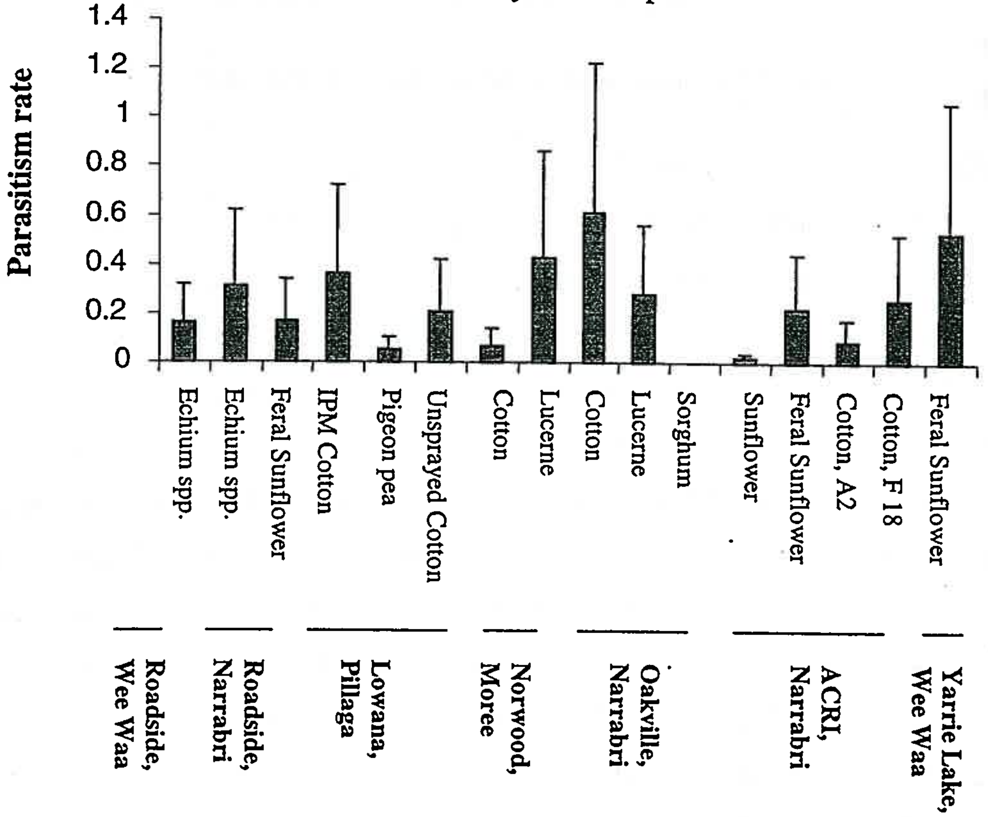

Figure 2. Egg parasitism rate from different crops and non-crops at several locations. The length of the bar next to the location represents the number of crops sampled.

Feeding on floral and extra-floral nectar

Our results show that wasps that fed on the honey solution live longer and parasitise more *H. armigera* than wasps that only had access to water or lucerne floral nectar (Table 1). Wasps that had access to cotton extra-floral nectar did not live as long as wasps that fed on honey solution. However, they did have a daily parasitism rate, and average parasitism rate that was more similar to wasps that fed on honey than wasps that fed on lucerne floral nectar or water (Table 1). Although, wasps were observed to spend similar amounts of time searching on lucerne (average of 52 seconds per visit) and cotton (average of 67 seconds per visit), they do not benefit from lucerne in terms of parasitism rate or life-time fecundity as they did from cotton extra-floral nectar.

Table 1. Results from feeding on floral and extra-floral nectar by the pupal parasitoid, *Ichneumon promissorius.*

	$\bar{X}$ Longevity	**$\bar{X}$ DPR[1]**	**$\bar{X}$ Parasitism rate**	**$\bar{X}$ LFT[2]**
Honey solution	19.6 a	0.18 a	0.16 a	12.5 a
Cotton extra-floral nectar	11.0 b	0.18 a	0.11 b	7.9 ab
Lucerne floral nectar	8.5 b	0.08 a	0.05 b	3.77 b
Water only	9.5 b	0.11 a	0.06 b	4.6 b

[1] DPR denotes daily parasitism rate

[2] LFT denotes life time fecundity

Letters that differ denote significant difference among treatments @ $P < 0.05$.

Discussion and conclusion

Larvae and pupae of *Helicoverpa* spp collected from lupin and pigeon pea were found to have the highest parasitism rate on the spring and summer plants sampled, respectively. Lucerne was also shown to have high parasitism, but larvae densities were very low and virus incidence was relatively high. However, lucerne has been shown to provide a good habitat for predators (Mensah 1999, Walker and Fitt 1998). These findings suggests that the most logical rotation crops to use to generate on-farm source populations of parasitoids are lupin in the winter and spring, before cotton is planted, and pigeon pea in the summer while cotton is growing. There are additional rotation crops that may also be suitable to use to generate parasitoid populations. Previous sampling by Walker and Fitt (1998) found that faba beans may also be a good crop to generate spring populations of parasitoids, and adzuki bean, mung beans, sorghum and sunflowers may generate summer populations of parasitoids. However, none of these crops produced parasitism rates as high as lupin and pigeon pea. It is not specifically known at this time what is responsible for the high parasitism rate on lupin and pigeon pea compared to other plants. The higher parasitism rate is most likely due to different plant characters: morphology of the plants (i.e. wasps may have better access to *Helicoverpa* larvae on lupin or pigeon pea than on other plants), the leaf surface (i.e. chick peas are known to produce an acid on the leaves which may make searching for hosts difficult) and cues produced by *Helicoverpa* larvae feeding on plants such as the specific frass or waste material produced by the larvae feeding on lupin compared to sunflowers (i.e. wasps use the frass to locate hosts (Quicke 1997). There may also be seasonal variation.

Egg parasitoids are fairly ubiquitous in the Namoi valley from spring to late summer in crops and non-crops that were not sprayed with OP's and SP's. Once OP's and SP's were sprayed on the back section of Australian Cotton Research Institute in Narrabri, we no longer found parasitised eggs at that location. However, in unsprayed crops or crops that use "soft options" they are rare and parasitism is highly variable among plants within a crop and among crops. One of the more puzzling questions was why we did not find parasitised eggs that were naturally oviposited at the same location that we found parasitised eggs from our "baited- eggs"? The most likely explanation is due to our method of baiting. Tricosane (a chemical in moth scales) is a contact Kairmone (a chemical produced by *Helicoverpa* that wasps use to locate their hosts, i.e. *Helicoverpa* egg or larvae) and is known to increase searching by wasps for hosts (Lewis et al. 1975). When we bag a moth on a plant for up to 18 hours, she probably leaves a considerable amount of scales on the plant, more so than a moth that is laying eggs "naturally". Therefore, when a wasp does land on the plant with the additional moth scales, she probably searches longer, and subsequently has a higher probability of parasitising an egg, then when she lands on a plant with a naturally oviposited egg. The difference in egg parasitism rates between the naturally oviposited eggs and "baited-eggs" contributes to our conclusion that egg parasitoids are rare in the Namoi Valley.

Trichogramma spp. are known to be quite common and fairly effective in agricultural systems (including cotton) in the Darling Downs, Queensland (personal communication Brad Scholz and Dave Murry, Queensland, DPI). Based on research to date, we can only speculate on the possible explanations for the rarity of egg parasitoids in the Namoi Valley. There are three possible explanations, none of which are mutually exclusive. The first is that the Namoi Valley may be near the southern end of the geographical distribution of *Trichogramma* spp. This explanation is based on our egg baiting results from Warren, NSW (further west and a little south) where 744 viable eggs were recovered from unsprayed pigeon pea and cotton crops and only seven eggs were parasitised. We purposely chose these spots because they had extremely high densities of larval and pupal parasitoids. A species geographic distribution is most frequently controlled by environmental factors such as temperature and humidity, for example. The second explanation is that compared to the Darling Downs, where a variety of crops are grown throughout the year, the Namoi Valley predominately grows cotton and autumn and winter are often characterized by large expanses of bare ground that has been prepared for the next cotton growing season. The poor availability of habitats during autumn and winter may attribute to the low population density of *Trichogramma* spp. in the spring. The third possible explanation is that the combination of broad scale insecticide use (especially OP's'and SP's') in cotton and the absence of on-farm nursery crops for egg parasitoids to avoid the spray may exacerbate the decline of a population that is already at a low density. Although egg parasitoids are one of many options to increase mortality of *Helicoverpa*, it may be of interest to conduct a few inundative releases on farms where we control for autumn and winter habitats, and frequent placements of on-farm nursery crops. Regardless, all 3 of the explanations are speculative and may be investigated further.

Parasitic wasps that have access to a high quality sugar source (i.e. honey or nectar) live longer and parasitise more *Helicoverpa* spp. Our results suggest that nectar sources vary in quality and wasps that have access to higher quality nectar may live longer and kill more pests. Because cotton appears to provide an adequate source of sugar in the extra-floral nectar, the implications of our findings are more important to identify plants that provide a food source early in the season, before cotton is sown.

Identifying crops that generate populations of parasitoids (and predators), and small populations of *Helicoverpa* spp. is only part of our research objectives. However, the next step (and critical point) is to determine whether those parasitoids (and predators) generated on-farm move into the adjacent cotton crop and cause mortality to *Helicoverpa* spp. And, if they do move into cotton and cause mortality to *Helicoverpa* spp, then we need to determine the spatial arrangement (i.e. size of the source population crop and placements) within the farm landscape that best directs beneficial insect movement into cotton. During our next field season we will continue to monitor parasitoid populations on a variety of crops and non-crops, but we will concentrate on the questions posed above. Conservation biological control and augmentation of parasitoids of *Helicoverpa* spp.

requires both discriminate use of insecticides, and active management to increase parasitoid populations and direct their movement. This finding has implications for how we design on-farm landscapes in cotton IPM systems. The crops chosen as nursery crops to build-up parasitoid populations (and predators) in the spring and early summer should provide hosts for wasps to parasitise and subsequently increase their populations, and a high quality food source. These systems will have to be monitored carefully to determine the response of both parasitoid and *Helicoverpa* spp. populations in the spring and early summer. On-farm crop diversity will play a role in cotton IPM systems, however, the right type of diversity will be important.

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In the previous issue of The Australian Cottongrower, there was an overview of CSIRO Entomology's research on insects in cotton. This article gives a more detailed look at CSIRO's work on conservation biocontrol.

Parasitoids in cotton

By Nancy Schellhorn

Helicoverpa moths are the main insect pests of cotton and controlling them is an ongoing problem. With the cotton industry keen to reduce its use of insecticides, attention has turned to the use of beneficial insects as part of Integrated Pest Management (IPM) strategies.

At the Australian Cotton Research Institute in Narrabri, CSIRO Entomology scientists are putting in long hours in the field looking for the most efficient beneficials and how to improve their effectiveness.

Classical biocontrol, which is better known than conservation biocontrol, involves introduction of an exotic biocontrol agent (parasitoid or predator) which is then left to fend for itself or be regularly reintroduced. Conservation biocontrol, on the other hand, is the conservation and augmentation of biocontrol agents already present in an area, and involves manipulation of the local environment to improve survival and reproduction of the agents relative to the pests.

The wasp, *Heteropelma scaposum*, attacking a *Helicoverpa armigera* larva. Photo by N. Schellhorn.

As a result, pest population growth rates are slowed and their numbers reduced. This can be achieved by enhancing the conditions which favour biocontrol agents, primarily by reducing insecticide use and minimising disturbance to the environment.

For conservation biocontrol to be effective, the following conditions must be met:

- Beneficial insects must be present;
- They must arrive on time;
- They must be sufficiently abundant; and,
- They must attack the pests of interest.

The last condition is particularly important, because when alternative hosts or prey items are available, the parasitoids or predators may avoid attacking the pests of interest. Therefore, although conditions

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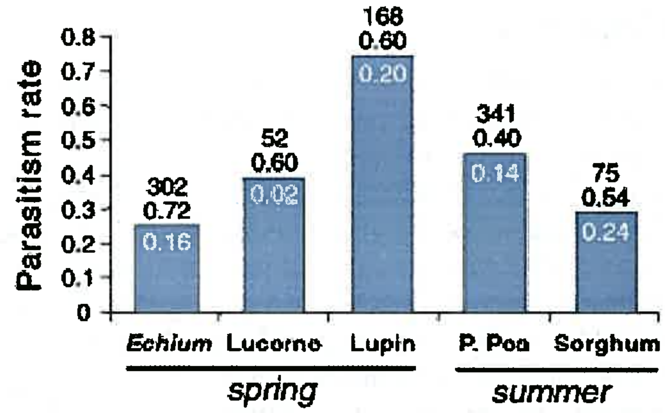
FIGURE 1: Larval parasite abundance in crops and non-crops



The top number above each bar is the number of larvae collected, the middle number is the proportion of the collected larvae that survived to assess parasitism, disease or healthy moths, and the bottom number is the average density of larvae per metre.

PARASITES AND PARASITOIDS

Most people are familiar with the term parasite. This is an animal or plant living in or on another species (its host) and obtaining its food from that host e.g. fleas and tapeworms. The host is usually harmed in some way by the parasite but is not killed.

In contrast, a parasitoid also lives in or on its host and feeds on it, but eventually kills it. This is what makes them successful biocontrol agents. The parasitoids found in cotton are wasps or flies that "attack" other insects by laying an egg in or on the eggs, larvae or pupae of a host such as Helicoverpa.

The larva which emerges from this egg feeds on the internal organs of the host eventually killing it. Parasitoids almost always have a free living adult stage and attack organisms in their own taxa i.e. insects attack insects as opposed to the flea on the dog.

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one to three may be met, the parasitoids and predators preferentially attack the alternative hosts or prey.

Cotton is potentially an excellent cropping system for conservation biocontrol of *Helicoverpa spp.* as there are numerous native Australian predators and parasitic wasps and flies which attack both the native *Helicoverpa punctigera* and the cosmopolitan (and more difficult to control) *Helicoverpa armigera*. Many also attack other pests of cotton.

Our research so far has concentrated on parasitic wasps in the Namoi Valley. Helicoverpa eggs, larvae and pupae are attacked by several native parasitoids that rely on it for survival. Several of the native parasitoids are pictured in *The Cotton Pest and Beneficial Guide* which is available from the Technology Resource Centre at the Australian Cotton CRC.

BEST CONDITIONS FOR PARASITIC WASPS

Specifically, our team is investigating the conditions under which parasitic wasps become more abundant in cotton, and therefore more effective at controlling Helicoverpa. Work began last season to identify spring and early summer crops that are good sources of the wasps but are not good sources of Helicoverpa. The aim is to plant these crops to generate on-farm, early season populations of biocontrol agents. These would then be available to attack Helicoverpa larvae as well as other pests, when they appear.

Crops (lucerne, lupin, pigeon pea, sorghum, sunflower, unsprayed cotton and wheat) and non-crops (native plants and various weeds) were sampled for Helicoverpa egg, larval and pupal parasitoids. Our work concentrated on the larvae and pupae of Helicoverpa as we found that, in the Namoi Valley, egg parasitoids were often rare and highly variable in their density.

Liz Barker sampling for parasitoids in the field. Photo by N. Schellhorn.

After collection, the larvae and pupae were taken to the laboratory to assess whether they were healthy, parasitised or infected by virus. The wasp, *Heteropelma scaposum* was the most abundant species reared from this field collected material followed by another wasp, *Microplitis demolitor*. Both preferentially attack 'small' larvae.

In spring, the majority of larval and pupal parasitoids were found in lupin,

A Helicoverpa larva on a cotton boll. Photo by C. Mares.

lucerne, and Paterson's curse with lupin providing the highest parasitism rate. Most of these larvae were *H. punctigera*. In summer, the majority of parasites were found in pigeon pea and sorghum with pigeon pea having the highest numbers. Most of these larvae were *H. armigera*.

From these experiments, it is clear that agents that attack Helicoverpa are present in cotton and surrounding habitats. In field trials this season, we will use lupin (early season) and pigeon pea (summer) as part of the cotton rotation to build up populations of biocontrol agents. The research planned will determine:

- Whether these crops produce large populations of parasites and predators;
- Whether these move from the crops into cotton early in the growing season;
- Whether they cause sufficient mortality to *Helicoverpa spp.* to provide effective suppression; and,
- The optimum size and placement of these crops on-farm to give the highest density of biocontrol agents moving into cotton.

In addition to the field studies, our team has also been conducting experiments to investigate how parasitoid nutritional requirements and behaviour patterns determine their effectiveness in the field. An Australian Cotton CRC summer scholarship student studied the effect of nectar quality on parasitoid abundance and parasitism rates.

PARASITOID BEHAVIOUR

In a laboratory study, he found that wasps feeding on honey solution or cotton extra-floral nectar live longer and parasitise more *H. armigera* than wasps that only have access to water or lucerne floral nectar. Nectar sources obviously vary in quality and the ability of wasps to access the nectar also varies. Wasps that have access to the best nectar may live longer and kill more pests.

These results have lead to a collaboration with Dr Mike Keller, at the Waite Campus of the University of Adelaide, to explore parasitoid behaviour and foraging for food. This research has important implications for the design of on-farm landscapes in cotton IPM systems. These landscapes will need to provide necessary habitat and nutrition for parasitoids, but not provide a nursery for pests.

To date, these findings from field and laboratory studies suggest that some plants may be better than others at generating source populations of and sustaining biocontrol agents. The key finding is that maintaining the right kind of on-farm crop diversity will allow safer, more profitable cotton IPM systems.

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Australian Cotton CRC summer scholarship student, Andrew Manners, setting up glasshouse experiments on wasp nectar feeding. Photo by N. Schellhorn.