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).

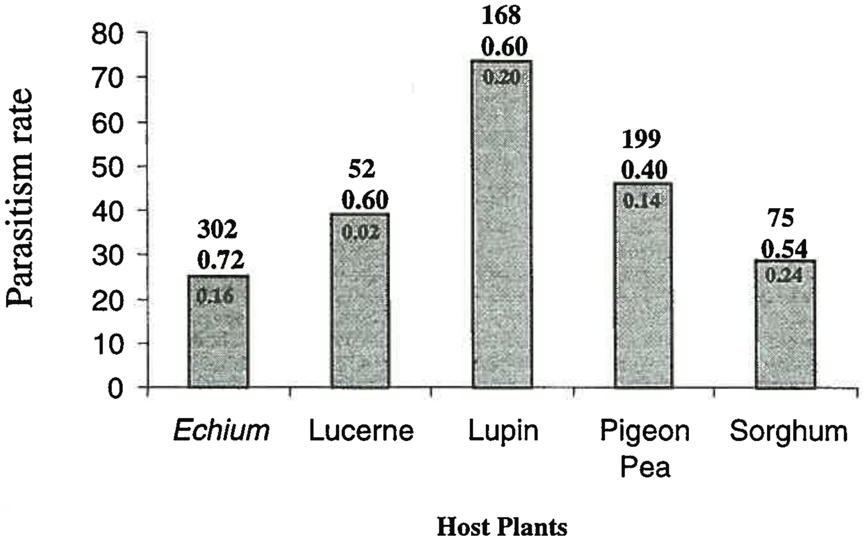

Figure 1. Larval parasitoid abundance in crops and non-crops represented as parasitism rate of *Helicoverpa* larvae. The top number above each bar is the number of larvae collected, the middle number is the proportion of the collected larvae that survived to assess parasitism, disease, or healthy individuals,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

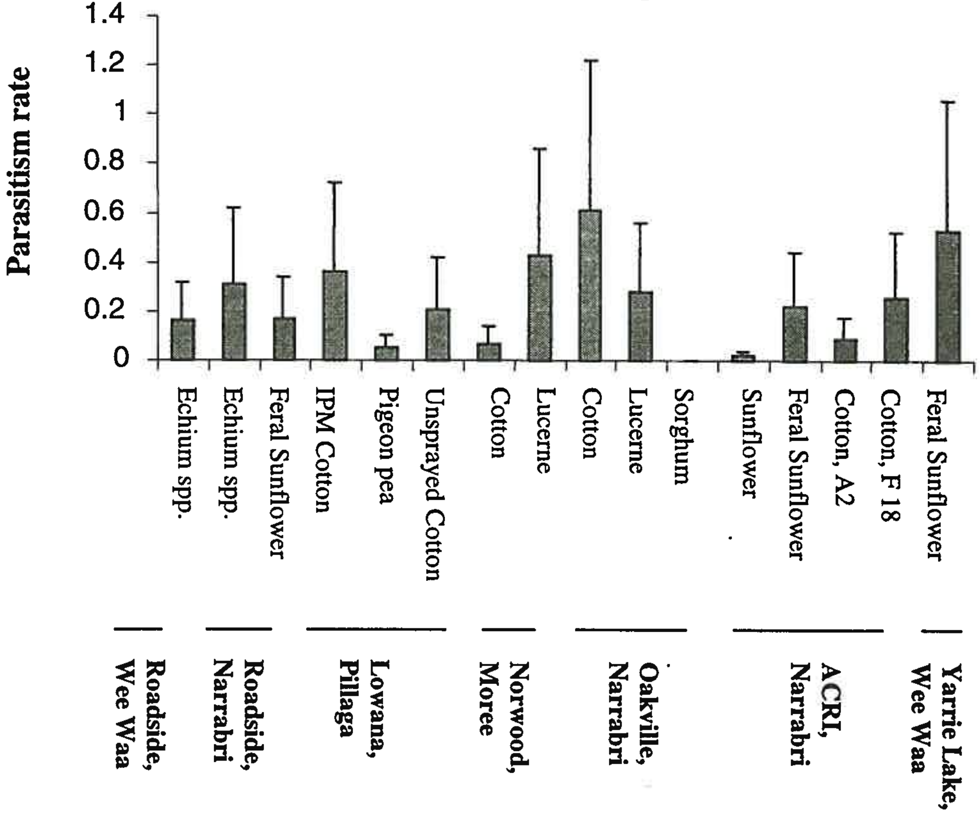

Figure 2. Egg parasitism rate from different crops and non-crops at several locations. The length of the bar next to the location represents the number of crops sampled.

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*.

	$\overline{X}$ Longevity	**$\overline{X}$ DPR[1]**	**$\overline{X}$ Parasitism rate**	**$\overline{X}$ 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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Acknowledgements

We are grateful to Tracey Parker for the maintenance of the *Helicoverpa* colonies which allowed us to have access to female moths for assessing egg parasitism, and *Helicoverpa* eggs, larvae and pupae to maintain our parasitoid colonies. We are grateful to Karen Stanford for her technical assistance in laboratory and field experiments. We would like to thank all of the growers who allowed us to sample on their properties. There are too many to mention by name, but we would like to specifically mention, Peter and Stan Getts at Lynebrae in Wee Waa, Mike Logan at Oakville in Narrabri, Peter Glennie at Norwood in Moree, Phil Lawrence at Cumberdeen in Wee Waa. Most of all we'd like to thank the Platt family at Lowana in Pillaga. We are grateful to them for accommodating our experiments on their property and their hospitality. This work was supported by the Cotton Research and Development Corporation, and Australian Cotton Cooperative Research Center Summer Scholarship to Andrew Manners.