Cotton
RESEARCH & DEVELOPMENT

Annual, Progress and Final Reports

Part 1 - Summary Details

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

CRDC Project Number: **UQ30C**

Annual Report: ☐ Due 30-September

Progress Report: ☐ Due 31-January

Final Report: ☒ Due 30-September
(or within 3 months of completion of project)

Project Title: *Understanding the behaviour of egg laying Helicoverpa moths: New designs for integrated control in cotton.*

Project Commencement Date: july2000 **Project Completion Date:** july2003

Research Program: 3 Crop Protection

Part 2 – Contact Details

Administrator: Kerry Johnston
Organisation: Research Admin, University of Queensland
Postal Address: St Lucia, Brisbane 4072
Ph: 33657493 **Fax:** **E-mail:** k.johnston@research.uq.edu.au

Principal Researcher: Dr Paul Cunningham
Organisation: University of Queensland
Postal Address: St Lucia, Brisbane 4072
Ph: 33651876 **Fax:** **E-mail:** p.cunningham@mailbox.uq.edu.au

Supervisor: Professor Myron Zalucki
Organisation: University of Queensland
Postal Address: St Lucia, Brisbane 4072.
Ph: 33651747 **Fax:** **E-mail:** m.zalucki@mailbox.uq.edu.au

Researcher 2 (Name & position of additional researcher or supervisor).
Organisation:
Postal Address:
Ph: **Fax:** **E-mail:**

Signature of Research Provider Representative:

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Part 4 – Final Report Executive Summary

This project has led to a significant advance in our understanding of the feeding behaviour of the adult heliothis moth. It has used detailed studies in ecology and behaviour of *H. armigera* to examine novel control strategies based around luring and killing adult moths. Our results will assist the design of more effective lures through consideration of important behavioural effects and odour preferences. We designed and executed a series of detailed experiments using odour lures and extended these experiments to allow moths to forage freely on flowers; testing their preferences for odours. Our results demonstrated odour learning on a number of levels, and provided evidence that flower visiting history influences attraction to odours. In addition to this we discovered that the enantiomeric forms of odour compounds may differ in their attractiveness to adult female moths, a finding that may improve the selection of synthetic odours for lures. Our results have been submitted as 3 key papers to international scientific journals.

We show that the location of feeding sites for adult moths influences the distribution of heliothis eggs. Our results imply that adult feeding sites have the potential to draw in adult moths to feed and that moths may then lay on nearby cotton plants. In addition, we show that male and female adult *H. armigera* moths may have differences in nectar feeding preferences. Our findings help elucidate why certain crops may be more attractive than others and why certain stages of the plant life cycle receive more heliothis eggs than others.

We have collected data on nectar foraging moths, which suggests that virgin female and male moths may be flying specifically to certain crops to feed. Specifically, we have shown that pigeonpea, used as a refuge and trap crop, is highly attractive as a host for feeding adult moths. We have backed field studies with controlled experiments carried out in outdoor flight cages. Our findings could be utilised to improve control methods and also cautions the use of some crops as refuges and trap crops, in that they may extend the lifespan and fecundity of this pest species.

In conclusion this work has contributed to the design of new heliothis control strategies, providing information that will help the improvement of lure and kill, trap cropping, refuge cropping and population monitoring strategies and the future production of new varieties of cotton with lower attractiveness to moths. It will benefit the Australian cotton industry and the Australian community by aiding the design and development of new economic and environmentally friendly methods of insect pest control.

Understanding the behaviour of egg laying *Helicoverpa* moths: New designs for integrated control in cotton.

SECTION 3: FIELD STUDIES ON THE FEEDING AND OVIPOSITION BEHAVIOUR OF ADULT *HELICOVERPA* MOTHS.

PROJECT SUMMARY

SUMMARY

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We have collected data on nectar foraging moths, which suggests that virgin female and male moths may be flying specifically to certain crops to feed. Specifically, we have shown that pigeonpea (*Cajanus cajan*), used as a refuge and trap crop, is highly attractive as a host for feeding adult moths. We have backed field studies with controlled experiments carried out in outdoor flight cages. Our findings could be utilised to improve control methods and also cautions the use of some crops as refuges and trap crops, in that they may extend the lifespan and fecundity of this pest species.

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PROJECT OBJECTIVES

Laboratory study of odour attraction in *H. armigera* (Section 1 of report).
The project aimed to study in detail, factors that might influence the attraction of adult *H. armigera* moths to host plant volatiles. In particular, we investigated how feeding experience (flower visiting) in adult moths might influence attraction to host plant volatiles. These studies are of key importance in the design of odour lures to lure and kill, or monitor adult moth populations. If experience strongly influences attraction to odours, significant improvements to lure formulations may be possible by considering adult feeding sites in the field. We also study specific host odours in detail, and show that different enantiomers of a single odour compound may differ in attractiveness to adult moths.

Flight cage studies on *H.armigera* feeding and oviposition behaviour (Section 2 of report):

These studies used controlled experiments in outdoor cages to investigate (1) the extent to which the feeding behaviour of adult moths influences egg distribution and (2) whether male and female moths prefer to feed on the same plants. These studies improve our understanding of why adult females prefer to lay eggs on flowering crops and may lead to improved lure formulations, which maximise attraction of female moths. In addition we aimed to investigate the suitability of using pigeonpea, in combination with lure and kill formulations, as a trap species to draw in male and female moths.

Collection of field data on feeding activity, movement and oviposition of adult moths within and between cotton, trap crops and refuges (Section 3 of report).

Data were collected in 2001/2 to examine adult heliothis behaviour within pigeonpea trap crops and refugia and neighbouring cotton crops. This involved following adult moths and recording behaviours and catching feeding and free flying moths in order to determine their sex and mating status. The study was aimed at investigating whether pigeonpea was attracting adult moths to feed. The results have crucial implications regarding the use of pigeonpea as a trap crop, a potential "lure and kill" crop and as a refuge crop.

Objectives not Achieved: Extremely low insect numbers during our field study testing (2001 & 2002) severely compromised sufficient data collection in a number of planned trials and prevented certain field objectives from being achieved.
These were: (1) field study of the influence of learning: Does laying on cotton affect the extent to which females will lay on other crops? (2) Pilot testing a lure and kill trap crop: Can the combination of trap crops and insecticidal feeding baits be used to lure and kill adult moths.

Section one: Experimental work on attraction to odours in adult *H. armigera* moths

OVERVIEW:

A potential new *H. armigera* control strategy currently being piloted by the CRDC is a "lure and kill" strategy. This consists of a sprayable bait, containing attractants, sugar and a small quantity of tank mixed insecticide. The idea is to attract adult moths using a special formulation of flower and crop odours mixed with a feeding stimulant (sugar) and then kill off the moths as they land and feed, using a low, but extremely effective dose of insecticide.

The success of this strategy lies crucially on the attraction of adult moths to the volatiles odours in the bait. In a number of detailed laboratory studies we looked in greater detail at the attraction of moths to odours and investigated ways in which lures may be significantly improved.

LEARNING, ODOUR PREFERENCE AND FLOWER FORAGING IN MOTHS.

Aim: The use of synthetic floral odours to lure adult heliothis moths in lure and kill or population monitoring strategies relies implicitly on the selection of odours that are highly attractive to both male and female moths. The aim of this study was to test how important previous experience of odours during adult feeding is in influencing response to odours.

MATERIALS AND METHODS

Insect and Plant Culturing

Helicoverpa armigera moths were obtained as pupae from a laboratory culture reared at QDPI Toowoomba, Queensland, Australia. Larvae had been reared on a soyflour based artificial diet for *Helicoverpa* spp., minimising any possible influence of experience of host plants at this stage (Jermy et al., 1968). Pupae were sexed and male moths were placed in a separate holding cage (200mm x 150mm x 150mm) until eclosion. Newly emerged adult males were transferred to either sealed 120mm diameter plastic containers (Experiment 1) or to new holding cages (Experiment 2) two hours before sunset each day in order to obtain discrete age groups. Moths were deprived of food until used in experiments.

In Experiment 1a and 1b, adult moths were kept in a laboratory at 25 °C under ambient light conditions. In Experiment 2, moths were transferred to new holding cages and placed outdoors, under shelter. To prevent the insects from dehydrating in Experiment 2, cages were sprayed lightly with water (using a hand held sprayer) at noon each day.

Tobacco (*Nicotiana tabacum*) was cultivated from seed under glasshouse conditions. To maintain new floral growth, maturing fruits were removed, preventing seed production.

Volatiles
The odours phenylacetaldehyde and α-pinene (obtained from Sigma-Aldrich reagents) were used in the conditioning experiments. We used (racemic) α-pinene, which is a mixture of two (+ and -) enantiomers. Previous Electroantennogram (EAG) studies have demonstrated that these compounds elicit a peripheral olfactory response in *H. armigera* (Bruce and Cork, 2001; Burguiere et al., 2001).

Wind Tunnel Trials
Dual-choice preference tests were carried out in a wind tunnel with a Perspex flight chamber measuring 1600mm x 650mm x 650mm. Air was circulated through the flight chamber at 0.7m/s (as measured at the centre of the chamber) using a fan system. A clean airstream was maintained by passing the circulated air through an activated charcoal filter and a dust filter before allowing it to enter the chamber. A laminar airflow was obtained by directing air through a honeycomb of soda straws and then a fine stainless steel screen (1.25 mm aperture) prior to entering the chamber.

Male *H. armigera* moths show a characteristic surge in activity commencing at sunset, which corresponds with location of feeding sites (Topper, 1987). Feeding behaviour subsides around 90 minutes later (Cunningham, 1996). In all experiments, trials commenced 15 minutes after sunset and were confined to a 90 minute testing period. Moths were exposed to changing ambient light conditions associated with sunset in order to instigate and maintain a regular pattern of behaviour. Additional lighting (for observation) was supplied using a diffuse light source, with the light intensity in the flight chamber measuring less then 1lux throughout the experiment. The temperature inside the wind tunnel during the experiment was 24.4 °C (± 0.12 S.E.).

Three and four day old moths which had been held in individual plastic containers (120mm diameter) without access to food or water, were used in experiments. An antenna of the moth was gently touched with a cotton wool bud which had been soaked in 25% w/v sucrose solution, in order to test feeding responsiveness. Only moths which extended their proboscis once the cotton wool bud had made contact with an antenna were used in conditioning trials. Each moth was only used once.

Conditioning trials
Moths were randomly allocated to one of three treatment groups: (1) *Conditioned*; moths exposed to a volatile (phenylacetaldehyde or α-pinene) whilst feeding on sucrose solution. (2) *Expose*d; moths exposed to volatiles without allowing feeding or (3) *No exposure;* moths given no exposure to volatiles and left unfed. The groups were constructed in order to ascertain whether feeding was required to initiate any changes in preferences, and whether any innate odour preferences existed. We did not look in detail at the precise nature of the paring between the unconditioned stimulus (sucrose) and the conditioned stimulus (volatile) involved in odour conditioning. This has been covered by previous studies on *Helicoverpa* species using proboscis extension tests (Hartlieb, 1996; Hartlieb et al., 1999).

Treatment (1) Conditioned
Odour sources (*lures* hereafter), were created by inserting a 15mm absorbent cotton wool plug to a depth of 25mm below the wide end (5mm diameter) of a 145mm glass pipette. 2µl of either phenylacetaldehyde or α-pinene were pipetted onto the cotton wool no more than 15 minutes before the start of each experiment. The narrow end of the pipette was inserted into a 40mm x 50mm x 50mm block of floral foam (*Smithers-Oasis Ltd.*) positioning the odour source at a height of 145mm above the floor of the wind tunnel. Feeding sites were constructed similarly by

plugging the end of a glass pipette with a cotton wool wick soaked in 25%w/v sucrose solution. This second pipette was positioned such that the sucrose wick was situated 2cm downwind from the lure. New feeding sites and lures were used in each experiment.

Conditioning trials commenced by placing an individual moth on the sucrose wick and allowing a 30s feeding bout. Feeding was identified as contact of the extended proboscis with the sucrose wick. In this way the moth fed approximately 2cm downwind from the lure. After 30s, the moth was removed with a wooden toothpick and placed 400mm directly downwind from the lure / feeding site. Moths were then allowed to fly freely back to the feeding source. Upon contact with the sucrose wick, the moth was allowed to feed for a further 20s and returned to the downwind starting position. This process was repeated until moths had been given a total of 4 feeding visits in the presence of the volatile; one initial 30s feed and 3 x 20s return feeds.

Treatment (2) Exposed
Moths were exposed to either phenylacetaldehyde or α-pinene without being allowed to feed in order to test whether any differences in response between treatments may have occurred through exposure to the volatile, irrespective of feeding. Each insect was placed into a 50mm x 50mm black mesh bag clipped (using a fold back clip) to a wooden skewer at a height of 130mm. To expose the insect to the odour, the base of the skewer was inserted into floral foam immediately downwind from the odour source holding the insect at the same height and position relative to the lures as insects used in the conditioning trials. To match the exposure time in this treatment group with the conditioning trials, each moth was placed in this downwind position for four bouts (1 x 30s and 3 x 20s). Intervals of 1 minute were allowed between each exposure bout. During this interval the moth was placed 30cm upwind of the lure in the centre of the wind tunnel.

Treatment (3) No Exposure
We used unfed male moths with no previous exposure to either volatile to determine the innate odour preferences. Adult moths were placed into individual sealed (120mm diameter) plastic pots upon emergence and kept until testing at three to four days old. Preference for either α-pinene or phenylacetaldehyde was determined using the dual-choice testing procedure described below.

Preference testing
Each preference test comprised a dual-choice test using one α-pinene and one phenylacetaldehyde lure, the same procedure being employed for all three treatments. The lures were placed 300mm apart at the upwind end of the wind tunnel. Smoke tests (titanium tetra-chloride) showed that at a wind speed of 0.7m/s these plumes remained separate within the wind tunnel. Two 200mm x 150mm x 150mm Perspex wedges were placed at the downwind end of the wind tunnel bringing odour plumes together at a distance of 800mm from the lures and leaving a 200mm gap through which the odours were directed into the rear 350mm portion of the flight chamber.

Experiment 1a: Preference testing (immediate)
Immediately following the conditioning treatment, the lure and feeding source were removed. The two odour lures were placed in position only when moths were in the 350mm long section at the downwind end of the wind tunnel, where both the plumes had merged. This method was used in preference to catching moths and placing them at the downwind end; disturbing moths in this way often instigated avoidance behaviours and erratic looping movements. In the absence of an odour plume moths generally relocated to the downwind end of the wind tunnel, making it easy to position the lures. If a moth remained in the upwind end of the tunnel after a 3 minute period it

was caught and released downwind once the lures were in position. In the exposed and no exposure treatments, moths could be released directly into the downwind end of the wind tunnel.

Preference for a volatile was seen as a characteristic upwind flight pattern in the odour plume to within 100mm of a lure. Once a lure had been approached, the odour source (lure type) was recorded and the test was terminated. If moths failed to approach either lure within a 5 minute period the test was terminated. The position of the feeding lure and odour source in the conditioning trials (centrally placed, 325mm from either wall) differed from the position of either lure in the preference trials (200mm from either the right or left hand side wall); learning the position of the feeding lure would not influence the choice of lure in the test. The position of each lure (nearest to the right or left hand wall of the chamber) was allocated randomly throughout the experiment to avoid positional biases. The volatile used in conditioning treatments was alternated throughout the experiment.

Experiment 1b: Preference testing (24 hours after conditioning)
Moths were conditioned to either odour source as in Experiment 1a. Once the conditioning treatment was completed, the moth was placed into a 120mm diameter airtight plastic container where it was held in the laboratory at 25 °C, under ambient light conditions, for 24 hours. The following night, preference tests were carried out as in Experiment 1a. Moths were released individually into the downwind end of the flight chamber and the lure approached was recorded.

Experiment 2: Odour learning using odour enhanced flowers.

Tobacco flowers attract feeding adult *H. armigera* (Cunningham et al., 1998). We used standard volatiles-trapping techniques followed by GC-MS analysis to establish that no phenylacetaldehyde or α-pinene was present among the volatile odour compounds of cut tobacco flowers. This is consistent with published data (Loughrin et al., 1990).Our choice of plant and of test volatiles was directed in part by the desire to augment the natural flower odour with compounds which were normally absent.

Fig. 2. Odour augmentation of tobacco flowers in Experiment 2.

(2A) Corolla tube partially removed to display the location of the additional single odour (①) and sucrose feeding site (②) in augmented flowers.

(2B) Standardised inflorescence used in conditioning trials and preference tests.

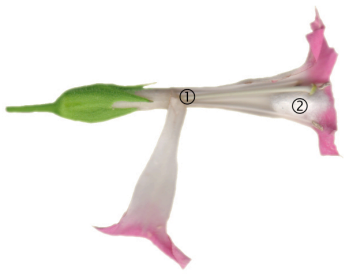

Fig 2A

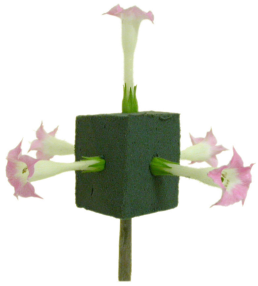
Fig 2B

Odour profiles of tobacco flowers were augmented by adding one of the additional volatiles into the base of the corolla tube. In this way two types of flower were created, these flowers being identical in visual cues, but differing in specific olfactory components detected by foraging moths. Tobacco flowers were picked one hour before dusk from plants reared in the glasshouse. Using a micropipette, 2µl of either phenylacetaldehyde or α-pinene were added into the inside base of the corolla tube. A third group of flowers, to which neither volatile was added, was prepared. The corolla tube was partially plugged using a small absorbent cotton wool plug which was lodged between the stamens at the lip of the corolla (Fig. 2A). The cotton wool plug was moistened with three drops (approx 0.1ml) of 25% (w/v) sucrose solution administered from a

pipette. This procedure provided sufficient sucrose solution for the duration of the conditioning experiments and prevented the insects from contacting either the floral nectar or added volatiles. Previous results have shown that moths that are fed from the top of the corolla will not attempt to enter the corolla tube in order to probe deeper (Cunningham et al., 1998).

To construct a standardised inflorescence, 5 flowers (from the same treatment group) were inserted, to the depth of the calyx, into a block of *Oasis* floral foam, (80mm x 60mm x 40mm) such that a single flower protruded from each of 5 faces of the block (Fig. 2B).

Conditioning experiments

On any one night, moths were conditioned using a single treatment group of flowers; odour enhanced using (1) phenylacetaldehyde or (2) α-pinene or (3) flowers with no added volatiles (non-enhanced). The standardised inflorescence bearing flowers was positioned at a height of 1m on a bamboo cane in the centre of an outdoor flight cage (1.8m x 1.8m x 1.8m).

To begin each conditioning trial, a single male was removed from the holding cage and encouraged to commence feeding using a cotton wool bud moistened with 25% w/v sucrose. Once proboscis extension was observed, the moth was placed onto the corolla lip of one of the tobacco flowers where it was allowed to feed on the sucrose wick for 30s. The moth was then removed using a wooden toothpick, and held at a distance of 200mm from the flowerhead. Insects were allowed three return visits to the flowers, with 20s feeding at each return. After the third return visit (insects having had a total of 90s of feeding) the moth was caught and held in a plastic container. Moths were conditioned in this manner until feeding activity subsided (around 90 minutes after the experiment commenced).

Preference Testing

Preference testing of flower conditioned moths was carried out on the following night. Two standardised inflorescences were constructed as previously described; one inflorescence using odour-enhanced flowers augmented with phenylacetaldehyde, the other with flowers augmented with α-pinene. Flower corolla tubes were partially blocked with cotton wool as in conditioning trials, but no sucrose solution was added to the cotton wool. Conditioned moths were released into the flight cage one hour before sunset. Fifteen minutes before sunset, the inflorescences were placed on 1m canes at a distance of 1m apart.

Moths which approached and landed on flowers were captured and the treatment group of the inflorescence visited was recorded. Once captured, moths were not re-released. The experiment was continued until all moths had been captured or until flight activity ceased, around 90 minutes later.

The experiment was repeated over 22 nights (7 trials for each odour-enhanced flower treatment and 8 trials for the non-enhanced flower treatment). On each night of conditioning, the treatment group was assigned randomly. In preference tests, the position of the flower head within the cage was assigned randomly using a grid . Each insect was only used once.

Statistical Analysis

Data were analysed using generalised linear modelling techniques (McCullagh and Nelder, 1989) in the GLIM statistical package (Crawley, 1993). Choice test outcomes were analysed as proportions, with the number of moths selecting a particular odour as the response variable and

the total number of moths selecting either host as the binomial denominator. Binomially distributed error variances were assumed and a logit link function employed. In all cases, we initially fitted a maximal model to the data, with all explanatory variables and experimental treatments. We then used the process of stepwise deletion (see Crawley 1993) to remove terms from the model until a minimal model was obtained. Hypothesis testing was carried out using a G -test on differences in deviance. Differences in treatments were assessed by testing whether grouping them caused a significant change in the deviance explained.

In Experiment 1a and Experiment 1b, due to the low number of moths tested each night (4-6 moths), data was pooled over the 27 nights of testing. Treatment order was randomised to prevent any biasing which may have resulted from night of testing. In Experiment 2, night of testing was included in the analysis to avoid pseudo-replication.

RESULTS

Experiment 1: Odour preference in conditioned and unconditioned moths
In total 160 adult male moths displayed upwind flight towards odour lures in the dual choice preference tests, carried out in the wind tunnel over 27 nights (mean moths/night = 5.93 ± 0.32 S.E.). Of these moths, 80 were in the *conditioned* treatment group (odour+feeding), 40 in the *no exposure* (no odour + no feeding) and 40 in the *exposed* (odour + no feeding). Preferences for α–pinene and phenylacetaldehyde lures for all treatment groups are summarised in Table 1. Differences between treatment groups are presented in Table 2 as G values determined by GLIM (G-test). Preferences between treatment groups were significantly different ($G_{(6\ df)} = 82.5$. $P<0.001$). Position of the odour lures (left or right side of wind tunnel) did not influence odour plume choice ($G_{(1\ df)} = 0.20$; $P>0.05$).

Table 1. *Results of Experiments 1a and 1b: Number of moths selecting each lure for each treatment group.*

Treatment group	Number of moths tested	Lure Selected aldehyde	pinene	% selecting pinene
1a: aldehyde	20	20	0	0
1a: pinene	20	0	20	100
1b: aldehyde	20	17	3	15
1b: pinene	20	3	17	85
No exposure	40	26	14	35
Exposed aldehyde	20	12	8	40
Exposed pinene	20	15	5	25

The table displays the number of moths selecting each lure (phenylacetaldehyde and a-pinene) in dual-choice preference tests. The percentage of moths selecting phenylacetaldehyde in each treatment group is also displayed (percentages selecting α-pinene = 100-value for each treatment). Each treatment group represents a new set of moths (total 160 moths). **Treatment Groups:** 1a = Experiment 1a (immediate test) ; 1b = Experiment 1b (24 hour test). pinene / aldehyde = moths in "conditioned" group, using α-pinene or phenylacetaldehyde respectively as the conditioning stimulus. No exposure = moths given no exposure to either volatile and left unfed. Exposed pinene / aldehyde = moths exposed to a-pinene or phenylacetaldehyde respectively without feeding.

Statistical analysis of these data is presented in Table 2.

The innate preferences of the adult male moths for either phenylacetaldehyde or α-pinene were determined by testing moths which had been given no experience of volatiles and no feeding

experience before testing (*no exposure* treatment). These moths showed a significant preference for the phenylacetaldehyde lure ($G_{(1\ df)} = 7.312$; $P<0.01$).

All moths (N=40) tested on the same night as conditioning (Experiment 1a) flew to the lure emitting the volatile on which they had been conditioned. Feeding experience in the presence of a volatile therefore led to significant differences in odour choice ($G_{(1\ df)} = 55.45$; $P<0.001$). No differences were found between moths given no experience of volatiles or feeding (*no exposure* group) and moths exposed to volatiles for the same amount of time as in conditioning trials but without pairing this with feeding (*exposed* group) ($G_{(2\ df)} = 1.09$). Thus changes in preference were attributed to classical conditioning; pairing of odour with feeding.

When moths were tested 24 hours after conditioning (Experiment 1b), preference for the conditioned odour was significantly lower compared to Experiment 1a moths ($G_{(1\ df)} = 4.40$, $P<0.025$). When the proportion of "errors" (moths choosing the non-conditioned odour) per night was examined for Experiment 1b moths, night of testing was not found to be significant ($G_{(6\ df)} = 5.288$); therefore the decrease in preference in this group was not attributed to greater error on any one night. Moths conditioned on α-pinene showed a significantly higher preference for the α-pinene lure compared to moths conditioned on phenylacetaldehyde ($G_{(1\ df)} = 21.64$, $P<0.001$) and moths without associative conditioning (unconditioned moths and exposed moths) ($G_{(1\ df)} = 17.98$, $P<0.001$). Preferences of Experiment 1b moths conditioned on phenylacetaldehyde were not significantly different from moths without associative conditioning ($G_{(1\ df)} = 2.97$, $P> 0.05$).

Table 2. *Summary of results of hypothesis testing (G-Test) to determine the significance of differences between treatments.*

Test	$G_{(df)}$	*P*
All Treatments	$82.5_{(6)}$	<0.001
Exposure vs no exposure	$1.09_{(2)}$	ns
aldehyde vs pinene	$7.312_{(1)}$	<0.01
1a: pinene vs aldehyde	$55.45_{(1)}$	<0.001
1a: pinene vs no exposure	$35.97_{(1)}$	<0.001
1a: aldehyde vs no exposure	$14.35_{(1)}$	<0.001
1b: pinene vs aldehyde	$21.64_{(1)}$	<0.001
1b: pinene vs no exposure	$17.98_{(1)}$	<0.001
1b: aldehyde vs no exposure	$2.97_{(1)}$	ns
1a pinene vs 1b pinene	$4.402_{(1)}$	<0.025
1a aldehyde vs 1b aldehyde	$4.402_{(1)}$	<0.025

See table 1 and methods for explanation of treatment groups.
ns = not significantly different (*P*>0.05)

The data are presented in Table 1.

Experiment 2: Odour learning in manipulated plants

We carried out 22 trials using 111 adult male *H. armigera* . Moths trained on α-pinene and phenylacetaldehyde enhanced flowers (41 moths per treatment) were trained over 14 trials (7 trials for each flower type, 5.86±0.48 SE moths/trial). Moths trained on non-enhanced flowers (N=29 moths) were trained over 8 nights (3.63±0.56 moths/trial). Treatment groups showed significant differences in preference for flowers ($G_{(2\ df)} = 19.5$, $P<0.001$).

Moths conditioned on the α-pinene enhanced flowers showed a greater preference for these flowers when compared with moths trained on phenylacetaldehyde enhanced flowers ($G_{(1\ df)}$ = 18.8, P<0.001) and moths trained on non-enhanced flowers ($G_{(1\ df)}$ = 8.0, P<0.005). Moths trained on phenylacetaldehyde showed a preference for the flowers containing phenylacetaldehyde (Fig. 1) but this was not significantly different from the moths trained on non-enhanced flowers ($G_{(1\ df)}$ = 1.41, P>0.05). Moths trained on non-enhanced flowers showed no difference in preference for either flower type ($G_{(1\ df)}$ = 0.07, P>0.05).

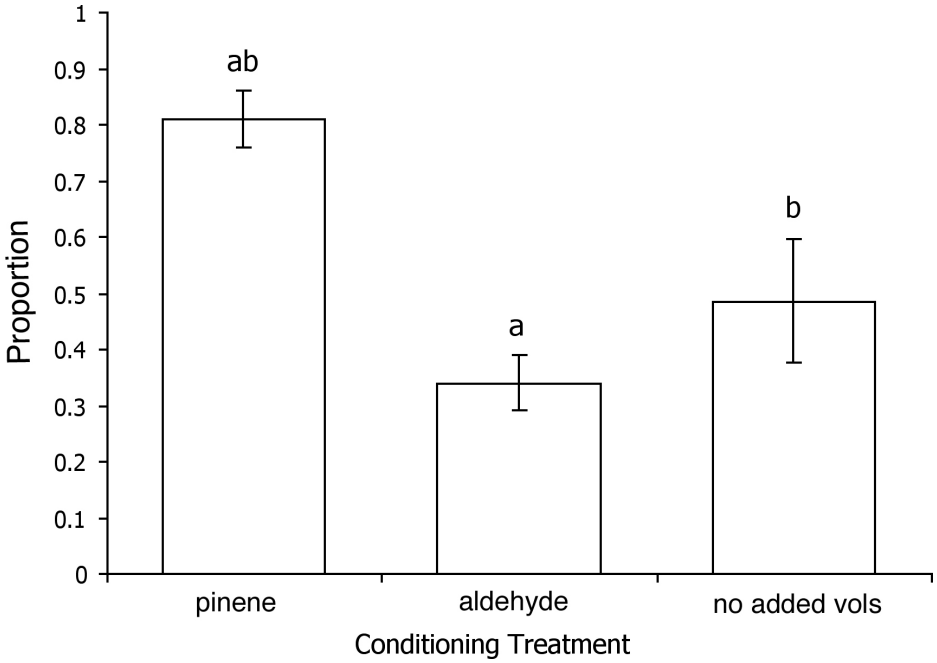

Fig. 1. The mean proportions of insects in Experiment 2 which selected tobacco flowers (± SE) augmented with α-pinene in each treatment group. Moths had been conditioned on flowers augmented with either α-pinene (pinene), phenylacetaldehyde (aldehyde), or no extra volatiles. Corresponding proportions of insects selecting flowers augmented with phenylacetaldehyde are 1-mean proportions shown for each treatment. Common letters beside bars denote significance of differences between treatments (G-test, GLIM). a = P<0.001, b = P<0.005.

DISCUSSION

This study shows that the upwind flight of male *H. armigera* moths towards different odour sources is strongly influenced by previous odour experience. In wind tunnel dual-choice bioassays, moths which were fed in the presence of a single volatile showed a preference for that odour compared to a second volatile which they had not experienced. Moths with no experience of the volatiles did not differ in their relative preferences for either odour source from those exposed to volatiles without association with a food reward. The results demonstrate that associative conditioning influences preferences for host odours in foraging moths. Studies on the proboscis extension reflex (PER) in *H. armigera* have looked more closely at the nature of the pairing in this type of learning (Hartlieb, 1996).

Moths flew upwind to both odours in the absence of conditioning which implies that an innate attraction to these odours exists. An innate preference for phenylacetaldehyde over α-pinene was

demonstrated in this treatment group, which suggests that attraction to odours is hierarchical, with certain odours being more attractive than others. These innate preferences then become modified through experience. Strictly speaking, conditioning to the odours in this form is termed an α-response, as a prior response to the conditioning stimulus (odour) already exists (Menzel et al., 1993).

Following a 24 hour period without reinforcement, a strong preference for the odour on which the moths were conditioned the previous night was still evident. This suggests that foraging decisions which occur during one night of feeding influence behaviour on the following night. The fidelity to the learned odour after 24 hours was lower than on the initial night of conditioning. A decline in the strength of the conditioned stimulus in eliciting a response with the absence of reinforcement is typical of classical conditioning (Papaj and Prokopy, 1989). Differences between the immediate test and the 24 hour test may also be related to the changes associated with short and long-term learning and memory (Menzel et al., 1993).

When the odour of tobacco flowers was enhanced with either phenylacetaldehyde or α- pinene, feeding experience again lead to significant differences in flower visiting. Moths preferred to visit flowers enhanced with the same odour as the flowers on which they were trained. Moths could therefore discriminate between flowers that differed in a single volatile compound. We therefore show that the discrimination and learning of odours is not solely a product of a "stimulus deficient" wind tunnel environment, where only a single conditioning stimulus (odour) is present. Moths can detect differences in odours which may exist between flowers with many common visual and olfactory stimuli. These differences are learned associatively with feeding. Moths with experience of the non-enhanced flowers show no preference for either enhanced flower type. Here, differences in preference may reflect natural variations in odour output of individual flowers.

The odour profile of flowers within a single species can vary in the presence, concentration and relative proportions of their constituents at different times of day (Baldwin et al., 1997; De Moraes et al., 2001; Heath et al., 1992; Shaver et al., 1997). Such variations in odour output have been linked to the attraction of pollinators and deterrence of pests (De Moraes et al., 2001; Heath et al., 1992). Other variables, such as insect damage (De Moraes et al., 2001; Kessler and Baldwin, 2001; McCall et al., 1994) and the onset of pollination (Schiestl and Ayasse, 2001) can lead to variations in the odour among plants of the same species . Where such odour signals are consistent with changes in nectar rewards from flowers, recognising such correlations between odour and reward will have fitness benefits to foraging insects. Associative learning of these subtle differences in odour would be advantageous to the generalist forager.

In conclusion, we demonstrate that both innate and learned behaviours are playing important roles in attraction to the individual volatile components of a floral blend. Innate responses to odours predict the expected environment and will have a strong influence on floral choice in newly emerged adult insects. Learning shapes the insects responses to odours to its local environment, increasing the response to odours which have previously led to successful foraging. Thus the role of odours in plant-insect communication cannot be determined by concentrating solely on the behavioural responses of naive moths. The "attractiveness" of volatiles to moths in nature is likely to depend as much on ecological factors such as host abundance as on inherited odour preferences (Cunningham et al., 2001; West and Cunningham, 2002). Where learning has a strong influence on the preference for floral odours, the volatiles emitted from the most frequently visited rewarding host species will be those towards which the insect will be the most attracted. Clearly, response to odour is a dynamic system that is as dependent on an ever-

changing environmental and behavioural context as it is on a highly evolved system of odour recognition and response.

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DETECTION OF ENANTIOMERS OF α-PINENE BY *HELICOVERPA ARMIGERA*: ELECTROPHYSIOLOGY AND BEHAVIOUR.

AIM: Synthetic floral odours form the basis of lure and kill strategies for heliothis moths. However, where these odours occur both naturally and through synthetic analogues as different enantiomers, little is known about whether moths detect and show preferences for the different forms of these compounds. The aim of this study was to improve our knowledge of the response of female moths to different enantiomers of the volatile α-pinene, a common volatile in many of the host plants of this moth. This knowledge could be important in the selection of volatiles to attract adult moths.

This study was carried out in collaboration with Dr Craig Hull (University of Queensland) who performed the electrophysiology studies.

METHODS AND MATERIALS

Electrophysiology

Insects. Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) were obtained as pupae from the Queensland Department of Primary Industries, Toowoomba. On emergence moths were individually placed into 50 mL plastic containers and given unlimited access to water. Female moths were tested between 2 to 5 days of age.

Test chemicals. The (+) form of α-pinene was obtained from Sigma Chemical Company and had a claimed purity of 98% and optical purity of 91% ee (enantiomeric excess). The (-) form was obtained from Aldrich Chemical Company and had a claimed purity of 99% and optical purity of 97% ee.

Electroantennograms (EAGs). This technique was used to assess the summed response of antennal receptors and determine response profiles across receptor cell fields. The method used follows Hull and Cribb (2001a) with minor changes: The glass capillaries were filled with a physiological saline (Chen and Friedman, 1975); the antenna was cut off at the base, and secured on Blu-tack (Bostik (Australia) Pty Ltd). A drop of physiological saline was placed over the base of the antenna to prevent desiccation. The indifferent electrode was inserted into the base of the antenna. The tip of the recording electrode was cut so that it could be placed over the tip of the antenna - which was not cut - as adequate electrical contact could be made this way.

Odour delivery. Humidified analytical grade compressed air was continuously blown over the moth at a rate of 400 cm^3/min, with the nozzle for the air-stream placed 1 cm from, and directly in front of, the antenna. The tube carrying the air flow was 3 mm internal diameter teflon tubing, connected to a glass nozzle (same internal diameter). Test odour samples were taken as saturated vapour, at room temperature, using gas-tight syringes. They were manually injected into the air-stream through a rubber septum, 8 cm from the delivery point. Injection time was between 0.5 and 1.0 secs.

Experimental procedure. In all experiments the responses were compared to a standard of 400 µl hexanol vapour. The method of standardisation, as well as the establishment of the saturating volumes followed that of Hull and Cribb (2001a). Chemicals were tested as binary mixtures, using 800 µL of each form, to determine receptor neuron types. The order of presentation of the mixture series and the chemicals within a mixture series were randomised.

Mixture experiments were analysed using paired sample *t*-tests. Initially a 1-way test was conducted to determine if the response to the mixture of chemicals was greater than the response of the larger of the two individual chemicals (*i.e.* a summating response). If the response to the mixture was summating, then a 2-way test was conducted to determine if the response to the mixture was equal to the calculated additive response of the two individual chemicals (*i.e.* fully summating). Before analysis, the mean result of stimulation with a control injection of clean air was subtracted from the antennal response in both the dose response and mixture experiments. To eliminate the possibility that some or all of the signals were artefacts due to electrode potentials, control experiments with dead antennae were conducted. The moths were frozen at approximately - 20 °C. After removal from the freezer, the moths were allowed to return to normal room temperature before the antennae were tested as above with both (+) and (-) α-pinene.

Single unit electrophysiology. This technique was used to record from individual receptor cells and determine their response profiles. Methodology followed Hull and Cribb (2001b) with these modifications: the excised antenna was secured onto Blu Tack adhesive, and a drop of saline placed over the base to prevent desiccation. The indifferent electrode was inserted into the base of the antenna. The nerve impulses were counted for the first 0.5 sec of stimulation.

Behaviour

Insects. Pupae were sexed and female moths were placed in a separate holding cage (200 mm x 150 mm x 150 mm) until eclosion. Newly emerged adult females were transferred to sealed 120 mm diameter plastic containers two hours before sunset each day in order to obtain discrete age groups. Moths were deprived of food until used in experiments. Adult moths were kept in a laboratory at 25 °C under ambient light conditions. Moths were tested between 3 to 5 days of age.

Wind tunnel trials. Dual-choice preference tests were carried out in a wind tunnel with a central Perspex flight chamber measuring 1600 mm x 650 mm x 650 mm (see Cunningham *et al*. Submitted, for further details). A laminar flow of clean air was circulated through the flight chamber at 0.7 m/s (as measured at the centre of the chamber using a fan system).

Preference tests. Procedures for conditioning and testing moths in the wind tunnel have been described in detail previously (Cunningham *et al*., Submitted). Odour sources (lures hereafter) were created by inserting an absorbent cotton wool plug to a depth of 25 mm below the wide end of a 145 mm glass pipette. 2 μL of either (+) or (-) α-pinene were pipetted onto the cotton wool no more than 15 mins before the start of each trial. The narrow end of the pipette was pushed into a block of floral foam, positioning the odour source at a height of 145 mm above the floor of the wind tunnel. To test the preference of moths for either the (+) or (-) α-pinene enantiomer, lures were placed 300 mm apart at the upwind end of the wind tunnel. Smoke tests (titanium tetra-chloride) showed that at a wind speed of 0.7 m/s these plumes remained separate within the wind tunnel. Two perspex wedges positioned in the downwind end of the wind tunnel brought the odour plumes together at a distance of 800 mm from the lures and left a 200 mm gap through which the odours were directed into the rear portion of the flight chamber.

Moths having previously undergone one of three treatments (associative conditioning with either (+) or (-) α-pinene, or no conditioning) were allowed to relocate into the downwind end of the wind tunnel before odour lures were placed in position. Preference for a particular odour was seen as a characteristic upwind flight pattern in the odour plume to within 100 mm of a lure. Once a lure had been approached, the odour type was recorded and the test terminated.

If moths failed to approach either lure within a 5 min period the preference test was terminated. The position of the feeding lure and odour source in the conditioning trials (centrally placed, 325 mm from either wall) differed from the position of either lure in the preference trials (200 mm from either the right or left hand side wall) so that learning the position of the feeding lure would not influence the choice on lure in the test. The position of each lure (*i.e*. nearest to the right or

left hand wall of the chamber) was allocated randomly throughout the experiment to avoid positional biases. The volatile used in conditioning treatments was alternated throughout the experiment.

Associative conditioning treatments. Associative conditioning trials were used to determine whether learning of one enantiomer would lead to a preference for that enantiomer in a dual choice test. The ability to learn to prefer one enantiomer would imply that moths can distinguish between the (+) and (-) α-pinene forms. Feeding sites were constructed similarly by plugging the end of a glass pipette with a cotton wool wick which had been soaked in 25% w/v sucrose solution. This second pipette was placed in the same block of floral foam, such that the sucrose wick was situated 2 cm downwind from the lure. New feeding sites and lures were used in each experiment.

Conditioning trials commenced by placing an individual moth on the sucrose wick and allowing a 30 sec feeding bout. Feeding was identified as contact of the extended proboscis with the sucrose wick. In this way the moth fed approximately 2 cm downwind from the lure. After 30 sec, the moth was removed with a wooden toothpick and placed 400 mm directly downwind from the lure/feeding site. Moths were then allowed to fly freely back to the feeding source. Upon contact with the sucrose wick, the moth was allowed to feed for a further 20 sec and returned to the downwind starting position. This process was repeated until moths had been given a total of four feeding visits in the presence of the volatile; one initial 30 sec feed and 3 x 20 sec return feeds. This procedure has previously been shown to lead to associative conditioning in male *H. armigera* (Cunningham *et al*., Submitted).

Conditioning trials using phenylacetaldehyde. To confirm that female moths could learn enantiomers of α-pinene, we compared the odour preferences of female moths conditioned on (-) α-pinene with moths conditioned on the single floral odour phenylacetaldehyde in a dual choice preference test using (-) α-pinene and phenylacetaldehyde lures (2 µl odour per lure). Conditioning trials and preference testing procedures for this experiment were identical to those describe for the α-pinene enantiomer trials.

Innate preference treatment. We used unfed female moths with no previous exposure to either α-pinene enantiomer to determine the innate odour preferences. Adult moths were placed into individual sealed (120 mm diameter) plastic pots upon emergence and kept until testing at three to four days old. Preference to (+) or (-) α-pinene was determined using the dual-choice testing procedure described above.

Statistical Analysis. Data were analysed using generalized linear modelling techniques (McCullagh and Nelder, 1989) in the GLIM statistical package (Crawley, 1993). Choice test outcomes were analysed as proportions, with the number of moths selecting a particular odour as the response variable and the total number of moths selecting either host as the binomial denominator. Binomially distributed error variances were assumed and a logit link function employed. Hypothesis testing was carried out using the χ^2 test on differences in deviance. Treatment order was randomised to prevent any biasing which may have related to night of testing.

RESULTS

ELECTROPHYSIOLOGY

Dose response curves. For the (+) and (-) forms of α-pinene the (-) form gave larger responses at all of the tested concentrations. The saturation point for both forms was reached by 400 µL. No signals were detected in the control experiments, showing that the signals recorded were solely due to physiological activity within the antenna.

Mixture experiments. The lack of additivity indicates that the two isomers are being detected by the same sensory receptor cell. The response to the mixture of the two isomers was compared to the larger response of the individual chemicals; the (-) form. The response to the mixture was not larger than the response to the minus form (1 way *t*-test between the mixture and the (-) form, t = 1.419, 9df, p = 0.0948).
Single unit electrophysiology. The α-pinene cell responded to both the (+) and (-) forms (in both recordings of an α-pinene cell).The cells responded in a dose dependant fashion, similar to that seen in the EAG response. One consistent spike height was present, indicating one cell type only was responding. There were no instances of double-height spikes, which would have indicated that two or more cells with a similar spike height were present.

BEHAVIOUR
We tested the enantiomer preference of 84 moths from three treatments. In dual-choice preference tests (Figure 1) treatment groups showed significant differences in enantiomer preference (G =7.832, 2df, P<0.05). Moths with no odour conditioning showed an innate preference for (+) α-pinene (χ^2 = 8.708, 1df, P<0.005). This preference displayed by naïve moths was not significantly different from the preferences of moths conditioned on (+) α-pinene (G =1.057, 1df, P>0.05). However, we found a significant difference in preference between moths conditioned on the (-) enantiomer compared to naïve moths and moths conditioned on (+) α-pinene (G = 6.776, 1df, P<0.01).

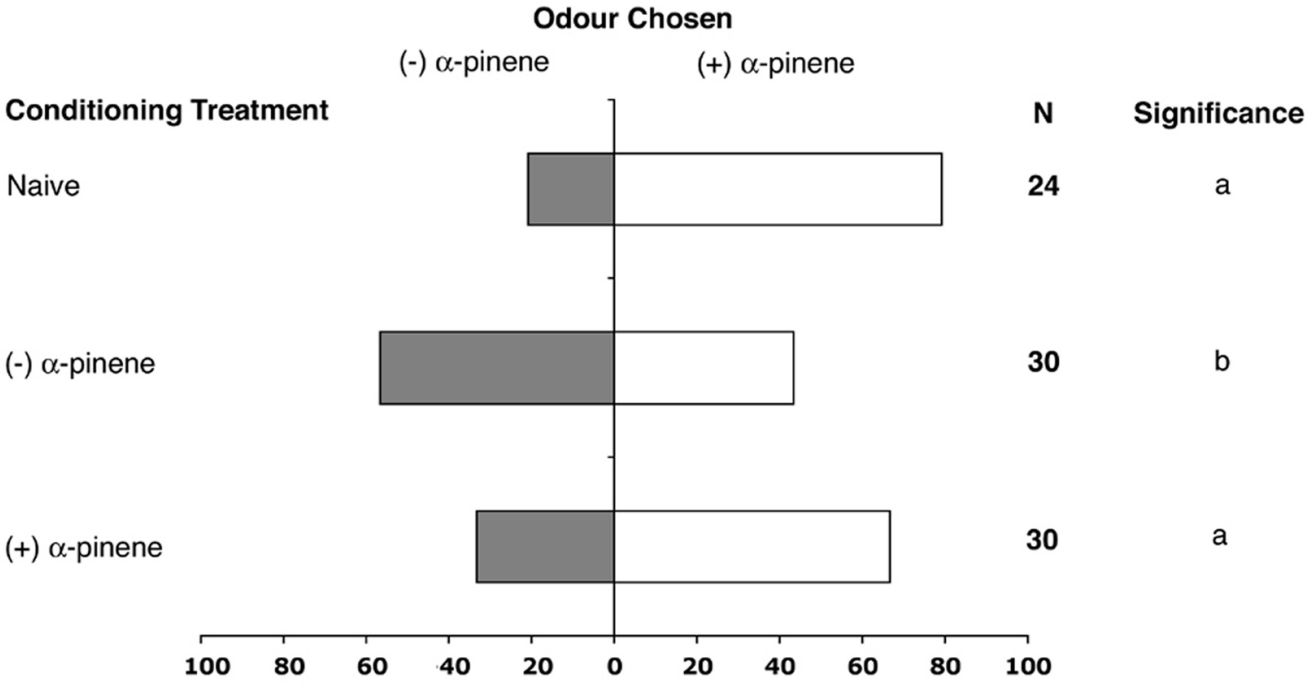

Figure 1. Percentages of moths selecting either (+) or (-) α-pinene in dual-choice preference tests. Treatments with common letters are not significantly different (P>0.05). Different letters denotes a significant difference (P<0.01) as determined by G-tests.

No change in preference after conditioning on an odour implies either that the insect is not capable of differentiating between the odours in the preference test, or that the odour itself cannot be learned under these test conditions. To confirm that the latter hypothesis was not the case here

(*i.e*. that neither enantiomer was learned) we compared the odour preferences of moths (male or female) conditioned on (-) α-pinene with moths conditioned on the single floral odour phenylacetaldehyde in a preference test using these two odours. Results of this test (N = 24 moths) showed significant differences in preference between moths conditioned on phenylacetaldehyde and moths conditioned on (-) α-pinene. All moths (12/12) trained on phenylacetaldehyde showed a preference for this odour compared with 1/12 moths trained on (-) α-pinene (i.e. 11/12 moths showed a preference for the α-pinene lure). This significant difference in preference ($G = 26.22$, 1df, $P<0.001$) demonstrates that when given two distinct odours, moths show strong differences in preference.
Comparison of the learning ability showed that moths trained on the two different odour compounds (phenylacetaldehyde and (-) α-pinene) showed a greater preference for the learned odour compared to the moths trained on two enantiomers of the same odour ($G = 12.31$, 1df, $P<0.001$) suggesting that although learning changed preferences in both these experiments, the ability to learn the differences between different enantiomers of the same odour was lower than when different odours were learnt.

DISCUSSION

α-Pinene is a monoterpene (10-carbon) compound given off by several host plant species of *H. armigera* (Rembold *et al*., 1989) and has been shown to play a role in behavioural attraction of the female moths towards artificial lures (Rembold *et al*., 1991). Our electrophysiological results indicate that the two enantiomers of α-pinene are detected by the same receptor cell dendrites: When mixtures of chemicals at saturating levels are used, a response to the mixture that is higher than for either of the individual chemicals indicates separate receptor cells are being used (Borst, 1984; Hull and Cribb, 2001a). Because no additivity was found in our EAG experiments, the same cells are likely to receive both enantiomers. Single unit recording confirms this hypothesis. Such a result is consistent with that of Stranden *et al*. (2002), who found that the enantiomers of a different plant chemical, the sesquiterpene (15-carbon) germacrene-D, were also received by the same receptor cells. The initial dose response EAG experiments showed that the (-) form of α-pinene produced a higher level of activation of the receptor cell dendrites. This was not mirrored in the single unit recordings where responses appeared similar for (-) and (+) forms, however the small number of receptors directly recorded from does not necessarily provide the average response across the sensillar field: for this information the EAG data are more reliable. The most likely explanation for a higher average response to the (-) form is that the (+) form does not bind as efficiently with the molecular receptor in many of the sensilla; although less efficiency in other steps of the transduction process such as transport to the receptor site cannot be discounted. A difference in electrophysiological response to the enantiomers of germacrene-D was also found (Stranden *et al*., 2002). However, this difference was seen only in single unit recordings so the average response for germacrene-D across the sensillar field of *H. armigera* is not yet known. The behavioural experiments demonstrate that *H. armigera* is able to distinguish between the enantiomers of α-pinene despite their being received on the same receptor. Additionally, the innate preference for one enantiomer is greater than for the other but the results are counter-intuitive. The moths show an innate behavioural preference for the (+) form of α-pinene which provides the smaller physiological signal in EAGs. This result shows that behavioural decisions are not necessarily based simply on the largest physiological response of the receptors, and once again indicates the complexity of the decision making process. For the studies using germacrene-D, Mozuraitis *et al*. (2002) only tested the behavioural response to the enantiomer that gave the larger electrophysiological response (in single unit recordings). Our results suggest that further studies with the (+) form of germacrene-D need to be undertaken.

An important outcome from our study is that moths can change their response to enantiomers as a result of experience: In other words they can learn to discriminate in favour of an enantiomer. This occurred when moths were able to increase their response to the (-) form over the innate response. However, the ability to learn to distinguish between the (+) and (-) enantiomers was low when compared to learning to distinguish (-) α-pinene from the single odour phenylacetaldehyde. Stranden *et al*. (2002) hypothesise that separate receptor neuron types for enantiomers will be needed if an insect is to be able to distinguish a plant based on differences in enantiomeric composition of specific compounds. We have shown that this is not the case for α-pinene.

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DOE *H. ARMIGERA* LEARN ODOUR COMPONENTS IN AN ECOLOGICAL CONTEXT?

AIM:

The aim of this study was to further investigate the influence of experience on attraction to odours. Here, we allowed adult moths to freely forage on a number of different flower species and tested how this experience influenced odour preferences. If moths change their preferences for odours with experience of different flowers, this has crucial implications regarding the use of different odours for lure and kill and monitoring programmes in the field.

METHODS

Insect Culturing

Helicoverpa armigera moths were obtained as pupae from a laboratory culture reared at QDPI Toowoomba, Queensland, Australia. Larvae had been reared on a soyflour based artificial diet for *Helicoverpa* spp., minimising any possible influence of experience of host plants at this stage (Jermy et al., 1968). Pupae were sexed and male moths were placed in a separate holding cage (200mm x 150mm x 150mm) until eclosion. Newly emerged adult males were transferred to either sealed 120mm diameter plastic containers two hours before sunset each day in order to obtain discrete age groups. Moths were deprived of food until used in experiments.

Plants

Tobacco (*Nicotiana tabacum*) and Pigeonpea (*Cajanus cajan*) were cultivated from seed under glasshouse conditions. Federation Daisies (*Argyranthemum frutescens*) were obtained as flowering plants. Flowers used in the behavioural experiments and chemical analysis were cut not more than one hour before the start of the experiment and kept hydrated in floral foam (*Smithers-Oasis Ltd.*) until used.

Odours

The odours linalool (95% purity, Sigma-Aldrich reagents), phenylacetaldehyde (90% purity Sigma-Aldrich reagents) and β-caryophyllene (CA Aromatics Company, >80% purity) were used in the conditioning experiments. Previous Electroantennogram (EAG) studies have demonstrated that these compounds elicit a peripheral olfactory response in *H. armigera* (Bruce and Cork, 2001; Burguiere et al., 2001). Two microlitres of the odour compound were added to 1 mil of paraffin oil (Sigma-Aldrich reagents) to create a 0.2% solution.

Odour lures

Odour sources (*lures* hereafter) were created by inserting a 15mm absorbent cotton wool plug to a depth of 25mm below the wide end (5mm diameter) of a 145mm glass pipette. 2μl of either phenylacetaldehyde or linalool solution (equivalent to 0.004μl of odour) were pipetted onto the cotton wool no more than 2 minutes before the start of each preference test. New lures were used in each preference test.

Flower odour profiles

We used standard volatiles-trapping techniques followed by GC-MS analysis to establish the odours present in cut tobacco, daisy and pigeonpea flowers. Cut flowers were sampled over a two hour period commencing at sunset. We ranked the context within which a particular odour component appeared within the floral blend by two factors (1) the predominance of the

component relative to all other flower odours present in the sample (2) The total number of volatile components in the blend. We classed the absence of a component (for use in preference tests) as one that could not be detected at a level of 0.01% of the total area

Conditioning Experiments

Wind tunnel trials. Conditioning treatments and Dual-choice preference tests were carried out in a wind tunnel with a central Perspex flight chamber measuring 1600 mm x 650 mm x 650 mm (see (Cunningham et al., (submitted) for further details). A laminar flow of clean air was circulated through the flight chamber at 0.7 m/s (as measured at the centre of the chamber using a fan system).

In all experiments, trials commenced 15 minutes after sunset and were confined to a 90 minute testing period corresponding to the natural peak feeding times for this species (Cunningham, 1996; Topper, 1987). Moths were exposed to changing ambient light conditions associated with sunset and temperature was held at 27°C Additional lighting (for observation) was supplied using a diffuse light source, with the light intensity in the flight chamber 1lux throughout the experiment.

Three day old moths which had been held in individual plastic containers (120mm diameter) without access to food or water, were used in experiments. An antenna of the moth was gently touched with a cotton wool bud which had been soaked in 25% w/v sucrose solution, in order to test feeding responsiveness. Moths which extended their proboscis once the cotton wool bud had made contact with an antenna were used in conditioning trials. Each moth was only used once.

Flower conditioning

Flowers were clipped to the top of wooden splints, holding them at a height of 10cm above the floor of the wind tunnel. A single tobacco flower or daisy flower was used for each conditioning treatment, using new flowers in each trial. In pigeonpea treatments, two flowers were used in each trial to allow for size difference. Splints were held upright by placing them in blocks of dry floral foam (50mmx40mmx20mm). Sucrose feeding sources were used to prevent any foraging biases, which may have been caused by differences in handling times(Cunningham et al., 1998b). Feeding sources comprised of cotton wool wicks immersed in sucrose solution (25%w/v). Wicks were placed at the entrance to the corolla in tobacco flowers, on the top of the daisy inflorescence and suspended between two adjacent pigeonpea flowers. All moths located the sucrose feeding source on alighting.

Conditioning trials commenced by placing an individual moth on the flower and allowing a 30s feeding bout. Feeding was identified as contact of the extended proboscis with the sucrose wick. After 30s, the moth was removed with a wooden toothpick and placed 400mm directly downwind from the lure / feeding site. Moths were then allowed to fly freely back to the feeding source. Upon contact with the sucrose wick (on the flower), the moth was allowed to feed for a further 20s and returned to the downwind starting position. This process was repeated until moths had been given a total of 4 feeding visits; one initial 30s feed and 3 x 20s return feeds.

Preference testing

Each preference test comprised a dual-choice test using two odour lures. In experiment 1, the odours used in lures were phenylacetaldehyde and linalool; for each flower tested one of the lures

would be an odour found to be in the floral blend, the second lure would be an odour not found in the floral blend. In Experiment 2, both odours used, linalool and β-caryophyllene, were present in the floral blend (tobacco) (Table 1). The lures were placed 300mm apart at the upwind end of the wind tunnel. The narrow end of the pipette was inserted into a 40mm x 50mm x 50mm block of floral foam (*Smithers-Oasis Ltd.*) to hold the lure mouth at a height of 10cm. Smoke tests (titanium tetra-chloride) showed that at a wind speed of 0.7m/s these plumes remained separate within the wind tunnel. Two 200mm x 150mm x 150mm Perspex wedges were placed at the downwind end of the wind tunnel bringing odour plumes together at a distance of 800mm from the lures and leaving a 200mm gap through which the odours were directed into the rear 350mm portion of the flight chamber.

Immediately following the conditioning treatment, the flower was removed. The two odour lures were placed in position only when moths were in the 350mm long section at the downwind end of the wind tunnel, where both the plumes had merged. If a moth remained in the upwind end of the tunnel after a 2 minute period it was caught and released downwind once the lures were in position.

Preference for a volatile was seen as a characteristic upwind flight pattern in the odour plume to within 100mm of a lure. Once a lure had been approached, the odour source (lure type) was recorded and the test was terminated. If moths failed to approach either lure within a 10 minute period the test was terminated and absence of choice recorded. The position of the flower in the conditioning trials (centrally placed, 325mm from either wall) differed from the position of either lure in the preference trials (200mm from either the right or left hand side wall); learning the position of the feeding lure would not influence the choice of lure in the test. The position of each lure (nearest to the right or left hand wall of the chamber) was allocated randomly throughout the experiment to avoid positional biases. The flower used in conditioning treatments was changed in sequence throughout the experiment. We continued preference tests until 20 moths from each treatment group flew to an odour source, noting the number of moths that did not make a choice. For moths trained on odours only (experiment 2) we trained 10 moths on each of the two different odours.

Flower	Odour phenylacetaldehyde	linalool	β-caryophyllene
Daisy (Expt 1)	⭘✓	⭘×	×
Pigeonpea (Expt 1)	⭘×	⭘✓	×
Tobacco (Expt 1)	⭘×	⭘✓	✓
Tobacco (Expt 2)	×	⭘✓	⭘✓

Table 1: Summary of lures used and odours present in the flowers used in Experiments 1&2. ⭘; lure used in preference test. ✓/× odour present / absent in the flower odour.

Odour conditioning trials & No exposure trials

In experiment 1, we tested the preference of unfed moths for the odours phenylacetaldehyde and linalool. These moths had not been conditioned on flowers. Lures were constructed and positioned as in the preference test and male moths were released into the downwind end of the wind tunnel. Lure choice was noted and each moth was used only once.

In Experiment 2, it was necessary to confirm that moths could learn to distinguish between the two odours used in the preference test (linalool and β-caryophyllene). This was achieved by conditioning moths to each odour by allowing them to feed from sucrose wicks placed 2cm downwind from an odour lure. Training moths to visit artificial lures in this way is described in detail elsewhere (Cunningham et al., (submitted)).The lures contained 0.2υl of odour. Moths were allowed one initial 30s feed and 3 x 20s return feeds and then tested for preference of the linalool or β-caryophyllene using lures constructed as in flower conditioning trials (ie odours diluted in paraffin solution). If moths could successfully learn to choose the odour lure on which they had been tested, we could conclude that they were capable of distinguishing between these odours. We also used this experiment to compare the number of moths that did not make a choice when trained on odours alone, with moths that were trained on flowers. We trained a total of 20 moths (N=10 per odour treatment).

Statistical Analysis. Data were analysed using generalized linear modelling techniques (McCullagh and Nelder, 1989) in the GLIM statistical package (Crawley, 1993). Choice test outcomes were analysed as proportions, with the number of moths selecting a particular odour as the response variable and the total number of moths selecting either host as the binomial denominator. Binomially distributed error variances were assumed and a logit link function employed. Within treatment preferences for a particular odour was determined using Poisson errors and a log link function. Hypothesis testing was carried out using the χ^2 test on differences in deviance. Treatment order was randomised to prevent any biasing which may have related to night of testing.

RESULTS

In total, we trained and tested the odour preferences of 159 moths in trials on 44 days. Mean moths/day tested = 3.63(±0.34) and 4± (0.34) for Experiments 1 and 2 and 2.86 (±0.80) moths/day for unconditioned moths.

Experiment One

The results of preference tests are displayed in figure 2. Flower feeding had a significant effect on odour choice (G-test, $P<0.01$). In all cases, once moths had been trained to locate and feed from a flower, they showed a significant difference in odour preference compared with unconditioned moths (daisy and tobacco; G-test $P<0.001$, pigeonpea; G-test, $P<0.025$). Unconditioned moths showed no preference for either odour (χ^2 test = 0, $P>0.05$). All moths that had fed on flowers showed a significant preference for the odour component that was present in the floral odour (χ^2 test = $P<0.001$).

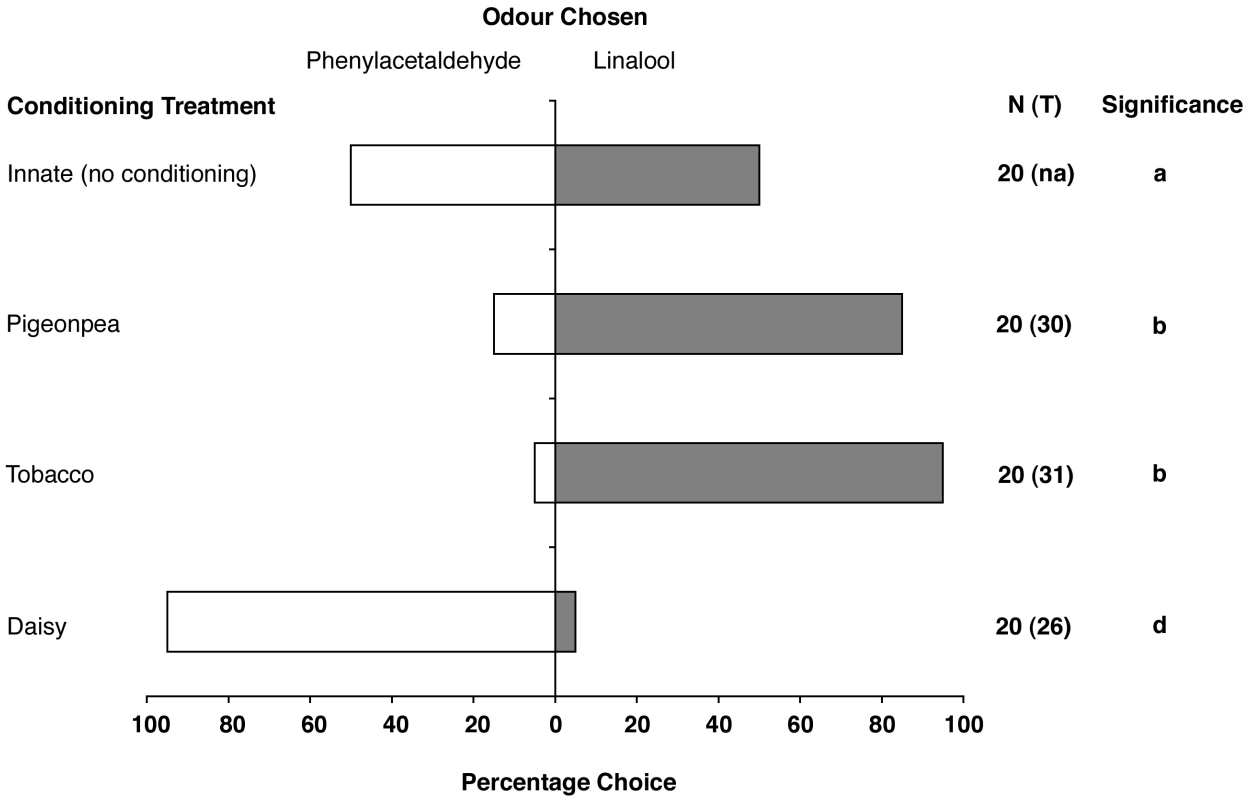

Figure 2. Percentages of moths selecting either phenylacetaldehyde or linalool in dual-choice preference tests after having feeding experience on one of 3 species of flower (pigeonpea, tobacco or daisy) or no experience (innate). N(T) = Number of moths responding to an odour (N) out of total number of moths trained (T). Treatments with common letters are not significantly different (P>0.05).

Floral odour context and odour response.

The floral odours of the three flower species used in Experiment 1 show differences with regards to the context in which the learned odour (used in preference tests) was presented. We ranked the odour phenylacetaldehyde in daisy as the simplest context for learning (highest relative concentration, lowest rank, fewest components in odour) and linalool in pigeonpea as the most complex context (lowest relative concentration, lowest rank, greatest number of components). In tobacco, where two odour compounds (β-caryophyllene and linalool) predominated we considered that the context for learning the odour linalool was intermediate between the daisy and pigeonpea.

There was no evidence from this study that the ability to learn a specific odour component in flowers differed with the context in which the odour was presented within the floral blend. In preference tests, moths trained on the different flower species showed no difference in their degree of response towards the odour component present in floral aroma (G-test =1.631, 1df, P>0.05).

In flower training experiments, the number of moths that did not respond to either odour during the preference tests was lower in the daisy treatment (N=6) compared to the tobacco (N=11 & N=12) and pigeonpea treatments (N= 10) although this difference was not statistically significant (G-test =1.49, 1df, $P>0.05$). In flower trained moths, frequently observed making looping movements and short flights part way up odour plumes before aborting upwind flight and returning to the back of the wind tunnel. This process would be repeated for up to 10 minutes (after which the experiment was terminated). When moths were trained on odour lures containing single odours (Experiment 2), this behaviour was less common and no moths failed to make a choice in these trials. The difference in "no choice" trials between flower feeding and lure feeding experiments was statistically significant (G-test, $P<0.001$).

Experiment 2

The results of preference tests using the odours β-caryophyllene and linalool (both present in tobacco flowers) are displayed in figure 3. Although moths showed a preference for β-caryophyllene, this was not statistically significant ((χ^2 test =1.83, 1df). In tobacco trained moths, preferences for linalool were significantly lower when the second odour used in the test was β-caryophyllene, compared with when the second odour was phenylacetaldehyde (an odour not present in tobacco) in Experiment 1 (G-test; $P<0.001$)

The lack of preference for either odour component in Experiment 2 could be explained by moths learning both components equally, or because they could not distinguish between the 2 odour components. Figure 3 clearly shows that moths can distinguish between components. All moths showed a preference for the odour on which they were trained and consequently a significant difference in preference occurred both within and between the two odour treatment groups (G-test; $P<0.001$).

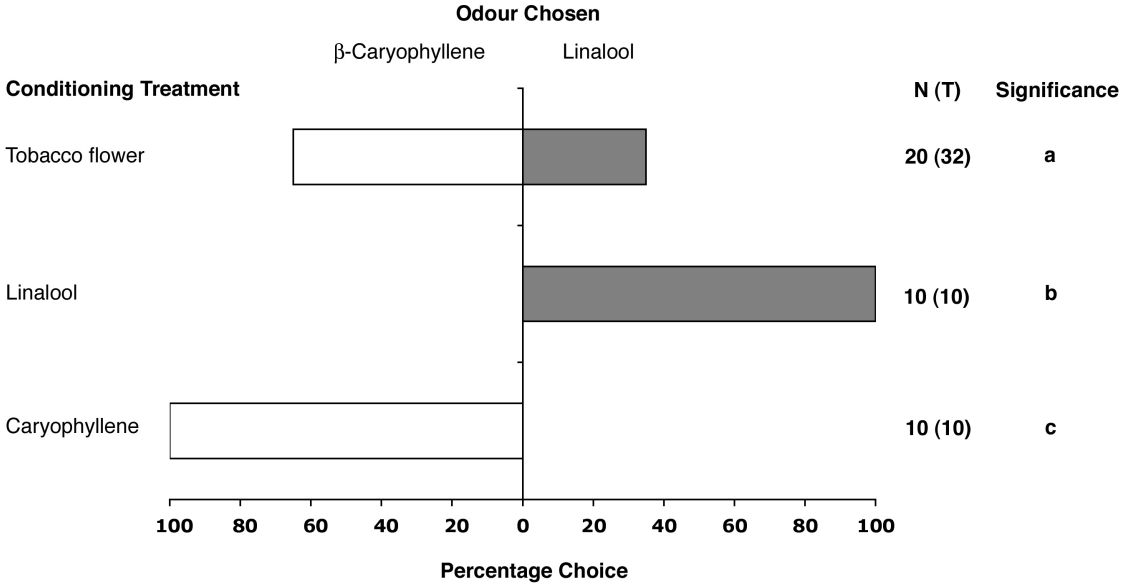

Figure 3. Percentages of moths selecting either β-caryophyllene or linalool in dual-choice preference tests after having feeding experience feeding on either tobacco flowers, β-caryophyllene odour or linalool odour. N(T) = Number of moths responding to an odour (N) out of total number of moths trained (T). Treatments with common letters are not significantly different ($P>0.05$).

DISCUSSION

Our results clearly show that moths can learn individual components of a floral blend whilst foraging on flowers. Our findings make an important step between studies which are elucidating the nature and mechanism of blend learning using synthetic blends and their validity to insect flower visiting ecology.

We tested the ability of male moths to learn a single volatile component of a particular flowers odour, whilst foraging on that flower. In the three flower species we used, the chosen volatile varied in the context in which it was presented from being the predominant volatile compound (daisy) to being one of two predominant compounds (tobacco) to being one of the less predominant compounds of a more complex blend (pigeonpea). In all three flowers, moths were capable of learning the component, and no differences in learning ability between the different flowers were found.

We then tested whether the two major compounds in tobacco were learned equally. Previous work has predicted that "key" components may be learned during flower learning, that blocking between components may influence learning, and that the similarity between odour components may prevent learning of either components. We found no evidence for this here; moths trained on tobacco flowers showed no preference for either component (β-caryophyllene or linalool) even though both components could be learned, suggesting that both components were learned equally.

The ability to learn individual components does not imply that blends are learned simply as a sum of individual components. Neurophysiological studies on honey bees have recently shown that at the level of the antennal lobe, inhibition and reinforcement between glomeruli stimulated by specific or multiple components can change the "pattern" of odour stimuli. Thus specific blends may have specific patterns. The ability to recognise a single odour may be that it bears a greater similarity to this pattern compared with a novel odour. Conversely, the lack of response towards an odour, seen only in flower trained and not in odour trained moths, may be due to lack of similarity between the single component and the components in the floral blend. Lack of visual cues may also be important here.

In all experiments, previous experience with any odour was minimised by isolating newly emerged moths in sealed pots until use in the training and preference trials. Consequently the dramatic changes in preference for odours witnessed may have been due predominantly to the pronounced effect of a single experience. This striking difference in response nevertheless shows the fundamental effect that experience has on shaping behaviour. Newly emerged adult moths may have innate preferences for certain odours. However, a single visit to a flower is capable of shifting these preferences. Flowers of both related and unrelated plant species share many similar odour components. The demonstration that flower visiting influences odour preferences, together with experiments using synthetic blends that show how insects show preferences of learned components within new blends suggests that learning a particular flower changes preferences towards that flower and to other species sharing odour components. Flower visiting ecology is thus a dynamic system where choices and preferences of individuals and populations for experienced and novel flowers are shaped by the abundance and distribution of all flower species within their local environment.

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SECTION 2: Flight Cage Experiments on *Helicoverpa* Feeding and Oviposition Behaviour

OVERVIEW:

The aim of these experiments was to investigate the extent to which feeding behaviour influences the egg laying behaviour of female moths and whether feeding behaviour differs between male and female moths. The study was designed to improve our understanding of why this insect may find particular stages of the plant life cycle and particular crop species attractive. In addition, it provides information that may improve the selection of host plants in a lure and kill trap crop strategy and the selection of odours to maximise female *H. armigera* attraction.

HOW DOES ADULT FEEDING BEHAVIOUR INFLUENCE HOST CHOICE IN EGG LAYING MOTHS.

AIM

Our field studies have shown that adult feeding behaviour may be important in determining the distribution of *H. armigera* populations among different crops. This flight cage study aimed to test whether adult female moths are more likely to lay eggs on cotton plants that are in the proximity of non-host flowering plants, which provide a feeding site (floral nectar).

METHODS

Insects & Plant Culturing

Helicoverpa armigera moths were obtained as pupae from a laboratory culture reared at QDPI Toowoomba, Queensland, Australia. Pupae were sexed and male moths were placed in a separate holding cage (200mm x 150mm x 150mm) until eclosion. We used a nectariless variety of cotton, obtained from CRDC, Narrabri. Cotton plants were grown in 200mm pots in a glasshouse until squaring. Plants were tied to bamboo canes for support. For nectar feeding sites we selected two flowering plant species which have not been reported as host of *H. armigera*; Lantana (*Lantana montevidensis*) and Mexican Heather (*Cuphea hyssopifolia*).

Flight Cage

We placed 6 nectariless cotton plants in 2 distinct patches within an outdoor flight cage (2×2×4.6m). Plants were randomly allocated in each trial and patches were place at opposite ends (4m apart). One patch (Host Only = HO) consisted solely of the three cotton plants and the second patch (Host plus Feeding Site - HFS) consisted of a mix of 3 cotton plants and 4 plants from one of the flowering non-host species. In each trial the position of HO and HFS patches were alternated to control for positional effects.

On the first night after emergence 6-10 female *H. armigera* moths, were placed into 200mm x 150mm x 150mm emergence cage for a single night. The following day, feeding was initiated (proboscis extension) in each female by touching the antennae with a cotton wool bud soaked in 20%w/v sucrose solution and then allowing the insect to commence feeding. Females were then

placed on the flowers of a feeding host held within a holding cage (450mm x 450mm x 450mm). Upon being positioned on the feeding host plant, most females would accept the host and start to feed. In order to insecure that The moths were housed with the feeding host for one night to improve attraction to the feeding host through experience.

On the third day the feeding host was removed and an excess of males added to ensure mating success. The screen of the cage was misted with water to avoid dehydration and reduced feeding that can result from water deprivation. On the fourth day, six gravid females were released in the centre of the flight cage containing the two host patches (HOS & HFS) where they were left to feed and oviposit for one night. The following morning all moths were recaptured and removed from the cage. The number of eggs deposited on each plant within the two patches was recorded. Care was taken to remove all eggs from the plants and to avoid eggs being displaced from upper leaves falling on to lower leaves. Eggs on delicate leaves, buds and bolls were removed using a moist cotton wool bud. The feeding hosts were checked for eggs and any found were counted, recorded and removed.

Behavioural observations of moths feeding and ovipositing were made by recording the activity of each moth at 4 minute intervals. This was done to ensure the moths were feeding from the feeding hosts during the trials and to validate the final egg distributions and discover any patterns in oviposition behaviour.

RESULTS

We carried out 24 trials, 13 with *Cuphea* as a feeding host and 11 with *Lantana*. Results are summarised in figure 1 and figure 2.

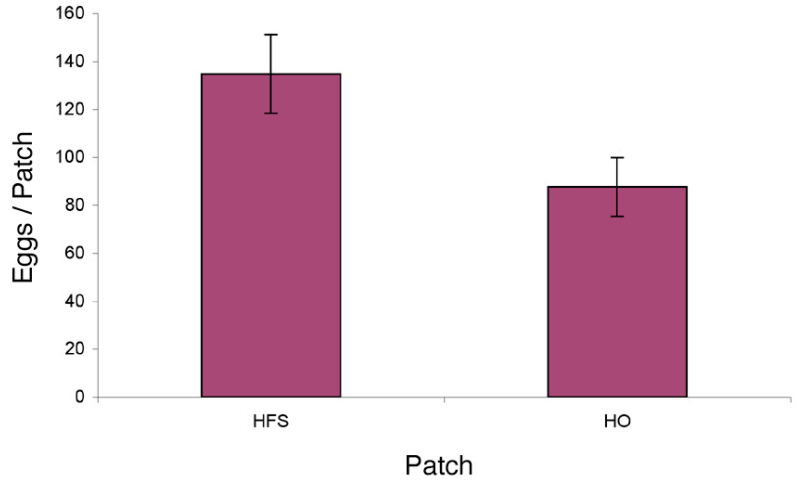


Figure 1.
Mean eggs / patch in Host Only (HO) and Host+Feeding Site (HFS) patches. Eggs/patch are significantly different (t-test, $P<0.05$)

Cotton plants that were within patches of flowering non-hosts received significantly more eggs than cotton plants without flowering non-hosts (t-test, $P<0.05$, figure 1). Figure 2 displays the result of individual trials. A greater proportion of eggs were laid on the cotton plants within flowering host patches (HFS) on 21 out of 24 nights ($P<0.001$, Wilcoxon matched pairs analysis). Throughout the study, no eggs were found on the Mexican heather (*C.hyssopifolia*). Eggs were noticed on the lantana flowers on some nights, indicating that although larval survival on this host has not been recorded, it may be attractive as an oviposition host to egg laying females. However, no significance difference in egg counts or proportions within patches was found between trials using *Cuphea* and those using *Lantana* as the feeding host ($P>0.05$, GlmStat, binomial errors).

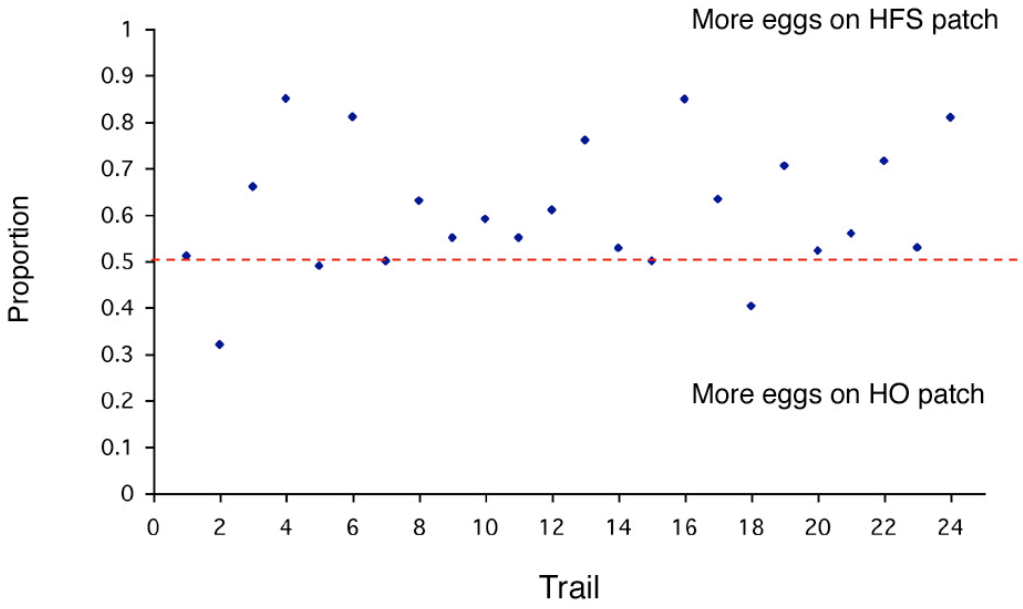


Figure 2. Proportions of eggs laid on cotton plants in patches with non-host feeding sites (HFS) compared to patches without non-host feeding sites (HO). Proportion = proportion of eggs laid on the **HFS patch** in each trial over 24 trials. Feeding host in HFS patches in trials 1-13 = *Cuphea hyssopifolia* and in 24-24 = *Lantana montevidenses*. Broken line at 0.5 represents equal proportion on both patches. Proportions laid on the two patches are significantly different ($P<0.001$ Wilcoxon, Matched Pairs).

CONCLUSION

We have shown that the presence of a feeding host increases the attractiveness of cotton crops to egg laying *H. armigera* females. The presence of flowers, may be attracting female moths for nectar feeding, this may then concentrate females within specific sites and thus increase the likelihood that they will lay eggs within the near vicinity of these hosts. Alternatively, odour components in flowers of non-host species may act as pre-alighting cues to ovipositing moths. The post-alighting (e.g. surface chemistry) cues of these hosts may be unfavourable, leading to females laying eggs on nearby cotton. The study shows that feeding and oviposition behaviour may be closely linked and that some of the attractiveness of particular host plant species and at particular stages of the plants development (eg flowering / extra floral nectary development) may be a result of increased attractiveness to adult female moths for the purpose of feeding.

HOW ATTRACTIVE IS PIGEONPEA AS A FEEDING SITE FOR ADULT MALE AND FEMALE *H. ARMIGERA* MOTHS?

AIM:

Our field studies have shown that pigeonpea is an attractive host to nectar feeding adult heliothis moths. Our flight cage experiments have shown that the proximity of a feeding host to cotton plants can influence egg distribution. This study aimed to investigate how attractive pigeonpea plants were relative to the plants used in the flight cage study and whether there was any difference in attraction between male and female moths.

METHODS

Helicoverpa armigera moths were obtained as pupae from a laboratory culture reared at QDPI Toowoomba, Queensland, Australia. Pupae were sexed and male moths were placed in a separate holding cage (200mm x 150mm x 150mm) until eclosion. Between 6 and 15 unmated moths of the same sex were transferred to a flight cage (2m x 2m x 2m). The following day, one flowering pigeonpea plant (*C.cajan*) and one flowering cuphea plant (*C.hyssopifolia*) were positioned 1.5m apart in within the cage. The height of the plants was manipulated to be the equivalent by raising on upturned plant pots. The positions of the plants were rotated between trials to avoid positional effects. Observations began at dust and continues until no further feeding behaviour was recoded for a 15min period. Upon initiation of feeding, individual moths were trapped in plastic containers and labelled according to the plant they had been feeding from. Each night, the total number of individuals caught from the two feeding hosts was recorded. New plants were used on each night of testing.

RESULTS

We recorded the feeding preferences of 34 female and 29 male *H. armigera* moths over 10 nights. Figure 3 shows the preferences of male and female moths for each species. Preferences of unmated male and female moths were significantly different ($P<0.05$, GLMStat). Female moths showed a preference for pigeonpea (χ^2 test, $P<0.05$), whereas male moths showed no preference for either species (χ^2 test = 0.31 $p>0.05$).

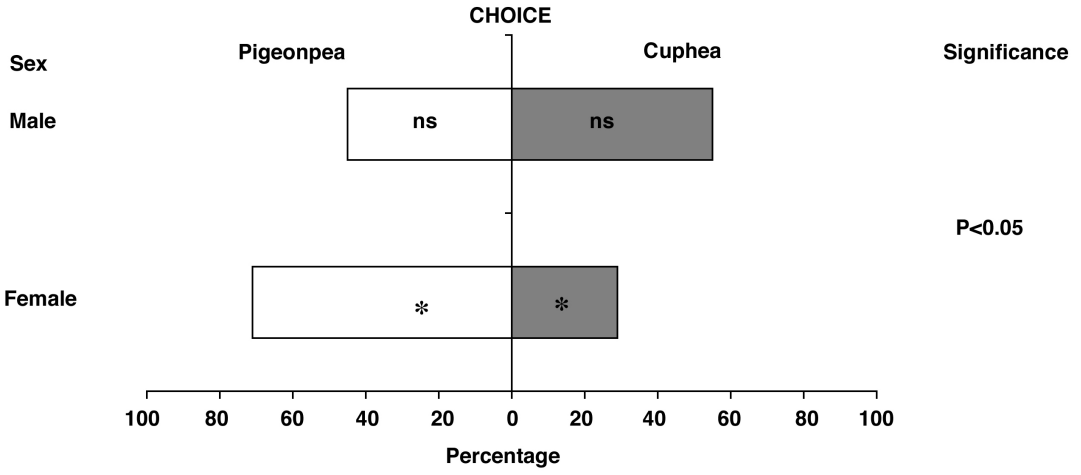


Figure 3. Preferences of male and female moths for pigeonpea versus cuphea. Within group preferences; ns = no significant preference for either host, * = significant preference at $P<0.05$. Significance = signficance of difference in between group (male vs female) preferences.

CONCLUSIONS

Male and female adult *H. armigera* moths showed significant differences in nectar feeding preferences. Unmated female moths showed a preference for feeding on the plant species that is also a host for egg laying (pigeopea). Male moths showed equal preference for the host and non-host species. The results imply that the feeding preference of female moths may be influenced by the suitability of these plants for oviposition, suggesting that an interaction between feeding and oviposition exists. In order to maximise attraction of female moths in lure and kill strategies and in proposed lure and kill trap crops, a plant species that acts as both a feeding and oviposition host should be used.

Section 3: Field studies on the feeding and oviposition behaviour of adult *H. armigera* moths.

OVERVIEW

The aim of this study was to investigate the feeding behaviour of adult heliothis moth populations in cotton crops and pigeonpea crops. Pigeonpea is highly attractive to egg laying heliothis moths and is used as a trap crop and refuge crop. This study specifically investigated the extent to which pigeonpea was attracting moths to feed. The results of the study have two very important implications (1) Pigeonpea may be ideal for using as a trap crop species in conjunction with specific adult lure and kill strategies (the primary objective of this project) and (2) The use of pigeonpea as a trap and refuge species should be cautioned – provision of an abundant feeding site may serve to increase the fecundity and longevity of adult moths.

METHODS

Field sampling of adult heliothis moth populations was carried out throughout the 2001/2002 cotton season in Emerald, Central Queensland in three trials: 26th November 2001 – 04th December 2001, 12th December 2001 – 14th December 2001, 30th-31st January 2002. Trials varied in length because of changing weather conditions and crop spraying schedules. Rain, and particularly evening thunderstorms frequently prevented sampling on a single night. Further sampling trials were also planned for the 2002/2003 season, but extremely low heliothis numbers prevented the collection of sufficient moth numbers.

Sampling in pigeonpea and cotton was restricted to periods when the crops were in flower, and where comparisons were made between crops, we used adjacent fields where both crops were in flower. Egg counts were made throughout adult moth sampling dates, examining the whole plant for eggs and calculating eggs/m over 10m on each sampling day. An estimate of flowering status of the crop was also made on these days (flowers/m).

Sampling commenced at 19:00 and continued until 21:00 or until no adult moths were observed feeding for a period of 15-20minutes. Moths were caught using standard butterfly nets, head torches were used once light intensity was too low to locate moths. No moths were caught within 20m of the field boundaries. Once moths were caught, they were transferred to individual plastic containers. The activity of the moth caught (either feeding on flowers or flying actively within the crop) was noted.

RESULTS

We caught a total of 92 moths feeding in pigeonpea over 8 nights sampling. A greater proportion of male moths compared to female moths were caught in the flowering pigeon pea (flower/plant =4.43(±0.47)) on all nights of sampling (P<0.001 Glmstat, figure1). We tested whether the male biased sex ratio was limited to feeding moths by comparing these data with results of 64 moths caught over the same 8 nights, which were actively flying in the crop. Figure 2 shows that a greater proportion of males compared to females were active within the crop (P<0.001, Glmstat). Proportions of male:female moths caught feeding on flowers compared to those active within the crop were not significantly different (χ^2 =12.01, 9df, P>0.05).

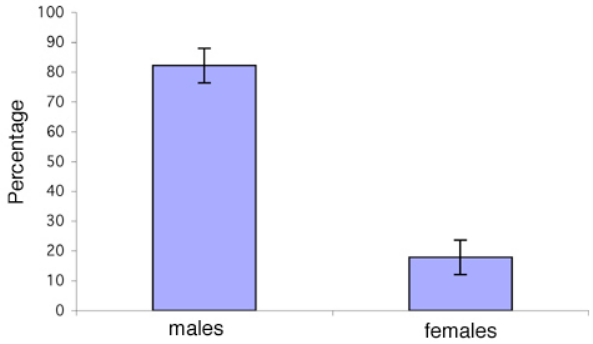


Figure 1. Feeding Moths
Moths caught feeding in pigeonpea crop on 6 consecutive nights. Difference between male and females caught is significant (p<0.001, Glmstat)

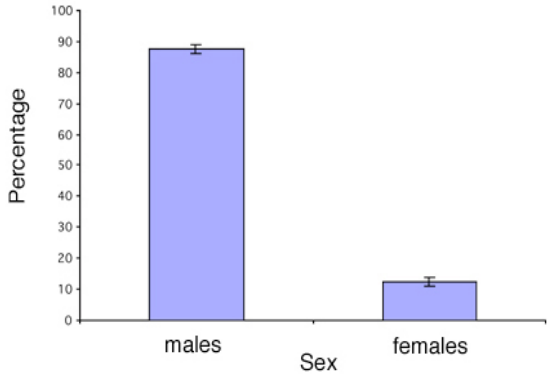


Figure 2. Active Moths.
Moths caught active in pigeonpea crop on 6 consecutive nights.
Difference between male and females caught is significant (p<0.001, Glmstat)

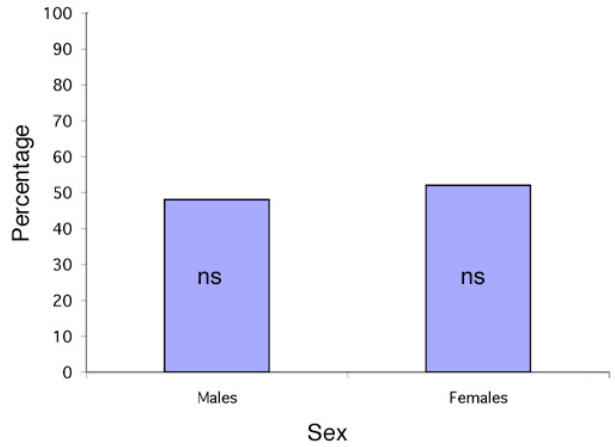


Figure 3. Active Moths in cotton.
Moths caught on a single night.
N=23 moths. Percentage of males and females caught were not significantly different. (χ^2 test, P>0.05)

We caught 23 moths in a nearby flowering cotton field (250m from pigeonpea crop, 2.12±0.41 open flowers/m) (figure 3). Data could not be collected pertaining to moths feeding in cotton, these moths were difficult to catch once identified (only 3 moths witnessed feeding were caught). Percentages of male and female moths caught in cotton were not significantly different (χ^2 =0.04, *P*>0.05). Sex ratios of moths caught in cotton and pigeonpea were significantly different (*P*>0.001, G-test).

To exclude possible effects due to sampling night, and to compare moth activity in pigeonpea with activity in cotton, we carried out sampling in the pigeonpea crop and in an adjacent cotton crop over 3 consecutive nights (12-14/12/02). Figure 4 shows that the sex ratio's of moths caught in an adjacent patch of cotton were significantly different from those caught in pigeonpea (*P*>0.05, G-test). Within the pigeonpea patch, a male:female bias still occurred in active moths (*P*<0.001, χ^2 test), whereas in cotton, there was no significant difference in male:female sex ratio (χ^2 test = 0.133, *P*>0.05). When feeding moths only were caught, a greater proportion of moths caught were males, however the differences in sex ratios were not statistically significant (χ^2 test =2.663, *P*>0.05).

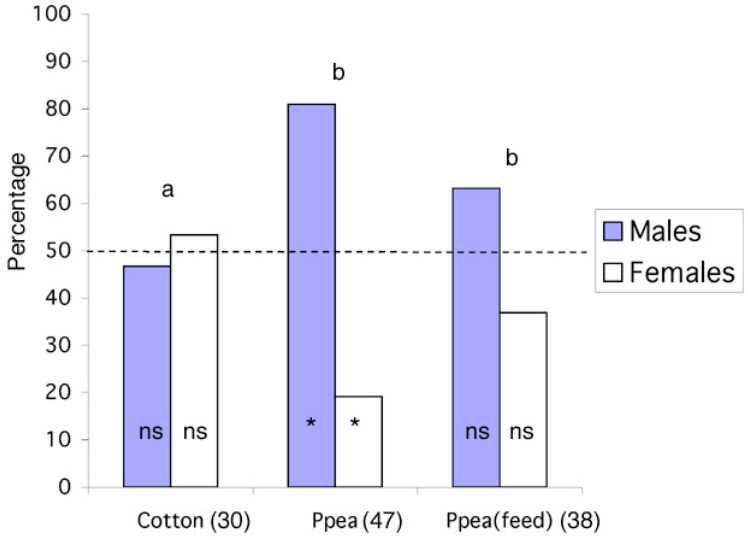


Figure 4. Moth sampling within 2 adjacent crops (cotton and pigeonpea). Terms within bars denote significance of difference in sex ratio's within groups (ns = not significant, * = significant at P<0.001). Groups with common letters above bars are not significantly different (P>0.05). Numbers in brackets next to groups denotes number of moths caught.

Differences in sex ratios within the pigeonpea could be a result of an influx of male moths or fewer female moths relative to cotton. Netting moths in cotton was considerably more difficult compared to pigeonpea and therefore we could not resolve this by comparing absolute numbers caught. When comparing eggs/m in the two adjacent crops; pigeonpea (1.33±0.5) and cotton (0.77±0.24) the egg numbers were not significantly different (χ^2 = 2.6, P>0.05, Glmstat, Poisson errors). This suggests that the differences in sex ratios were due to a greater number of males within the pigeonpea crop.

Mating Status

We compared the mating status of female moths caught in cotton and pigeonpea. (Figure 5). In both cotton and pigeonpea, of the moths caught flying withing the crop (active) approximately half (56%) of the female moths were mated. However, all the females caught feeding in the crop were unmated. The difference in mating status between feeding and active moths in pigeonpea was statistically significant (*P*<0.001, G-test).

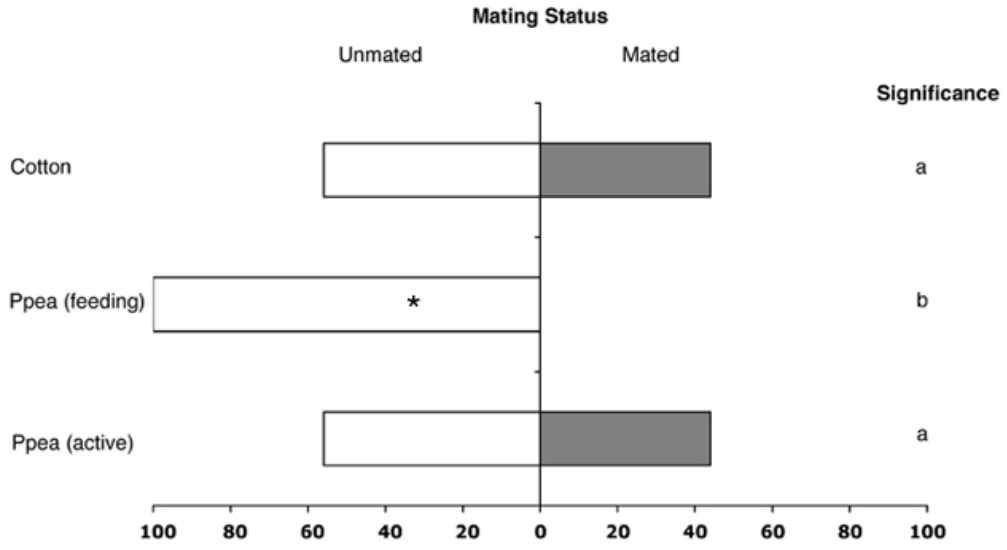


Figure 5. Mating status of moths caught in cotton and pigeonpea on sampling dates 12-14/12/02 (see also figure 4). Between group significance; Common letters are not significantly different (P>0.05, G-test). Within group significance; * = P<0.001, χ^2 test (all other within group differences are not significant, *P*>0.05)

Pupae

We collected 34 pupae from the within the pigeonpea crop during the sampling dates 12-14/12/02. 27 of these pupae were male (84%). Thus the sex ratios of moths found within the crop may be explained in part by the sex ratios of moths emerging within the pigeonpea field. However, the duration of the studies (8 days for trial one and 3 days for trial two: carried out 8 days after trial one) are inconsistent with the hypothesis that this was soley a result of males emerging within the crop; the lag time between female emergence and male emergence being 2-3 days. No pupae were found in the adjacent cotton.

Repeated Trials

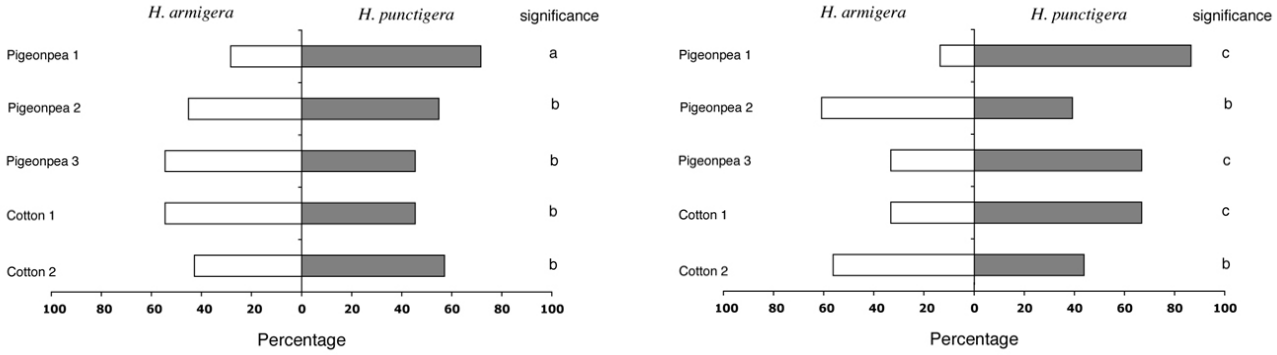
We attempted to repeat the above trial on 3 further occasions (Jan 2002, Nov 2002, Jan 2003) in order to determine the repeatability of our findings. Unfortunately on 2 of these occasions (Nov 2002, Jan 2003) extremely low moth numbers during the time of peak pigeonpea flowering prevented the possibility of data collection. The trial planned for Jan2002 had limited success, with a total of 24 moths caught over 3 days. Because of low numbers, both active and feeding moths were combined into a single group. 79% (19/24) of the moths caught in this trial were males. This compares with 86% males in the trials 26/11-14/12 and 73% in sampling trial 12-14/12/02 (5.9 2df)..

Wild Hosts.

Throughout sampling dates in all trials we noted any adult heliothis behaviour around wild hosts within crops and around the crop margins. Moths were observed feeding on Bladder Ketmia (*Hibiscus trionum*), and 3 cornered Jack *Tribulus* spp. We caught 14 moths, all of which were feeding on the flowers of this plant. 71% of the moths (10/14) were males, and of the females caught, 3 were unmated and 1 was mated.

Helicoverpa Species

We determine the Helicoverpa species for all moths caught. Only *H. armigera* and *H. punctigera* were caught. Proportions of each species for each different sampling trial are shown in figure 6a (males) and 6b (females)



6a) Male Moths

6b (female moths)

Figure 6. Percentages of *H. armigera* to *H. punctigera* adult moths caught in each sampling trial. 6a) male moths 6b) female moths. Pigeonpea 1 = 26/11/01 - 04/12/01, Pigeonpea2 = 12-14/12/01, Pigeonpea 3 = 30/31 Jan. Cotton 1 = 26/11/01 - 04/12/01, Cotton 2 = 12-14/12/01. Significance; between group significance as determined by GLMstat; groups with common letters are not significantly different ($P<0.05$).

In all sampling trials bar pigeonpea trial one, ratio's of *H. punctigera* to *H. armigera* male moths caught were similar. In trial one, a higher proportion of the males caught were *H. punctigera* compared to other trials ($P<0.05$). We observed that during these trials, moths caught in pigeonpea had a high degree of wing damage, suggesting that they were not recently emerged moths. Figure 6b shows that in trial 2 (12-14/12/01), ratios of moths caught differed in both cotton and pigeonpea crops, with higher number of *H. armigera* caught during these trials.

CONCLUSIONS

Our results show that pigeonpea is highly attractive to adult moths, predominantly as a nectar feeding host. Once cotton was in flower, there was little difference in the egg counts (eggs/m) between the pigeonpea crop and an adjacent cotton field, however, there were large differences in the numbers and population structure of moths caught in the two crops. In cotton and pigeonpea, approximately equal numbers of mated and unmated female moths were caught in this study. In pigeonpea, large numbers of moths were observed and caught feeding. These moths were predominantly virgin females, suggesting that newly emerged / unmated female moths use flowering pigeonpea crops as a nectar source. Moreover, large numbers of males were caught in pigeonpea (significantly more than females on all sampling trials) again suggesting the importance of this crop as a feeding source and potential as an aggregation site for males and unmated females. In cotton, equal numbers of male and female moths were caught. Adult moths were also observed feeding on a number of wild host species. Heliothis populations were made up of both *H. armigera* and *H. punctigera.*

Two important conclusions can be drawn from this field study.

(*1) Use of pigeonpea as a lure and kill host.*

Pigeonpea is highly attractive to feeding moths. One of the main objectives of this study was to identify a potential feeding host that may be capable of attracting nectar feeding adult moths away from cotton. Combination of the use of this species, planted in strips either within or at the boundaries of cotton crops, together with a lure and kill formulation (adult moth insecticide, feeding substrate and additional floral odours) may be an effective novel control strategy and a significant improvement on current lure and kill initiatives. Use of this crop has the added advantage over other non host species in that it is also attracts significant numbers of egg laying female moths. Further field studies should be carried out to test the repeatability of these findings and to test the proposed new strategy.

(2) Cautionary note on the use of trap and refuge crops

There is a wealth of literature showing that adult feeding increases the fecundity and longevity of adult moths. Pigeonpea is highly attractive to feeding adult moths and provides a nutritional nectar source. Consequently, the use of flowering pigeonpea as a refuge crop and trap crop may be attracting moths into the locality (and possibly retaining moths within a field), for the purpose of nectar feeding and providing an ideal, available nectar source. Depending on the abundance of other feeding sources, pigeonpea crops may be increasing the accessibility of available nectar and increasing the fecundity and longevity of local moth populations, as well as providing a feeding source for moths which are migrating into the area. Further studies should be carried out to assess the impact of these findings.

SUMMARY OF PROJECT CONCLUSIONS

(1) There is clear evidence that egg laying behaviour is influenced by adult feeding behaviour. Manipulating adult feeding behaviour should be considered in control strategies that include

- Lure and Kill strategies
- Population monitoring
- Trap cropping / refuge crops
- Production of new cotton varieties

(2) The design of these strategies should carefully consider a number of important findings

- Experience and learning by insects in both egg laying and feeding behaviour can fundamentally alter their attraction to host plants and odours. Design of lure and kill strategies in particular may benefit from considering the local abundance of host plants which provide a feeding source
- Enantiomers of volatile odours differ in their attractiveness to adult moths. Elucidation of the enantiomeric form of a volatile may be important in chemical analysis of the odour constituents of host plants.
- Male and female heliothis moths may show differences in attraction to odours and to host plants for feeding.
- Our results suggest that pigeonpea is a powerful attractant to feeding adult heliothis moths, even when neighbouring cotton crops are in flower. It may be an ideal plant to combine with current lure & kill formulations in a "lure and kill trap crop" strategy.

(3) Pigeonpea is used as a refuge crop and trap crop for heliothis. This crop attracts large numbers of virgin female moths and provides moths with nectar for egg maturation and longevity. It may also act as an aggregation site that improves mating success. Careful consideration of the possible downsides of having these crops (in terms of increased survival and fecundity of the pests) should be included in any assessment of these strategies.

The results of this study have been submitted to the Cotton CRC Semiochemicals Working Group. This project has made a number of important findings that apply directly to the objectives of this group and the design and implementation of novel heliothis control strategies.

We strongly advise that the CRDC considers the importance of these studies on the behaviour and ecology of cotton pest species in designing new control programmes and continues to fund research in this field in the future.

FUTURE RECOMMENDATIONS:

- Field testing of findings and development of pilot studies to test the proposed "lure and kill trap crops" using pigeonpea crops (combined with lure and kill formulations) as a feeding attractant
- Further laboratory work on key odours influencing adult behaviour including unmated and ovipositing moths.

- Manipulation of plant varieties through conventional breeding and genetic transformation to modify odour production

PUBLICATIONS ARISING FROM THE PROJECT

Cunningham, J. P., Moore, C. J., Zalucki, M. P. and Cribb, B. C. (submitted). Learning odour components in complex contexts: Tests with moths and flowers. *Proceedings of the Royal Society of London Series B-Biological Sciences*.

Hull, C. D., Cunningham, J. P., Moore, C. J., Zalucki, M. P. and Cribb, B. C. (submitted). Detection of enantiomers of α-pinene by *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae): Electrophysiology and behaviour. *Journal of Chemical Ecology*.

Cunningham, J. P., Moore, C. J., Zalucki, M. P. and West, S. A. (in press). Learning, odour preference and flower foraging in moths. *Journal of Experimental Biology*

Cunningham, J.P. Andrews, S Zalucki, M.P & Wright D, J (in prep.). Interactions between feeding and egg laying in a polyphagous moth. (*Bull. Ent. Res.*)