

Final report for 2006 Science and Innovation Awards for Young People in  
Agriculture, Fisheries and Forestry

Recipient: Sharon Downes  
CSIRO Entomology  
ACRI, Locked Bag 59  
Narrabri 2390 NSW  
(02) 6799 1576  
[Sharon.Downes@csiro.au](mailto:Sharon.Downes@csiro.au)

## **The role of mating in managing resistance by *Helicoverpa armigera* to GM cotton**

### **Introduction**

A major challenge for insect pest management is responding to resistance. The spread of resistance 'genes' throughout a population depends upon initial frequencies, dominance, fitness consequences, and the mating system of the pest. The latter is often ignored in pest management strategies, which assume that insects mate randomly and disregard the possibility that females exercise some choice in mating partner. There is now widespread evidence that females both actively select mating partners and, in those species in which females mate with more than one male, influence the paternity of their offspring.

Transgenic cotton that expresses genes from a soil bacterium (*Bacillus thuringiensis*) that encode insecticidal proteins was first used in the USA and Australia in the mid-1990's. The technology has been widely adopted and delivers enormous benefits to several aspects of the cotton industry. It presents an opportunity to significantly reduce the amount of pesticides applied as sprays to crops. In turn, this technology reduces both the amount of insecticides that run off into the environment and the cost of using insecticides to control a significant target pest. The consequences of these processes include a safer and cleaner environment for rural people, support for an industry that is committed to being environmentally friendly, and an opportunity to maintain profitability and competitiveness against world markets.

Prior to, and since, its commercial release, there has been concern about the resistance risk to this important technology. This concern is particularly relevant to the Australian cotton industry due to the unique biology of the cotton bollworm, *Helicoverpa armigera*. This species is one of Australia's most damaging agricultural pests and the major pest of cotton. Its status reflects a remarkable ability to rapidly evolve resistance to synthetic pesticides, and a relative tolerance to the Bt toxins within transgenic cotton. This situation is in contrast to that in the New World where the major pests of cotton are very susceptible to the toxins or do not have a record of developing resistance to conventional insecticides.

To prolong the efficacy of transgenic cotton against *H. armigera* in Australia, a resistance management plan (RMP) is implemented throughout the industry. The resistance management plan (RMP) employed in Australia for Bt-cotton is largely based on information from studies of the ecology and population genetics of *H. armigera*, and the outputs of computer simulation models that use biological information to predict the likelihood of resistance under different scenarios. A key assumption of the RMP is that resistant insects selected on transgenic crops mate randomly, and hopefully, with susceptible insects produced on non-transgenic (refuge) crops. Two recent studies in different insect systems provide evidence that supports and refutes this hypothesis. In the Colorado Potato Beetle, resistance to Bt enhances male reproductive success, whereas in the Pink Bollworm resistance to Bt decreases male reproductive success.

I tested one aspect of this hypothesis for *H. armigera* using a series of laboratory experiments to investigate patterns of mate choice as well as differences among resistant versus susceptible moths in fecundity and fertility. Female *H. armigera* can mate with up to six different males during their lifetime. There is evidence that male accessory gland products inhibit female receptivity and enhance egg laying in this species. In current work we are testing if the order of mating affects paternity. In the present study, I used direct observations under laboratory conditions to test if resistance genotype affects (a) mating frequency, (b) mate choice (measured as time until first mating), and (c) fecundity and fertility.

The GM cotton currently available in Australia contains a pyramid of the Bt genes *cry1Ac* and *cry2Ab* (registered as 'Bollgard II'). This product improves on its predecessor ('INGARD') which contained only *cry1Ac*. INGARD became commercially available in Australia in 1996 and since then the CRDC have funded a Bt resistance monitoring study. The results from this program suggest that high level resistance by *H. armigera* to Cry1Ac is rare, at between 1 in 1000 and 1 in 10,000. However, CSIRO Entomology have isolated several strains of *H. armigera* that carry an allele conferring high level resistance to Cry2Ab, and estimate its frequency at 2 in 100. Since conducting work on both proteins is beyond the scope of this study, I focussed on strains that are resistant to Cry2Ab and are allelic with the first isolated strain (designated SP15) that has been well characterised.

## Methods

### *Helicoverpa armigera strains*

Three laboratory strains were used in this study: ANGR, HA405 and HA738.

The general laboratory strain used in our assays, designated ANGR, is susceptible to Cry2Ab toxin. This susceptibility was monitored regularly as part of another project. ANGR has been in culture since 1998 and is derived from a strain designated GR and a strain designated ANO2, both of which were derived from material collected from cotton fields in the Namoi Valley, northern NSW Australia. This strain was used as a standard background for testing against Cry2Ab resistant strains.

The two Cry2Ab resistant lines used in this study, designated HA405 and HA738, were identified by an F<sub>2</sub> screen in the 2005/06 season. Isofemale lines of *H. armigera* rapidly exhibit loss of fitness through inbreeding depression. Therefore, within one generation of the F<sub>2</sub> screen, HA405 and HA738 were outcrossed to ANGR to improve vigour before re-selection. At the time of this study, each strain had been outcrossed three times and selected twice. This procedure generated colonies that are presumed to be 87% compatible with ANGR.

When evaluated herein, HA405 and HA738 were believed to be homozygous for the Cry2Ab resistance allele. Both strains are allelic with the first identified Cry2Ab resistant line, designated SP15, which has been characterised.

### *Rearing methods*

The rearing methods used to maintain *H. armigera* were the standard procedures. Rearing trays were covered and heat-sealed by a perforated lid. Moths were provided with a small pot of 4% honey/sugar solution that was fed through a cotton wick, and housed in containers that were open at the top and covered with nappy liners secured around their lip.

### *Laboratory experiments*

The following experiments took place within CSIRO laboratories during July-September 2007. Leading up to the experimental period, larvae were reared individually to pupae on artificial diet in the laboratory that did not contain Bt proteins. On maturation, pupae were collected, washed, sexed and set up in cages that contained pupae of the same sex from the same colony. Emerged adult moths were removed twice daily and placed into holding containers located in a cool room set to 10°C. The following day they were utilised in experiments as described below.

During the experimental periods pairs of moths were housed individually in plastic containers (750 ml) maintained in a room at 25°C and 70% RH. Unless otherwise stated, the containers were covered with a nappy liner secured to the lip of the container by the outer rim of the lid. Each container was provided with a small pot of 4% honey/sugar solution that was fed through a cotton wick. *Helicoverpa armigera* is primarily active at night. We reversed the natural photoperiod in the experimental room to enable observations to be made during the day on moths that experienced nocturnal light levels. The photoperiod was kept constant at L:D of 11:13, which approximated the corresponding cycle in nature.

At the conclusion of our mating experiments female moths were preserved in 100% alcohol. Where relevant (see below) we subsequently counted the number of spermatophores lodged within female moths by dissection.

Occasionally, the genitalia of moths “locked” together during mating and pairs were unable to separate post-copulation. Data from these pairs of moths was disregarded in our analyses.

#### (a) Mating frequency

In this experiment I determined the effect of resistance status on mating frequency by partnering single pairs of same-age virgin moths for 9 days and dissecting preserved females to count the number of spermatophores. A pilot study with 124 single-mated *H. armigera* female moths (28 female ANGR x male ANGR, 32 male HA738 x female ANGR, 32 female HA738 x male HA738, 32 female HA738 x male ANGR) demonstrated that in all but 2 cases one spermatophore was retained for at least 10 days post-copulation.

Virgin male and female moths were partnered within 24-36 hours of emergence in the following treatment groups: (1) female ANGR x male ANGR; (2) female ANGR x male HA405; (3) female ANGR x male HA738; (4) female HA405 x male ANGR; (5) female HA405 x male HA405; (6) HA405 x male HA738; (7) female HA738 x male ANGR; (8) female HA738 x male HA405; and (9) female HA738 x male HA738. In terms of resulting offspring, these treatments correspond to the following Cry2Ab resistance genotypes: (1) SS; (2) SR<sub>1</sub>; (3) SR<sub>2</sub>; (4) R<sub>1</sub>S; (5) R<sub>1</sub>R<sub>1</sub>; (6) R<sub>1</sub>R<sub>2</sub>; (7) R<sub>2</sub>S; (8) R<sub>2</sub>R<sub>1</sub>; and (9) R<sub>2</sub>R<sub>2</sub>.

I used 2-factor ANOVA, with repeat number and treatment as factors, to examine variation in mating frequency. Initially I performed tests of whether there was significant variation among treatments that consisted of pairs of the same genotype from different replicate colonies. These tests demonstrated no significant variation in mating frequency among the treatment groups comprised of a susceptible and resistant moth (i.e., 2, 3, 4 and 7 above; main effect of treatment, ANOVA,  $df = 3, 230$ ,  $F = 0.6$ ,  $P = 0.62$ ) or the treatment groups comprised of two resistant moths (i.e., 5, 6, 8 and 9 above; main effect of treatment, ANOVA,  $df = 3, 238$ ,  $F = 1.5$ ,  $P = 0.22$ ). We therefore pooled data within each of these two treatment groups and used 2-factor ANOVA, with repeat (1, 2 or 3) and treatment (SS pairs, RS pairs, RR pairs) as factors, to examine the impact of resistance genotype on mating frequency.

If there was no significant interaction between the two factors in the ANOVA I removed the interaction term from the final model. If there was a significant main effect I used Tukey-Kramer Honestly Significant Difference (T-K HSD) tests to explore specific differences among the treatments within that factor.

#### (b) Mate choice

In this experiment I inferred the effect of resistance status on mate choice by observing the duration before first mating of virgin male and female moths. I ran the experiment for 5 consecutive days, because our pilot work on 25 pairs showed that the bulk (69%) of first matings occurred during this time. Individuals were partnered within 24-36 hours of emergence in the following treatment groups: (1) female ANGR x male ANGR; (2) female HA738 x male ANGR; (3) female ANGR x male HA738; and (4) female HA738 x male HA738. In terms of resulting offspring, these treatments correspond to the following Cry2Ab resistance genotypes: (1) SS; (2) R<sub>2</sub>S; (3) SR<sub>2</sub>; and (4) R<sub>2</sub> R<sub>2</sub>.

The experimental containers were covered with transparent plastic cling wrap that was perforated with holes for air. We scanned the containers of paired moths every 15 minutes and collected copulating pairs in small plastic pots that were sealed with lids. Copulating moths were left in the experimental room undisturbed within the pots until they separated.

Transmission of spermatophores by male to female *Helicoverpa armigera* moths is successful only after copulation for at least 30 minutes (S. Downes, unpublished data). We therefore defined pairs of moths as having “mated” if copulation took place for at least 30 consecutive minutes (i.e., two consecutive checks 15 mins apart). To ensure that mating did not occur during the daylight non-observation period, at the end of the night cycle we placed a transparent plastic division between the pairs in each container. This division was removed at the end of the daylight cycle immediately prior to observations commencing on the following day. In all cases, the females in this experiment that were recorded as “mated” contained one spermatophore, and the females recorded as “not mated” contained no spermatophore.

We recorded the time of first mating and the time of completed copulation (both to the nearest 15 minute interval). We subtracted the time of first mating from the time that pairs were first placed together to determine “duration until first mating”. We did not include the time that pairs were separated by the division during daylight in this calculation. We subtracted the time that copulating pairs separated from the time that copulating pairs were collected to determine “duration of copulation”.

I used a Fisher’s Exact Test to examine whether there was an interaction between pair treatment and the frequency of mating during the 5 day experiment. I used ANOVA, with pair treatment as the factor, to examine differences in duration until first mating and duration of copulation, and T-K HSD tests were used to explore any specific differences among the pair treatments. Exploratory analysis revealed that untransformed measures of duration until first mating were not normally distributed. I therefore used log transformed data in the analysis.

#### (b) Fecundity and fertility

In this experiment I measured the fecundity and fertility of the single-mated female moths from the mate choice experiment. Upon ceasing copulation, females were relocated to a clean container that was lined, and covered, with nappy liners. Eggs of *H. armigera* hatch after three days at 25°C. Infertile eggs remain white after this time, whereas fertile eggs gradually turn dark brown as the neonate develops.

The liners from the containers were collected at 24hrs, 48hrs and 72hrs post-copulation, and placed into a plastic bag and stored at 25°C until any fertile eggs turned dark brown. The bags containing the eggs were then placed into a freezer to process at a latter date. Processing involved using a dissecting microscope to sight and count the number of black versus white eggs. The sighted eggs were marked with a highlighter pen to avoid recounting.

For each collection we summed the number of fertile (brown) eggs and number of infertile (white) eggs to determine the “total number of eggs”. For each collection we divided the total number of fertile eggs by the total number of eggs to determine the “proportion of fertile eggs”.

I used Repeated Measures ANOVA, with day of egg lay (time) as the repeat and pair treatment as the factor, to examine variation in percentage of fertile eggs. The initial analysis demonstrated a significant interaction between day of egg lay and pair treatment. I therefore used separate ANOVA, with pair treatment as the factor, on the dataset for each egg lay to examine variation in percentage of fertile eggs. T-K HSD tests were used to explore any specific differences among the pair treatments.

## **Results**

### *Mating frequency*

The effect of genotype on mating frequency was consistent among the three repeat tests (ANOVA,  $F_{4,540} = 1.5$ ,  $P = 0.50$ ; Table 1). However, the genotypic makeup of pairs significantly affected mating frequency (ANOVA,  $F_{2,540} = 8.1$ ,  $P = 0.0003$ ; Figure 1). Pairs consisting of two resistant individuals mated significantly less often than did pairs comprised of a resistant and susceptible individual (T-K HSD, Diff. = 0.55, Crit. Diff. = 0.28) or pairs comprised of two susceptible individuals (T-K HSD, Diff. = 0.55, Crit. Diff. = 0.45). There was no significant difference in the mating frequency of pairs comprised of two susceptible individuals versus pairs comprised of a resistant and a susceptible individual (T-K HSD, Diff. = 0.04, Crit. Diff. = 0.46; Figure 1).

This result does not reflect differences in the tendency of susceptible females to mate compared to resistant females. When females are matched with a partner of the opposite genotype, there is no significant difference in mating frequency between females that are susceptible versus females that are resistant (Mean  $\pm$  SE number of spermatophores: susceptible,  $1.71 \pm 0.13$ , resistant,  $1.71 \pm 0.12$ , ANOVA,  $F_{1,240} = 3.4$ ,  $P = 0.98$ ).

### *Mate choice*

There was no significant difference among pair treatment in the tendency of females to mate at least once during the 5 day experiment (No. of matings: female ANGR x male ANGR = 39/52; female ANGR x male HA738 = 35/52; female HA738 x male HA738 = 37/51; female HA738 x male ANGR = 36/52; Fisher's Exact Test:  $df = 3$ ,  $\chi^2 = 0.82$ ,  $P = 0.89$ ).

There was a significant effect of pair treatment on duration until first mating (ANOVA:  $F_{3,142} = 3.53$ ,  $P = 0.017$ ; Figure 2). Pairs comprised of two susceptible individuals mated significantly sooner for the first time than did pairs comprised of two resistant individuals (T-K HSD, Diff. = 0.064, Crit. Diff. = 0.045) or pairs comprised of a resistant female and susceptible male (T-K HSD, Diff. = 0.046, Crit. Diff. = 0.045). Pairs comprised of a susceptible female and resistant male took significantly less time to mate for the first time than did pairs comprised of two resistant individuals (T-K HSD, Diff. = 0.055, Crit. Diff. = 0.046) and mated significantly sooner for the first time than did pairs comprised of two susceptible individuals (T-K HSD, Diff. = 0.009, Crit. Diff. = 0.045). There was no significant difference in duration until first mating for pairs comprised of a resistant female and susceptible male compared to two resistant individuals (T-K HSD, Diff. = 0.018, Crit. Diff. = 0.045) or a susceptible female and resistant male (T-K HSD, Diff. = 0.037, Crit. Diff. = 0.046).

There was no significant effect of pair treatment on the time that mating moths remained in copula (Mean  $\pm$  SE duration of copulation (minutes): female ANGR x male ANGR =  $184.1 \pm 22.6$ ; female ANGR x male HA738 =  $214.0 \pm 20.1$ ; female HA738 x male HA738 =  $191.1 \pm 16.1$ ; female HA738 x male ANGR =  $214.6 \pm 18.3$ ; ANOVA:  $F_{3,140} = 0.64$ ,  $P = 0.59$ ).

### *Fecundity and fertility*

Pair treatment significantly affected the proportion of a female's eggs that were fertile but this trend was not consistent among the different egg lays (RM ANOVA:  $F_{6,282} = 4.1$ ,  $P = 0.0006$ ). For eggs produced 24hrs and 48hrs after a single mating there was no significant effect of pair treatment on the proportion of a female's eggs that were fertile (in both cases ANOVA:  $F_{3,141} < 0.83$ ,  $P > 0.47$ ; see Table 2).

For eggs produced 72hrs after a single mating there was a significant effect of pair treatment on the proportion of a female's eggs that were fertile (Mean  $\pm$  SE fertility (%): female ANGR x male ANGR =  $0.32 \pm 0.07$ ; female ANGR x male HA738 =  $0.25 \pm 0.06$ ; female HA738 x male HA738 =  $0.21 \pm 0.34$ ; female HA738 x male ANGR =  $0.09 \pm 0.03$ ; ANOVA:  $F_{3,141} = 3.1$ ,  $P = 0.027$ ). Pairs comprised of a resistant female and susceptible male produced a significantly lower proportion of fertile eggs 72hrs after mating than did those comprised of two susceptible individuals (T-K HSD, Diff. = 0.230, Crit. Diff. = 0.153) or those comprised of a susceptible female and a resistant male (T-K HSD, Diff. = 0.187, Crit. Diff. = 0.155).

### **Discussion**

A major challenge for insect pest management is responding to resistance. The spread of resistance 'genes' throughout a pest population depends in part on the fitness consequences of various life-history and behavioural traits, including subtle changes to mating behaviour. The latter is often overlooked in pest management strategies, which assume that insects mate randomly and disregard the possibility that resistance status can affect mating opportunities. This study is a first step in testing a key assumption of the resistance management plan (RMP) employed in Australia for Bt-cotton; that resistant insects selected on Bt crops mate randomly or preferentially with susceptible insects preserved on non-Bt 'refuge' crops.

The results demonstrate the potential for the Bt resistant status of *Helicoverpa armigera* to impact on some aspects of mating behaviour. Resistance status did not impact on the tendency of moths to mate for the first time, the time that moths remained joined during their first copulation, or fecundity. However, the resistant status of a moth significantly affected the frequency of mating during a defined period and mate choice. Resistance status also affected the fertility of eggs produced by females at certain periods after her first mating.

I used number of spermatophores as a surrogate for mating frequency. My pilot trials and previous work demonstrates that the number of spermatophores lodged within a female gives a reliable count of the number of times that a female has mated (see Methods).

I used duration until first mating as a surrogate for mate choice. The traditional method of determining female mating preference is through simultaneous choice tests but male-male competition may influence any increased mating success for some males. It is difficult to adequately exclude male-male interactions without restricting interactions among individuals or altering male behaviour. Measuring attractiveness as mating latency allows females to base their choice on the entire physical and chemical cues males offer, and female mating preference can be determined from actual mating.

In Lepidoptera, females from long established colonies are often more likely to mate and successfully reproduce in the laboratory than are females from recently formed colonies. This is the case for our *H. armigera* colonies. Through out-crossing and reselection, I ensured that the resistant colonies used herein (HA405 and HA738) shared a genetic background that is presumptively 87% compatible with the susceptible ANGR strain. Nevertheless it is possible that differences in mating behaviour between resistant and susceptible strains may result from differences between colonies in the presumptively 13% of genes that do not code for Bt resistance.

The results from two tests presented herein support the notion that variation in mating behaviour among treatment pairs reflects true differences due to genes that code for Bt resistance. First, when females are matched with a partner of the opposite genotype, there is no significant difference in mating frequency between females that are susceptible versus females that are

resistant (see Results). This result holds for both of the resistant colonies (HA405 and HA738) used in this study. Second, there was no significant difference in duration until first mating for pairs comprised of a resistant female and susceptible male compared to a susceptible female and resistant male (see Results).

Resistance status did not affect the tendency of single pairs of moths of different genotypes to mate for the first time. However pairs of resistant moths mated significantly fewer times during the same period than did pairs which included at least one susceptible individual. There are at least two different ways to interpret these data both of which require additional tests. These interpretations are not mutually exclusive.

In a number of species mating frequency is positively correlated with fecundity and fertility and hence reproductive success. My results may indicate a reduced (or increased) fitness of resistant moths relative to susceptible moths. However, I do not know how mating frequency affects the fecundity and fertility of *H. armigera*.

Second, it is possible that once a female has mated for the first time, those that carry a gene conferring resistance to Cry2Ab are less likely to re-mate. My results suggest that this hypothesis may hold only if the resistant female's partner also carries a resistance gene. In my single-pair experiments a female only had an opportunity to re-mate with the same male but our results may hold to situations where females are presented with different males. If so, this scenario could have positive implications for the evolution of resistance because a resistant female may be more likely to re-mate if she encounters a susceptible partner compared to a resistant partner.

My study also indicates that resistance status may affect the mating preference of *H. armigera* moths (as measured by time until first mating). Susceptible moths preferred partners that were susceptible compared to those that were resistant, and this result held for males and females. Furthermore, resistant female moths did not show a significant preference for partners according to resistance status. These findings suggest that among resistant and susceptible genotypes, a preference may exist that could result in a greater than expected proportion of matings between susceptible – susceptible pairs. However, although these trends for preference were statistically significant, we do not know how they translate to actual matings in a field situation.

My results on the impact of resistance status on fecundity and fertility are similar to those obtained by Rod Mahon (CSIRO Entomology) for another strain of Cry2Ab resistant *H. armigera* (SP15) that is allelic with HA738. A difference between the previous work and my study is that I controlled the number of matings for tested females; they were all mated once. In both studies there was little impact of resistance status on the number of eggs produced by a female or the proportion of laid eggs that successfully hatched.

The current RMP for Bt-cotton in Australia is largely based on information from studies of the ecology and population genetics of *H. armigera*, and the outputs of computer simulation models that use biological information to predict the likelihood of resistance under different scenarios. My work has generated a number of testable hypotheses about the impact of resistance status on the close range mating behaviour of *H. armigera*. The information from these further studies, as well as work that considers the long-range mating behaviour of *Helicoverpa*, will be important for refining future versions of the RMP.

## Tables

Table 1: Mean  $\pm$  SE number of spermatophores for the three pair genotypes presented separately for each repeat test. There is no statistically significant difference in number of spermatophores among the reciprocal pairs or replicate strains within each pair genotype (see main text), thus the data within each of these broad treatments were pooled.

Repeat	Susceptible x Susceptible	Susceptible x Resistant	Resistant x Resistant
1	2.29 $\pm$ 0.50	2.00 $\pm$ 0.18	1.85 $\pm$ 0.20
2	1.80 $\pm$ 0.30	1.56 $\pm$ 0.17	1.01 $\pm$ 0.11
3	1.46 $\pm$ 0.20	1.63 $\pm$ 0.13	1.01 $\pm$ 0.11

Table 2: Mean  $\pm$  SE proportion of fertile eggs from females of the four pair types 24hrs and 48hrs after mating. ANGR = homozygous susceptible; HA738 = homozygous resistant.

Pair type	24hrs	48hrs
♀ANGR x ♂ANGR	0.365 $\pm$ 0.069	0.403 $\pm$ 0.072
♀ANGR x ♂HA738	0.385 $\pm$ 0.071	0.301 $\pm$ 0.063
♀HA738 x ♂HA738	0.504 $\pm$ 0.070	0.346 $\pm$ 0.066
♀HA738 x ♂ANGR	0.398 $\pm$ 0.065	0.278 $\pm$ 0.052



## Figures

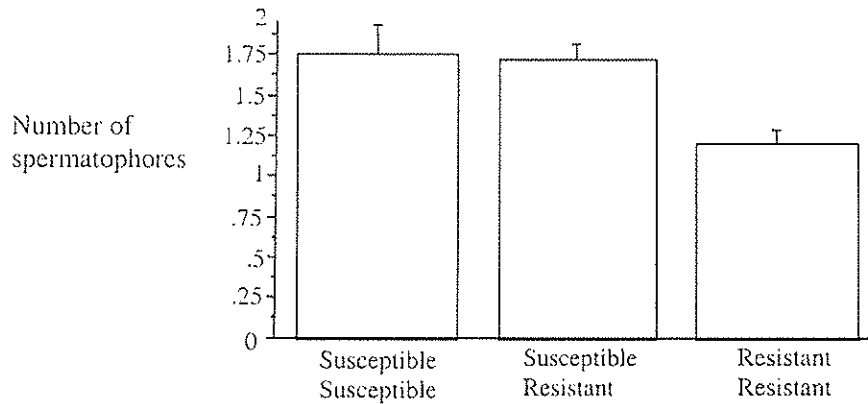


Figure 1: Mean  $\pm$  standard error number of spermatophores for the three pair genotypes pooled across the three repeat tests. There is no statistically significant difference in number of spermatophores among the reciprocal pairs or replicate strains within each pair genotype (see main text), thus the data within each of these broad treatments were pooled.

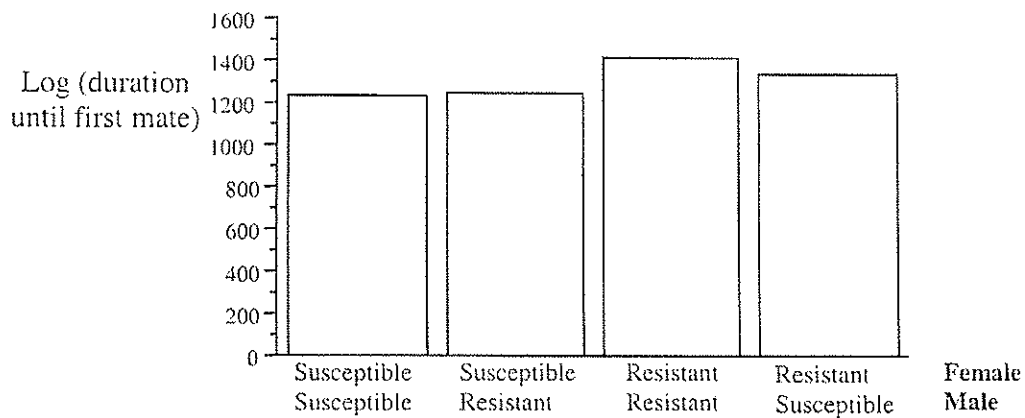


Figure 2: Mean  $\pm$  SE duration until first mating for the four pair treatments used in the study. The first line for each bar indicates the female in the pair whereas the second line indicates the male in the pair.

## **Expenditure of the grant**

The main equipment and facilities required to complete the experimental component of this project were available within the CSIRO Division of Entomology. However, a full-time technician for 8 weeks was essential to help set up and run the intensive laboratory experiments.

Mating pairs of *H. armigera* copulate for at least 30 minutes. The proposed project required that large numbers of moths be observed for copulating pairs every 15 minutes for 8 hours per day for ten-day periods throughout our experimental blocks. The project also required that female moths were dissected, eggs from matings counted, and neonates from egg collections scored. The hired person had experience and skills in working under laboratory conditions, and capturing, handling and maintaining moths. The technician was employed by CSIRO Entomology (CSOF2M @ \$8634).

This project was a joint effort among researchers in CSIRO Entomology and the University of Melbourne. I undertook one trip to Melbourne in May to meet for two days with Assoc. Prof. Elgar to source relevant literature, and finalise the design and proposed analysis of experiments. During the preparation of this work for publication we will correspond by phone and email. The cost of \$900 for the trip included return economy airfare from Narrabri to Melbourne (@ \$700), as well as accommodation in Melbourne on the evening that I arrived and the night following our first days meeting (@ \$100 per night for two nights = \$200).

---

Total requested budget = \$9534

Total expended budget = \$9534

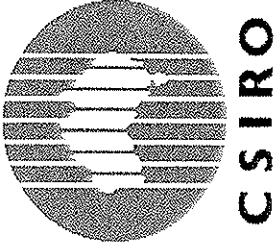
Balance = 0.00

Please see the attached certified statement of receipts and expenditure incurred



COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION

Department of Agriculture Fisheries and Forestry



Certified statement of receipts and expenditure incurred by CSIRO Entomology on : DAFF: Science and Innovation Award for Young People in Agriculture, Fisheries and Forestry - Sharon Downes. The role of mating in managing resistance by Helicoverpa armigera to GM cotton

RECEIPTS

Balance brought forward

\$0.00

Contributions received from DAFF

\$9,534.00

TOTAL Contributions

\$9,534.00

EXPENDITURE

Salaries

\$8,634.00

Travel

\$900.00

Equipment

\$0.00

Operating

\$0.00

TOTAL Expenditure

\$9,534.00

BALANCE TO BE CARRIED FORWARD

\$0.00

Compliance Statement:

I certify that all of the Funds were received and expended for the purpose of and in accordance with the signed Agreement

Ian Sakkara  
Finance Manager  
CPA

22/ 05/2008

Certified Correct

Robyn Noel  
Divisional Accountant

22/ 05 /2008