The Influence of Host Plants on the Mating Success of the Cotton Bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

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ABSTRACT

The chances of a moth mating successfully may depend on the type of crop or other vegetation surrounding it. Mating trays were used to determine the mating success of Helicoverpa armigera (Hübner) in various crops in the Darling Downs, Queensland, Australia during the 1996/97 and 1667/98 seasons. Moth abundance was assessed with pheromone and light traps. A series of comparisons of three or four crops was used, including mature maize (corn), immature, flowering and post flowering cotton and pre flowering and post flowering sunflower, sorghum and soybeans and fallow land. Laboratory reared virgin females with their wings clipped were placed in mating trays in the various crops and remained there until they were mated. The studies indicate that host plants did not significantly influence the chance of being mated, despite substantial variation in moth abundance between crops. Preliminary studies included a comparison of wing-clipping methods. The removal of the right fore and hind wings proved most effective. No consistent pattern emerged suggesting the multiple moths could be used in each tray. A comparison of females aged two to five days old showed no significant effect of moth age on the chance of being mated. The response of females to host volatiles and male response to females in the presence of host volatiles is being investigated in laboratory studies.

Introduction

Species in the noctuid genus *Helicoverpa* are significant agricultural pests world-wide (Fitt**,** 1989). In Australia, the cotton bollworm *H. armigera* (Hübner) is becoming increasingly significant due to the development of resistance to a wide variety of insecticides, including synthetic pyrethroids and organophosphates. In Australian cotton, the control costs and residual damage amount to $130 million per annum nation-wide (Fitt, 1997). Transgenic cotton is expected to reduce this pesticide requirement by 60-80%, thus significantly reducing costs as well as environmental damage inflicted by pesticides (Fitt, 1997). The success of transgenic cotton that is genetically engineered to contain the *Bacillus thuringiensis,* (Bt) toxin (Llewellyn *et al.*, 1994), depends on managing resistance of *Helicoverpa* moths to this toxin. *Helicoverpa armigera* is likely to develop resistance to Bt cotton (commercially known in Australia as INGARDR) due to its population ecology, association with cropping areas and consequent exposure to selection (Zalucki *et al.*, 1986; Fitt, 1989; Gregg *et al.*, 1993; Fitt, 1997). A resistance management strategy based on refugia and used to maintain susceptible individuals in a population is believed to be an effective measure in delaying the development of resistance (Roush, 1996). The mating success of moths within a refugia and in nearby crops or other vegetation may have a major impact on the dilution of resistant alleles from moths emerging from transgenic cotton.

It is possible that the chance of a moth successfully mating depends on the type of crop or other vegetation surrounding that moth. For example, in the American species, *Helicoverpa. zea,* which is closely related to *H. armigera,* it was shown that in moths collected as larvae from corn, pheromone production in the laboratory was 20 to 30 fold greater in those exposed to corn silks or their volatiles (ethylene and long-chain alcohols) than in those not exposed (Raina *et al.***,** 1992). In another American species, *Heliothis virescens*, female moths produced very little sex pheromone unless exposed to a host plant (Raina *et al.*, 1997). This paper describes preliminary studies to investigate the mating success of *H. armigera* in the field in the presence of various host crops and on fallow land. Techniques enabling mating success to be determined are also presented.

Materials and methods

Insect rearing

A culture of *H. armigera* was maintained in a reverse-light-cycle insectory at 25°C and 16:8 light:dark (L:D) regime with the scotophase during 0930 - 1730h Australian Eastern Standard Time (AEST). Individual larvae were reared in 35-ml plastic containers and fed on soybean-based artificial diet (Shorey and Hale, 1965). Pupae were sexed. Adult moths were held in 100-ml plastic containers provided with dental wicks soaked in 5% sucrose solution as food.

Wing-clipped versus normal- winged females

A number of methods were tried to determine a suitable wing-clipping technique for laboratory-reared female *H. armigera* moths that were to be used in the field in mating experiments. These included superglueing the fore- and hind- wings together above the centre of the body, clipping all the fore and hind wings, clipping both forewings and clipping the fore and hind wings on the right hand side. It was decided that clipping both the fore and hind wings on the right hand side was the most suitable method as very few moths died and they were not able to escape readily.

A laboratory experiment was set up to determine if wing-clipping affected the chance of a female moth mating in the presence of normal winged females. This was conducted with and without a host plant (flowering cotton), in a cage measuring 1m x 0.5m x 1m constructed of a metal frame and covered in wire gauze. Wing-clipped females were placed in nine mating trays held by 3 steel posts (3 trays per post) inside the cage. A mating tray consisted of a 20cm x 20cm x 7cm metal frame welded together with a metal gauze base and an open top. The vertical sides were coated in fluon (Dupont, Sydney, Australia) and plastic lips placed on the edges to ensure the female moths could not escape by climbing out or by males dragging them over the edge. Thirty laboratory-reared, wing-clipped unmated females were distributed in the mating trays, each with three or four females. Thirty normal winged females and 70 normal-winged males were also released into the cage. A dental wick soaked in 5% sucrose solution was placed in each mating tray as food. Moths were placed in the cage one hour prior to scotophase. All the females were collected in the morning and dissected to determine if spermatophores were present, indicating that they had mated.

Mating success in females of different ages

The effect of moth age on mating success of wing-clipped females in mating trays was investigated in three field cages. A field cage was 2.5m high covering a basal area of 1m2, supported by a metal frame and covered with white voile cloth fastened with Velcro® around the base of the poles and around access openings (Stanley, 1997*)*. Each cage had three steel poles, each holding six mating trays, giving a total of 18 mating trays per field cage.

Two laboratory-reared, unmated wing-clipped females were placed in each mating tray, giving a total of 36 females per field cage, one hour prior to scotophase. Each mating tray was provided with a dental wick soaked in 5% sucrose solution. Females of different ages (2-5 days) were placed randomly in the mating trays, and the ages of individuals in each mating tray in each field cage were recorded. A total of 36 males (aged 2-5 days old) were also released into each field cage. All females were collected at dawn (0530 AEST) for the field experiments and at 1730 AEST for the laboratory experiment and killed in alcohol. They were then dissected to determine mating status.

Single versus multiple females in mating trays

Two experiments compared mating success of single and multiple females in mating trays. One, two or three female moths were placed in each mating tray one hour prior to scotophase. A total of 36 females (aged 2-5 days) were placed in the mating trays in each cage, six singles, six doubles and six triples. A total of 36 males were also released in each cage.

Field experiments and moth trapping

Using the information obtained from the above experiments to study mating success in captive females, field experiments were conducted to investigate the influence of various host crops on mating success in *H. armigera at* a diverse cropping site near Cecil Plains, Queensland (27° 30'S, 151° 11'E). The experiments were conducted from September to May over two cotton seasons. In 1996/97, six experiments were completed and in 1997/98, four experiments were completed.

For each experiment, three or four crops at different growth stages and fallow land were selected. They included mature maize, pre-flowering, flowering and post flowering cotton and pre-flowering and post-flowering sunflower, sorghum and soybeans. Twenty to 30 mating trays were placed in each field. Laboratory-reared, wing-clipped, unmated females aged two to five days old were randomly placed as singles, doubles or triples in each mating tray approximately one hour prior to scotophase (approx. 1830-1930 AEST). They were collected into alcohol near dawn when they were still inactive and dissected to determine whether they had mated.

To assess *H. armigera* abundance, three light and three pheromone traps were operated in each field. The light traps comprised inverted fiberglass cones, made from airport runway markers, 75cm in diameter at the top, 7cm diameter at the base and 45cm deep. These were painted white and polished on the inside surface. The cones were placed on 60L plastic garbage bins and a 12-volt caravan light, with two 8-watt NECFL 8BL blacklight tubes fitted, was placed inside the cone. Moths attracted to the light fell down the cone into a 4L jar containing 70% ethanol. This light trap design caught only moths flying above the trap as light could only be seen from above and was therefore less likely to attract moths from a distance. The pheromone trap used was the Agrisense trap (Dunluce International, Pty, Ltd., PO Box 44, Killara, NSW 2071, Australia), referred to as the International Pheromone dry funnel trap by Gregg and Wilson (1991). This trap used a killing agent (Sureguard Ministrips®) to kill moths caught in the canister.

Results and discussion

Wing-clipped versus normal winged female moths

In both the presence and absence of cotton, there were significantly more normal winged females mated than wing-clipped females (mean of 10 of 30 wing-clipped females mated when cotton was present, and 5 of 30 when it was not, compared to 25 of 30 normal females with cotton present and 23 of 30 without it). This indicates that wing-clipping decreases the chance of a moth successfully mating. The lack of mating by wing-clipped females may be due to fluctuating asymmetry, defined as deviations from perfect bilateral symmetry in a morphological trait (Thornhill, 1992). A number of studies have revealed that in some species fluctuating asymmetry is important in sexual selection. For example, in the Japanese scorpionfly, *Panorpa japonica* (Mecoptera: Panorpidae) it was found that males with relatively low fluctuating asymmetry of the fore wings were favoured by the females for mating in a population (Thornhill, 1992). It is possible in *H. armigera*, where the female releases a pheromone in order to attract a mate, that the male is making a choice on the asymmetry of the female's wings. Another possibility is that the winged female's ability to call from more easily accessible or more favourable places, enables males to more readily locate them.

Age of female moths

In all three field cages, no consistent pattern was detected, suggesting that age did not affect the chance of a female moth being mated. This allowed females aged two to five days to be used in the field experiments.

Singles versus multiple females

The results indicated no consistent pattern, suggesting that single or multiple females per mating tray did not affect the chance of being mated. Thus single or multiple females could be used in the field experiments.

Field trial of mating success

The results of the light trap catches; the total catch and percentage of females caught and the pheromone catches for *H. armigera* are shown in Table 1.

The results from the nights of the 10th February 1997 and the 9th December 1997 will be discussed in detail. On 10th February 1997, the light trap catches indicated a significant difference for the total catch between crops. Significantly larger numbers of moths were present in the flowering soybean crop compared with non-flowering cotton and fallow land. The percentage of females caught in the light trap catches was not significantly different. There were no significant differences between the pheromone trap catches in different crops on this night, though on other nights this was not the case (Table 1).

On the 9th December 1997, the light trap catches showed a significant difference for the total catch between crops. Significantly more moths were present in the flowering sunflower, indicating a preference for flowering sunflowers compared with fallow land. The percentage of females in the light trap catches was not significantly different between crops. The pheromone trap catches for this night were significantly different between crops, a result consistent with those from light traps. The greatest catch was in flowering sunflower and the lowest in the fallow land. If pheromone catches can be regarded as an indicator of a female's chances of mating, this suggests that this chance was greatest in flowering sunflower.

A summary of the effects of host crops on mating success, the percentage of females mated and the mean mating frequency for *H. armigera* is shown in Table 2.

Host crops influence many aspects of the behaviour of *Helicoverpa* spp., including feeding, oviposition and flight (Fitt, 1991; Fitt and Boyan, 1993). As mating is energetically expensive for both females (the production of pheromones) and males (flight required to find females) as well as exposing them to the risk of predation prior to and during mating, it is logical to believe the investment in mating should be related to the proximity of suitable hosts for the development of the resulting larvae. However, despite this and differences in the abundance and sex ratio of moths caught in traps, the results from the mating trays indicate that host plants did not significantly influence the chance of captive wing-clipped females being mated, with the exception of the night of the 10th February 1997. On this night, female moths were significantly more likely to be mated in flowering soybeans than fallow land or mature cotton. The percentage of females mated and the mean mating frequency from light trap catches revealed no significant differences between crops on all nights. These results are consistent with those from the mating trays, indicating that females were no more likely to be mated in one crop than another.

These results suggest that none of the crops and fallow land tested influenced *H. armigera* mating success. These results may appear to conflict with those of Raina (1992, 1997), who found greater pheromone production in the presence of host plants. However, in our studies suitable host crops usually had greater moth densities, as indicated by light trapping. It is possible that in such environments, females need to produce more pheromone simply to compete with their neighbours. In a less favourable environment such as fallow land, smaller amounts of pheromone may be needed to attract males because there are fewer pheromone plumes competing for the available males. Thus, increased pheromone production may not automatically increase mating.

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Table 1. The effects of host crops on the light trap catches for total catch and percentage of females caught and the pheromone catches for *H. armigera.*

Date	Crops	Light trap		Pheromone catches
		Total catch	% females	
10/12/96	F, N-Fl C, N-Fl Su, Fl Su	***	**	***
20/12/96	F, N-Fl C, N-Fl Sg, Fl Sg	***	NS	*
21/12/96	F, N-Fl C, N-Fl Sg, Fl Sg	***	NS	**
09/02/97	F, N-Fl C, N-Fl Sb, Fl Sb	***	NS	**
10/02/97	F, N-Fl C, N-Fl Sb, Fl Sb	***	NS	NS
02/04/97	F, N-Fl C, N-Fl Sb	**	NS	NS
09/12/97	F, N-Fl C, N-Fl Su, Fl Su	***	NS	*
11/12/97	F, N-Fl C, N-Fl Su, Fl Su	**	NS	**
08/02/98	F, Fl C, M-M	***	NS	**
09/02/98	F, Fl C, M-M	***	NS	NS

Fl – Flowering , N-Fl - Non-flowering, M - Mature,
F – Fallow, C – Cotton, Sg - Sorghum , Sb – Soybeans, Su – Sunflower and M – Maize

Table 2. The effects of host crops for *H. armigera* on mating success, percentage of females mated and the mean mating frequency.

Date	Crops	Mating success	Light trap catch mating	
		(mating trays)	% females mated	mean frequency
10/12/96	F, N-Fl C, N-Fl Su, Fl Su	NS	NS	NS
20/12/96	F, N-Fl C, N-Fl Sg, Fl Sg	NS	NS	NS
21/12/96	F, N-Fl C, N-Fl Sg, Fl Sg	NS	NS	NS
09/02/97	F, N-Fl C, N-Fl Sb, Fl Sb	NS	NS	NS
10/02/97	F, N-Fl C, N-Fl Sb, Fl Sb	**	NS	NS
02/04/97	F, N-Fl C, N-Fl Sb	NS	NS	NS
09/12/97	F, N-Fl C, N-Fl Su, Fl Su	NS	NS	NS
11/12/97	F, N-Fl C, N-Fl Su, Fl Su	NS	NS	NS
08/02/98	F, Fl C, M-M	NS	NS	NS
09/02/98	F, Fl C, M-M	NS	NS	NS

Fl – Flowering , N-Fl - Non-flowering, M - Mature,
F – Fallow, C – Cotton, Sg - Sorghum , Sb – Soybeans, Su – Sunflower and M – Maize