Plant volatiles as attractants for *Helicoverpa* moths

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Introduction

Helicoverpa spp. remain the key pests of cotton in Australia. The cotton bollworm, *H. armigera* is becoming resistant to many insecticides. With this increasing resistance as well as environmental concerns with insecticide use, we need to look into viable alternative tools that can be useful in the integrated approach to manage this pest. Semiochemicals or insect behaviour-modifying chemicals fit well in an integrated pest management system due to their specificity and low toxicity. Sex pheromones, have been successfully used in pest monitoring systems and mating disruption techniques for a number of orchard pests. In Egypt, attract-and-kill methods using sex pheromones have successfully contributed to the management of another cotton pest, the pink bollworm, *Pectinophora gossypiella* (Mafra-Neto & Habib 1996).

Kairomones are another type of semiochemicals that can be exploited for the management of *Helicoverpa* in cotton, and include the chemical volatiles emitted by many plants that are attractive to these insects. Researchers in Texas, USA have patented a chemical blend based on the floral volatiles emitted by the night-blooming *Gaura* spp. as attractants for noctuid pests like *Helicoverpa zea* (Lopez *et al* 2000). Floral compounds identified in the African marigold, *Tagetes erecta*, and their synthetic equivalents were found to be attractive to *H. armigera* females (Bruce & Cork, 2001, Burguiere *et al* 2000). Volatiles from pigeonpea plants were also recorded to be attractive to *H. armigera* moths (Hartlieb & Rembold 1996). Xiao *et al* (2002) reported the attraction of *H. armigera* to wilted leaves of the Chinese wingnut tree, *Pterocarya stenoptera*.

Our group is investigating attractants for *Helicoverpa* based on chemical volatiles of host and non-host plants. The project was initiated in 1997 by the former CRC for Sustainable Cotton Production and a similar one followed under the new Australian Cotton CRC. Plant-based attractants could be useful in several ways. Plant volatiles are attractive to adult moths of both sexes, and thus have advantages over sex pheromones that catch only males. These volatiles can also be used in attract-and-kill systems, where the attractant volatiles are treated with small amounts of insecticide. The insect is lured by the attractant and upon contact or ingestion, eventually dies. Similarly, moths might be lured by attractants treated with other biological control agents such as selective pathogens of *Helicoverpa*, and then released to disseminate the pathogen. Another potential use of attractants is to enhance trap crops planted next to cotton as foraging sources for *Helicoverpa*.

In this paper, we discuss the approaches we employed to evaluate the attractiveness of plants and synthetic plant odours to *H. armigera* moths in the laboratory and in the field.

Bioassay of plants

Many plants are attractive to *Helicoverpa* for adult foraging and female oviposition. Zalucki *et al* (1994) recorded *H. armigera* on 101 plants and *H. punctigera* on 172 plants in 40 families. Analysis of pollen carried on moths also indicated adult foraging on native species (*Eucalyptus* spp.) and weeds (Brassicaceae) (Gregg 1993, Del Socorro & Gregg unpublished).

Our initial approach was to screen selected plants for attractiveness to unmated moths using a two-choice olfactometer system in the laboratory. The olfactometer was based on the design by Beerwinkle *et al* (1996), and described in detail by Gregg *et al* (1998). It consisted of a 'test' chamber which contained the plant or volatiles to be tested, and a 'blank' or the control chamber. Fifty moths of each sex aged 1-4d old were used for each run in the olfactometer. Each run was replicated three times for each sex (N=150). The number of moths that went into the test and blank chambers were counted at the end of an 8-hr dark period. The attractiveness of the plant was determined by calculating the percentage of the total 150 moths that went into the test chamber ('test %) and the number entering the test chamber relative to those entering the blank chamber ('success rate'). The former is our primary criterion. Data were analysed using Zar's procedure for multiple comparison of proportions. With N=150, the minimum significant different between two percentages using this procedure is about 8%.

We have screened a total of 40 plants in the olfactometer. These plants included host and non-host crops, weeds, Australian natives, trees and ornamentals. A fresh bouquet of the plant (flowers and leaves) to be tested was held in a subchamber below the test chamber, in a small container of water. Volatiles from these plants were collected from the test airstream of the olfactometer using a solid phase micro-extraction (SPME) technique, followed by thermal desorption from the SPME fibre directly into the analytical instrument. Volatiles were then analysed by gas chromatography and mass spectrometry (GC-MS) on a Hewlett Packard 6890. We identified about 170 compounds from these plants. A matrix of the profile of volatiles collected from each plant was constructed to determine what volatiles were in common in the least to the most attractive plants.

Bioassay of single volatiles and blends

Based on the profile of the volatiles that were found in the most attractive plants, we selected 30 chemicals to be tested singly in the olfactometer. Synthetic equivalents of these volatiles were formulated in canola oil at a concentration of 10%. The lure consisted of 200μl of the formulation absorbed into a cotton wick and placed in the test airstream of the olfactometer. The lure was changed every 2 h during olfactometer runs.

Most published moth attractants are usually mimics of attractive plants. This approach may not work for *H. armigera* for several reasons. Firstly, *H. armigera* is highly polyphagous and is therefore attracted to so many plants. The question is: which plant do we then mimic? Secondly, the volatiles that were identified in the most attractive plants might not necessarily be attractive. They might even be repellents. Another possible reason is that, with the reported learned behaviour in this species (Cunningham *et al* 1999), the volatile blend might not work in the field if the moths have been previously conditioned to the existing plant volatiles in that area. Our main approach, therefore, was to combine volatiles that were in common in the most attractive plants into "super blends" rather than mimic the volatiles present in a single attractive plant. These blends consisted of between 2 and 6 volatile compounds.

Volatile blends were formulated in Sirene®. This is a polymeric substance containing anti-oxidants and UV protectants to slow down release of volatiles, and is marketed by IPM Technologies Ltd, USA. The synthetic volatiles were mixed into Sirene® in a barrel of syringe using a slow (4rpm) broad-bladed propeller stirring the viscous material for 3 h. Sirene® formulations were held in air-tight 20ml syringes and stored at 4°C for periods of up to 2 weeks. About 100mg of this formulation was placed on a 1 x 1 cm square of plastic (Corflute®) held by a pin in the test airstream of the olfactometer. Sirene® blends contained 2-8% attractant volatiles.

Field studies

Field trapping trials were conducted to test volatile blends that were found to be attractive to moths in the olfactometer. About 200mg of Sirene® containing volatiles at concentrations of 6-8% was placed in AgriSense® canister type traps (Gregg & Wilson 1991). The traps were cleared three times a week, and moths sexed and dissected to determine mated status. Traps were spaced 50m apart and were rotated, within a row, between catch intervals. The experiments were analysed as Latin squares. In all trials, pheromone traps were included for comparison with our volatile blends. Field trapping trials were conducted on various crops such as cotton, soybeans, chickpeas, wheat, and sunflower at Cecil Plains and Bowen, Queensland, Kununurra, WA, and in China.

Field wind tunnel experiments were conducted to test attract-and-kill formulations in semi-natural conditions. The wind tunnel was 4m long and 1.2m in diameter, and made of disposable plastic. Wind tunnels were hung just above ground level and oriented parallel to the prevailing wind. Volatile blends were formulated in 'sloppy' Sirene® (normal Sirene® plus Spraytech® oil) combined with a feeding stimulant (sucrose) and either 0.5% methomyl or 0.5 and 1% carbaryl as the toxicant. Lures for the tunnel consisted of three bottle tops each with 100-200μl of the blend, which were held on a plastic dinner plate in the upwind end. For each trial, between 20 and 50 moths aged 1-4d were released downwind of the tunnel before sunset. The number of dead moths in the tunnel were counted the following morning.

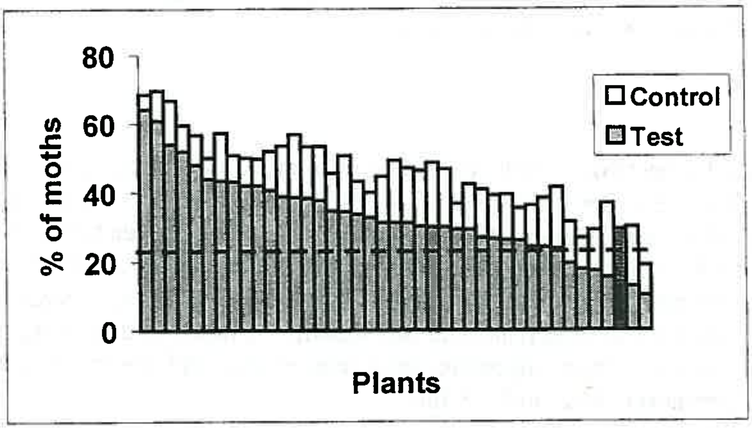
Preliminary open field trials were done to test if our attract-and-kill formulations would kill wild moths. The formulations were based on canola oil with the addition of feeding stimulant, thickener, emulsifying agent and antioxidants, and mixed with 0.5% methomyl as the toxicant. Three trials were done in Bowen, Queensland on vegetative sweet corn and French beans. In all the trials, we included a previously published attractant blend (Texas blend, Lopez *et al* 2000) for comparison with our own blends, along with a formulation containing no attractant chemicals and an unsprayed section as control. Each treatment used 500ml formulation that was sprayed before sunset on 50m sections. Treatments were replicated 3 or 4 times. The numbers of dead moths on 5 rows upwind and 5 rows downwind of the treated rows were counted every morning for up to 6 d after spraying.

Results and discussion

Bioassays of plants

The names of plants and volatiles mentioned in this section are not given for commercial reasons. All except 6 of the 40 plants we tested in the olfactometer were significantly attractive to *H. armigera* female moths (Fig. 1). The first five most attractive plants were not necessarily hosts of *Helicoverpa*. There appeared to be no correlation between attractiveness in the olfactometer and suitability as larval hosts. The olfactometer probably measures attractiveness for adult foraging rather than oviposition.

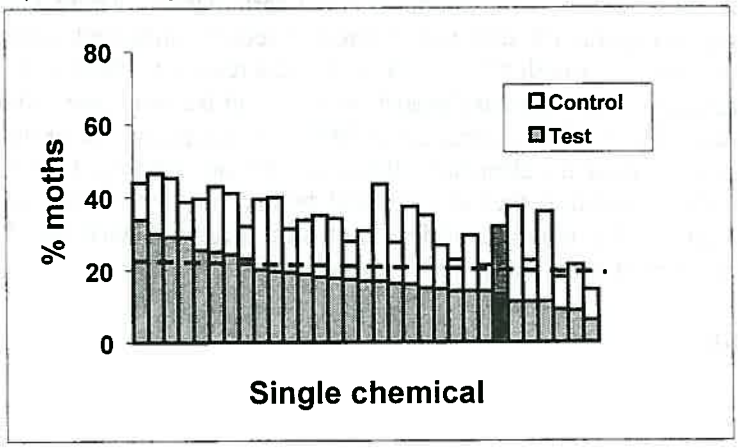
Figure 1. Attractiveness of plants to *H. armigera* females in the olfactometer. The dark bars represent the % of moths that entered the test chamber and the light bars above these for those that went into the control chamber. The dotted line represents the minimum test % level to be significantly different from the blank olfactometer (dashed bar). For attraction to be statistically significant, the dark bar should be above this dotted line.



Bioassay of single volatiles and blends

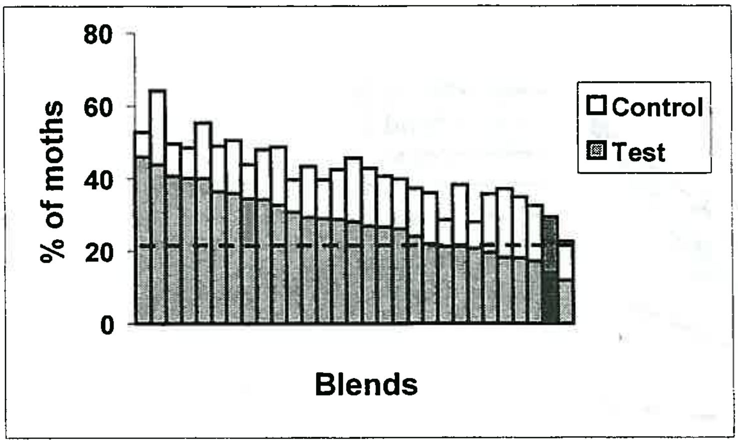
We tested a total of 30 single volatiles in the olfactometer. Very few chemicals were attractive on their own. Only 8 of the 30 chemicals tested were significantly attractive to female moths (Fig. 2). These results suggest that moths might be respond more to blends than single chemicals.

Figure 2. Attractiveness of single volatile compounds to *H. armigera* females in the olfactometer. Explanation of graph is the same as in Fig. 1.



On the other hand, volatile blends were more attractive than single chemicals. Twenty of the 28 blends we tested in the olfactometer were significantly attractive to female moths (Fig. 3). Some of these blends were comparable to highly attractive plants. These attractive blends were usually a mixture of floral and leaf volatiles. In nature, moths are likely to recognise plants by a combination of both their leaf and floral volatiles.

Figure 3. Attractiveness of volatile blends to *H. armigera* females in the olfactometer. Explanation of graph is the same as in Fig. 1.



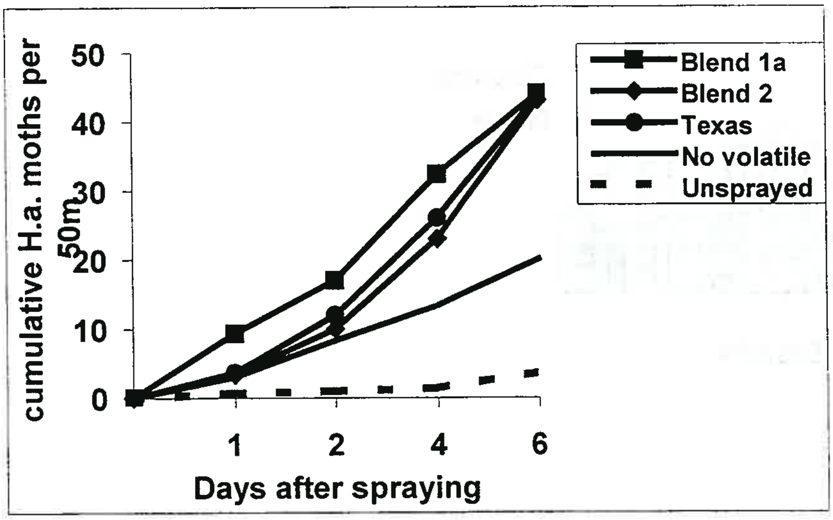
Field studies

A total of 23 field trapping experiments was conducted on various crops. Typically, plant volatile blends caught only 5-10% of pheromone catches. The sex ratio of moths was highly variable, and often, catches tended to be mostly females. It might be that the volatiles are inherently more attractive to females or that adjacent pheromone traps (which only catch males) are "robbing" the volatile traps of males. On the other hand, in some cases volatile traps caught mostly males. Whether this variation reflects a bias in the real sex ratio of the population, or just behavioural differences between sexes, is not clear. Some of our blends have also caught substantial numbers of other pest species like armyworms and loopers.

We had a total of 82 field wind tunnel nights in Cecil Plains and Bowen, Queensland to test different attract-and-kill volatile blends, including types of ingestible formulations, concentrations, feeding stimulants, and artificial flowers as visual cues to enhance close-range attractiveness. As with our olfactometer results, blends consisting both floral and leaf volatiles were more attractive than blends with either floral or leaf volatiles alone. In general, we obtained between 60-80% kill during warm and moderately windy nights. Between the two types of toxicants, 0.5% methomyl killed more moths than either 0.5 or 1% carbaryl. This was probably due to the higher contact activity of methomyl than carbaryl which is a stomach insecticide. Artificial flowers as visual cues for close-range attractiveness did not significantly increase the attractiveness of the volatile chemical lures. On the other hand, taste stimuli such as sucrose or molasses appeared to be important in keeping the moths in the vicinity of the lure.

Results from the preliminary open field trials in Bowen, Queensland, showed that our attract-and-kill formulations did kill wild *H. armigera* moths. Up to about 50 *H. armigera* moths per 50m of treated row were killed in sweet corn (Fig. 4), despite the low numbers during the trial (5-10 males/night in pheromone traps, and ~2 eggs/m). In all the three trials, the sex ratios ranged from 50 to 80% females. The formulations remained active for up to 6 days. We observed that although the droplets dried up during the day, they re-absorbed moisture from dew on subsequent evenings making them active again for contact or ingestion. We dyed the formulations with blue food dye. Moth dissections showed that most of the dead moths ingested the lure as indicated by the presence of blue dye in the guts.

Figure 4. Cumulative mean numbers of dead *H. armigera* moths per 50m sprayed with attract-and-kill formulations on vegetative sweet corn, Bowen, Queensland.



Wind affected the kills obtained from the volatile treatments. Treatments placed on the downwind edges of fields killed more moths than those on the upwind edges. The volatile formulations also killed other noctuid pests such as armyworms and loopers. In the first corn trial higher numbers (79-108 per 50m) of the common armyworm, *Mythimna convecta*, were killed than *H. armigera.* Other species killed were *M. loreyimima* (sugarcane armyworm), *Spodoptera litura* (cluster caterpillar) and *Chrysodeixis spp.* (false loopers).

Conclusions

We now have a suite of plant volatile chemicals that are attractive to *H. armigera* females. We understand the principles of how to combine these chemicals in blends which are comparable in attractiveness to real plants, to *H. armigera* and other pest moths. We understand some aspects of moth behaviour in relation to these lures, and how we could use them in the field. We have patented these concepts and are looking for commercial partners to develop the technology.

Our open field trials killed considerable numbers of wild *H. armigera* and other pests such as armyworms and loopers of both sexes. Volatile attractants used in an attract-and-kill strategy could be useful in the integrated approach to manage and control this pest in cotton. We do not envisage attractants to be substitutes for insecticides. They might be applied any time from planting to fruit setting with the objective of reducing *Helicoverpa* pressure. We plan to conduct more large-scale field trials during the 2002-2003 season. The next step is to determine the impact of the attract-and-kill approach on actual oviposition and on non-target beneficials in the field. Research is also needed to determine application rates, and the proportion of a field which should be treated.

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References

Beerwinkle KR, Shaver TN, Lingren PD, Raulston JR. 1996. Free-choice olfactometer bioassay system for evaluating the attractiveness of plant volatiles to adult *Helicoverpa zea. Southwestern Entomologist*, 21:395-405.

Bruce TJ, Cork A. 2001. Electrophysiological and behavioral responses of female *Helicoverpa armigera* to compounds identified in flowers of African marigold, *Tagetes erecta. Journal of Chemical Ecology*, 27:1119-1131.

Burguiere L, Marion-Poll F, Cork A. 2000. Electrophysiological responses of female *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) to synthetic host odours. *Journal of Insect Physiology*, 47:509-514.

Cunningham JP, Zalucki MP, West SA. 1999. Learning in *Helicoverpa armigera* (Lepidoptera: Noctuidae): a new look at the behaviour and control of a polyphagous pest. *Bulletin of Entomological Research*, 89:201-207.

Gregg PC. 1993. Pollen as a marker for migration of *Helicoverpa armigera* and *H. punctigera* (Lepidoptera: Noctuidae) from western Queensland. *Australian Journal of Ecology*, 18:209-219.

Gregg PC, Del Socorro AP, Henderson GS, Forrester NW, Moore C. 1998. Plant-based attractants for adult *Helicoverpa* spp. In MP Zalucki, RAI Drew & GG White (eds) *Pest Management – Future Challenges*, Proceedings of the Sixth Australasian Applied Entomological Research Conference, Brisbane, pp 341-347.

Gregg PC, Wilson AGL. 1991. Trapping methods for adults. In MP Zalucki (ed) *Heliothis: Research Methods and Prospects*. Springer-Verlag, Berlin, pp 30-48.

Hartlieb E, Rembold H. 1996. Behavioural response of female *Helicoverpa (Heliothis) armigera* Hb. (Lepidoptera: Noctuidae) moths to synthetic pigeonpea (*Cajanus cajan* L.) kairomone. *Journal of Chemical Ecology*, 22:821-837.

Lopez JD, Shaver TN, Beerwinkle KR, Lingren PD. 2000. Feeding attractant and stimulant for adult control of noctuid and/or other lepidopteran species. US Patent No. 6,074,634.

Mafra-Neto A, Habib M. 1996. Evidence that mass trapping suppresses pink bollworm populations in cotton fields. *Entomologia Experimentalis et Applicata*, 81:315-323.

Xiao C, Gregg PC, Hu W, Yang Z, Zhang Z (2002). Attraction of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) to volatiles from wilted leaves of a non-host plant, *Pterocarya stenoptera. Applied Entomology and Zoology*, 37:1-6.

Zalucki MP, Murray DAH, Gregg PC, Fitt GP, Twine PH, Jones C. 1994. Ecology of *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) in the inland of Australia: larval sampling and host plant relationships during winter and spring. *Australian Journal of Zoology*, 42:329-346.