Attract-and-kill for *Helicoverpa* moths - a new tool for area-wide pest management?

Alice Del Socorro and Peter Gregg
Australian Cotton CRC and School of Rural Science and Agriculture
University of New England, Armidale NSW 2351

Introduction

Airborne volatiles emitted by flowers and other vegetative plant parts that attract insects for feeding and egg-laying could be useful in pest management. Over the last 6 years or so, we have developed blends of plant volatiles that can be used as attract-and-kill for *Helicoverpa* moths in cotton and other crops. Our first approach to developing attractants was to screen various plants for attractiveness to these moths in the laboratory using an olfactometer (Beerwinkle *et al* 1996), and to identify the compounds emitted by these plants (Gregg *et al* 1998). Our attractant blends were not mimics of certain attractive plants but mixtures or "super blends" of compounds that were in common in the most attractive plants.

Small-scale field trials on sweet corn and green beans in Bowen, Qld showed that attractant blends including 0.5% methomyl killed substantial numbers of *Helicoverpa* and other noctuid pest moths for up to 6 days after spraying (Del Socorro *et al* 2002). In this paper, we will describe the two large-scale field trials of attract-and kill in cotton during the 2002-2003 and 2003-2004 seasons on the property "Wamara", near Cecil Plains, Qld, including the impact of these trials on moth numbers and oviposition, and the implications of attract-and-kill technology as a novel tool in the area-wide management of *Helicoverpa.*

Materials and methods

2002-2003 Field Trial

In the first large-scale trial, the treated field consisted of 42 ha of conventional cotton (cv S-80), and an adjacent cotton field of similar area (~500m away) was used as the control field. These fields were intensively monitored by the UNE team. Visual checks, beat sheet counts and D-vac samples (large back-pack style) were taken at intervals of about 1 week or more often if possible. Visual checks from these two fields were also provided by the consultants. These checks were not replicated, and were used only to fill longer intervals when no checks were made by the UNE team.

Since our attractants target mobile stages of the pest, it is possible that they may protect nearby fields (including controls) as well as the treated field. To check for such effects we also included a regional control area consisting of six fields (totalling about 250 ha) which was located about 6-8 km to the north-east of the treated field. This control was compared with a group of 5 fields totalling about 200 ha which surrounded the treated field. It included both the treated and local control fields. Data on visual checks on these fields were provided to us by the consultants.

The treated field which consisted of about 730 rows, was sprayed every 72nd row (i.e. about 1.5% of the field) on 30 Nov 2002, 15 Dec 2002, and 6 Jan 2003. The total volatile concentration in the final spray of the attractant was 3.1%. The attractant was applied at 400ml per 100m, with 0.5% methomyl (Electra 225®) as the toxicant. The attractant formulation also had sucrose (20%) as a feeding stimulant, and various emulsifying agents and anti-oxidants, and blue food dye to mark moths which had fed on the material. The formulation was applied using a low pressure 12 volt pump, through a nozzle designed for applying liquid fertilizer. A motor cycle fitted with a third wheel to allow operation in row crops was used.

Dead moths were collected every morning for up to four days after each spray from a 50m section around each sprayed row. These 50m sections were staggered across the field in a "Z" pattern, and in each 50m stretch, the furrows 1, 3, 10 and 35 rows away from the sprayed row, in both directions, were searched. All noctuid moths were collected, and all *Helicoverpa* spp. were dissected to determine their species, sex, mated status (for females) and whether they contained blue dye.

2003-2004 Field Trial

For this season, the treated field which consisted of 40ha of irrigated conventional cotton (cv S-80), was sprayed on 19 Dec 2003 and 27 Dec 2003. The adjacent 24 ha planted to the same cotton variety was used as the local field control. The grower also sprayed an adjacent 40 ha of dryland S-80 cotton on 23 Dec 2003. We used the commercial attractant formulation, Magnet®, which contained the same attractant volatiles as used in the 2002/03 season. Magnet ® is licensed by the Australian Cotton CRC to Ag Biotech Pty Ltd. It has been approved by the Australian Pesticides and Veterinary Medicines Authority (APVMA) for commercial release under a Product Evaluation Permit, and our 2003/04 trial was one of several conducted under this permit.

During the first spray, we also compared two row spacings of attractant treatment - 1 in 72 rows and 1 in 36 rows. As in the 2002-2003 trial, a 50m section on each sprayed row was checked for dead moths, at 3,10 and 35 rows away in 1/72 spacing, and at 3 and 10 rows away at 1/36 spacing. Flush counts of moths resembling *Helicoverpa* in size and colour, were done on 300m transects on the treated and control fields. This was done by an observer on a motor cycle fitted with a bar on which rubber straps were suspended so that they brushed the cotton canopy about 3m in front of the observer. Egg counts were provided by the consultants for the treated and control field, and for another 73 fields of cotton within a radius of about 11km of the treated field, for the period 15 Dec 2003 to 15 Jan 2004.

Results and Discussion

2203-2003 Field Trial

Figure 1 shows the numbers of dead *H. armigera* moths collected over the four days after the first spray. The highest numbers of moths were collected in the furrows immediately adjacent to the sprayed rows, indicating that some moths were killed quickly by the insecticide. However.

significant numbers were also found at 3, 10 and 35 rows away, indicating that moths which are not killed immediately by the insecticide can move considerable distances before they die. The highest numbers of dead moths were found in the first two days, although substantial numbers were still being killed after 4 days. We estimated to have killed about 28,500 *H. armigera* and about 3,500 *H. punctigera* after the first spray. Of the *H. armigera* killed, about 54% were females. We also estimated a theoretical total of about 13.2 million eggs not laid by *Helicoverpa* moths as a result of the first spray. If they had been spread evenly across the treated field, this would have amounted to 3-4 eggs per plant. Subsequent sprays did not kill as many moths as the first spray. This might have been due to natural decline of moth population or perhaps because of the effects of the first spray.

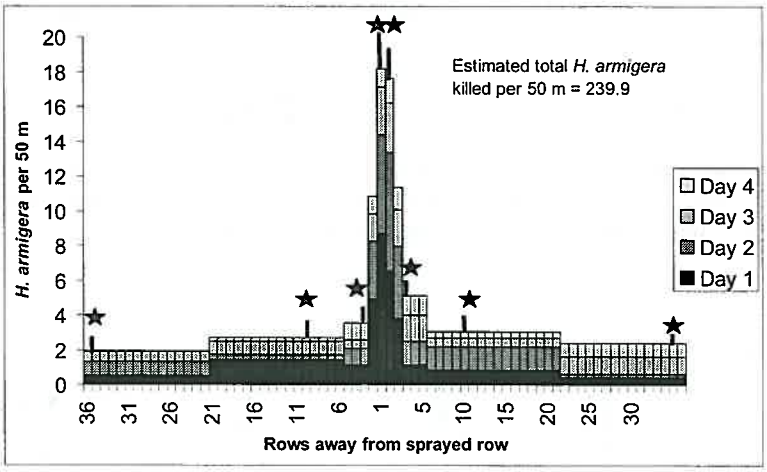

Figure 1. Numbers of dead *H. armigera* moths collected over four days after the first spray at "Wamara", 2002-2003. Sampled rows are indicated by stars, bars are standard errors of the mean.

Figure 2 shows the numbers of *Helicoverpa* egg and larvae per plant on the treated field at "Wamara" versus the adjacent control field. Following each application of the attractant, there was a consistent pattern of lower *Helicoverpa* numbers in the treated field than in the control field. As the effect of each attractant spray wore off, egg numbers rose in the treated field so that after about 2 weeks it had higher counts than the control. We believe this reflects greater attractiveness of the treated field due to better timing of irrigation compared to the control field. Apparently, however, the effects of the attractant were able to counter this difference.

We estimated the possible district impact of the attractant by comparing the consultants' data on total *Helicoverpa* numbers per plant in the treated region (ie, 5 cotton fields in "Wamara" and an adjacent farm) with the control region (ie, 5 cotton fields, 6-8km away). Though the two areas started out at the same pest levels, the treated region showed similar pattern of declines following attractant treatment (Fig. 3). There was the same pattern of a decline in the treated district relative to the control after the application of each spray. There was a marked and progressive decrease in pressure in the treated area compared to the control.

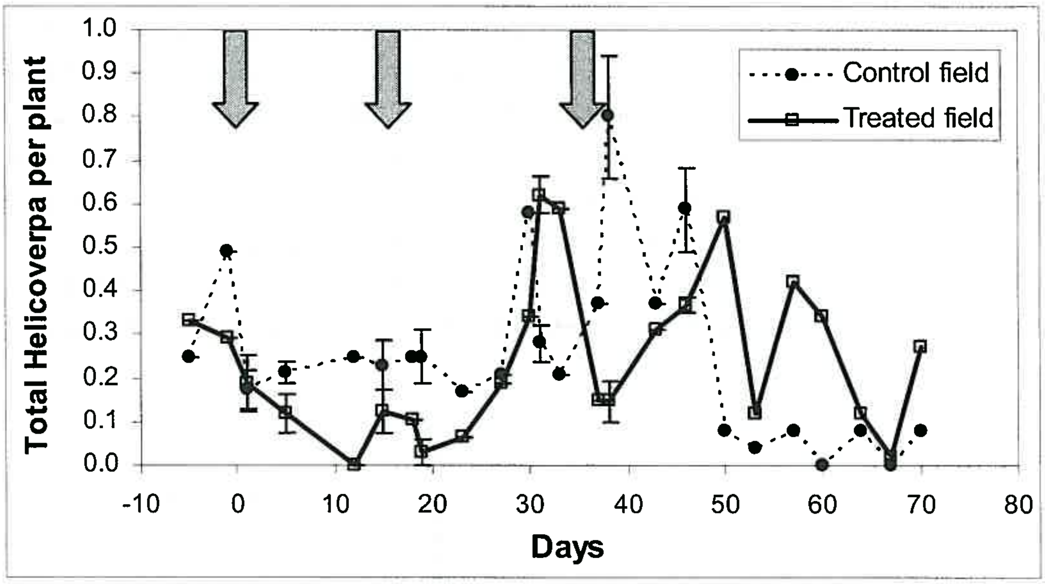

Figure 2. Numbers of *Helicoverpa* spp. eggs and larvae per plant in the treated field and control field, "Wamara", 2002-2003. Bars are standard errors of the means; where none are present the counts are unreplicated data from consultants. Arrows represent Magnet applications.

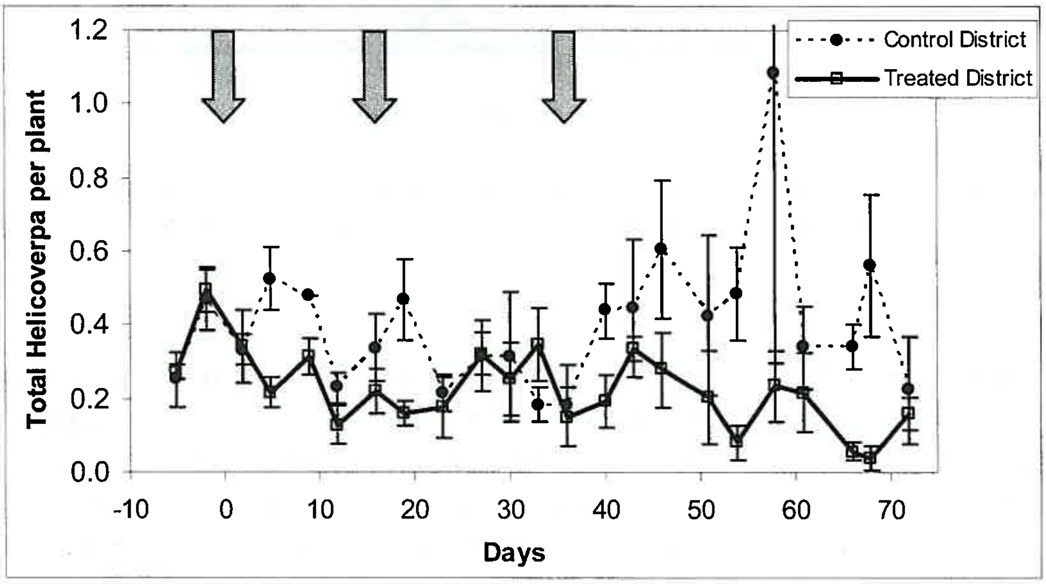

Figure 3. Numbers of *Helicoverpa* eggs and larvae per plant in the treated district versus the distant control district. Bars are standard errors of the means; where none are present the counts are unreplicated data from consultants. Arrows represent Magnet applications.

2003-2004 Field Trial

In comparison with the 2003-03 season, moth numbers and egg pressure were higher in 2003-04 season, and the dominant species was *H. punctigera* rather than *H. armigera*. Our trial was conducted on the same property as the previous one. In this trial we estimated to have killed over 85,000 *Helicoverpa* moths in the 6 days after the first spray, of which about 75% were *H.*

punctigera. The closer spacing treatment (1/36) killed 70-80% more moths than normal (1/72) spacing. This suggests that there was not much competition for moths between treated rows at the higher spacing, that is, the numbers of moths available to be killed each night were not limiting. Over 20,000 moths were estimated to have been killed by the subsequent spray on the treated field and on the grower's treated field.

Flush counts (Table 1) indicated that there were only about 100 moths/ha before Magnet® was applied. Numbers fell to 20.8/ha on the first day after spraying, but rose to 45.8/ha on the second and 158.3/ha on the fourth day. In the control field, similar numbers of moths were present on the day of spraying, but numbers were significantly higher (225/ha) on the first day, and 158.3/ha on the 4th day, before dropping back to numbers on the 5th day which were not significantly different from the treated field. These estimates of the spot density of moths were quite low compared with the estimates of the numbers killed in the treated field on these days, which averaged about 400/ha per night over the first 5 nights. This suggests that there must have been substantial immigration to the treated field each night, a conclusion which is supported by the increased numbers of moths killed at the denser row spacing compared to the wider spacing. If the treatment was progressively killing off a population which was resident within the field, it would be expected that there would be a high flush count at the start that would progressively decline over the life of the Magnet® application. Also, as the population fell, there would be competition between treated rows for the remaining moths, so the numbers killed per hectare would grow closer in the two spacings.

Days after spraying	Treated field	Control field
0	100.0 ± 8.5 (36)	112.5 ± 18.8 (8)
1	20.8 ± 6.0 (16)	225.0 ± 20.9 (4)
2	45.8 ± 10.5 (16)	158.3 ± 27.3 (8)
4	158.3 ± 43.7 (4)	
5	100.0 ± 36.0 (4)	58.3 ± 16.0 (4)
7	161.8 ± 23.4 (14)	

Table 1. Estimates (mean ± s.e.) of moth population density (moths/ha) from 300 m flush counts at various times before and after spraying on control and treated fields. The number of 300m transects is given in brackets.

There were no significant differences in flush counts within the treated field, in relation to the spacing of treated rows, on any day. Again, this suggests that there was extensive mobility within the field as well as between fields, so that any depletion of moths in the denser spacing relative to the wider one was quickly negated.

Comparison of the dead moth counts and flush counts of the treated and control fields suggest that this experiment was started early in the course of a large influx of *H. punctigera* moths, whether from immigration or local emergence. Moth activity at night (eg in car headlights and around house lights) was high, as were pheromone trap catches in the early stages of the experiment.

To determine the area-wide impact of the attractant on oviposition, we investigated egg counts from all the fields in the district for which consultants' data were available. For the purposes of analysis the data were grouped into (1) treated field, (2) fields within 3 km of the treated field, (3) fields between 3 and 6 km of the treated field, (4) fields more than 6 km from the treated field. Estimates of the egg density at intervals of 2 days were calculated as percentage infestation (eggs/plant x 100) at each check interval; if fields were not checked within a given interval it was assumed that there had been no change from the previous check

To illustrate the area-wide effects more clearly, mean egg densities were calculated for the four distance categories as a proportion of the starting density (calculated from the mean of the three check intervals before the first spray). Results are shown in Fig. 4. Egg numbers in the treated field fell to almost zero after 2 weeks from the first spray, and then rose to about half their previous levels as the effect of the attractants wore off. There was also a difference in the trends among fields close to the treated fields, where egg numbers were almost halved during the period when the sprays were applied, while in fields remote from the treated area they were roughly doubled. Examination of the detailed data shows that this is not due to any directional effects, such as a localized outbreak in one quadrant of the region. Rather, it appeared that there was a strong central sink for moths operating in the Magnet®-treated inner part of the region.

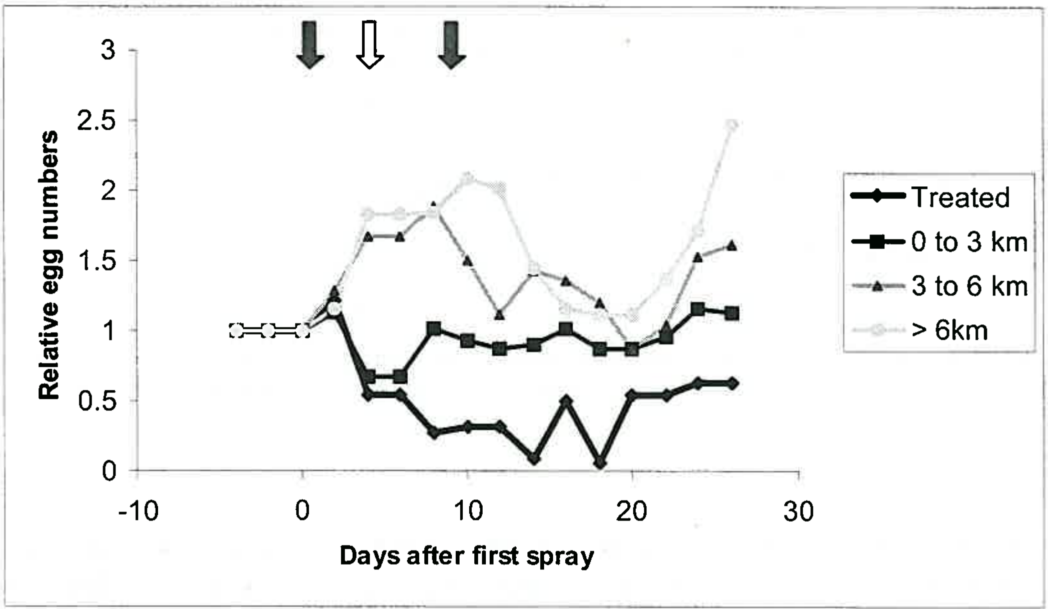

Figure 4. Mean egg densities for the treated field and three distance categories (0-3km, 3-6km and > 6km from the treated field), expressed as a proportion of the starting density. Solid arrows indicate Magnet applications to the treated field; open arrow indicates the grower application to a neighbouring field.

It is clear from the comparison shown in Fig. 4 that the Magnet® sprays had substantial effects on egg lays over a wide area. The degree of suppression was greatest closest to the applications. However, fields within 3 km of the treated area showed no rise in egg density at times when there were substantial rises in fields further away, in all directions. This suggests that the influence of Magnet® might extend some kilometers from a treated field. There are even indications in the data that effects were felt out to the edges of the sample region, up to 10 km away. There were temporary drops in the numbers of eggs laid at 3-6 km and over 6 km away, about 8 and 12 days after the first spray. The relative timing and magnitude of these drops are what might be expected if the Magnet® treated areas were acting as a central sink for moths from the whole region.

In both trials, there were substantial reductions in egg density in the treated and the adjacent control fields during and shortly after the time when Magnet® was active. The reduction in egg pressure extended up to several kms away from the treated region. It is likely that these area-wide effects are not due to attraction by Magnet® over a long range, but because moths are extensively mobile within and between fields each night and eventually discover a Magnet® treated area where they are retained and eventually killed. In the 2003-04 trial, the dominant species of *Helicoverpa* killed was *H. punctigera*, which is generally believed to be more mobile than *H. armigera*. It is likely that in this season there were large "waves" of *H. punctigera* moths coming into the fields in our study area, from surrounding non-crop vegetation or perhaps from other regions. The ability of Magnet® to produce measurable reductions in egg density in this situation is very encouraging.

The fact that the impact of Magnet® is not confined to the field it is applied in raises certain difficulties in assessing its effects, compared to conventional insecticides targeted against larvae. It is not appropriate to compare Magnet® treated areas with untreated ones within a field, or to compare a treated field with an adjacent control field. Replication must be on spatial scales of some kilometres. As with all area-wide management techniques, this makes evaluation difficult, and suggests that repeated large-scale unreplicated experiments, rather than smaller-scale replicated experiments, may be more suitable.

Conclusions

The area-wide impacts of Magnet® appear remarkable, given the relatively small areas treated. It suggests that such attractants would be very useful as a component of area-wide management. Treatment of larger areas might give substantial reductions in egg lays over an entire district. Such reductions in egg pressure, even if they did not result in sub-threshold levels and directly save conventional insecticide applications, would improve predator-prey ratios and reduce the incentive for growers to use "hard" insecticides. This would give other components of AWM, such as biopesticides, beneficial insects and trap cropping, more chance to work. For these reasons we believe that the most useful role of Magnet® and similar products which may be developed in the future will be as a tool in area-wide management, to manipulate *Helicoverpa* populations in a region rather than within a field.

Acknowledgments

We thank the following for their support and assistance in our field trials: Richard Tennant, Dave Armstrong, Stuart Armitage, Dimity Armitage, Steve Hanlon, Graham Boulton, Stuart Mason, Matthew Holding, Gary Chesterfield and Anthony Hawes.

References

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

Del Socorro, AP, Gregg, PC, Tennant, R, Alter, D and Moore, C. 2002. Plant volatiles as attractants for *Helicoverpa* moths. Eleventh Australian Cotton Research Conference, Brisbane, 13-15 August.

Gregg, PC, Del Socorro, AP, Henderson, GS, Forrester, NW and Moore, C. 1998. Plant-based attractants for adult *Helicoverpa* spp. In MP Zalucki, RAI Drew and GG White (eds) *Pest management – future challenges, Vol 1*. University of Queensland, Brisbane. pp.342-348.