

Attract-and-kill heliothis for low pressure every season

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The past few seasons have had fairly low pressure from *Helicoverpa* spp (heliothis), even on conventional cotton. Many people are becoming convinced that area-wide management (AWM) is working, but the skeptics still want to see how AWM will perform in a high-pressure season.

But what if we could make every season a low-pressure one? What if we had a tool that would reduce egg pressure by taking adult female moths out of the population, on an area-wide level?

Recent research by our group in the Australian Cotton CRC, based at the University of New England, suggests that we may have such a tool in the very near future — attract-and-kill for female *Helicoverpa* moths, using plant volatile chemicals.

Plants emit volatile compounds that attract insects for foraging and oviposition (egg laying). Insects perceive these compounds primarily by olfaction (smell). Overseas research on such compounds has been done in the US, UK, Germany, India and China.

For example, US scientists developed an attractant for the corn earworm, *Helicoverpa zea*, based on the volatiles emitted by night-flowering plants which are native to the southwestern US. We embarked on similar work for Australia's major cotton pest, *H. armigera*, about five years ago, through the Australian Cotton CRC. Our aim was to develop an attractant for the adults — especially female moths.

Attract-and-kill system

Plant-based attractants can be used in an attract-and-kill system in combination with a small amount of toxicant. Attract-and-kill systems using sex pheromones for the pink bollworm and some orchard pests have been developed in Europe and North America. Moths are lured to the attractant and, upon contact or ingestion, are killed by the toxicant.

With this system, blanket coverage of the crop is not necessary, and so the amount of insecticide can be significantly reduced.

But when pheromones are used as the attractant, only males are killed. For a species like *Helicoverpa*, which can mate several times, it is necessary to kill a very high proportion of the males before any effect on egg laying is observed. Attractants for females would avoid this difficulty — every female killed would mean fewer eggs laid.

Another potential use of attractants is in an attract-and-disseminate system, where instead of a toxicant, a specific pathogen is used. Once the moths are contaminated with the pathogen, they are released and spread the pathogen. We can also envisage applications for attractants in conjunction with trap crops, repellants or anti-feedants to manipulate *Helicoverpa* populations. But attract-and-kill is our initial approach — and if we can get that to work, other applications will follow.

We decided to research attractants for *Helicoverpa armigera* from scratch, rather than adapting the US results. Our approach involved screening about 40 plants (hosts and non-hosts for *Helicoverpa* larvae) for attractiveness to *H. armigera* moths in the laboratory, using a two-choice olfactometer.

We then analysed and identified the chemical compounds found in the attractive plants. Synthetic equivalents of the compounds that were in common between the most attractive plants were then combined into blends, which were tested in the olfactometer.

We identified a number of synthetic blends which gave levels of attraction comparable to some of

the most attractive plants. It is important to note that our blends are not mimics of particular plants, as are those from the US. We have combined the best components from a number of different plants, producing what we call a super-blend — a combination of plant volatiles not normally found in nature.

Over the past two years we have conducted field trials under closed conditions to evaluate the effects of these blends. Field wind tunnels were used to test the blends in semi-natural conditions, using captive female moths.

To see if we could kill wild moths, we initiated small-scale open field trials on sweet corn and French beans in Bowen, Queensland in April last year. The 2002–03 season is the first in which we have treated whole fields of cotton with our attractant blends and attempted to assess the impact in terms of the numbers of moths killed in the field, and the effects on egg oviposition. The rest of this article will discuss the results of these trials.

Trial sites

Our trials have been on the Darling Downs, as this area consistently produces large numbers of *H. armigera* moths. Trials on conventional cotton were conducted in two properties near Cecil Plains ('Wamara' and 'Yanco Farms') and one at Oakey ('Glen Shee').

The 'Wamara' field site was about 42 hectares, fully irrigated, and was sprayed with our attractants three times. The 'Yanco Farms' site was about 51 hectares, irrigated only once, and sprayed three times. The 'Glen Shee' site was about 20 hectares, fully irrigated and sprayed once.

All sites were managed using conventional chemicals as well as our attractants, and management decisions were left in the hands of growers and consultants. The most extensive trial was on 'Wamara' and discussion in this article will focus on the methods we used and the results we obtained for this site.

Formulations, application and monitoring

We sprayed every 72nd row at 'Wamara' (about 1.5 per cent of the field) on November, 30, December 15 and January 6.

The attractant was formulated in canola oil in combination with various emulsifiers, thickeners, anti-oxidants and feeding stimulants. Blue food colouring was also added to mark moths which had ingested the material. Application was done using a low pressure 12 volt pump through a nozzle designed for applying liquid fertiliser. A motor cycle fitted with a third wheel, allowing operation in the row crops, was used .

With an operating speed of 20 km/h and treating only every 72nd row, it took less than an hour to treat a 40–50 hectare field. The material was present on leaves as coarse splashes rather than a fine spray — coverage is not important for an attractant.

In our trials, 0.5 per cent methomyl was used as the toxicant to ensure that moths that contact the attractant are quickly killed. We need quick kills so we can find the dead moths near the sprayed row to assess the impact of the spray.

But we don't envisage using methomyl in future commercial applications because of its toxicity to applicators and to beneficial insects. We will be testing other types of insecticides that would better fit with current insecticide resistance management recommendations.

On each morning for three to four days after each treatment, dead moths were collected from 50 metre sections around each sprayed row. These sections were staggered across the whole field in a "Z" pattern.

In each 50 metre section, the furrows one, three, 10 and 35 metres (rows) from the treated row, in both directions, were searched. The *Helicoverpa* moths found were dissected to determine the species, sex, mated status of females, and whether they contained the blue dye.

We then used the numbers of moths from these furrows to estimate the total number we killed in the whole field for each attractant spray (Figure

1 and Table 1). But we may have substantially underestimated the kills, particularly later in the season. It is often difficult to find all the dead moths because of factors such as size of the cotton plants, cracks in the soil and the presence of predators such as ants which might remove the corpses before we find them.

We are also using these trials to assess the impact of attractant sprays on other pests, and on beneficial insects. We collected samples from five locations across the field using rows well away from the sprayed ones. Similar sampling was also done in an adjacent control field of about 42 hectares, located about 500 metres from the treated field.

As well as this local control, we compared five surrounding fields, totalling about 200 hectares, within one km of the treated field with a similar group of five fields located six to eight km away. So we had a local control to check for effects between fields, and a district control to check for larger, area-wide effects. For the latter, bug-checking data were provided by local consultants Graeme Boulton and Stewart Mason of Black Earth Cotton Co.

Moth kills and egg lays

The highest numbers of moths killed were found in the furrows immediately adjacent to the treated rows (Figure 1). But significant numbers were also found 35 rows away — almost as many as there were at three and 10 rows away. This indicates that while many moths are killed very quickly, some moved considerable distances after contacting the attractant.

Counting only the rows adjacent to the sprayed one is likely to substantially underestimate the kill. In the case of the first spray at 'Wamara', we estimated that about 226 *H. armigera* moths were killed per 50 metres of treated row. Since there were 126 such 50 metre sections, the total kill was estimated at 28,500 moths.

Though the highest numbers of dead moths were found in the first two days, substantial numbers were still being killed after four days. In previous small-scale experiments, our formulations

remained active for four to six days, depending on the weather. Most dead moths contained blue dye, indicating that they were killed by our attractants, rather than by other chemicals or natural mortality.

In addition to the 28,500 *H. armigera*, we also estimated about 3,500 *H. punctigera* moths were killed in the 42 hectare field. Of the *H. armigera* killed, about 54 per cent were females. We estimated a theoretical total of about 13.2 million eggs not laid by *Helicoverpa* moths as a result of our spray (eggs that probably would have been laid if we had not killed the female moths).

If they had been spread evenly across the treated field, this would have amounted to three to four eggs per plant. Neither the treated nor the control field had egg lays of this magnitude — either before or after the attractant spray. Combined with evidence from flush counts (estimates of the moth population made by flushing them from the plants), this suggests that our attractant was killing moths that were not resident in the treated field.

For subsequent sprays at 'Wamara', and the sprays at other sites, the estimates of moths killed and eggs not laid due to the attractants are given in Table 1. Later sprays did not kill as many moths as the first spray. Obviously, the number of moths we can kill will depend on the numbers which are present.

In the second spray at 'Wamara', for example, the numbers of *H. armigera* killed were very much lower than for the first spray, while the numbers of *H. punctigera* were similar. There were probably fewer *H. armigera* moths present, perhaps because of the effects of the first spray, and perhaps because of natural decline.

In contrast, there were probably similar numbers of *H. punctigera* moths present, because their numbers had been replenished by movement in from nearby fields. Timing of attractant sprays, in relation to the local ecology of *Helicoverpa* species, is likely to be a critical factor in their effectiveness.

Local (field) impact

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 the attractant wore off after about two weeks, the treated field had higher *Helicoverpa* numbers 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.

The comparisons of these measured differences and the theoretical estimates for each spray are given in Table 2.

District impact

We attempted to estimate the possible district impact by comparing the consultants' data on total *Helicoverpa* numbers per plant in five conventional fields for the treated region (on 'Wamara' and an adjacent farm) with the control region (five similar fields owned by two farmers, six to eight km away). Though the two areas started out at the same pest levels, the treated region showed similar pattern of declines following each attractant treatment (Figure 3).

Following the third spray, numbers initially declined in the treated field compared to the control, but then rose after the effects of the spray would have worn off. But at the district level, there was a marked and progressive decrease in pressure in the treated area compared to the control.

Impact – theoretical and measured

Table 2 shows the numbers of eggs which should, in theory, not have been laid because of the three sprays at 'Wamara'. Also shown are the measured differences between treated and control areas, at the local and district level.

For the first spray, there is good agreement between the theoretical and measured numbers.

It is apparent that most of the difference is at the district level. That is, the attractant is protecting not only the sprayed field but also adjacent fields. We do not believe this is because moths are attracted from a long distance. Rather, we think that in the course of their local exploratory movements, they fly into the treated field where they are retained (and eventually killed) by the attractant.

The same pattern occurred with the second spray, although both the theoretical and measured impacts were lower than for the first. For this spray, the measured difference was somewhat greater than the predicted one, and this may be because we substantially underestimated the numbers of moths killed.

The *Helicoverpa* pressure following the third spray showed a very different pattern. Though there was a small increase in numbers at the local field level, there was a marked and sustained reduction in *Helicoverpa* numbers in the treated area, at the district level.

This may have been due to the direct effects of the third spray plus carry-over effects from reducing the previous generation with the first two sprays. But we cannot exclude the possibility that there might have been some other difference between the two areas — such as a source of immigrant moths to the control area but not the treated area.

Number of sprays

At the local level, there was little difference in the number of sprays. The treated field received eight sprays and the control field received seven.

But at the district level, there was a marked difference. The five fields in the control area received an average of 9.8 sprays, of which 8.4 were directed primarily against *Helicoverpa*.

The five fields of the treated area received an average of 6.8 sprays, of which 6.0 were directed mostly against *Helicoverpa*. This is a

reduction of around 30 per cent. Most of this difference arose late in the season, after our third attractant spray.

Again, we need to be cautious about ascribing the difference solely to our attractants. We will need to show the effect is repeatable. But the results are most encouraging. Both the pattern of effects at the local level and the overall difference between the districts suggest that it may be possible to obtain regional impacts from our attractants.

This would be very useful as a component of area-wide management. Reductions in egg pressure would give other components of AWM — such as beneficial insects, soft insecticides (including biologicals) and trap cropping — more chance to work. When calculated over the whole treatment district (about 200 hectares), we sprayed only about 0.3 per cent of the host crop area. If we have achieved impacts on this scale with such a low percentage of the area treated, what might have happened if the whole area had been sprayed?

Effects on beneficials

A question we have frequently been asked is whether our materials attract beneficial insects. If they do, we will have to choose a selective insecticide to avoid damaging beneficial populations. We have done a small-scale experiment at Goondiwindi with the cooperation of Iain Macpherson, using attractants without insecticide added.

The results suggest our material had some weak attraction to ladybirds, but none to lacewings, wasps, spiders and some other beneficial groups. We are currently analysing data on beneficials from the 'Wamara' experiments, and so far we have no obvious effects on most species.

We believe our attractants will be quite compatible with the natural enemy component of area-wide management. But there is one group which might be at risk from moth attractants — ants. We frequently observed ants dismembering dead moths and carrying pieces of them back to their nests.

At 'Wamara' there were significantly fewer ants in the treated field compared to the control, though we are not yet certain whether this is due to our attractants or some other difference between the fields, such as prior cropping history. In any case, it is obvious that the insecticides we choose for use with the attractants should be ant-friendly.

Where to from here?

The results of these first large-scale trials were encouraging. Our attractants can regularly kill significant numbers of both *Helicoverpa* species and other noctuid pests (among the other species we recorded were cutworms, armyworms, loopers and rough bollworms).

The numbers killed are likely to reflect the local moth population, so we need to determine how best to time applications. The detection of both local and district impacts on *Helicoverpa* pressure suggests that if larger areas were treated, perhaps starting earlier in the season, we might be able to substantially reduce egg pressure over a wide area and for a long period.

As with research on any technique in area-wide management, it is difficult to provide adequate replication for field trials when we are considering mobile insects on large areas. We need to repeat these trials on a larger scale and over several seasons before we fully understand the impact that attract-and-kill techniques might have, and how best to incorporate them in area-wide management.

We will be applying to the Australian Pesticides and Veterinary Medicines Authority (previously the NRA) for permits to conduct such expanded trials next season. We are also patenting our attractant blends, and negotiating with potential commercial partners to register, develop and market them.

We are aware of at least one commercial company conducting research on similar attractant blends, so it is likely that growers will soon have a range of such products to choose from. We anticipate that such products will be

realistically priced. Commercial-scale trials are expected as early as next season.

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