Australian Cotton Cooperative Research Centre

November, May & Final Reports

Part 1 - Summary Details

REPORTS

Please use your TAB key to complete part 1 & 2.

COTTON CRC Project Number: **2.2.3**

November Report:	☐	Due 14-November-03
May Report:	☐	Due 29-May-03
Final Report:	x	Due within 3 months of project completion

Project Title: Semiochemical approaches to control *Helicoverpa* spp.

Project Commencement Date: 1 July 2000 **Project Completion Date:** 30 June 2003

Research Program: 2. Innovative Technology

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Part 6 – Final Report Format

The points below are to be used as a guideline when completing your final report.

1. Outline the background to the project.

In recent years, work has been undertaken in the USA, Germany, UK, China and India towards developing attractants for adult moths based on plant volatiles. We initiated similar work in Australia in 1997 through the former CRC for Sustainable Cotton Production (Project 2.2.14 - "Attractants for adult *Helicoverpa* spp."). This work continued with Project 2.2.3 ("Semiochemical approaches to control of *Helicoverpa* spp.") in the Australian Cotton CRC in 2000, and Project 2.2.9 ("Plant-based attractants for *Helicoverpa* moths and sucking pests of cotton"), beginning in 2003.

Plant-based attractants combined with feeding stimulants and insecticides could be used in attract-and–kill strategies to reduce oviposition on an area-wide basis. The reduced levels of insecticides needed, and the ability to selectively place them in the field and to avoid drift, would offer significant environmental advantages over current insecticide control methods. Attractants which catch both male and female moths have advantages over sex pheromones which catch only males. Another potential use for an adult attractant would be to assist the dissemination of selective pathogens of *Helicoverpa* spp. In this role, moths would be lured to a trap, contaminated with the pathogen, and then released. Attractants could also be used in conjunction with trap crops, by encouraging *Helicoverpa* females to an area of trap crop where they remain and oviposit. The trap crop is then destroyed. Trap cropping is becoming widely used in the cotton industry, with the most common trap crops being chickpeas in spring, and pigeon peas in summer and autumn.

2. List the project objectives and the extent to which these have been achieved.

Aims:

(1) To develop improved attractants based on plant volatile chemicals, for adult *Helicoverpa* spp., especially females.
(2) To conduct large-scale field trials of attract-and-kill techniques using female attractants

Milestones:

Year 1
(1) Develop improved female attractants from lab and field trials
(2) Characterise moth responses around lures using night vision and wind tunnel studies
(3) Determine best formulations to use in large-scale field trials, including insecticides
(4) Conduct negotiations with commercial companies, file patent claim(s) if warranted

Year 2
(1) Continue to improve female attractants
(2) Assess impact of attract-and-kill in large-scale field trial
(3) Modify attractant formulations according to field experience

Year 3

(1) Continue to improve female attractants
(2) Repeat large-scale field trial using modified formulations
(3) Conclude commercial arrangements if warranted

We achieved all the above objectives/milestones of the project. We continued improving our attractants by combining floral volatiles with leaf volatiles in our blends and tested them in the laboratory using the olfactometer, and in the field. We designed a disposable field wind tunnel to observe the behaviour of moths around plant volatile lures using night-vision glasses, and to assess the potential for attract-and-kill methods in semi-natural conditions using captive moths. Prior to the large-scale field trials, we conducted small-scale field trials (50m sections) on maize and beans to see if our attract-and-kill formulations would kill wild moths. Large-scale field trials in cotton using two of our best attract-and-kill lures were conducted in the Darling Downs in the 2002-2003 season, and in Kununurra in April 2003.

A provisional patent application entitled 'Attractants for moths" (Patent No. PR4797) was lodged with the Australian Patent Office in May 2001. The International Patent was published in August 2003. A license agreement with AgBiotech Pty Ltd, to commercialise the attract-and-kill technology, was finalised in August 2003.

3. How has your research addressed the Corporations three outputs: Sustainability, profitability and international competitiveness, and/or people and community?

Sustainability

Insect pests remain one of the greatest threats to the sustainability of cotton production in Australia. The results from this project provide the basis for a new tool in IPM of the key pests, *Helicoverpa* spp., and possibly other important pests such as green mirids. Attractants should complement other techniques in IPM, including the use of biopesticides, soft chemicals, and manipulating the effects of natural enemies of pests.

Profitability and international competitiveness

Modern selective insecticides in cotton are quite expensive ($50-70/ha). By contrast, it should be possible to commercialise attractants for about $20/ha, largely because only a small fraction of the area is treated. No other cotton-producing country has yet commercialised products of this nature. Our world leadership in this field will also provide significant opportunities for support industries associated with cotton, especially small-medium enterprises which will commercialise our technology. There will also be significant royalties which will flow back into further research on cotton pests.

People and community

Attractants are designed to be applied in small quantities, from the ground, and are applied as a very coarse spray which does not drift. There should therefore be few environmental problems associated with their use, and this will improve relationships between the industry and the general public.

4. Detail the methodology and justify the methodology used.

(a) Olfactometer experiments

In Project 2.2.14, we developed the protocol for laboratory bioassays of blends of volatile compounds using a two-choice olfactometer, based on the design of Beerwinkle *et al* (1996). We used it to assess the attractiveness of a wide range of host and non-host plants, and to test the attractiveness of about 30 volatile chemicals produced by these plants. In the current project, we investigated the effects of systematically adding complexity to chemical blends, either within the groups of leaf volatiles or floral volatiles, or by combining the two.

As a result of our collaboration with the researchers at the Norwegian University of Science & Technology we met at the International Society of Chemical Ecology (ISCE) Conference in Hamburg, Germany in 2002, we identified a new compound (Germacrene D) that might considerably improve our blends. We were able to source this compound although not in pure form.

(b) Field trapping

Two designs of traps were used – the AgriSense® pheromone traps of the canister type (Gregg and Wilson 1991), and the Zap Trap, which we designed at UNE. In this design, the lure is held inside an electric grid powered by a 12-volt gel-type battery. When the moth gets close to the grid, it is zapped but not killed, then falls down in a bowl with soapy water.

(c) Feeding experiments

Feeding experiments under laboratory and field conditions were conducted to determine if moths would ingest a formulation containing the volatiles, a feeding stimulant and a toxicant, and to see if ingestion of the formulation would kill moths. Two types of formulation were compared– aqueous (oil/water mixture) and 'sloppy' Sirene® (diluted with vegetable oil). A histological dye, Brilliant Green, was added so that moths could later be dissected to determine if they had ingested the material. Finely sieved icing sugar was added at 10%w/w as the feeding stimulant to the 'sloppy' Sirene. Sirene® is a polymeric matrix containing UV-protectants, antioxidants used in attract-and-kill using pheromone for orchard pests in America, and marketed by IPM Technologies in the USA).

Two types of toxicants to be used with the attractant were compared – carbaryl and methomyl, at rates of 0.5-1%. In some experiments laboratory-reared moths were used in a constant 25^{o}C reverse-cycle system. In others natural cycle laboratory moths were used in containers placed outdoors. Moths were observed at regular intervals and dead ones removed for dissection. At the end of the scotophase all surviving moths were removed and dissected. On dissection under a binocular microscope, the presence of green dye in the digestive tract indicates that moths have ingested the material.

(d) Field wind tunnel experiments

We designed an innovative disposable field wind tunnel to observe the behaviour of moths around plant volatile lures, and to assess the potential for attract-and-kill methods in semi-natural conditions. We also used the wind tunnel to compare the two types of formulation (aqueous and sloppy Sirene) and the two types of toxicants (carbaryl and methomyl). Field wind tunnels were also used to compare and to test whether attractiveness is increased by the presence of visual (artificial flowers) and gustatory (molasses) stimuli.

Clear polythene was stretched around a series of steel hoops 1.2 m in diameter which were suspended from a horizontal pipe. Flywire was placed over each end. The wind tunnels were just above ground level and oriented parallel to the prevailing wind. Lures were placed in the upwind end and 20-50 moths were released into the downwind end about 1 h before sunset. Behavioural responses were observed using night vision glasses. In the morning both live and dead moths were collected and dissected to determine from the presence of green dye whether they had fed on the lure.

(e) *Small-scale field trials*

We obtained a research permit from the NRA (now APVMA) in October 2002. Thus sprayed plants in earlier trials were destroyed after the trials, in accordance with NRA's generic small-scale trial requirements.

To see if our attract-and-kill formulations would kill wild moths, we conducted small-scale field trials on maize and French beans in April 2002, and on cotton during the 2002-2003 season in the Darling Downs. The formulations were based on canola oil with the addition of sugar as the feeding stimulant, thickeners, emulsifiers, and antioxidants. Blue food dye was also added as a marker that moths have ingested the formulation. All blends contained 0.5% methomyl. The trials involved comparing different attract-and-kill blends with no-volatile (toxicant only) and control (unsprayed) treatments in 50m-sections divided by buffer zones between treatments. The crops were sprayed at sunset. Dead moths were collected at rows 1, 2, 3, 4, and 5 on either side of the treated rows for up to 6 days after application. All moths were dissected to determine sex, mating status and presence of blue dye.

(f) Field trial to determine effects of attractants on beneficials

We conducted an experiment on post-flowering faba beans at "Carbucky", Goondiwindi in October 2002 to determine if PBELo (one of our attractant blends) affects beneficial and other insects. We sprayed 50 m row sections with PBELo at 500ml per 50m, with no insecticide present but including sucrose. Unsprayed rows at 5m, 20m and 50m from the sprayed row were sampled for comparison. There were four replicates of each treatment. PBELo was applied just before sunset, and rows were sampled the next morning at 0800h (14h post spray) and at the same time the following day (38h post-spray), and 2 days later (86h post-spray), using a large back-pack style D-vac.

(g) *Large-scale field trials to assess impact of attract-and-kill on Helicoverpa oviposition*

Darling Downs, Queensland

Three large-scale trials were conducted in 2002/03 on two properties near Cecil Plains – 'Wamara' and 'Yanco' – and one at Oakey – 'Glen Shee'.

In all these trials, 0.5% methomyl was used as the toxicant to ensure quick kill of moths that came in contact with the attractant. The attractant was applied using a low pressure 12-volt pump, through a nozzle designed for applying liquid fertilizers. A three-wheeled motorcycle was used to allow operation in row crops, with a speed of about 20k/h. Dead moths were collected for 3-4 days after spraying, from rows 1, 3, 10 and 35 on either side of the sprayed row. All dead moths were dissected to determine sex, mating status and presence of blue dye.

Standard bug checks were conducted using visual methods for *Helicoverpa* eggs and larvae, and other insects, and suction sampling using the large D-vac. For each of 5 locations in the field location, a visual sample of 15 plants in three sets of five, two one-meter beat sheet samples and a 50m D-vac sample were taken. Similar sampling was also done in an adjacent control field of about 42 ha, located about 500m from the treated field. We also obtained consultants' bug-checking data for the treated fields and designated adjacent control fields for the whole season. These data were obtained from samples which doubled the normal consultants' sampling intensity. We also obtained normal consultants' data for many other fields in the surrounding region. Thus, we had a local control to check for effects between fields, and a district control to check for larger, area-wide effects.

Kununurra, WA

In April 2003, a trial was conducted in Kununurra, WA. The aims of the trial were to test moth attractants on dry-season cotton at Kununurra, to determine whether kills of *Helicoverpa* spp. and other pest moths obtained during summer field work on the Darling Downs could be repeated in the northern system. Supplementary aims included trialing three insecticides (potential alternatives to methomyl) for inclusion with the attractants.

The main trial was conducted using the BMP block, consisting of 9 ha of Bollgard® cotton. Eight rows spaced at intervals of 56 rows apart were treated with the attractant PF_3Hs, with a total volatile concentration of 3.1%. There were four insecticide treatments, each of which was sprayed on 2 rows:

Deltamethrin (Decis Options®) at 0.2% a.i.

Fipronil (Regent®) at 0.2% a.i.
Spinosad (Success®) at 0.5% a.i.
Methomyl (Electra 225®) at 0.5% a.i.

The remaining excipient components (carriers, sugar, emulsifying agents, antioxidants and thickeners) were as previously used in our Darling Downs trials. Blue food dye was added to our standard (methomyl) mixture to detect ingestion by moths. Other coloured food dyes (yellow for deltamethrin, green for fipronil and pink for spinosad) were added to detect ingestion of those formulations.

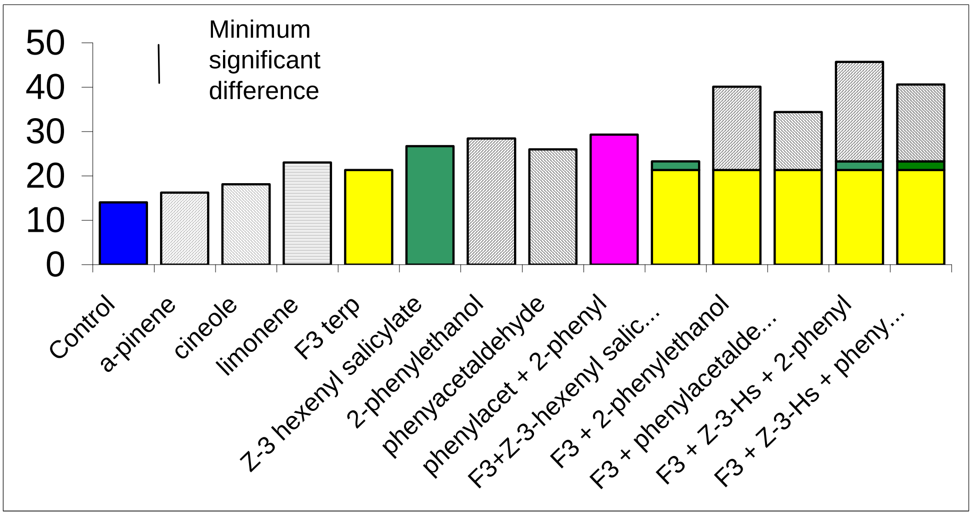
The material was applied by hand (shaken from bottles). The application rate was 500 ml per 100m. Two sections of 50 m were marked in each row (ie 4 replicates per treatment), and the furrows 1, 3, 10 and 25 meters away, on either side, from the sprayed row in each section were searched for dead moths early on subsequent mornings. All moths collected were dissected to determine their sex, mated status and whether they contained coloured dye.

5. Detail results including the statistical analysis of results.

(a) Olfactometer experiments with improved blends

We systematically investigated the effects of adding complexity to blends, either within the groups of leaf volatiles or flower volatiles, or by combining the two. (Fig. 1). The three dominant terpenoids in the most attractive plant *Angophora floribunda* had only weak activity (yellow bars). The only one which was statistically significant was limonene. When combined in the F3 blend, their attractiveness was about the same as limonene. Likewise, the two floral volatiles phenylacetaldehyde and 2-phenylethanol (purple bars) were significantly attractive, but when combined they were not more attractive than either alone. On the other hand, combination across the classes (ie floral volatiles and terpenoids) does result in significantly increasedattractiveness. The green leaf volatile Z-3-hexenyl salicylate was significantly attractive on its own, and when added to the terpenoid/floral volatile mix it slightly increased attractiveness (though the difference was not statististically significant).

We continued improving our attractants by incorporating a new compound (Germacrene D) to the two best attractant blends (PF_3Hs and PBLLo) that we have used in small-scale and large-scale field trials. Germacrene D proved highly attractive on its own. Both laboratory olfactometer experiments and field trials have shown that Germacrene D did improve the attractiveness of the modified blends although this improvement was not statistically significant. Given the cost of germacrene D, however, it is unlikely that this additional attractiveness will be of commercial value.



<u>Fig. 1.</u> Effects of adding complexity to blends in the olfactometer, either within or across the classes of floral volatiles, terpenoids and green leaf volatiles. Yellow represents terpenoids, green is green leaf volatiles and purple is floral volatiles.

(b) Field trapping

Although the blends that were tested in the field were found to be highly attractive to both sexes in the olfactometer, the numbers caught in traps in the field have been very low relative to those caught by pheromone traps in the same experiments. Typically, plant volatiles in AgriSense® traps caught only about 5-10% of pheromone catches. We designed a new trap at UNE, the Zap Trap (see section 4b). With pheromones, the Zap Trap was 5x more efficient than the AgriSense trap (O'Keefe 2001), but with plant volatiles, similar results with AgriSense traps were obtained. So, we did not further pursue field trapping with plant volatiles, and consider it unlikely that the use of plant volatiles in traps will be a commercially useful technique.

However, the trapping experiments did show that some of our blends also caught substantial numbers of other pest moth species. The catch of other moths appeared to depend on the relative abundance of these species during the trial. During spring trials in the Darling Downs, most formulations caught significant numbers of the native budworm, *Helicoverpa punctigera*, the false looper, *Chrysodeixis argentifera*, and the common armyworm *Mythimna convecta*. During summer and autumn on the Downs, large numbers of soybean loopers, *Thysanoplusia orichalcea,* were caught. Like the false looper, this species seems to respond to any blend containing phenylacetaldehyde. The cotton looper *Anomis flava* was caught in large numbers in one trapping trial at Goondiwindi.

(c) Feeding experiments

In our experimental conditions most moths ingested droplets containing attractants and insecticides. Kill rates of around 70-100% were obtained, except in one trial when the formulation did not contain an insecticide, when no mortality occurred. Formulations with 1% methomyl killed all moths; for carbaryl it was 69-94%. Ingestion of the formulation, as indicated by the presence of green dye, was always lethal. No surviving moths (carbaryl only) were found to have internal dye. On the other hand, not all dead moths had ingested the material. Most of those which had not (as indicated by the absence of internal dye) carried external dye, on the legs, proboscis or tip of the abdomen. These moths were presumed to have contacted the lure either accidentally or when attempting to feed, and been killed by the contact activity of the insecticide. In the case of carbaryl, which has relatively weak contact activity, there were only a few such moths. In the case of methomyl, a much more contact-active carbamate, all the dead moths carried external dye, sometimes only in very small traces. There were very few with internal dye, and then only in small quantities. It is likely that these moths were killed by contact before they were able to ingest much of the formulation. 1% methomyl is probably an gross overkill; in subsequent experiments, we reduced the concentration to 0.5%. All surviving moths had not ingested the material. These moths, up to about 30% of the total, were mostly young, and it is likely that they had not yet exhausted their readily available energy supplies from the pupal stage and therefore were not stimulated to feed.

(d) Field wind tunnel experiments

We conducted field wind tunnel studies in the Darling Downs. In general, moth mortality depended heavily on weather. We had 60-80% mortality on warm and moderately windy nights. Also, we had higher mortality with blends consisting of a combination of floral and leaf volatiles, confirming our olfactometer results that blend complexity is important. We killed more moths when the attractant contained 0.5% methomyl than 0.5 or 1% carbaryl. Observations with night vision glasses showed that feeding stimulants such as sugar and molasses were important to keep moths in the vicinity of the lures.

(e) Small-scale field trials

We conducted three open field trials in Bowen, Queensland, two in vegetative sweet corn and one in French beans. In all the trials, we included a previously published attractant blend (the Texas blend of Lopez *et al.* 2001) for comparison with our own blends, along with a formulation containing no attractant chemicals and an unsprayed section as controls. The formulations were based on canola oil with the addition of feeding stimulant, thickener, emulsifying agent and antioxidants. All blends contained insecticide (0.5% methomyl). Fig. 2 shows the cumulative numbers of *H. armigera* per 50m of row in the second sweet corn trial, and the French bean trial. Up to 50 moths per 50 m were killed, although the pest pressure from *Helicov erpa* spp. at the time was low. In all trials, between 50 and 84% of the *H. armigera* killed were females. Other pests killed were *Mythimna convecta* (common armyworm), *M. loreyimima* (sugarcane armyworm), *Spodoptera litura* (cluster caterpillar) and *Chrysodeixis* spp. (false loopers). The effects of the attractant formulations persisted for up to 6 days, a result which surprised us because we thought both the volatiles and the insecticide would dissipate quickly, or that the formulations would dry on the leaves and be unavailable for ingestion. While the latter problem did occur, it was apparent that high humidity and dew led to the re-hydration of droplets on successive nights.

Blends containing attractrant volatiles killed 2-4 times as many *H. armigera* as those containing sugar only, and our best blends were at least as good as the published Texas blend. We calculated that if the numbers of moths killed in these small-scale trials could be obtained when larger areas were treated, significant reductions in the numbers of eggs laid should be possible.

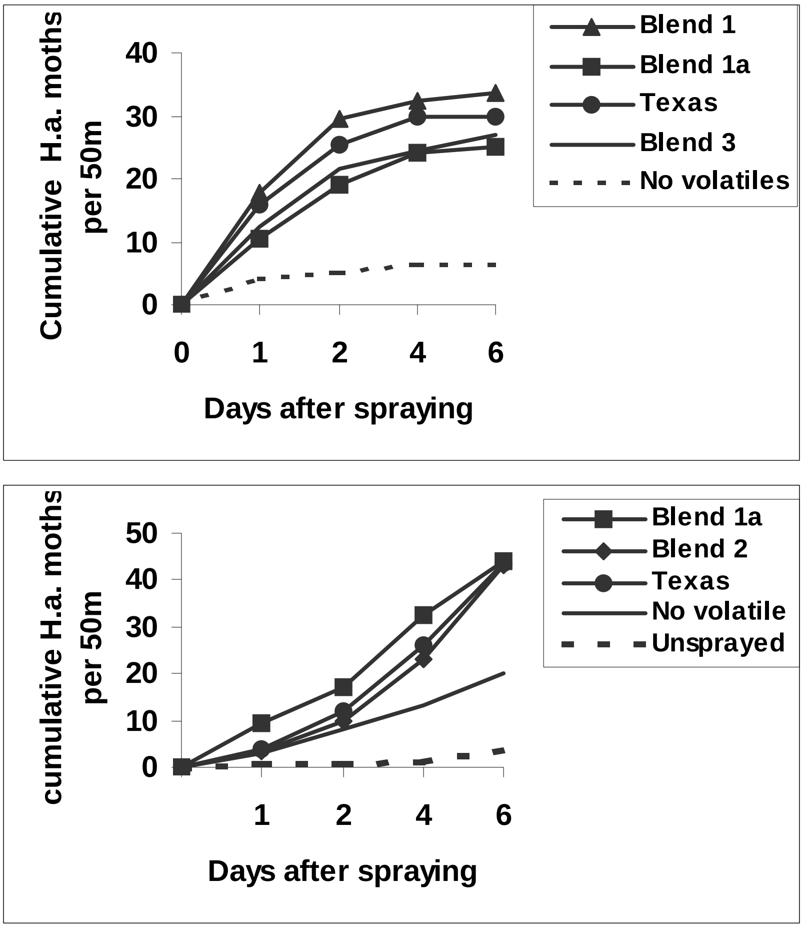


Fig. 2. Cumulative number of *H. armigera* moths killed per 50m on sweet corn (top) and French beans (bottom).

(f) Field trial to determine effects on beneficials.

This trial was conducted on "Carbucky", Goondiwindi, in October 2002. The primary aim was to determine whether one of our moth attractants, PBELo, also attracted beneficial insects. If so, it might compromise the value of attractants in IPM. A second aim was to determine attractiveness to other pests, including sucking insects. We used suction sampling on rows treated with the attractants (without insecticide) compared to other rows, so that accumulation on the treated rows indicated attractiveness.

Principle findings with respect to beneficials and other insects were:

Minute pirate bugs - The adults were not seen in the first sample at 14h, but at 38h they were present in sprayed rows at 11 times the numbers seen in unsprayed rows ($p < 0.001$). The effect disappeared at 86h. Nymphs were not seen.

Ladybirds - Hippodamia variegata was the only ladybird present in sufficient numbers to give a good indication. At 38h after spraying, there were twice as many adults in the sprayed row compared with other rows, but the effect seemed to be weakening at 86h. There were no significant differences in larval numbers, as would be expected in view of the limited mobility of larvae.

Brown lacewings - Large numbers of brown lacewings were present. There were no significant differences in the numbers of adults or larvae. Larval numbers declined with repeated sampling.

Green lacewings – Adults were rare at 14 and 38h after spraying, but at 86h there were 6.7 times as many in the sprayed rows ($p < 0.01$). Larvae were not seen.

Spiders and wasps - Many different groups of spiders and wasps were seen. There were no significant differences related to the spray.

Flies – Many different groups of flies were seen. On all sampling occasions there were about twice as many in the sprayed compared to unsprayed rows.

Results related to other pests were:

Apple dimpling bugs (Campylomma liebknechtii) - were significantly higher at all times in the sprayed rows ($P < 0.001$). They were 21 times higher in the sprayed rows at 86h. In contrast to the adults, there were no significant differences in the numbers of nymphs.

Leptocoris spp. - These are plant feeding bugs in the family Rhopalidae, minor pests specific to the Fabaceae. They were hardly seen at 14h, but at 38 and 86h, they were present in sprayed rows at 50 to 60 times the numbers of the unsprayed rows ($p < 0.001$).

Thrips - very large numbers of thrips (several species) were present. Their numbers declined with repeated sampling. There were no significant differences related to the spray.

Jassids - green jassids (probably *Austroasca* sp.) and brown jassids (probably *Orosius* sp.) were both present in large numbers. Neither were significantly affected by the spray.

Other pests (green mirids, green vegetable bugs, Rutherglen bugs) and beneficials (damsel bugs, predatory shield bugs, broken back bugs, red and blue beetles, hover flies, ants, ladybirds other than *H. variegata*) were found. None of them were significantly affected by the spray, but the numbers were too low to draw conclusions.

(g) Large-scale field trials

<u>Darling Downs, Queensland</u>

Our trials have been in the Darling Downs region, as we have found that 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 ha, fully irrigated, and was sprayed with our attractants three times, between 30 November 2002 and 6 January 2003. The "Yanco Farms" site was about 51 ha, irrigated only once, and sprayed with attractants three times between 6 January and 13 February 2003. The "Glen Shee" site was about 20 ha, fully irrigated and sprayed once on 20 January 2003. 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" near Cecil Plains, Qld. About 1.4% (one row in 72) of the field was sprayed with the PF_3Hs blend three times (30 Nov and 16 Dec 2002, 6 Jan 2003). On each treated row, 50m sections were checked for dead moths at 1, 3, 10 and 35 rows on either side. These 50-m sections were stretched along a Z-pattern across the whole field. We also used these trials to assess the impact of attractant sprays on other pests, and on beneficial insects, using our own and consultants' data as detailed under section 4g.

The highest numbers of moths killed were found in the furrows immediately adjacent to the treated rows (Fig. 3). However, significant numbers were also found at 35 rows away, and there were almost as many at this distance as there were at 3 and 10 rows away. This indicates that while many moths are killed very quickly, some can move 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 m of treated row. Since there were 126 such 50 m sections, the total kill was estimated at 28,500 moths.

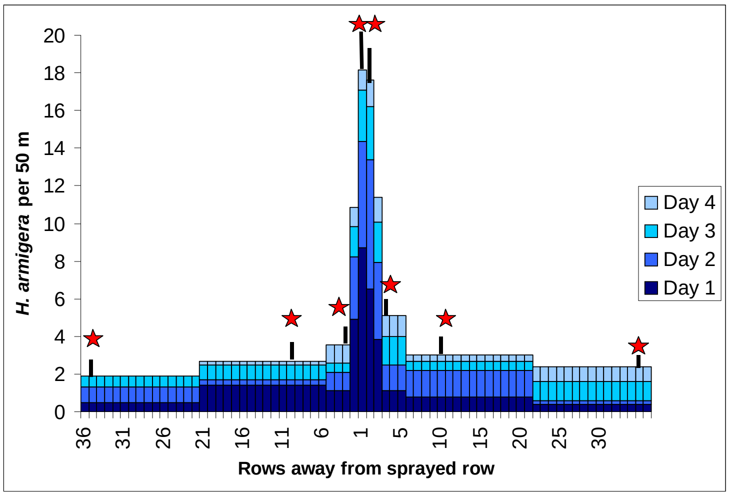


Fig. 3. Estimated numbers of moths killed after first spray. Stars indicate rows where 50m-sections checked for dead moths.

Though the highest numbers of dead moths were found in the first two days, substantial numbers were still being killed after 4 days. Most dead moths contained blue dye, indicating that they were killed by our attractants, rather than by other chemicals or natural mortality. About 54% of these moths were females. We dissected these females to determine, based on the number of spermatophores, how many times they had been mated.

In addition to the 28,500 *H. armigera*, we also estimated about 3,500 *H. punctigera* moths were killed in the 42-ha field. Of the *H. armigera* killed, about 54% were females. To calculate the numbers of "eggs not laid" (ie eggs that would probably have been laid by the females if we had not killed them with the attractant), we assumed that virgin females had not laid any of their eggs. We assumed that those mated between one and five times had already laid progressively more of their eggs, up to the time they were killed. We also assumed that the potential fecundity in *Helicoverpa* females is 1500 eggs per female. On this basis, we estimated a theoretical total of about 13.2 million eggs not laid by *Helicoverpa* moths as a result of our spray. If they had been spread evenly across the treated field, this would have amounted to 3-4 eggs per plant. We wrote two small computer programs (in QuickBasic) to facilitate the calculation of total moths killed and estimated eggs not laid.

Neither the treated nor the control field had egg lays of close to the estimate of eggs not laid, 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.

Table 1. Numbers of moths killed and estimates of eggs not laid following applications of attractants at three sites

Location	Spray date	Moths killed		Eggs not laid
		H. armigera	*H. punctigera*	(x 10^6)
Wamara	30/11/2002	28,500	3,500	13.2
Wamara	16/12/2002	1,900	4,250	2.8
Wamara	5/1/2003	14,210	4,400	5.5
Yanco Farms	5/1/2003	6,920	3,350	4.4
Yanco Farms	14/1/2003	6,300	440	2.4
Yanco Farms	13/2/2003	5,150	1,520	6.6
Glen Shee	20/1/2003	3,200	3,980	3.3

Later sprays at Wamara 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. We thought this was because there were 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.

Local (field) impact on oviposition

Fig. 4 shows the numbers of *Helicoverpa* (eggs and larvae) per plant on the treated field at "Wamara" versus the adjacent control field, as recorded by the UNE team and the consultants. 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 2 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 temporarily counter this difference.

After each spray, we estimated the reduction in egg numbers between the two fields for the 12 days following spraying of attractant. We calculated the number of egg-days represented by the difference between the two fields, and these are the shaded areas in Fig. 3, colour-coded to match the three attractant sprays. To estimate the differences between the two fields and compare them with the theoretical numbers of eggs not laid given in Table 1, we multiplied the number of egg-days by the total number of plants in the treated field (estimated at 4.2 million), then divide by 4, assuming that the average life of *Helicoverpa* eggs in the field is 4 days. The comparisons of these measured differences and the theoretical estimates for each spray are given in Table 2.

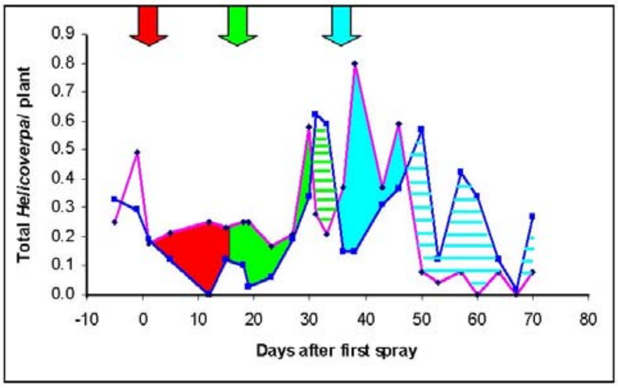


Fig 4. Numbers of *Helicoverpa* spp. eggs and larvae per plant in the treated field and control field in the "Wamara" trials. Solid shading represents fewer eggs in the treated field than the control field, lined shading indicates the reverse. Colours associate the difference in *Helicoverpa* numbers with attractant sprays indicated by the arrows.

District impact on oviposition

We attempted to estimate the possible district impact by comparing the consultants' data on total *Helicoverpa* numbers per plant in 5 conventional fields for the treated region (ie, "Wamara" and an adjacent farm) with the control region (5 similar fields owned by two farmers, 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. 5). There was the same pattern of a decline in the treated district relative to the control after the application of each spray. There was, between the second and third sprays, a late rise in the numbers in the treated region compared with the control region. However, it was delayed and smaller, which is what one might expect if the effect was due to increased attractiveness of the treated field, but was buffered by greater areas and distances between the treatment and control, in relation to the two-field comparison. 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. At the district level, however, there was a marked and progressive decrease in pressure in the treated area compared to the control.

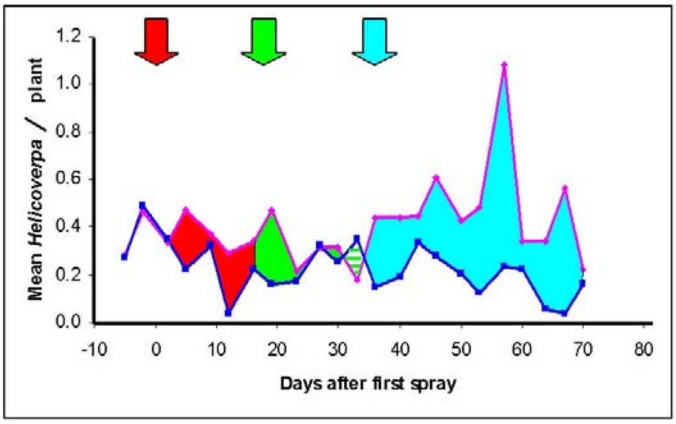


Fig. 5. Numbers of *Helicoverp*a eggs and larvae per plant in the treated district versus the distant control district. Colour coding and arrows are the same as for Fig. 3.

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. These figures have been derived from the graphs (Figs. 4 and 5), and are net figures, that is, corrected for occasions when the *Helicoverpa* numbers found in the treated areas exceeded those in the control. 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.

Table 2. Theoretical and measured reductions in *Helicoverpa* pressure at the local and district level following applications of attractants at "Wamara".

Treatment	Theoretical eggs not laid	Measured difference (control-treated) Local	District
30/11/2002	13.2	2.1	11.4
15/12/2002	2.8	0.9	5.8
6/1/2003	5.5	-1.2	52.4

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 *Helicoverp*a pressure following the third spray showed a very different pattern. Though there was a small net negative effect (ie numbers increased) at the local field level, there was a marked and sustained reduction in *Helicoverpa* numbers in the treated area, at the district level. We can only ascribe the first 10-14 days of this to the direct effects of the third spray, since this is about the lifetime of *Helicoverp*a moths plus the 4-day average life of *Helicoverpa* eggs and larvae in the field. We believe the rest of this prolonged effect may be due to carry-over effects from reducing the previous generation by the first two sprays. However, we cannot exclude the possibility that there might have been some other difference between the two areas, for example, a source of immigrant moths to the control area but not the treated area. About 30% fewer insecticide applications were needed in the treated region compared to the control, so if the effect was due to our attractants, it is a remarkable result considering that only one 42 ha field was treated.

The Wamara trial showed no significant differences between the treated field and the control in the numbers of beneficial insects, either in total or of individual species. The one exception to this was ants, which were lower in the treated field. However, we do not know if this difference was due to the attractants or to other differences between the two fields, such as prior cropping history and irrigation management.

Kununurra, WA

Moth kills

This trial involved the comparison of a number of different insecticides. Fig. 6 shows the mean numbers of *Helicoverpa* moths killed in each searched row of each insecticide treatment.

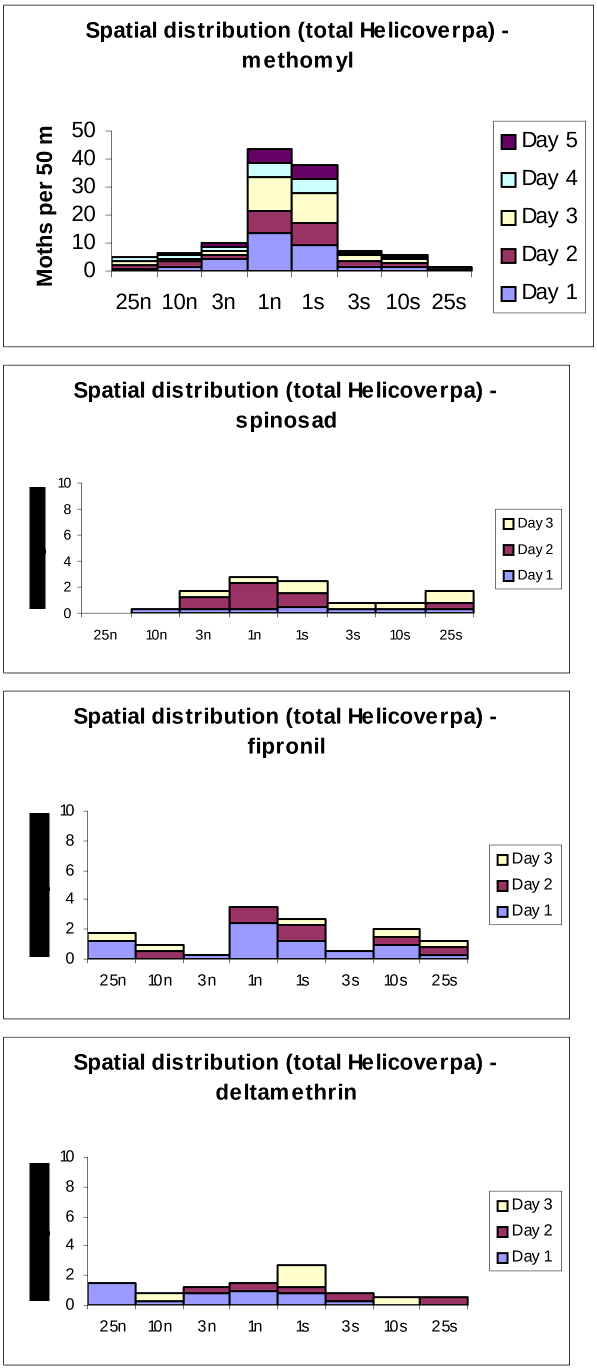


__Fig.6.__ Mean *Helicoverpa* moth kills in the four insecticide treatments, in furrows 1, 3, 10 and 25 in each direction (N and S) away from the treated row, on each day of collection. Note the scale is different for methomyl, and there are 5 collection days for this treatment compared to only three for the others.

Our standard insecticide, methomyl, performed as expected. Substantial numbers of moths were killed. The highest numbers were in the furrows adjacent to the sprayed rows, but significant numbers were found out to 25 furrows away. However, very few dead moths were found in the furrows near the rows treated with the other three insecticides, and there was little sign of the sharply peaked bell curve we typically get with methomyl.

We then ran the numbers through our QuickBasic model to estimates total kills for the whole area treated with each insecticide (about 2.3 ha), on the basis of the spatial distributions described by the graphs above. Cumulative estimates of the kill are shown for the two *Helicoverpa* species, and for other moths we collected, in Fig. 7.

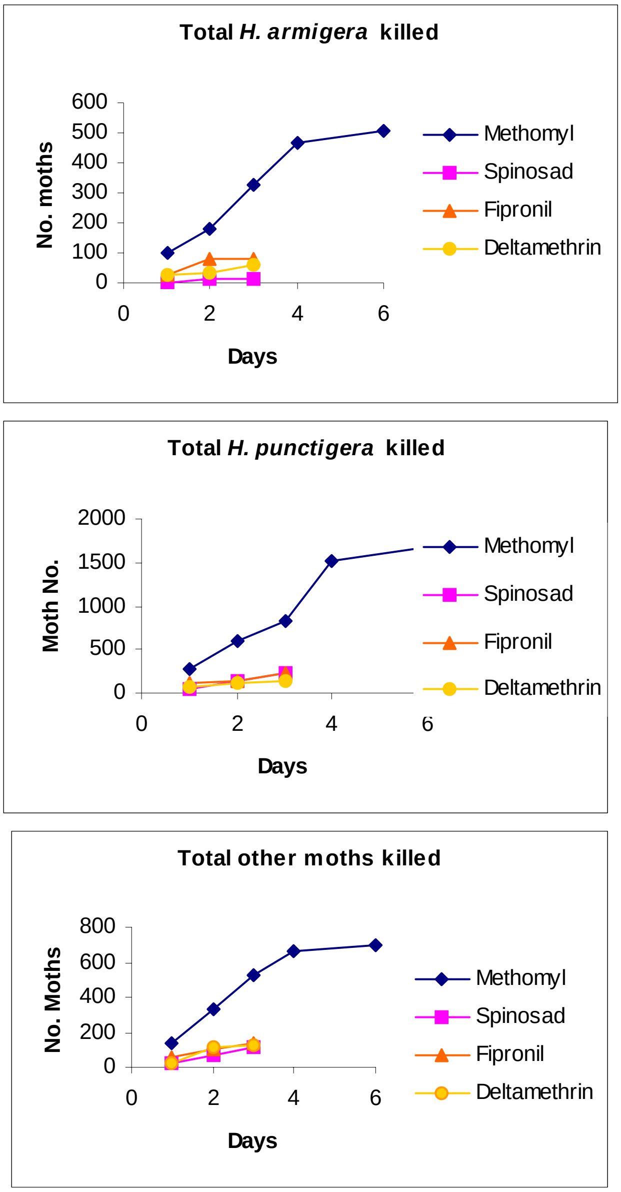


__Fig. 7.__ Estimates of *H. armigera*, *H. punctigera* and other moths killed by each insecticide treatment in the Kununurra experiment.

For methomyl, the estimates were comparable to our best result in the Darling Downs. We killed approximately 1600 *H. punctigera* and 500 *H. armigera* from the total of 400 m of row we sprayed with this formulation, plus several hundred other moths. However, for the other three insecticides, the kills were less than a quarter of those with methomyl. We gave up counting on these treatments after 3 days because it was not worth continuing to check them. Moth kills with methomyl continued to increase steadily for 4 days, and then flattened off, in a pattern similar to that we have seen previously.

Overall kills, sex ratios, mated status and estimated impact

We estimated total kills for the whole field of 2265 *H. punctigera* and 665 *H. armigera*. This estimate is conservative because (apart from the usual factors affecting the efficiency of searching) the whole field was not searched on all 6 days, only the methomyl treated areas. Also, the moths killed on the 5th night would have been lost due to the cultivation of the field.

Of the *H. punctigera*, 69% were females, and for *H. armigera* there were 63% females. Approximately 25% of the females were virgins, and another 40% had mated once only. Most of the remainder had mated twice, with very few more than this. These distributions of mating frequency were similar for both species. When we ran these figures through our model which estimates the total number of eggs which might not have been laid because of the attractants, the estimate was slightly over 2 million, which when spread over the 9 ha field amounted to an egg lay of about 2 eggs/plant. Given that the *Helicoverpa* pressure was low as judged by both pheromone trap catches and egg counts, and given that probably only 400m of row was effectively treated due to the poor performance of three of the four insecticides, this represents a substantial impact.

About 700 other moths were also killed. These were mostly pest species (not all cotton pests) including: *Spodoptera exigua*, *S. litura*, *Agrotis munda*, *Chrysodiexis* spp., *Mythimna convecta*, *M. loreyimima* and *Mocis* sp. There were also significant numbers of small pyralid moths, but these were not collected.

6. Discuss the results, and include an analysis of research outcomes compared with objectives.

The results of this project indicate that attract-and-kill using plant volatiles is a viable strategy for *Helicoverpa* spp., which might find a useful place in integrated pest management and area-wide pest management in cotton. In the laboratory olfactometer system, our best synthetic blends (section 5a , Fig. 1) were as attractive as the most attractive crop and weed plants in cotton ecosystems. This, together with the understanding we developed of the principle of super-blending and the basic rules governing the composition of successful blending of attractants, enabled us to produce effective attractants at reasonable cost.

These blends were not very effective in traps (section 5c), and our work with field wind tunnels (section 5d) indicated why – because of the importance of visual stimuli in close-range moth responses. It therefore seems unlikely that trapping using plant volatiles will contribute much to practical management of *Helicoverpa* spp. However, the development of methods of formulating plant volatiles with insecticides for direct application to plants, combined with our understanding of how moths feed on these materials (section 5c and 5e) has enabled a practical sprayable formulation to be developed, which overcomes the difficulties with inappropriate visual stimuli associated with traps.

Initial small-scale field trials (section 5e) were very encouraging. They showed that substantial numbers of moths could be killed, even under conditions of low *Helicoverpa* pressure, and that the addition of plant volatiles greatly increased the kill compared to sugar alone. They also gave an unexpected positive result – the persistence of the material was much longer than expected, and moths could be killed for several days following a single application. They also demonstrated that the attractants were effective on other species of pest moths.

Work on beneficial insects (section 5f) showed that while there was some attraction to some species of beneficials, the overall impacts were likely to be relatively small. Species and life cycle stages with limited mobility were not attracted (as expected) and some mobile species also seemed to be not attracted. We cannot claim that there are no effects on beneficials, and further work is required to determine the effects of our current blends on some beneficials. However, it is likely that attractants will be a relatively soft option in IPM, and there are opportunities to make them softer through choosing softer insecticides, or tailoring attractants to be less attractive to beneficials (section 7).

Results from the large-scale experiments (section 5g) were even more encouraging. There were no major problems in scaling up the production and application methods to commercial levels, and many thousands of moths were killed. Theoretical calculations indicated that there were enough moths killed to have a significant impact on oviposition (Table 2), and measured egg densities (Figs. 4 and 5) were roughly comparable to the theoretical estimates. It was particularly encouraging that the major impact seemed to be on the district level (ie over several km), and that there seemed to be a generational carry-over effect late in the season (Fig. 5). This effect led to a significant (30%) reduction in conventional sprays in the treated area. If these effects can be realised (and perhaps even improved) by area-wide treatment, it is clear that attractants could significantly impact on the pest status of *Helicoverpa* spp. However, some caution is needed in interpreting the results of these large-scale trials. They were unreplicated – as with all area-wide management techniques, it is almost impossible to provide adequate replication on the scale needed for such trials. This means it is always possible to think of other explanations for the reduced pest pressure in the treated area. It is only by extensive repetition of trials such as the Wamara experiment that we can be sure the effects are due to the treatment, and providing such repetition is a major focus of our current Project 2.2.9.

The Kununurra experiment confirmed the results of the Wamara experiment in that it demonstrated that large numbers of moths could be killed by attractants. It also pointed to a limitation of the technology – very few insecticides will produce the rapid kills necessary for evaluating the impact of treatments. Some insecticides which kill larval *Helicoverpa* spp., such as deltamethrin and spinosad, either do not kill moths, or do so too slowly to enable the corpses to be easily found. Further research is required to identify more insecticides, and especially ones which fit with insecticide resistance management strategies and with preservation of beneficial insects. These issues are also being addressed in our current project.

In general we believe this has been a very successful project. It has achieved all the objectives we set, and it has proceeded further towards commercialisation than we anticipated. It has been one of those rare projects in which the results have appeared more promising as we moved from laboratory to small-scale and then to large-scale trials, and finally commercialisation. We hope that, by the conclusion of our current project, attract-and-kill with plant volatiles will become firmly established as a useful component of IPM in Australian cotton – and if so, it will be a world first.

7. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. Where possible include a statement of the costs and potential benefits to the Australian cotton industry and future research needs.

Costs and benefits to the Australian cotton industry

The large-scale trials described in this report suggest that plant-based attractants can reduce the need for conventional insecticides in the cotton industry by about 30%. It might be even higher if these attractants were applied on an area-wide basis rather than on single fields as our trials have so far used. Insecticides cost cotton growers around $200 million annually (though it has been less in recent years due to lower areas in drought conditions, and to lower insect pressure). Balanced against this possible reduction in costs is the cost of the attractants, but this is much less than current insecticides – about $15 per hectare compared with $50-70 for the most recent insecticides.

Of potentially greater significance than this rough cost/benefit analysis is the fact that attractants are compatible with other components of IPM, such as biopesticides and natural enemies. By reducing oviposition pressure, they will encourage growers to use soft options more frequently, thereby conserving beneficials, reducing selection pressure for resistance, and contributing to the change in mind-set on pest management which is currently occurring in the industry.

Future research needs

This technology is very new, not only for the Australian industry but in the world. There are many research questions to be answered, and some are being addressed in the on-going Project 2.2.9. They include:

- what will be the impact of area-wide application of attract-and-kill technology, on a scale of thousands of hectares? Is it possible that, when combined with large areas of Bollgard cotton, attractants might greatly diminish *Helicoverpa* populations in entire districts?

- what are the impacts on beneficial insects? Initial results suggest that they will be minor, but this needs to be confirmed on a wider scale. There are many beneficial groups for which we have very limited data, including ants, parasitic wasps, and several important generalist predators. Can we tailor attractants that will not attract (and will maybe even repel) beneficials? We know from initial experiments that some components of our blends, in isolation, will repel some beneficial species.

- are there alternatives to methomyl as the toxicant? At present we are using this chemical because it kills quickly enough to enable dead moths to be found, which is necessary to enable the impact of the treatments to be assessed. But methomyl is hard on beneficials, and has high mammalian toxicity. In Project 2.2.9 we are studying other insecticides in the laboratory and already have several possible alternatives. But all are slow killers, and will growers accept a control method which does not produce immediately noticeable results? And what are the implications for resistance management? This question will need to be considered urgently by TIMS.

- can attractants be used in other ways, such as enhancing trap crops? We believe our attractants are primarily for adult feeding – if we can attract a female moth for that purpose, will she remain in the crop for oviposition? What about suicide crops – can we make a female oviposit on crops which will not support the larvae? Could we use Bollgard cotton in this way? What about the implications for selection pressure for resistance?

- can we develop attract-and-kill of other pests of cotton and other crops? We know our current blends will attract and kill some important sucking pests, such as green mirids and apple dimpling bugs. We do not yet know whether this translates into effective control. Our current Project 2.2.9 aims to design blends specifically for these pests, which will become increasingly important in the era of transgenic cotton, and for which we currently have no soft options.

8. Describe the project technology (eg. commercially significant developments, patents applied for or granted licenses etc).

We have applied for a patent (PCT/AU02/00554, "Attractants for moths") covering the most important aspects of the attractant technology developed in this project. It is in the name of the University of New England, on behalf of the Australian Cotton CRC, with the inventors listed as Peter Gregg and Alice Del Socorro, and a priority date of 3 May 2001. National phase applications are currently in progress for Australia, Europe, the USA, Brazil and India.

Reports from the International Preliminary Examination of the patent application have been mixed. We attempted to patent some broad principles of developing attractants, such as the idea of combining attractants found in different plants rather than mimicking a particular plant (section 9), and the principle of combining floral and leaf volatiles as described in section 5a. The Australian patent examiner rejected these claims, on grounds which we vigorously disputed. However, he did approve narrower claims which protect our current best blends. These views are currently being tested by the patent examiners in the countries in which we have sought National Phase entry. There are few precedents for products of this nature. If the

Australian view prevails, there will be very little patent protection, either for us or potential competitors, since it will be possible to develop a large range of attractant blends which meet the criteria described in section 5a.

The technology has since been licensed and commercialised. Most of these developments occurred after the term of Project 2.2.3, so they are described below in section 10.

9. Provide a technical summary of any other information developed as part of the research project. Include discoveries in methodology, equipment design, etc.

An important conceptual development from this project has been the principle of super-blending, in which volatile compounds found to be in common between attractive plants are combined in blends which do not necessarily mimic any one plant. When combined in ways which meet a minimum level of complexity, combine floral and leaf volatiles, and include one critical compound, phenylacetaldehyde, such blends were at least as attractive as the best host plants we tested. This approach represents a significant advance over previous approaches which involve mimicking a particular plant, and will give our blends generality, and perhaps help overcome complications due to learned responses of moths in the field.

There are also a number of innovative technical spin-offs which have arisen from the needs of this project. They include:

- the electrified "Zap-trap" (section 5b), which is much more efficient than current designs because it does not require moths to enter the trap. It has applications where low thresholds are important, for example, in detecting insects of quarantine importance. Since it stuns rather than kills moths, it may also be useful for attract-contaminate-release applications, for example with pathogens. We elected not to patent this trap, but it has attracted considerable interest from overseas. An example was sent to IPM Technologies Ltd in the USA for evaluation.

- the field wind tunnel (section 5d) which allows behavioural experiments with moths to be conducted in more natural field conditions than the usual laboratory wind tunnels. The design is cheap, quickly assembled and readily modified to suit changing conditions, eg wind direction.

- the development and validation of the flush counting method to estimate moth numbers in row crops such as cotton. This is a potential tool for fundamental ecological studies of adult *Helicoverpa* and other moth pests.

- methods for testing the toxicity of ingested material in adult *Helicoverpa* moths, which in our current project have been further developed to assess the effectiveness of a range of insecticides in combination with attractants. These methods could be used to study adult resistance, thus providing useful information to guide resistance management.

10. Detail a plan for the activities or other steps that may be taken;

(a) to further develop or to exploit the project technology.

In August 2003, the University of New England, on behalf of the Australian Cotton CRC, signed a technology license agreement with Ag Biotech Australia Pty Ltd., a small Australian company with close links with the cotton industry. This agreement gives Ag Biotech an exclusive 5 year license to exploit the technology represented by our current patent application, and first negotiation rights for technology arising from our current research project. In return, Ag Biotech will provide significant royalties, and assistance (both cash and in-kind) to our on-going research.

In conjunction with Ag Biotech we have obtained a Product Evaluation permit from APVMA which allows them to sell our best blend, PF_3Hs, for use in large-scale trials on up to 20,000 ha of cotton, plus 1,000 ha of several other crops including grain legumes, corn, sorghum and horticultural crops. A commercial manufacturing plant has been established in Sydney, and the material is being sold as Magnet®. Interest from consultants and growers is high.

If results of the product evaluation trials are satisfactory, Ag Biotech will seek full registration for Magnet before the expiry of the Product Evaluation permit in June 2005.

We are in the first year of a follow-up research project funded by the Cotton CRC (Project 2.2.9) which will continue the work of this project as well as exploring some of the new directions outlined in section 7. However, this project is not fully funded by the CRC, owing budgetary constraints imposed by the ramp-down in government funding, and will depend on inputs from our commercial partner in 2004/05 and 2005/06. However, semiochemical research is also a component of the new CRC bid currently being prepared.

(b) for the future presentation and dissemination of the project outcomes.

Results of the project have been extensively communicated to the cotton industry through presentations at seminars and reviews, and in grower and consultant meetings, as well as articles in the Australian Cotton Grower and the proceedings of the ACGRA Cotton Conference, and numerous press releases. We will continue to do this, but the major need at present is to communicate the findings to the national and international scientific community. To some extent this has been done at scientific conferences such as the International Society for Chemical Ecology. However, we have not been able to publish in refereed scientific journals because important aspects of the project have been commercial-in-confidence. Now that the patent has been published this is no longer the case, and we anticipate submitting papers to such journals shortly.

11. List the publications arising from the research project.

Del Socorro, A., Gregg,P, Tennant,R. and Moore,C. (2003) Attract-and-kill heliothis for low pressure every season. *Australian Cotton Grower* 24 (2) 14-19.

Gregg,P.C. and Del Socorro,A.P. (2002) Attractants for moths. Australian and International Patent No. PCT/AU02/00554, 67 pp.

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

Del Socorro,A.P. and Gregg,P.C. (2002) Bioassay methods for plant volatiules as attractants for *Helicoverpa* moths. *International Society of Chemical Ecology, 19th Annual Meeting, University of Hamburg, Germany, 3-7 August 2002.*

Del Socorro,A.P., Gregg,P.C., Tennant,R., Alter,D. and Moore,C. (2002) Plant volatiles as attractants for helicoverpa moths. *Eleventh Australian Cotton Research Conference, Brisbane, 13-15 August. Australian Cotton Growers Research Association, Brisbane, pp. 805-811.*

Britton,D.R., Gregg,P.C. and Del Socorro,A.P. (2002) Attract-and-kill formulations for *Helicoverpa armigera* males (Lepidoptera: Noctuidae). *Eleventh Australian Cotton Research Conference, Brisbane, 13-15 August. Australian Cotton Growers Research Association, Brisbane.*

Britton,D.R., Gregg,P.C. and Del Socorro,A.P. (2001) Developing an attracticide for male cotton bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae): visual and chemical cues. *International Society for Chemical Ecology, 18th Annual Meeting, Lake Tahoe, USA, July 7-12.*

Kvedaras,O., Gregg,P., Del Socorro,A., Alter,D. and Moore,C. (2001) The influence of host plant volatiles on mating behaviour in the cotton bollworm, *Helicoverpa armigera* (Hubner). *Australian Entomological Society, 32ndt Scientific Conference and A.G.M., Sydney.*

Britton, D. and Gregg, P. (2000) Attractive from a distance, but up close, not so good…. Developing attract and kill lures to control the cotton bollworm *Helicoverpa armigera. Australian Entomological Society, 31st Scientific Conference and A.G.M., Darwin.*

Britton,D., Gregg,P. and Del Socorro, A. (2000) Attract and kill formulations for *Helicoverpa armigera* males (Lepdioptera: Noctuidae). Proceedings of the farming Systems Forum, Dalby, 5-6 December 2000. Cotton Research and Development Corporation, Narrabri. 11 pp.

Kvedaras,O.L., Gregg,P.C., Del Socorro,A.P., Alter,D. and Moore,C. (2000) The effects of host plant odours on the calling behaviour , egg maturation and mating success of the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Proceedings of the XXI International Congress of Entomology, Iguassu Falls, Brazil, 20-26 August 2000. A687*

Gregg,P.C. and Del Socorro, A.P. (2001) Developing attractants for *Helicoverpa armigera* moths. Confidential report to the Australian Cotton CRC. 39 pp.

12. Are changes to the Intellectual Property register required?

This will be discussed within the Australian Cotton CRC.