Development of a synthetic plant volatile-based attracticide for female noctuid moths. III. Insecticides for adult *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

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Abstract We investigated the efficacy of insecticides combined with a plant volatile-based attractant for *Helicoverpa armigera* moths, under laboratory and field conditions. In the laboratory, 16 insecticides were assessed by the level of mortality and time to incapacitate and kill moths. The proboscis extension reflex technique was used for dosing moths. The pyrethroids, bifenthrin (only when synergised by the addition of piperonyl butoxide (PBO) but not without it) and cyfluthrin (with or without PBO), endosulfan, the carbamates methomyl and thiodicarb, and spinosad killed all moths tested at rates equivalent to, or less than, those which would be applied in cover sprays targeting larvae. The shortest time to moth incapacitation and death was observed with methomyl and thiodicarb. Spinosad produced very high mortality but moths took much longer to die. The two pyrethroids gave relatively slow kills, as did endosulfan. In a field trial, four insecticides were combined with the attractant and dead moths were collected daily from 1 to 4 days after application of the attracticide on 50 m rows of cotton. Significantly more dead moths (*H. armigera*, *H. punctigera* and other noctuids) were found near the rows treated with attracticide containing methomyl compared with spinosad, fipronil and deltamethrin. For determining the impact of attracticides by recovering dead moths, quick acting insecticides are required to prevent moths flying away from the treated area to locations where they cannot be found. Methomyl and thiodicarb are suitable for this, but other insecticides especially spinosad could be used where quick action is not needed. Large numbers of moths were killed in the field trial, suggesting that attracticides for female *Helicoverpa* spp. moths could have significant impacts on local populations of these pests.

Key words attract-and-kill, attracticide, insecticide, noctuid moth, plant volatile.

INTRODUCTION In previous papers (Del Socorro et al. 2010; Gregg et al. 2010) we have described work leading to the development of an attracticide for noctuid moths, especially *Helicoverpa* spp., based on plant volatile compounds. Insecticides active by ingestion could be used with such attractants if moths could be induced to feed on the attractant, perhaps by the inclusion of a feeding stimulant. Such a formulation could effectively reduce the moth population, especially of females, thereby reducing oviposition, decreasing the need for broad spectrum insecticides, and giving other components of Integrated Pest Management more opportunity to work.

Heliothine larvae are among the most serious insect pests of crops worldwide. Consequently, there is an extensive literature on the effectiveness of various insecticides (Ma et al. 2000; Farrell 2008), and resistance to them (Goodyer & Greenup 1980; Gunning et al. 1984; Daly 1988; Gunning et al. 1992; Forrester et al. 1993; Gunning & Easton 1994; Armes et al. 1996; Ahmad et al. 1997). Almost all of this literature deals with the larvae or (to a lesser extent) eggs, because these are the stages usually targeted with insecticides. There are few references on the toxicity or behavioural effects of insecticides to adults, though there are many anecdotal reports of toxicity and repellence of some groups. Information is available on the contact effects of synthetic pyrethroids on adult *Heliothis virescens* (Fabricius) (Campanhola & Plapp 1989) and *H. armigera* (Daly & Fitt 1990) using the adult vial technique for monitoring resistance. A diagnostic dose of 90 μg per vial of fenvalerate was used to distinguish susceptible from resistant moths, though it was later found that the expression of resistance declined with age, so the technique was not reliable for field-caught *H. armigera* (Daly 1992). Similarly, contact toxicity of methomyl has been evaluated using a vial test for *Helicoverpa zea* (Boddie) (Herbert et al. 2008). A dose of 25 μg active ingredient (ai) per vial killed all laboratory-reared susceptible moths tested. Forrester et al. (1993)
reported on the toxicity of endosulfan, dieldrin and cypermethrin to *H. armigera* moths when administered by topical contact with the eye, and of fenvalerate by topical contact with the tarsi. Cypermethrin and fenvalerate were the most effective insecticides when administered by these routes.

Less information is available for oral toxicity in adult heliothine moths. This is partly due to the difficulties of ensuring repeatable, quantitative ingestion of the insecticide through the proboscis of a moth. The only relevant studies appear to be the briefly reported work of Lopez and Clemens (1997) and Lopez and Latheef (1999). Lopez and Clemens (1997) combined various insecticides with a feeding stimulant (2.5 M sucrose) and fed them to *H. zea* collected from pheromone and light traps. Effective insecticides (LC$_{50}$ < 10 p.p.m., measured at 24 and 48 h) included lambda-cyhalothrin, methomyl, profenofos, thiodicarb, cyfluthrin, cypermethrin, acephate, chlorpyrifos and spinosad. Later, Lopez and Latheef (1999) confirmed the high toxicity of spinosad and noted that it killed slowly but had no apparent feeding deterrent even at very high concentrations.

Here we describe a technique for laboratory feeding of moths with an attractant formulation containing plant volatiles, sucrose, various excipients including marker dyes, and insecticides. We tested 20 insecticides in the laboratory using this technique, and then tested four in preliminary field tests in which the attracticide formulations were applied to cotton in the early evening and dead moths collected from the vicinity of the plants the following morning. We describe the characteristics required for suitable insecticides for use with plant volatile-based adult attractants and identify some that are suitable for continued development in this field.

**MATERIALS AND METHODS**

**Experimental insects**

For all insecticides except rynaxypyr, moths were sourced from a laboratory colony originating from collections made on chickpeas in the Darling Downs in September 2003, and maintained in culture for two generations using techniques described by Del Socorro et al. (2010). In the case of rynaxypyr, which was not available at the time of the original experiments, a new culture was established from moths collected from chickpeas in the Darling Downs in September 2008. Female moths 3 days after emergence, which were unmated and had been allowed access to distilled water on a dental wick, were used for testing all insecticides.

**Laboratory dosing techniques**

The insecticides we tested and their suppliers are listed in Table 1. Insecticides listed in Table 1 were presented in a candidate attractant blend consisting of 9.08 g/L phenylacetaldehyde, 5.68 g/L α-pinene, 5.07 g/L cineole, 1.88 g/L limonene and 10.4 g/L (Z)-3-hexenyl salicylate (plant volatile components), dissolved in 76.15 g canola oil, emulsified with 560.29 mL water using 17.06 g/L sorbitan monostearate as an emulsifying agent, with 304.62 g/L sucrose added as feeding stimulant. Xanthan gum (thickening agent) was added at 0.75 g/L, as was Vitamin E (anti-oxidant) at 1.5 g/L. A Brilliant Blue food dye (Queen Fine Foods, Enoggera, Queensland) was added at 12 g/L to enable visual assessment of the mixture in the digestive tract of moths. In regard to the plant volatile components, this formulation was comparable to the PF3Hs blend described by Gregg et al. (2010), which was the most attractive blend identified. The insecticides tested were sourced from commercial preparations rather than technical ingredients, because the aim was to identify potential insecticide partners for field use in attractants, rather than to investigate the mechanisms of, or to precisely quantify, toxicity. For similar reasons, each insecticide was first used at a concentration such that, if applied in 5 mL of attractant blend per metre of crop row, a treated row would receive an amount of ai equivalent to that which would be received if a cover spray, targeting larvae, was applied at the highest rate registered in Australia for cotton. Only insecticides which produced 100% mortality at this rate were further investigated at lower concentrations.

To facilitate ingestion of the attracticide formulations we exploited the proboscis extension reflex, commonly used to study learning in insects (e.g. Fan et al. 1997). Moths were restrained in 1.5 mL Eppendorf tubes from which the lid was removed and the tip was cut so that moths could be inserted head first, with only their head including antennae and proboscis projecting through the hole. Their exit to the rear was blocked by a wad of cotton wool. One antenna was briefly touched with a toothpick moistened with 20% sucrose. Moths usually extended their proboscis in response to this stimulus. Any which did not had their proboscis gently extended with a probe. Following extension a spatula bearing approximately 100 μL of the attracticide was placed at the tip of the proboscis. The spatula was left in place for 30 s, a time comparable to the maximum period that moths were observed to spend in a single feed on nectar from various flowers under field conditions (PC Gregg & AP Del Socorro unpubl. data 1998). Moths were then returned to their individual holding cups, which contained moist dental wicks, and observed for signs of intoxication. Normally 20 moths were used for each insecticide–rate treatment, but in some treatments fewer moths were used due to limited supply from the culture. Moths were examined almost continuously for the first hour after dosing, then at intervals of approximately 15 min for the next 2 h, and then at hourly intervals until 8 h after dosing. They were finally examined the next morning, about 24 h after dosing. Moths were considered incapacitated when they were unable to right themselves when turned upside down. They were considered dead when no movement of any body part could be elicited in response to touching with a probe.

In a preliminary study, moths in Eppendorf tubes were weighed prior to and immediately after ingestion of the attractant formulation without insecticide, to determine the amount of formulation which had been ingested. They were dissected...
Table 1  Insecticides, with concentrations (%v/v) of active ingredients (ai) in the attractant, trade names and suppliers, numbers of moths used and not used (due to lack of ingestion), percentage mortality and mean time to incapacitation (Time I) and time to death (Time D) in min

<table>
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<th>Insecticide</th>
<th>Dose% ai</th>
<th>Source</th>
<th>N not used</th>
<th>N used</th>
<th>% dead</th>
<th>Time I ± SE</th>
<th>Time D ± SE</th>
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<td>Rogor</td>
<td>3</td>
<td>17</td>
<td>52.9</td>
<td>140.8 ± 31.2</td>
<td>543.7 ± 149.8</td>
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<td>Rogor</td>
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<td>577.8 ± 36.9</td>
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<td>25.8 ± 4.3</td>
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<td>100.0</td>
<td>65.0 ± 2.7</td>
<td>423.4 ± 43.6</td>
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</table>

Suppliers: Bayer, Bayer Crop Science AG, Monheim, Germany; Crop Care, Crop Care Australasia Pty Ltd, Murrarie, Qld; Dow, Dow Chemical (Australia) Ltd, Altona, Vic; Dupont, Dupont (Australia) Ltd, Macquarie Park, NSW; Farmoz, Farmoz Pty Ltd, St. Leonards, NSW; FMC, FMC International AG, Marrurrie, Qld; Nufarm, Nufarm Australia Ltd, Laverton, Vic; Sipcam, Sipcam Pacific Australia, Geelong, Vic; Syngenta, Syngenta Crop Protection Pty Ltd, Macquarie Park, NSW.

Field testing

The field test was conducted at the Frank Wise Institute, Western Australian Department of Agriculture and Food, Kununurra, Western Australia, on a 9 ha field of squaring cotton in April 2003. Insecticides used were methomyl at 0.5% ai (sourced from Electra 225®; Farmoz), spinosad 0.5% (sourced from Success®; Dow), fipronil 0.25% ai (sourced from Regent®; Nufarm) and deltamethrin 0.2% (sourced from Decis Options®; Bayer). Methomyl and spinosad were both found to be effective in laboratory studies. Deltamethrin, which was not tested in the laboratory, was included in the field study as a substitute for cyfluthrin when the latter was unexpectedly not available at the field site. It is a synthetic pyrethroid with similar mode of action and similar levels of activity on Helicoverpa spp. larvae (Forrester et al. 1993). Fipronil had limited activity in laboratory trials, but was considered worthy of inclusion in the field trial because it is 8 h later, and the extent of blue dye in their digestive tracts was scored on a scale of 0, +, ++ and +++. In subsequent work, moths were dissected within 1 h of their death or after 24 h if they were still alive. Data from moths which were scored at less than ++ on this scale were discarded from the analysis as it was considered that they may not have ingested enough insecticide to be affected. These moths are listed in Table 1, and accounted for less than 5% of the total moths used in the experiments.

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among the most common insecticides used in transgenic (Bollgard®) cotton in Australia (Whitehouse 2006).

All insecticides were added to the same formulation of attractant volatiles and excipients used for laboratory studies, except that different food dyes (all obtained from Queen Fine Foods) were added in an attempt to distinguish moths which had been killed by the different insecticides. Blue dye was added to the methomyl formulation, pink dye to the spinosad formulation, green dye to the fipronil formulation, and yellow dye to the deltamethrin formulation.

Treatments were applied to 50 m sections of single cotton rows. Rows were spaced 1 m apart. There were four replicates of each treatment, arranged in a 4 \times 4 Latin square design with buffer zones of 50 m along the rows, and 50 rows across the rows. The formulations were sprinkled from plastic bottles with ‘pop-top’ lids, so as to form small pools on the top of the crop canopy. A high level of foliage coverage was not considered important because the formulations were expected to be attractive. Treatments were applied about 30 min before sunset. Each morning, between dawn and 3 h later, furrows between the rows were carefully searched for dead moths, which were collected and dissected later the same day, to identify the species, sex, mated status (determined by the presence of spermatophores in the bursa copulatrix, and classified as none, 1, 2, 3 or more than 3) and presence or absence of dye in the digestive tract. Furrows located at intervals of 1, 3, 10 and 25 rows (metres) in each direction from the treated row were searched. The searches were repeated 1, 2 and 3 days later for all treatments, with an additional search on day 4 for the methomyl treatment only.

RESULTS

Preliminary studies without insecticides

Moths which consumed the attractant formulation to which no insecticide was added showed no ill effects. All 14 were still alive after 24 h (Table 1), and showed no signs of intoxication. In an additional sample of 15 moths which were weighed to determine consumption and then dissected after 8 h, there were likewise no ill effects. Of these moths, two showed weight gains of <0.3 mg and were scored 0 for the presence of blue dye. These two were considered not to have fed. Another moth, which had an abnormally formed proboscis, had a weight gain of 2.0 mg and a dye score of ++. The remaining 12 had a mean weight gain of 4.0 ± 0.2 mg (range 2.8–5.1), and dye scores of +++ (10 moths) or ++ (2 moths).

Laboratory dosing studies

The percentages of moths which were dead after 24 h, and the mean time (±SE) to incapacitation and death of those which died, are shown in Table 1. Also shown are the numbers of moths not used due to failure to ingest sufficient formulation. There were only 29/639 (4.5%) of these, and they did not seem to be concentrated in particular treatments. Insecticides which produced 100% mortality included the synthetic pyrethroids bifenthrin (only when synergised by the addition of piperonyl butoxide (PBO) but not without it), cyfluthrin (with or without PBO), endosulfan, the carbamates methomyl and thiocarb, and spinosad. Of these, by far the quickest to incapacitate and then kill moths were methomyl and thiocarb. These two insecticides produced 100% mortality at 0.05% ai, which is 10% of the maximum registered rate for cover sprays, and 88.8% and 89.5% at 0.0015 (3% of the maximum registered rate). Spinosad also produced very high mortality at rates down to 0.02% ai (12.5% of the maximum registered rate), but was much slower to kill. Of the two synthetic pyrethroids tested, cyfluthrin gave the quickest kill and did not require synergism with PBO, which produced only marginal improvements in the speed of kill. Endosulfan produced complete mortality at 0.7% ai (48% of the maximum registered rate) but was also a slow killer. Moths poisoned with endosulfan ‘dumped’ eggs on the walls of their plastic cages before they died, even though these eggs were unfertilised because the moths had not been mated. This behaviour was not recorded to any great extent with other insecticides. The new insecticide rynaxypyr (Altacor®) did not kill moths within 24 h, or incapacitate them according to our criteria (inability to recover from being inverted). However, it did make all moths dosed with it extremely sluggish and unable to fly, and they remained so at 24 h. Imidacloprid produced temporary incapacity, in which many moths were affected within 2–3 h after dosing, but subsequently recovered and were still alive after 24 h. For all other insecticides, almost all moths recorded as incapacitated died within 24 h.

Field trial

The heliothine population at Kununurra at the time of the experiment was dominated by H. punctigera. A total of 386 dead moths of this species, and 169 of H. armigera, were collected by searching the furrows surrounding the treated row. The distribution of these moths in relation to distance away from the treated rows and the time after treatment is shown in Figure 1. The great majority of moths were collected from the methomyl treatment, with the highest numbers found in furrows adjacent to the treated rows. Numbers declined progressively with distance from the treated row, but some were still found 25 m away. Only low numbers of dead moths were collected around the other three insecticide treatments, and the bell shaped distribution was much less obvious. In the methomyl treatment, similar numbers of moths were collected on all 4 days, indicating that the activity of the formulation did not decline during this period. In the other treatments (which were not searched on day 4), the numbers were too low for reliable assessment of the duration of activity.

An encouraging feature of the field trial results was that moth mortality occurred over more than one night. The period of activity (at least 4 days for the methomyl treatment) is consistent with observations of the rate of dissipation of volatiles from leaves of cotton plants in glasshouse conditions (AP Del Socorro & PC Gregg unpubl. data 2001). However, it
might be expected that oil-water based formulations would not
remain liquid enough for ingestion for this period. The persis-
tent activity in this trial can be explained by the observation
that, although the deposits on the foliage dried during the day,
at night when the humidity rose the deposits became at least
partially liquid again, perhaps due to the hygroscopic effect of
high sucrose concentrations.

It was not possible to dissect a few moths because the
abdomen was missing, probably due to scavenging by ants
before they were collected. The percentages of females and the
mated status of the female moths among the remainder are
shown in Table 2. Over 70% of the dead moths were females.
The majority of females were mated, many more than once
and some up to five times. Generalised Linear Modelling
analyses of the proportions of females and of the various
mating status categories, weighted using the number of moths
dissected and the numbers of females dissected, respectively,
indicated no significant effects of the insecticide treatment on
any parameter for either species. When these proportions were
similarly analysed with respect to the day of collection, no
significant effects were obtained for *H. armigera*, but for *H.
punctigera*, an increase in the proportion of mated moths was
found in the third and fourth days compared with the first and
second days after the formulations were applied (Fig. 3). An
additional 267 moths of other species, mainly noctuid pests
such as *Anomis flava* (Fabricius), *Spodoptera litura* (Fabri-
cius), *Mythimna convecta* (Walker) and *Helicoverpa assulta*
(Guenèe) were also collected.

The percentages of dead moths of both heliothine species
that were marked with various coloured dyes on each day after
treatment are shown in Figure 2. On the first day, slightly over
half the moths of both species were marked, indicating methomyl
consumption. Over time the percentage marked declined to
about 20% by day 4, and marks other than blue were very rare.

![Distribution of dead Helicoverpa armigera and H. punctigera moths in space and time around rows treated with attractant containing methomyl, spinosad, fipronil and deltamethrin.](image)

<table>
<thead>
<tr>
<th>Attracticides for <em>H. armigera</em> moths</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Methomyl</strong></td>
</tr>
<tr>
<td><strong>Spinosad</strong></td>
</tr>
<tr>
<td><strong>Fipronil</strong></td>
</tr>
<tr>
<td><strong>Deltamethrin</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>H. armigera</th>
<th>H. punctigera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number dissected</td>
<td>161</td>
</tr>
<tr>
<td>% female</td>
<td>72.5</td>
</tr>
<tr>
<td>% of females with no spermatophores</td>
<td>26.3</td>
</tr>
<tr>
<td>% of females with 1 spermatophore</td>
<td>43.2</td>
</tr>
<tr>
<td>% of females with 2 spermatophores</td>
<td>20.0</td>
</tr>
<tr>
<td>% of females with 3 spermatophores</td>
<td>7.4</td>
</tr>
<tr>
<td>% of females with &gt;3 spermatophores</td>
<td>3.0</td>
</tr>
</tbody>
</table>

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unpubl. data 2003) showed that dyes other than blue tended to dissipate from moths, both dead and alive, within a few hours. This was particularly the case for pink (spinosad) and yellow (deltamethrin) dyes. Further, dyes of all colours tended to dissipate from foliage after the first day. It is therefore possible that the unmarked moths were killed by insecticides other than methomyl on day 1, and by any insecticides after day 1. It is unlikely that many moths found dead but lacking dye were killed by contact activity alone. While moths were readily killed by 5 ml of formulation containing 0.5% methomyl when it was applied to the eye, they were not killed by an equivalent application to the abdomen, probably because of the protective effect of scales. Nor were they killed by dipping one foreleg in the formulation (PC Gregg & AP Del Socorro unpubl. data 2003). Moreover, observation of moth feeding in the laboratory and the field indicates that moths do not blunder into pools of the formulation on leaves, nor do they stand in them. They alight nearby, and stand at the edge of the pool to insert their proboscis.

**DISCUSSION**

**Efficacy of insecticides on adult *H. armigera***

The insecticides used in this study were chosen because they were registered in cotton in Australia, often but not always for *Helicoverpa* spp. larvae. The moths used in the preliminary study with insecticide-free attractant weighed 209 ± 8.6 mg and were estimated to have consumed 4.0 ± 0.2 mg of formulation. On the assumption that the moths fed attractant containing insecticides consumed similar amounts (an assumption supported by the internal dye scores) it is possible to determine approximate LD₉₉ values for some insecticides, and compare them with discriminating topical doses which have been used for resistance testing of larvae (R Gunning & L Rossiter, unpubl. data 2008). On this basis moths appear to be less susceptible to bifenthrin, about equally susceptible to methomyl, and more susceptible to spinosad, compared with larvae. These differences may be related to inherent differences in susceptibility between adults and larvae. They may also be influenced by the route of dosing and by possible resistance in our moth culture (which was sourced from the Darling Downs region at a time when resistance to endosulfan, pyrethroids and carbamates was prevalent). We lack sufficient data to make meaningful comparisons for other insecticides.

**Suitability of insecticides for inclusion with attractants***

Desirable properties for an insecticide for inclusion in attracticide formulations based on plant volatiles include: (1) efficacy at concentrations which produce application rates equal to or lower than those resulting from cover sprays at registered rates; (2) lack of repellent or deterrent effects on moth behaviour; (3) persistence beyond the life of the plant volatile components of the attracticide; (4) rapid incapacitation and/or killing of moths; and (5) limited toxicity to non-target organisms, both vertebrate and invertebrate. The first characteristic is desirable because it means that attracticides will not produce higher residues in agricultural commodities than those which have already been accepted by regulatory authorities. The second characteristic is desirable because repellence or deterrence would compromise efficacy. The third characteristic means there is no risk of attracting moths to a field but failing to kill them, which might result in higher levels of oviposition and crop damage than would occur without the attracticide. The fourth characteristic may not be important for commercial applications, but it is crucial in evaluating the impact of attracticides during the developmental phase. In our field trial, for example, it was possible to estimate the impact of the attracticide including methomyl, because it killed within a few minutes (Table 1), which meant that dead moths were concentrated around the treated rows (Fig. 1a). However, it was not possible to do this with spinosad, because although the formulation should have been lethal on the basis of laboratory experiments (Table 1), the much slower speed of kill may have allowed many moths to escape the vicinity of the treated rows before they died. Limited toxicity to non-target organisms is desirable because it is unlikely that plant volatile-based attractants will be as specific as pheromones, so formulations should pose as little risk to non-target organisms (including beneficial insects and persons applying them) as possible.

On the basis of these criteria, there is a limited range of candidate insecticides for inclusion in attractant blends. Of those we tested, only the pyrethroids bifenthrin (synergised with PBO) and cyfluthrin together with endosulfan, spinosad and the carbamates methomyl and thiodicarb meet the first
criterion, efficacy at realistic concentrations. Many pyre-throids have repellent or deterrent effects on a wide range of insects including adult moths (reviewed by Forrester et al. 1993). While our laboratory studies did not indicate those we used had such effects (dye scores in moths ingesting them were equivalent to those in moths ingesting the formulation with no insecticide), it remains possible that these effects might occur under field conditions. This might be one reason why few moths were found marked with green dye (indicating deltamethrin consumption) in the field trial (Fig. 2). However, another possible explanation is that the pyrethroids we tested in the laboratory (which did not include deltamethrin) were relatively slow killers, and in the field moths may have had time to move away from the deltamethrin treatment before dying. Endosulfan is another insecticide which met the efficacy criterion, but was a slow killer. Moreover, it is relatively toxic to non-target organisms (Farrell 2008), and the egg-dumping behaviour it produced in laboratory trials, if replicated in the field with moths carrying fertile eggs, could result in temporary increases in oviposition. Spinosad met the efficacy criteria, has no known deterrent effects, and has good characteristics in relation to toxicity towards most non-target organisms. It is an extremely slow killer, and in the field trial very few moths could be found around treated rows.

Spinosad is an unsuitable insecticide for evaluating the impact of attracticides or comparing different attracticides in the field, but it may be an ideal insecticide for commercial use if locating the dead moths is not important. As a stomach poison, spinosad is relatively safe for beneficial insects, and compatible with IPM (Farrell 2008). Lopez and Latheef (1999) also concluded that spinosad could be valuable in this role. The carbamates methomyl and thiodicarb were highly effective and fast killers, especially methomyl. While methomyl is not very persistent, the wide margin between the maximum and minimum concentrations which could be used (Table 1) suggests minimal risks of attracting moths to a field but failing to kill them, with consequent increases in oviposition. Carbamates, especially methomyl, are relatively toxic to non-target organisms (Farrell 2008), but they are likely to be the insecticides of choice for evaluating different attractant formulations and for determining the impact of attract-and-kill in the field.

None of the other insecticides, including the organophosphates and the newer, more specific insecticides such as chlorfenapyr, emamectin benzoate and indoxacarb, met the efficacy criterion, nor did insecticides which are primarily targeted at pests other than Helicoverpa spp. larvae, such as abamectin, acetamiprid, fipronil and imidacloprid. The new insecticide rynaxypyr warrants further investigation in the field, because although it did not kill moths within 24 h, it rendered them sluggish and unable to fly, which may be sufficient to eventually kill them. However, it is unlikely to be useful for field trials aimed at comparative evaluation of candidate attractant blends.

Whatever insecticide is selected for use in commercial attracticides, resistance management must be considered. In the case of Australian cotton, an extensive resistance management scheme with both curative and preventative dimensions has been adopted within the industry (Farrell 2008). It is updated yearly as new larvicidal products come onto the market, and adherence by growers is virtually total. Clearly, as adult attracticides are commercialised, their use will be regulated by this scheme.

Impact of attractants in the field trial

While the primary purpose of the Kununurra trial was to compare insecticides, the numbers of dead moths collected allow us to make at least preliminary estimates of the likely impact of treating cotton fields with attracticides similar to those used here. We recorded a total of 555 dead heliothine moths, of which most were H. punctigera. While the plant volatile blends we developed were targeted at H. armigera (Gregg et al. 2010), it is not surprising that the PF3Hs blend also attracted H. punctigera given the taxonomic and ecological similarities between the two species (Zalucki et al. 1986, 1994; Fitt 1989, 1994; Matthews 1999). In the absence of an independent measure of the relative abundance of the two species at the time of the trial, it is not possible to determine whether the formulations were more or less attractive to one or other of the species. Similarly, it is not possible to determine what fraction of the resident populations of both species were killed, or from how far moths were being drawn.

The sex ratio of the moths found dead was consistently biased to females (overall, more than 70%, Table 2). This could mean that the formulation was differentially attracting and killing female moths, although our olfactometer studies (Gregg et al. 2010) suggest that it is not intrinsically more attractive to females. It is possible that differences in female behaviour in the field, such as an increased propensity for feeding, may cause female biased moth kills. It is also possible that the population in the general area was female biased, but it is very difficult to determine this because most methods of sampling noctuid moth numbers (such as light traps) may also be biased. Among the females killed, moths with one or more spermatophores in the bursa copulatrix, indicating mating, outnumbered unmated moths which lacked spermatophores. In the absence of an independent measure of the mated status of the population as a whole, it is difficult to determine whether the formulations were more attractive to mated females than unmated ones. Among the H. punctigera (but not H. armigera) females, the percentage of mated moths increased as the trial progressed (Fig. 3). This could mean that successive nights’ moth kills were being drawn from the same population, which was ageing and therefore becoming more mated as the experiment progressed. However, immigration from a nearby, more mated, population is another possibility. It is clear, however, that although the attractant formulation was developed using olfactometer studies with unmated moths (Gregg et al. 2010), it also attracts mated moths, which would increase its potential utility in pest management.
The 10th furrow and after collecting ceased on day 4 following treatment. In rows moths killed in these treatments might not have been recovered tamethrin and spinosad are likely to be slow killers, additional dye, especially on the first day after application. As both del- the substantial numbers of dead moths recovered which lacked dyes they contained, but it is the most plausible explanation for treatments was not possible due to the poor persistence of the thines, comparable to cyfluthrin which produced complete the laboratory, it is a highly active pyrethroid on larval helio- tial numbers of moths. While deltamethrin was not tested in Similarly, the deltamethrin treatment may have killed substan- been killed by the spinosad treatment as by the methomyl. mortalities in the 50 surrounding each treatment were searched (the 1st, 3rd, 10th and 25th furrows away from the treated row). An approximation of the total kill for the methomyl treatments for all days can be made by representing the uncol- lected furrows using a model which is consistent with the spatial distribution shown in Figure 1:

\[
y = 2x_1 + 12x_3 + 16x_{10} + 20x_{25}
\]

where \(y\) = total kill, \(x_1\) = numbers found in the 1st furrow, \(x_3\) = numbers found in the 3rd furrow, \(x_{10}\) = numbers found in the 10th furrow and \(x_{25}\) = numbers found in the 25th furrow.

This model yields an estimated total kill of 1722 heliothine moths across all treatments. This estimate remains conservative because it does not count moths which may have been killed by the other insecticide treatments from meth- omyl, but not found because they dispersed. Dispersal before death may even have occurred beyond the boundaries of the relatively small field; numerous dead moths were observed outside the field but were not counted in the total. The laboratory studies suggest similar numbers of moths should have been killed by the spinosad treatment as by the methomyl. Similarly, the deltamethrin treatment may have killed substan- tial numbers of moths. While deltamethrin was not tested in the laboratory, it is a highly active pyrethroid on larval helio- thines, comparable to cyfluthrin which produced complete mortality in moths. Confirmation of the effectiveness of these treatments was not possible due to the poor persistence of the dyes they contained, but it is the most plausible explanation for the substantial numbers of dead moths recovered which lacked dye, especially on the first day after application. As both del- tamethrin and spinosad are likely to be slow killers, additional moths killed in these treatments might not have been recovered because they flew too far before the insecticides took effect. Nor does the model count moths which may have been killed after collecting ceased on day 4 following treatment. In rows surrounding the methomyl treatment, there was no apparent decline in kills during the 4 days of collection. Further investiga- tion of the persistence of attracticide formulations beyond 4 days is required.

Considering these factors, it is likely that the overall helio- thine moth kill totalled several thousand. As over 70% of the kill comprised females, and each female can lay between 1000 and 2000 eggs (Zalucki et al. 1986), it is possible that the number of eggs that were not laid due to the effects of the attracticides was in the region of several million. This is a substantial reduction in a field as small as 9 ha, where as few as 180 000 eggs of either species would be considered an economic threshold if they all hatched (Farrell 2008). This suggests that the use of plant volatile-based attracticides such as those described here, spaced at around one row in every 50, to target female moths may be a viable alternative to cover sprays targeting larvae. Confirmation of this would require measurements of oviposition and larval numbers in the next generation. This was not the purpose of the trial reported here, but in future papers we will describe trials in which this type of impact was measured.

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