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Integrated pest management in cotton: exploiting behaviour-modifying (semiochemical) compounds for managing cotton pests

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Abstract. We review here research on semiochemicals for cotton pest management carried out in successive Cotton Co-operative Research Centres from 1998 to 2012. Australian cotton is now dominated by transgenic (Bt) varieties, which provide a strong platform for integrated pest management of key pests such as *Helicoverpa* spp., but new technologies are required to manage the development of resistance in *Helicoverpa* spp. to transgenic cotton and the problems posed by emerging and secondary pests, especially sucking insects. A long-range attractant for *Helicoverpa* moths, based on plant volatiles, has been commercialised as Magnet®. The product has substantial area-wide impacts on moth populations, and only limited effects on beneficial insects. Potential roles are being investigated for this product in resistance management of *Helicoverpa* spp. on transgenic cotton. Short-range, non-volatile compounds on organ surfaces of plants that do not support development of *Helicoverpa* spp. have been identified; these compounds deter feeding or oviposition, or are toxic to insect pests. One such product, Sero X®, is effective on *Helicoverpa* spp. and sucking pests such as whiteflies (*Bemisia tabaci*), green mirids (*Creontiades dilutus*), and other hemipteran insects, and is in the advanced stages of commercialisation.

Additional keywords: attractants, deterrents, Australia, *Helicoverpa*, insect behaviour, sucking pests.

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Introduction

With the commercial release of transgenic Bt (*Bacillus thuringiensis*) (Bollgard II®) cotton, insecticide use to control *Helicoverpa* spp. has declined. However, early-season applications of synthetic insecticides against *Helicoverpa* also suppressed the populations of sucking pests such as *Creontiades dilutus* (green mirids), *Nezara viridula* (green vegetable bugs), *Bemisia tabaci* (silverleaf whiteflies), and *Aphis gossypii* (cotton aphid). This reduction in insecticide applications and the ineffectiveness of the Bt toxin against sucking pests has resulted in a significant increase in sucking pest populations as well as an increase in the use of insecticides aimed at controlling these sucking pests. Issues of insecticide resistance, disruption of beneficial species, high production costs, and environmental impacts now require the development of alternative strategies for managing and controlling sucking pests (Gregg and Wilson 2008).

Although Bollgard II® currently provides good control of *Helicoverpa* spp. except for occasional surviving larvae (Lu *et al*. 2011), the development of resistance remains a threat. *Helicoverpa armigera* has a long history of resistance to synthetic insecticides, and might be expected to respond similarly to selection with Bt toxins. Recent trends in the frequency of resistant alleles in this species and (surprisingly) in *H. punctigera* mean that comprehensive resistance-management strategies are required for both species (Downes *et al*. 2010*a*, 2010*b*). These strategies require novel ways of reducing the numbers of resistant *Helicoverpa* spp. while increasing the relative numbers of susceptible individuals in the population.

Thus, for both managing resistance to transgenics and dealing with pests that arise from their use, we need new tactics in integrated pest management (IPM). Such tactics include (but are not limited to) biopesticides and behaviour-modifying compounds (semiochemicals). The latter approach has great potential to revolutionise the way insect pests are managed in broadacre crops such as cotton. The natural plant chemical compounds that influence the behaviour of insects are described as secondary plant compounds (SPCs) (Rhoades and Coates 1976). As well as functioning as cues affecting insect behaviour, many SPCs have evolved in plants to protect against pest infestation. This has led to several examples of SPCs being used as botanical insecticides to reduce pest damage when applied to crop plants. Some SPCs extracted from non-host plants and then sprayed on host plants can change the behaviour of a pest, particularly moths, which then avoid the host plant (Tingle and Mitchell 1984). Unfortunately, numerous studies into the effects of SPCs on pests have used the paradigm for insecticide screening—focusing on compounds that kill pests, not compounds with potential to modify and/or ameliorate damaging pest behaviours. Consequently, potentially useful compounds with more subtle modes of action that could lead to novel products have been overlooked. Such compounds attract or repel pests over considerable distances, or stimulate or deter both feeding and egg-laying following contact.

Behaviour-modifying chemicals that improve control and management of sucking pests and *Helicoverpa* spp. on cotton are some of the novel non-chemical and natural chemical pest-control tools that are required to complement beneficial insect activity against cotton pests and thus support IPM programs in cotton. Semiochemicals may be used to attract, stimulate, repel, or deter oviposition and/or feeding of pest species. By combining the use of attractants and repellents, push–pull strategies can be developed to modify pest behaviour and move insect populations around crops. Feeding stimulants can be used as adjuvants or in mixtures with conventional pesticides and biopesticides to improve efficacy against pests. On the other hand, because deterrents may directly suppress oviposition and feeding by insects, these deterrents may be considered by farmers to be more important than stimulants, and in fact, a deterrent effect is more commonly noted in SPCs by farmers (Bernays and Chapman 1994). It is plausible that the efficacy of a deterrent would be increased when used in combination with an attractant/stimulant applied to a trap, refuge, or suicidal crops (Miller and Cowles 1990) in a push–pull strategy (Pyke *et al*. 1987).

In Australia, SPCs might be used for mid- to late-season control of *Helicoverpa* on transgenic Bt cotton crops to reduce *H. armigera* and *H. punctigera* populations that may be exposed primarily to the Cry2Ab toxin in Bollgard II® plants (Fitt *et al.* 1998). This is because the level of the other toxin in Bollgard II® plants (Cry1Ac) declines with plant age (Greenplate *et al*. 2003; Olsen *et al*. 2005). Using the SPCs in this way may reduce the risk of resistance to the transgenic crops (Downes *et al*. 2010*a*). Thus, semiochemical products to modify egg-lay and/or feeding behaviour as well as causing direct mortality to cotton pests are novel products for pest management on cotton and other crops due to their unique mode of action compared with synthetic insecticides. These novel products can offer potentially very significant benefits to the cotton industry in terms of reduced insecticide use, delayed pest resistance to synthetic insecticides and transgenic (Bt) cotton, and also complementing IPM through conservation of beneficial insects and their biological activities.

The Cotton Research and Development Corporation (CRDC) commissioned a research review of the role of semiochemicals in pest management (Mensah and Moore 1999). The recommendation of the review adopted by successive Cotton Co-operative Research Centres (CRCs) was to undertake chemical ecology research to better elucidate the nature of variation in host-plant utilisation by *Helicoverpa* and other cotton pests in the field, with longer term objectives including the identification of plant characters and the role of volatile plant chemicals (attractant, deterrents, and anti-feedants) in pre-alighting host selection, and of both leaf texture and leaf-surface chemistry in post-alighting behaviour that would render the cotton plant a less suitable host to *Helicoverpa* spp. and other pests. Beyond this, the CRCs (CRC for Sustainable Cotton Production, Australian Cotton CRC, and Cotton Catchment Communities CRC) sought, in conjunction with commercial partners, to develop and exploit semiochemicals as stimulants, deterrents, attractants, and repellents as part of IPM for sustainable cotton production in Australia.

This paper reviews the development and effectiveness of semiochemicals as insect behaviour modifying compounds for managing cotton pests. It describes efforts (*1*) to develop novel, long-range attractants (based on plant volatile chemicals) that might be used for push–pull or for attract-and-kill of *Helicoverpa* spp. moths; and (*2*) to investigate the influence of leaf chemistry (particularly short-range, non-volatile compounds on organ surfaces of plant shoot system) on the behaviour of *Helicoverpa* spp. and other pests.

Two Australian Cotton CRC chemical ecology projects were set up in 1998 and 2000 that were designed to develop and test these compounds. Additionally, the projects were to utilise these plant secondary compounds to develop a commercial products that might be used as behaviour-modifying compounds such as feeding deterrents or anti-feedants, oviposition deterrents, attractants, repellents, mating disruptants, as well as toxic compounds against *Helicoverpa* spp. and other pest species in agricultural crops. The results of the experiments are considered as part of a general review of semiochemicals in the *Discussion*.

Materials and methods

Development of long-range attractants

The study used a two-choice olfactometer based on the design of Beerwinkle *et al*. (1996) to test 38 plants including hosts and non-hosts, native to Australia and exotic, for attractiveness to unmated male and female *H. armigera* (Del Socorro *et al*. 2010*a*). From this range of plants, volatiles were collected in the same olfactometer apparatus using solid phase micro-extraction techniques, and analysed by gas chromatography–mass spectrometry. Approximately 100 volatiles were identified, and based on commonality between attractive plants, several green-leaf volatiles, floral volatiles, aromatic compounds, and terpenoids were suggested as potential candidates for attractants. Gregg *et al*. (2010*b*) then tested these compounds, individually and in combinations (blends) in the olfactometer. Of 34 chemicals tested, only seven were significantly attractive on their own, but when combined in blends with volatiles not necessarily attractive on their own, attractiveness of the blends was greatly increased compared with any of the single chemicals. Subsequently, field trials of promising blends were undertaken in collaboration with Ag Biotech Australia Pty Ltd (now AgBiTech Pty Ltd). Sixteen synthetic insecticides, already registered against *Helicoverpa* spp. or other pests in cotton, were tested in the laboratory using a modification of the proboscis extension-reflex technique (Fan *et al*. 1997). Insecticide was administered orally in an oil–water homogenate containing plant volatiles, sugar, and other excipient ingredients (Del Socorro *et al*. 2010*b*). Only insecticides that produced high mortality at application rates equivalent to those present in larvicidal cover sprays already registered for use in cotton in Australia were considered. The carbamates methomyl and thiodicarb met these requirements, and killed moths quickly, so that they could be found near treated sections of cotton, in order to evaluate the impact of field treatments. Spinosad was also effective, but killed more slowly. Other insecticides, including some groups such as synthetic pyrethroids and organophosphates, which are effective by contact and ingestion for larvae, were less effective.

Regulatory requirements were considered at an early stage. The material could not be registered as a biological product since its active ingredients, although nature-identical, were produced synthetically. The Australian registration system allows for a category of chemicals whose active ingredients are 'commonly used household/industrial chemicals with a history of safe use', and imposes fewer requirements for toxicological and environmental data on such products (Gregg *et al*. 2010*a*). These considerations, along with cost of the volatiles and their persistence, were taken into account along with their attractiveness when present in blends. Eventually, a registration package was prepared for a five-component blend consisting of d-limonene, α-pinene, cineole, phenylacetaldehyde, and (Z)-3-hexenyl salicylate, with the trade name Magnet®. An area-wide field trial consisted of 12 fields, averaging ~110 ha, that were treated with Magnet® on 13 occasions from November to February 2005, at intervals of 5–8 days, using a row spacing of 1 in 72. Egg counts were conducted by independent commercial consultants at intervals of 3–7 days on these fields and another 10 fields of similar size interspersed with the treated fields, on the same farm. Similar egg counts were made on another farm ~40 km away.

Studies were also conducted on the attractiveness of Magnet® to non-target organisms, especially beneficial insects (predators and parasites). Suction sampling on rows treated with Magnet® but omitting the insecticide was used to determine whether beneficial insects accumulated on or near Magnet®-treated sections of the field.

The five-component blend Magnet® encountered regulatory difficulties with one of the volatiles in the formulation, (Z)-3-hexenyl salicylate, because the Australian Pesticides and Veterinary Medicines Authority (APVMA) did not consider it to be a 'commonly used household/industrial chemical with a history of safe use', since it was not on the Generally Recognised as Safe (GRAS) list of the Flavour and Extract Manufacturer's Association of the USA (FEMA). As a result, experiments were conducted to find substitutes that were on the GRAS list of FEMA. Eventually, butyl salicylate was selected, and an additional volatile, anisyl alcohol, was added to the blend. The modified six-component blend was tested in field trials similar to those with the original five- component blend and proved more attractive than the original.

Development of short-range, bioactive, novel semiochemical products

The working hypothesis used in this study for the screening and identification of potential plants that may have insect behaviour modifying properties was based on the evolution of oviposition behaviour of insect females that tend to select plant species to maximise the survival of the larvae (Rausher 1982; Thompson and Pellmyr 1991). For example, ovipositing moths such as *Helicoverpa* spp. that select a host plant to support larval survival and performance use surface cues of the plant in the final decision to oviposit or not (Schultz 1988).

Cotton genotypes and other plants originally selected to be part of this study were chosen based on a demonstrated range of susceptibilities to oviposition by *H. armigera* (Jallow and Zalucki 1995, 1996; Jallow 1998; Jallow *et al*. 1999*a*, Jallow *et al*. 1999*b*) and feeding by other sucking pests (Mensah and Khan 1997). The genotypes of cotton (*Gossypium hirsutum*) selected were Sicala VII, Multiple Host-plant Resistance (MHR11), and Lumein. Other crops used in the study included sorghum (*Sorghum bicolor*), sweet corn (*Zea mays* convar. *saccharata* var. *rugosa*), pigeon pea (*Cajanus cajan*), and lucerne (*Medicago sativa*). These crops are used as trap and refuge crops for Bt resistance management and IPM of *Helicoverpa* spp. in transgenic and conventional cotton crops (Mensah and Khan 1997). In addition, *Clitoria ternatea* (referred to as 'Plant X' during the developmental stage of the work), a native herbaceous plant species with anecdotally observed insect behaviour modifying properties (Mensah 1999), was included in the investigation of behaviour-modifying properties against *Helicoverpa* spp. oviposition and feeding and toxicity in a mesh house and commercial cotton trials.

Between 2001 and 2002, the *C. ternatea*, cotton genotypes, and the selected refuge crops were planted in 12-m-row strips within commercial cotton fields at the cotton farm of BS Glennie and Son at Norwood in Moree, New South Wales. Counts of *Helicoverpa* spp. eggs and larvae on these crops were carried out fortnightly throughout the cotton season. The study identified *C. ternatea* as deterring insect egg-lay and feeding, as well as causing direct mortality to *Helicoverpa* spp. and other cotton pests. Thus, the plant was thought to contain some SPCs that may either kill, or modify the behaviour of, insect pests. Since *C. ternatea* had not previously received attention in terms of identification of SPCs and use of these SPCs to control pests, it was anticipated that preparation of crude extracts from *C. ternatea* for bioactivity testing and subsequent bioassay-directed fractionalisation of the plant or bioactive crude extract may reveal compounds or fractions for biological pest control that were previously unknown.

Between 2003 and 2004, crude extracts from *C. ternatea* were prepared by the project's collaborating chemist from Queensland Department of Primary Industries at Yeerongpilly, Brisbane, using three solvents with different polarity: water (high polarity), methanol (intermediate polarity), and hexane (apolar). Both surface rinsing and homogenisation of *C. ternatea* leaves were employed in the early stages of the study, and the crude extracts were bio-assayed for bioactivity against *Helicoverpa* spp. on cotton plants in the laboratory and mesh house at the Australian Cotton Research Institute (ACRI), Narrabri. The most promising crude extracts (particularly non-volatiles) that demonstrated biological activity against *Helicoverpa* spp. were identified and later fractionated.

A solid phase extraction technique similar to that used by Sharma *et al.* (2001) and Green *et al.* (2003) to identify feeding stimulants from pigeon pea pods towards *H. armigera* was used to fractionate extracts and to provide fractions for biological assays against insects. Six fractions were prepared and bioassayed for efficacy against *Helicoverpa* spp. oviposition and feeding and mortality on cotton plants.

The bioassay studies identified fractions 2, 3, 4, and 6 as having antibiotic effects on *H. armigera* larvae. Fractions 2, 3, 4, and 6 were also found to be toxic to the larvae. Thus, following the bioassay studies, the promising *C. ternatea* fractions were mixed and formulated in hexane and then bioassayed for efficacy against *Helicoverpa* spp. adult oviposition and larval feeding and mortality on potted cotton plants in the mesh house. The hexane formulation was found not to be stable when applied to cotton plants under natural field conditions. The next stage was to develop the *C. ternatea* fraction mixture into a more stable spray product, and so it was formulated in horticultural oil. The formulation was tested in small-scale field trials against *Helicoverpa* spp. and sucking pests on cotton crops under APVMA Permit 7250. The field trial was very successful and the *C. ternatea* formulation was found to control *Helicoverpa* spp. and other pests such as *Bemisia tabaci* (silverleaf whitefly) with efficacy similar to the conventional insecticide products used by growers to manage these pests on cotton in Australia.

Results

Long-range attractants

The levels of attraction obtained in the olfactometer with some individual volatiles and blends are shown in Fig. 1; (*Z*)-3-hexenyl salicylate ((*Z*)-3Hs) and the floral volatiles phenylacetaldehyde (PAA) and 2-phenyl ethanol (2P) produced significant attraction, but the terpenoids α-pinene, cineole, and limonene, which are prominent in *Eucalyptus* and *Angophora* spp., did not. Blending these compounds in the ratios in which they occurred in one particularly attractive eucalypt (the F3 blend) was also ineffective. However, addition of the F3 blend to PAA or 2P produced blends with enhanced attraction in the olfactometer (the four blends on the right of Fig. 1).

The PF3Hs blend, consisting of the three eucalypt terpenoids plus phenylacetaldehyde and (Z)-3-hexenyl salicylate became the first Magnet® blend, which ultimately proved problematic for registration, but was tested extensively in field-scale trials under an APVMA Product Evaluation Permit. Results from one such trial are shown in Table 1. The trial site was a large isolated farm Miralwyn near Walgett, NSW (30°01′29″S, 148°06′56″E), which was growing roughly equal areas of transgenic and non-transgenic cotton. Only the conventional fields (12 fields, totalling 1475 ha) were sprayed from the air with the attractant plus thiodicarb as the insecticide partner. The numbers of *Helicoverpa* spp. eggs per meter were recorded by independent consultants for all 12 conventional fields, and compared with 10 transgenic fields that were intermixed with the conventional fields, and with five fields from a distant control farm 40 km away. Prior to the first application of the attract-and-kill formulation on 24 November, the numbers of eggs were steadily rising in both treated and untreated fields, and on the control farm. Immediately following the first attractant application, egg numbers fell in the treated fields and (less rapidly) in the neighbouring untreated fields. Throughout the next six applications (29 November–26 December), numbers remained very low (up to 50 times lower) compared with the distant control farm. Thereafter, egg numbers remained low in all fields, and although there were occasional statistically significant differences, these would have been of no consequence for pest management.

In this trial, replication was provided only within farms. There are considerable difficulties in providing adequate replication when working with semiochemicals, which may have effects well beyond the location where they are applied, due to the large study areas required (~1600 km^2 in this trial). However, the

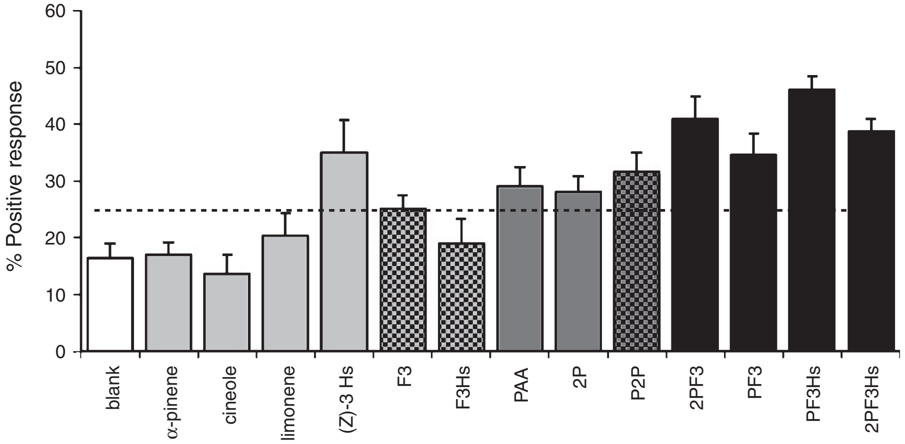

Fig. 1. Attractiveness of individual volatiles and blends to *Helicoverpa armigera* females in a two-choice olfactometer. Percentage positive response is the mean proportion of 50 individuals, from four replications, that went to the test chamber. White bar is a blank olfactometer. Light grey bars are individual leaf volatiles: (Z)-3Hs, (Z)-3-hexenyl salicylate; F3, a blend of α-pinene, cineole, and limonene; F3Hs, F3 + (Z)-3Hs. Mid-grey bars are floral volatiles: PAA, phenylacetaldehyde; 2P, 2-phenyl ethanol. Black bars are complex four- and five-component blends of floral and leaf volatiles. Error bars are standard errors of the means, and the dotted line represents a significant difference ($P < 0.05$) from the blank olfactometer.

Table 1. *Helicoverpa* spp. egg counts (eggs/m) in fields on the commercial cotton farm Miralwyn, treated with attract-and-kill formulation using the PF3Hs blend of volatiles (Fig. 1)

Egg counts from control fields on the same farm and distant fields on an untreated farm 40 km away are also shown. For days after first spray, the first value is the sequential number of the spray, and the value in parentheses is days after that spray when the count was made. Number of fields sampled varied due to difficulties of accessing some fields following rain or irrigation. Within rows, means followed by the same letter are not significantly different ($P > 0.05$) using one-way analysis of variance on $\log_{10}(x + 1)$ transformed data followed by Fisher's l.s.d. tests

Date	Days after last spray	No. of fields sampled			Mean no. of eggs/m		
		Treated	Control	Distant	Treated	Control	Distant
6 November	NA	12	6	0	3.9a	0.9b	
11 November	NA	12	6	0	6.8a	5.7a	
15 November	NA	9	10	0	14.6a	23.7b	
19 November	NA	9	10	5	14.7a	29.5b	11.7a
22 November	NA	9	6	5	29.5a	45.0a	67.1b
26 November	1 (2)	0	10	0		14.3	
29 November	1 (5)	12	10	5	6.1a	17.9b	13.3b
3 December	2 (4)	0	8	0		1.4	
6 December	2 (7)	12	10	5	2.8a	3.5a	14.1b
10 December	3 (3)	12	10	0	5.1a	3.1b	
17 December	4 (3)	12	9	5	1.9a	1.8a	36.5b
20 December	5 (1)	12	10	5	0.9a	1.6b	52.6c
24 December	5 (5)	11	10	0	1.3a	0.5b	
27 December	6 (3)	12	10	0	1.4a	0.6a	
31 December	7 (2)	12	10	0	5.3a	1.0b	
3 January	7 (5)	9	10	0	0.9a	2.5b	
7 January	8 (3)	12	10	4	1.8a	2.5a	5.6b
10 January	9 (1)	12	0	0	0.6		
14 January	9 (5)	9	7	0	3.2a	1.6a	
17 January	9 (8)	12	10	0	2.2a	0.6a	
21 January	10 (4)	9	10	5	5.8a	2.7b	1.5b
24 January	11 (2)	12	10	0	2.3a	0.9a	
28 January	11 (6)	12	10	0	2.9a	2.0a	
31 January	12 (3)	5	10	0	3.2a	2.3a	
7 February	12 (5)	10	10	0	1.0a	0.3a	
11 February	13 (3)	9	0	0	2.3		
14 February	13 (6)	12	10	0	0.6a	0.1a	
21 February	13 (13)	12	9	0	0.7a	0.1a	

experienced consultants who collected the egg data, and the farmers, regarded the reduction in egg laying during December and thereafter as highly unusual, and ascribed it to the attract-and-kill treatment. Similar area-wide studies have been conducted on a further seven occasions, and all have shown major reductions in *Helicoverpa* spp. populations on spatial scales of ≥1 km.

Short-range bioactive novel semiochemical products

Infestation by *Helicoverpa* spp. and other cotton pest was significantly lower on *C. ternatea* than on cotton and the other refuge crops (Fig. 2). Thus, *C. ternatea* was thought to contain some SPCs that may either kill or modify the behaviour of insect pests. Since *C. ternatea* had not received previous attention in terms of identification of SPCs and use of these SPCs to control pests, it was anticipated that a bioassay-directed fractionation of the plant might reveal compounds or fractions for biological pest control that were previously unknown.

The solid phase extraction technique yielded six fractions for bioactivity tests against *Helicoverpa* spp. on cotton plants. Cotton leaf disks treated with *C. ternatea* fractions 2, 4, and 6 were consumed by *Helicoverpa* spp. second-instar larvae at lower levels, resulting in lower weight gains by the larvae, compared with the other fractions and the control tested (Fig. 2). Fraction 2 appeared to have a stronger deterrent effect than fraction 4 and 6, so that the second instar resulted in a weight loss (Fig. 3).

The mixture of *C. ternatea* fractions 2, 4, and 6 in horticultural oil was found to reduce *Helicoverpa* spp. egg-lay on conventional cotton crops (Table 2). The extract was also found to be efficacious against *Helicoverpa* spp. first to third instar larvae on conventional cotton crops (Table 3).

In addition to *Helicoverpa* spp., the *C. ternatea* extract was found to control *Bemisia tabaci* (silver leaf whitefly) adults and nymphs (Fig. 4) when applied to infested cotton crops in the field. No significant difference in the number of *B. tabaci* nymphs per leaf was found among plots treated with *C. ternatea* and conventional insecticides (Fig. 4).

Discussion

The study on long-range, plant volatile-based attractants was initiated following a scientific exchange visit to USDA-ARS in Texas in 1996 by P. C. Gregg. During this visit, work was in progress on identifying the volatiles that made native American wildflowers, *Gaura drummondi*, *G. suffulta*, and *G. longiflora*, particularly attractive to foraging *Helicoverpa zea* (Shaver *et al.* 1998). There was also early collaboration by P. C. Gregg with a Chinese research group attempting to identify the volatiles that made wilted leaves of the Chinese wingnut tree and certain poplar species attractive to *Helicoverpa armigera* (Xiao *et al.* 2002; Wang *et al.* 2003). However, it was soon recognised that the unique species complex of Australian *Helicoverpa* spp., and the unique flora with which they had co-evolved, meant that attractive volatiles would have to be identified from Australian plants (Gregg *et al.* 1998).

The 38 plants tested in an olfactometer in this study were identified from Australian plants and were found to be attractive, but there was little correlation between attractiveness in the olfactometer and suitability as hosts for larval development. Thus, Gregg *et al.* (2010*b*) proposed a novel hypothesis for odour recognition in highly polyphagous moths such as *Helicoverpa* spp., and outlined some important principles for developing volatile blends that might serve as commercially viable attractants. Significantly, mimicking an attractive host did not seem necessary for *Helicoverpa* spp., as would be predicted by the dominant paradigm of insect attraction to host plants (e.g. Bruce *et al.* 2005). However, converting an attractant blend into attract-and-kill technology requires that a suitable toxicant be identified, and that issues of cost, persistence, and formulation be addressed.

The testing of 16 synthetic insecticides identified methomyl, thiodicarb, and spinosad as insecticide partners, as an attract-and-kill system for Magnet® for the management of *Helicoverpa* spp. The modified Magnet® formulation was eventually

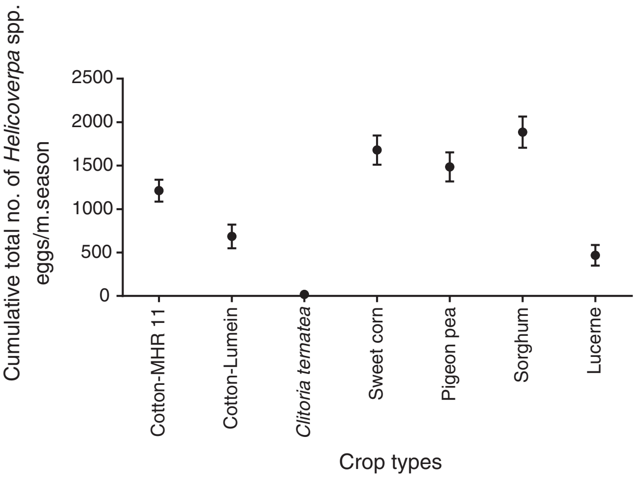

Fig. 2. Cumulative total number of *Helicoverpa* spp. eggs recorded on cotton and refuge crops inter-planted in commercial cotton field in Norwood near Moree in the 2001–02 season. Bars are standard errors of means.

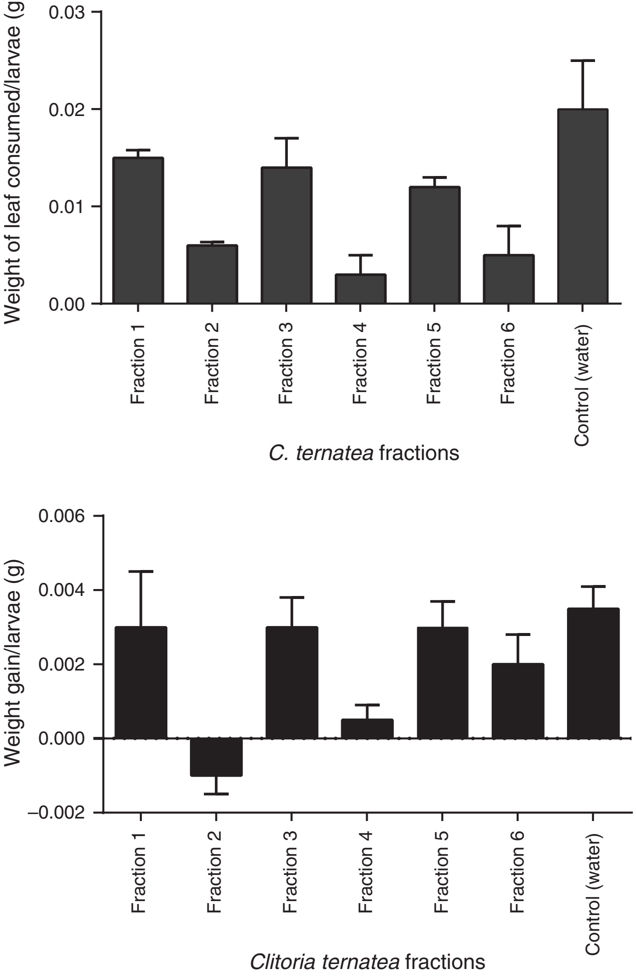

Fig. 3. Feeding response of *Helicoverpa armigera* third instar larvae on cotton leaves treated with *C. ternatea* fractions (no-choice tests) at ACRI in Narrabri, 2004–05

Table 2. Efficacy of *Clitoria ternatea* extracts and conventional insecticides on *Helicoverpa* spp. eggs (no. of eggs/m) on commercial conventional cotton crops at the Australian Cotton Research Institute, Narrabri, 15 February–1 March 2010

DAT, Days after treatment. Within columns, means followed by the same letter are not significantly different ($P > 0.05$); Tukey-Kramer multiple comparison test. Odour emanating from the semiochemical plots may have resulted in reduced egg lay on the unsprayed plots

Treatments	Pre-treatment counts	3 DAT	7 DAT	14 DAT
C. ternatea, 1.0 L/ha	6.67 ± 0.56a	3.67 ± 0.21a	4.33 ± 0.21a	0.00 ± 0.00a
C. ternatea, 1.5 L/ha	3.67 ± 0.56b	3.00 ± 0.37a	4.33 ± 0.76a	0.00 ± 0.00a
C. ternatea, 2.0 L/ha	3.33 ± 0.21b	3.00 ± 0.37a	4.33 ± 0.21a	0.00 ± 0.00a
Spinosad, 0.80 L/ha, conventional	3.33 ± 0.21b	3.67 ± 0.21a	8.67 ± 0.84b	9.67 ± 1.04b
Unsprayed (control)	8.00 ± 0.37a	11.00 ± 1.32b	6.33 ± 0.56ab	11.00 ± 1.37b
Level of significance	$P < 0.0001$	$P < 0.0001$	$P < 0.025$	$P < 0.0001$

Table 3. Efficacy of *Clitoria ternatea* and conventional insecticides on *Helicoverpa* spp. very small and small larvae (first to third instar; no. of larvae/m) on commercial cotton crops the Australian Cotton Research Institute (ACRI), Narrabri, 15 February–1 March 2010

DAT, Days after treatment. Within columns, means followed by the same letter are not significantly different ($P > 0.05$); Tukey-Kramer multiple comparison test

Treatments	Pre-treatment counts	3 DAT	7 DAT	14 DAT
C. ternatea, 1.0 L/ha	2.67 ± 0.21a	1.33 ± 0.21a	1.33 ± 0.21a	1.00 ± 0.37a
C. ternatea, 1.5 L/ha	2.67 ± 0.42a	1.33 ± 0.21a	1.00 ± 0.36a	0.67 ± 0.21a
C. ternatea, 2.0 L/ha	1.33 ± 0.21b	0.33 ± 0.21b	0.33 ± 0.21b	0.67 ± 0.21a
Spinosad, 0.80 L/ha, conventional	1.33 ± 0.21b	0.00 ± 0.21b	0.33 ± 0.21b	0.33 ± 0.21a
Unsprayed	3.00 ± 0.37a	1.67 ± 0.42a	2.33 ± 0.21c	3.33 ± 0.21b
Level of significance	$P < 0.001$	$P < 0.0001$	$P < 0.0002$	$P < 0.0001$

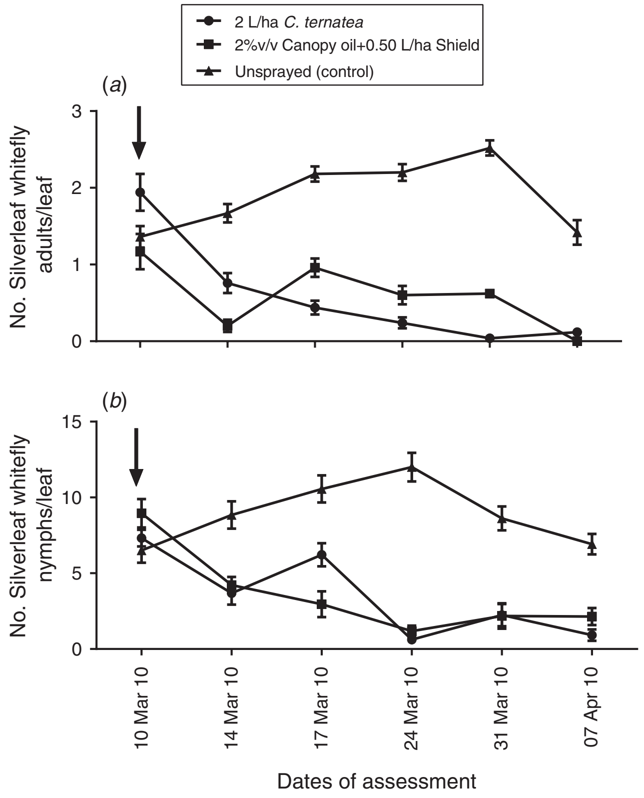

Fig. 4. Efficacy of *Clitoria ternatea* against silverleaf whitefly (*Bemisia tabaci*) (*a*) adults and (*b*) nymphs per leaf on commercial cotton crops at Norwood near Moree, 2009–10. Arrows indicate spray application dates.

registered for use on cotton, corn, and beans in 2009, with methomyl, thiodicarb, and spinosad as insecticide partners, as an attract-and-kill system for *Helicoverpa* spp. Prior to registration it was realised that the mobility of *Helicoverpa* spp. moths meant that Magnet® might have area-wide impacts. Treatments of individual fields (tens of hectares) or groups of fields (hundreds of hectares) could produce reductions in moth populations and subsequent oviposition extending for several kilometres (Del Socorro *et al*. 2003; Grundy *et al*. 2006; Mensah and Macpherson 2010). It was only necessary to treat one row in every 72, or one in every 144, to kill many moths and produce substantial reductions in egg pressure that extended well beyond the treated fields.

Only limited attraction to a few beneficial species was detected (P. C. Gregg and A. P. Del Socorro, unpublished data 2009), and the fact that ~99% of the field was untreated allowed high survival of beneficials, especially the non-mobile immature stages. Field studies have shown no significant changes in beneficial numbers (Mensah and Macpherson 2010), meaning that Magnet® should have a good fit in IPM schemes.

Investigations to find a substitute to (*Z*)-3-hexenyl salicylate, which APVMA did not consider a 'commonly used household/industrial chemical with a history of safe use' since it was not on the GRAS list of FEMA, delayed registration of the Magnet® product by about 2 years. This was because it was necessary to repeat many of the efficacy, non-target impact, and residual activity studies that were part of the original five-component Magnet® registration package. The lessons from this experience are discussed in a review paper on commercialisation of novel pest-management products (Gregg *et al*. 2010*a*).

The modified Magnet® formulation, as registered in 2009, was used on up to 50% of conventional (non-Bt) cotton, and there was significant use on sweet corn. However, from 2009, the acreage of conventional cotton fell markedly as Bollgard II® cotton increasingly dominated the market. Bollgard II® cotton occasionally needs treatment due to the survival of *Helicoverpa* larvae (Lu *et al*. 2011), but it does not need the systematic, area-wide protection provided by Magnet®, if resistance to the toxins in Bollgard II® cotton remains manageable. For this reason, current research on Magnet® focuses on its potential to contribute to the resistance-management plans that are obligatory for growers of Bollgard II® cotton. Work has been done on the potential for improving the productivity of refuges that produce susceptible moths (Addison 2010), but the gains from this approach appear limited, probably because feeding and oviposition can be spatially separated in *Helicoverpa* spp. (P. C. Gregg, A. Del Socorro, A. Hawes, unpubl. data, 2005). The use of Magnet® to directly target potentially resistant moths emerging from Bollgard II® cotton late in the season offers more promise. This approach, termed 'moth busting' (Gregg and Del Socorro 2010; Gregg *et al*. 2012), is being investigated as a potential substitute for 'pupae busting'. Pupae busting is cultivation of the soil to kill overwintering pupae, and is currently a requirement of resistance management plans, but poses many agronomic and environmental problems, especially for dryland cotton growers (Ceeney *et al*. 2013).

The foundation of the research to develop short-range, bioactive, novel semiochemical products was a CRDC

Occasional paper (Jallow *et al*. 1999*a*) and a CRDC-commissioned review (Mensah and Moore 1999). Jallow *et al*. (1999*a*) recommended, *inter alia*, that the CRDC commission research to better elucidate the nature of variation in host-plant utilisation by *Helicoverpa* in the field, with longer term objectives including the identification of plant characters that would render the cotton plant a less suitable host to *Helicoverpa* species. The role of volatile plant chemicals in pre-alighting host selection, and of both leaf texture and leaf-surface chemistry in in post-alighting behaviour, were highlighted.

The review by Mensah and Moore (1999) also recommended that the CRDC commission research to assess and compare chemical components of the organ surfaces and inner tissues of cotton plants, other hosts, non-hosts, and potential trap crops. The review envisaged exploitation of such chemicals as stimulants, deterrents, attractants, and repellents in conventional spray programs, either alone or in combination with biopesticides or synthetic insecticides.

When the project was formulated, it was anticipated that other projects funded by the cotton industry in host plant resistance, trap cropping, conservation of beneficial insects, insecticide resistance, and others would benefit from the better understanding of the chemical ecology of insect–plant interactions. Thus, the project sought to isolate and identify natural plant chemicals that demonstrate stimulant or deterrent/repellent activity towards *Helicoverpa* spp. on cotton and other relevant crops, with both oviposition and larval feeding being targeted.

Although a thorough literature search preceded this study, as reflected in Mensah and Moore (1999), the study was approached with no preconceptions about the nature of behaviourally significant chemicals. There was no intention to focus on compounds that are of known structure, properties, or general behaviour-modifying characteristics that were already in the public domain. However, if these compounds emerged as particularly relevant during the bioassay and chemical identification studies in various plants or plant parts, etc., as significant behaviour determinants, then they would be studied in detail. Nevertheless, focusing on the analysis of known compounds logically precludes the discovery of new secondary plant compounds. Thus, the project focused on the selection and screening of plants for which there was anecdotally observed preference or non-preference by insect pests, potentially due to SPCs able to modify the behaviour of *Helicoverpa* spp. and sucking pests when the plant's extracts are applied to primary crops such as cotton. Bioassay-directed fractionation of the plant materials could reveal compounds of previously unknown structure, particularly in the case of plants that had received little or no previous attention.

So far, eight different formulations of individual *C. ternatea* fractions and mixtures have been developed and evaluated against *Helicoverpa* spp., mirids, shield bugs, and silverleaf whiteflies in small-scale field trials on cotton under APVMA permit 7250 in collaboration with Growth Agriculture Pty Ltd (commercial partners). A new biopesticide product now known as Sero X® has been developed from *C. ternatea*. The study found minimal impact of the *C. ternatea* product on beneficial insects, less than the commercial synthetic insecticides, indicating that the *C. ternatea* product should be compatible with IPM.

We considered regulatory requirements during the fractionation stage of the project. The United States Environmental Protection Agency, Office of Pesticide Program in 1979 recognised that semiochemicals were inherently different from synthetic insecticides and so made a policy statement encouraging the development and registration of semiochemicals as safer alternatives to conventional pesticide products (Tinsworth 1990). Unfortunately, initial Australian regulatory difficulties have been encountered, with the APVMA classifying the product as a pesticide, and thus, many years of mammalian and environmental toxicology tests are to be undertaken. Collaboration with APVMA and their advisors, along with formulation changes, have been undertaken to minimise these studies and costs, although considerable toxicology tests are still required. The delayed registration of the Magnet® product for 2 years (Gregg *et al*. 2010*a*) provided us with experience in the pursuance of registration of Sero X®. However, Sero X® was developed from a plant used as fodder for cattle that contained many compounds, some of which had no previous commercial uses and for which toxicological data were not available, whereas Magnet® was developed from a blend of a few synthetically produced and well-known compounds.

It is anticipated that the aim for use of Sero X® in cotton pest management is as part of the industry's IPM program to modify the behaviour of insect pests on cotton crops by deterring pest egg-lay and feeding, as well as causing mortalities to pests to reduce/suppress populations of *Helicoverpa* spp. and other sucking pests. It is also anticipated that Sero X® might be used, in conjunction with attractants such as Magnet® or attractive crops, in push–pull systems that manipulate *Helicoverpa* spp. populations in cotton landscapes for the purposes of IPM and/or Bt resistance management.

In cotton cropping systems, Magnet® (moth attractant) and Sero X® (oviposition, feeding deterrents, and semiochemical) can be integrated and exploited as behavioural manipulation methods of pests to manage *Helicoverpa* spp. The use of these products will elicit some changes in pest behaviour. Additionally, Sero X® used as a mixture with reduced rates of insecticides can enhance synergism while reducing the quantity of synthetic insecticide actives used on the cotton crops without sacrificing product efficacy. On the other hand, Magnet® can be applied to alternative crops bordering cotton crops to divert *Helicoverpa* spp. moths from the primary crop (cotton) to the alternative crops where they can be controlled using synthetic insecticides, or used to genetically dilute moth populations to manage insecticide and Bt resistance on conventional and transgenic (Bt) cotton crops. Magnet® can also be used in an attract-and-kill strategy to control *Helicoverpa* spp. moths to reduce infestation levels on conventional cotton crops. Therefore, these two semiochemical products can be exploited in the context of IPM to manage *Helicoverpa* spp. and other pests in agricultural crops such as cotton. The strategy to integrate the long- and short-range semiochemicals is yet to be developed on transgenic (Bt) and non-Bt cotton systems in Australia.

The studies reviewed here indicate that semiochemicals operating over both long and short range have considerable potential for the management of key pests of cotton such as *Helicoverpa* spp., and emerging or secondary pests such as whiteflies and green mirids. The products described here have taken a decade to bring to commercial use, which is comparable to the time required for new conventional insecticides (Gregg *et al*. 2010*a*). In this period, many changes can affect the likely markets for such products. For the Australian cotton industry, the major change over the last decade has been the increasing dominance of transgenic cotton. This has meant that the roles originally envisaged for these semiochemical products, as key platforms for IPM in conventional cotton, are now less relevant. Nevertheless, history has repeatedly shown the risks in depending too heavily on one pest management tactic (Fitt 1994), and it is prudent to develop new tactics which complement transgenic technology. The use of deterrents and anti-feedants against changing pest complexes is one example of this strategy. Similarly, potential use of long-range attractants for resistance management might help prolong the life of key transgenes.

In principle, there are several strategies for exploiting semiochemicals in managing pests, especially *Helicoverpa* spp., on transgenic or conventional cotton crops. These include: (*i*) use semiochemical lures with insecticides to attract and kill pests, (*ii*) apply semiochemicals onto cotton plants to deter pest oviposition and feeding, (*iii*) apply semiochemical lures with insecticides to stimulate oviposition and feeding on another crop, (*iv*) apply semiochemicals directly to pests to cause direct mortality, and (*v*) mix semiochemicals with insecticides to enhance synergism (through exploitation of semiochemicals as stimulants, deterrents, attractants, or repellents in conventional spray programs to manipulate the behaviour of the pest or cause direct additional mortality of the pest to protect the resource). The studies reported here have only begun to explore these possibilities.

Research aimed at developing commercial products can often provide new insights into some fundamental questions of insect chemical ecology, which in turn open new possibilities for pest management. For example, the dominant paradigms of how insects are attracted to plants by olfaction have been developed with monophagous or oligophagous species. They emphasise the importance of either unique volatile compounds (Fraenkel 1959) or species-specific ratios (Bruce *et al*. 2005). Neither of these hypotheses is compatible with the wide range of blends, none of which resembled a real host, that are attractive to *Helicoverpa* spp. (Gregg *et al*. 2010*b*). The adoption of 'super-blending', as proposed by Gregg *et al*. (2010*b*), opens many options and helps prevent the narrow focus on the importance of particular volatiles that has hampered many previous attempts to develop commercial attractants. This helps to avoid volatiles that may prove too expensive to compete with cheaper, broad-spectrum insecticides (such as synthetic pyrethroids), or may pose regulatory difficulties because of the absence of toxicological data. We believe this is a major reason why Magnet® remains, as far as we know, the only registered, plant volatile-based attract-and-kill technology that can be applied directly to crops.

In the study of insect chemical ecology to develop a semiochemical product, understanding of the sequence of behavioural events (such as searching, orientation, encounter, landing, surface evaluation, etc.) leading to host find or acceptance (Kogan 1977; Renwick and Chew 1994) is very important. All stages of the host finding and acceptance sequence depend on a wide variety of cues, both sensory (Renwick and Chew 1994) and chemical (Renwick 1989). After the insect alights on a plant, contact perception of both physical and chemical characteristics of the leaf or other organ surface becomes the most important factor in determining the suitability of the host (Blaney and Chapman 1970). Therefore, if a plant was found to deter oviposition or feeding or to be toxic to insect pests, then the plant's insect behaviour modifying properties may not be due to only one or two unique bioactive compounds alone but there may be a whole range of chemical compounds in the plant's extract acting together to cause that effect and protect the plant from predation. The identification and isolation of a single bioactive compound from a plant's crude or fractionalised extracts to develop a semiochemical product may not be as effective in managing pests as the crude or fractionated extracts developed into a product. Thus, the development of an effective semiochemical product based on a single active ingredient (similar to synthetic insecticides) may not be difficult to achieve, but may not have the whole range of chemical compounds acting together to manage the target pests. Therefore, the adoption of a strategy whereby a fractionated extract that contains a whole range of a plant's SPCs (e.g. Sero-X) can be exploited to manage pests such as *Helicoverpa* spp. and other pests in agricultural crops such as cotton. Ultimately, the success of any particular strategy to manage *Helicoverpa* spp. in cotton will depend on the efficacy of the semiochemical product on the oviposition and feeding behaviour as well as its toxicity to the pest on the host plant.

Conclusion

In light of the evidence provided in this study, exploitation of semiochemicals as stimulants, deterrents, attractants, repellents, or synergists in conventional spray programs, either alone or in combination with biopesticides or synthetic insecticides, has the potential to manipulate the behaviour of the pest or cause direct mortality of the pest to protect the target crop. For polyphagous species such as *Helicoverpa* spp. that are attracted as adults to nectar-bearing plants, long-range attractants based on super-blended mixtures that are not found in nature might form the basis of effective attract-and-kill technologies. Similarly, for ovipositing insects such as *Helicoverpa* spp. that do not contact the inner tissues of the plant (i.e. feed on the plant), recognition and selection of the host plant after landing could be determined by small quantities of many types of chemical substances that come from the inner tissues of the plant and are present on the plant surface. Masking plant surface cues with semiochemicals can play a major role in host selection and acceptance for oviposition and feeding of *Helicoverpa* spp. Hence, semiochemicals sprayed on host plants can change the behaviour of the pest, which may avoid the host plant or lay fewer eggs, feed less, or die from the spray.

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