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Developing Bisexual Attract-and-Kill for Polyphagous Insects: Ecological Rationale versus Pragmatics

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Abstract We discuss the principles of bisexual attract-and-kill, in which females as well as males are targeted with an attractant, such as a blend of plant volatiles, combined with a toxicant. While the advantages of this strategy have been apparent for over a century, there are few products available to farmers for inclusion in integrated pest management schemes. We describe the development, registration, and commercialization of one such product, Magnet®, which was targeted against *Helicoverpa armigera* and *H. punctigera* in Australian cotton. We advocate an empirical rather than theoretical approach to selecting and blending plant volatiles for such products, and emphasise the importance of field studies on ecologically realistic scales of time and space. The properties required of insecticide partners also are discussed. We describe the studies that were necessary to provide data for registration of the Magnet® product. These included evidence of efficacy, including local and area-wide impacts on the target pest, non-target impacts, and safety for consumers and applicators. In the decade required for commercial development, the target market for Magnet® has been greatly reduced by the widespread adoption of transgenic insect-resistant cotton in Australia. We discuss potential applications in resistance management for transgenic cotton, and for other pests in cotton and other crops.

Keywords Attract-and-kill · Plant volatiles · *Helicoverpa* spp. · Registration · Market development

Background – Recent Developments in Attract-and-Kill

The paradigm of integrated pest management (IPM) currently embodies the theory and practice of arthropod pest management (Way and van Emden 2000), especially in the Australian cotton industry, which is now dominated by transgenic insect resistant (Bt) varieties, notably Bollgard II® (Wilson et al. 2013). However, there remains a need for new and selective techniques for managing *Helicoverpa* spp. in cotton and other crops, as well as for managing emerging and secondary pests. Behavioral manipulation using semiochemicals is at the forefront of this effort (Mensah et al. 2013).

Attract-and-kill, targeting the adult stages, is one promising approach. Most products available for this purpose have been based on pheromones, especially sex attractants (El-Sayed et al. 2006; Witzgall et al. 2010). These are usually highly specific, but in most cases they attract only males. The object is to remove so many males from the local population that females are unable to find mates, and numbers in the subsequent generations are reduced. This approach has been successful with some orchard pests, such as the codling moth *Cydia pomonella* (L.) (Charmillot et al. 2000), the Oriental fruit moth *Cydia molesta* (Busck) (Evenden and McLaughlin 2004), and the light brown apple moth *Epiphyas postvittana* (Walker) (Brockerhoff and Suckling 1999). However, for insects such as *Helicoverpa* spp. which are capable of multiple mating (Baker and Tann 2013), a high proportion of the males must be removed. Immigration of females that have already mated outside the treated area may negate any local shortage of males, which adds to the

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difficulties of attract-and-kill with pheromones for highly polyphagous and mobile insects such as *Helicoverpa* spp. These problems are shared with mass trapping and mating disruption, and mean that successes with male-attracting pheromones are largely restricted to species with limited levels of polyandry, polyphagy, and mobility, and have been most common in islands or ecologically isolated areas (Witzgall et al. 2010).

In contrast, female-specific or bisexual attractants offer the possibility of more direct impacts on pest populations, especially those not suited to strategies involving male attractants. Removing a female also removes her potential fecundity. The advantages of this, and possible approaches to exploiting them, have long been recognized. Consider the prescient remarks of Trägårdh (1913): "*…we ought… to be able to discover in the chemotropical reactions of insects in many cases a superb weapon in the fight against noxious species. For it has always been considered that prevention is better than cure, and of all methods in preventing devastation it is undoubtedly nearest to the ideal in which we succeed in capturing females ere they have had an opportunity of ovipositing. And we shall probably be able to effect this if we succeed in isolating the organic substances in food-plants of larvae, towards which the females react with positive chemotropism*". Over a hundred years later, with the detour in the philosophy of pest management associated with synthetic chemical insecticides now showing abundantly obvious limitations, and after a century of theoretical and empirical progress in "isolating the organic substances", are we much closer to realising the potential of female or bisexual attractants?

Here we focus on the development of one product, Magnet®, which is based on a blend of plant volatiles and was registered in Australia in 2009, for control of *Helicoverpa* spp. in cotton, corn, and beans (Gregg et al. 2010b). This registration, which we believe to be the first of its kind in the world was the culmination of over a decade of research under the Australian Cooperative Research Centres scheme (Australian Government 2016). This scheme supports collaborative projects between public sector organizations and industry, in our case AgBitech Pty. Ltd. (AgBiTech 2016a). We describe the work from initial concepts through laboratory research, small- and large- scale field trials to registration and market development.

Ecological Theory Versus Pragmatics

Our work was inspired by research in Texas on the attractive components of wildflowers, *Gaura* spp., to *Helicoverpa zea* (Boddie) (Kint et al. 1993; Shaver et al. 1998), and its aim of developing a bisexual attract-and-kill product that could be sprayed on a field crop (Lopez et al. 2000). This research reflected contemporary (and still widely current) thinking about the ways in which insects use plant volatiles to recognize their hosts. Earlier ideas about unique volatiles specific to particular plants, foreshadowed by Trägårdh (1913) (see above) and elaborated by Fraenkel (1959), suggested that such volatiles might be used alone for attract-and-kill. However, there are few such volatiles even at the plant family level, and in general blends produce greater attraction than single volatiles (Szendrei and Rodriguez-Saona 2010).

The unique volatile hypothesis has largely been supplanted by the ratio-specific hypothesis (Bruce and Pickett 2011; Bruce et al. 2005). This postulates that a template of key volatiles, present in specific ratios, provides a model for host recognition. The implication is that plant volatile blends for attracting females should mimic these templates. The work with *Gaura* spp. followed this approach, as did earlier work on *Helicoverpa armigera* (Hübner), mimicking the volatile profile of pigeon peas (Rembold et al. 1991), and later work on the same species which used marigolds as a model (Bruce and Cork 2001).

While plausible for oligophagous insects, the ratio-specific model poses obvious difficulties for highly polyphagous species such as *H. armigera*, which has larval hosts in at least 35 plant families. The other major heliothine pest of Australia, *H. punctigera*, has hosts in at least 49 families (Cunningham and Zalucki 2014). Both these species also feed as adults on nectar from many hosts that do not necessarily support larvae (Gregg 1993). The question therefore arises: which host do we mimic? Should it be a host for oviposition or for adult feeding? How would we recognize a suitable model?

It has been suggested that even such highly polyphagous insects as the heliothine moths have primary or key hosts, with which they share a long co-evolutionary history and which are particularly important in their population dynamics (Walter 2005). It might seem these would be good models for mimic blends (Rajapakse et al. 2006), but the difficulties of identifying them are considerable, and they may not always be ecologically important. For *H. punctigera* it was suggested that the daisy *Ixiolaena brevicompta* F. Meull was a primary host (Walter and Benfield 1994), but this species is absent from non-cropping areas in inland Australia, which are major sources of immigrants to cropping areas, and where host availability is highly variable. It is more likely that, if there are any meaningful primary hosts for *H. punctigera* in this region, they are the legumes *Cullen* spp. (Gregg et al. 2016).

Even where primary hosts can be identified, there are theoretical difficulties in mimicking. Volatile profiles vary considerably with intrinsic factors, such as plant phenology (Bengtsson et al. 2001) and extrinsic factors, such as the time of day (Shaver et al. 1997). Blend activity may be greatly affected by components that are minor both quantitatively in volatile profiles and in their behavioral effects in isolation. This has been noted at both physiological levels (Pinero et al. 2008) and in behavior, to the point where blend attraction may even be enhanced by components that are repellent in isolation (Collatz and Dorn 2013).

Mimicking also has pragmatic disadvantages. It may promote the idea that certain components are essential, and if those components are expensive or problematic for regulatory reasons, it may prove difficult to commercialize a product. This can be the case for volatiles that are not widely used in other industries (such as fragrances, foods, or cleaning products), and so have not been thoroughly investigated for their toxicological and environmental properties. It also is a risk for volatiles that are expensive to synthesize, such as enantiomers.

Identifying Potential Volatiles for Magnet®

Given the difficulties of identifying a potential model to mimic in an attractant blend for *Helicoverpa* spp., we began by testing the attractiveness of a wide range of plants for *H. armigera* (Gregg et al. 1998). We used a two-choice olfactometer based on the design of Beerwinkle et al. (1996) and tested responses of unmated male and female moths to a total of 38 plants. Of these, only five were not attractive. There was a strong correlation between attractiveness to males and attractiveness to females (Del Socorro et al. 2010a). There was no correlation between attractiveness to adults and suitability as a host for larvae. Four of the five most attractive species were eucalypt trees, which do not support larvae but are among the most abundant sources of nectar in many Australian landscapes. Pollen from such species frequently is abundant on the proboscis of both *H. armigera* and *H. punctigera* (Gregg 1993), indicating their importance for adult feeding and probably reflecting a co-evolutionary relationship dating from well before the introduction of European agriculture. These observations suggested that attraction in the olfactometer was for feeding rather than oviposition.

We next profiled the volatiles emitted by our test plants, using solid- phase microextraction (SPME) followed by gas chromatography- mass spectrometry (GC-MS). The SPME fibre was inserted into the airstream of the olfactometer, so that it would have been absorbing the same volatiles that the moths were responding to. In this way, we identified 80 volatiles that were present in more than one of the plants (Del Socorro et al. 2010a). We then ranked these volatiles, which included green leaf volatiles, terpenoids, and aromatics, according to the number of plants in which they were found, and the relative attractiveness of those plants. This resulted in a total of 34 volatiles that were considered worth testing as potential attractants.

We also collaborated with researchers using electrophysiological techniques, including EAG (Cribb et al. 2007) and single cell responses (GC-SCR) (Stranden et al. 2003b). These collaborations did not provide any new volatiles for testing, other than the sesquiterpene germacrene D. They did, however, assist our thinking on which volatiles to prioritize for further testing in the olfactometer.

Combining Volatiles into Blends

When we tested the 34 candidate volatiles on their own in the olfactometer, only seven showed statistically significant attraction (Gregg et al. 2010a). Even the best of these (phenylacetaldehyde, 2-phenylethanol, and (*Z*)-3 hexenyl salicylate) were much less attractive than the majority of plants that we had tested previously.

Blends generally are more attractive than single volatiles (Szendrei and Rodriguez-Saona 2010), although there seem to be few gains from more than four components. For testing blends, we abandoned the mimicking approach, for the reasons discussed above. Instead, we adopted an approach we termed "superblending": the combinations of volatiles in blends that do not necessarily resemble any real plant, but are based on empirical determination of attractiveness (Gregg et al. 2010a). Comparisons of 31 blends, each with from two to seven components, in olfactometer studies indicated some general guidelines for blending. The best blends were characterized by a minimum of four components, with a diversity of chemical types and tissues of origin (leaf vs. flower). One or both of the floral volatiles phenylacetaldehyde and 2-phenylethanol were essential and performed best with a background of terpenoids common in eucalypts, including cineole, limonene, and α-pinene.

At this point we engaged a commercial partner, AgBitech Pty. Ltd., who provided advice on pragmatic considerations. Some volatiles were excluded from further consideration because their toxicological profiles were either problematic or insufficiently understood. They would have presented obstacles to registration. Other volatiles were excluded because they were too expensive. For example, there are more receptor neurones in heliothine moths that are tuned to germacrene D than to any other volatile (Stranden et al. 2003a), but they are tuned specifically to the (−) enantiomer. Relatively pure (−) germacrene D is extremely expensive, and products that included significant amounts of it would not be cost-competitive with cheap broad spectrum insecticides such as pyrethroids. A partially purified mixture of germacrene D enantiomers, which also contained various terpenoids, was tested and shown to be very attractive (Gregg et al. 2010a), but was nevertheless excluded on the grounds of cost and toxicological uncertainties.

Insecticides and Other Components

We envisaged an attract-and-kill formulation that would be lethal after ingestion by moths. This required the additions of a feeding stimulant and a toxicant, since the plant volatiles we tested were unlikely to be sufficiently toxic on their own. Sucrose is a cheap and effective feeding stimulant for many adult lepidopterans, including heliothine moths (Lopez and Lingren 1994). We included it at 30 % *w*/*v* in formulations for testing potential insecticide partners. We utilized the proboscis extension reflex (Fan et al. 1997) to facilitate ingestion (Fig. 1). We recorded the percentage mortality and the time taken to incapacitate, and then kill, each moth. In this way, we tested 16 potential insecticide partners. Two carbamates, methomyl and thiodicarb, produced high kills at low concentrations, and quickly incapacitated moths. The fermentation derivative spinosad also was effective at low concentration, but took much longer to incapacitate moths, as did the pyrethroids cyfluthrin and bifenthrin [the latter only when synergized with piperonyl butoxide (PBO)], and the organochlorine endosulfan. The remaining ten insecticides were not effective enough to warrant further consideration, even though many of them were registered for control of *Helicoverpa* spp. larvae.

Desirable properties in an insecticide partner for bisexual attract-and-kill include effectiveness at appropriate concentrations, rapid killing (or at least, incapacitation), lack of deterrent or repellent effects, and lack of toxicity to non-target organisms, including applicators. We anticipated that the product registration would be facilitated if the active ingredient(s) was already registered in cover sprays for the target crops (especially cotton), and was effective in our tests at concentrations that would produce a loading, per unit area of crop, that would result in levels at or below those received in a cover spray at the highest registered dosages. For the two carbamates, this was 0.5 % a.i. Rapid incapacitation also was desirable, as moths that were incapacitated or killed within a few seconds of ingesting the formulation could be found adjacent to treated areas, while slower-killing insecticides would have allowed them to fly away. For small-scale field trials, it was essential to find dead moths in order to evaluate attractiveness and impact. The insecticide that acted fastest was methomyl, but it has high mammalian toxicity, so we restricted its use to field trials by experienced researchers (Del Socorro et al. 2010b). Commercial-scale trials used thiodicarb. Spinosad has the least impact on non-target organisms, but does not allow ready location of dead insects, and was used only when this was not important. Subsequent work has shown that the synthetic analogue of spinosad, spinetoram, is also effective, and it has been used in field trials against diamondback moth, *Plutella xylostella* (L.) (P.C. Gregg, A.P. Del Socorro and M.R. Binns, unpublished data).

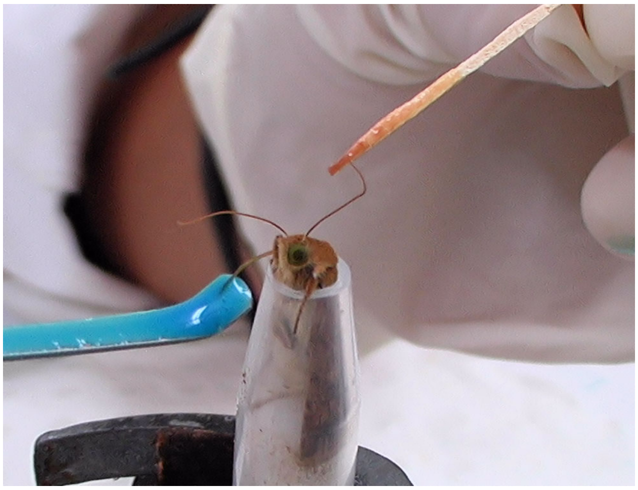

Fig. 1 The proboscis extension reflex method for testing insecticide partners for Magnet® (Del Socorro et al. 2010b). The moth was restrained in an Eppendorf tube with the tip cut out so the head protruded, and prevented from backing out of the tube by a plug of cotton wool. When the antenna was touched with a toothpick dipped in 30 % sucrose, the moth extended its proboscis and fed on Magnet® containing insecticide, presented on a spatula. The quantity ingested could be determined by weighing before and after feeding, or by scoring the extent of blue dye in the digestive tract. Moths were kept for 24 h after dosing, and the time to incapacitation and death was recorded

Small-Scale Field Tests

We used traps in initial field trials. While mass trapping with plant volatiles is effective in some situations (Camelo et al. 2007), we did not believe it would be ecologically or economically feasible against highly mobile pests of broad acre crops such as cotton, so we envisaged traps as only a test platform for initial evaluation of blend attractiveness in the field. Our standard experimental design was a 4 × 4 Latin Square, in which four treatments were replicated four times, with traps spaced 50 m apart and rotated between locations. Universal pheromone traps (AgriSense BCS Pty. Ltd., UK) were used. Plant volatiles were incorporated in Sirene®, a slow-release matrix used for pheromone-based attract-and-kill (Charmillot et al. 2000). In each experiment one treatment was a control (blank traps), and another consisted of the standard pheromone used in Australia for monitoring *H. armigera* (Gregg and Wilson 1991). The other two treatments were candidate volatile blends.

The trapping studies generally were disappointing, although a few *Helicoverpa* spp. moths of both sexes were caught. Most of the work was not published, although the results of 21 such trials were summarized in a patent application (Gregg and Del Socorro 2002). Usually the numbers of moths in traps with plant volatiles were vastly lower than those in pheromone-baited traps, and often too low to allow statistical comparison of the blends. Observations with night vision glasses (P.C. Gregg and D.R. Britton, unpublished data) showed that moths would approach a pheromone baited trap more closely than one with plant volatiles, suggesting that visual trap avoidance affected catches more if the attractant was a blend of plant volatiles than if it was a pheromone. Comparison with pheromone trap catches can, therefore, give an unduly pessimistic indication of the chance of success with plant volatile mixtures.

Following the poor results from trap studies, we moved to trials where the attractants were sprayed on crops. This required a Research Permit from the regulator, the Australian Pesticides and Veterinary Medicines Authority (APVMA 2016). Generic small-scale permits were available with minimal data requirements, but only if the treated crop was destroyed in a manner that prevented entry into the food chain. To avoid this it was necessary to provide toxicological and other data on all potential active ingredients, both attractants and toxicants. Our initial Research Permit covered 20 volatiles and two insecticides, for all of which pre-existing toxicological data were available. Trials were permitted on 2–50 ha of cotton, beans and sweet corn.

Using this permit we carried out over 20 trials that involved spraying sections of crop rows with formulations containing various plant volatile blends and including a toxicant. These trials also are largely unpublished, although a detailed description of the methodology is provided by Del Socorro et al. (2010b) and summaries of the results of some trials are given in Gregg and Del Socorro (2002) and Hawes et al. (2008). Fixed sections of row, usually 50 m, were treated with 250–500 ml of oil and water emulsion formulations containing the candidate plant volatiles along with sucrose, methomyl (0.5 % a.i.) and various other excipients such as anti-oxidants and thickeners. Since coverage of the foliage was not necessary for an attractant, coarse sprays or droplets shaken from "pop-top" water bottles were applied. Dead moths were collected from furrows surrounding the treated row, early in the morning for 4–6 d after treatment. They were identified to species and, in the case of *Helicoverpa* spp., dissected to determine their sex and mated status.

Typically, 4 × 4 Latin Square designs were used, and one treatment always was a blank formulation, that is, with sugar, insecticide and various excipients, but no plant volatiles. This treatment usually killed some moths, but only 15–25 % of those killed by good volatile blends. Another control treatment was the current lead blend, and there were usually two other new candidate blends. Blends were evaluated using a "leap-frogging" approach, where, if a candidate blend outperformed the lead blend in two successive trials, it became the new lead.

It soon became apparent that sprayed trials resulted in much higher moth kills than trapping, with kills up to several hundred per 50 m section often recorded. Generally, 50–70 % of the kill consisted of females, both mated and unmated. Kills of non-target species also were recorded, including other noctuid pests that may have been potential targets. An unexpected advantage of the sprayable formulation was that it lasted for more than one night. Such formulations need to be liquid for ingestion, but while the droplets on foliage did dry out during the day to form a dry or tacky deposit, at night when the humidity rose they absorbed moisture, probably because of the high concentration of sucrose, and became liquid again. In this way, a single application could remain active for 4–6 nights, depending on temperature and humidity.

Large-Scale Field Tests and Area-Wide Impacts

At the conclusion of the small-scale trials, a five component blend was identified as the most promising for commercialization. It consisted of (*Z*)-3 hexenyl salicylate (10.4 g/L), phenylacetaldehyde (9.08 g/L), α-pinene (5.68 g/L), cineole (5.07 g/L), and D-limonene (1.88 g/L). The trade name Magnet® was registered, and the blend was patented in Australia and internationally (Gregg and Del Socorro 2002). A Product Evaluation Permit was sought to enable commercial-scale trials.

Product Evaluation Permits were a valuable feature of the Australian regulatory system. They allowed trials on ecologically realistic spatial scales, which for an attract-and-kill product targeted at highly mobile insects may be many thousands of hectares, and they allowed limited sale of the product to defray trial costs and to gauge likely market response. Data requirements were greater than for small-scale Research Permits. Our permit covered 20,000 ha of cotton, and 1000 ha of several other crops.

Our first commercial-scale trial involved treating a 40 ha field of cotton with bands of Magnet®, which covered one row (about 1 m wide) and were spaced 72 m apart, meaning that about 1.5 % of the field was treated. Numbers of moths killed were estimated using the same methods as for small-scale trials. Subsequent oviposition was monitored with techniques used by commercial cotton scouts, in the treated field, a neighboring control field, and ten other fields at a range of distances from the treated field (Del Socorro and Gregg 2003). Oviposition on the treated field fell substantially after Magnet® was applied, but it was immediately apparent that the impacts were not confined to that field. The neighboring control field and other fields within 1 km showed similarly reduced oviposition, which led to a 30 % reduction in the need for conventional insecticides. In contrast, oviposition increased in the distant control (untreated) fields. These changes were attributed to area-wide impacts due, not to long-range attraction to Magnet®, but to high levels of inter-field movement by the moths, combined with an arresting and locally attracting function of Magnet®.

Such area-wide impacts, which were repeatedly seen in other commercial-scale trials, indicated the potential of Magnet®, but they also led to problems in rigorously demonstrating efficacy. It is extremely difficult to provide adequate replication when plot size is measured in kilometres rather than metres. Apart from the logistical problems, spatial variation due to factors such as topography, nearby crop and non-crop vegetation, and farmer actions may lead to extreme variability between replicates. This problem affects any attempts to measure the impact of control measures involving behavioral manipulation for highly mobile species. Our response to this was to ensure ample pre-treatment measurements on both treated and control fields, and to rely more on repetition than replication to provide convincing evidence of efficacy.

Several further area-wide trials were conducted, on increasing spatial scales. A method of aerial application was devised to allow large-scale application, and application in crops where extensive canopy cover prohibited ground application (Fig. 2). By using aerial application, a trial was conducted on a large but isolated cotton farm (Mensah et al. 2013). Twelve fields of conventional (non-Bt) cotton, totalling 1475 ha, were sprayed on 13 occasions at intervals of 1–2 weeks during the cotton season. Oviposition was monitored at intervals of 3–7 days on these fields, and on 10 fields of Bt cotton that were interspersed with them, but not sprayed. Distant control fields were located on another farm approximately 40 km away. When Magnet® applications began, there was a rapid decline in egg numbers of more than 90 %. This also occurred on the untreated Bt fields that were interspersed between treated fields, but it did not occur on the distant control farm. These trends were attributed to area-wide impacts of Magnet®.

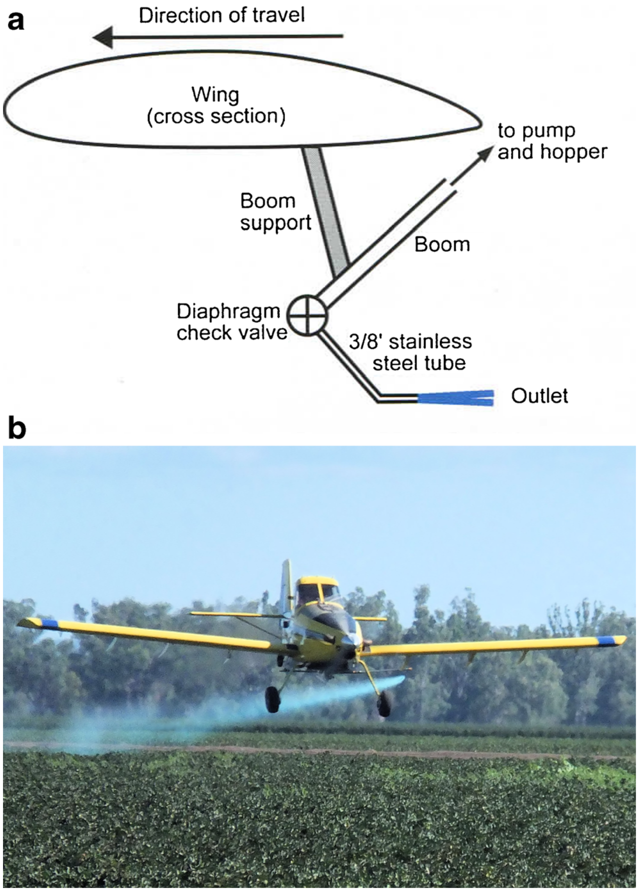

Fig. 2 **a** Schematic diagram of the apparatus used for aerial application of Magnet® **b** Spraying in progress. Since large droplets were required, nozzles were not appropriate, and the formulation was simply pumped through a pipe under the wing, at a speed which matched the forward speed of the aircraft (AgBiTech 2016b). This produced droplets from 1 to 5 mm in diam, large enough for moths to feed on, in bands 1–2 m wide, spaced between 36 and 144 m apart, depending on anticipated pest pressure. Application by air was very quick, allowing 1000–2000 ha per hour to be treated, depending on band spacing

Non-Target Impacts

Data on non-target impacts were required for two purposes: to satisfy regulatory requirements for environmental safety, and to demonstrate compatibility with IPM for farmers. For the former, regulatory requirements emphasised potential impact on rare and threatened species. These were predominantly vertebrates, but some invertebrates (mostly charismatic Lepidoptera and Coleoptera from non-arable areas) also were on the list of endangered species (Department of the Environment 2016). No dead or sick vertebrates were noted during the extensive collections of dead moths that were made in small- and large-scale trials, and this evidence, together with theoretical calculations on the quantity of Magnet® that would need to be ingested before lethal doses of the included insecticides were reached, was submitted to regulatory authorities.

For invertebrates, in one study, all the dead lepidopterans in surrounding rows were collected and identified to species (D.R. Britton, P.C. Gregg, and A.P. Del Socorro, unpublished data). Of 1711 specimens, 1346 (80 %) were the primary targets, *Helicoverpa armigera* and *H. punctigera*. A further 198 (12 %) were incidental pest species, mostly noctuids, leaving only 8 % of non-pest species. Of the latter, most were common pyralid or crambid moths, and none was a threatened species. In another study (Gregg et al., this issue), suction sampling was used on and around rows treated with Magnet® with no insecticide added. This method was intended to detect accumulation or depletion of insects on the treated row relative to nearby and distant untreated rows, which was taken to reflect attraction and repellence, respectively. In this way, effects on species that were too small to find after being killed could be assessed. Of seven generalist predators (Coleoptera, Hemiptera, and Neuroptera) studied, only one apparently was attracted, five apparently were repelled, and one was not affected. Repellence of non-target organisms also appears to extend to the Hymenoptera, being observed in the laboratory for the parasitic wasp *Diadegma semiclausum*

(Hell'en) (M. Yazdani and G. Baker, unpublished data), and in the laboratory and field for honey bees *Apis mellifera* L. (R. Spooner-Hart, P. Gregg and A.P. Del Socorro, unpublished data).

These observations suggest that Magnet® should be compatible with IPM in most situations. In Australian cotton, commercial scouting methods usually have shown no significant differences in the numbers of generalist predators between treated and nearby untreated fields (Del Socorro and Gregg 2003; Mensah and Macpherson 2010; A.J. Hawes unpublished data).

Registration

Experiences in registering Magnet® have been described in detail by Gregg et al. (2010b). In all developed countries, and most developing countries, attract-and-kill formulations to be sprayed on crops require registration as pesticides. The Australian registration system was designed for regulation of pesticides applied as cover sprays against relatively immobile, mostly immature pests. These sprays contain toxicants, mostly of synthetic origin. The registration system mostly has been applied to new formulations, or new uses of existing formulations, containing active ingredients developed by large multi-national agrochemical companies that have already been registered elsewhere. This system, as with those in the USA and other countries (Weatherston and Minks 1995) poses difficulties for registration of semiochemical products, which often are developed by smaller companies in association with public sector researchers. The high costs of providing toxicological and environmental data, combined with limited market opportunities, often threaten the commercial viability of these products. However, in some cases, special classes of pesticides have lower data requirements. In Australia, one such class was defined by possession of active ingredients that were "commonly used household/industrial chemicals with a history of safe use" (Gregg et al. 2010b), and in 2004 we attempted to register Magnet® in this category. All the plant volatiles had uses in other industries, and the insecticide components were at levels which would have produced residues at or below those already registered in the target crops (cotton, corn, and beans). To facilitate the application we provided data on product chemistry, efficacy, environmental impacts, residue chemistry, toxicology, and occupational health and safety considerations for producers, transporters, retailers, and applicators. After considering the application for about a year, APVMA rejected it on the grounds that one volatile, (*Z*)-3 hexenyl salicylate, did not fit the criteria of a commonly used chemical with a history of safe use. This compound is an aromatic derivative of a green leaf volatile, not widely distributed in nature but present in trace amounts in some melons and fruits (Pino et al. 2005). It is found commonly in the catalogues of fragrance suppliers, where it often is described as having a "grassy, green" odor. It has been widely used in the cosmetics industry, with an estimated 50 t consumed worldwide in 2000, rising to 140 t in 2010 (Gaudin 2014). However, unlike our other four volatiles, it did not appear on the Generally Recognized as Safe (GRAS) lists of the Flavour and Extract Manufacturers Association (FEMA 2016) or the UN Joint Expert Committee on Food Additives (FAO 2016), and was, therefore, deemed to have inadequate toxicological data. Faced with the alternative of expensive vertebrate and invertebrate toxicity studies, and given our approach to blending described above, we believed that substitutes could be found for (*Z*)-3 hexenyl salicylate. Small-scale trials were conducted using the methodology described earlier. New blends containing the other four volatiles plus one or two potential substitutes for (*Z*)-3 hexenyl salicylate were compared against the original Magnet® formulation. It was found that addition of butyl salicylate (10.4 g/L) and anisyl alcohol (4-methoxybenzyl alcohol) at 5.2 g/L gave a blend that was as attractive as the original formulation in all trials, and more attractive in some. The registration application was re-submitted in 2006, and eventually granted in 2009. A patent was granted for the new blend (Hawes et al. 2008) and it assumed the Magnet® trade name.

Market Development

The development of Magnet® from initial research to product registration took 11 years, a period comparable to that required to commercialize a new synthetic insecticide. During this time, extensive changes occurred in the Australian cotton industry. At the time the research began, only single-gene (Ingard®) Bt cotton was available. It had limited efficacy, especially late in the season, and the acreage was capped at 30 % of the crop for the purposes of resistance management. *Helicoverpa* spp. were the key pests, and broad spectrum insecticides were used extensively (Wilson et al. 2013). By 2012/13, two-gene (Bollgard II®) cotton, which gave much more effective control, had grown to over 96 % of the cotton acreage, and most of the non-Bt cotton was in refuges (components of the Resistance Management Plans) that could not be sprayed for *Helicoverpa* spp. Consequently, *Helicoverpa* spp. had become minor pests and the cotton market had been virtually eliminated, for Magnet® and its competitors among conventional insecticides. Even the use of Magnet® on Bollgard II® crops, aimed at protecting nearby conventional crops (Mensah and Macpherson 2010) has not been widely adopted.

Of the crops on which Magnet® was originally registered, only sweet corn, which is a minor crop in Australia, currently provides a small market. Our recent work has focused on exploring opportunities to use Magnet® in resistance management for Bt cotton, as a source of unrelated mortality that might be used strategically against potentially resistant moths (P.C. Gregg, A.P. Del Socorro, M.R. Binns and S. Downes, unpublished data). While there is some potential for including Magnet® as a contingency tactic, current strategies appear to be containing resistance at present (Downes and Mahon 2012). Another potential use in resistance management is application of Magnet®, without insecticide, to refuges in order to increase oviposition on them (Addison 2009).

Other work has examined the potential for use of Magnet® against other pests, in cotton and other crops. Among the noctuid pest moths that have been attracted in small-scale trials with sprayable formulations are *Agrotis infusa* (Boisduval), *A. munda* Walker, *Anomis flava* (Fabricius), *Chrysodeixis argentifera* (Guenée), *C. eriosoma* (Doubleday), *Earias huegeliana* Gaede, *Spodoptera litura* (Fabricius), *S. exempta* (Walker), and *Thysanoplusia orichalcea* (Fabricius) in Australia and New Zealand; *Agrotis ipsilon* Hufnagel, *Chrysodeixis includens* (Walker), *Helicoverpa zea* (Boddie), *Heliothis virescens* (Fabricius), *Spodoptera frugiperda* (JE Smith), and *Trichoplusia ni* (Hübner) in the USA. Some of these are major pests, but using Magnet® against them would require further registration applications.

Mixed results have been obtained with trials against non-noctuid moths. There appears to be little if any attraction to the navel orange worm *Amyelois transitella* (Walker) in California, despite it being a pyralid, the family that appeared to be most attracted, after noctuids, in Australian trials. Similarly, the potato tuber moth *Pthorimaea opercullela* (Zeller) (Gelechiidae) does not respond. However, the diamondback moth *Plutella xylostella* (L.) does respond (P.C. Gregg, A.P. Del Socorro, M.R. Binns and N. Myers, unpublished data), and trials against this pest in Australian canola have reached the commercial stage, with registration anticipated in the near future.

Lessons from the Magnet® Experience

Despite recognition for over a century of the potential of bisexual attract-and-kill (Trägårdh 1913), Magnet® appears to be the first product of its kind (a sprayable bisexual attract-and-kill product for broad acre crops) registered anywhere in the world. We are aware of only one other, Bio-attract Heli®, which was registered in South Africa recently (Bioglobal 2015), and is based on the same mimic of *Gaura* spp. that inspired us to begin this work (Lopez et al. 2000). Work is proceeding on a similar product in the USA (personal communication, A Mafra-Neto 2015).

The long and difficult path for Magnet®, from initial laboratory research in 1998 to registration in 2009, and continued efforts to develop markets to the present, indicates some of the reasons why there are so few registered products. Some lessons we have learned along the way include:

1. Researchers must be prepared to give primacy to empirical and pragmatic considerations over theory. While our superblending approach can be criticized for lack of ecological rationale, it has at least resulted in a product. The rationale for mimicking is less convincing than it first seems, and it may lead to including volatiles that will threaten the commercial viability of the product.
2. Early engagement of a commercial partner that understands the market and the economic and regulatory constraints is essential. The objective is not necessarily to produce the best attractant – it is to produce one that is good enough, at a price that is competitive with competing technologies such as broad spectrum insecticides, and that will clear regulatory hurdles. Very few researchers will understand how to adapt their scientific knowledge to this paradigm, at least in the initial stages.
3. There is no substitute for field trials, on ecologically realistic scales of time and space that fit the mobility of the insect, the range of its host crops, and the nature of the agricultural ecosystem. Electrophysiological studies, although intellectually satisfying and potentially less logistically challenging than field work, can only suggest future directions.
4. Using traps in the field may give misleading indications of the potential of sprayable formulations. Had we not overlooked disappointing results from trapping studies and proceeded to small-scale sprayed trials, we may never have developed a product. Even small-scale field trials of sprayable formulations may fail to detect area-wide impacts that only become apparent in commercial scale trials.
5. Obstacles with regulatory systems must be anticipated, at all stages from field trials to registration. These systems are designed to facilitate the safe adoption of synthetic pesticides applied over the whole crop, and for new synthetic active ingredients that are developed by large companies with extensive resources. Regulators are not accustomed to products that work by behavioral manipulation, use commonly available chemicals, and originate in public sector and/or small company research. Similarly, researchers usually are not familiar with the requirements of regulators. In these circumstances, a conservative approach is needed. After our experience with (*Z*)-3 hexenyl salicylate, we now believe that FEMA or JECFA GRAS status should be the first, not the last, question that we ask about a candidate volatile.
6. Given the likely time scales between research and realization, a long term view of the market is essential. The extent to which transgenic varieties would dominate the Australian cotton market and reduce the need for both conventional chemicals and attract-and-kill might be obvious in hindsight. We were not alone in failing to recognize it at the time. While specificity often is promoted as

an advantage of semiochemical products, it also is a double-edged sword, because it restricts market potential. With products such as Magnet®, which are more specific than most conventional insecticides but less specific than pheromones, opportunities for alternative markets are likely to be available; these should be investigated at the same time as the main target market.

Summary

We conclude that Trägårdh's (1913) assessment of the potential for bisexual attract-and-kill was correct: there is great potential for the technology as a component of integrated pest management, for many pests in many crops. This potential should encourage further efforts in applied research and commercial development. However, for researchers, willingness to challenge orthodox theory, plus early consultation with commercial partners and regulators is essential. For commercial developers, foresight, patience, clever marketing, and understanding the needs of farmers are the qualities that will be needed.

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