Attract and kill formulations for *Helicoverpa armigera* males (Lepidoptera: Noctuidae)

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Abstract

Attract and kill formulations are ideal tools for inclusion in IPM strategies to control insect pests, as they can be targeted at a single pest species without affecting beneficial insects, or creating secondary pest outbreaks. Field studies with attract and kill formulations using pheromone for *H. armigera* male moths indicate that contact rates with the basic formulation were very low, but manipulation of visual stimuli and lure presentation could greatly increase contact rates. There were no deterrent effects caused by inclusion of a pyrethroid insecticide in the pheromone formulation.

Introduction

Attract and kill techniques which utilise a chemical attractant to lure pest species to a pesticide-laden formulation have been successful with a number of pest insects such as fruit flies (Cunningham & Steiner 1972, Steiner *et al.* 1965, Steiner *et al.* 1961), pink bollworm *Pectinophora gossypiella* (Haynes *et al.* 1986, Miller *et al.* 1990), codling moth *Cydia pomonella* (Charmillot *et al.* 2000) and light brown apple moth *Epiphyas postvittana* (Brockerhoff & Suckling 1999, Suckling & Brockerhoff 1999). Attractants used in these formulations usually mimic female sex pheromones, attracting male moths to the lure, killing the males and thereby reducing mating in the field. The effects of attract and kill are similar to mating disruption, where the cropping area and surrounds are permeated with high concentrations of pheromone, which prevents male moths finding the females (Valeur 1998). The high pheromone concentrations required for successful mating disruption for moth species has meant that the cost of using this technique is prohibitive in some farming systems (Suckling & Shaw 1995). Attracticides for pink bollworm use 80% less pheromone to achieve the same control as mating disruption in Egyptian cotton fields (Hofer 1994), so attract and kill may offer an economic alternative to mating disruption in some cases.

Attract and kill formulations can use toxins (eg. De Souza *et al.* 1992), sterilants (eg. Langley *et al.* 1990) or pathogens (eg. Pell *et al.* 1993). Pyrethroids are the most popular toxins in use, mainly due to their high contact activity, quick knockdown, availability, and low mammalian toxicity (Haynes *et al.* 1986, Miller *et al.* 1990, De Souza *et al.* 1992, Downham *et al.* 1995). In addition to this, the formulation needs to provide a slow controlled release of pheromone for the desired control period. An early formulation used for attract and kill of pink bollworm included the insecticide into a sticker that was used with the application of hollow fibres containing pheromone for mating disruption (Butler & Las 1983). Later versions of attract and kill have used castor oil (Lösel *et al.* 2000 for codling moth), and a sticky polymeric substance, Sirene®[1] (Charmillot *et al.* 2000, Hofer 1997, Santos & Hofer 1996, Brockerhoff & Suckling 1999, Suckling & Brockerhoff

[1] The "®" status is implied where Sirene is mentioned in the rest of this paper

1999). Sirene contains antioxidants and UV protectants which prevent degradation of pheromone components, whilst the polymer allows a slow release of the pheromone components over time. Existing commercial formulations of Sirene used for control of codling moth and pink bollworm contain relatively high concentrations of permethrin (usually about 5-6 % w/w) to achieve control (Charmillot *et al.*2000, Hofer 1994). Despite these high concentrations the overall amount of insecticide applied to the crop is usually much less than conventional sprays (eg. nine times less for pink bollworm control in Egyptian cotton, Hofer 1994), and is targeted only at the pest. The absence of non-target effects means that attract and kill is an ideal tool for part of an IPM program.

This paper presents results of our initial field research into Sirene-based formulations for *H. armigera* males to determine the proportion of moths approaching lures that contact the lure, and factors which might alter this proportion. The effects of visual stimuli, 6% bifenthrin, and lure presentation on the proportion and number of moths contacting the lures are also discussed.

Materials and Methods

Formulations

Formulations were made with Sirene supplied by IPM Technologies, USA and consisted of 1% w/w pheromone components (10:1 ratio of Z-11-hexadecenal and Z-9-hexadecenal, 95% purity, Sigma-Aldrich). The pheromone concentration is equivalent to that used in commercial applications for monitoring purposes (eg. AgriSense-BCS Ltd. laminate lures). The insecticide used was 6% bifenthrin (Technical grade, 93.3% purity, dissolved in LR grade acetone, 1g/1ml). Lures consisted of 100-200mg droplets of the formulation on 2.0 x 1.5cm white plastic Corflute® rectangles.

Behavioural Observations

Field observations were made from October, 1999 to October, 2001 on flowering sunflower, early-stage flowering soybean, silking corn, cotton and immature chickpeas near Nangewee, 9km southeast of Cecil Plains, Queensland (27°36'S 151°17'E). Crops were chosen on the basis on their perceived general attractiveness to *H. armigera*, so that as many males as possible could be observed per night. Night vision goggles and a hand torch with an infrared filter were used to observe behaviour of male moths approaching the lures. Single droplets of the test formulation were placed onto 2.5 x 2cm white plastic Corflute® rectangles held by white curtain rods between plants in a row at a similar height to the top of the crop. To avoid competition between lures only one lure was used for observation each time.

To get a full view of the moth behaviour, a 4m high trailer-mounted mobile observation tower was used. Observations were made over 10-15min intervals per treatment, recorded onto a cassette recorder and later transcribed into a behavioural analysis program, The Observer v. 3.0 (Noldus Information Technology b.v. 1995). Moth behaviours were scored as approaching, near or contact. Moths coming to the lure with a directed flight were noted as "approaching". Those that approached within 1m of the lure were recorded as getting "near", and any contact with the lure was noted as "contact". The percentage and number of moths observed in these three behavioural states were estimated. Errors associated with percentages are 95% confidence limits.

Visual Stimuli

H. armigera female moths were used to test the effects of visual stimuli on male behaviour to the pheromone formulation. Female moths were obtained from a laboratory culture at the University. They were frozen, pinned and set in a position that approximated the live female calling posture adopted when soliciting mating with a male moth. The pinned moths were oven-dried at 40°C for one week prior to use. To determine what qualities of the pinned female moth were attractive to the approaching males, female moths were dissected into component parts, assembled and glued onto 2.0 x 1.5cm white Corflute® plastic rectangles, giving five treatments including the plain pheromone lure, a pair of fore wings, a pair of hind wings, both pairs of wings, and a synthetic "female" consisting of a black stripe 15mm x 2mm on white plastic. All of the wings were glued facing upwards. Lures were placed in flowering sunflower. Visual cues were supplied with the same amount of formulation as provided for previous observations. The set and dried females were pinned into the centre of the formulation, whilst the glued wings etc. had a strip (100-200mg) of formulation placed between the paired wings.

Presentation

To investigate substrate effects Sirene formulation was placed directly on the top of the sunflower stem just under the flower head, where the stem forms a 90° bend.

The effect of increasing the surface area of the lure was investigated by comparing three treatments on 10 x 10cm squares of Corflute®, with 1ml of formulation in a single droplet in the centre of the square, 1ml in a 5 x 4 grid of small droplets over the entire square, and 1ml smeared over the entire square.

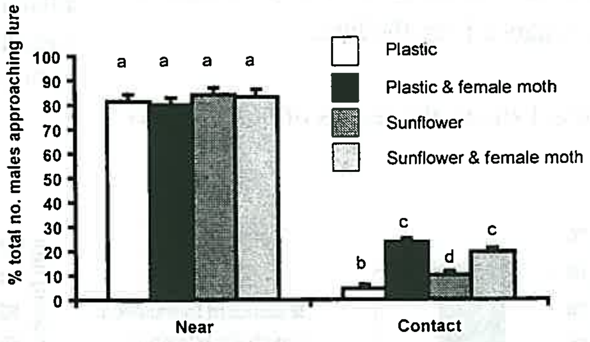

Figure 1 Percentage of *H. armigera* males approaching 1% pheromone in Sirene formulation for four treatments, (3 nights data combined, n=2,196). Different letters indicate that there are significant differences between treatments ($p<0.01$) with paired proportions tests using Pearson's chi-squared statistic (S-Plus, 2000).

Results

Large numbers of males approached the lures in the flowering sunflower field. Figure 1 shows the aggregate results for three consecutive nights of observation (18-20 January, 2000) on both the plastic substrate and the sunflower stalk, with a total of 2,196 male moths approaching the lures. The percentage of moths which contacted the lures on the plastic substrate (1% pheromone) was very low. Only about 5% of the moths that got near the lure contacted the lure. With lures on the sunflower substrate, about 10% of the moths that approached contacted the lure. The sunflower substrate significantly increased the number of contacts ($p<0.01$). Addition of the pinned female moth significantly increased ($p<0.01$) the percentage of contacts for both substrates (24% for the plastic substrate; 20% for the sunflower substrate), but removed any effect of substrate.

For the observations on the dissected female moths a total of 3,219 moths were observed flying to the lures. Figure 2 shows the percentage of males that got near and contacted the lure at these five treatments. Lures with both pairs of wings, and fore wings present were significantly more attractive (p<0.01), with a greater proportion of the moths getting near the lures and contacting the lures.

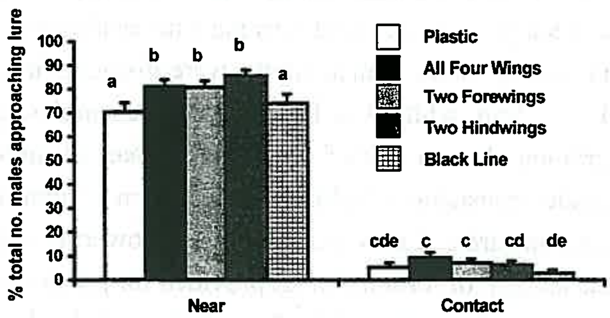

Figure 2 Percentage of *H. armigera* males approaching 1% pheromone in Sirene formulation for five treatments, (3 nights data combined, n=3,219). Different letters indicate that there are significant differences between treatments (p<0.01) with paired proportions tests using Pearson's chi-squared statistic (S-Plus, 2000).

The effect of 6% bifenthrin in the Sirene lures was investigated in soybean, pigeon pea, corn and cotton, with a total of 8 nights observation of 1,293 moths for the bifenthrin treatments, and 13 nights with 3,322 moths for the control treatment. Figure 3 indicates that there is no significant deterrent effect on males at any distance from the lure.

Figure 4 shows the results of observations

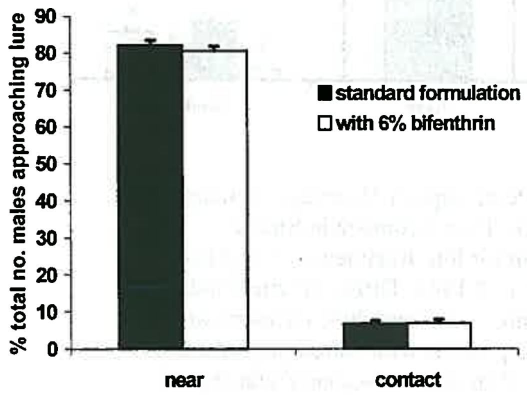

Figure 3 Percentage of total number of *H. armigera* males approaching 1% pheromone in Sirene and the same with 6% bifenthrin

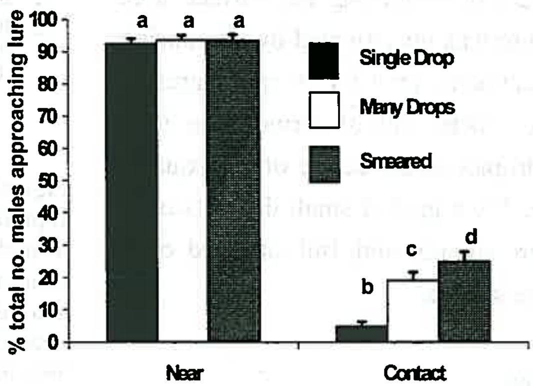

Figure 4 The effect of lure presentation on the percentage of approaching moths that got near and contacted the lure. Different letters indicate that there are significant differences between treatments (p<0.01) with paired proportions tests using Pearson's chi-squared statistic (S-Plus, 2000).

on three ways of presenting the lure conducted on immature chick peas. The surface area of lure presented to the moths made a significant difference to the percentage contact, with all three treatments significantly different to each other. Up to 19% of males contacted the multiple small droplet treatment, and 25% of males contacted the smeared lure, compared with only 5% for the single droplet.

Discussion

Of the male moths approaching the pheromone lures, only a very low proportion contacted the lures. One possible reason for this might be the absence of a calling female moth at the source of the pheromone plume. Male moths responding to a pheromone stimulus are likely to need visual cues such as calling females to complete their sexual behavioural repertoire. The full reproductive behavioural sequence of a related North American species *Heliothis virescens* has been documented in laboratory conditions (Teal *et al.* 1981). The precourtship behaviours prior to the male alighting next to the female are stereotyped and unidirectional. The female produces sex pheromone, which the male follows until he reaches the female. The situation after landing (arriving next to the female) requires more committed interactions between both sexes, and it is this stage where the Sirene formulation is possibly ineffective. Some of the post-landing behaviours rely on males extruding hair pencil organs. In *H. virescens* these male pheromones seem to turn off female sex pheromone production (Hendricks & Shaver 1975) and possibly initiate other visual behaviours in female moths, such as wing-fanning (Teal *et al.* 1981). If males do not perceive a reactive female, there may be a subsequent failure to contact the lure in the case of a Sirene-based formulation.

It is also possible that there may be minor pheromonal components missing from the formulation. At least four other volatile compounds have been isolated in small quantities from female *H. armigera* extracts (Mayer & McLaughlin 1991), (Arn *et al.* 2000). These are Z-11-hexadecenol, hexadecanol, hexadecenal and Z-9-tetradecenal. These compounds may be associated with close-range communication between sexes and may be required to complete the landing and subsequent behavioural sequence leading to a successful mating. Future studies may include these minor components of the female sex pheromone to determine if these increase contacts with formulations.

Pyrethroids are often noted in the literature as having a repellent effect on insects (Haynes *et al.* 1986, Reith & Levin 1988), although this has not always been the case (Floyd & Crowder 1981). Our formulations used a highly active pyrethroid, bifenthrin, at 6% w/w in Sirene. Our studies showed that the addition of 6% bifenthrin in the pheromone formulations did not deter moths from contacting the formulations.

Increasing the surface area of the pheromone lure significantly increased moth contacts compared to a single droplet. Unlike visual cues it may well be possible to include presentation differences similar to this in the final application of the lures in field trials. Weathering studies should be done to determine if smeared lures lose their efficacy much faster in the field than single droplets as smearing may result in more rapid loss of the active volatiles in the lure.

Whilst it seems unlikely that visual cues can be successfully incorporated into an Sirene-based attract and kill system, it does seem possible that appropriate presentation of the lure may enhance and increase the efficacy of this formulation in field trials. Even with only 25-30% contacts per lure it may be possible to control relatively large populations of male *H. armigera* simply by putting out more lures in the crop. Whether the degree of control which can be achieved is sufficient to make an impact on oviposition remains to be determined. Future studies of the population ecology of *H. armigera* are required to shed light on the feasibility of attract and kill in cotton and associated agroecosystems.

Acknowledgements

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References:

Arn, H, Tóth, M & Priesner, E. 2000. The Pherolist: List of Sex Pheromones of Lepidoptera . http://www.nysaes.cornell.edu/pheronet/index.html

Brockerhoff, EG & Suckling, DM. 1999. Development of an attracticide against light brown apple moth (Lepidoptera : Tortricidae). *Journal of Economic Entomology.* **92**, 853-859.

Butler, GD & Las, AS. 1983. Predaceous insects: effect of adding permethrin to sticker used in gossyplure applications. *Journal of Economic Entomology* **76**, 1448-1451.

Charmillot, PJ, Hofer, D & Pasquier, D. 2000. Attract and kill: a new method for control of the codling moth *Cydia pomonella* . *Entomologia Experimentalis et Applicata.* **94**, 211-216.

Cunningham, RT & Steiner, LF. 1972. Field trial of Cue-Lure + Naled on saturated fiberboard blocks for control of the melon fly by the male-annihilation technique. *Journal of Economic Entomology* **65**, 505-507.

De Souza, KR, McVeigh, LJ & Wright, DJ. 1992. Selection of insecticides for lure and kill studies against *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* **85**, 2100-2106.

Downham, MCA, McVeigh, LJ & Moawad, GM. 1995. Field investigation of an attracticide control technique using the sex pheromone of the Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Bulletin of Entomological Research.* **85**, 463-472.

Floyd, JP & Crowder, CA. 1981. Sublethal effects of permethrin on pheromone response and mating of male pink bollworm moths. *Journal of Economic Entomology* **74**, 634-637.

Haynes, KF, Li, W-G & Baker, TC. 1986. Control of pink bollworm moth (Lepidoptera: Gelechiidae) with insecticides and pheromones (attracticide): Lethal and sublethal effects. *Journal of Economic Entomology* **79**, 1466-1471.

Hendricks, DE & Shaver, TN. 1975. Tobacco budworm: Male pheromone suppressed emission of sex pheromone by the female. *Environmental Entomology* **4**, 555-558.

Hofer D. 1994. Sirene®: A new approach to pink bollworm control in cotton. In: *Insect Control Newsletter* (ed R Scheurer) pp. 2-5. Ciba Crop Protection Division, Basle, Switzerland.

Hofer D. 1997. Attract and kill: another step forward - first sales of Sirene® CM. In: *Insecticides Newsletter* (ed R Scheurer) pp. 8-9. Novartis Crop Protection, Basle, Switzerland.

Langley, PA, Felton, T, Stafford, K & Oouchi, H. 1990. Formulation of pyriproxifen, a juvenile hormone mimic, for tsetse control. *Medical and Veterinary Entomology* **4**, 127-133.

Lösel, PM, Penners, G, Potting, RPJ, Ebbinghaus, D, Elbert, A & Scherkenbeck, J. 2000. Laboratory and field experiments towards the development of an attract and kill strategy for the control of the codling moth, *Cydia pomonella. Entomologia Experimentalis et Applicata.* **95**, 39-46.

Mayer MS & McLaughlin JR. 1991. *Handbook of Insect Pheromones and Sex Attractants*. CRC Press, Boca Raton.

Miller, ER, Staten, RT, Nowell, C & Gourd, J. 1990. Pink bollworm (Lepidoptera: Gelechiidae): point source density and its relationship to efficacy in attracticide formulations of gossyplure. *Journal of Economic Entomology.* **83**, 1321-1325.

Noldus Information Technology b.v.. 1995. The Observer® System for collection and analysis of observational data, Version 3.0 DOS, Wageningen, Netherlands

Pell, JK, Macaulay, EDM & Wilding, N. 1993. A pheromone trap for the dispersal of the pathogen *Zoophthora radicans* Brefeld (Entomophthorales) amongst populations of the diamondback moth *Plutella xylostella* L., (Lepidoptera: Yponomeutidae). *Biocontrol Science and Technology* **3**, 315-320.

Reith, JP & Levin, MD. 1988. The repellent effect of two pyrethroid insecticides on the honey bee. *Physiological Entomology* **13**, 213-218.

Santos, WJ dos & Hofer, D. 1996. Study of the effectiveness of boll weevil control applying drops of a mixture of sex pheromone (Grandlure) and cypermethrin through a pistol. *Proceedings of the Beltwide Cotton Conferences* 712-715.

Steiner, LF, Mitchell, WC, Harris, EJ, Kozuma, TT & Fujimoto, MS. 1965. Oriental fruit fly eradication by male annihilation. *Journal of Economic Entomology* **58**, 961-964.

Steiner, LF, Rohwer, GG, Ayers, EL & Christenson, LD. 1961. The role of attractants in the recent mediterranean fruit fly eradication program in Florida. *Journal of Economic Entomology* **54**, 30-35.

Suckling, DM & Brockerhoff, EG. 1999. Control of light brown apple moth (Lepidoptera : Tortricidae) using an attracticide. *Journal of Economic Entomology.* **92**, 367-372.

Suckling, DM & Shaw, PW. 1995. Large scale trials of mating disruption of light brown apple moth. *New Zealand Journal of Crop and Horticultural Science* **23**, 127-137.

Teal, PEA, McLaughlin, JR & Tumlinson, JH. 1981. Analysis of the reproductive behavior of *Heliothis virescens* (F.) under laboratory conditions. *Annals of the Entomological Society of America* **74**, 324-330.

Valeur, P. 1998. Male moth behaviour and perception in pheromone plumes. PhD. dissertation, Lund University, Sweden.