Using sex pheromone to control male *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) in cotton.

David R. Britton
BSc. Hons. (Uni. of Melb.) MSc. (La Trobe Uni.)

A thesis submitted for the degree of Doctor of Philosophy of the University of New England

March 2005

Candidate's certification

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

.....

Acknowledgements

I would like to acknowledge the following for their assistance:

My supervisors, Peter Gregg and Alice del Socorro.

Letitia Silberbauer.

The Cotton Research and Development Corporation who provided the generous scholarship as well as an excellent professional development program.

The Cotton CRC who fostered contacts with other postgraduates and researchers throughout Australia, and provided a forum for exchanging ideas and research findings.

Horticulture Australia Limited and SP Exports Pty. Ltd., Childers, Qld
for funding the mating disruption project.

Emma Smith from SP Exports Pty. Ltd.

Philipp Kirsch of IPM Technologies Pty. Ltd., Portland, Oregon, USA.

Robin Gunning of NSW Agriculture, Tamworth, NSW.

Jeff and Marilyn Bidstrup, Bidstrup Biologicals Pty. Ltd. "Warra", Qld.

My fellow postgraduate students from the University of New England

UNE technical staff, especially George Henderson, Duncan Mackay, Dan Alter, the Science and Engineering Workshop.

The Australian Museum for providing study leave during the writing of this thesis.

The many farmers listed below who allowed access to their properties, as well as advice, generous assistance in accommodation and equipment.

Darling Downs:
The Armitages
The Armstrongs
Clapham Farms
The Ladners
The Glovers
Matilda Farms
Harley Bligh

Bonshaw:
Tim & Mack Ramsay

Mulgowie:
Mulgowie Farms

Bowen:
Wright Pack
Mulgowie Farms

Abstract

The aim of this thesis was to develop and test slow-release sex pheromone-based formulations for *Helicoverpa armigera* (Hübner) (Noctuidae: Heliothinae) for use as part of an integrated pest management (IPM) program in cotton and associated crops. *H. armigera* is considered to be the most significant pest of cotton and is destructive in many other crops. It readily develops resistance to insecticides, hence alternative methods of control are highly desirable, especially those which are selective and environmentally safe. This thesis briefly reviews the pest status of *H. armigera*, then looks at the sex pheromones of heliothines. The use of sex pheromones for attract and kill and mating disruption of *H. armigera* and other insect pests are discussed in detail.

Two main formulations of *H. armigera* sex pheromones were considered in this thesis. The first one was for attract and kill. The basis for the formulation was a gel-like matrix called Sirene® which protects semiochemical components such as pheromones, whilst allowing their slow release into the environment. This matrix can also be laced with insecticide to make a formulation which kills insects attracted to it. Synthetic blends of *H. armigera* sex pheromone used here were based on existing blends reported in the literature, and consisted of a 10:1 ratio of (*Z*)-11-hexadecenal:(*Z*)-9-hexadecenal loaded at 1% in the Sirene® matrix. The second one was the commercially available formulation for mating disruption (Selibate HA, AgriSense BCS Pty. Ltd., Pontypridd, South Wales, UK) which was used for a mating disruption trial. This contained the same blend of components at 5% in an extruded polymer dispenser. Both methods work by preventing mating by either killing male moths (attract and kill), or by preventing male location (mating disruption).

Sex pheromones attract only male moths. Male behaviour at sex pheromone sources was studied in detail. Male activity at the lures was influenced by diel periodicity, type of and stage of crop present, season, % relative humidity, wind run and overall climatic conditions. Of these factors the type of crop and seasonal factors were associated with the largest changes in number of moths flying to lures, and the percentage of moths contacting the lures. The effect of lure formulation, appearance and presentation method was evaluated by varying these factors and observing male behaviour in the field. The synthetic lure was compared with captive female *H. armigera* to determine if the synthetic blend was adequately attractive. Observations indicated that the synthetic blend was adequate, and was as least as attractive as calling females. Contact rates with standardised 200 mg droplets with 1% pheromone were relatively low, with only 10% of approaching males contacting the lure. The percentages of contacting males could be

significantly increased by placing a dead decoy female next to the lure, by placing the lure on a natural substrate and by increasing the active surface of the lure by smearing it or placing it as many small droplets. The addition of a synthetic pyrethroid (bifenthrin) did not significantly repel males from lures.

The laboratory toxicology of the pyrethroid contact insecticide bifenthrin was evaluated for formulations made in Sirene®. A concentration of 6% bifenthrin gave close to 100% mortality over 4 h. Concentrations less than 1.5% were also effective, but took over 14 h to achieve > 90% mortality. Sublethal effects on reproductive behaviour were also tested. Treating males with concentrations of >0.01% bifenthrin in Sirene® reduced the chance of successfully mating by more than 50%. The toxicity of formulations was compared in field conditions using a passive field wind tunnel. The 6% bifenthrin and 1% pheromone formulation gave the best results when associated with dead decoy female moth, but mortality in the field wind tunnels was much lower than expected. This appeared to be due to the experimental conditions of the field wind tunnel altering the male behaviour.

Weathering of the Sirene®-based pheromone formulations was studied under field conditions. The estimated life-span of 200 mg droplets was between 4-6 weeks. The life-span of the formulation was more than halved by smearing the lure over substrates compared to leaving formulations as a single droplet.

A mark-recapture study of male behavior in flowering sorghum found that populations of male *H. armigera* were non-resident in the crop. Population estimates in the field range from 97 to 4,008 males per hectare per night. Extrapolation from population estimates obtained in this study indicate that with only 12 pheromone sources in a 21 ha field it was possible to remove 10% of males present when population densities were very low. However, the number of pheromone sources would have to be increased to successfully control the large numbers (>4,000) seen on some nights. The technique used in this study allowed for an estimate of moth turnover on a nightly basis, and would be useful in other studies of insect movement.

A full-scale mating disruption trial of *H. armigera* was carried out in an isolated cropping area. Mating was reduced to almost zero in treated areas, and there was a significant reduction in the number of eggs laid in capsicum crops, and in the number of spermatophores per female. However, there was not a sufficient decrease in egg lays in any of the treated areas that would allow any decrease in conventional sprays. Mating was occurring on non-host plants adjacent to

the treated areas, and mated females were flying back into treated areas to lay eggs. This is in contrast to published results of noctuid moths mating only on host plants.

Information from the mark-recapture study was used in conjunction with a model developed from observing contact rates of moths with pheromone lures in the field. From this a recommended rate of 75–100 200 mg droplets per ha was estimated for the attract and kill formulation. The final recommended formulation based on laboratory and field testing used an existing protective base (Sirene®), 6% bifenthrin with 1% of the *H. armigera* pheromone blend.

The local movements of male moths and mobility and labile nature of mating behaviour in *H. armigera* indicate that any sex pheromone-based pest management program such as attract and kill or mating disruption is unlikely to succeed as a stand-alone system. It is possible that attract and kill may be a very useful tool when combined with mating disruption and other semiochemical techniques in an IPM program. The implications and future uses of sex pheromone for control of *H. armigera* are discussed.

Table of contents

Figures

Tables

1 General Introduction

This chapter reviews the pest status of *H. armigera*, chemical communication in heliothines, attract and kill techniques and mating disruption for heliothines. An overview of the thesis structure, and the rationale behind the thesis is also provided.

1.1 *Pest status of Helicoverpa armigera: Taxonomy, life-history, ecology and management*

The genus *Helicoverpa*[1] (Lepidoptera: Noctuidae: Heliothinae) is distributed throughout the tropical and warm temperate regions of the world. There are 20 described species, some of which have very large geographical ranges, and are among the most serious pests of agriculture (Matthews 1999). *H. armigera* has one of the widest distributions of any agricultural pest, ranging throughout the Old World from the Canary Islands in the west to various Pacific Islands in the east, occurring in both the northern and southern hemispheres (Common 1953, Hardwick 1965, Zalucki *et al.* 1986). The species is highly polyphagous, feeding on many crop and non-crop plants. Because of this polyphagy and broad distribution the moth is referred to by a large variety of common names in the English literature, such as Old World bollworm (Piccardi *et al.* 1977), cotton bollworm (Parsons 1939), tomato fruitworm (Cameron *et al.* 1995), American bollworm (Chamberlain *et al.* 2000), and in the United Kingdom where the moth is a rare migrant, the scarce bordered straw (Buleza *et al.* 1983).

The species is sometimes referred to as *H. obsoleta* in older publications, a name that has also been (incorrectly) associated with the similar North American corn earworm *H. zea* (Parsons 1939). Older publications (up until the early 1990s) often place *H. armigera*, and the related Australian endemic *H. punctigera* in the genus *Heliothis* (eg. Cox & Forrester 1992, Hassan & Wilson 1993, Mallett *et al.* 1993)), and many farmers and non-entomologists still refer to *H. armigera* as "heliothis". Species belonging to the genus *Helicoverpa* can readily be separated from those in *Heliothis* by the very long, coiled vesica with a strip of cornuti along its length in the male genitalia, and in the female, by the long, alternately dilated and constricted appendix bursae, the membrane of which is thickened, opaque, and appears "leathery" (Matthews 1999). *H. armigera* has also been confused with *H. punctigera*; the two species occur in similar crops and have overlapping distributions, with *H. punctigera* being restricted to Australia. The superficially similar adults can be separated by wing colours and patterns in good quality dry specimens, or by dissection and examination of the genitalia in other cases (Matthews 1999).

[1] Taxonomic authorities are cited in the Appendix 12.1 for all fauna and flora mentioned in this thesis.

The life-history, biology and ecology of *H. armigera* contribute to the high pest status of this species (Fitt 1989). The larvae of *H. armigera* are extremely polyphagous, with known host plant records including 34 plant families and over 130 plant species (Zalucki *et al.* 1986, Zalucki *et al.* 1994, Matthews 1999). This polyphagy enhances their pest status by increasing the range of available hosts, allowing simultaneous development on a number of hosts in any given region, allowing continuous development within a region, and allowing the persistence of populations in areas which are fallow in regards to crop hosts (Fitt 1989). Many commercial crops in Australia and throughout other parts of the species' range are damaged. Some of the more important crops include cotton *Gossypium hirsutum*, sweet corn *Zea mays*, tomato *Lycopersicon esculentum*, capsicum and chillies *Capsicum* spp., strawberries *Fragaria* x *ananassa*, and various legumes including green beans *Phaseolus vulgaris* and garden peas *Pisum sativum* (from list compiled in (Matthews 1999).

The adult females are extremely fecund and can produce large numbers of eggs, with estimates between 1,000 to 2,000 eggs laid per female (Hardwick 1965). This fecundity allows for rapid increase of larvae in suitable conditions. Eggs are usually laid in small groups of 1 to 6 on the developing shoots, flowers and fruit of the host plants. By feeding on these parts larvae have access to the plant structures that are often most rich in nitrogen, which both allows for rapid development of the larvae and increases the severity of economic damage in the case of crops (Hardwick 1965).

Adults of both sexes of *H. armigera* are highly mobile, exhibiting both local or trivial movement, and long range movement as defined by Taylor (1986). They are capable of flying up to 10km per night as part of normal or "trivial" local movement. Movements between crops between 1 and 10 km have been reported for *H. armigera* in the Sudan Gezira (Topper 1987). In a diverse cropping landscape movements of up to 6km were recorded using sunflower pollen as a marker (Del Socorro & Gregg 2001). Under certain conditions *H. armigera* may undergo long distance migration and can fly between regions which may be separated by as much as 2000km (Bowden & Johnson 1976). This facultative migration is in contrast with *H. punctigera* which is an obligate migrant, and exhibits an annual migration pattern between winter breeding locations in inland Australia and the summer coastal cropping regions. Farrow and Daly (1987) rank *H. armigera* as "lowest" when comparing the migratory behaviours to those observed in *H. punctigera*, *H. zea* and *Heliothis virescens.* Wholesale migration of *H. armigera* populations is thought to be a relatively rare occurrence, but genetic data suggests that long distance migration

probably occurs over much of the Australian continent from time to time (Daly & Gregg 1985, Scott *et al.* 2003).

Migration in heliothines is thought to be triggered by unfavourable conditions, such as the senescence of winter annuals in the case of *H. punctigera* (Farrow & McDonald 1987, Gregg *et al.* 1995). These migratory movements are distinct from the short-range local movements between crops and other vegetation within a region, occurring at much higher altitudes in synoptic scale wind systems (Drake & Farrow 1985, Gregg *et al.* 1995). They are probably associated with behavioural features which characterize long distance migration, such as the suppression of normal appetitive responses such as mating and feeding (Johnson 1969). Females which undergo migration normally remain unmated until they reach their destination (Colvin & Gatehouse 1993b, Coombs *et al.* 1993).

This mobility of adult *H. armigera* has several consequences in relation to the pest status of this species. The most obvious is that the moth can readily colonise cropping areas as they become available. An additional consequence is that large scale or area-wide management techniques have to adopt a scale that is appropriate given the movements of the adults. This topic is dealt with in more detail in section 1.6 of this Chapter. A further consequence is that the development and prevalence of insecticide resistance genes in the population is greatly assisted by the rapid movement of resistant individuals between cropping regions (Daly & Gregg 1985).

The winter pupal diapause of *H. armigera* in its range may also act to increase the pest potential of this species. By passing the winter in diapause the moths can emerge in spring whilst avoiding unfavourable conditions such as cold temperatures and absence of suitable host plants (Fitt 1989). This spring emergence is often synchronous or concentrated due to the convergence of pupal development status over the diapause period, so that emergence is no longer linked to the initial date of pupation (Wilson *et al.* 1978).

The final factor affecting the pest status of *H. armigera* is the rapid development of resistance of this species to many of the broad spectrum insecticides commonly used in agricultural systems. This led to the collapse of cotton growing in the Ord River Scheme in northern Australia in the 1970s due to the increasing costs of multiple DDT applications and the subsequent control failures due to resistance (Michael & Woods 1980). The species has subsequently developed resistance to the newer synthetic pyrethroids in the cropping regions on the southeast of Australia (Gunning *et al.* 1984). There are now resistance management strategies for Australian cotton production

which aim to delay the onset of resistance to some of the more selective insecticides now available (Johnson & Farrell 2004).

The cost from damage and control measures for *H. armigera* and *H. punctigera* in Australia is conservatively estimated to be in the region of $227,000,000 per annum (Adamson 1997) (based on data collected between 1989-1990 and 1993-1994). In addition to these costs there is the potential cost to the environment due to off-target effects of insecticide, health issues, and insecticide residues. Alternative control methods for *H. armigera* involving area-wide regional management, plant breeding (especially the use of transgenic varieties expressing Bt toxins) and many other techniques are now seen to be answer for long term sustainable crop production in Australian cotton and many other vulnerable crops. Pheromones and other semiochemicals are among the techniques which might contribute significantly to these new approaches.

1.2 *Heliothine sex pheromones*

Table 1.1 lists compounds found in the gland and effluvial analyses of fourteen species of heliothine moths (adapted from The Pherolist (Arn *et al.* 2000). A total of 16 compounds have been identified from analyses of heliothine sex pheromones to date (Arn *et al.* 2000). Most of the compounds are simple straight chain aldehydes, alcohols and acetates of either 16 or 14 carbon length. Gland and effluvial extracts from heliothine females often turn up compounds which do not seem to have any attractive properties to male moths, or are, in some cases, repellant to male moths (Kehat & Dunkelblum 1990). Note that the isomeric configuration of the points of unsaturation within those molecules which have double bonds in the chain is always *(Z)* rather than *(E)*. Non-pest species such as *Schina bina*, *S. meadi* and others have been included to highlight phylogenetic conservatism in the range of compounds present. The frequency with which these compounds are found in heliothine species is shown in Table 1.2. The most commonly extracted components are *(Z)*-11-hexadecenal and *(Z)*-11-hexadecen-1-ol. Unique occurrences of components are rare, with only three of the sixteen recorded components extracted from one species only. Some of these unique extractions may also represent geographic variation or misidentification, as in *(Z)*-11-tetradecenal extracted from *Heliothis armigera* which has only been found in one (Konyukhov *et al.* 1984) of the eleven studies of this species (see also Table 1.1).

Table 1.1 Compounds present in analyses of heliothine gland extracts and effluvial collections. * Species with an asterisk are known to have behavioural responses to the compound. Source: (Arn *et al.* 2000, Cork & Lobos 2003).

Compound	*Species*
tetradecanal	*Heliothis peltigera* *Hs. subflexa* *Hs. virescens**
(Z)-9-tetradecenal	*Helicoverpa armigera* *H. punctigera* *Hs. peltigera** *Hs. subflexa* *Hs. virescens**
(Z)-11-tetradecenal	*H. armigera**
(Z)-9-tetradecen-1-ol	*Hs. peltigera* *Hs. virescens*
(Z)-11-tetradecen-1-ol	*H. armigera*
(Z)-9-tetradecenyl acetate	*Hs. peltigera*
hexadecanal	*H. armigera* *H. assulta** *H. gelotopoeon** *H. zea** *Hs. maritima* *Hs. peltigera* *Hs. phloxiphaga* *Hs. subflexa* *Hs. virescens**
(Z)-7-hexadecenal	*H. armigera** *H. assulta* *H. punctigera* *H. zea** *Hs. peltigera* *Hs. subflexa* *Hs. virescens**
(Z)-9-hexadecenal	*H. armigera** *H. assulta** *H. gelotopoeon** *H. zea** *Hs. maritima** *Hs. peltigera* *Hs. phloxiphaga** *Hs. subflexa** *Hs. virescens**

Table 1.1 continued	
(Z)-11-hexadecenal	*H. armigera** *H. assulta** *H. punctigera** *H. zea** *Hs. maritima** *Hs. ononis* *Hs. peltigera** *Hs. phloxiphaga** *Hs. subflexa** *Hs. virescens** *Schinia bina* *S. meadi*
hexadecan-1-ol	*H. armigera* *H. assulta* *Hs. virescens*
(Z)-11-hexadecen-1-ol	*H. armigera** *H. assulta* *H. punctigera** *H. zea* *Hs. maritima** *Hs. peltigera** *Hs. phloxiphaga** *Hs. subflexa* *Hs. virescens** *Protoschinia scutosa*
hexadecyl acetate	*H. assulta* *Hs. subflexa*
(Z)-7-hexadecenyl acetate	*H. assulta* *Hs. subflexa** *Hs. virescens*
(Z)-9-hexadecenyl acetate	*H. assulta** *Hs. subflexa** *Hs. virescens*
(Z)-11-hexadecenyl acetate	*H. armigera* *H. assulta** *H. punctigera** *Hs. peltigera* *Hs. subflexa** *Hs. virescens* *Pyrrhia umbra* *S. bina*

Table 1.2 Number of times compounds have been isolated from heliothines (15 species examined). Data based on (Arn *et al.* 2000, Cork & Lobos, 2003).

Compound	Presence (out of 15 species)
(Z)-11-hexadecenal	12
(Z)-11-hexadecen-1-ol	10
(Z)-11-hexadecenyl acetate	8
(Z)-9-hexadecenal	9
hexadecanal	9
(Z)-7-hexadecenal	7
(Z)-9-tetradecenal	5
(Z)-7-hexadecenyl acetate	3
(Z)-9-hexadecenyl acetate	3
hexadecan-1-ol	3
tetradecanal	3
(Z)-9-tetradecen-1-ol	2
hexadecyl acetate	2
(Z)-11-tetradecen-1-ol	1
(Z)-11-tetradecenal	1
(Z)-9-tetradecenyl acetate	1

Table 1.3 lists eleven potential pheromone components from 10 studies of *Helicoverpa armigera*. Eight compounds have been identified from female gland and effluvial extractions alone, with an additional two compounds identified from field assay (Mayer & McLaughlin 1991, Arn *et al.* 2000). Two components, *(Z)*-11-hexadecenal and *(Z)*-9-hexadecenal, are currently used in synthetic blends. Blend ratios for these binary components vary between 10:1 (Buleza *et al.* 1983) and 100:2.5 (Kehat & Dunkelblum 1990). Blends used for monitoring male moth activity vary between 97:3 to 10:1, although there seems to be little published experimental evidence for Australian *H. armigera* populations that blends with these reduced proportion of *(Z)*-9-hexadecenal are any more or less effective than the 10:1 blend for attracting males. Tamhankar *et al.* (2003) tested a range of blend ratios between 0: 100 and 15: 85 *(Z)*-11-hexadecenal and *(Z)*-9-hexadecenal at various locations in India. Their results that indicated geographical variation in the response of *H. armigera* males to varying blends of the two sex pheromone components, suggesting male sex pheromone response polymorphism.

Although the commonly accepted blend is the binary mixture of *(Z)*-11-hexadecenal and *(Z)*-9-hexadecenal there are other possible components which may make up the full blend. The components *(Z)*-11-tetradecenal and *(Z)*-7-hexadecenal have been identified as attractants in field and lab bioassay (Gothilf *et al.* 1978a,b, Konyukhov *et al.* 1984, Kehat & Dunkelblum 1990). As mentioned above, it seems unlikely that *(Z)*-11-tetradecenal as isolated from Russian *H. armigera* occurs in the pheromone blend of most populations of *H. armigera*, as none of the studies of moths from the Mediterranean, African, Australian and Middle Eastern regions have isolated and identified this component. The component *(Z)*-7-hexadecenal has only been detected from one analysis of *H. armigera* (Kehat & Dunkelblum 1990), and was found to have repellant qualities when included at 1% of 2mg of the 97.5:2.5 *(Z)*-11-hexadecenal and *(Z)*-9-hexadecenal typical

synthetic blend. The addition of the same compound at 1% of a 10μg loading of the typical blend increased copulatory response of the male moths.

Table 1.3 Compounds detected from eleven analyses of *Helicoverpa armigera*. Compounds currently used in synthetic blends are italicized. The asterisk refers to compounds which have elicited a behavioural response. Original compilation of references sourced from the Pherolist (Arn *et al.* 2000) and Mayer and McLaughlin (1991).

Pheromones	Presence in analyses	Attractant response	Repellent response	Ratios of compounds						Titres (ng)	
References:				1	2	3	4	5	6	7	8
Aldehydes											
(Z)-11-Hexadecenal	11*	5		1	87	25	10	5	100	22	43
(Z)-9-Hexadecenal	6*	3			3	1	1		2.5		<3
Hexadecanal	4				4				3.5	3	<21
(Z)-11-Tetradecenal	3*	1		3				3			
(Z)-9-Tetradecenal	2	1[11]	1[6]						1.5		
(Z)-7-Hexadecenal	1*	1(low rates)[6]	1(high rates)[6]						0.6		
Alcohols											
(Z)-11-Hexadecen-1-ol	4		3[6,9,10]					2	5.5	0.8	<9
Hexadecan-1-ol	3				6					1.6	trace
(Z)-11-Tetradecen-1-ol	1							1			
Acetates											
(Z)-11-Hexadecenyl acetate	1							1			

References:

1. (Konyukhov *et al.* 1978)
2. (Dunkelblum *et al.* 1980)
3. (Kehat *et al.* 1980) (as an optimized field blend)
4. (Buleza *et al.* 1983) (as an optimized field blend)
5. (Konyukhov *et al.* 1984)
6. (Kehat & Dunkelblum 1990)
7. (Nesbitt *et al.* 1979)
8. (Nesbitt *et al.* 1980)
9. (Rotundo & Tremblay 1985)
10. (Huang *et al.* 1996a)
11. (Rothschild 1978)

1.3 *Attract and kill formulations for insect pest management- definitions and history.*

Mass-trapping and attract and kill are two very similar techniques for insect control which rely on attracting the insect pest with a lure and subsequently trapping or removing that insect from the effective pest population. These techniques require two components, an attractant, which may be a volatile chemical, visual cue (or both) which attracts the insect to the formulation, and an affector, which removes the insect from the population. The affector may be a trap, such as a container, or a sticky surface in the case of mass-trapping. For attract and kill, affectors may contain an insecticide, may prevent effective mating by sterilizing the insect, or may disseminate a pathogen (Lanier 1990, Jones 1998a). The majority of this thesis will be concerned with the technique of attract and kill; for discussions on mass-trapping see Lanier (1990) and Jones (1998b).

Attract and kill formulations (attracticides) have a relatively recent history in insect pest management compared to mass-trapping, which dates back to Roman antiquity (Snetsinger & Shelar, 1982, cited in Lanier 1990). Attractive poison baits were used in attempt to control grasshoppers in California in 1885 (Dethier 1947). Phillips and Lincoln (1968) mention that sugar baits mixed with arsenicals and/or tartar emetic as insecticides were tested in the 1930s for bollworm (*Helicoverpa zea*) control. The laced sugar baits were successful in killing substantial numbers of moths, but did not appear give effective control.

Many of the early formulations were attempts to make normal broad-acre spraying more efficient, with the attracticide effect used as a way of monitoring pest activity as much as controlling pests. Such treatments include molasses and arsenicals for (Ditman 1937) and molasses and carbaryl mixtures (Lincoln *et al.* 1966) (both for *H. zea* in Arkansas, USA) and molasses and carbaryl for *H. armigera* in Zimbabwe (Tunstall 1968). In these cases the spray mixture was directed at killing larvae in the crop, whilst adults attracted to the molasses and killed were used as an indication of moth and the potential larval numbers present in the crop.

Development of synthetic insecticides, and the isolation and subsequent synthesis of sex pheromones from insects have enabled much more effective attract and kill formulations. The post-1945 development of effective synthetic organic insecticides allowed the potential development of much more effective formulations for attracticides, but further development of useful attract and kill formulations was delayed until suitable semiochemicals were elucidated.

One of the earliest applied uses of more specialized semiochemicals was the control of tephritid flies, such as Mediterranean fruit fly, *Ceratitis capitata*, in Florida, USA (Steiner *et al.* 1961), Oriental fruit fly, *Bactrocera dorsalis* on Rota Island, north of Guam (Steiner *et al.* 1965), and melon fly, *Bactrocera cucurbitae* in Hawaii (Cunningham & Steiner 1972). These control programs used a range of semiochemical attractants in both trapping and attracticide applications. Angelica seed oil was initially used for mass-trapping of male *C. capitata* in Florida, but was replaced by a synthetic blend (siglure) (Steiner *et al.* 1961). DDVP (2,2-dichloroethenyl dimethyl phosphate or dichlorvos) was added to the attractants in the trap to ensure that flies entering the trap were killed before they could escape. Later trials in Florida for *C. capitata* used bait-spraying techniques developed in Hawaii which are similar to modern "splash" lures for fruit fly. Hydrolysable proteins were used as the bait, malathion (diethyl [(dimethoxyphosphinothioyl)thio]butanedioate) as the insecticide and both sexes of the fly were attracted and killed (Steiner *et al.* 1961). For the eradication of *B. cucurbitae* in Hawaii fibreboard squares were impregnated with the insecticide, naled (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) and the attractant, cue-lure. Only males were killed, so this technique worked well on islands where the influx of mated female flies was minimal or non-existent (Cunningham & Steiner 1972). *B. dorsalis* was eradicated from the 33 square mile (85.5 square km) Rota Island by using this technique, indicating how successful male removal can be when used on isolated populations (Steiner *et al.* 1965). Fruit fly control by attract and kill is still considered to be critical to management of these pests (Mazomenos *et al.* 2000).

Following the successful extraction of bombykol from the silkmoth, *Bombyx mori* in Germany (Butenandt *et al.* 1959) and the coining of a class of biologically active chemicals called "pheromones" (Karlson & Lüscher 1959) there was an expectation that these semiochemicals would contribute greatly to pest management programs though out the world (Schneider 1999), especially following concerns about the environmental damage caused by broad spectrum insecticides such as DDT (Carson 1962). The modern perspective on the contribution of pheromones and other semiochemicals to pest management in commercial sectors is summarized in Table 1.4 adapted from Kirsch (1997a).

Table 1.4 Commercial applications of semiochemical technology across user sectors (adapted from Kirsch (1997a). The majority are sex pheromone-based technologies unless otherwise stated.

Sector	Monitoring (including detection)	Mass Trapping	Repellents	Attract and Kill	Mating Disruption
Consumer	detection	stink bugs, wasps, flies (muscids and calliphorids)	mammalian repellants	muscids and tephritids, cockroaches, Indian meal moth	
Pest Control Operators	detection	cockroaches		cockroaches	
Turf producers, Landscapers, Ornamental Nurserymen	detection	Japanese beetle	mammalian repellents, bark beetle		
Government detection and quarantine programs	detection; population delineation	management & eradication of tephritid flies, boll weevil		tephritid bait sprays	gypsy moth
Agriculture	detection; population monitoring and forecasting	tropical weevils; armyworms (*Spodoptera* spp.); aphids; leafminers; stem borers	mammalian repellents; aphid alarm pheromones	tephritid bait sprays; corn rootworm (*Diabrotica* spp.) feeding stimulant bait; pink bollworm; codling moth, light brown apple moth	cotton; fruit and nut orchards; vegetable crops; rice; sugar cane (estimated >30 pest spp. successfully controlled)
Animal production	detection	flies (muscids, tabanids, calliphorids)		screwworm, tsetse fly	
Forestry	detection; population monitoring and forecasting	bark beetles	bark beetle anti-aggregation	bark beetle tree baits	defoliators; tip moths
Stored products	detection	Indian meal moth; flour beetles		Indian meal moth	Indian meal moth

1.3.1 Sirene®[2]-based management systems

Sirene was developed by the Ciba-Geigy Crop Protection Division (now Novartis Crop Protection) mainly for insect attract and kill formulations. The objective was to develop a novel product carrying a lethal dose of a contact insecticide and an attractant which could be placed with minimal effort within crops or orchards. Two major developmental hurdles had to be overcome. The first was that the formulation had to be UV-protected, as pheromone, and to a lesser extent, insecticide components are rapidly degraded and rendered inactive by UV light. This was achieved by the addition of a liquid UV-absorber which helped prevent pheromone components from isomerisation. The final formulation is a viscous slow-release formulation containing the insecticide and pheromone components. The other major hurdle was the parallel development of delivery and application systems which would allow precise and quick application of attracticide droplets in the field; for Sirene CM (a variant used for Codling Moth hence the "CM") this was the adaptation of a 100g container with a simple pump system which allowed the user to dispense 50 µl droplets with each pump activation (Hofer 1997).

In 1988 the first successful Sirene field trials were carried out for control of codling moth *Cydia pomonella* in orchards (using 6% permethrin and 0.16% codelmone[3] – Sirene CM in Europe, Last Call CM® in the USA and Canada) (Charmillot *et al.* 1996, Hofer *et al.* 1996). The technology was then adapted for pink bollworm *Pectinophora gossypiella* in Egyptian cotton, and in 1993/94 entered the market there under the trade name of Sirene® (Hofer & Angst 1995). The development of more practical application techniques in cotton prompted further research into codling moth control (Hofer & Angst 1995, Hofer *et al.* 1996), leading to the first commercial sales of Sirene CM in 1997 (Hofer 1997). Most of the Sirene CM/Last Call CM is applied to apple and pear orchards in the United States, Switzerland and South Africa (Kirsch *et al.* 2001).

These successful trials of the Sirene technology have encouraged research on a number of other pest insect species, including boll weevil *Anthonomis grandis* on cotton in the southern United States (as Sirene BW) (Santos & Hofer 1996), light brown apple moth *Epiphyas postvittana* on apple in New Zealand (Brockerhoff & Suckling 1999, Suckling & Brockerhoff 1999) and diamond back moth *Plutella xylostella* in the southern United States (as Last Call DBM) (Mitchell 2002). In the case of *E. postvittana* the insecticide used was 6% permethrin with 1% synthetic *E. postvittana* pheromone blend[4] (Brockerhoff & Suckling 1999). Cypermethrin and grandlure[5]

[2] The "®" symbol associated with Sirene is implied hereafter
[3] (E,E)-8,10-dodecadien-1-ol
[4] 95% *(E)*-11-tetradecenyl acetate and 5% (E,E)-9,11-tetradecadienyl acetate

were used for the boll weevil study in the US (Santos & Hofer 1996). For diamond back moth the formulation resembled that for codling moth, with 0.16% w/w diamond back moth pheromone blend[6] and 6% w/w permethrin (Mitchell 2002). Formulations using sex pheromones[7,8] of other species such as potato tuber moth *Phthorimaea operculella* (Gelechiidae) in South African solanaceous crops and false codling moth *Cryptophlebia leucotreta* (Tortricidae: Olethreutinae) in South African citrus, litchii and other subtropical fruits (Sack *et al.* 2001b) have also reached the commercial stage (Kirsch 2001). Further experimental data are being gathered in trials of Sirene-pheromone formulations for the western pine shoot borer *Eucosma sonomana* and the European pine shoot moth *Rhyacionia buoliana*, (both Tortricidae: Olethreutinae) (Czokajlo *et al.* 2001), pecan nut casebearer *Acrobasis nuxvorella* (Pyralidae) (Kirsch 2001), obliquebanded leafroller, pandemis leafroller and pear leafroller (Kirsch *et al.* 2003), loopers (Noctuidae: Plusiinae) and fruitworms fall and beet armyworm, cabbage looper, tobacco budworm and corn earworm on vegetables and Douglas fir cone gall midge *Contarinia oregonensis* (Diptera: Cecidomyiidae) in fir seed orchards (Kirsch 2001). These applications are not currently available on the market, but all have shown promise in field trials.

The Sirene matrix has also been used successfully for non-pheromone attractants, such as plant-based kairomones[9] for three species of *Ceratitis* fruit flies, Mediterranean fruit fly, *C. capitata*, Natal fruit fly, *C. rosa*, and Marula fruit fly, *C. cosyra* (Diptera: Tephritidae) in South African citrus, (Sack *et al.* 2001a), and for host odors extracted from the tarsal gland secretions of white-tail deer for three species of tick, *Ixodes scapularis*, *Dermacentor variabilis* and *Amblyomma americanum* (Acarina: Ixodidae) (McLaughlin *et al.* 2001).

---

[5] (3,3-dimethylcyclohexylidene)acetaldehyde mixture with *(Z)*-2-(3,3-dimethylcyclohexylidene)ethanol and (1*R-cis*)-1-methyl-2-(1-methylethenyl)cyclobutane ethanol

[6] 27% *(Z)*-11-hexadecenyl acetate, 1% *(Z)*-11-hexadecen-1-ol, 9% *(Z)*-11-tetradecen-1-ol and 63% *(Z)*-11-hexadecenal

[7] sex pheromone used for *P. operculella* (E,Z)-4,7-Tridecadienyl acetate, (E,Z,Z)-4,7,10-Tridecatrienyl acetate in ratios between 1:1 to 4:6 (Arn *et al.* 2000)

[8] sex pheromone used for *C. leucotreta* is a 50:50 mixture of *(Z)*:*(E)*-8-dodecen-1-ol acetate

[9] alpha-copaene based natural extracts (Sack *et al.* 2001b)

1.3.2 Pesticides for use in attract and kill

There are four basic modes of action for attract and kill formulations, some of which may be combined within a single formulation:

1. Contact toxicity. An insecticide is incorporated that provides a toxic or incapacitating dose when the insect contacts the lure. The insecticide must be easily able to penetrate the insect cuticle, and thus have a high contact toxicity. Typically these are pyrethroids, such as permethrin (Floyd & Crowder 1981, Hofer & Angst 1995, Angeli & Ioriatti 2000, Charmillot *et al.* 2000a), cyfluthrin (Lösel *et al.* 2000), cypermethrin (Hofer 1996, Santos & Hofer 1996, Angeli & Ioriatti 2000) and λ-cyhalothrin (De Souza *et al.* 1992, Downham *et al.* 1995).
2. Ingestion toxicity. An insecticide and feeding stimulant are combined in the formulation, which the insect ingests after being attracted to the lure. Ingestion toxicants used include carbaryl (Tunstall 1968), methomyl and thiodiacarb (Gregg & Del Socorro 2002), carbofuran (Weissling & Meinke 1991), imidacloprid (Prokopy *et al.* 2000), naled (Steiner *et al.* 1965, Cunningham & Steiner 1972) and dimethoate (Mazomenos *et al.* 2000). Other potential toxicants for which successful laboratory trials for *H. armigera* have been undertaken include spinosad, endosulfan, bifenthrin and other pyrethroids (Gregg & Del Socorro pers. comm.).
3. Sterilization. The males contact or imbibe the attracticide and are sterilized, but not killed. These toxins may also be horizontally transmitted to females that mate with males that have visited the attracticide. Toxicants such as fenoxycarb (Charmillot *et al.* 2000b) and pyriproxifen (Langley *et al.* 1990) have been used.
4. Horizontal transmission of pathogens. Males visiting the pheromone source are contaminated with pathogen propagules; these are transmitted to females that mate with the males, and potentially to eggs that the females subsequently lay. (Pell *et al.* 1993, Furlong & Pell 2001) used an entomopathogenic fungus *Zoophthora radicans* (Entomophthorales) against diamondback moth, *Plutella xylostella*.

Formulations using Sirene are limited to those insecticides and chemosterilants which show high levels of contact activity. In addition to contact activity there are a number of other desirable characteristics for toxicants in a Sirene-based attracticide for *Helicoverpa armigera*. Table 1.5 lists a range of insecticides and insect pathogens and their properties which might influence their use in attracticides for *H. armigera* (data from Tomlin 1997)). An ideal insecticide for this purpose would have no existing problems with resistance, high contact toxicity, no deterrent effects, low mammalian toxicity for both oral and dermal tests, high stability in field conditions,

and low solubility in water. An additional desirable property is low cost of production; this will be determined by market pressures as well as cost of synthesis and production, and cannot be included on Table 1.5. With the exception of the neem extract azadirachtin, all of the insecticides listed are currently approved as active constituents for chemical products as of May 2002 within Australia (Australian Pesticides and Veterinary Medicines Authority 2004), although some of the broader groupings such as the pyrethroids, organophosphates and carbamates may be limited in part. This does not necessarily mean that these are all available for use with *H. armigera*. As of May 2002 the nuclear polyhedrosis virus is exempt from the requirements of the Australian Pesticides and Veterinary Medicines Authority.

The main constraint for the majority of chemicals listed in Table 1.5 is the lack of contact activity. Most of the more recent lepidopteran-specific insecticides are larger molecules which are unable to penetrate the insect cuticle, and are mainly targeted at larvae and eggs where they are ingested by the immatures feeding on sprayed food plants or egg chorions. Of the listed chemicals only pyrethroids and some carbamates and organophosphates exhibit high contact activity for adult heliothines (Plapp & Vinson 1977, Daly 1992). Pyrethroids are also recorded as having repellant or deterrent effects for some insects (Rieth & Levin 1988), and have existing problems with resistance in field populations of *Helicoverpa armigera* within Australia (Gunning *et al.* 1984). Later generations of pyrethroids such as bifenthrin and λ-cyhalothrin give better results against resistant populations of larval *H. armigera*, particularly when synergised with organophosphates such as ethion or with piperonyl butoxide (Gunning *et al.* 1999).

Most data for insecticidal contact activity on heliothine pests comes from assays of early instar larvae rather than adult moths, as it is the early larval stages that farmers attempt to control with broad-acre spraying. Vial tests of insecticides where adult moths are placed in a glass vial coated with a known, usually discriminating, dose of the test compound provide some information on the contact activity of insecticides such as pyrethroids (Plapp & Vinson 1977, Daly 1992, Cameron *et al.* 1995), but these tests are only useful for testing moths of a known age, as insecticide-induced mortality is strongly linked with age (Daly 1992).

Table 1.5 Insecticides and insect pathogens and their properties which might influence their use in attracticides for *H. armigera*. Data from (Tomlin 1997)

Insecticide	Mode Of Action (binding or otherwise)	Group	Resistance	Contact Toxicity	Deterrent	Other Effects	WHO Classifications and LD_{50} Oral Mammalian[1]	LD_{50} Dermal Mammalian[2]	Stability[3]	Solubility[4]
Endosulfan	GABA-gated Cl channel	cyclodiene organochlorine	+	+			II (70mg in aq.sol./kg)	359mg/kg (in oil)	stable	water insoluble, soluble in organic solvents
Fipronil*	GABA-gated Cl channel	phenyl pyrazoles	+	+			II (100mg/kg)	354mg/kg	stable	almost insoluble in water, soluble in organic solvents
Foliar Bt	Crystals damage gut epithelium	bacterium		low			non-toxic	non-toxic	unstable	insoluble, but may be carried
Spinosad	Nicotinic acetyl choline receptor	spinosyns		++			non-toxic (>5,000mg/kg)	low (>2,000mg/kg)	stable	slightly soluble in water
Pyrethroids	Voltage-gated sodium channel	pyrethroids	+	+++	+++		II (15->5,000mg/kg), mean = 132.7mg/kg	low, but may cause sensitivity problems (>2,000mg/kg)	stable-unstable	slightly soluble in water, soluble in organic solvents
Abamectin	GABA & glutamate Cl channels	avermectins		low			Ib (10mg in oil/kg)	low (>2,000mg/kg)	unstable	water insoluble, soluble in organic solvents
Chlorfenapyr	Disruption of mitochondrial respiration	pyrazole analogue		low			II (441mg/kg)	low (>2,000mg/kg)		water insoluble, soluble in organic solvents
Carbamates	Acetylcholinesterase receptor	oxime carbamates	+				Ib,II (17-120mg/kg), mean = 67.7mg/kg	low (>2,000->5,000mg/kg)	stable-unstable	slightly soluble in water & org. solvents (not oils)
Amitraz	Octopamine receptor	formamidines		+		+ (antifeedant)	III (650mg/kg)	>200mg/kg	stable	water insoluble, soluble in organic solvents
Organophosphates	Acetylcholinesterase receptor	organophosphates	+				Ia,Ib,II,III (3->3,000mg/kg), mean = 270.8mg/kg[†]	45->2,000mg/kg	stable	very variable depending on compound
Nuclear Polyhedrosis Virus	Occlusion bodies from gut to rest of insect	virus		none			non-toxic	non-toxic	unstable	
Indoxacarb	Binds to nerve sites	oxadiazines		low			non-toxic (>5,000mg/kg)	low (>2,000mg/kg)		
Emamectin benzoate	Binds chloride channels	avermectins		low			1,500mg/kg	low (>2,000mg/kg)	unstable	
Methoxyfenozide	Hormone regulation & moulting	diacyhydrazines					non-toxic (>5,000mg/kg)	low (>2,000mg/kg)	moderately stable	slightly soluble in water
Tebufenozide*	Hormone regulation & moulting	diacyhydrazines					non-toxic (>5,000mg/kg)	low (>5,000mg/kg) (rat)	stable	insoluble in water & org. solvents
Novaluron*, Lufenuron*	Chitin synthesis inhibitors; affects moulting	benzoylphenylureas		+			non-toxic (>5,000mg/kg)	low (>2,000mg/kg) (rat)	stable	water insoluble, soluble in organic solvents
Azadirachtin*	Disrupts moulting	extract of Neem tree			+++	+ (anti-feedant)	non-toxic (>5,000mg/kg)	low (>2,000mg/kg)		

*Not registered for use on "heliothis" in cotton (*Helicoverpa armigera* & *H. punctigera*) by the APVMA (Australian Pesticides and Veterinary Medicines Authority updated nightly)

[1]Oral LD_{50} figures are those obtained for adult male rats unless stated otherwise

[†]omitting data for chlorpyrifos-methyl which has extremely low mammalian toxicity (>3,000mg/kg)

[2]Dermal LD_{50} figures are those obtained for rabbits unless stated otherwise

[3]Unstable compounds are those which degrade under field conditions to inactive compounds within 2 days; stable compounds persist in active form for >2 days

[4]solubility determined by weight dissolved into solution, with functional insolubility determined as being less than 1mg/litre

1.4 *Mating disruption and comparisons with attract and kill*

The main emphasis of this thesis is on attracticides, but because many of the ecological factors important in achieving a result with attract and kill also apply to mating disruption it is important to compare and contrast the two techniques. Mating disruption has been the subject of several recent and comprehensive reviews (Bartell 1982, Cardé 1990, Cardé & Minks 1995, Sanders 1996, Valeur 1998) and it would be inappropriate to attempt to cover the vast body of literature available on the subject here. However, the basics of mating disruption are outlined, and the published information in relation to mating disruption and heliothine moths is reviewed.

Mating disruption is achieved by permeating a treated area with compounds which interact with male moths so that they are unable to mate with females and egg lay is reduced in the crop. These can include sex pheromones, behavioural antagonists and pheromone analogues. For this thesis I am only considering applications which use synthetic blends which mimic sex pheromones of the target species. The correct choice of pheromone dispensers is critical for successful mating disruption. Designs include slow-release pheromone lures and dispensers which contain stabilizers and UV filters to prevent premature degradation of the formulations (Cardé 1990, Weatherston 1990), spraying micro-encapsulated pheromones and other formulations (Weatherston 1990), or by putting out active high emission sprayers (MSTRS) (Mafra-Neto & Baker 1996, Baker *et al.* 1997) and passive high emission devices (Baker 2004, AC. Oehlschlager, pers. comm.).

The mechanisms which inhibit mate location and mating in this situation are the subject of considerable debate, and different mechanisms may apply depending on the moth species (Valeur 1998). Valeur (1998) summarizes the proposed mechanisms. Based on an extensive review of the literature and his own findings, he concluded that the most important mechanisms are short-term adaptation/habituation and false trail following. Adaptation/habituation occurs after males are exposed to elevated concentrations of sex pheromone. Their physiological response thresholds are raised so that the males can no longer detect the lower concentration of natural plumes. False trail following occurs when males repeatedly follow sex pheromone plumes from the mating disruption sources rather than natural plumes.

The success of pheromone control methods such as mating disruption and attract and kill is often highly dependent on the dispersal ecology of the pest species. Heliothine moths are highly mobile

insects (Gregg 1995), and as such would seem to be poor choices for control with pheromonal techniques (McLaughlin & Mitchell 1982). Mating disruption trials for *Helicoverpa armigera* and other heliothine species have shown that it is possible to prevent mating within a pheromone-treated area (Betts *et al.* 1993, Kehat & Dunkelblum 1993, Park *et al.* 1999, Chamberlain *et al.* 2000, Toyoshima *et al.* 2001). However, significant levels of control within treated crops are rarely obtained with only two studies claiming success (Toyoshima *et al.* 2001 for *H. armigera* in lettuce, Park *et al.* 1999 for the more host plant specific *H. assulta* in capsicum). Lack of control in the studies which report failures has been attributed to the influx of mated females from untreated areas (Chamberlain *et al.* 2000, Betts *et al.* 1993).

Attract and kill using sex pheromones for lepidopteran pests functions in a similar way to mating disruption by preventing successful mating of females within a treated area. However, the aim with attract and kill is to get the males to contact the pheromone source associated with the attracticide, whereas mating disruption does not require males to locate the dispensers.

The principle concern when comparing and designing the attract and kill application is that an attract and kill program which also causes mating disruption through elevated levels of pheromone may fail to work because insufficient males are locating and contacting the lure to receive an incapacitating dose. This will only be a problem if this mating disruption effect does not give equivalent control as a properly designed mating disruption program.

As mentioned before adaptation and/or habituation may result from elevated levels of synthetic pheromone. The male physiological response thresholds are raised to such a level that males cannot detect naturally produced female sources of pheromone. This can result from adaptation of peripheral receptor neurones or from habituation at higher levels of neuronal processing. Habituation has been demonstrated in the laboratory wind tunnel for *Heliothis virescens* (Daly & Figueredo 2000) and for *Trichoplusia ni* (Kuenen & Baker 1981) with the later study separating habituation from sensory adaptation as a principal mechanism resulting in reduced upwind flight and close approach to lures. Kuenen and Baker (1981) found that sensory adaptation of the receptor neurones in the *T. ni* antennae was temporary; once the pheromone source was removed, the neurones returned to normal function after a minute. Continuous exposure to high concentrations of pheromone may inhibit central nervous system habituation because of this adaptation. Habituation can subsequently result in arrestment of upwind flight (Cardé & Minks 1995) and arrestment may prevent males contacting an attract and kill lure if it has been formulated at too high an overall concentration.

When false-trail following is operative males may be able to successfully orient and locate point sources of pheromone, and the ratio of synthetic pheromone sources to calling females in the field may lead to the synthetic sources out-competing the calling females. When female numbers are high mating disruption can sometimes fail as the competitive balance may be overturned. This may represent a general constraint to mating disruption and attract and kill (Cardé & Minks 1995).

Successful mating disruption or attract and kill programs will have to overcome the problem of long-distance movement of moths by increasing the treated area to such a size that mated females from untreated regions are no longer reaching the cropping areas within the treated area. Aside from the extension problems that would arise from having to coordinate and enforce such an area-wide treatment regime, the amount of active ingredients, dispenser/lure production and deployment of lures may become prohibitively expensive.

Attract and kill offers some advantages over mating disruption by using less pheromone per hectare for the same result (Nakasuji & Fujita 1980). Although direct comparisons are not yet published, orchard trials of Sirene CM/Last Call CM applied to apple and pear in the United States, Canada, Switzerland, and Syria (Charmillot & Hofer 1997, Charmillot *et al.* 2000a, Kirsch *et al.* 2003, Mansour *et al.* 2003, Smith *et al.* 2003). Using attract and kill in a Sirene-based formulation allows for ease of application and reduction of potential crop residue in the form of plastic or rubber pheromone dispensers. The greater efficiency and ease of application may enable much larger areas of crop to be treated than with traditional rubber and laminate dispensers for mating disruption.

The use of Sirene based attract and kill for codling moth *C. pomonella* is probably the most prevalent of all the attract and kill systems currently available on the market, yet Last Call CM/Sirene CM still has a small market share in the large market of North America. Small plot trials have been run in Washington and the Pacific Northwest, where the perception is that this system may be more effective than mating disruption in small blocks (< 4 ha), and less affected by conditions such as steep slopes, uneven canopies, irregularly shaped blocks, or windy conditions, all of which can limit the success of mating disruption (Alway 1998).

Attract and kill based on Sirene requires considerably less pheromone. For example Hofer and Angst (1995) found that for control of pink bollworm in Egyptian cotton up to 50 times less pheromone is used compared to comparable mating disruption.

Comparisons with the history of farmer acceptance of mating disruption techniques can explain part of the slow uptake of attract and kill. Mating disruption for orchard pests was demonstrated to be commercially effective compared to conventional insecticides, and resulted in significant reduction in environmental and IPM problems caused by conventional spraying. Despite this it took decades before mating disruption was viewed as a conventional and reliable treatment. Attract and kill may well face the same problem, even though there is adequate data to show that it is as robust or better than mating disruption, with added cost savings (Charmillot & Hofer 1997, Charmillot *et al.* 2000a, Kirsch *et al.* 2003, Mansour *et al.* 2003, Smith *et al.* 2003).

There are some disadvantages in attract and kill. One of the principal issues is that formulations use insecticide which may conflict with organic production values, or interfere with insecticide resistance management (IRM) plans for the targeted pests. There are economic issues to be addressed in the production and marketing of attracticides. Chemical companies which may have invested considerable research and development money in developing attract and kill often need to recoup the costs in the short term, which may in turn result in over-inflated prices for their product at the farm gate, and delay or prevent farmers adopting their product.

Mating disruption and attract and kill systems should be viewed as components of an integrated pest management system which may also involve a complex of other components, including insecticides, cultural techniques, trap cropping and other methods. For example, an insecticide application may be required prior to application of a mating disruption treatment in order to reduce an insect population to a level where mating disruption works. A more productive viewpoint than some of the conflicts mentioned in the previous paragraphs is to view the various techniques as potentially complementary.

1.5 *Thesis outline and rationale*

The following chapters form the body of this thesis:

- **Chapter 1 General Introduction**

  Reviews the pest status of *H. armigera*, chemical communication in heliothines, attract and kill techniques and mating disruption for heliothines.

- **Chapter 2 General Methodology**

An outline of common procedures and locations used throughout the thesis which is necessary for interpretation of all results chapters.

- **Chapter 3 Effects of environmental factors on male behaviour at lures**

Male behaviour was observed around synthetic pheromone lures and the influence of environmental factors on this behaviour measured.

- **Chapter 4 Effects of lure formulation and appearance on male behaviour**

The behaviour of males around synthetic lures is observed after manipulating various aspects of the lures.

- **Chapter 5 Laboratory toxicology of bifenthrin in Sirene®**

The lethal and sublethal effects of insecticides in a Sirene formulation were examined in laboratory conditions.

- **Chapter 6 Field toxicology of bifenthrin in Sirene®**

The efficacy of the attract and kill formulations in Sirene was examined in field conditions.

- **Chapter 7 Weathering of Sirene® formulations in the field**

Life span and weather rates of pheromone formulation in Sirene were measured.

- **Chapter 8 A mark-recapture study of *Helicoverpa armigera* males using pheromone traps**

Fluorescent dye powder and non-lethal traps were employed to study male movement in crops.

- **Chapter 9 Mating disruption in an isolated cropping region**

A full scale commercial mating disruption trial in an isolated cropping region was used to examine the feasibility of using sex pheromone to control *H. armigera*.

- **Chapter 10 General discussion**

A synthesis of the results of the thesis which outlines the future of pheromones for managing *H. armigera*.

Thesis Rationale:

One of the principal aims involved in developing a pest management program which uses behaviour-altering compounds is to make direct field observations of the effects of these compounds on the behaviour of the pest (Lingren *et al.* 1982, Lingren *et al.* 1986). These observations provide essential data so that later observations of experimental manipulations observations can be related back to these original observations. These data also allow for more informed interpretation of monitoring data from field trials and mark-recapture studies. Chapter 3 describes the basic behaviour of *Helicoverpa armigera* males flying to synthetic pheromone lures and how this changes with time after dusk, season, climate and crop type.

An attract and kill formulation based on female sex pheromone will only be successful if it is as attractive as a calling female, particularly when it comes to making the male contact or ingest the formulation (Jones 1998a, Lanier 1990). There are a diverse range of potential factors which may alter the attractiveness of a synthetic lure, some of which might possibly be included in a commercially viable formulation. Other factors which might affect contact can also help to explain what is lacking from a synthetic formulation. Chapter 4 observes the differences in male behaviour in response to different visual cues, presentation methods, pheromone component changes, and inclusion of insecticide and adjuncts.

The effectiveness of the attract and kill formulation will also rely on the insecticide included in the formulation. Males coming in contact with the formulation should be reliably killed at least 95% of the time. Chapter 5 details the laboratory toxicology tests used to determine the appropriate dosage and formulation of insecticide in the Sirene formulation.

Laboratory toxicological tests provide basic and useful information on the potency of insecticide formulations, but this information must be supplemented by field observations on efficacy.

Chapter 6 describes the experiments used to try and determine efficacy of Sirene formulated with bifenthrin under near-field conditions.

The longevity of attracticides is dependent mainly on the release rate of the attractive principals, in this case the sex pheromone components. Other factors such as presentation may alter this release rate. Chapter 7 assesses the weathering of Sirene formulations under field conditions.

The assessment of attracticides up to this point has focussed on individual lures, so has not provided information on how many attracticide droplets should be placed in the field, and over what area of crop. To attempt to provide some answers Chapter 8 describes a mark recapture experiment which provides information on male movement in cropping areas to assist in planning for attract and kill and mating disruption.

Chapter 9 is a description of a full-scale commercial mating disruption trial for *H. armigera*. Similar same factors which lead to success or failure of mating disruption may also be critical for success of attract and kill, hence the findings of this trial have considerable relevance to the rest of this thesis. A large horticultural company, SP Exports Pty. Ltd. provided the funding and field location for this study.

2 General methodology

This chapter outlines common and shared methodologies used for this thesis. Additional statistical, observation and experimental methods are described elsewhere in the relevant chapters where appropriate.

2.1 Study sites

The major study sites used in this study were Nangwee, Bowen, and Promised Land (Queensland) and Bonshaw and Armidale (New South Wales. Figure 2.1 shows the location of these sites.

Figure 2.1 Map of Australia showing location of study sites

Nangwee is located near Cecil Plains in the Darling Downs (27°33'S 151°17'E). This plains region is predominately cropped with wheat (in winter) and sorghum and cotton (in summer), although the relative area put down to these crops varies between years with market demands. Other crops grown include summer crops like sunflower, corn, sweet corn, soybeans and pigeon pea (as a trap crop for *H. armigera*), and winter crops like chick peas, oats, barley and various *Brassica* spp. (as vegetable crops). Soils are heavy grey cracking clays, and there is both dry-land and flood-irrigated cotton grown. Most of the rainfall occurs over summer. Adult *Helicoverpa* spp. are present from early September through to late April.

Bonshaw is located on the Dumaresq River on the New South Wales/Queensland border (29°03'S 151°16'). There are some irrigated cropping regions within 1 km of the river with corn, peanuts, lucerne and some market vegetables grown. The soils are sandy river loam. Irrigation is via overhead sprinklers and is mostly by centre-pivot systems. Cotton is a new crop for this region with about 300 ha grown in two farms since 1999/2000.

Bowen is a coastal town in northern Queensland (20°00'S 148°14'). Bowen mainly produces tomatoes and many other market vegetables during winter. Other agricultural activities include grazing and mango orchards. Soils are sandy river loams and all crops are irrigated with drip irrigation. Rainfall is largely restricted to the summer months, with scant rainfall from April to October.

Study sites at Armidale were at the Laureldale Rural Research Station (30°29'S 151°40'E). This is a University of New England research station used for teaching and research purposes. Crops include some horticultural and orchard plantings, but the area is predominantly pasture. Most rainfall occurs during the summer months.

Promised Land is a cropping region near Cordalba, Queensland (25°10'S 152°13'E). Most of the cultivated area is planted to sugar cane, with the remainder being tomato, capsicum, melon, citrus, avocado and other fruit trees. This area is described in detail in Chapter 9.

2.2 Field observations

Field observations were made with night vision goggles (binocular, Litton Electron Devices Night Vision Goggles, Model M912A, Arizona USA, monocular, ITT Industries 6015 Night Vision

Monocular, Model NQ6015UL). Hand torches (3 V) with an infrared filter (Hoya R72) were used to illuminate the observation area around the lures. The binocular goggles enabled better resolution of the three dimensional flight behaviour of moths, whilst the monocular device gave better results at low light levels, as well as reducing operator fatigue due their light weight, ease of use with prescription glasses and hands-free operation.

To get a full view of the moth behaviour, a mobile observation tower was used. Earlier observations were from a 4 m welded-frame tower on a trailer, with later observations from an adjustable (2 to 4.5 m high) scaffolding tower on the back of a tray-top 1 tonne utility truck. These towers are shown in Figure 2.2. The average height of the observer was 5.5 m above the crop level depending on crop type.

Figure 2.2 The two towers used for night observations for this study. The welded steel frame tower was a fixed height of 4 m (main photograph), whilst the scaffolding tower could be adjusted between 2 and 4.5 m high (insert).

Observations in late spring, 1999 indicated that male moths could be deterred from approaching lures which were set up too close to the observation tower, or downwind of the observation tower and associated vehicle. It was not clear whether this was because of the visual perception of the tower and vehicle, or from the disturbance to the pheromone plume from interruption of the natural wind flow. When setting up for observations the vehicle and observation tower were always parked in such a way as to allow prevailing winds to come from across the surrounding

crop area and reach the lure unimpeded by the observation point. Pheromone lures and other treatments under observation were placed at the same height on white plastic-coated hollow steel curtain rods. Each observation was 10-15 minutes long. Normally multiple moths would be observed within each observation period (Chapter 3). As male moths approached the lure their behaviour was noted by recording the observer's voice onto a cassette recorder. Observations were transcribed into a behavioural analysis program, The Observer v. 3.0 (Noldus 1995). Moth behaviours were scored as approaching, near or contact. In general three behavioural parameters were measured, although variations occurred due to the nature of some treatments used in observations. These were:

1) Approaching: The male moth entered the field of vision of the observer and exhibited directed flight towards the lure. The range of this observation was dependent upon the surrounding vegetation. It was usually possible to monitor moths approaching from a distance of at least 10m.
2) Near: The moth is within 1m of the lure
3) Contact: The moth contacts the treatment

2.3 Statistical analysis

Data compilation of observations was performed using The Observer v 3.0 program (Noldus 1995). From these data summaries of the percentages and numbers of moths in behavioural states observed for each observation period and the mean duration/time spent in the behavioural states were subjected to further analysis in S-Plus 2000 Professional (MathSoft 1999). Data were analysed for normalcy and homogeneity of variance depending on the tests which were used. Common tests used include the following:

- Pearson's χ^2 statistic is used throughout to analyse proportional (ie. percentage) data; this is abbreviated to χ^2 in the text where test results are presented.
- For data which were normally distributed, one-way ANOVA, Studentized t-test, for absolute numbers of moths in different behavioural states. If there were multiple treatments and the ANOVA indicated a significant result, contrasts were used to extract information as to the direction of these significant results.
- For non-normal data with multiple treatments the non-parametric Kruskal-Wallis Rank Sum Test was used to compare treatments.
- Where multiple means were compared (for example, behavioural variables versus crop type) a multiple comparison of simultaneous confidence limits was used to determine if there were

significant differences between means. For these tests a critical point was derived from the Tukey studentized range quantile, and this was used to calculate the intervals. Intervals derived from these comparisons of pairs of means which excluded zero from their range were deemed significant at the comparison-wise error rate of alpha < 0.05 (MathSoft 1999). This procedure is a relatively conservative multiple comparison test, which allows for an overall Type I error rate of p = 0.02 for a comparison of six treatments, as is the case for comparing crop types in this dataset (ie. the chance of incorrectly obtaining a significant result is 2% for a comparison over six treatments) (Jones 1984).

- Proportional (percentage) data were arcsine (square root) transformed before analysis to avoid violating assumptions in regards to normality.

Other more specialised and targeted tests are used throughout the thesis; these are outlined in the relevant chapters when they are used. When data are presented graphically or in tables, the errors associated with proportional/percentage data are 95% confidence limits. Errors associated with means are the standard errors of means.

2.4 Weather data

Data logged weather data were collected for Darling Downs observations. Data came from a Queensland Department of Primary Industry weather station located at "Clapham Farms" 27°34'S 151°20'E). Data were collected at 15 min intervals, and included wet and dry bulb temperatures, soil temperature (at 10 cm deep), wind run, wind direction and humidity. Direct readings of the average wind speed (over the observation period), air temperature at crop level and relative humidity were made from a Kestrel handheld Weather Meter (Nielsen-Kellerman, USA, Model K3000).

2.5 Laboratory cultures

Laboratory cultures of *H. armigera* were raised on a soybean-based artificial diet similar to that of Teakle & Jensen (1991) at 25±2°C 16:8 h light:dark (L.D.) reverse-cycle conditions. Individual larvae were reared until emergence in 35 ml-plastic cups (Solo P101M, Urbana, Illinois, USA), and adults were either directly used in experiments, or transferred to 150 ml plastic containers (Polarcup (Australia) Ltd, Bankstown, NSW). Water was supplied via a dental wick soaked in distilled water for those moths which were used within a day after emergence, and 5% sucrose for those used in longer term experiments. Laboratory cultures were refreshed annually by

interbreeding with moths reared from spring-collected wild larvae from the Darling Downs in Queensland.

2.6 Formulations

Blank Sirene (containing neither pheromone nor pesticide) was supplied by Phillipp Kirsch of IPM Technologies, Portland, Oregon, USA and stored at 5°C until needed. The viscous nature of Sirene required the use of a specialized mixer in order to uniformly distribute the small quantities of pheromone and pesticide components. A double-vane paddle which fitted into the base of a 50 ml plastic syringe and an electric motor taken from a microwave oven geared to 4 rpm was used to mix 10 to 20 ml of Sirene for 2-3 h. An alternative method of using two 25 ml syringes was devised, where the Sirene and pheromone/insecticide components were placed in one syringe and then joined to the second syringe by a short length of non-reactive plastic fuel tube. Passing the formulation between the two syringes twenty times gave satisfactory results in a shorter time compared to the mechanical stirrer. Smaller syringes (eg. 5 ml) were used for small quantities of test formulation. Adequate mixing by both methods was judged either by comparing sub-samples of a formulation in the GC-MS (Chapter 7), or by adding a fluorescent yellow water-soluble dye powder (Radiant, USA) and visually assessing the homogeneity of the mixture.

Earlier pheromone observations (1999-2000 and 2000-2001 field seasons) used (*Z*)-11-hexadecenal (95%) and (Z)-9-hexadecanal (~95%) obtained from Sigma-Aldrich (Castle Hill, NSW). Later formulations (2001-2002) used (*Z*)-11-hexadecenal (±95%) and (*Z*)-9-hexadecenal (±95%) and rubber septa lures from Pherobank, Plant Research International Wageningen, The Netherlands. Pheromone components were stored at -20°C.

Rubber septa lures for general monitoring and observation work were formulated by placing 80 septa in 40 ml hexane (~99% GC purity, Fluka, Sigma-Aldrich, Castle Hill, NSW) with 130 mg (*Z*)-11-hexadecenal, 15 mg (*Z*)-9-hexadecenal, and 7 mg 2,6-di-tert-butyl-4-methyl phenol (butylated hydroxytoluene, BHT) (99%, Lancaster, Bio-Scientific Pty. Ltd., Kirrawee, NSW) as an antioxidant. This gave a loading of ~1.8 mg/septum of a 10:1 ratio of the two pheromone components, which approximates the loading found in commercially supplied lures.

A "standard" Sirene lure was a droplet of ~ 200 mg and contained 1% w/w loading of the two pheromone components in the same ratio as for the rubber septa lures. Unless otherwise stated this lure was used for most of the observations in this thesis. Many recent publications cite a smaller 50mg droplet size for Sirene/Last Call (eg. Evenden & McLaughlin 2004), but these

applications are for much smaller moths, and were not used in the context of direct observation of males at the lure where droplet size assisted in observation of behaviour. Additionally, the dispensers used for factory-formulated Last Call are calibrated to deliver droplets of this size. In this study we did not use these dispensers, as we formulated our pheromone and additives directly into blank Sirene.

Technical grade bifenthrin (93.3% active ingredient, FMC Corporation, Agricultural Chemicals Group, Maryland, USA) was used for all insecticide assays. Formulated concentrations were based on the amount of the active ingredient present in the formulation. Technical grade bifenthrin is a waxy solid and could not be evenly incorporated into the Sirene. To overcome this bifenthrin was dissolved to make a stock solution of 1:1 weight of active ingredient to volume of acetone (AR grade, APS Pty. Ltd., Seven Hills, NSW) and the appropriate volume incorporated in the Sirene formulation.

2.7 Pheromone traps

Green universal funnel traps (AgriSense BCS Pty. Ltd., Pontypridd, South Wales, UK), commonly known in Australia as AgriSense traps or dry funnel traps, were used for general monitoring and experimental purposes. These are referred to throughout the thesis as "AgriSense traps". Figure 2. 3 illustrates an example with a clear plastic base which shows the pest strip (Sureguard Ministrips, Kiwi Brands Pty. Ltd., Clayton South, Victoria) inside the trap; the ones used in this thesis were identical except they had an opaque green base. A non-killing inverted funnel trap was also used when it was necessary to obtain live males for field experiments. These are referred to as "Texas traps" in this thesis. Gregg and Wilson (1991) describe the design and use of both of these traps in detail in respect to monitoring heliothine populations.

2.8 Laboratory wind tunnel observations

The wind tunnel at the University of New England was made of transparent Plexiglass®, and was 260 cm long, 60 cm wide and 60 cm high, with a pulling fan that provides a 0.3 to 0.4 ms^{-1} air flow. Temperature conditions in the wind tunnel were between 24-27°C, and lighting was provided by two fluorescent red photographic safe lights (Encapsulite, Type R10) above the tunnel. Bubble wrap on the top of the tunnel acted as a diffuser for the light. The far side and half of the upper surface of the wind tunnel were covered with white cardboard so that moths were clearly visible during flight.

Figure 2.3 Dry or universal funnel trap ("AgriSense trap") showing pest strip in base. Traps used in this thesis had an opaque green base rather than the clear base shown here.

3 Effects of environmental factors on male behaviour at lures

The process of chemical communication between female and male moths occurs in a spatially complex and temporally fluid environment. To maximise the chances of successful mating both sexes may adopt a number of behavioural strategies which are responses to this changing environment.

3.1 Introduction

Circadian rhythms in sexual behaviour are present in all moths which have been studied (Cardé *et al.* 1996). Female moths produce pheromone at specific times of day, with the timing of calling behaviour at least partially controlled by an endogenous body clock (Traynier 1970, Sower *et al.* 1970). The timing of calling can be modified by exogenous events such as photoperiod (Traynier 1970, Kanno 1981b, Delisle & McNeil 1986, Del Socorro & Gregg 1997), humidity (Kanno & Sato 1980) and temperature (Kanno & Sato 1979, Haynes & Birch 1984, Delisle & McNeil 1987, Kou & Chow 1987, Webster 1988, Del Socorro & Gregg 1997).

Male moths also exhibit diel periodicity in that they are more responsive to pheromone plumes at certain times of the day. As with female moths, these rhythms can also be modified by factors such as season (Batiste 1970, Saario *et al.* 1970, Kaster *et al.* 1989), photoperiod (Traynier 1970, Kanno 1981b) and temperature (Kanno 1981a, Kanno & Sato 1979, Cardé & Roelofs 1973). This periodicity is not strongly linked to circadian rhythms, and is more dependent on exogenous factors such as time after lights off/dusk For most species this timing of peak male responsiveness is primarily controlled by the female circadian rhythms and coincides with mating in the field (Shorey 1966). The periodicity of sexual behaviour in both sexes also changes with the age of the individual insect (Traynier 1970, Delisle & McNeil 1986, Delisle & McNeil 1987, Kou & Chow 1987, Del Socorro & Gregg 1997).

Females may emit sex pheromones at certain times of day that do not conflict with other biological functions such as feeding, or oviposition, or at times which reduce the risk of exposure to potential predators. Being reproductively active within a certain time window may also increase the chance of finding a suitable partner, and may act to enforce reproductive isolation between closely related sympatric species which share a common sex attractant system (Cardé & Roelofs 1973, Roelofs & Cardé 1974).

For many species females are only receptive and emitting pheromone for a short period of time, with males actively seeking females for the time leading up to, during and immediately after this receptive period, so that their searching behaviour overlaps the female calling period (Shorey 1966, Sower *et al.* 1970, Gemeno & Haynes 2000). Male gypsy moths *Lymantria dispar* (Lymantriidae) show a bimodal pattern of attraction to synthetic pheromone lures which is congruent with the bimodal pattern of female emergence, suggesting that male activity patterns are synchronized with emerging and pheromone-emitting females (Cardé *et al.* 1996). Similar coordination of female calling and male response has been recorded for the artichoke moth *Platyptilia carduidactyla* (Pterophoridae) (Haynes & Birch 1984), *Holomelina immaculata* (Arctiidae) (Cardé & Roelofs 1973), and *Agrotis ipsilon* (Noctuidae) (Gemeno & Haynes 2000).

For exothermic organisms such as insects, it may not be possible to be active when temperatures are below or above certain thresholds. A further constraint is whether the organism has diurnal, crepuscular or nocturnal activity patterns, as this limits the total time available for reproductive behaviours. This circadian pattern of mate-finding behaviour may be further modified by a number of other factors that impinge upon a species that uses an airborne pheromone mate-location. The pheromone plume structure and density may be altered by wind speed, wind direction, geophysical characteristics and crop type. The air temperature may influence the volatility and release of pheromone components (Ioriatti *et al.* 1987), including the re-release of pheromone from the surrounding vegetation (Wall & Perry 1983), the reception of the pheromone (Charlton *et al.* 1993), and the ability of the male to fly (Coombs 1993). Females may not call once the temperature drops below a certain threshold, or may call for shorter periods of time (Hou & Sheng 2000).

Over longer time scales further variation in mate-finding behaviour might be expected. Adult *Helicoverpa armigera* are active in cropping areas of southern Queensland and northern New South Wales from early October until late April, when the shortened autumn photoperiod and decreasing temperatures trigger a facultative diapause in late larval and prepupal stages (Wilson *et al.* 1978). For example, minimum temperatures from the Darling Downs cropping region vary from early spring mean daily minimums of 12 °C in October, rising to 18 °C in January/February, finally cooling down to 12 °C in April. Mean daily maximum temperatures range from 27 °C to 32 °C to 26 °C for the same times (Dalby Post Office weather station, data from 30 year database, 1961 to 1990, Australian Bureau of Meteorology, http://www.bom.gov.au/). This wide range of temperature is matched by corresponding changes in humidity, rainfall and wind run and wind direction.

Mate finding behaviour is likely to be altered by these factors on a broader level than on a night to night basis. Female *H. armigera* that have emerged from over-wintering pupae exhibit different patterns of calling behaviour compared to subsequent generations (Hou & Sheng 2000). Some Lepidoptera react to long hot summer periods by undergoing some form of reproductive diapause. Adults of the bogong moth *Agrotis infusa* (Noctuidae) migrate in late spring from their inland breeding grounds and spend summer in the cooler montane areas closer to the coast. During this time they do not mate or reproduce, but when cooler times come to the inland breeding regions they migrate back, mate, then lay eggs (Common 1954). Available food plants during the hot dry summer months are unsuitable for larval development, but later in summer and in autumn when there is a flush of new growth suitable for larval feeding the moths migrate back from the montane regions to the inland regions to mate and lay eggs. This is probably an obligate summer diapause for *A. infusa*. There is no evidence for such a mechanism in *H. armigera* but it is possible that long hot and dry periods may alter female calling behaviour or change male response to pheromone.

A form of temperature-dependent summer pupal diapause has been reported for the North American species *Heliothis virescens* (Noctuidae) where pupae exposed to high temperatures (at or above 43°C) entered summer diapause and did not emerge until autumn (Henneberry & Butler 1986). A similar diapause has been reported for pupae of *H. armigera* in the Sudan Gezira (Hackett & Gatehouse 1982), although this has not been observed for *H. armigera* in Australia (Fitt 1989).

Summer diapause/aestivation in adults is often associated with migratory behaviour, as with *A. infusa*, but the effect of this migratory behaviour/summer diapause on pheromone related mate-finding behaviour in *A. infusa* is yet to be investigated. The correlates between migratory behaviour, summer diapause and mating behaviour have been studied in some detail in black cutworm *Agrotis ipsilon* (Gemeno & Haynes 2001, Gemeno & Haynes 2000, Kaster *et al.* 1989).

Asaro and Berisford (2001) tested the hypothesis that male nantucket pine tip moth *Rhyacionia frustrana* (Tortricidae) numbers were reduced in pheromone trap catches due to seasonal changes in adult longevity. Tip moth catches in pheromone traps decrease in the middle of summer despite there being no apparent drop in population densities or in damage to trees. Male life spans may have been reduced in the middle of summer compared to early or late summer, which in turn may reduce the number of males able to visit pheromone sources. Spessa (1991) found a negative

relationship between temperature and female longevity; whether this occurs in males is unclear. Other potential factors for seasonal variation in male activity at pheromone sources may come from changes in food plant availability and quality and factors induced by changing moth population densities, such as potential competition from calling females in the field (Kvedaras 2002).

This chapter analyses the behaviour of male *H. armigera* flying to synthetic lures in the field. The general flight behaviour near the lures is described, and this behaviour is correlated with time of day, crop type, time of year and climatic conditions. From this it may be possible to better understand the dynamics of an attract and kill system in different crops, at different times of the day and the year, and in different climatic conditions given the variation in behaviour of males around synthetic pheromone lures.

3.2 Methodology

Observations of male *Helicoverpa armigera* behaviour were made with a droplet of the standard Sirene formulation (Chapter 2.6) placed on a 3 x 2 cm piece of white plastic (Corflute®, Signwave, Parramatta Australia) with lures replaced nightly. Observation techniques and climatic conditions were made and data collected and analysed as described in the general methods section (Chapter 2.2). Data were from a total of 60 observation periods (10 – 15 minutes each) with 18 observation periods from 6 nights at Bowen and the remainder from Nangwee. Observation periods were usually 30 - 40 minutes apart.

Observations were made in a variety of crops which were chosen on the basis of their perceived attractiveness to adult moths (both for oviposition and nectaring) to maximise the number of males attracted to the lure. If a crop was flowering it was preferred over a post- or pre-flowering stage of the same crop. Observations were made in sunflower, pigeon pea, cotton, soy bean, tomato, and corn. Observations were also made in corn and pigeon pea as strip crops around cotton (referred to as "corn and cotton" and "pigeon pea and cotton" in the results).

The observation tower was moved every two nights to a new location in the crop to avoid problems with pheromone contamination of the crop surrounding the lure. Males contacting the lure often spread traces of the Sirene formulation with pheromone onto surrounding plants, which caused other males to fly to the contaminated plants, or to display plume-following behaviour which was not directed at the lure. This may have also occurred due to the surrounding vegetation

absorbing airborne pheromone from the Sirene formulation and re-emitting, a phenomenon which has been observed for synthetic lures placed in the field for other species such as the pea moth *Cydia nigricana* (Tortricidae) (Wall *et al.* 1981).

Behavioural variables measured at the lure were:

1) Percentage of approaching males within 1m of the lure (near)
2) Percentage of approaching males contacting the lure
3) Number of males per second approaching the lure
4) Number of males per second within 1 m of the lure (near)
5) Number of males per second contacting the lure
6) The time spent by each male approaching the lure
7) The time spent by each male within 1 m of the lure (near)

The environmental variables collected during the observations were:

1) Air temperature at crop level (recorded at the end of each observation)
2) Relative percentage humidity
3) Average wind speed at crop level during the observation period
4) Time of season (month)
5) Time after sunset (hours)
6) Crop type

Not every observation had a full set of the three climate-related variables; the number depended on availability of equipment.

The statistical analyses used are described in Chapter 2.3.

3.3 Results

3.3.1 General observations of behaviour around lures

Male *H. armigera* displayed a stereotyped set of behaviours when viewed flying close (within 5 m) to lures. Male behaviour approaching the lure from distances further than 5 m was variable, with some males flying directly towards the lure at a slight angle to the line of the wind direction while other males performed wide casting (zigzag) movements. Low wind speed often seemed to induce casting behaviour. When males were within 5 m of the lure these differences largely disappeared, with males hovering in line with the pheromone plume from the lure, and

approaching close to, and slightly below, the downwind side of the lure. Often males would approach no closer than 10-20 cm, and would remain hovering at this distance downwind from the lure prior to flying away. Males often half crawled/flew up the curtain rod from below and downwind of the lure before either contacting the lure, or flying away upwind. Figure 3.1 and 3.2 are flash photographs of a male exhibiting this crawling/flying behaviour at a lure. Note the large amount of scales left on the lure by moths contacting the formulation. Some males displayed advanced stages of sexual arousal, and treated the pheromone lure as if it was a female moth. Figure 3.3 shows a male at a Sirene and pheromone lure with extended hair pencils, as if it was in the advanced stage of courtship with a female *H. armigera*. Other males were observed attempting to copulate with the source, but this was not a commonly observed behaviour with the synthetic lures.

Most males left in the upwind direction, with few looping back to return and approach the lure for a second time. It was not possible to determine if the same males were visiting the lure many times over if they were flying out of the visual range of the night vision equipment. An added complication which prevented some observation of behaviour at a distance from the lure was that approaching males often flew from below the cover of the crop canopy, only becoming visible when they got within 10 m or so of the lure.

Groups of males often flew to the lure. The number of males in a group ranged from two to ten. Grouping such as this appeared to modify the behaviour of males within the group, although this was difficult to quantify. A typical observation was that the lead male would fly to the lure, and either get very close, or contact it. The remaining males in the group would hover close behind the lure, and would leave without contacting or getting very close to the lure.

Casual observations of males in the field often indicated that other factors which were difficult to quantify may also alter male behaviour. When the number of males flying was reduced, such as in early or late season, and observations were made in low open cropping systems such as in green beans or soybeans, male behaviour was often altered by insectivorous bats attempting to capture the moths. As a bat approached its sonar pulses seemed to trigger avoidance behaviour on the part of the male moths, interrupting their normal approach behaviour to the pheromone lure, and causing them to spiral towards the ground. These conditions often reduced the number of legitimate observations of males per observation period. When this happened observations were temporarily terminated, reducing the overall number of observations per period.

Figure 3.1 Male *H. armigera* crawling/flying up a curtain rod to a Sirene and pheromone lure on a Corflute plastic square. Note the scales left adhering to the lure after previous contacts from males. The lure was placed in a field adjacent to flowering sunflower in the Darling Downs.

Figure 3.2 Male *H. armigera* near the same lure as for Figure 3.1. Typical proximate behaviour of males at these lures consisted of males approaching from below the height of the lure and flying/climbing up to the level of the lure. Contact with the lure was scored conservatively when viewed from the distance of the observation tower; this male may not have been scored as having contacted the lure.

Figure 3.3 Male *H. armigera* near a fresh Sirene and pheromone lure adjacent to a field of flowering sunflower in the Darling Downs. A yellow fluorescent dye has been added to this formulation. The extended hair pencil scales on the tip of the abdomen indicate that the synthetic sex pheromone has aroused the male to a state where it is responding to the lure as if it is a female moth.

Table 3.1 lists the ranges, means and standard error for the seven behavioral variables. The overall numbers of males at lures varied greatly over the range of environmental conditions encountered. A large proportion of males that approached the lure subsequently got near it, but after this there was considerable variation in male behaviour. Subsequent sections of this Chapter attempt to explain why this variation occurred.

Table 3.1 Ranges, means and standard errors of the seven behavioural variables measured for males flying to standard lures based on 60 observations.

Behavioural Variable	Range	Mean & Standard Error
% males approaching that got near the lure	50-100	82.1 (1.7)
% males approaching that contacted the lure	0-58.3	11.0 (1.3)
Mean number of males per observation	6-325	66.7 (9)
Mean number of males per second approaching	0.0084-0.4693	0.0961 (0.0126)
Mean number of males per second near	0.0083-0.4196	0.0794 (0.0111)
Mean number of males per second contacting	0-0.0266	0.0077 (0.0009)
Mean time spent approaching the lure per male (sec)	0.1737-15.3167	3.3277 (0.3504)
Mean time spent near the lure per male (sec)	0.1896-18.3800	5.3761 (0.4266)

3.3.2 Diel variation in behaviour at lures

Observations were made from between 28 min to 8 h 56 min after dusk. Figure 3.4 is a histogram of the number of observations made over 9 h after dusk throughout this study. At least three observations were made during the course of this study for every hour up to nine hours after dusk.

Figures 3.5 and 3.6 show the mean percentage of approaching male moths that flew near and contacted the lure. There did not seem to be a strong relationship between measured behaviour of the males at the lure and time since dusk, although there was some evidence for bimodal peaks in the mean percentage of approaching males that contact the lure (Figure 3.6). The peaks occurred at between 3-4 hrs after dusk, and 6-7 hrs after dusk. The number of moths per second near and contacting the lure shows similar, but more variable trends, whilst there were no observed trends in other measured behaviour.

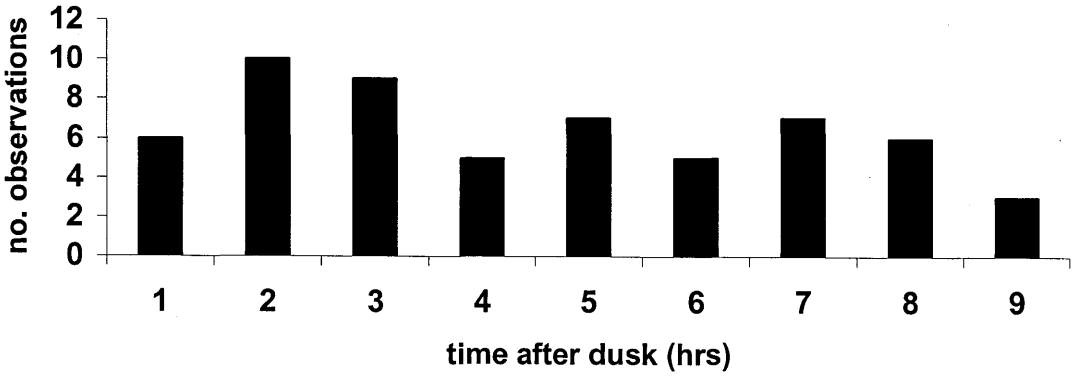

Figure 3.4 Numbers of observation periods of males at standard pheromone lures for each of nine hours after dusk

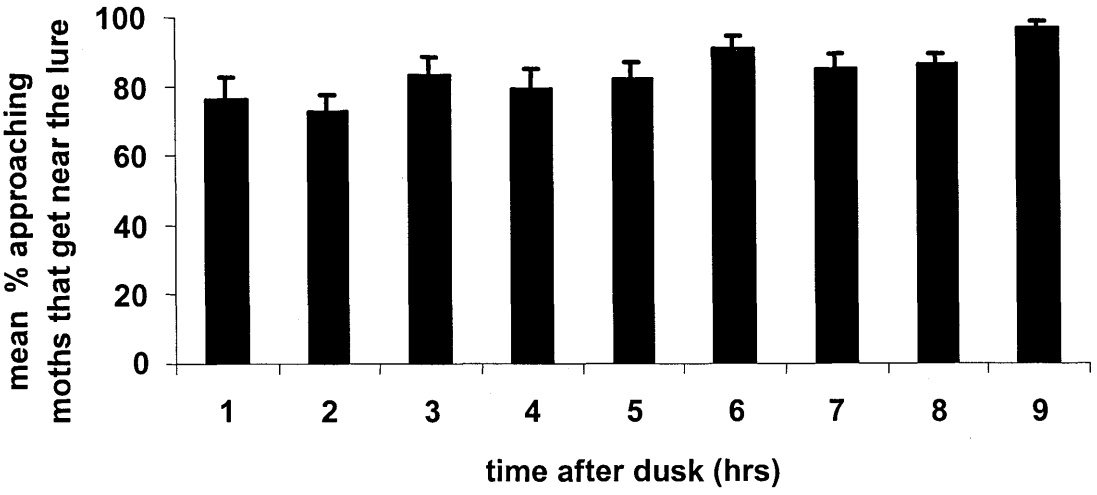

Figure 3.5 Mean percentages of males approaching the lure that got near the lure for each hour after dusk. Error bars are the standard errors of the means.

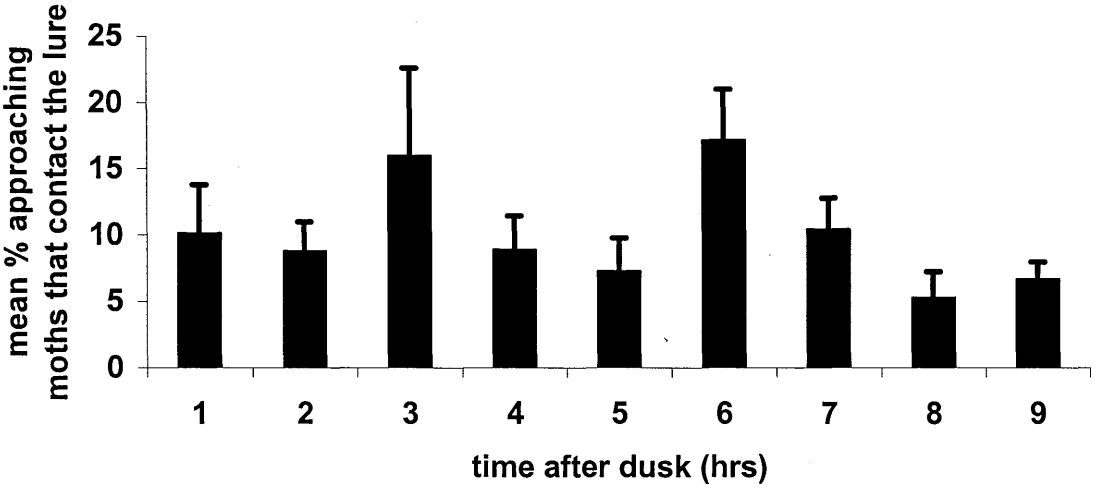

Figure 3.6 Mean percentages of males approaching the lure that contacted the lure for each hour after dusk. Error bars are the standard errors of the means.

3.3.3 Variation associated with crop type

The number of observations on different crops are shown in Figure 3.7. Crop type had a significant effect on many of the behavioural variables. Figures 3.8, 3.9 and 3.10 show the mean number of males per second approaching, getting near and contacting the lure. Significantly more males per second were observed approaching the lure in sunflower compared to the other crops (with the exception of pigeonpea) (Figure 3.8). Similar outcomes were observed for the number of males per second getting near the lure, with significantly more males near sunflower compared

to tomato, pigeonpea strip next to cotton, corn strip next to cotton and corn (Figure 3.9). The pigeon pea by itself also resulted in significantly more males near the lure compared to tomato. These significant differences were not expressed in the numbers of males per second contacting the lure; there were no significant differences between crops for this behavioural variable (Figure 3.10).

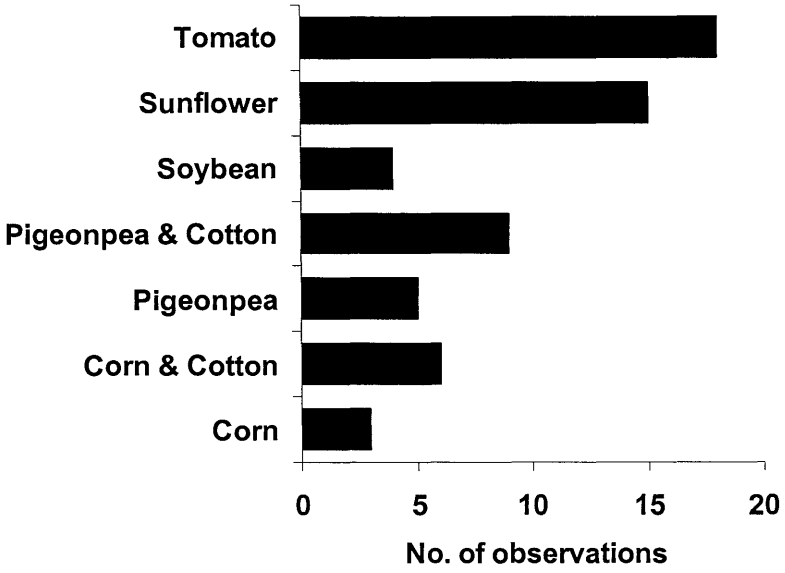

Figure 3.7 Numbers of observation sessions made in different crop types

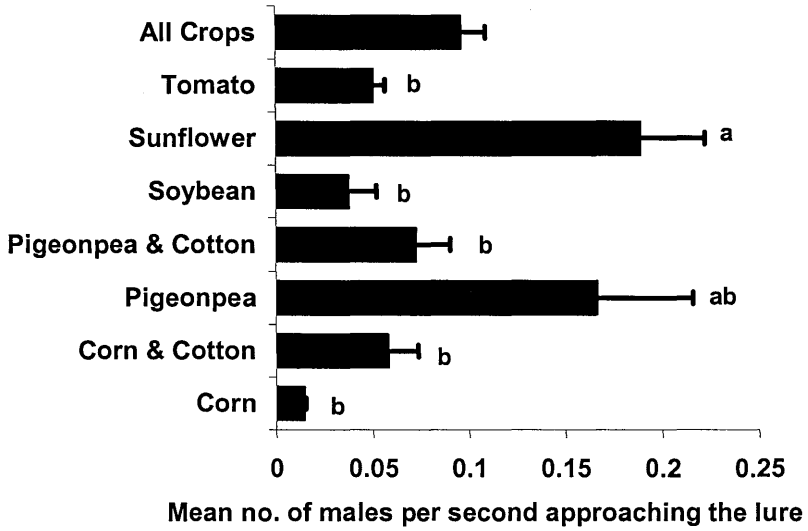

Figure 3.8 Mean numbers of males per second approaching the lure in different crop types. Error bars are standard errors of the means. Bars with the same letters are not significantly different.

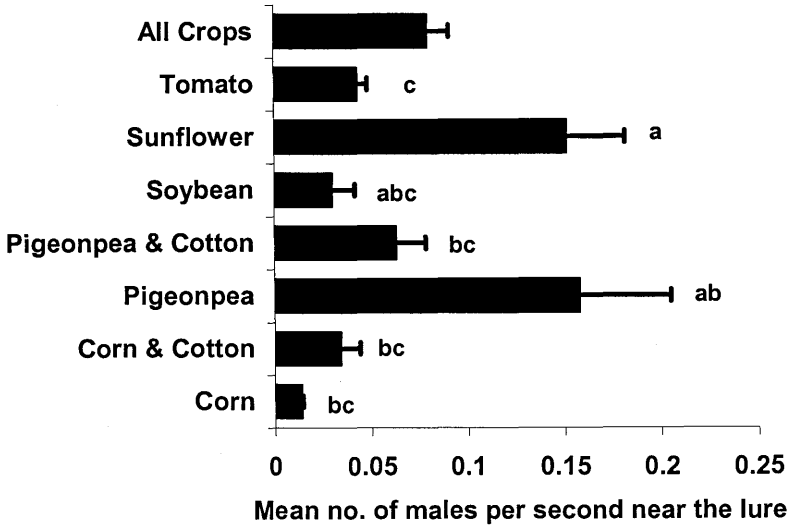

Figure 3.9 Mean numbers of males per second near the lure in different crop types. Error bars are standard errors of the means. Bars with the same letters are not significantly different.

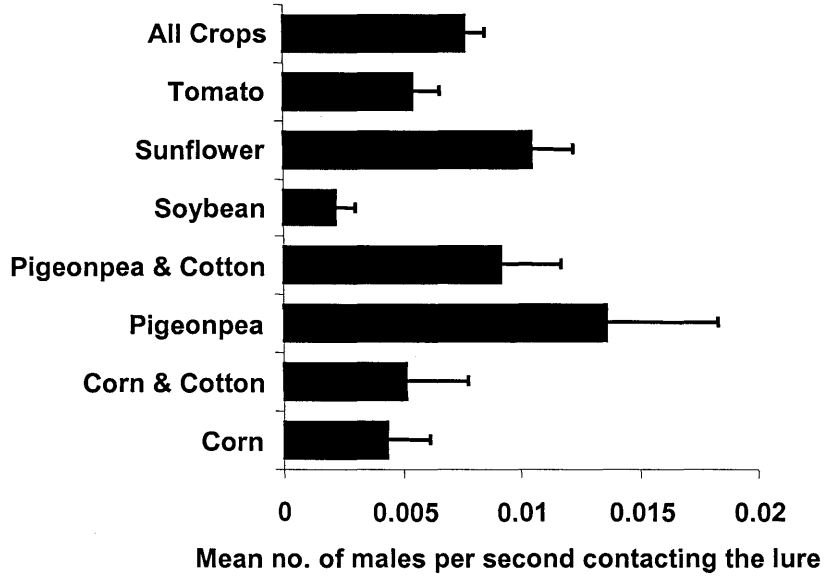

Figure 3.10 Mean numbers of males per second contacting the lure in different crop types. Error bars are standard errors of the means. Scale of x-axis is an order of magnitude less than Figures 3.10 and 3.11.

Figures 3.11 and 3.12 show the mean percentages of approaching males that got near and contacted the lure for the different crops. The percentages of approaching males that got near the lure were somewhat similar for most of the crops (80-90%), although the proportion of males getting near the lure was much lower (~60%) for the observations made in corn bordering a cotton crop. Multiple comparisons using arcsine-square root transformed proportions revealed however,

that there were significant differences between crops. A significantly greater proportion got near the lure in corn compared to all the other crops, whilst the corn strip next to cotton resulted in a significantly reduced proportion of males getting near compared to the other crops. Other less pronounced significant differences were observed between the remainder of the crops. The proportion of males that contacted the lure was similar for most crops (~10%) with exception of males observed in corn (~30%) (Figure 3.12), although this difference was not significant when compared to pigeon pea, a pigeon pea strip next to cotton, soybean and tomato.

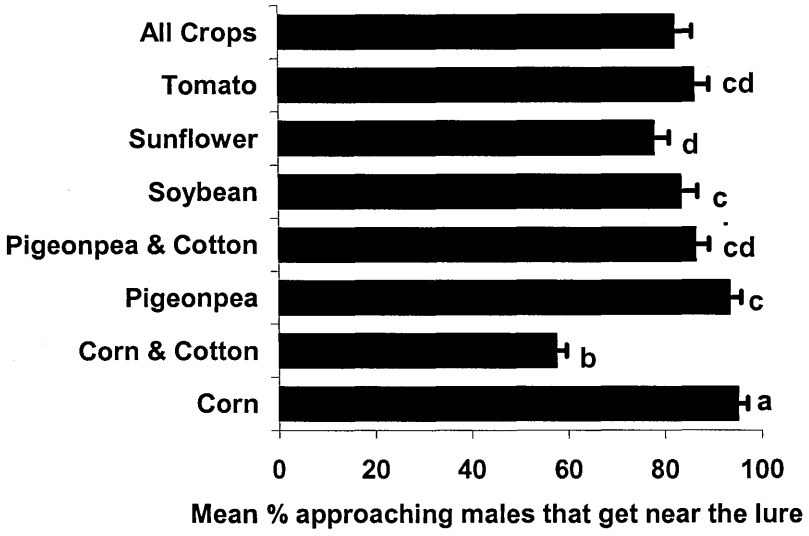

Figure 3.11 Mean percentages of approaching males that get near the lure in different crop types. Error bars are standard errors of the means. Bars with the same letters are not significantly different.

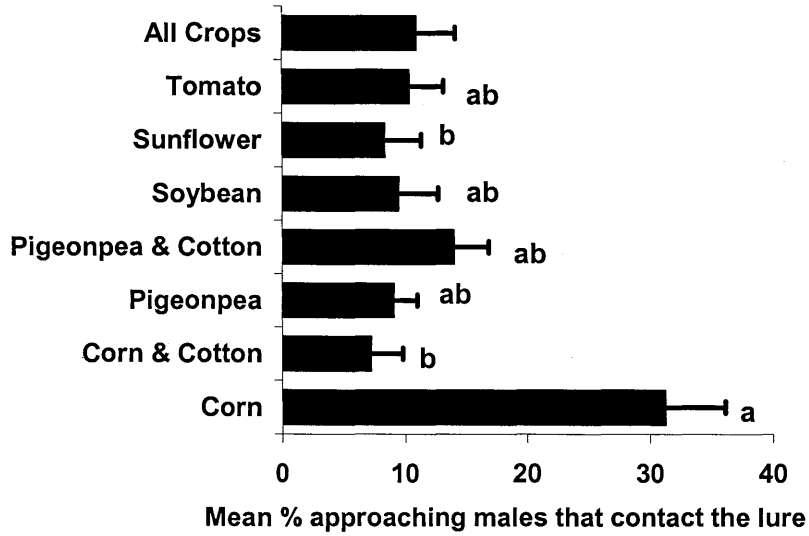

Figure 3.12 Mean percentages of approaching males that contacted the lure in different crop types. Error bars are standards error of the means. Bars with the same letters are not significantly different.

3.3.4 *Variation associated with date of observation*

Figure 3.13 is a histogram of the number of observations per month. Note that there are fewer observations in November, December and March. General conclusions based on the data from these months must be treated with some caution.

Seasonal variation in moth numbers and behaviour was evident, with much higher numbers of moths approaching the lure during the warmer months, December, January and February, a time when farmers often experience the worst problems with egg lay and larvae in crops. Figures 3.14, 3.15, 3.16, 3.17 and 3.18 plot the means for the five behavioural variables for each month. The observations from Bowen are excluded from this analysis because they were made in August in a very different climatic zone.

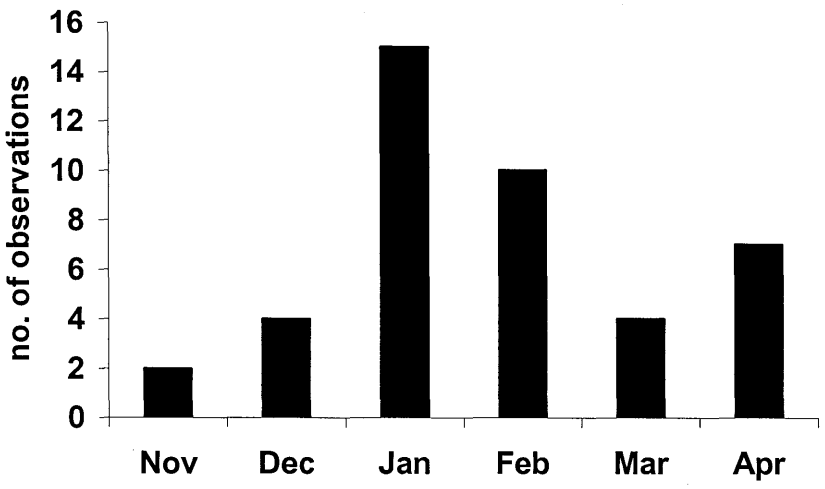

Figure 3.13 Numbers of observations made per month (Darling Downs sites only). Note low number of observations for November, December and March.

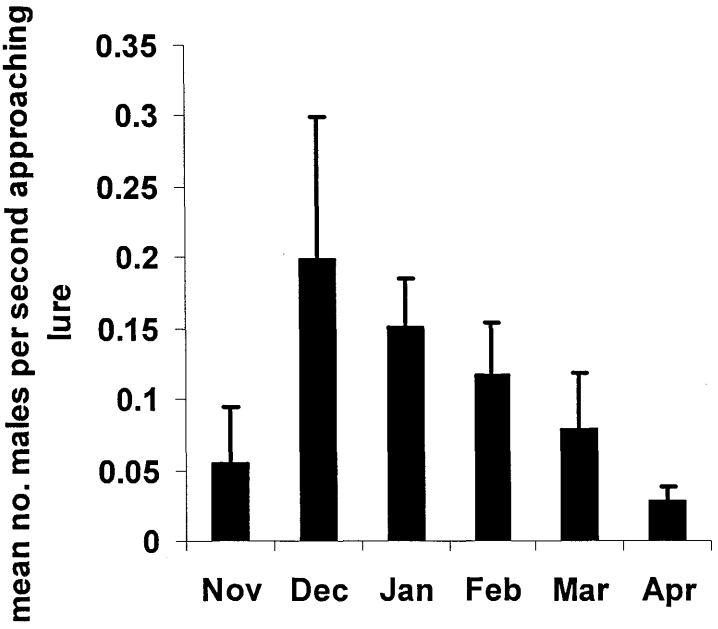

Figure 3.14 Mean numbers of approaching males per second for each month of observations. Error bars are standard errors of the means.

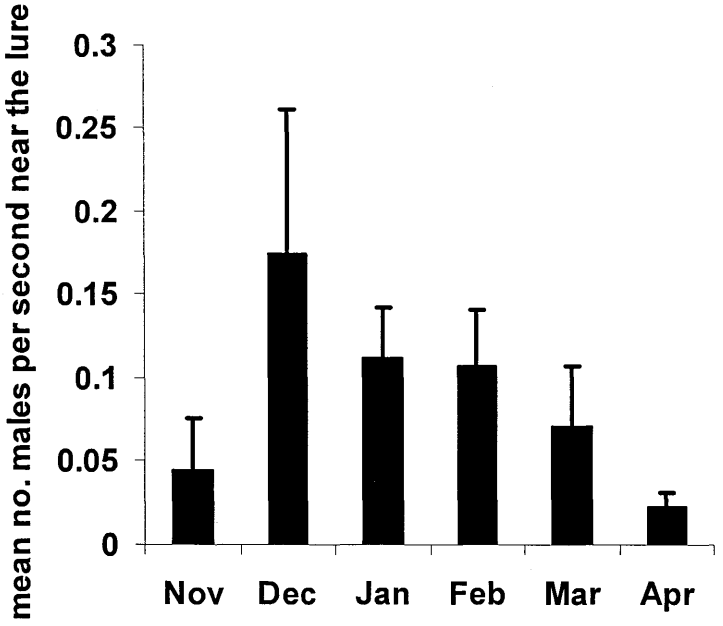

Figure 3.15 Mean numbers of males per second near the lure for each month of observations. Error bars are standard errors of the means.

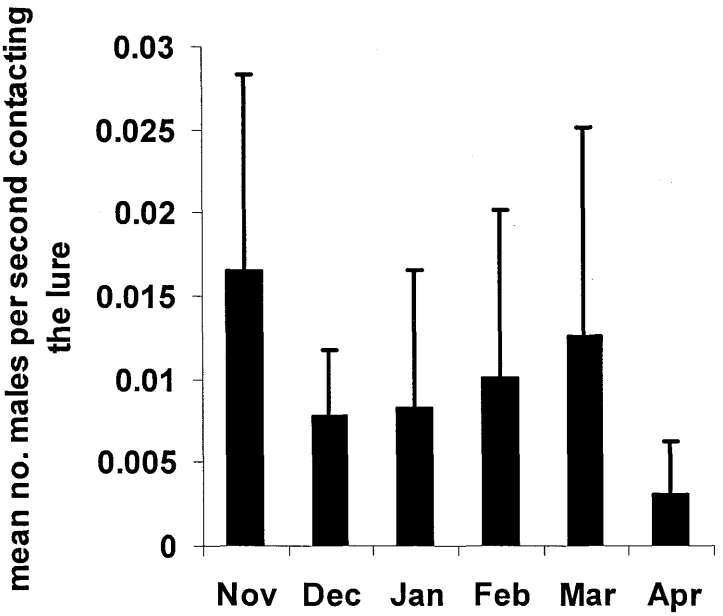

Figure 3.16 Mean numbers of males per second contacting the lure for each month of observations. Error bars are standard errors of the means.

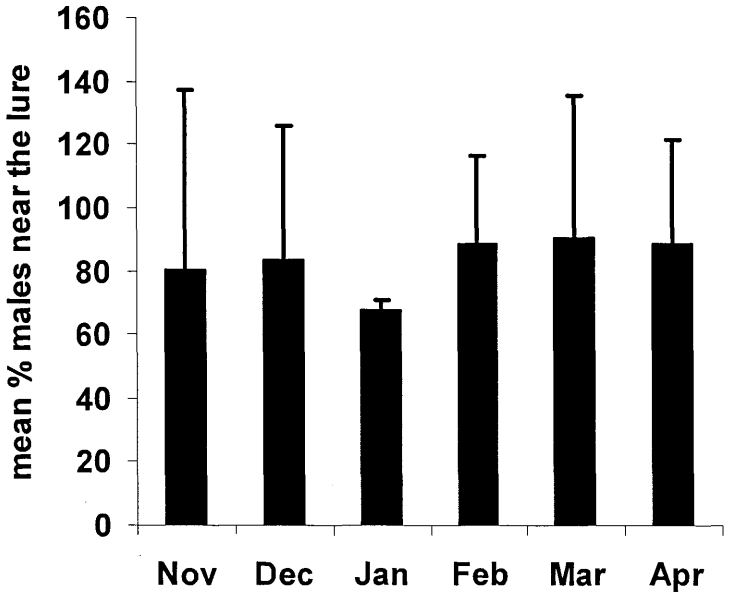

Figure 3.17 Mean percentages of approaching males that got near the lure for each month of observations. Error bars are standard errors of the means.

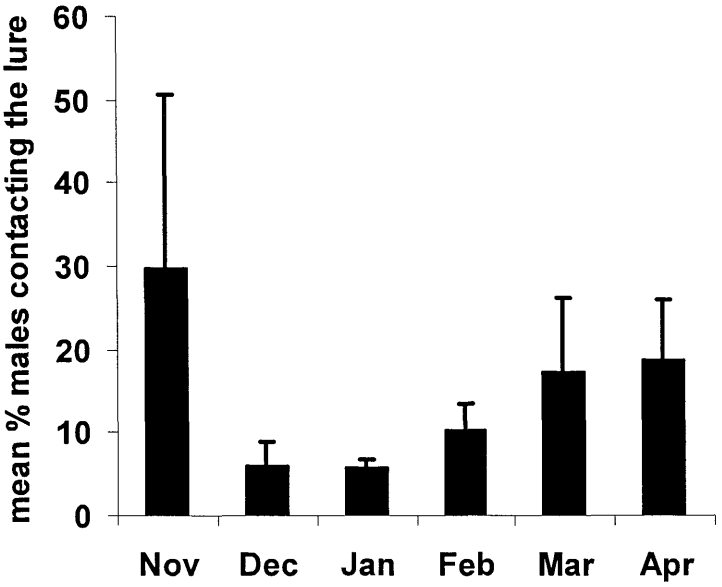

Figure 3.18 Mean percentages of approaching males that contacted the lure for each month of observations. Error bars are standard errors of the means.

The mean number of males per second approaching lures tended to decrease after December (Figure 3.14). This reduction in overall numbers was often reflected in pheromone trap catches during January, February and March, where catches from both AgriSense and Texas traps were reduced compared to November and December catches (pers. obs.). These trends were reflected in the mean number of males per second near the lures but not the mean number of males per second contacting the lure (Figures 3.15 & 3.16).

The mean percentages of approaching males that got near the lure dropped slightly in January observations, but was consistently around 85-90% for the remaining months (Figure 3.17). The mean percentages of males that contacted the lure were much more variable with month, with the highest percentages recorded in early and late season observations (Figure 3.18). In December, January and February only a small percentage of approaching males seemed to contact the lure during the observations

3.3.5 Variation associated with weather

The three climate variables were treated separately in an attempt to tease out relationships between moth behaviour and climate. A multivariate analysis was also used to test the combined

effects of the three variables. Note that climatic data were not available for every observation, so most regressions used a subset of the 60 available observations.

3.3.5.1 Temperature

Temperature at the crop level was not significantly correlated with any of the behavioural parameters. Table 3.2 lists the results of least squares regression for each the seven behavioural parameters with temperature as the explanatory variable. As there were no significant relationships between temperature and the variables the details of the line and the degree of fit have been omitted. The slopes and intercepts were calculated from arcsin (square root) transformed proportional data.

Table 3.2 The results of least squares regression for each of the seven behavioural variables measured, with temperature as the explanatory variable. Where there is no significant relationship the details of slope and intercept and the degree of fit have been omitted. N is the number of observations, F is the F-statistic.

Behavioural Variable	N	F	Probability	Slope	Intercept	R^2
Mean number of males per second approaching	49	0.35	0.57	n/a	n/a	n/a
Mean number of males per second near	49	0.28	0.60	n/a	n/a	n/a
Mean number of males per second contacting	49	1.37	0.25	n/a	n/a	n/a
Proportion of males that got near the lure	49	1.58	0.21	n/a	n/a	n/a
Proportion of males that contacted the lure	49	0.03	0.87	n/a	n/a	n/a
Mean time spent approaching the lure per male	47	0.23	0.64	n/a	n/a	n/a
Mean time spent near the lure per male	47	2.87	0.10	n/a	n/a	n/a

3.3.5.2 % Relative humidity

The number of moths approaching, getting near and contacting the lure tended to have negative relationships with increasing humidity, so as the % relative humidity approached 100% the mean number of males approaching, getting near and contacting the lure decreased. The proportion of approaching males that got near the lure was positively correlated with % relative humidity. However, this was not reflected in the proportion of males that subsequently contacted the lure, where there was no significant correlation. In addition to this, males were staying longer near the lure when the % relative humidity was higher.

The % relative humidity was significantly correlated with five of the behavioural variables. Figures 3.19, 3.20, 3.21, 3.22, 3.23, 3.24 and 3.25 are scattergrams with % relative humidity on the x axis and the behavioural variable on the y axis. A trend line is included when there was a significant linear correlation between the behavioural variable and % relative humidity. Note that

the percentage of approaching males that got near and contacted the lure are graphed, whilst the regression analysis of these data used the arcsin (square root) transformed proportion from the same data (Figures 3.22 & 3.23). Table 3.3 lists the results of least squares regression for each the seven behavioural variables with % relative humidity as the explanatory variable. The slopes and intercepts in Table 3.3 also use the arcsin (square root) transformed proportion. Where there is no significant relationship the details of the line and the degree of fit have been omitted.

Table 3.3 The results of least squares regression for each of the seven measured behavioural variables, with % relative humidity as the explanatory variable. Where there is no significant relationship the details of slope and intercept and the degree of fit have been omitted. N is the number of observations, F is the F-statistic.

Behavioural Variable	N	F	Probability	Slope	Intercept	R^2
Mean number of males per second approaching	31	15.20	<0.01	-0.0024	0.2749	0.34
Mean number of males per second near	31	9.20	<0.01	-0.0017	0.2087	0.24
Mean number of males per second contacting	31	5.69	<0.05	-0.0001	0.0128	0.16
Proportion of males that got near the lure	31	11.44	<0.01	0.0030	0.9619	0.28
Proportion of males that contacted the lure	31	1.44	0.24	n/a	n/a	n/a
Mean time spent approaching the lure per male	31	0.08	0.77	n/a	n/a	n/a
Mean time spent near the lure per male	31	18.1	<0.01	0.0593	1.5302	0.38

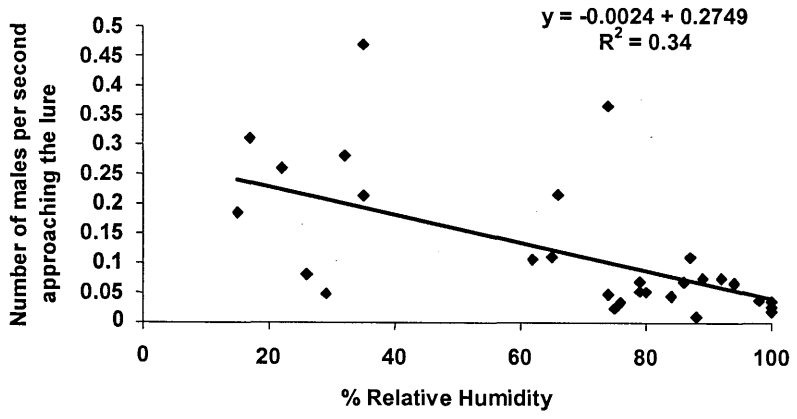

Figure 3.19 Scatterplot of the mean numbers of males per second approaching the lure in relation to % relative humidity for each observation period.

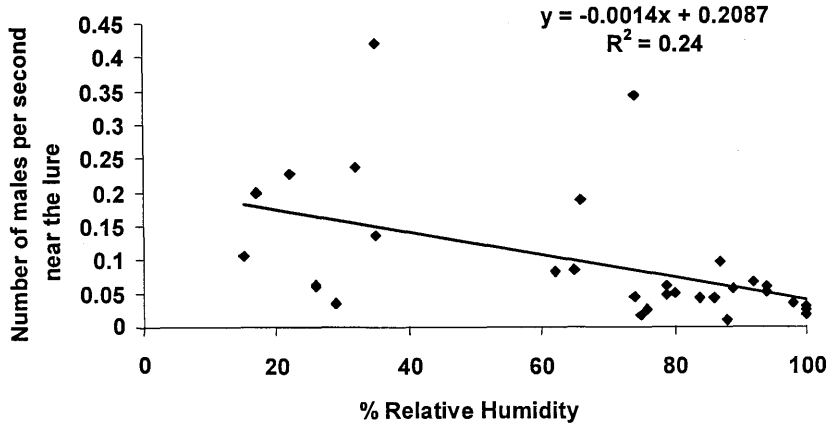

Figure 3.20 Scatterplot of the mean numbers of males per second near the lure in relation to % relative humidity for each observation period.

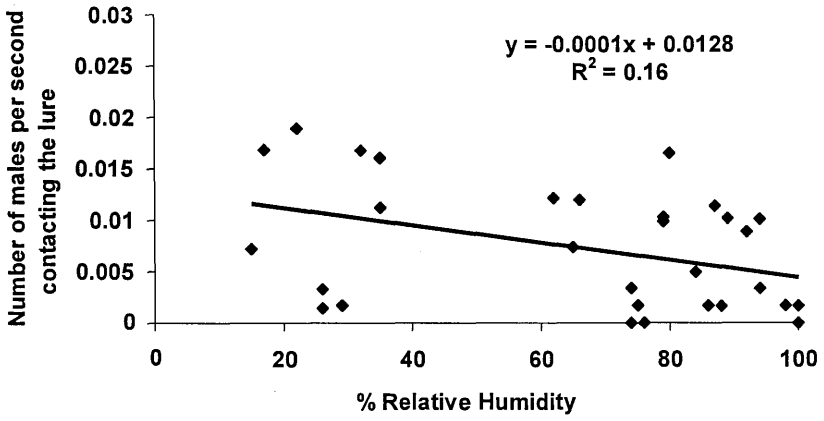

Figure 3.21 Scatterplot of the mean numbers of males per second contacting the lure in relation to % relative humidity for each observation period.

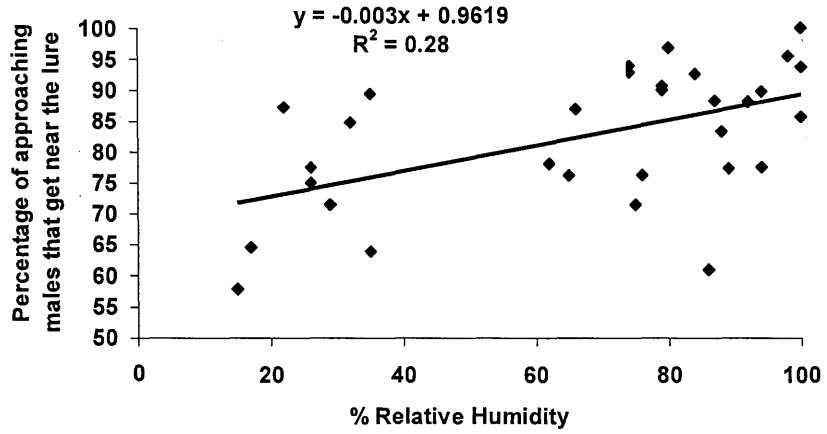

Figure 3.22 Scatterplot of the percentages of approaching males that get near the lure in relation to % relative humidity for each observation period. The linear equation is based on arcsine (square root) transformed data.

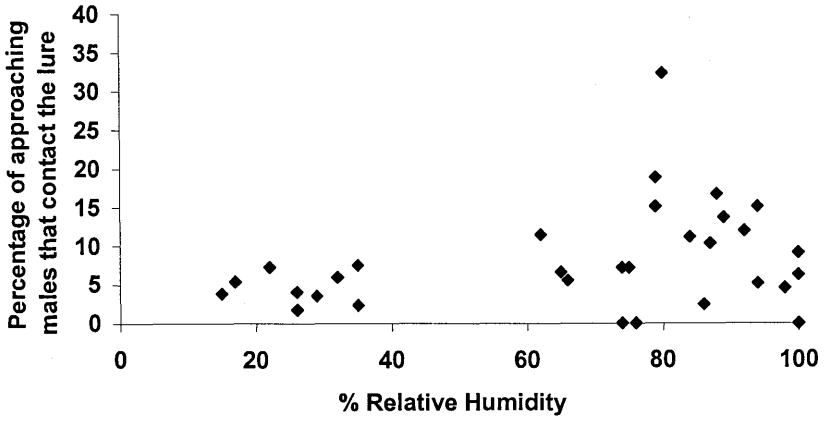

Figure 3.23 Scatterplot of the percentages of approaching males that contact the lure in relation to % relative humidity for each observation period.

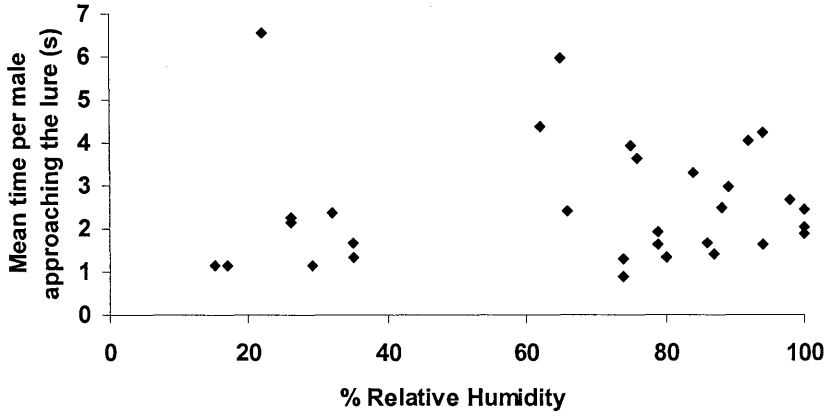

Figure 3.24 Scatterplot of the mean time per male approaching the lure in relation to % relative humidity for each observation period.

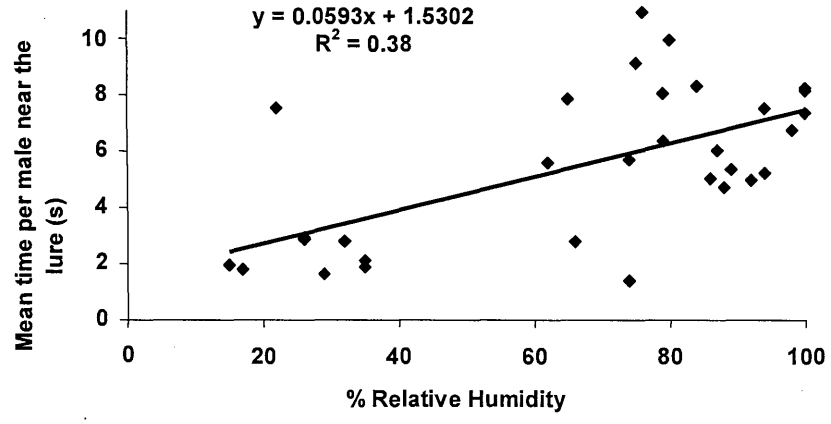

Figure 3.25 Scatterplot of the mean time per male near the lure in relation to % relative humidity for each observation period.

3.3.5.3 *Wind run*

Wind run was weakly correlated with two of the behavioural variables. The mean number of males approaching and getting near the lure was positively correlated with wind run. As wind run increased, there was a corresponding slight increase in the number of males that approached. This increase was not seen in the mean number of males per second contacting the lure. Increased wind run did not significantly increase the proportion of males that contacted the lures.

Figures 3.26 and 3.27 are scattergrams with wind run on the x axis and the behavioural variable on the y axis with trend lines included. Table 3.4 lists the results of least squares regression for each the seven behavioural parameters with wind run as the explanatory variable. The slopes and intercepts in Table 3.4 also used the arcsin (square root) transformed proportion. Where there is no significant relationship the details of the line and the degree of fit have been omitted.

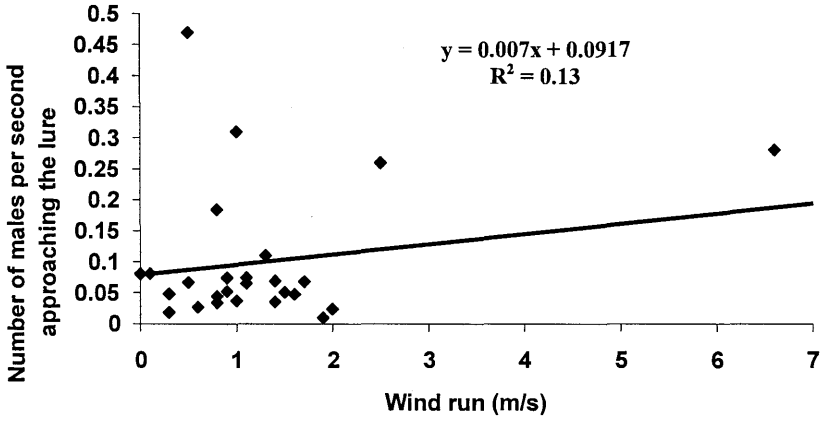

Figure 3.26 Scatterplot of the mean numbers of males per second approaching the lure in relation to wind run for each observation period.

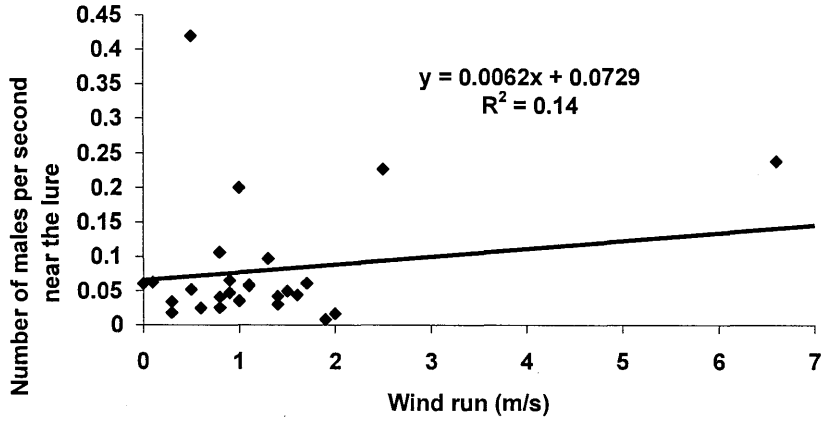

Figure 3.27 Scatterplot of the mean numbers of males per second near the lure in relation to wind run for each observation period.

Table 3.4 The results of least squares regression for each of the seven behavioural variables measured, with wind run as the explanatory variable. Where there is no significant relationship the details of slope and intercept and the degree of fit have been omitted. N is the number of observations, F is the F-statistic.

Behavioural Variable	N	F	Probability	Slope	Intercept	R^2
Mean number of males per second approaching	31	4.25	<0.05	0.0070	0.0917	0.13
Mean number of males per second near	31	4.54	<0.05	0.0062	0.0729	0.14
Mean number of males per second contacting	31	1.50	0.23	n/a	n/a	n/a
Proportion of males that got near the lure	31	0.11	0.74	n/a	n/a	n/a
Proportion of males that contacted the lure	31	0.35	0.56	n/a	n/a	n/a
Mean time spent approaching the lure per male	31	1.60	0.22	n/a	n/a	n/a
Mean time spent near the lure per male	31	1.62	0.21	n/a	n/a	n/a

3.3.5.4 Interactions between the three climatic variables

Figure 3.28 depicts the relationship between temperature and % relative humidity. Although there was a strong linear relationship between these two climate parameters as demonstrated by this figure this was not reflected in the behavioural variables when these two parameters were considered as univariate and independent as in the above analyses. A multivariate approach using MANOVA with the three climate variables allowed further insight into how the climatic variables affected the behaviour of male moths at the lures. The models for MANOVA assumed that all of the variables were gaussian in distribution, with grouped dependent responses (see below) with a general additive multiple regression model for the climatic variables. Of the four significance tests available in the S-Plus statistics package for MANOVA the default Pillai-Bartlett trace test was chosen on the basis that it was the most robust if there were potential violations of homogeneity of covariance (MathSoft 1999). From this an F-statistic can be calculated and a probability value assigned to each of the climatic variables. A significant result indicates that the climatic variable in question exerted a significant influence over the behaviour of the males when compared to the other climatic variables.

The behavioural responses were grouped as follows:

1. The frequency responses of mean number of males per second approaching, near and contacting the lure
2. The proportional responses of number of approaching males that got near and contacted the lures (the proportions were transformed using arcsine(square root) transformation).
3. The duration responses of mean time spent approaching and near the lure

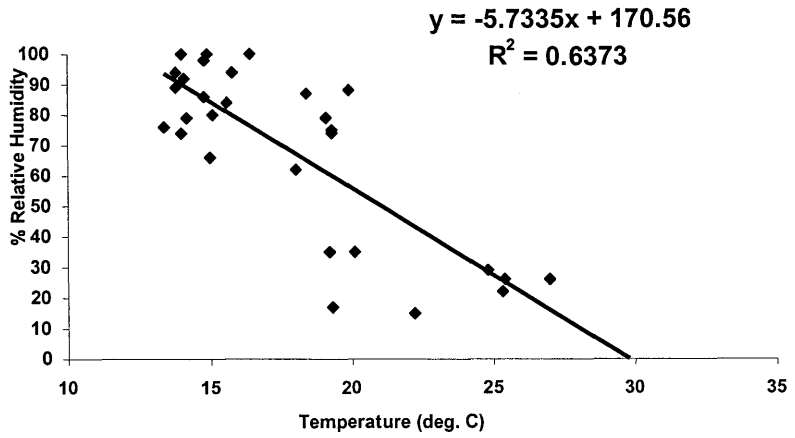

Figure 3.28 Scatterplot of % relative humidity in relation to temperature for each observation period.

Table 3.5 summarizes the results of the three MANOVAs. The % relative humidity still emerged as the most significant climatic variable in relation to its influence on the recorded male behaviours at the lure, particularly when the mean numbers of males approaching, near and contacting the lure were considered. However, temperature and % relative humidity were both significant variables when the time spent approaching and near the lure was considered. Wind run was not found to be significant compared to the other two climate variables in this model. The proportion of approaching males which got near and contacted the lure were not significantly affected by any of the climate variables. The results of this analysis should be considered as highlighting the overall significance of changes in climatic conditions rather than negating the findings of the other univariate analyses made above.

Table 3.5 Results of a MANOVA of grouped behavioural responses in relation to a general additive model of the three climatic variables. * indicates a response that was significant at the 0.05 level.

Grouped behavioural responses	Climatic Variables	df	Pillai-Bartlett trace	Approximate F-Statistic	Num. df/ Denom. df	Prob.
Mean no. males/sec approaching, near & contacting lure	Temp	1	0.26664	2.78746	3/23	0.06346
	% RH	1	0.72238	19.94871	3/23	0*
	Wind Run	1	0.10453	0.8949	3/23	0.45873
Approaching males that get near & contact lure	Temp	1	0.19455	2.89859	2/24	0.07455
	% RH	1	0.18095	2.6512	2/24	0.09114
	Wind Run	1	0.01065	0.12922	2/24	0.87939
Mean time spent approaching and near the lure	Temp	1	0.31592	5.54191	2/24	0.0105*
	% RH	1	0.31655	5.55796	2/24	0.01039*
	Wind Run	1	0.18139	2.659	2/24	0.09056

3.4 Discussion

The behaviour of male *H. armigera* flying to synthetic pheromone formulated in Sirene was affected by several factors. A difficulty of any field study which attempts to isolate the effects of environmental factors on behavioural patterns is that environmental factors are likely to be highly autocorrelated. For instance, diel periodicity may be correlated with temperature, humidity and wind run, simply because, generally, temperatures are lower, humidity is higher, and the air is still late in the evening compared to early evening. Similarly, the effects of crop type may be confounded with those of seasonal factors simply because different crops are planted and grown at different times of the year, and the flowering response of these crops is also linked to seasonal factors. It is not feasible to attempt a statistical comparison which includes all of the temporal and other environmental variables. The approach here is to conservatively interpret those variables that appeared to have a significant effect on moth behaviour and compare them to other similar factors. Table 3.6 summarises these effects of these variables, and their relative importance in respect to attract and kill based on a subjective judgement of the changes in male behaviour. For example, factors which influenced the number and proportion of males contacting the lure are likely to be critical, whilst factors which influenced the overall numbers of males visiting the lure may also be important.

Table 3.6 Table of field factors and their relative importance in influencing male behaviour at synthetic lures.

Factor	Effect	Relative Importance
Diel Periodicity	Bimodal increases in % contacting & no. males/sec contacting peaking at 3-4 hrs and 6-7 hrs after dusk, increasing % near with time after dusk, probably correlated with the effects of weather variables	High
Crop Type	Increasing no. males/sec approaching & near lure in sunflower & pigeonpea compared to other crops	High
Seasonal	Decreasing % contacting during mid-summer, decreasing no. males/sec approaching mid to late summer, increasing no. males/sec near early to late summer, probably correlated with effects of crop type	High
Temperature	No effect	Nil
% Relative Humidity	Decreasing no. males/sec approaching, near & contacting, increasing % near, time spent near (for increasing humidity)	Low
Wind run	Increasing no. males/sec approaching & near with increasing wind run	Low
Weather overall	Increasing % relative humidity decreases the no. males/sec approaching, near & contacting, and increases the amount of time spent approaching & near, increasing temperature decreases the time spent approaching & near	Low

The observations made on behaviour of male *H. armigera* flying to synthetic pheromone lures were mostly similar to those observed in other studies of noctuid moths responding to sex

pheromone both in the laboratory and in the field, for example Murlis and Bettany (1977) for *Spodoptera littoralis*. Many early studies in this area used calling females as a pheromone source, which does not readily permit separation of female pheromone-related sexual behaviour from male behaviours (Batiste 1970), but with a synthetic pheromone source it is possible to determine male activity patterns in the field.

General night observations by Lingren *et al.* (1978) of *Heliothis virescens* and *Trichoplusia ni* in southern USA indicate that moths of both sexes foraged for nectar, and in the case of females, oviposited on plants during the early part of the evening. This movement began about 30 min after sundown and peaked about an hour later. Later in the night (at about 2 hrs after sunset) males of these species began to move rapidly crosswind at heights up to 10 m above the crop canopy, presumably searching for pheromone plumes from calling females. Hourly pheromone trap catches for *Helicoverpa zea* were highest between about 40 min to 1 h 40 min after sunset (Latheef *et al.* 1993), but this does not necessarily seem to coincide with frequency of mating within the crop and adult activity patterns. Sparks *et al.* (1979) observed *H. virescens*, noting that searching males fly rapidly 0.1 to 1 m above the crop at an oblique angle to the prevailing wind. When the males reach the pheromone plume they slow down, and approach the lure from a downwind direction, and appear to "assess" the pheromone source, and at this stage may either proceed to the source, or reject the source and continue flying upwind. Coombs (1992) examined diel periodicity in *H. punctigera*, and found that adult feeding activity was greatest at sunset, and that temperature and light influenced the numbers of moths found feeding. The timing of these behaviours may be modified by field conditions such as temperature and humidity.

The observation of groups of males flying to the lure is similar to that made by Lingren *et al.* (1978) for a number of moth species including corn earworm *Helicoverpa zea*, tobacco budworm *Heliothis virescens*, fall armyworm *Spodoptera frugiperda*, cabbage looper *Trichoplusia ni* (all Noctuidae), and pink bollworm *Pectinophora gossypiella* (Gelechiidae). These authors attributed this behaviour to a partial synchronizing effect which occurs when a number of spatially distant males in the field fly into a pheromone plume, and are attracted upwind to the source. Once at the source only one male can mate (if the source is a calling female moth), so the remaining males leave, flying closer together than they would have earlier, resulting in a "clumping" effect when they fly into the next pheromone plume. This movement of males in groups peaked in conjunction with the peak mating period within the crop. Later studies by Raulston *et al.* (1975, 1979) found that this grouping behaviour could also be related to competition for females. Mated females are less likely to call, and are reluctant to mate compared with virgin females. As the

percentage of mated females increases within the crop over time, the number of calling and sexually active females diminishes, and searching males are forced to compete for any available calling female. This leads to a larger proportion of males adopting the searching behaviour mentioned earlier, and in conjunction with the "clumping" effect, this leads to groups of actively searching and competing males. Sparks *et al.* (1979) found that this competition for females can increase the number of males entering a pheromone trap, but in this present study, it seemed to decrease the number of males contacting the Sirene droplet. One corollary of this behaviour is that the proportion of males contacting the Sirene droplet may be dependent upon the number of receptive female moths present, with a smaller proportion of males contacting after the peak emergence of female moths. Similarly, a large number of virgin females calling in the field may also compete for male attention (Lingren *et al.* 1982), and may reduce the number of males visiting the Sirene droplet.

Changes in field temperature did not seem to elicit a strong response in male *H. armigera* flying to the synthetic sex pheromone. Temperature appears to change the calling response in female moths by altering the time at which the females call. Sower *et al.*(1971) found that cooler temperatures advanced the time at which female *Trichoplusia ni* called. From this, it may be expected that males would also time their peak responses to sex pheromone using temperature as a cue. Temperature may also be physiologically limiting. Cooler temperatures may cause males to expend more energy flying than in warmer temperatures. Coombs (1993) found *H. armigera* can warm their thoracic muscles prior to flight to temperatures between 21-32°C, and that a proportion of a given laboratory population can do this from an initial 5°C. The same threshold of 5°C has been observed for moths flying to pheromone traps (Gregg & Wilson 1991). This implies that there would be no physiological limitation to males flying to the sex pheromones in the conditions under which observations were made in this thesis, but there may be behavioural reasons linked to energy expenditure that still limit male activity at both low and high field temperatures.

Vickers and Baker (1997) using a wind tunnel with relatively low wind speeds found that male *Heliothis virescens* flying to synthetic pheromone lures maintained ground speed at *c.* 200 cm s^{-1} regardless of the wind run by adjusting their airspeed. Similarly they maintained track angles (the angle which the moth must turn at to reach the pheromone source) at 33° regardless of wind run. This is reflected in the observations here for *Helicoverpa armigera* where there was no significant relationship between wind run and the time spent near or approaching the lure. An earlier study (Murlis *et al.* 1982) found a similar result for Egyptian cotton leafworm moths *Spodoptera*

littoralis (Noctuidae), where males compensated for changes in wind run by varying their air speed. This is only possible when wind run is relatively low compared to the maximum air speed of the moth as was the case in this thesis where most observations were made at wind speeds less than 200 cm s^{-1} (Figure 3.26).

Crop type may affect male behaviour at synthetic pheromone in many ways. The presence of suitable larval host plants may mean that many more females are present in these crops, and these females may be producing sex pheromone which attracts more males, which may in turn visit synthetic lures placed near or in this crop, although females may compete for males. One of the principal uses of synthetic sex pheromone for moths is as a monitoring tool where presence and number of male moths in the traps baited with the sex pheromone acts as an indication of female oviposition activity within the crop. There have been many studies of *H. armigera* examining the link between pheromone trap catches and oviposition activity (as measured by egg and larval counts), and the use of pheromone trap thresholds as a trigger for crop management decisions (Kehat *et al.* 1982, Nyambo 1989, Wilson & Morton 1989, Srivastava *et al.*1992, Kehat & Dunkelblum 1993, Herman *et al.* 1995, Izquierdo 1996, Reddy & Manjunatha 2000). In general, the short-term temporal linkage between pheromone trap catches and egg or larval counts is poor, and pheromone traps by themselves are inadequate for monitoring of *H. armigera* for the purpose of making spraying decisions (Kehat *et al.* 1982). Comparisons between pheromone trap catches of male *H. zea* and yield in Bt cotton indicate that pheromone traps can be linked over longer spatial scales to overall moth population levels (Micinski 2001), and some authors have found that pheromone trap catches can predict egg counts if factors such as trap placement, weather and time lag in oviposition (Wilson & Morton 1989), and crop phenology (Slosser *et al.*1978) are taken into account. The inherent complexity of most cropping systems where there are multiple host plants available over any cropping season appears to introduce variation both in trap catches and egg counts which confounds what might otherwise be a straightforward relationship (Izquierdo 1996).

H. armigera is highly polyphagous, and within Australia larvae have been found feeding on numerous plant species [141 species from 34 plant families (Matthews, 1999)]. Females are largely responsible for determining which host plant is selected. Females preferentially oviposit on certain plant species. Roome (1975) found that *H. armigera* preferred maize and grain sorghum over other species, whilst Jallow and Zalucki (1995) and Jallow *et al.* (1999) found that maize, sorghum, tobacco and sunflower were preferred. This oviposition preference only holds if the plants are at a similar stage of development. The phenology of the crop exerts a strong

influence on oviposition behaviour, with the most attractive stage generally being the flowering stage (Parsons 1940, Johnson *et al.* 1975, Roome 1975, Wardhaugh *et al.*1980), and normally preferred hosts which are not flowering will often be rejected in favour of a flowering host plant of a less suitable nature. In any event, the likelihood of male *H. armigera* encountering a calling female may be enhanced by the presence of a flowering host plant, and this may lead to increased numbers of male moths flying to synthetic pheromones in traps located on crops which are flowering and attractive, but in turn may also increase female competition for males. Flowering crops may be attractive to large numbers of males simply as a nectar resource, and these males may be then attracted to any pheromone sources placed in the crop, but again this may increase female competition.

This relationship between phenology, host plant species, and oviposition is further complicated by learned responses of moths. Cunningham *et al.* (1998) observed that *H. armigera* females exposed to two host plants (tobacco or tomato) selected the host for which they had been exposed to for oviposition more frequently than female moths exposed to other hosts, or than inexperienced females. Learning for nectaring in *H. armigera* has also been experimentally demonstrated by Cunningham *et al.* (1998). Unmated moths of both sexes showed a preference for locating host plant species with which they had previous experience in nectar feeding, which may in turn bias the probability of females choosing those hosts, or similar hosts, as oviposition targets. This may also encourage aggregation of males in those crops in which males have already experienced positive reinforcement through nectaring.

Mark-recapture studies (King *et al.* 1990, Fitt *et al.* 1995) indicate that whilst the majority of moths arising from a large generation within a crop may not move very far, only a small proportion are captured in the crops where they originated. Attractive and suitable crops for oviposition and larval development may not necessarily mean that large numbers of males from the subsequent generation will be present and attracted to pheromone sources within these crops.

The direct effect of plant volatiles on the perception and response to sex pheromones is still controversial. The presence of green leaf plant volatiles such as linalool and (*Z*)-3-hexenol have been shown to have synergistic effects on pheromone reception in male *Helicoverpa zea* (Ochieng *et al.* 2002), lowering the threshold at which the major pheromone component can be physiologically detected by antennal sensillae. Green leaf volatiles such as (*Z*)-3-hexenyl acetate and (*Z*)-3-hexenol can increase trap catches when added to synthetic pheromone blends for *Heliothis virescens*, (Dickens *et al.* 1993) and *Helicoverpa zea* and *Cydia pomonella* (Light *et al.*

1993). However, some plant volatiles appear to suppress pheromone trap catches for fall army worm (Meagher 2001b) and *H. armigera* (Kvedaras 2002). This sensitivity to plant volatiles may mean that different crops may cause changes in male responsiveness due to the release of volatiles from the surrounding crop, although the response may be positive or negative depending on the insect species in question.

4 Effects of lure formulation and appearance on male behaviour

The low rates of contact at the standard Sirene with synthetic pheromone lure (~ 11 % of males overall, see Table 3.1 in Chapter 3) observed in the field indicate that there may be a number of attractive factors that are absent from a single droplet lure presented on a plastic substrate, or that there may be some deterrent factors that prevent males from contacting the lures. This chapter investigates a variety of factors that may act as attractants or deterrents to male moths approaching Sirene formulations in the field, and how these may affect successful field deployment of an attract and kill management program. The lures were compared to caged calling females, and the effects of visual cues, lure presentation and composition were considered. The response of males to the addition of insecticide to lures was analysed for potential deterrent effects.

4.1 Field comparisons with calling females

4.1.1 Are synthetic lures chemically as attractive as calling females?

Some early attempts to characterize and synthesize pheromone blends often focused on major components, resulting in incomplete blends, or blends containing mixtures of both attractive and antagonistic components. Technical advances in analytical equipment and more detailed studies have sometimes revealed the presence of new pheromone components in apparently well-studied species such as the turnip moth *Agrotis segetum* (Gemeno & Haynes 1998) and the codling moth *Cydia pomonella* (El-Sayed *et al.* 1999, Witzgall *et al.* 2001), although these may or may not influence the effectiveness of the blend for attract and kill (see Vickers *et al.* 2000 for an example using diamondback moth, *P. xylostella*, where an additional compound significantly increased attractiveness).

The low contact rates observed for synthetic blends for *H. armigera* (Table 3.1, Chapter 3), along with the number of components observed in analysis of gland and effluvial extracts that are not included in the current synthetic blend (Table 1.3, Chapter 1) suggests that the synthetic blend may be lacking components that initiate short-range male behaviours. The following observations were designed to investigate whether there were significant differences in the behaviour of male moths when flying to synthetic lures compared to calling female moths. Preliminary experiments

were conducted where the ratios between pheromone components of the current blend were altered, and an additional component added.

4.1.2 Methodology

Night vision observations, formulations and analyses of the behavioural data were made as described in the General Methods section (Chapter 2.2). Because some of these observations were made comparing live female pheromone sources to synthetic sources the behavioural event of "contact" could not be included in the analysis. For experiments where synthetic lures were compared to calling females analyses attempted to detect differences in the mean time spent approaching and near the lure, the mean number of males per second approaching and getting near the lure (see Chapter 2.2 for definitions of these terms), and the percentage of males that approached that subsequently got near the lure.

For the comparisons between synthetic lures and females three treatments were tested:

a) A single 200 mg droplet of standard 1% pheromone in Sirene formulation on Corflute® plastic. See Chapter 7 for information on pheromone release rates from similar droplets.
b) The same as for (a), but in the container as used in (c).
c) Three 3 day old virgin female *H. armigera* from a laboratory culture. Three females were used to maximize the chances that at least one female would be calling at any given time. The females were set up in the field half and hour before sunset so that they could acclimatize to field conditions. Figure 4.1 shows the container used to hold the females or the synthetic lure used in (b). Visual cues from the females were eliminated by placing them in the upper half of an upside down 800 ml round plastic takeaway food container, the sides of which were covered in opaque brown masking tape. Natural light occurring in the field was able to illuminate the females via the top of the container, but approaching male moths were unable to see the female from the side. A stainless steel mesh prevented females from accessing the bottom half of the container which was perforated with 2 mm holes to allow the free passage of air and pheromone. Females were given water from wet dental wicks.

Observations were made in early-stage flowering round tomato at Wright Pack Farms, Bowen, Queensland from the 20th to 22nd, August 2001. The observations were made from 2300 h to 0200 h when the caged females were most likely to be calling (Hou & Sheng 2000 for Taiwanese *H. armigera*, Kvedaras 2002 for eastern Australian *H. armigera*). Whilst the two synthetic pheromone treatments were observed the cage with the females was moved 50 m away to avoid

any competition effects. This movement sometimes caused females to cease calling behaviour. Observations of males flying to females were made after a 15 min resting period to allow the females to settle back into calling behaviour

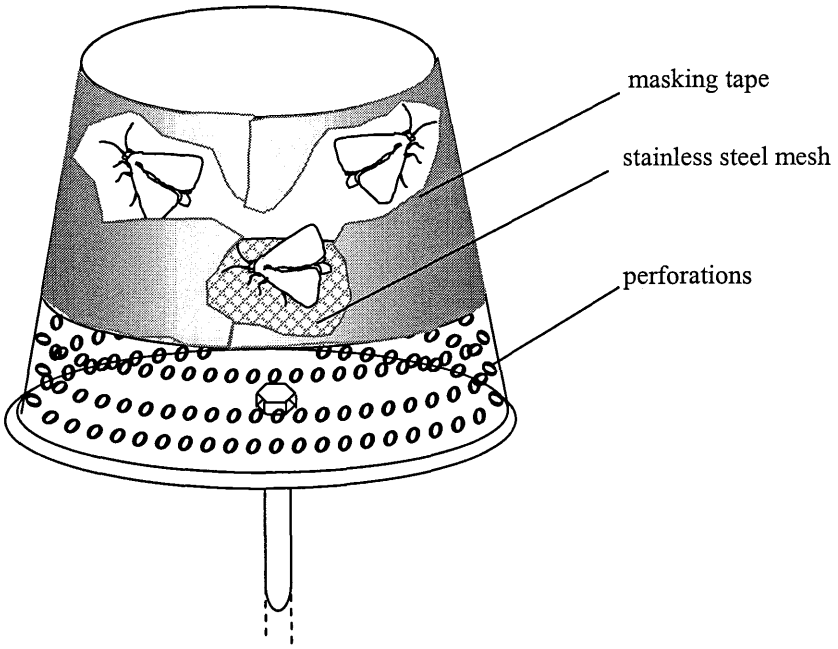

Figure 4.1 "Blind" cage used to provide a natural pheromone source from calling female *H. armigera* whilst excluding visual cues. The sides of the top half of the cage would normally be completely masked; here the cut-away shows calling females and mesh barrier.

For experiments where pheromone ratios and components were altered male behaviour was observed around the following three treatments:

(a) A single 200 mg droplet of standard two-component pheromone (10:1) at 1% in the Sirene formulation on Corflute® plastic. ("10:1:0")

(b) As for (a), but with the two components in a 97:3 ratio. ("97:3:0")

(c) As for (a), but with the addition of (n)-hexadecanal to the two standard components to make a 10:1:1 ratio. ("10:1:1")

The choice of (n)-hexadecanal (~95%, Shin-Etsu Chemical Company, Japan) was made on the basis of the number of times this component was recorded in published analyses of female *H. armigera* gland and effluvial extracts (Table 1.3, Chapter 1), and the absence of antagonistic effects of this component in published trap and wind tunnel data (compare with (*Z*)-11-hexadecen-1-ol, see also (Kehat & Dunkelblum 1990). Observations were made in round tomato at Wright Pack Farms, Bowen, Queensland, from the 17th-18th August 2001.

4.1.3 Results

Females could be observed calling through the tops of the cages. They called consistently allowing observation from midnight to 02:00 h. Males approached the container from the side, and would have been unable to obtain a line of sight to the female. Figure 4.2 shows the mean time males spent approaching and near the pheromone sources. Figure 4.3 shows the mean numbers of males per second approaching and getting near the sources. There was no significant difference between the mean time spent by males approaching the three treatments, but males spent significantly more time near the caged calling females compared to the synthetic lure (paired t-test, $df = 1$, $F = 6.67$, $p < 0.05$). There was no significant difference in mean time spent by males near the caged synthetic lure compared with the exposed synthetic lure or the caged females.

Figure 4.3 shows the mean number of males per second approaching and near the three treatments. There was a significant effect of treatments on the mean number of males per second approaching. (ANOVA, $df = 2$, $F = 16.59$, $p < 0.01$) and getting near the synthetic lure (one way ANOVA, $df = 2$, $F = 22.40$, $p < 0.01$). The mean number of males per second approaching and near was significantly greater for males approaching the synthetic lure. There was no significant difference in the percentage of approaching males that got near the different sources. Appendix 12.2, Table 1 details the totals and means for the three analysed parameters.

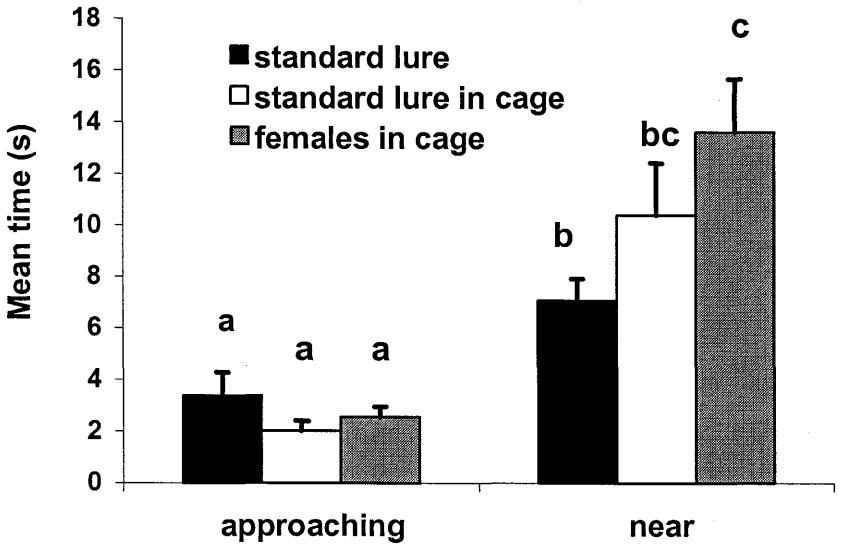

Figure 4.2 Mean time spent by male *H. armigera* approaching and near three treatments. Different letters indicate significant differences for each behavioural category ($p < 0.05$), one-way ANOVA.

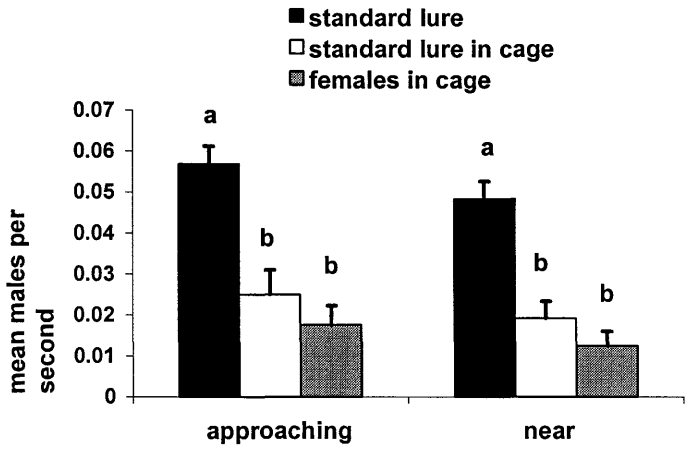

Figure 4.3 Mean numbers of male *H. armigera* per second approaching and near three treatments. Different letters indicate significant differences for each behavioural category ($p < 0.05$), one-way ANOVA.

Varying the blend ratio (10:1 compared to 97:3) made no significant differences to the percentages of males that got near and contacted the lures, to the number of males per second approaching, near and contacting the lures and to the mean time spent by males approaching and near the lure. The addition of (n)-hexadecanal made no significant difference for the same parameters when compared to either the 10:1 or 97:3 two component blends. Figures 4.4, 4.5 and 4.6 show the results for the observations. Appendix 12.2, Table 2 details the totals and means for the observations.

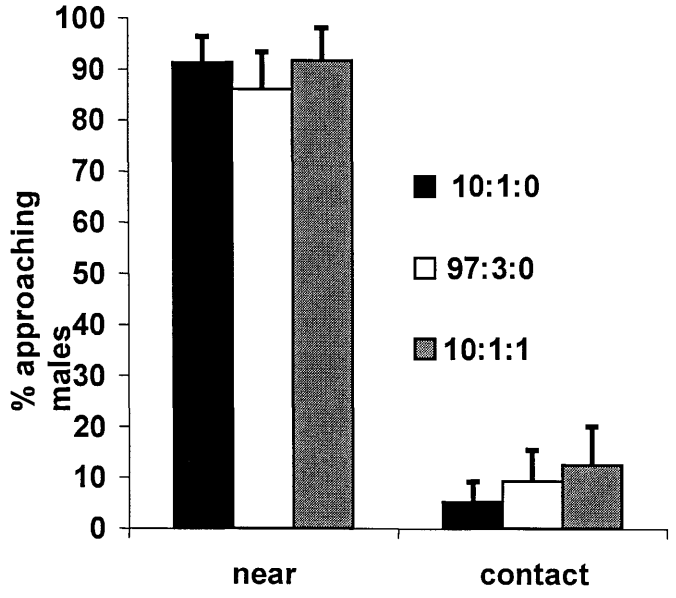

Figure 4.4 Percentages of approaching male *H. armigera* that got near and contacted three treatments. No significant differences were observed.

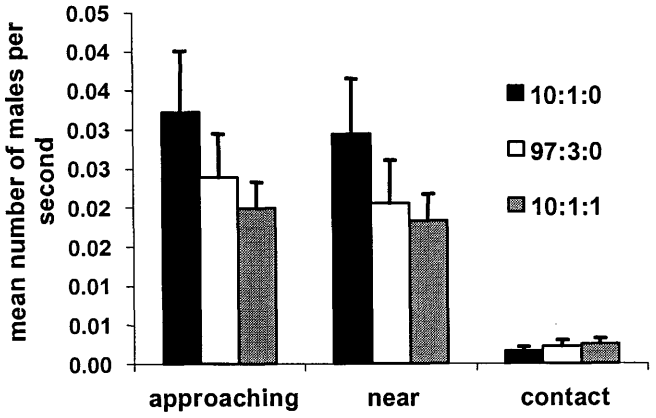

Figure 4.5 Mean numbers of male *H. armigera* per second approaching, near and contacting for three treatments. No significant differences were observed.

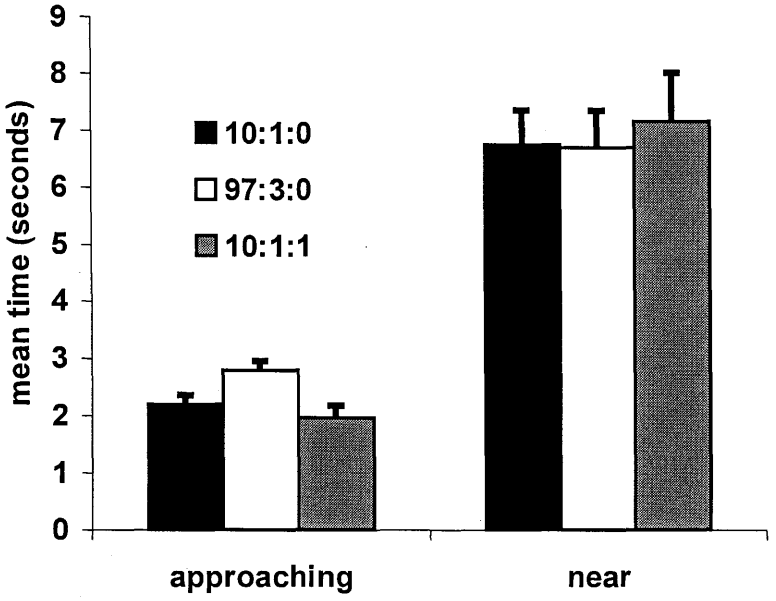

Figure 4.6 Mean time spent by male *H. armigera* approaching and near for three treatments. No significant differences were observed.

4.1.4 Discussion

Male moths may react to incomplete or incorrect sex pheromone blends in many ways. These reactions can be broadly divided into failure to elicit critical steps in the repertoire of mating

behaviours and/or the reduction in the number of males fully responding to the blend. A complete blend presented to responsive male moths in the correct environmental conditions should elicit a response resulting in a close approach and initiation of other mating behaviours, where other factors such as visual cues, substrate choice, and mutual exchange of behavioural cues may also be required for the male to successfully mate.

Observations of close range behaviour around pheromone sources are required to fully understand male responses. However, because of necessity of caging the females, the crucial behaviour of contact with the pheromone source was not assessed. The mean time spent near the source can be related to a number of factors which may or may not indicate that the best possible blend of pheromone components is present. If a male spends more time near or approaching a lure it can be interpreted as being less attractive. A more attractive blend might be expected to cause the male to fly in a more direct path to the lure. An alternative interpretation might be that if a male is spending more time near a pheromone source, there may be components in that source that are maintaining a certain level of response in the male which cause it to remain near the source.

The larger numbers of males flying to the exposed synthetic lure in these observations may be an artifact of the presentation of the different treatments. There is presumably a limited pool of receptive males present in the field during the observation period. The lures and females in the cage were not visible, so males were unable to make a rapid assessment of the nature of the pheromone source and hence spent more time at the lure. When the exposed lure was presented males could quickly assess that there was no female present. These males may have left the lure, only to return when they located the pheromone plume again, inflating the number of males observed per second at the lure. It was not possible to determine whether males approaching lures were first-time visitors, or males which were returning by looping back through the field out of range of the night-vision glasses. When this possibility is considered alongside the mean time males spent approaching the lure, it would seem that the increased mean time spent near the caged female pheromone sources did not necessarily indicate that the synthetic blends were inferior, but may have instead indicated that males were more able to rapidly locate and assess an exposed pheromone source.

Attempts were made to measure the extent to which individual marked moths returned to a pheromone source over the course of an evening. The results of these observations were inconclusive because recapture methods altered behaviour at the pheromone source, and marked males could not be reliably recaptured.

If this interpretation of the increased number of males at the exposed lures, and the increased time spent around near the concealed treatments is correct, it suggests that the 10:1 two component blend used here is close to being the correct pheromone blend, and it is other cues (visual, presentation) which are important in enabling males to locate receptive females.

The addition of the third component (n)-hexadecanal made no difference to the number of males approaching, getting near and contacting the lure when compared with the existing blend. Nesbitt *et al.* (1980) hypothesized that this component may be a precursor in the biosynthesis of the other pheromone components. It is possible that other minor pheromone components may yet be missing from the formulation. At least four other volatile compounds have been isolated in small quantities from female *H. armigera* extracts and may be active (Mayer & McLaughlin 1991). These are (*Z*)-11-tetradecenal, (*Z*)-11-hexadecen-1-ol, hexadecen-1-ol, and (*Z*)-9-tetradecenal (see Section 1.2 in Chapter 1 for a discussion of isolated and identified components found in *H. armigera*). These compounds may be associated with close-range communication between sexes and may be required to complete the landing and initiate the behavioural sequences leading to a successful mating, and may allow for further improvement of contact rates with synthetic lures. Alternatively, these compounds may act as repellants eg. Huang *et al.* (1996a, 1996b, 1997). Further research to clarify the role of minor components would be desirable, but a systematic study of this nature was beyond the scope of this thesis. The existing 10:1 blend was considered sufficiently attractive to continue with the studies planned for the remainder of the thesis, and is the blend currently used in commercially available pheromone products associated with H. armigera (eg. Selibate HA dispensers manufactured by AgriSense BCS Pty. Ltd., Pontypridd, South Wales, UK).

The release rate of pheromone from the three sources could explain some of the variation observed, particularly in the number of males per second approaching the pheromone sources. Release rate of both pheromone components for the freshly exposed 1% synthetic blend in a 200 mg Sirene droplet was at least 315 ng/day or 13 ng/h (estimated by calculating back from weathering data, Chapter 7). Release rates of individual females may be comparable. Pheromone titres washed from captive female *H. armigera* ovipositors range between 27.4 and 76 ng per female (Nesbitt *et al.* 1979, 1980, Section 1.2, Chapter 1). Release rates of 7.22 ng per 10 mins from individual females have been estimated by Centner (1983) for South African populations of *H. armigera*. Females call in short bouts, with a maximum total time spent calling of less than one hour per evening (M. Betts & PC. Gregg, unpubl. data). The behavioural significance of any

differences in release rates between the synthetic sources and the caged females did not seem to be important given that the response was not significantly different when caged synthetic sources were compared to the caged females. Pheromone loadings for the Sirene lures used in this thesis were higher than those used in commercial applications for other species eg. the 0.16% loading for Oriental fruit moth *Grapholita molesta* (Evenden & Mc Laughlin 2004). However, the repellency observed with a 1.6% loading in their study was not observed when the 1% w/w loading was compared to lower concentrations in field wind tunnel studies (See Chapter 6.3). It may be possible to make a more economical formulation with less than 1% loading, but it was considered unlikely that the 1% loading would influence the observed results in this thesis.

A possible reason for the differences in behaviour around exposed synthetic pheromone source compared to concealed synthetic and natural sources might be the absence of appropriate visual cues at the source of the pheromone plume. This is examined in more detail in Chapter 4.3. Male moths responding to a pheromone stimulus are likely to need visual cues to complete their sexual behavioural repertoire. The full reproductive behavioural sequence of the related heliothine *Heliothis virescens* has been documented in laboratory conditions (Teal *et al.* 1981). The pre-courtship 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 sequence after landing (arriving next to the female) requires more committed interactions between both sexes, and it is at this stage where the Sirene formulations are possibly lacking.

Some of the post-landing behaviours may rely on males extruding hairpencil organs which release male pheromones which in *H. virescens* seem to turn off female sex pheromone production (Hendricks & Shaver 1975) and possibly initiate other more 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-type formulation. Compounds released from male hairpencils in the noctuid *Trichoplusia ni* appear to act as an aphrodisiac to females (Grant 1970), but similar chemicals isolated from males of the noctuid *Pseudaletia unipuncta* failed to stimulate female antennae in electroantennograms (Grant *et al.* 1972). Elsewhere male pheromones have been reported in other lepidopteran families such as. Arctiidae, (Birch & Hefetz 1987) and Pyralidae (Kimura & Honda 1999). The potential inhibitory role of chemicals produced from male hairpencils has also been investigated. Huang *et al.* (1996a) extracted ten components from male hairpencils of Chinese populations of *H. armigera*, including the alcohol (*Z*)-11-hexadecen-1-ol, which has also been extracted from female glands (Nesbitt *et*

al. 1979, Konyukhov *et al.* 1984, Kehat & Dunkelblum 1990). They found that the alcohol (*Z*)-11-hexadecan-1-ol from male hairpencils interfered with male orientation behaviour in wind tunnel observations, and decreased the egg hatch rate when sprayed in field enclosures containing male and female *H. armigera*. There is currently no evidence for (or against) any close range chemical communication in the process of mating for males and females of *H. armigera*.

Male-male competition in the field could also reduce the percentage of males which contact the Sirene-based pheromone lure. Males often flew in groups ranging in size from two to twelve. Within each group often only the leading male in the group contacted the lure (see Chapter 3.1.1 for additional details of this observation). Although it could not be determined that this was due to male-male competition, it does seem possible that for every group of males, only one will contact that particular lure. This may occur naturally when a male moth attempting to locate a female must deal with the other males that are trying to mate with the same female. This male-male competition has been reported in *Grapholita molesta* where males which are copulating or about to engage in copulation actively exclude other males (Baker 1983). There is a possibility that males may actively inhibit this competition by releasing inhibitory chemicals from their hairpencils, which would give a biological explanation for the phenomenon observed by Huang *et al.* (1996a) (see previous paragraph). Such inhibitory effects have not been observed in another noctuid *Pseudaletia unipuncta* (Fitzpatrick *et al.* 1988).

A lack of appropriate acoustic cues may also reduce the attractiveness of the synthetic lure. The presence or absence of intraspecific acoustic communication in *H. armigera* has not been actively researched. *H. armigera* of both sexes certainly have the ability to perceive airborne vibrations. The superfamily Noctuoidea, which contains the Noctuidae, is largely defined by the presence of metathoracic tympanal organs in both sexes of the adult moths (Common 1990). In most cases these organs have the ability to perceive ultrasonic sounds. This sensory ability seems to be associated with detecting and avoiding nocturnal predators such as bats (Common 1990). Males of the African species in the heliothine genus *Heliocheilus* have a thickening halfway along the costa in the forewing which can produce a buzzing noise which seems to be part of the sexual behaviour of these moths (Matthews 1987). Although no such costal thickening is present in *Helicoverpa* spp. (Matthews 1999) there may be other means of sound (particularly ultrasonic sound) production that are not immediately obvious such as clapping wing surfaces together in flight (Agee 1971). Further research is required to determine if this acoustic behaviour exists, and whether it is part of the sexual behaviour of *H. armigera*.

Differences between the synthetic pheromone blends and the natural blends are a key consideration when considering operating principles in attract and kill, as a blend which is less attractive than the existing natural female pheromone sources will be unlikely to suppress pest populations in the field (Lanier 1990). Although it was not possible to observe differences in the numbers of contacts between females and synthetic lures in the present study, the results suggest that the critical blend components are present in the synthetic formulations in correct ratios. The behavioural patterns of males around exposed and concealed pheromone sources suggest that visual and presentation factors are important in eliciting the full behavioural repertoire of males. The following chapter sections explore these aspects in greater depth.

4.2 Presentation of Sirene-based lures

4.2.1 Why might presentation make a difference?

Sirene-based lures are usually presented to the target pest as a small 50 to 200 mg droplet placed on a crop substrate (Hofer & Angst 1995, Hofer *et al.* 1996), and most of the observations in this thesis have been of Sirene formulations presented as 200 mg droplets (see Chapter 2.6). Sex pheromone studies of moths, both in wind tunnels and in the field, tend to use small point sources similar to the small droplet of Sirene as the method of presenting pheromone to the males, presumably because this mimics the presentation method of the female moth. Calling females are normally stationary whilst producing pheromone, allowing the active components to volatilize from the tip of the abdomen which represents a single point source. Whilst point sources are biologically realistic the potential for improved attractiveness and increase in the rate of contact by artificially presenting larger surface areas requires investigation.

Plume-following behaviour of male moths is predominantly determined by the fine-scale structure of the plume, which is in turn determined by both the nature of the air currents present, structures around the pheromone source, and the pheromone source (Baker & Haynes 1989, (Mafra-Neto & Cardé 1994). Pheromone point sources may cause arrested flight in male moths where males terminate the upwind flight without reaching the source (Cardé & Hagaman 1979, Charlton *et al.* 1993). Flight arrestment is not a universal phenomenon (Sanders 2000). A point source which releases a narrow concentrated plume may also prevent males from locating the source. For example about 50% of *Cadra cautella* males do not find the source when presented with a "ribbon" plume of synthetic pheromone in a wind tunnel (Mafra-Neto 1993 cited in Cardé & Mafra-Neto 1996). Sources which produce a low-concentration diffuse plume may not promote this close-range searching behaviour as in the light brown apple moth *Epiphyas postvittana*

(Foster *et al.* 1991) and the pea moth *Cydia nigricana* (Lewis & Macaulay 1976). Conversely, male gypsy moths exhibit more direct flight and greater net velocities, air and ground speeds when flown to a diffuse turbulent plume compared to a narrow plume from a point source (Willis *et al.* 1994). Males of *Heliothis virescens* observed in wind tunnel conditions will only commence the straighter, upwind surging behaviour which characterizes close-range location behaviour if there are sufficient strands of pheromone to maintain this frequency-dependent behaviour (Vickers & Baker 1994). This phenomenon has also been observed for *C. cautella* (Mafra-Neto & Cardé 1994, Mafra-Neto & Cardé 1995a).

The studies presented here involved manipulating the presentation of the Sirene formulation to determine if alternative methods of presentation are more efficacious than the standard 200 mg droplet method observed elsewhere in this chapter and in Chapter 3.

4.2.2 Methodology

Night vision observations, formulations and analyses of the behavioural data were made as described in the General Methods section (Chapter 2).

Three treatments were observed:

(a) a 10 x 10 cm square of Corflute® plastic with a single droplet in the centre of 1 ml of Sirene with 1% pheromone ("droplet")

(b) a 10 x 10 cm square of Corflute® plastic with 1 ml of Sirene with 1% pheromone arranged in a 4 x 5 pattern of small droplets over the entire area of the square ("multiple droplets")

(c) a 10 x 10 cm square of Corflute® plastic with 1 ml of Sirene with 1% pheromone smeared over the entire area of the square ("smear")

Observations were made on immature chickpea near Nangwee on the Darling Downs, Queensland on the 23rd, 24th & 25th of October 2001.

4.2.3 Results

Figures 4.7, 4.8 and 4.9 show the mean numbers of males per second approaching, getting near and contacting the lure, the percentage of approaching males which got near and contacted the lures, and the mean time spent approaching and near the lures for the three treatments respectively (see Appendix 12.2, Table 3 for details of the total numbers and mean values for the behavioural parameters). The mean number of males that contacted the lure per second was significantly

increased for the smear and multiple droplets treatments compared to the droplet (one-way ANOVA with contrasts, df = 2, F = 5.99, $p < 0.05$); the difference between the multiple droplets and smeared treatments was not significant. There was a significant increase in the number of males per second contacting when the droplet was compared to the multiple droplets (paired t-test, df = 1, F = 4.96, p = 0.05). The smeared and the multiple droplets treatments significantly increased the proportion of approaching males that contacted the lures ($\chi^2 = 1013.106$, df = 2, $p < 0.001$) when compared to the droplet, and the smeared treatment significantly increased the proportion of moths that contacted compared to the multiple droplets treatment ($\chi^2 = 14.57614$, df = 1, $p < 0.01$). The droplet tended to increase the mean amount of time the males spent approaching and near the lure, but the trends were not significant (Kruskal-Wallis Rank Sum Test $\chi^2 = 0.4103$, df = 1).

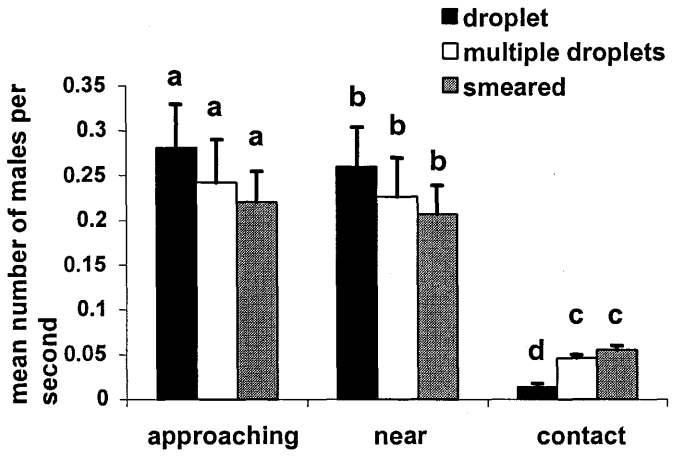

Figure 4.7 Mean numbers of male *H. armigera* per second approaching, near and contacting for three treatments. Different letters indicate treatments that are significantly different ($p < 0.05$)

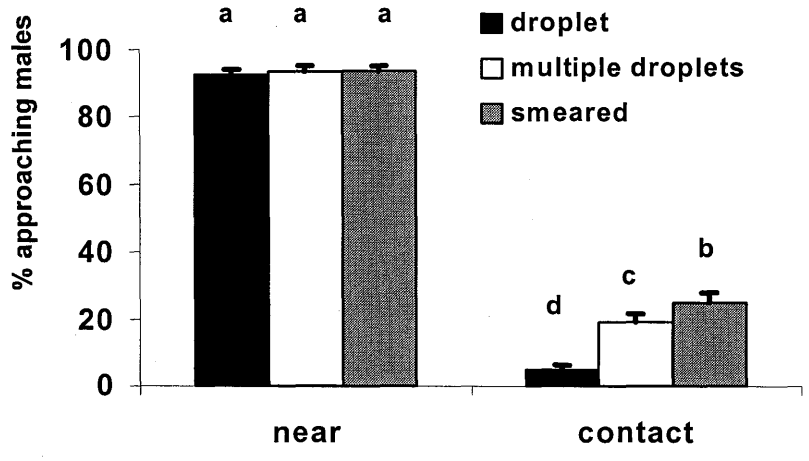

Figure 4.8 Percentages of approaching male *H. armigera* that got near and contacted three treatments. Different letters indicate treatments that are significantly different ($p < 0.05$)

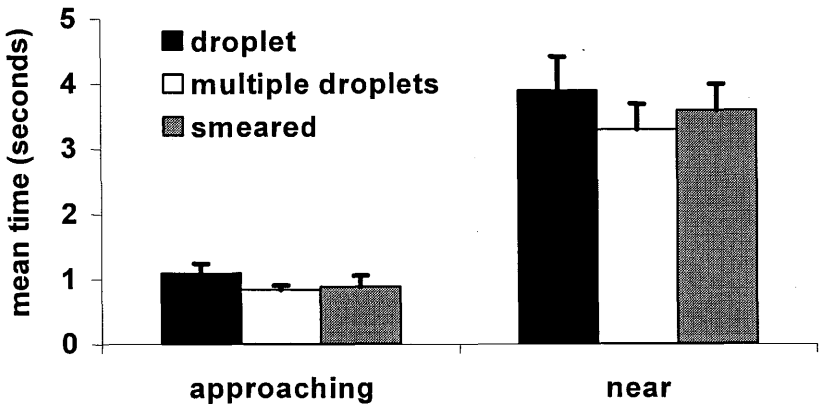

Figure 4.9 Mean time spent by male *H. armigera* approaching and near for three treatments. No significant differences were observed.

4.2.4 Discussion

Presentation factors often result in considerable changes in the behaviour of moths around lures. (Willis *et al.* 1994) found that *Lymantria dispar* males display significantly more direct flight to the lure and increased net velocities, air and ground speeds when flown to a pheromone point source with baffles around it, indicating that a diffuse plume may be more attractive than a single point source. The effect of having the multiple droplets probably mimics a diffuse source, making it more attractive at close range than the single large droplet. When the source is a uniform square surface as in the smeared treatment this effect is enhanced. If this is the case we would expect to see an increase in the number of moths getting within 1 m of the lure, but this was not observed. In the present study, however, *H. armigera* males did spend much more time near the single large droplet treatment than the other two treatments; this may equate with having a more indirect path to the single droplet lure, along with reduced net velocity, air and ground speed. Further detailed observation and video analysis such as those used by Willis *et al.* (1994) may be necessary to determine if there is such an interaction between source shape, plume structure and moth behaviour in *H. armigera.*

Another potential reason for the increased efficacy of lures when presented as multiple droplets or a smear may be the change in release rate of pheromone. An increased surface area of formulation results in an increased release of volatiles. Chapter 7 measures the weathering of Sirene and compares the loss of active pheromone components when the formulation is either a droplet or a smear. Smearing a 200 mg Sirene formulation over a 2 x 2 cm area resulted in a significant loss of pheromone components over 31 days compared with 200 mg Sirene left as a

droplet. Daily release rate over the first 31 days was 231 ng/day for the droplet compared to 3,271 ng/day for a smeared droplet, more than tenfold difference in release rate.

Increased release rate alone may not be sufficient to explain an increase in the proportion of contacts. Another explanation may be that the increased surface area of formulation increases the chances of a male contacting the lure, either by accident, or by providing more landing area. (Lanier 1990) refers to this area of active surface as the "insect-affector interface", which is equivalent to the cross-sectional area of the trap entrance in a funnel-type pheromone trap (Lanier 1990), or the area of adhesive surface in a sticky pheromone trap (Lewis & Macaulay 1976). Accidental contacts might be increased due to increased surface area of the lure in the smeared and multiple droplet treatments but in view of the observed flight behaviour of *H. armigera* this would seem unlikely. Night vision observations at close distance (< 2 m) showed that males rarely blunder into lures, but were generally in full control of their flight. Adults of *H. armigera* are strong flyers and are capable of hovering close to nectar and pheromone sources, so it is unlikely that any of the contacts observed with the different pheromone sources could be the result of errors in flight.

An alternative explanation may be the increase in the perimeter of the patch which provides more potential locations for contact with the formulation. This may explain most of the increase in the number and rate of contact in the smeared and multiple droplet treatments. The single droplet had 20% of outer perimeter available for contact compared to the smeared treatment (85 mm compared to 400 mm). This is similar to the difference in the proportion of contacts for these two treatments, as the single droplet received 20% of the contacts that the smeared treatment did (4.90% versus 24.97%).

Applications of these findings may be limited by the availability of suitable plant surfaces in the field, by the increase in the rate of weathering observed in smeared droplets and the subsequent reduction in the useful life-span of the lures (see Chapter 7 for details), and by the cost of any increase in the overall rate of application in the field. Additionally, there will eventually be a trade-off with increase in active area of the individual applications of Sirene in relation to the number of point sources present in a given area of crop, as increased surface area and release rate may lead to mating disruption rather than attract and kill (see Chapter 8.4, Chapter 9).

The physical shape of the crop, such as the size of the leaves and stems in the upper canopy will also influence the application method. If a plant has only a narrow stem, or small upper leaves,

there will be little opportunity to increase the active area of individual Sirene applications. An alternative way of increasing the available active area per individual Sirene application may be to apply the formulation as a smeared strip across a section of crop, but this will require further research into effects on approaching males, and on the weathering of the formulation.

4.3 Visual cues

4.3.1 The importance of visual cues in mate finding

Visual cues are poorly understood in relation to pheromonal communication. Most research on visual cues has concentrated on enhancing trap performance rather than lure formulations, but there have been some studies which have focused on the relative importance of visual cues to successful mating in moth species.

(Carpenter and Sparks (1982) associated artificial ("mock") females and dead male moths with the synthetic pheromone of *Helicoverpa zea* Boddie in a wind tunnel. They found that visual cues were important for mate location at distances between 16 and 22 cm, with males preferring to alight on pheromone sources with a mock female at distances less than 16 cm compared to pheromone sources without a specific visual stimulus. Males would also prefer to alight on an artificial female next to a pheromone source, but when the sources were separated by more than 12 cm they would only fly to the pheromone source. Gross *et al.* (1983) used trap catches to test the visual acuity of *H. zea* males. They found that 1.8 times more males of the corn earworm *H. zea* were trapped at pheromone sources which had a dead female glued adjacent to them compared to traps with just pheromone. Males were also able to discriminate between dead females and dead males or foam models of female *H. zea*, with more males caught in traps with dead female moths compared to the other two treatments. The presence of the foam models still gave significant increases in trap catches compared to traps with just pheromone.

However, research on mating systems in gypsy moth *Lymantria dispar* L. (Lymantriidae) demonstrates why care must be taken in interpretation of what seem to be visual cues associated with mating behaviour. Doane (1968) observed that male *L. dispar* would normally only mate with unmated females, and showed no interest in previously mated females. However, when a mated female was placed on a stake 15 cm downwind of an unmated calling female, males would attempt to mate with the mated female. This seemed to indicate that males used pheromone cues for long-distance location of virgin females, but used visual cues when close to the source of the

pheromone plume. Mated females placed on a stake 15 cm upwind of a virgin female were ignored, as was a mated female placed 7.5 cm below the virgin female.

Charlton and Cardé (1990) further investigated the role of visual cues in mate location in the field and found that male *L. dispar* located an isolated pheromone source in the field just as readily as one supplemented with visual cues. Wind tunnel experiments found that visual cues were only important over short distances (<5 cm) in the presence of the pheromone plume. If the visual cue was uncoupled from the pheromone plume by moving the pheromone source a short distance away from the female model, males simply responded to the loss of the pheromone rather than visually orientating to the female model. By using a tree trunk model in the wind tunnel they determined that the position of the pheromone source on the cylindrical surrogate tree trunk was more critical in eliciting close-range orientation, landing and walking rather than the visual cue of the female. This may have explained in part the observations made by Doane (1968). The stakes which Doane (1968) used to position females around the calling female may have influenced the behaviour of the males rather than the visual cues from the female.

Willis *et al.* (1994) made further field observations on male *L. dispar* responding to synthetic pheromone sources, and found that close-range orientation to the sources was enhanced by the shape of the tree trunks, rather than visual cues from the tree trunks. By placing a transparent cylindrical baffle in the field which mimicked the effect of a tree trunk on the pheromone plume shape they determined that the structure of the pheromone plume greatly influenced the behaviour of males flying to the lure, and that males were more direct and faster at locating sources which were on the baffle than a simple point source without any baffle.

Shorey and Gaston (1970) documented the short-range visual cues that are important in mating of the noctuid *Trichoplusia ni*. They found that *T. ni* males showed limited orientation behaviour towards pairs of visual cue models arranged 2 cm either side of a pheromone source, with 33% of males orientating towards models compared to 67% to the source. The frequency of attempted copulation was significantly increased with the presence of one or more models, with 86% of copulatory attempts directed towards a model. The models compared included dried *T. ni* females set and dissected in a variety of ways, and a series of models cut from black card, including one which mimics the outline of a female with partially spread wings. Models using set females were preferred over card models, although the female with wings removed was less attractive. A set female dyed black was less attractive than a normally coloured female, suggesting that both outline and colour are important criteria in stimulating attempted copulation.

Other studies on the plume-following behaviour of the Mediterranean flour moth *Ephestia (Anagasta) kuhniella* (Pyralidae) and the light brown apple moth *Epiphyas postvittana* (Tortricidae), also revealed the importance of visual cues in mate location (Traynier 1968, Foster *et al.* 1991, Rumbo 1993). Trematerra and Capizzi (1991) also looked at the importance of visual cues when developing an attracticide for *E. kuhniella*. They observed the mean number of wild males that flew to pheromone-baited sticky traps when these traps were accompanied by different shapes of brown cardboard. Sub-triangular shapes which resemble the outline of a resting moth were significantly more attractive than other shapes.

The importance of visual cues in mate location and mating has been demonstrated for male codling moths *Cydia pomonella* (Tortricidae) (Hutt & White 1977, Castrovillo & Cardé 1980). Only 59% of males which had their eyes covered in opaque paint were able to successfully mate with females in cage trials compared to 87% in the control group. Males which had their antenna excised managed to mate 46% of the time, which was not significantly different to painted eyes treatment, implying that close-range visual cues are almost as important as pheromone cues for successful mating in this species (Hutt & White 1977). However, mating frequency in caged moths will probably not reflect what happens in the field, and does not give much information on the reasons and cues which might influence the observed mating frequencies. Castrovillo and Cardé (1980) found that male codling moths spent a significantly increased time walking, wing fanning and attempting to copulate near the visual cue of a dead female codling moth in the presence of a synthetic pheromone source than they would in the presence of the pheromone source without the visual cue. The visual cue did not influence the persistence of orientation (ie. the moths were still able to find the pheromone source), but as in *T. ni*, a similar cue influenced the persistence and orientation of attempted copulation (Shorey & Gaston 1970).

Nocturnal insects have often been assumed to be colour-blind, but it has been conclusively demonstrated that several species of hawk moth (Sphingidae) utilise colours to discriminate between different coloured objects at very low light intensities corresponding to starlight levels at night (Kelber *et al.* 2002). Other studies have considered how trap colour and shape might affect trap catches. Mitchell *et al.* (1989) looked at how pheromone trap colour affects catches of two noctuid species, the fall armyworm *Spodoptera frugiperda* and the velvetbean caterpillar *Anticarsia gemmatalis*. The authors recorded significant differences in catches of *A. gemmatalis* associated with trap colour, whereas *S. frugiperda* was less sensitive. They attempted to relate these differences in catch size to the spectral sensitivity of the eyes, but electroretinograms

showed very few differences between the two species in sensitivity to visible wavelengths, implying that sensitivity to trap colour is the result of higher level neural processes. As with the Mitchell *et al.* (1989) study, Meagher (2001a) found that trap colour did not appear to exert a particularly large influence on catches of *S. frugiperda*. A New Zealand study of *H. armigera* found that all green pheromone traps caught fewer moths compared to the yellow and white trap type (Herman *et al.* 1995).

With the exception of the study of Trematerra and Capizzi (1991) on developing an attract and kill method to control a stored product pest there appear to be no published studies regarding the importance of visual cues, or lack of them, in attracticide formulations. Much of the field information available on visual stimuli use indirect data from trapping information rather than direct observations. In this chapter section a variety of visual stimuli are presented with Sirene formulations and male responses are directly recorded as the moths approach the pheromone source.

4.3.2 Methodology

Night vision observations, formulations used and analyses of the behavioural data were made as described in the General Methods section (Chapter 2).

Visual stimuli/treatments included the following:

(a) A single droplet of formulation on Corflute ("plastic" treatment).

(b) Dead female moths set in a position which approximates a live calling female, oven-dried for one week at 40°C. These were pinned through the middle of the Sirene droplet on the Corflute ("plastic + female" treatment).

(c) A single droplet placed directly on top of the sunflower stem just under the flower head, where the stem forms a 90° bend ("sunflower" treatment).

(d) The same as for (c), but with a similar dead pinned female as for (b) ("sunflower + female" treatment).

(e) The complete wings of a female moth dissected and glued, upperside up, on Corflute with the Sirene droplet in the position of where the body of the female moth would have been ("complete wings" treatment).

(f) The fore wings of a female moth dissected and glued as for (e) ("fore wings" treatment).

(g) The hind wings of a female moth dissected and glued (e) ("hind wings" treatment).

(h) A simple black line on Corflute designed to approximate the black hind wing margin with the Sirene droplet in the position of where the body of the female moth would have been ("black line" treatment).

Direct comparisons between (a) and (b) were made in flowering sunflower crops at Nangwee for three nights (18,19, 20 January 2000), and comparisons between (a) and (c)-(f) were made in the similar stage sunflowers at Nangwee also for three nights (20, 21, 22 January, 2000).

Observations on the effect of the decoy female moth on plume structure were made in the laboratory wind tunnel (Chapter 2.8). Plume images were captured using a digital video camera.

4.3.3 Results

4.3.3.1 Comparing standard lure with pinned decoy female on lure

Figures 4.10, 4.11 and 4.12 show the aggregate results for the three consecutive nights of observation on both the plastic substrate and the sunflower stalk, with a grand total of 2,196 male moths approaching the lures during the observations. Details of the total number of moths observed for each treatment approaching, near and contacting the lure, the percentage of approaching moths that got near and contacted the lures, the number of moths per second for each behavioural state, and the mean times taken by moths approaching and getting near the lures can be found in Table 4, Appendix 12.2.

Figure 4.10 shows the mean numbers of males per second that approached, got near and contacted the lure for the four treatments. The sunflower substrate significantly increased the proportion of contacts compared to the plastic substrate ($\chi^2 = 29.01$, df = 1, $p<0.01$), but the mean number of moths per second that contacted the lures was not significantly greater than the plastic. Figure 4.11 shows the percentages of the total numbers of moths approaching the lure that got near and contacted the lure. Addition of the pinned female moth significantly increased the percentage of contacts for both substrates compared to lures without the female ($\chi^2 = 426.07$, df = 1, $p<0.01$, plastic vs plastic + female, $\chi^2 = 270.02$, df = 1, $p<0.01$, plastic vs sunflower + female), but the effect of substrate was no longer evident ($\chi^2 \cong 0$, df = 1, sunflower + female vs sunflower + female). The mean number of contacts per second was significantly greater when the plain plastic substrate was compared with the plastic + female (Kruskal-Wallis Rank Sum Test $\chi^2 = 4.8109$, df

= 1, p-value = < 0.05), but there was no significant increase in the mean number of contacts for the sunflower substrate compared to the plastic substrate with or without the female decoy.

Figure 4.12 shows the mean times taken by moths approaching and getting near the lure for the four treatments. There was no significant overall effect of treatments on the mean time moths took approaching the lure (ANOVA, df = 3, F = 1.61), or getting near the lure (ANOVA, df = 3, F = 1.99), but the mean time of males approaching and getting near the sunflower substrate was significantly greater than that of the plastic substrate (paired t-test, df = 1, F = 8.35, $p < 0.05$ for approaching mean times, paired t-test, df = 1, F = 11.86, $p < 0.01$ for near mean times). Paired comparisons of the mean times of moths approaching and getting near the other treatments were all non-significant.

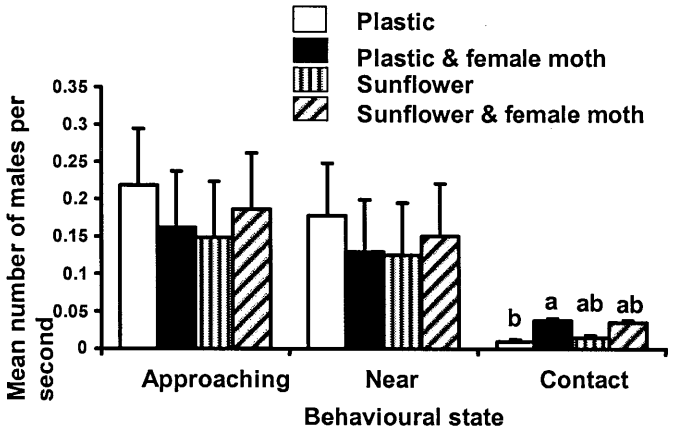

Figure 4.10 Numbers of male *H. armigera* per second that approached, got near, and contacted the lure for four treatments. In each category different letters above columns indicate that there are significant differences between treatments (p<0.01), Kruskal-Wallis Rank Sum Test. Categories without letters showed no significant differences.

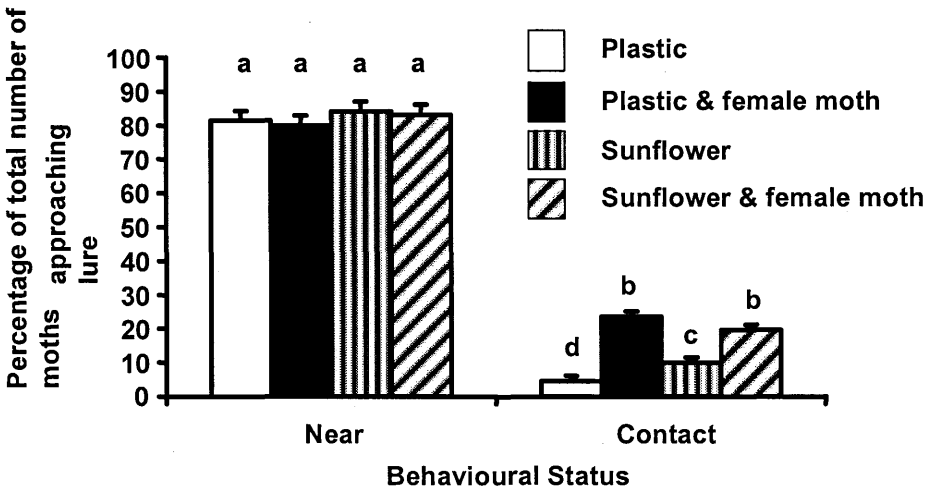

Figure 4.11 Percentages of the total numbers of *H. armigera* males that got near and contacted the lure for four treatments. In each category different letters above columns indicate that there are significant differences between treatments ($p<0.01$), Pearson's chi-squared statistic.

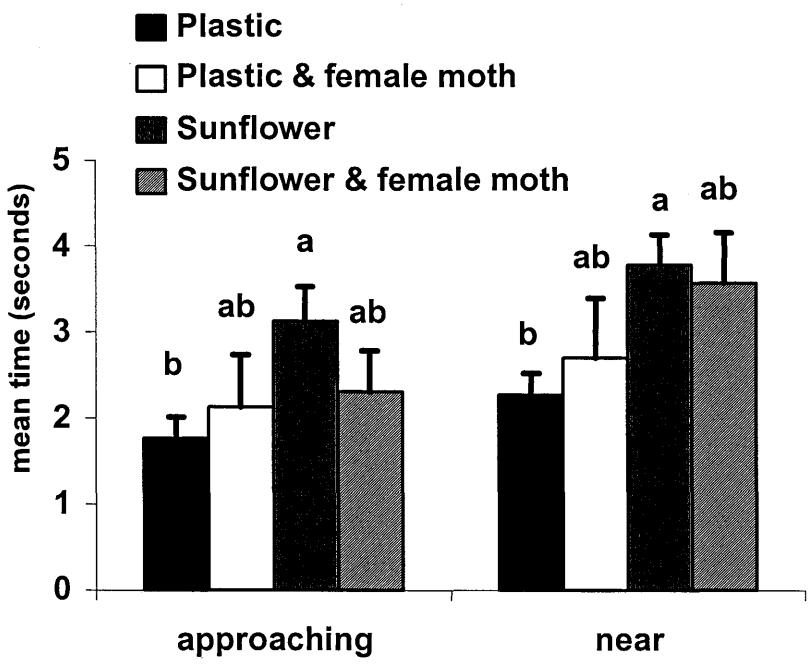

Figure 4.12 Mean time per male *H. armigera* spent approaching and near the lure for four treatments. In each category different letters above columns indicate that there are significant differences between treatments ($p<0.01$), paired t-test.

4.3.3.2 Comparing standard lure with dissected female and an artificial visual stimulus

Figures 4.13, 4.14 and 4.15 show the aggregate results for the two consecutive nights of observation. Details of the totals, percentages and mean data for the observations of the five treatments can be found in Table 5, Appendix 12.2. Figure 4.13 shows the mean number of males per second that approached, got near, and contacted the treatments, Figure 4.14 shows the percentage of approaching males that got near and contacted the five treatments, and Figure 4.15 shows the mean time males took approaching and getting near the lures.

Lures with complete wings, and fore wings present significantly increased the proportion of moths that contacted the lure compared to the unadorned lure, the hind-wings, and the black-line treatment (χ^2 statistic, $p<0.01$), with a greater proportion of the moths getting near the lures and contacting the lures. There were no significant differences in the mean numbers of moths per second between the five treatments, or in the mean time males took approaching and getting near the treatments.

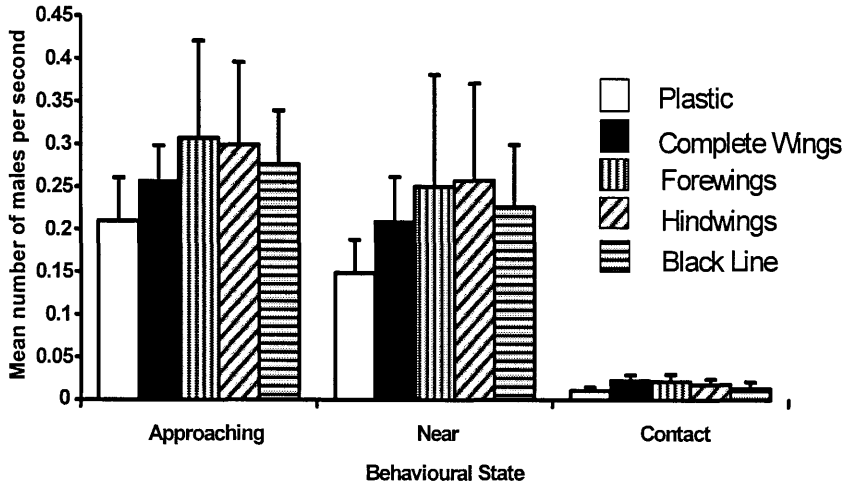

Figure 4.13 Numbers of male *H. armigera* per second that approached, got near, and contacted the lure for five treatments. No significant differences were observed.

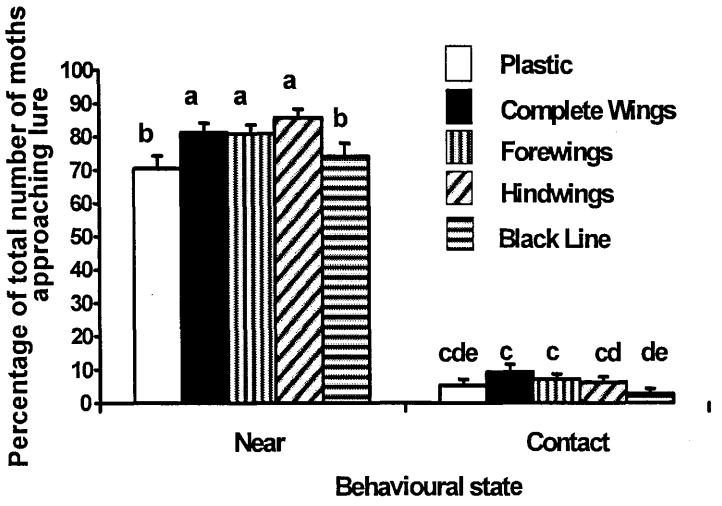

Figure 4.14 Percentages of the total numbers of *H. armigera* males that got near and contacted the lure for five treatments. In each category different letters above columns indicate that there are significant differences between treatments ($p<0.01$), Pearson's chi-squared statistic.

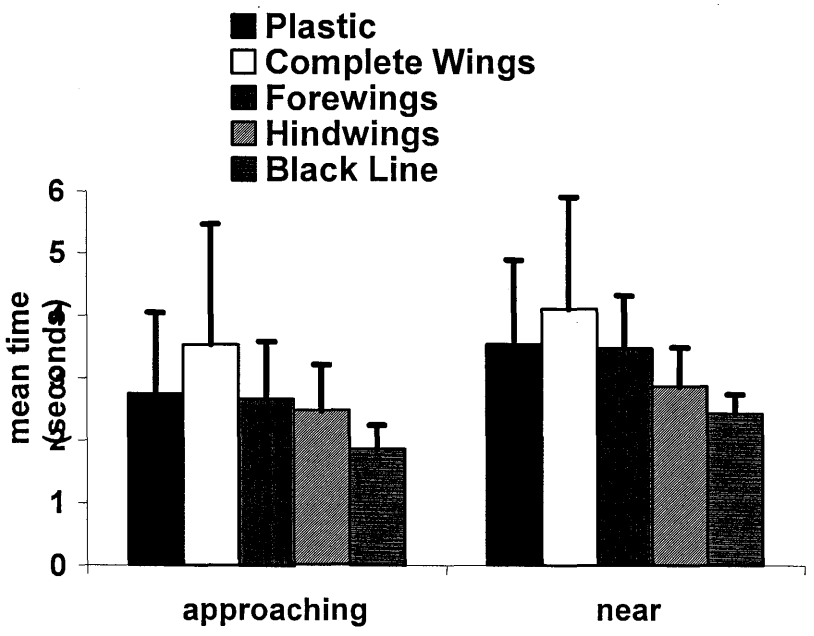

Figure 4.15 Mean time per male *H. armigera* spent approaching and near the lure for five treatments. No significant differences were observed.

4.3.4 Discussion

Visual cues appeared to increase the close-range attractiveness of synthetic pheromone lures to male *H. armigera*. Most of the observations made in this study were consistent with existing literature on wind tunnel observations and trapping experiments (Section 4.1.1). The visual stimuli involved would seem to be relatively complex, involving both wing patterns and colours as perceived by the male moth, as well as the outline of the female decoy. The dissected female observations did not invoke a strong reaction even when all four wings of the female moth were included, suggesting that the absence of additional cues such as the posture and outline of the female moth were necessary. This corresponds with the published observations of *Trichoplusia ni* males flying to lures with visual stimuli, where decoy females were preferred over black card models (Shorey & Gaston 1970), and with the measurements of Gross *et al.* (1983) of the visual acuity of *H. zea* males.

A potentially confounding effect of using any three dimensional substrate such as a female moth, or a sunflower plant is that it may cause turbulence within the pheromone plume coming from the synthetic lure which may affect its attractiveness independently of visual cues. Willis *et al.* (1994) found in field observations that a pheromone source that was diffused by a baffle was more attractive to males of the gypsy moth *Lymantria dispar* than the point source equivalent, inducing more direct flight to the lure and increasing net velocities, air and ground speeds. Mafra-Neto and Cardé (1994) showed that *Cadra cautella* (Pyralidae) males flying in turbulent or mechanically pulsed odour plumes flew faster and straighter upwind, and located sources more frequently than males following continuous narrow plumes. This effect on *C. cautella* was more important than a 1000-fold range in pheromone concentration in terms of influencing flight pattern (Mafra-Neto & Cardé 1995b). Similar effects can occur when male *C. cautella* fly in the company of other males when the wing beats of upwind males create turbulent pulses of pheromone (Mafra-Neto & Cardé 1995b).

A similar response has been observed in the male tobacco budworm *Heliothis virescens* (Noctuidae) in a wind tunnel, where rapidly pulsed pheromone plumes induced more upwind surges (Vickers & Baker 1994), with a frequency of four pulses per second required to elicit sustained upwind flight (Vickers & Baker 1992). These responses to increased turbulence appear to be due to fine-scale structure of plumes, and must be taken into account when placing objects on or around pheromone sources, such as in the visual cue experiments presented here.

The observations of the influence of fine-scale structure of plumes have been made across a broad phylogenetic range of moths, and this would indicate that these responses can be generalized to other moth species (Cardé & Mafra-Neto 1996). It would be expected that *H. armigera* would respond to alterations in plume structure. The behaviour of *H. armigera* in response to compact constant plumes compared to turbulent plumes is yet to be fully documented, but it is clear from this work that the pheromone plumes produced from a point source in a wind tunnel were different when a decoy moth was placed on the point source. Observations of digitized images of smoke plumes produced by a point source in a wind tunnel indicate that placing the decoy female on the lure does induce a significant change in the plume structure. Figures 4.16 and 4.17 show tracings of the plumes produced with and without the decoy. The decoy female produced a turbulent plume, resembling a series of pheromone clouds, whilst the Sirene droplet by itself produced a very narrow ribbon plume.

It would seem unlikely that changes in plume shape were responsible for all of the increases in contacts with the lure and decoy female at close range, as the observed behaviours up to 1 m away from the lure were similar in all treatments. Many males were observed to fly very close (<5 cm) to the plain lures without contacting the lure, and it was at this distance that the visual cues based on female decoys seemed to stimulate more lure contacts.

Figure 4.16 Smoke plume generated in wind tunnel upwind of a Sirene droplet. (a) = smoke source (b) = droplet.

Figure 4.17 Smoke plume generated in wind tunnel upwind of a Sirene droplet with a pinned dead female moth placed in the middle of the droplet. (a) = smoke source (b) = droplet and female.

The complexities of the visual cues which stimulate more contacts for male *H. armigera* would seem to preclude their incorporation into a Sirene-based attract and kill system. Mimicking these complex stimuli is likely to be too costly. Although further research may reveal small increases in

close-range attractiveness of lures which are dyed in appropriate colours it is unlikely that this will match the visual stimulus of an intact female.

Only one alternative substrate (sunflower) was examined in this thesis. Further research into the effect of different crop substrates on the behaviour of males around the Sirene lures would be useful. Differences in plant architecture between crop species may influence the shape of pheromone plumes, and the subsequent attractiveness of the Sirene applications on those plants. Attract and kill formulations will have to last up to three weeks or more in the field, which means that if they are applied to rapidly growing plants there may be differences in plant architecture within an individual crop type over time, as well as differences in the position of the droplets on the plant. Attracticide droplets which end up below the level of the crop canopy as the crop grows would probably be less effective, as males typically follow pheromone plumes at or slightly above the level of the crop canopy. Some of the failures of mating disruption programs to control codling moth in apple orchards have been attributed to the placement of pheromone dispensers below the canopy of the orchard (Witzgall *et al.* 1999). One solution to this problem is to use repeat applications throughout the growing season, which was the case for Sirene-based attracticide trials with pink bollworm in Egyptian cotton (Hofer 1994, Hofer & Angst 1995). Another solution may be the use of artificial substrates, such as wooden or plastic stakes, although these may be undesirable because of the additional cost, and because they have the potential to contaminate the harvested crop and/or damage harvesting machinery.

4.4 Addition of bifenthrin

4.4.1 Interactions between insecticides and chemical communication

Bifenthrin and other synthetic pyrethroids are often thought of as insect repellants. Examples include permethrin and cypermethrin to honey bees *Apis mellifera* (Hymenoptera: Apidae) (Rieth & Levin 1988), permethrin to stable flies *Stomoxys calcitrans* (Diptera: Muscidae) (Bartlett 1985) and larval diamondback moth *Plutella xylostella* (Plutellidae) (Kumar & Chapman 1984), λ-cyhalothrin, permethrin, cypermethrin to various species of mosquito (Diptera: Culicidae) (Arredondojimenez *et al.* 1997, Guillet *et al.* 2001), natural pyrethrum and synthetic pyrethroids (tetramethrin, *d*-pheronthrin), both with piperonyl butoxide, to brown lacewings *Micromus tasmaniae* (Neuroptera: Hemerobiidae) (Hodge & Longley 2000), deltamethrin to parasitic Hymenoptera (Longley & Jepson 1996) and to boll weevil *Anthonomus grandis* (Coleoptera: Curculionidae) (Moore 1980). Many commercial repellent products sold for medical, veterinary and general domestic use contain synthetic pyrethroids. However, the Australian Pesticides and

Veterinary Medicines Authority considers pyrethroids as insecticides rather than repellents, and has placed some restrictions on claims for repellent effects for these insecticides (see http://www.apvma.gov.au).

A form of repellency occurs when behavioural resistance to pyrethroids (and other insecticides) is developed. This is sometimes termed "stimulus-dependent resistance" where insects sense the presence of the insecticide and avoid it (Lockwood & Storey 1984). This has been observed for a number of insects that actively avoid contact with the treated areas (Uk & Dittrich 1986, Byford *et al.* 1987, Guillet *et al.* 2001). This form of behavioural resistance may extend to actively flying insects such as honey bees and moths avoiding treated crop areas; this may enhance pest management by deterring oviposition in treated crop areas, and by reducing lethal contact with insecticide by allowing otherwise susceptible individuals to avoid contact with insecticide. *H. armigera* is resistant to a number of pyrethroid insecticides. A variety of biochemical mechanisms are involved in this resistance (Gunning *et al.* 1995, 1996a,b, 1998a,b, 1999) but the possibility of behavioural resistance has not been discounted.

Moore (1988) observed that significantly fewer *Helicoverpa zea* were captured at sugar lines in a cotton field that had been treated with 0.11 kg permethrin (AI) per hectare the same day compared to an untreated field, and that significantly fewer moths were found during daylight hours in cotton fields that had been sprayed with 0.055 kg permethrin (AI) per hectare compared to unsprayed cotton. This may indicate a real reduction in numbers through mortality, but may also indicate a deterrent effect due to the presence of the pyrethroid.

There appears to be little evidence from field studies of pyrethroid-mediated repellency of attracticide formulations. Haynes *et al.* (1986) compared the wind tunnel responses of male pink bollworm *P. gossypiella* to five different types of gossyplure-laced formulations, four with insecticides (10% of cypermethrin, fenvalerate, permethrin and chlordimeform) and a control without any insecticide associated with the pheromone source. Of the four insecticide treatments only the non-pyrethroid chlordimeform decreased responses to the source compared to the control without insecticide, a result which had already been noted in oriental fruit moths, *G. molesta* (Linn & Roelofs 1984). Moore (1988) also noted that wild males of *H. zea* present in cotton fields treated with a foliar spray of 0.055 kg permethrin (AI) per hectare were captured at the same rate in pheromone traps as those in adjacent unsprayed cotton.

Direct field observations of male behaviour at pheromone lures with and without insecticides are not common in the literature. Using the observation techniques outlined in Chapter 2 the behaviour of male *H. armigera* around lures with and without bifenthrin and other adjuncts were studied to determine if there are potential repellant effects which might impact on the efficacy of these lures.

4.4.2 Methodology

Night vision observations and analyses of the behavioural data, chemicals used, formulation procedure and their sources and purity are described in Chapter 2.

The following formulations were compared:
(a) A single droplet of standard 1% pheromone in Sirene formulation on Corflute (as a control)
(b) The same as (a), but with 6% bifenthrin (AI)

Observations were made in early-stage flowering round tomato at Wright Pack Farms, Bowen, Queensland on the 19th and 23rd of August 2001, and in various properties near Nangwee, Queensland in corn (11th April 2000), soybean (12th April 2000), silking corn bordering mid-season cotton (29th,30th January 2001) and flowering pigeon pea (21st, 22nd, 27th, 28th February 2001), and at Wright Pack Farms, Bowen, Queensland in early-stage flowering round tomato (19th, 20th, 23rd August 2001). Only observations made on the same night were compared.

4.4.3 Results

Figures 4.18, 4.19 and 4.20 show the number of moths per second that approached, got near, and contacted the different treatments, the percentage of those moths that approached that got near and contacted the lure, and the mean time taken by males approaching and near the lure respectively. Table 6 (Appendix 12.2) details the same data. There was a trend for the mean number of moths per second to be less for the bifenthrin treatment, but this was not significant for all three measured behaviours (Kruskal-Wallis Rank Sum Test $\chi^2 = 0$, df = 1). There was no significant difference in the percentage of moths approaching lures with 6% bifenthrin in the Sirene that got near or contacted compared to lures without insecticide ($\chi^2 = 0.936$, df = 1, $\chi^2 = 3.124$, df = 1 respectively). There was no significant difference in the mean time taken by males approaching and near the lures.

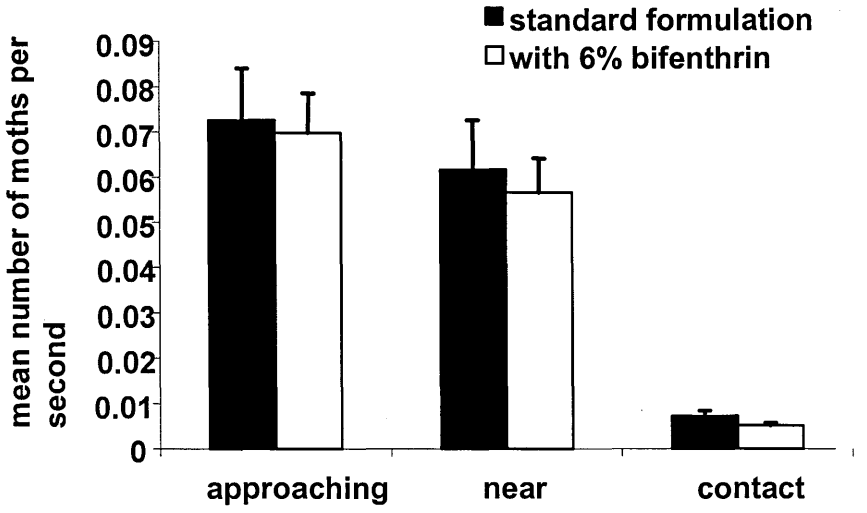

Figure 4.18 Mean numbers of male *H. armigera* per second that approached, got near, and contacted the lure with and without bifenthrin. No significant differences were observed.

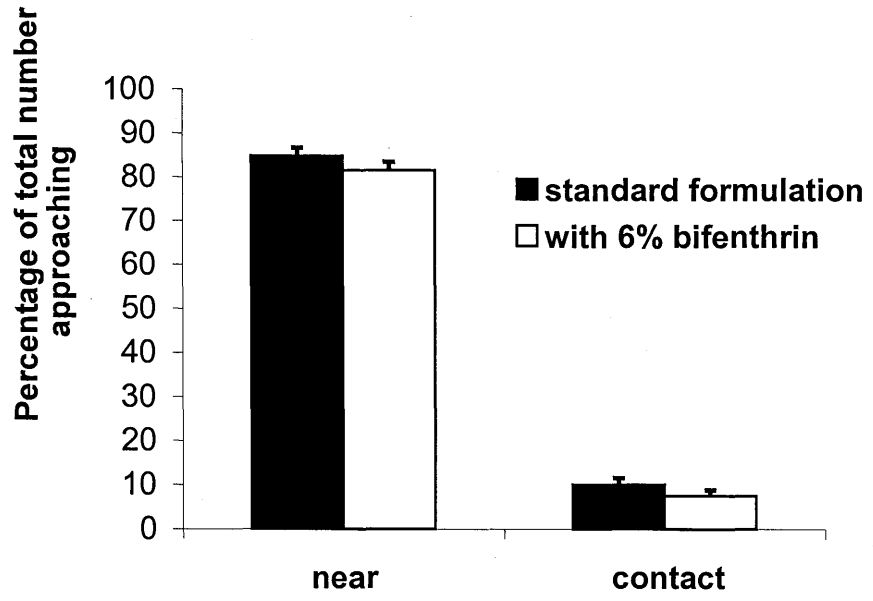

Figure 4.19 Percentages of the total numbers of *H. armigera* males that got near and contacted the lure with and without bifenthrin. No significant differences were observed.

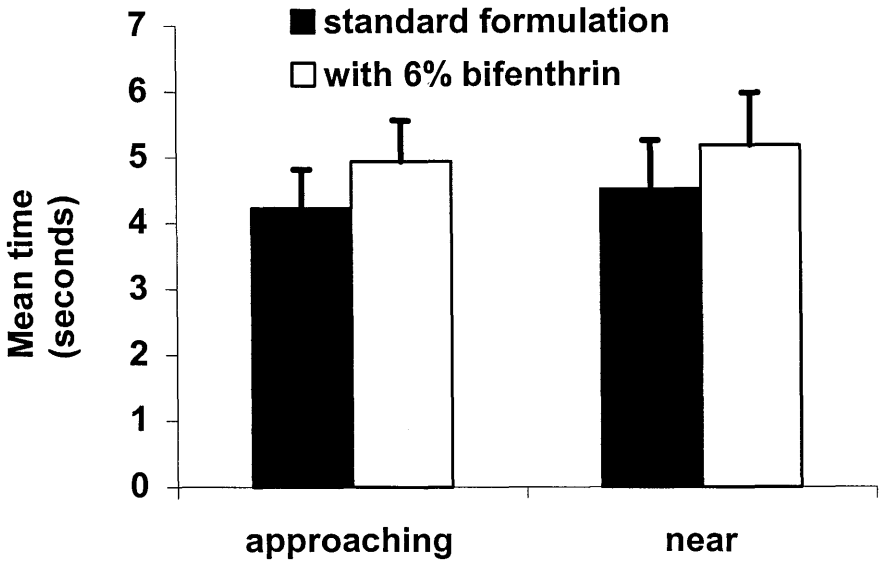

Figure 4.20 Mean time per male *H. armigera* spent approaching and near the lure with and without bifenthrin. No significant differences were observed.

4.4.4 Discussion

There was no evidence for repellency of the pyrethroid bifenthrin for male *H. armigera* attracted to synthetic pheromone sources, even though some pyrethroids are notable for interfering with neural processes and behaviour. These observations agree with those made for pink bollworm by Haynes *et al.* (1986) and with the field observations of *H. zea* by Moore (1988). The repellent effect of pyrethroids usually requires that an insect has intimate contact with the insecticide, either directly with a surface coated with the insecticide as is certainly the case for permethrin in stable fly *S. calcitrans* repellent (Bartlett 1985), or as an airborne vapour. Bifenthrin has a low vapour pressure of 2.41×10^{-4} mPa at 25°C compared to permethrin (4.5×10^{-2} mPa at the same temperature) (Extoxnet 2005). This may mean that the bifenthrin molecules present in the air stream may be insufficiently concentrated to interfere with plume-following behaviour of *H. armigera* males.

4.5 Concluding discussion

Various factors and cues which may or may not be important in increasing or decreasing the percentage of males contacting synthetic Sirene-based pheromone formulations were studied in this chapter. These include comparison with calling females, addition of a potential third component, variations in formulation presentation, substrate cues, visual cues and the addition of insecticide to the synthetic blend. Table 4.1 lists the most influential stimuli for increasing the percentage contact, and the increase in response compared to the typical single 200 mg droplet presented on a plastic substrate. The factors which induced the most change were the addition of a decoy female, the presentation of the Sirene droplet, and the substrate upon which the lure was placed.

Table 4.1 Summary table showing the percentages of males that contacted four different treatments where a significant increase over the standard presentation (italicized) of a 200 mg droplet 1% pheromone on a plastic substrate was noted. *Numbers in parentheses indicate the increase over the standard presentation. Note that the overall percentage contact for all observations at standard lures was approximately 11% rather than 7.3%.

Treatment	Percentage Contact *
Standard 1% synthetic pheromone on plastic (data which can be directly compared to experimental manipulations)	*7.3*
Inclusion of a female decoy	23.5 (16.2)
Natural substrate (Sunflower stem)	10 (2.7)
Increasing the active surface (presentation as many droplets)	19.1 (11.8)
Increasing the active surface (presentation as a smear)	25 (17.7)

Of these three factors, only manipulating the presentation (ie. the available area of active surface) and the choice of substrate are likely to be economically feasible for the applied use with attract and kill formulations. However, it may prove unnecessary to increase the number of contacts with the Sirene-based pheromone attracticide droplets provided there are sufficient lures in the field.

It is possible that a 10% contact rate will result in enough male mortality for successful attract and kill. This can be investigated using a simple mathematical modeling approach. This model assumes that 10% of males approaching a lure will contact it, and that moth experience does not alter this percentage. Therefore the 90% of approaching males that do not contact the attracticide lure the first time will fly to the next droplet, where an additional 10% will contact and receive a lethal dose, and so on. This can be described simplistically by an exponential function.

$$y = ne^{-0.1054x} \qquad \textbf{(Equation 4.1)}$$

Where y = number of surviving moths in the field

n = initial number of moths in the cohort

x = number of droplets encountered

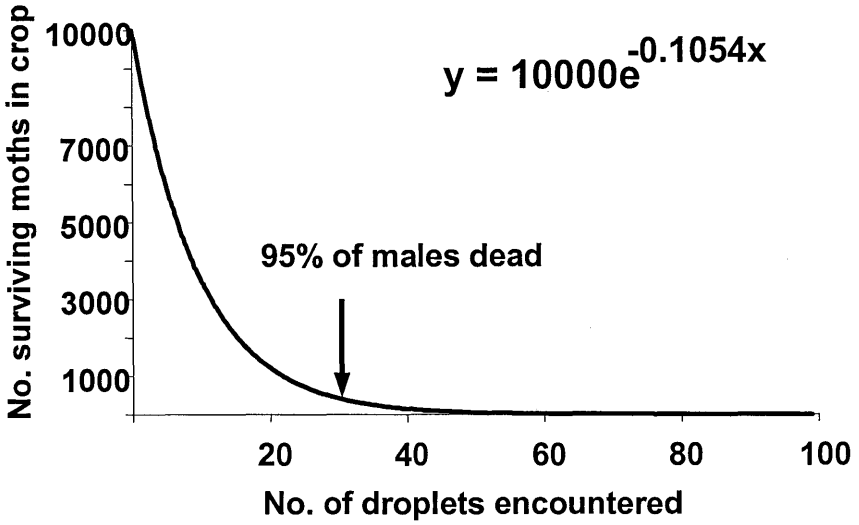

Figure 4.21 Model of number of males surviving from an initial cohort of 10,000 as they encounter attracticide droplets in the field assuming 90% of males survive their initial encounter with a droplet.

From equation 4.1, and assuming that a cohort of 10,000 moths has entered a treated area, by the time all of the surviving moths from each encounter had encountered 40 droplets there would be only 150 moths surviving from the initial 10,000. To achieve 95% effective control there would only have to be a maximum of 28 encounters by the few surviving moths, whilst close to 100% effective control would require 90 encounters. Figure 4.21 graphically depicts this model with a starting population of 10,000 males. This is a simplistic model as it does not included some of the potentially confounding effects mentioned above such as droplet density, male-male competition, attracticide placement in the crop, crop architecture, and time available for contact each evening. In the case of some current applications for Sirene (Last Call) such as for Oriental fruit moth in apples, droplet densities of up to 3,000 per hectare are suggested by field trials (Evenden & McLaughlin 2004b). For other crops this application density may be dependent on factors such as the mobility of the target species, and the nature of the crop. A more complex model would require information which is not yet available for this species. However, this simple model does indicate that attract and kill might be feasible even with an apparently low contact rate.

5 Laboratory toxicology of bifenthrin in Sirene

This chapter investigates the toxicology of bifenthrin in Sirene formulations to laboratory-reared *H. armigera* in a controlled laboratory situation. Information on lethal dosage and time until knockdown, time until death for a range of concentrations have been used to estimate appropriate concentrations of insecticide in formulations. Sublethal effects were also examined.

5.1 *Bifenthrin as a toxicant in attract and kill*

Pyrethroids offer the very useful property of high contact activity when used in attracticides (Chapter 1.1.2). This contact activity is acknowledged as the main pathway by which these insecticides work, and is often the only property assessed in toxicology studies on target species. This contact activity and the rapid knockdown achieved by pyrethroids makes them the predominant insecticide used in attracticides for lepidopterous pests (see Chapter 1.3) (De Souza *et al.* 1992, Downham *et al.* 1995), with permethrin or cypermethrin being used in the majority of Sirene-based formulations (Hofer & Angst 1995, Santos & Hofer 1996, Charmillot & Hofer 1997, Brockerhoff & Suckling 1999, Lösel *et al.* 2000, Czokajlo *et al.* 2001, Ioriatti & Angeli 2002, Krupke *et al.* 2002, Mitchell 2002).

A degree of field resistance to pyrethroids occurs in Australian populations of *H. armigera* (Gunning *et al.* 1984), and older synthetic pyrethroids such as permethrin are not very active against this species. There is no current registration of permethrin for use on heliothine pests on cotton in Australia (Australian Pesticides and Veterinary Medicines Authority, updated nightly, accessed 27 January 2005). New generation pyrethroids still maintain good activity against *H. armigera*. One of these is bifenthrin[1], a 4th generation Type I pyrethroid (Tomlin 1997, Ware 2000) which is currently used in cotton and a number of other crops to control *H. armigera* and other insects and mites (Johnson & Farrell 2004). Insecticides in Australia using bifenthrin include Talstar®, Brigade®, Rage® and Venom®, and the typical field formulations include sprayable emulsifiable concentrate and wettable powder. The manufacturer of the active ingredient is FMC Corporation, Agricultural Chemicals Group, Maryland, USA (EXTOXNET 1994).

[1] (2-methyl-1,1-biphenyl-3-yl)-methyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl cyclopropanecarboxylate

5.1.1 Bioassay techniques for attracticides

A variety of bioassays have been used for contact insecticides, tailored to suit the particular moth species and the formulation type. Modifications of standard insecticide residue bioassays such as the vial test are commonly used, but more novel methods are often required. A basic division occurs between these bioassay methods. The first type is forced-contact or no-choice bioassay where the insect is not allowed to choose between coming into contact with the formulation or evading the formulation, such as in most the bioassays reported in this chapter. Examples include the vial test for resistance levels (Plapp & Vinson 1977), the contact tests for Sirene-based formulations (Brockerhoff & Suckling 1999, Krupke *et al.* 2002), and other bioassays of contact attracticides (De Souza *et al.* 1992, Lösel *et al.* 2000, Ioriatti & Angeli 2002). "Choice" or "unforced" bioassays are those where the insecticide/attracticide efficacy is estimated by allowing the insect to contact the attracticide in a "natural" or unforced way (eg. Trematerra & Capizzi 1991, Mitchell 2002). In most cases the chances of the insects contacting the insecticide are somewhat enhanced by the bioassay method. "Unforced" bioassays are covered in detail in Chapter 6.

The vial test is normally used to determine resistance levels in adult moths in the field (eg. Plapp & Vinson 1977, Campanhola & Plapp 1989, Plapp *et al.* 1990 for *Heliothis virescens*) and laboratory (eg. Daly & Fitt 1990, Daly 1992 for *Helicoverpa armigera*). These tests use standard scintillation vials which have been coated internally with a mixture of the test pyrethroid and acetone and allowed to dry. Moths are introduced into the vials and scored 48 h (Daly & Fitt 1990) or 24 h (Campanhola & Plapp 1989) later as dead or alive. Daly and Fitt (1990) were able to distinguish between resistant and susceptible populations of *H. armigera* using discriminating dosages of fenvalerate in vial tests on laboratory populations, and for field-collected pupae which were allowed to emerge in the laboratory. The vial test was not found to be applicable to wild moths collected in pheromone traps as different aged males varied by as much as 95% in susceptibility, with 97% of older moths (~8 days old) dying at the discriminating dose compared to <5% of freshly emerged males (Daly 1992). These results indicate that different aged males might be differentially affected by a pyrethroid-based attracticide. Whilst the vial test has been used successfully for resistance monitoring by using pheromone trapped male *Heliothis virescens* in southern USA (Plapp *et al.* 1990), it is unlikely to be used for *Helicoverpa armigera* in Australia for this reason.

De Souza *et al.* (1992) used both a topical test and a residue bioassay whilst testing insecticides for an attracticide targeted at male cotton leafworms *Spodoptera littoralis* (Noctuidae). From their topical tests they selected λ-cyhalothrin on the basis of toxicity and speed of action. Their residual bioassay consisted of spraying cotton leaves with λ-cyhalothrin in various concentrations and formulations, then allowing the leaves to dry leaving a residue. Initial cage bioassays proved unsuccessful due to males failing to contact the treated leaves. A modified version was developed where males were held by the wings and "walked" across the treated leaf surface for 10 seconds, then assessed for knockdown and mortality.

Lösel *et al.* (2000) used a contact bioassay where male codling moths were immobilized with low temperatures and held by the scutum with a suction pipette. The fore tarsii of the moths were then allowed to come in contact with droplets of the test formulation containing cyfluthrin. This contact induced a landing response so that the tarsii of the remaining legs also came in contact with the droplet. These contacts were < 1 second in duration, with the moths released into a clean Petri dish immediately after the treatment.

Methods used for the determination of appropriate concentrations of pyrethroids in Sirene-based formulations are not readily available from the general scientific literature, as most published bioassays have used a pre-formulated product with 6% permethrin and tested whether this has worked for specific insect species, which have not included *H. armigera*. A range of laboratory-based methods have been used to test the efficacy of this single concentration.

In their sublethal dosage experiments on laboratory-reared codling moth *Cydia pomonella*, Krupke *et al.* (2002) briefly chilled male moths at 0°C then used forceps to grasp the forewings of the temporarily immobilized moths. Males were then moved near an attracticide droplet (Last Call CM, 6% permethrin and 0.16% codling moth pheromone in Sirene) until the tarsus of one leg touched the formulation. The males were then assessed for mortality and sublethal effects. Ioriatti and Angeli (2002) used a similar technique to determine field longevity of insecticide efficacy in Sirene (comparing permethrin and cypermethrin), but without cooling the *C. pomonella* males before handling them. Each male was held for 5 seconds on the insecticide droplet (compared to the brief contact used by the previous researchers).

Brockerhoff and Suckling (1999) did not attempt to test different concentrations of permethrin in Sirene on mortality in light brown apple moth *Epiphyas postvittana* (Tortricidae), but did test the speed of poisoning with the standard 6% permethrin formulation in Sirene CM. Individual males

were introduced into plastic cylinders and forced to contact an aluminium mesh floor (thinly coated with Sirene CM) by tapping the cylinder. The subsequent behaviour and time until knockdown was noted every 5min after the initial dose, and a time-mortality model derived from these data.

5.1.2 Sublethal effects of pyrethroids

Pyrethroid insecticides act upon both the peripheral and central nervous system in insects. Initially they cause repetitive discharges of axons, eventually resulting in paralysis of the insect. These effects appear to occur due to their actions on the sodium channel present in nerve axons. Excitation occurs when this channel is opened, allowing sodium ions to enter the nerve axon (Ware 1999). This mode of action would seem to have the potential to disrupt chemical communication and reproductive behaviours in moths, and this certainly seems to happen to moths that have received a sublethal dose of pyrethroids. Linn and Roelofs (1984) made wind tunnel observations on oriental fruit moth *Grapholita molesta* males treated with varying sublethal doses of permethrin. Treated males showed a significant decrease in orientation to the pheromone plume and in initiation of upwind flight. Following initiation of upwind flight these males often settled on the walls of the wind tunnel, and took significantly longer to reach the pheromone source. Males treated with higher concentrations (10^{-5}µg in 1µl) exhibited 22% mortality, with survivors unable to locate the pheromone source. Males treated with a very low concentration (10^{-7}µg in 1µl) exhibited no mortality, but were still significantly slower reaching the source compared to the control moths. An earlier study which did not consider all of the mate location repertoire found that there was a significant reduction in activation (wing-fanning) response to pheromone in pink bollworm *Pectinophora gossypiella* males which had received sublethal doses of permethrin (Floyd & Crowder 1981). Haynes and Baker (1985) also detected sublethal effects on the communication system of *P. gossypiella*, with both females and males affected by topical application of permethrin. Moore (1988) investigated the sublethal effects of permethrin on the chemical communication of both sexes of *Helicoverpa zea.* Laboratory reared males were divided into two groups, one as a control, and the other treated topically with sublethal doses of permethrin. These males were marked, released into the field, and the percentage recaptured compared for the two treatments. Significantly fewer of the permethrin-treated males were recaptured, suggesting that the pyrethroid insecticide inhibited successful mate finding in the field.

One aspect that has not been directly addressed by most studies looking at sublethal effects of pyrethroids is the effect of a range of lower than ideal doses. The experiments in this chapter

attempted to find out what happened when males receive a lower dosage than the ideal lethal dose of bifenthrin, as a way of mimicking weathering effects and micro-dosage events that might occur when moths are exposed to formulations in the field.

15.2 *Methodology*

Moths used for trials were reared on artificial diet as described in Chapter 2.5. Formulations were prepared as described in Chapter 2.6. Adult male moths 2-4 days post-emergence were placed in a portable car refrigerator and chilled at 5°C for 5 min. The quiescent males were taken one at a time and the Sirene formulation applied by allowing the moths to extend a foreleg into a 200-300 mg droplet of formulation. To determine application rates a sample of 25 male moths were killed in the freezer and their forelegs removed and weighed. Sirene was then applied to the fore tarsus of 25 forelegs to mimic the dosage received by male moths in the bioassay, and the mean weight of Sirene/foreleg estimated. All experiments were conducted in similar conditions to that which the moths were reared in.

Lethal Dose Experiments

Males were treated as above and placed individually in 160 ml clear plastic containers. A wet dental wick in the lid was provided for moisture. The status of the moths was checked at 12h intervals (1h intervals for some of the knockdown data) up to 160h after initial dosage. Determination of knockdown effects was based on the moth's inability to cling to the side of the container. Determination of a lethal dose was made when moths were either dead, or in convulsions, unable to fly, or suppurating fluid from intersegmental membranes, or losing legs. Estimates of percentage mortality were calculated using the formula from Abbott (1925), and LD_{50}, LD_{90}, KD_{50}, KD_{90} calculated using a probit analysis program (P-A MOD: A. Woods, C. Orton, and C. Virgona, 1987, University of New South Wales, Sydney, NSW). Lines generated in P-A MOD were tested for heterogeneity of slope by calculating χ^2 values, with probabilities $\geq$ 0.05 judged non-significant.

Sublethal Dose Experiments

The effect of sublethal doses of pyrethoids in Sirene is often determined by allowing treated males to mate with untreated females in containers in the laboratory with responses such as mating frequency and egg fertility assessed to determine if sublethal amounts of insecticide interfere with

these reproductive parameters (Brockerhoff & Suckling 1999). Most researchers have used the 6% permethrin-formulated factory product to determine if treated males caged with a female can still successfully mate (ie. before they are knocked down by the insecticide) (Brockerhoff & Suckling 1999, Krupke *et al.* 2002), although this assumes that the laboratory dosage method in a caged enclosure reflects field conditions. One aspect of these experiments is that the concentrations used are so effective at knocking down or killing male moths that it is often difficult to observe any sublethal effects.

In these experiments males were treated as for above, and each male was placed with a female moth in 800ml round plastic containers covered with an open gauze netting. Food was provided by placing 30ml of 10% sucrose solution in a 35ml plastic vial with a dental wick through the lid. The experiments ran for four days, after which the females were removed from each pair and frozen, then later dissected to determine mating status. The presence/absence and number of spermatophores was noted for each female. Two days after the females were removed from the containers the presence or absence of eggs was noted for each container. If eggs were present they were assessed as being fertile (dark brown/black) or infertile (yellow, often with collapsed chorion), and the number of eggs estimated as either less than about 100 or many when there were more than 100). The percentage of mated females, those with fertile eggs, and those producing many fertile eggs was calculated for each treatment, as well as the mean number of spermatophores. The effects of sublethal doses on the count parameters were analysed using a probit analysis program (P-A MOD: A. Woods, C. Orton, and C. Virgona, 1987, University of New South Wales, Sydney, NSW). Lines generated in P-A MOD were tested for heterogeneity of slope by calculating χ^2 values, with probabilities ≥ 0.05 judged non-significant.

5.3 Results

5.3.1 Lethal dosage/knockdown experiments

Dosage of Sirene formulation per male was estimated to be 1.2 ± 0.4 mg. With this dosage concentrations of 6% bifenthrin gave over 94% mortality after 4 hr, with 100% of moths dead by 30 hr. Concentrations as low as 1.5% still gave 100% mortality after 30 hr, but at lower concentrations the effectiveness decreased markedly over the short term. A range of lower concentrations between 0.2% and 1.5% were assayed to provide estimates of LD figures. LD_{50} after 12-14 hr was estimated to be 0.61% bifenthrin (fiducial limits 0.46-0.80), with LD_{90} after 12-14 hr at 1.62% (fiducial limits 0.97-2.71). Table 5.1 lists probit analysis results for these mid-

range concentrations over the course of a three day incubation period. After 38 hr there was little additional mortality, with no significant decrease in the LD_{50} concentration after this time.

Table 5.1 LD_{50} for bifenthrin in Sirene (0.19, 0.38, 0.75, 1.5%) over 80hr incubation. All χ^2 values non-significant, df=2, letters after LD_{50} values indicate whether there is significant overlap between fiducial limits between times after treatment.

Time after treatment (hr)	Slope	LD_{50} (fiducial limits) % bifenthrin	χ^2
14	3.00	0.61 (0.46-0.80)a	1.7
22.25	3.52	0.47 (0.37-0.60)ab	4.1
38	3.81	0.34 (0.27-0.43)abc	0.18
46.10	3.24	0.30 (0.22-0.39)bc	0.27
80.05	2.67	0.20 (0.13-0.31)c	1.5

An attracticide will be more effective if it kills quickly before treated insects have a chance to mate. Whilst lower concentrations in the order of 0.83-2.5% are effective, killing about 90% of moths after 14 hr, higher dosages result in more rapid knockdown (KD) of moths. Table 5.2 lists probit analysis results for KD_{50} over an eight hr observation period. After 3-4 hr the KD_{50} concentration of bifenthrin in Sirene was about 3.5%, and after the eight hr mark the KD_{50} was about 2.5%. Moths which were knocked down often exhibited leg autotomy similar to that reported for codling moth (Krupke *et al.* 2002) and diamondback moth (Mitchell 2002). Knockdown data was analysed for higher concentrations (0.75%, 1.5%, 3%, 6%) to determine KD_{50} concentrations.

Table 5.2 KD_{50} for bifenthrin in Sirene (0.75, 1.5, 3, 6%) over 8hr incubation. An asterisk indicates values significant at p=0.05, df=2, same letters after KD_{50} values indicate significant heterogeneity (χ^2-test, p<0.05).

Time after treatment (hr)	Slope	KD_{50} (fiducial limits) %	χ^2
1	4.33	6.92 (5.16-9.26)a	0.0878
2	5.35	4.70 (4.01-5.50)ab	0.1748
3	4.68	3.77 (3.20-4.44)b	2.8693
4	3.49	2.82 (2.01-3.96)bc	6.2100*
5	3.54	2.69 (2.23-3.26)bc	3.9308
6	3.57	2.63 (2.18-3.18)c	3.0114
7	3.65	2.52 (2.09-3.04)c	1.6000
8	3.73	2.41 (2.00-2.90)c	0.7625

5.3.2 The effect of sublethal doses of bifenthrin on mating success of captive H. armigera

Male moths exposed to low concentrations of bifenthrin in Sirene (<0.4%) often survive for up to 4-5 days without showing knockdown symptoms (Chapter 5.3.1). Male moths were treated with low concentrations (0.05, 0.10, 0.15, 0.20%) of bifenthrin and placed with females for a four day incubation. Table 5.3 lists the mortality and effects on reproduction for these treatments.

Estimated mortality data and comparisons between treated and control moths were subject to considerable error due to the low proportion of moths mating in the control. Less than half of the moths mated in the control treatments. When controls were compared to males given sublethal dosages there was a significant reduction in mating frequency for male moths treated with higher sublethal doses (probit analysis, slope = 0.7932, intercept = 1.5319, data not heterogeneous, χ^2 = 2.2046, df = 2). Back calculation from this relationship gave an estimate of 0.0117 % bifenthrin as the sublethal concentration which would result in a 50% reduction in mating. There was a trend towards reduction in the proportion of females which produced fertile eggs and large numbers of fertile eggs, but this was not significant. The mean number of spermatophores per female decreased significantly overall with increasing sublethal doses (one way ANOVA, F = 4.92, $p < 0.001$). Pair-wise comparisons indicate that this was largely due to the differences between the lowest dose and the highest dose, with intermediate doses giving variable responses (Fishers pairwise comparison, Critical value = 1.966).

Table 5.3 Mortality and reproductive parameters for females paired with males subjected to sublethal doses of bifenthrin at the end of a four day incubation.

% Bifenthrin	n	% Mortality of male moths*	% with fertile eggs ± 95% C.I.	% >100 eggs ± 95% C.I.	% females inseminated ± 95% C.I.	Mean no. of spermatophores ± SE
0.05	45	0.2	28.9 ± 13.3	22.2 ± 12.1	51.1 ± 14.6	0.71 ± 0.13
0.10	32	3.7	29.0 ± 16.0	25.0 ± 15.0	34.4 ± 16.5	0.61 ± 0.17
0.15	34	0.4	26.5 ± 14.8	14.7 ± 11.9	41.2 ± 16.5	0.56 ± 0.13
0.20	68	0.4	13.2 ± 8.1	11.8 ± 7.7	32.4 ± 11.1	0.40 ± 0.08
Summed Controls	181	0	30.9 ± 6.7	26.0 ± 6.4	47.5 ± 7.28	0.77 ± 0.07

* calculated using the mortality estimate from Abbott (1925).

5.4 Discussion:

Insecticide resistance related issues:

Resistance to pyrethroid insecticides is widespread in Australian populations of *H. armigera* (Gunning *et al.* 1984), with three mechanisms contributing to this resistance (Gunning *et al.* 1991). The most potent and labile mechanism is that induced by high order nerve insensitivity, which is similar to the Super-Kdr that has been identified in houseflies. The other mechanisms include reduced penetration through the cuticle of the insect and metabolic resistance associated with enzymatic activity. The Kdr-type nerve insensitivity was a common resistance mechanism associated with *H. armigera* field resistance during unrestricted usage of synthetic pyrethroids in Australian crops (prior to 1983). After restriction of periods when pyrethroids could be used in insect management programs this mechanism declined in field populations, and was difficult to

detect after 1986 (Forrester *et al.* 1993, Gunning *et al.* 1991). However, field resistance to pyrethroids is persistent, with other enzymatic/metabolic mechanisms prevalent in most field populations (Forrester *et al.* 1993, Gunning *et al.* 1995, Gunning *et al.* 1996a). These mechanisms may also be involved in the development of cross-resistance with other neurotoxic compounds such as organophosphorus, thiodicarb and carbamate insecticides (Gunning *et al.* 1996a, Gunning *et al.* 1998a, Gunning *et al.* 1998b).

Development or enhancement of existing resistance to pyrethroid insecticides used in attracticides for adult *H. armigera* could lead to these attracticides becoming ineffectual, as well as interfering with existing resistance management programs present in crops. Poullot *et al.* (2001) investigated the potency of attracticides to insecticide-resistant strains of the codling moth *Cydia pomonella*, where resistance to pyrethroids and other insecticides (due to direct spraying rather than attracticide) was occurring in field populations. They found that pyrethroid-resistant moths were only partially controlled by standard Sirene CM with 6% permethrin. There was no evidence of this resistance reducing or increasing male response to pheromone in wind tunnel trials. Resistant males which survived initial doses of Sirene CM were still able to fly to pheromone sources in the wind tunnel, and a very small proportion were still able to successfully mate in cage experiments. They also proposed that the resistance issue does not impact upon attracticides as it does with direct spraying, as there is no strong economic or environmental impediment to increasing the concentration of insecticides in the attracticide to cope with resistant moths. A direct spraying program which already uses many more times the amount of insecticide than an equivalent attracticide program would not be able to do this without being too costly in economic and environmental terms. However, there are practical constraints to increasing the concentration of insecticide in Sirene, as formulations with excessive insecticide lose the gel-like viscosity. Resistant *H. armigera* and other insect pests have also demonstrated tolerance to extremely large doses of insecticide to the extent that attracticides could lose functionality. The strains of *C. pomonella* used for these observations were resistant due to exposure of larvae to pyrethroids. The resistance that emerges in adult moths due to exposure to attracticides may be different to that induced by larval exposure.

Little is known about the development and retention of resistance in adult moths. The vial technique which is discussed in the introduction to this chapter has been used as a surrogate for measuring larval insecticide resistance, but as demonstrated by Daly (1992) it does not work reliably for *H. armigera*. If attracticides are to become part of IPM programs there will need to be

a concerted research effort to understand the development of resistance mechanisms in adults as distinct to larvae.

The resistance mechanism that is most likely to cause problems with an attracticide program would be Kdr-type resistance as it will affect the speed at which males are knocked down, allowing resistant males to potentially mate with females. Knockdown bioassays have been used with larval *H. armigera* as a means of assessing development of Kdr-type resistance (Gunning 1996) and it would seem likely that resistant adult moths could also be assessed in this manner, allowing monitoring of potential resistance problems within attracticide treated regions.

The laboratory reared *H. armigera* moths used in these experiments would be considered to be at least partially resistant as most native Australian populations demonstrate partial resistance to pyrethroids (Gunning *et al.* 1995, Gunning *et al.* 1996a). However, this did not appear to be a current issue with the suggested 6% concentration of bifenthrin in Sirene. If the formulations developed in this thesis are to be used in an IPM program for *H. armigera* in Australian cotton there will need to be ongoing consultation with farmers and the Transgenic and Insect Management Strategy (TIMS) committee. This committee develops and communicates Insecticide Resistance Management (IRM) strategies for the Australian cotton growing industry. IRM strategies aim to reduce the development of insecticide resistance to ensure that useful insecticides remain viable in the field. Use of an attracticide with bifenthrin will have to be integrated into existing IRM strategies as outlined in the Cotton Pest Management Guide {Johnson & Farrell #2870}.

Problems with laboratory assays:

Estimation of the appropriate concentration of bifenthrin to use in Sirene formulations based on laboratory observations is problematic for a number of reasons. The most difficult problem to overcome is the method of applying the formulation to the insect in an appropriate and repeatable manner. By simulating the event of a male flying to a lure and contacting the attracticide formulation with one of its forelegs it should be possible to mimic an average encounter with Sirene in the field. It is likely that the moths will receive either much smaller amounts or much larger amounts in the field. Observations of moths in the field approaching lures show that some moths will get larger doses compared to others (Chapter 3).

If this application technique is appropriate, the next problem is relating that to the dose the insect receives. Topical larval bioassays use a set volume (eg. 1 µl dissolved in acetone) and placed on the dorsum of a larva (Gunning *et al.* 1984). The application of a Sirene formulation is such that only the surface of the Sirene droplet directly in contact with the cuticle will transfer the insecticide. Krupke *et al.* (2002) noted this particular characteristic of Sirene in relation to control of codling moth. Future experiments noting the sublethal effect of Sirene formulations should emphasize this feature, perhaps by comparing direct sublethal doses of pyrethroids in acetone to the same doses in Sirene.

An additional experimental error is introduced when the moths are kept in plastic containers during the incubation period of the trial. Sirene that is initially a droplet on the foreleg is subsequently smeared onto the container, and when the moth moves around an additional contact with the formulation occurs. This means that moths may receive an excessive amount of insecticide when held in containers compared to a moth which contacts the formulation in the field.

Laboratory toxicology studies are often required to establish a starting point for field observations by determining physiologically significant concentrations of insecticides. Correlating data from laboratory studies of insecticides with what happens in the field is a difficult proposition, with field trials often giving very different results to those of laboratory trials (Robertson & Preisler 1992). Field dosage rates are extremely difficult to estimate accurately, and the behaviour of insects in the field is different to those in captivity.

Sirene was applied at an estimated 1.2 ± 0.4 mg rate to the moth legs. The standard error was relatively high. Given that it is difficult to estimate how much of the bifenthrin in that Sirene actually reaches the cuticle of the moth, the actual error induced by the application method could be even higher. This is further complicated by the presence of scales on the adult moth which may act as a barrier to transfer; this may in part be why freshly eclosed moths are so difficult to kill. Older moths which do not have the barrier of scales may receive a much greater dose than these freshly eclosed adults. Although the scales may act as a barrier this decline in survival with age is more likely to be related to the change in activity of mixed-function oxidases which are associated with resistance mechanisms in the moth (Daly 1992).

Despite the potential for large errors in this assay it was still possible to generate well-fitted dose-response curves for bifenthrin in Sirene. On this basis it seems that a suitable concentration for bifenthrin in Sirene would be between 1.5 and 6%.

Sublethal effects:

The low mating frequencies (< 51%) observed for captive pairs of moths in both treated and control cages were somewhat unexpected. Cage trials of mating frequency often induce unnatural behaviour. Laboratory mating cages for culturing of *H. armigera* may have up to fifty pairs of moths, but not all of the females will be fertilized (PC. Gregg, pers. comm.) whereas unmated non-migratory females are rarely observed in the field (Topper 1987). Colvin *et al.* (1994) commented on this reduction of mating in captive moths. Their explanation for this was that the caged moths from a laboratory culture tended to be asynchronous in relation to reproduction ability. This was due to the difference in time between the two sexes emerging from pupae, and difference in pre-reproductive periods. On the average *H. armigera* females emerge two days earlier than males, resulting in an average pre-reproductive period that is two days shorter than the male, which can potentially lead to a four day difference in reproductive status between the two sexes from a single brood of moths. This is further enhanced by the relatively predictable and stable conditions found in a laboratory culture, where moths emerge in a short period of time.

The sublethal experiment presented here used moths of the same age (3 days old) from over the duration of a culture's pupal emergence period so it was possible to get pairs of compatible reproductive status (eg. late emerging females were mated with the early emerging males). This makes it unlikely that age-related factors such as mating and insecticide-susceptibility would have affected the results seen here. Other factors involved in caging such as reduction in flight space, lack of normal stimuli such as plant volatiles, nocturnal light sources, and wind currents and many other factors may also play a role in reducing the mating frequency in caged adult moths.

6 Field toxicology of bifenthrin in Sirene®

Laboratory toxicological tests provide basic and useful information on the potency of insecticide formulations, but this information should be supplemented by field observations on efficacy. This chapter describes the experiments used to try and determine efficacy of Sirene formulated with bifenthrin under near-field conditions.

6.1 Introduction

6.1.1 Field efficacy of attracticides

Laboratory toxicological observations (Chapter 5) indicated that bifenthrin (6% active ingredient) in Sirene is sufficient to deliver at least 90% mortality to moths that make tarsal contact with the formulation. However, a number of other factors may prevent formulations from delivering this efficiency in field conditions. Laboratory assays (Chapter 5) required that the moth be physically manipulated into contacting the test formulation with one fore tarsus. Whilst this is proposed to be a realistic imitation of the contact moths make with formulations in field conditions, it is likely that wild moths in field conditions will receive much larger or much smaller doses of formulation (Chapter 5.6) which could result in different mortality of adult males in field conditions. The results from observations of males flying to lures with 6% bifenthrin (Chapter 4.4) indicate that there is no repellent effect associated with including this insecticide, but these observations did not provide any information on what happens after the male contacts a lure with bifenthrin. A further bioassay is required to measure field mortality of *H. armigera* males in the presence of attracticide.

Bioassays where the insect is allowed to come into contact with the attracticide in an "unforced" manner allow an estimate of how effective the attracticide will be in the field. Trematerra & Capizzi (1991) developed a laminate attract and kill system against male Mediterranean flour moth *Ephestia kuehniella* (Pyralidae) with cypermethrin as the toxicant. The laminates contained 2 mg of the pheromone component (Z,E)-9,12-tetradecadienyl acetate and 4 mg of cypermethrin. They used a cage bioassay where one face of the laminate dispenser was exposed within the cage and male and female moths were released into the cage. The moths were not "forced" to contact the laminate dispenser, so that the bioassay gave an estimate of field mortality.

For testing the efficacy of Last Call DBM (6% permethrin and 0.16% diamond back moth pheromone in Sirene) against male diamond back moth *Plutella xylostella* (Plutellidae) Mitchell

(2002) used an "unforced" bioassay. Five *P. xylostella* were released into 0.47 litre ice cream cartons with fibreglass screen tops. The cartons had a 0.15g droplet of Last Call DBM placed on the top of the fibreglass screen. Moths were held in greenhouse conditions under ambient light for 24hrs and the number of dead and live moths recorded.

Observations on formulations in field wind tunnels were carried out to determine the efficacy of formulations in an unforced bioassay under near field conditions, and to determine if changes in lure composition altered this efficacy.

6.1.2 Field wind tunnels

Field wind tunnels provide an opportunity to view moth behaviour in field-like conditions (Cardé *et al.* 1998a). The response of male moths to pheromone sources in the field may be modified by the presence of plant volatiles (Dickens *et al.* 1993, Light *et al.* 1993, Meagher 2001b, Kvedaras 2002), absorption and re-emitting of pheromones from foliage (Wall *et al.* 1981, Wall & Perry 1983), natural light and climatic regimes (Gemeno & Haynes 2001), the presence of other pheromone sources such as conspecifics or synthetic lures (Cardé *et al.* 1998), or volatiles produced by other organisms. The use of field wind tunnels allows these factors to be incorporated in ways which laboratory wind tunnel studies do not.

The field wind tunnels used by (Cardé *et al.* 1998) were active pushing/pulling tunnels equipped with a fan, where as the wind tunnels used for the following work were passive devices, relying on natural air flow to generate pheromone plumes within the tunnel. Rather than making extensive direct behavioral observations the wind tunnels in this study were used to estimate the efficacy of formulations as indicated by percentage mortality of moths left in the wind tunnel overnight.

6.2 Methodology

Field wind tunnels

Figure 6.1 is a schematic diagram of the field wind tunnel. It consisted of a 4m long , 127cm internal diameter polythene-sided tube suspended on six large metal rings which were in turn suspended from a steel frame. The polythene tube was formed from a 4.2 x 4m sheet of clear builders plastic sheeting by joining the long sides of the plastic sheet together with adhesive cloth tape. The steel hoops were placed at intervals of 90cm along the inside of the sheeting with the exception of the two upwind hoops where the hoop destined to support the test lure was 25cm in

from the end hoop. Steel eyelets were screwed into the top of the hoops through the plastic sheeting. These eyelets were then suspended from rings from a rectangular frame, and the hoops aligned perpendicular to the long axis of the wind tunnel and held in place with small plastic cable ties. Aluminium channel (25mm) was formed into hoops which clipped into the steel end rings of the tunnels. Plastic splines were used to fasten fly wire or coarse nylon gauze onto each aluminium end ring; these end rings could be easily removed to allow access to the interior of the tunnel. The entire frame and tunnel could then be orientated according to prevailing winds allowing an air flow through the tunnel via the mesh at either end. Formulations were supported on top of a 6mm diameter steel rod 60cm tall.

The steel frame was held upright by two metal star picket posts hammered into the ground. In the event of change of wind direction the vertical support at one end could be quickly detached from the metal star picket post in order to re-orientate the tunnel. Figure 6.2 shows a field wind tunnel set up in a soybean crop near Nangwee on the Darling Downs, Qld. This tunnel arrangement was an early prototype which did not have the lure supported on a rod within the tunnel.

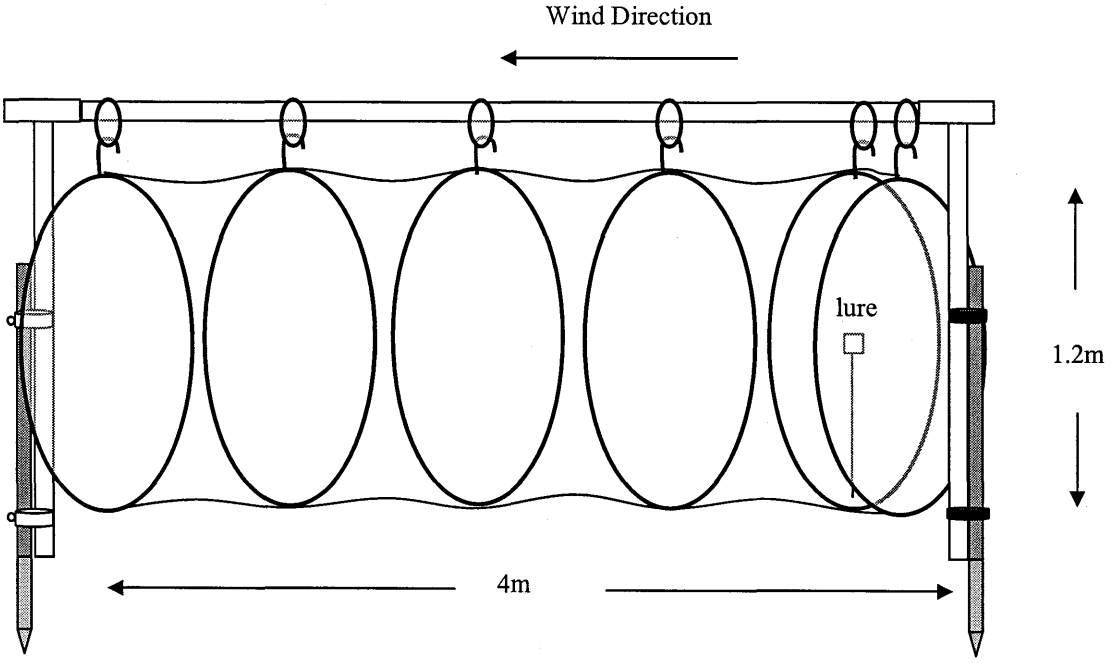

Figure 6.1 Schematic diagram of the field wind tunnel. The metal hoops were covered with clear builders plastic and either end of the tunnel is covered with a removable circular panel of fibreglass flywire to allow access and wind flow to the interior of the tunnel.

Figure 6.2 Field wind tunnel in soybean adjacent to flowering sunflower, Nangwee, Darling Downs.

Field Sites

Observations were made at "Bonnington", Nangwee, Qld., 13, 23, 25-27 October 2000, 16-17 November 2000, 4,6 December 2000 and at Laureldale Rural Research Station, Armidale, NSW., 17-19 December 2000. Table Weather observations for the Laureldale field site were taken from the University of New England/Bureau of Meteorology meteorological station at Armidale, approx. 4.9 km NW of the Laureldale field site. Localised weather observations were not available for the Nangwee trials.

Experimental Methods

Tunnels were set up and left in the field for the duration of each observation period; the plastic sheeting was replaced if it was obviously contaminated with formulations, or if it was torn or weathered. The Laureldale observations used laboratory-reared male *H. armigera* (Chapter 2.4), whilst the Bonnington observations used both laboratory and wild males. Wild males were collected in non-killing Texas traps or inverted cone traps (Gregg & Wilson 1991) using commercial laminate lures as the attractant, then stored in a shaded cool area during the day with moist dental wicks to maintain humidity, and used the following evening in the wind tunnel. Laboratory males were two to three days old. An average of 40 males/tunnel was tested, although sample size sometimes varied with availability of wild males.

Moths were released into the downwind end of the tunnel at about half an hour prior to sunset. Results were scored between 6:30 and 9:00am the following morning. Lethal and sublethal effects were assessed in the same way as laboratory experiments (Chapter 5). A control tunnel containing 1% Ha in Sirene without added insecticide was used during earlier trials (13, 23, 25, 26, 27 October 2000). Mortality in from treatments in trials were adjusted using the mean mortality from these control trials, adopting the method from (Abbott 1925).

Formulations and Treatments

Formulations were prepared as described in Chapter 2, with four mixtures tested:

1) "standard" lure 1% 10:1 (Z)-11-16Ald:(Z)-9-16Ald, 6% bifenthrin.
2) "low" lure 0.1% 10:1 (Z)-11-16Ald:(Z)-9-16Ald, 6% bifenthrin.
3) "very low" lure 0.01% 10:1 (Z)-11-16Ald:(Z)-9-16Ald, 6% bifenthrin.
4) "standard + female" lure, same as 1) with a set pinned female of *H. armigera* as a decoy on top of the lure
5) "control" Sirene without insecticide used for calculation of adjusted mortality (Abbott, 1925).

Table 6.1 lists the dates of trials for each treatment.

Table 6.1 Dates and number of trials for field wind tunnel treatments.

Treatment	Dates
standard	13, 23, 25, 26 October 2000
low	16, 17 November 2000
very low	17, 18, 19 December 2000
standard + female	13, 23, 25, 26 27 October 2000, 17 November 2000, 4, 17, 18, 19, December 2000
control	13, 23, 25, 26, 27 October 2000

6.3 Results

Overall efficacy of insecticide laden lures in the wind tunnel was poor, with the "standard" lure killing only 5% of males placed in the tunnel. Figure 6.3 shows the mean percentage adjusted mortality for the four treatments. The "standard + female" treatment greatly increased the success rate of the formulation, with 33% mortality. The poor efficacy of the "standard" lure was initially thought to be due to excessive amounts of airborne pheromone trapped within the wind tunnel, so the trials were repeated with the "low" and "very low" treatments. These two formulations with

lower pheromone concentrations did not kill significantly better than the standard blend, in fact the trend was for even fewer dead males with reduced pheromone concentration. The corrected mortality of the "very low" treatment indicated that the very low pheromone concentration was no better than presenting Sirene without insecticide added (control mortality = 3.6%). This "very low" treatment acted as an additional control against the possibility that mortality with the other three treatments was due to random contact with insecticide-laden lures rather than due to males being attracted to the lures then killed.

6.4 Discussion

Efficacy of the "standard" lure was much lower than expected, and given that the conservative estimate of the percentage of approaching males that contact an exposed lure was 11% (Table 3.1, Chapter 3), and that the inclusion of bifenthrin did not deter approaching moths (Chapter 4) it seemed that the environment of the field wind tunnel may have been adversely affecting male

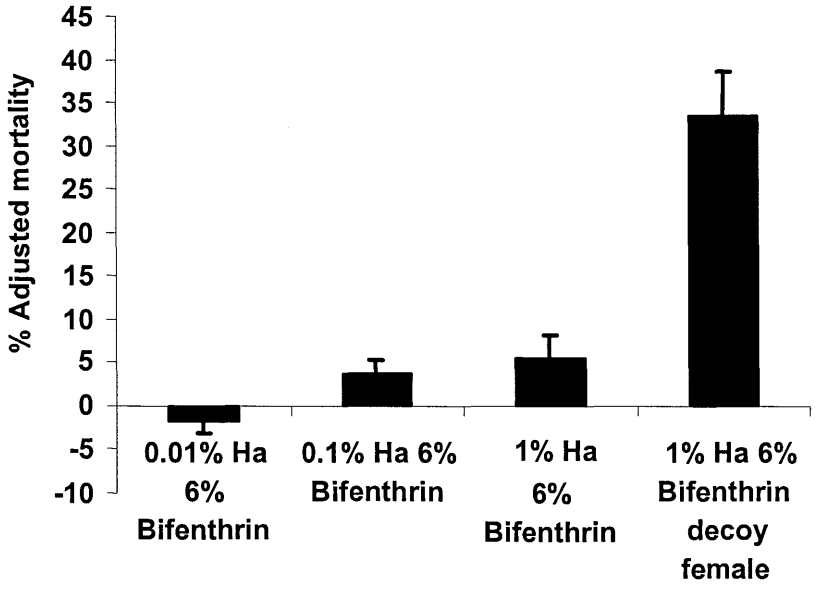

Figure 6.3 Percentage mortality for four different formulations presented in the wind tunnels. Mortalities have been corrected for control mortality (Abbott 1925).

behaviour, preventing them from normal plume-following. Night-vision observations at the field wind tunnel backed up this supposition. Males seemed unable to follow the pheromone plume. They flew from the downwind end of the tunnel, up to the top of the tunnel, landing on the mesh at the upwind end of the tunnel. The pheromone produced by the synthetic source was attractive in that wild males were frequently observed flying to the external side of the mesh on the downwind end of the tunnel and attempting to gain access to the wind tunnel.

One of the possible reasons proposed for the observed change of behaviour was that males were experiencing a form of mating disruption. The airborne concentration of pheromone within the tunnel was much higher than that encountered at an exposed lure in the field, but as explained above, lowering the pheromone concentration in the lures seemed to reduce the number of moths contacting the lure, indicating that the few males that were killed were attracted to the "standard" pheromone concentrations.

Another potentially correlated reason was that the pheromone plume within the tunnel was either amorphous, or was very narrow and ribbon-like, and lacking in sufficient structure to allow plume-following behaviour to occur (see discussion in Chapter 4.3.4 for a more detailed discussion on the effect of plume structure on male behaviour). Visible smoke plumes produced at the mesh on the upwind end of the wind tunnel tended to be initially very narrow, usually less than 1cm across when observed about 10 cm from source. These plumes became much broader, and lacking in distinct visible structure by the time they reached the mid-point of the tunnel, but they were certainly not amorphous nor were they narrow and ribbon-like.

The reduction in air flow inside the tunnel due to the mesh on either end may have meant that the pheromone plumes produced were not sufficiently well-structured to allow males to locate the lures. Preliminary measurements of wind speed inside the tunnel using a hot-wire anemometer indicate that airflow could be restricted by as much as an order of magnitude by the mesh (AP. Del Socorro, pers. comm.).

At this stage it is not clear why males fail to follow a plume within the field wind tunnel environment. The effect of wind speed reduction within the tunnel is likely to be the most important. The nocturnal light sources present in the field (celestial and man-made) may have been filtered through the plastic in such a way as to disturb the visual perception of male moths within the tunnel. Laboratory wind tunnels designed for sustained flight observations usually have a series of contrasting patterns such as black and white stripes painted on the floor of the tunnel to provide a visual stimulus for the flying insect (Hummel & Miller 1984). The steel hoops which were on the inside of the plastic lining may have provided some visual stimulus, but more may be required. Further detailed research on the reasons for these changes in male behaviour within the wind tunnel is required before these problems with using the field wind tunnel can be overcome.

Despite these apparent drawbacks for using the field wind tunnel for experimental observation of male moths at pheromone, the same apparatus has proved to be very useful for observations of *H. armigera* flying to plant-volatile derived attractants laced with insecticide (pers. comm. AP. Del Socorro). There may be additional correlations with time of evening, as moths of both sexes appear to forage more actively just after dusk, at a time when there is often more air movement in the field. Mortality of female moths placed in the tunnel with these plant volatile attracticides occurs in the first 2-4 hours after dusk (pers. comm. AP. Del Socorro, P.C. Gregg). Male response to pheromone tends to peak later in the evening (Chapter 3), when air flow may be greatly reduced within the tunnel, giving the results observed in these trials.

7 Weathering of Sirene® formulations in the field

This chapter describes the weathering characteristics of 1% *Helicoverpa armigera* pheromone formulated in Sirene under field conditions corresponding to the cotton growing season in the Australian summer.

7.1 Introduction

The successful deployment of pheromone-based pest management techniques for the strategies of monitoring, mating disruption and attract and kill relies heavily on the design of the formulation (Weatherston 1990). A successful formulation needs to deliver the correct amount of pheromone in the correct component ratio for the correct period of time. The design factor which is the critical determinant of this success is the rate of weathering of the lure. Weathering is determined by two main physical characteristics. These are the release rate of the active ingredients from the formulation and the longevity/stability of the active ingredients within the formulation. Both of these physical characteristics can change the amount of effective pheromone released from the lure, the ratio of the different pheromone components and associated volatiles (Weatherston 1990).

The Sirene pheromone-carrier system was originally designed for attract and kill system against the codling moth, *Cydia pomonella*. The original design brief was to fulfill the following conditions (Hofer 1994):

1. constant release rate of pheromone
2. four-week effectiveness period
3. imperviousness to rain
4. excellent male attraction rate
5. quick death of the pest after contact
6. direct application to the crop

The first three points are relevant to this discussion of weathering of pheromone formulations. In addition to these six points from Hofer (1994) Sirene was designed so that the formulation was UV-protected to prevent the degradation of the pheromone components by sunlight. This was achieved by the addition of a liquid UV-absorber which helped protect pheromone components from isomerisation (Hofer 1997). Sirene® CM for codling moth in Swiss apple orchards has a

recommended life-span of attracticide droplets in the field of 5-7 weeks (Hofer *et al.* 1996), which equates to two to three applications during the period from bud burst to harvest. Further research in apple and pear orchards in Oregon, USA indicated that Sirene® CM droplets should be replaced every 4-6 weeks, which is about two applications per generation time of the moth, although better suppression of late summer codling moth populations was gained from three applications within an orchard area (Kirsch 1997b). Sirene® 6.4 GS (A-8781) which was designed for control of pink bollworm in Egyptian cotton had a shorter life-span of about 3-4 weeks, which equates to four applications during the growing season of the crop, although it is not clear whether this was due to the release rate of pheromone from the droplets or degradation of the pheromone within the droplets (Hofer 1994). The more extreme climatic conditions encountered in cotton fields may have restricted the life span of the droplets in the Egyptian trials.

7.2 Methodology

Weathering Experiments

Two Sirene formulations were prepared using the syringe-mixing technique and components outlined in Chapter 2.5. The first formulation had 20 ml of Sirene blended with 1% of the 10:1 blend of (*Z*)-11-hexadecenal and (*Z*)-9-hexadecenal and 1% of the inert minimally volatile alkane n-eicosane ($C_{20}H_{42}$) (Sigma, 99% purity) as an internal standard. The other had 20 ml of Sirene containing the same pheromone components and internal standard, but with 6% bifenthrin added. Eicosane was chosen as an internal standard as its retention time in the gas chromatographic column was similar, but not identical to the two pheromone components, and because it is very stable, with low volatility and was considered unlikely to be lost from the Sirene droplet over the duration of the experiment. For each blend 5 ml samples were left in a –20 °C freezer, and the remaining 15 ml of each blend was deployed in the field at Bonshaw, NSW (See Chapter 2.1 for description of this locality). Four treatments were placed in the field on 15 December 2001:

1) Six pairs of 200 mg 1% pheromone and Sirene droplets in a cage
2) Six pairs of 200 mg 1% pheromone and Sirene smeared over 2 x 2 cm in a cage

The cage used for the enclosed droplets and smeared droplets was a light gauge galvanised steel frame 20 cm x 20 cm x 7 cm deep, with a sheet of zinc mesh attached on one side. The treatments (1) and (2) were placed on a sheet of Corflute® plastic which formed the base of the

cage. Figure 7.1 shows the cage and layout of the Sirene treatments. The cage was placed 1.2 m above the ground on a steel post in an open position in the field where the formulations inside would be exposed to normal daytime temperatures, air flow and rain. The mesh size (1.3 x1.5 mm, 65% open area) may have slightly reduced the impact of direct sunlight on the formulations but some direct exposure would occur. The mesh excluded male *H. armigera*, larger debris, and birds which have a tendency to perch on structures in the field such as cages and pheromone traps.

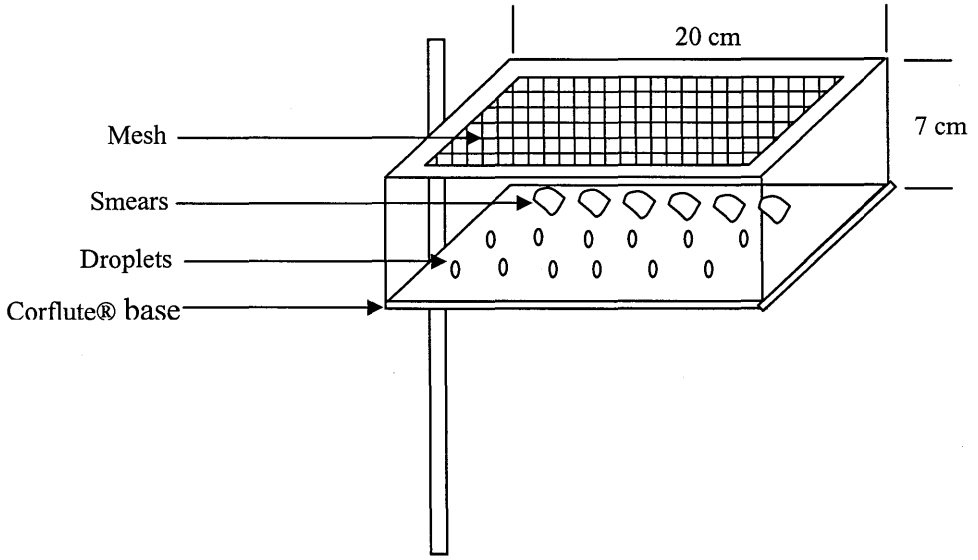

Figure 7.1 Cage used to house Sirene treatments for weathering study.

Analysis of Weathered Samples

A pair of Sirene samples from each treatment were collected at 31 and 46 days after being placed in the field, and kept in a freezer until they could be analysed. An additional pair of samples from the freezer standards was also obtained for the initial measurement, and for comparison to each of the subsequent weathered Sirene samples. Between 20 to 50 mg of each sample was weighed into 1.5 ml amber vials with Teflon liner (12 x 32 mm, Alltech Part # 95179 Alltech Associates Australia Pty. Ltd., Baulkham Hills NSW, Australia), and dissolved in hexane (Fluka ~99% purity, GC grade) equivalent in millilitres to ten times the weight (in grams) of the sample formulation in the vial (eg. 0.040 g of Sirene formulation was dissolved in 0.4 ml of hexane).

A sample volume of 1 µl was injected into a gas chromatograph equipped with a flame ionisation detector (Varian Star 3400) run in splitless mode with a capillary column (Alltech AT-35, 30 m x 0.25mm ID, 0.25 µm film thickness) using helium as a carrier gas at 1 ml/min. Injector and detector temperatures were maintained at 160 °C and 300 °C respectively. Column temperature was programmed as follows: An initial temperature of 100 °C held for 2 min, followed by two

temperature programs, the first which took the column up to 200 °C at 15.0 °C/min with a hold time of 1.34 min, and the second which raised the column to 270 °C at 25.0 °C/min with a hold time of 2.20 min. The relative quantities of pheromone and internal standard were expressed as a percentage of the total peak area. The amount remaining of the pheromone components was estimated by the ratio given when the percentage peak area of each individual pheromone component, or the summed components was divided by the percentage peak area of the internal standard n-eicosane. The results for each treatment and control are presented as the mean of each pair of data points, with variation expressed by the standard error of the mean.

7.3 Results

Figure 7.2 illustrates the mean ratio of the percentage peak area summed for both pheromone components divided by the percentage peak area for n-eicosane. There was an obvious effect of smearing the sample, as after 31 days there is approximately half of the amount of pheromone components left in the smeared droplets compared to that of the normal droplets, which retain the approximately the same amount of pheromone as that of the standards which had been left in the freezer. By 46 days the normal droplets had lost considerable amounts of pheromone and were similar in pheromone content to the smeared droplet samples which had lost this amount of pheromone after 31 days.

Figure 7.3 shows the ratio between percentage peak area of the dominant pheromone component (*Z*)-11-hexadecenal and n-eicosane for each sample date for the three treatments. Smearing the formulation significantly increased the release rate of (*Z*)-11-hexadecenal. After 31 days there was less than half the original amount of (*Z*)-11-hexadecenal remaining in the formulation, whilst the droplet retained a similar amount of (*Z*)-11-hexadecenal to that of the freezer standard. After 46 days the (*Z*)-11-hexadecenal in the normal droplet dropped to a similar level of that of the smeared droplet. It might be expected that this figure would be similar to Figure 7.2 as (*Z*)-11-hexadecenal is the major pheromone component, but the mean values at 46 days indicate that proportionally more (*Z*)-11-hexadecenal is lost compared to the minor component.

Figure 7.4 shows the ratio of the percentage peak area of (*Z*)-9-hexadecenal and n-eicosane for each sample date for the three treatments. After 31 days the amount of remaining (*Z*)-9-hexadecenal had declined in both the smeared and normal droplet treatments compared to the freezer standard, but after 46 days the situation was not readily interpretable with large variation

of peak areas of both the peak areas of (*Z*)-9-hexadecenal and n-eicosane within each paired sample per treatment.

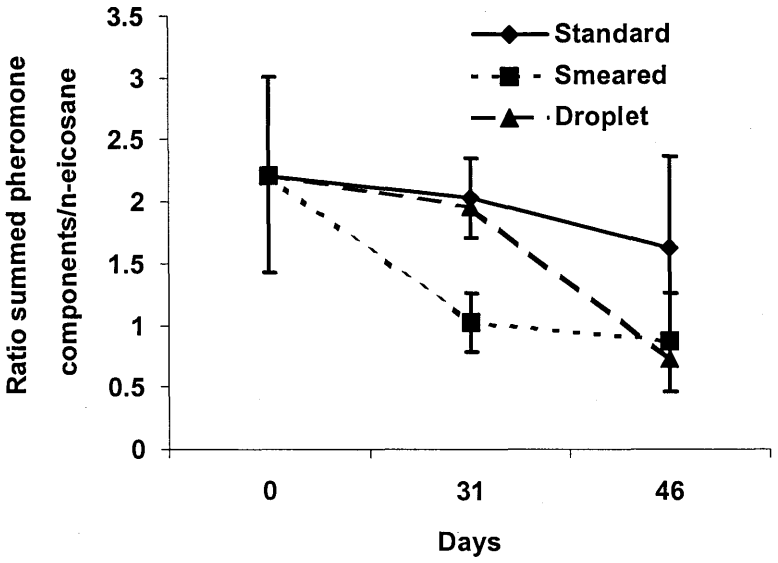

Figure 7.2 The percentage peak areas, summed for both pheromone components divided by the percentage peak areas of n-eicosane for three treatments sampled at 0, 31 and 46 days.

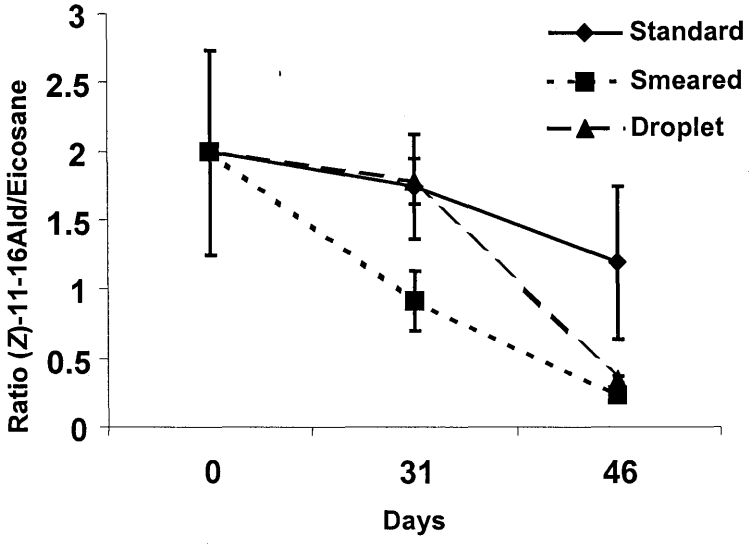

Figure 7.3 The percentage peak areas of (Z)-11-hexadecenal divided by the percentage peak area of n-eicosane for three treatments sampled at 0, 31 and 46 days.

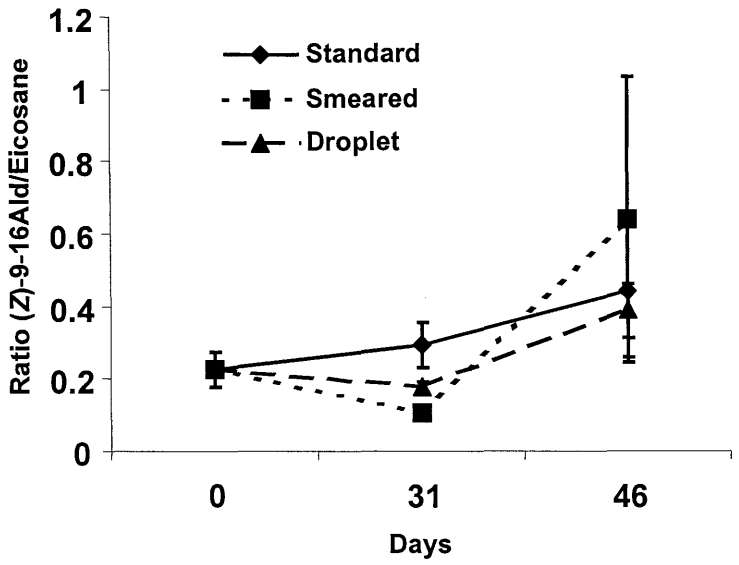

Figure 7.4 The percentage peak areas of (*Z*)-9-hexadecenal divided by the percentage peak area of n-eicosane for three treatments sampled at 0, 31 and 46 days.

Considerable error occurred in some measurements from the gas chromatograph. This is illustrated in Figures 7.5 and 7.6 which are traces from a single session using the gas chromatograph. Figure 7.5 shows the output for the first sample of the session, whilst Figure 7.6 shows the final sixth sample. The baseline of the first sample is flat, with clearly identifiable peaks, and with only one other non-target peak in the target region (between retention times of 11 to 13 min), whilst baseline for the sixth sample rises sharply towards the end of the run, with considerable noise introduced by the Sirene contamination of the column. The three peaks of (*Z*)-9-hexadecenal, (*Z*)-11-hexadecenal and (n)-eicosane are still identifiable, but there are many additional peaks present which were not found in the sample run on the clean column and injector. This error was also systematic. If a series of six samples was run through the machine the errors grew larger as the amount of contamination coming from the injector increased.

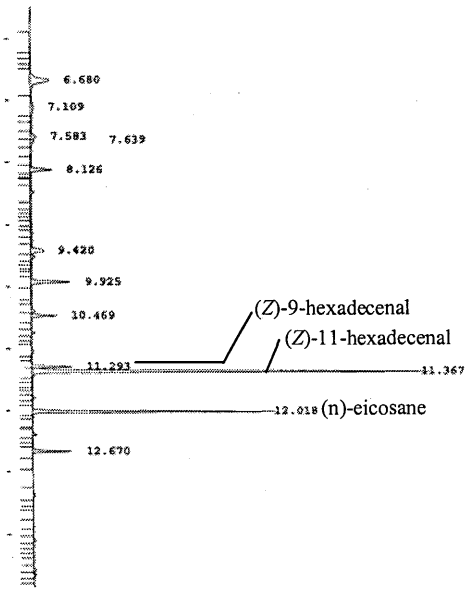

Figure 7.5 Gas chromatograph output for sample of Sirene and pheromone dissolved in hexane with the peaks corresponding to the pheromone components and the internal standard labelled. Run one of six. Note flat baseline over the entire trace.

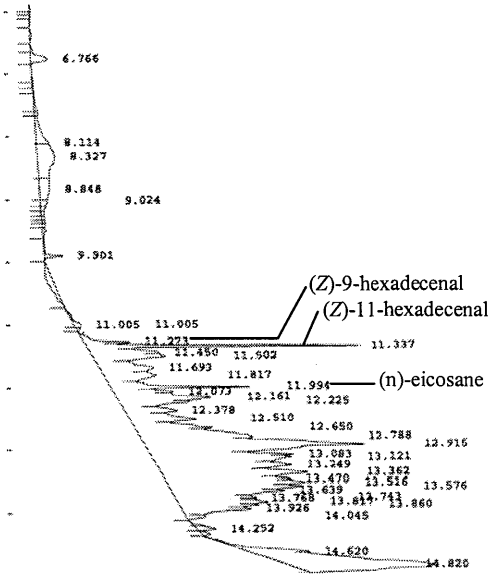

Figure 7.6 Gas chromatograph output for sample of Sirene and pheromone dissolved in hexane with the peaks corresponding to the pheromone components and the internal standard labelled. Run six of six.

7.4 Discussion

Several methodological problems were evident in this experiment. The most obvious problem was the large standard errors in peak area measurements produced by the gas chromatograph. The cause of this appeared to be the breakdown of the Sirene matrix in the injector and column of the chromatograph, leading to contamination of samples entering the column, and difficulties in accurately estimating the area under peaks. Several attempts were made to flush the injector and column to remove the Sirene contamination between individual sample runs, but this was not practical in terms of the time available for analyzing the samples. Another possible solution was to replace the injector liner prior to each sample, but this was also prohibitive in terms of downtime of the chromatograph and the expense of new parts. A possible solution which may be worth considering for future weathering studies of would be to use solid-phase micro-extraction (SPME) to measure headspace pheromone in a sealed system containing the Sirene sample. A precise amount of each Sirene sample could be introduced into a clean glass container where a SPME fibre is used to sample the airborne pheromone concentration. This measurement method would not allow exact measurement of remaining pheromone in the sample, but would measure the current release rate for pheromone from weathered samples to compare to freezer standards. There would be a number of methodological problems associated with such a measurement

system, such as maintaining a constant temperature and air volume in the sample system, and the interpretation of any errors introduced from the system, but it may offer a suitable alternative to dissolving the entire formulation in hexane as was done for this experiment.

An additional problem was encountered in the use of the internal standard (n-eicosane) and the measurement of the minor component (*Z*)-9-hexadecenal. The internal standard was added at 1% of the total weight of the original formulation, whereas the minor component was present at 0.09% of the total weight. When the ratio between the minor component and the internal standard was calculated the errors in measurement of the internal standard (see above) may have been as much as an order of magnitude greater than the total percentage peak area measured for the minor component, which would in turn obscure the response of the minor component to weathering.

The frequency with which samples were analyzed should have been greater in order to better understand the release rate profile and the relationship between release of the internal standard and the pheromone components. A suggested minimum period between samples would be 14 days.

Despite these problems with errors of measurement, it was clear that the field life span of Sirene formulated with 1% *H. armigera* pheromone would be at least 31days, and that smearing the formulation significantly reduces this life span. Over the first 31 days the release rates of the pheromone can be calculated from Figure 7.2 and correspond to 315 ng/day for droplets, and 1,453 ng/day for the smears. Published life spans of Sirene droplets for codling moth and pink bollworm pheromone (Hofer 1994, Hofer *et al.* 1996, Kirsch 1997b) indicate similar life spans. Although smearing the lure has great advantages in increasing the contact rate of male moths with the lure (Chapter 4.2), its use may make this strategy uneconomical due to added costs both from increased labour arising from additional applications, as well as the extra cost of active ingredients and Sirene.

8 A mark-recapture study of *Helicoverpa armigera* males using pheromone traps

Mark-recapture studies are widely used in ecology to measure population densities and to understand movement of animals. However, they can also be useful for examination of more specific questions relating to the likely success of insect control programs such as attracticides.

8.1 Rationale

One of the major limitations to our understanding of how mating disruption and attract and kill work in the field is the lack of information about local short-term movements and population densities of male moths within treated areas. This is particularly critical when it comes to designing large-scale field trials. The common approach to large-scale field testing of attract and kill or mating disruption formulations is to place pheromone sources at an arbitrary density in the field over an area usually matching the physical shape of the cropping or orchard area (Cardé 1990). Large-scale trials of this nature are expensive, difficult to interpret and difficult to replicate. If the trial results in failure to control the pest it is often difficult to say why this has happened. With more mobile targets such as *H. armigera*, the success of the treatment is likely to be closely linked to the increasing size of the treated area as well as the density of lures in the field, so these decisions become even more critical and costly to the researcher.

An additional complication in assessing efficacy from attract and kill field trials is that moths contacting the attracticide (as described in Chapters 3 & 4) in the field may not necessarily die near the lure, and may potentially fly a large distance away from the lure. This spatial displacement of the target species prevents accurate estimates of attracticide efficacy obtained by counting dead moths in the field. One way around this problem is to mimic the effect of a male moth contacting the attracticide droplet. Instead of the male receiving a lethal dose of insecticide, it is marked by a dye. Males can then be trapped from the general field population using pheromone traps, and assessed for dye marks. The percentage of recaptured marked moths in the trap catches then represents the proportion of moths which would have been killed by the attracticide.

Mark-recapture data can also be used to monitor immigration/emigration rates, and to estimate population size over short periods of time. This is basic ecological information which has a bearing on the likelihood of success of an attract and kill method. By using the mark-recapture technique I attempted to answer the following questions:

1) What proportion of the male moth population within a treatment area can be removed with a given density of attracticide point sources?
2) How many moths are there present from night to night within a treatment area?
3) How does varying the density of attracticide point sources affect the number of moths killed within a treatment area?

8.2 Methodology

Externally applied marker dusts have been used in a variety of field mark-recapture studies over the last eighty years, and are probably the most common materials used for external marking of insects (Hagler & Jackson 2001). A key factor in the success of mark-recapture is that the marked insects have equal opportunity to be recaptured as the unmarked insects. Physical handling of insects, or using laboratory-reared insects for mark-recapture experiments may affect the probability of recapture of the marked population. One way of minimising this is to use self-marking methods. Self-marking using field populations can potentially avoid the extensive costs and logistics involved in mass-rearing and marking large numbers of the target species for release. Another potential benefit is that it may reduce the negative effects of physically marking insects.

Self-marking with traps in conjunction with fluorescent dusts as a method of marking has been employed in several published studies on other insects, such as that of Gentry and Blythe (1978) who used it for lesser peachtree borers *Synanthedon pictipes* (Lepidoptera: Sesiidae), Harlan and Roberts (1976) who marked various species of march flies (Diptera: Tabanidae) and Hogsette (1983) who marked stable flies *Stomoxys calcitrans* (L.) (Diptera: Muscidae).

In this study, *H. armigera* male moths which visited synthetic pheromone sources were marked with fluorescent dye powder. The experiment required a method of mimicking contact with a pheromone source which marked the visiting moth with dye. For this purpose an electric grid trap was designed which temporarily incapacitated males contacting the grid. The males then fell into a dish containing the fluorescent dye, but soon recovered and were able to fly off. This trap is referred to as a marking "zap trap" and is described in detail below. Marked moths were recaptured in standard funnel traps (AgriSense). Killing versions of the zap traps (detergent and water is substituted for the dye) gave an estimate of the total number of moths marked by the marking traps. From this the estimate of the potential efficacy of the attracticide formulation was calculated. All lure formulations were based on the rubber septa described in Chapter 2.6.

Each section of the experiment is described in detail below with Figure 8.1 is a summarises the development of the experimental methodology for this mark-recapture study.

Marker dyes

Red, orange and chartreuse fluorescent dye powders (Radiant, USA) were used in traps and in the laboratory wind tunnel experiment. These dyes were non-toxic and water-soluble, and adhered strongly to moths without seeming to cause any deleterious effects. All colours fluoresced strongly when viewed with a black light (Sylvania, Black Light-Blue F18W/BLB) held 3-4 cm away from marked moths in darkroom conditions. The red colour could also be assayed with white light under a stereo dissecting microscope, but the other two colours were not reliably identifiable on marked moths this way. The black light was used to assay moths from the main mark-recapture experiment, whilst white light and the stereo microscope were used to assess moths in the marking fidelity experiment. Figure 8.2 shows three moths each marked with one of the colours, and a fourth unmarked moth under UV light, and under white (camera flash) light.

Marking fidelity experiment

A potential problem with using dyes in this mark-recapture experiment was that marked moths could transfer dye to other unmarked moths whilst in the recapture trap, giving a false estimate of the number of marked moths in the field. To assess how significant this might be, a preliminary trial was conducted over two nights to test marking fidelity. Three laboratory-reared male moths were marked by placing them in a container coated with the red dye powder, and by adding a black dot from a permanent marker pen on the costal area of one forewing. These test-marked moths were placed into an AgriSense funnel trap baited with *H. armigera* lure (Chapter 2.7). Three of these traps were placed in the field 50 m apart along side a flowering sunflower crop, and then cleared the following morning. All the moths caught in these traps were examined under a stereo dissecting microscope, and moths scored as being marked (*Marked*), secondarily marked (*2° Marked*), trace (*Trace*), and not marked (*Unmarked*). Percentage marking error was calculated as the ratio of the total number of marked moths in the total catch minus the three original marked moths divided by the square root of the total number of moths in the trap minus the three original marked moths. This last divisor compensated for the increased chance that a marking error would occur if there were more moths in the trap interacting with the three original marked moths.

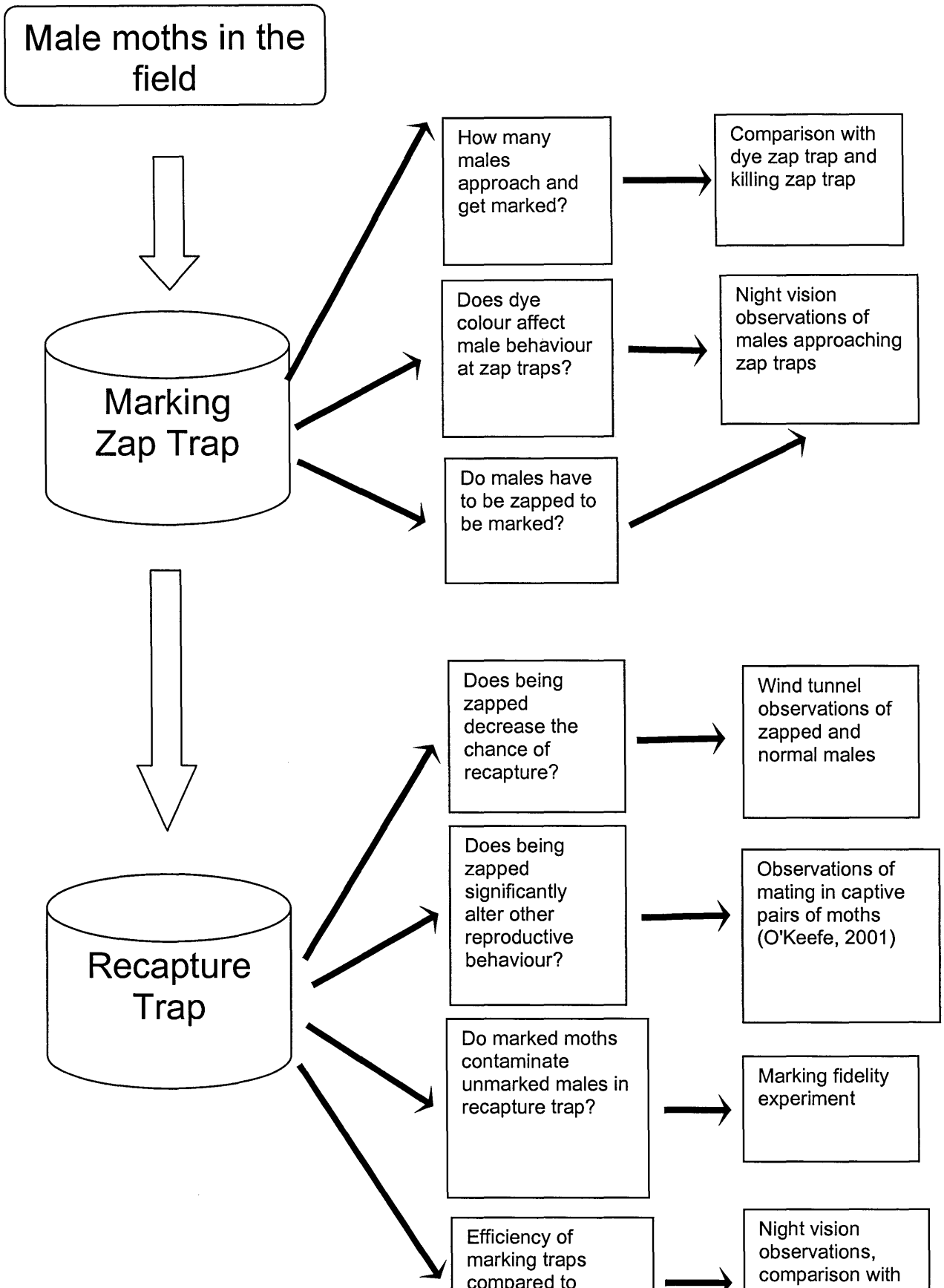

Figure 8.1 Diagram of the development of the experimental methodology for the mark recapture trial

$$MarkError = (\frac{Marked-3}{Marked} \div \sqrt{total-3}) \times 100$$

Individual moths caught in the funnel traps from this marking fidelity experiment were examined for the presence of the red dye to accurately define the scoring system of marked moths. *Marked* was defined as having dye particles on antennae, tarsii and wing tips. Moths with dye particles only on one of these body parts were scored as *2° Marked*; whilst this resulted in very conservative estimates of the total number of marked moths it was deemed necessary to determine which moths had been marked outside of the trap and which moths have been marked within the trap. Moths scored as *Trace* had only tiny traces of dye particles on one body part.

Figure 8.2 Male moths marked in the Darling Downs field trial of mark recapture under UV light (top). Dye colours are (from the left) orange, red and yellow. The moth on the right hand side is unmarked. The picture below shows the same moths under white light (a camera flash)

Marking traps (zap traps with dye)

Marking zap traps used an electric grid based system which had previously been designed for use as an alternative to the AgriSense funnel trap. Universal funnel traps of the AgriSense type are highly inefficient, catching less than 1% of moths which approach the pheromone lure (personal observations, GP. Fitt pers.comm.). The zap trap considerably improved catch efficiency, particularly when moths were not abundant in the field (O'Keeffe 2001). Figure 8.3 is a diagram of the zap trap. It consisted of a hemispherical wire grid 5 cm in diameter connected to a high voltage (~1,300 V) supply powered by a 12 V gel cell battery (11 amp/hr). The grid was placed on a small Teflon pedestal which had an upper cavity to hold lure formulations. Figure 8.4 shows a close up of the grid with a commercial laminate pheromone lure placed in the Teflon holder. This grid was held in the middle of a black-painted 420 mm diameter plastic planter bowl (Addis Replicotta brand, HomeLeisure Pty. Ltd., NSW, Australia). This bowl could be coated with dye

powder for marking, or partially filled with a weak solution of detergent in water to capture moths. Figures 8.5 and 8.6 show examples of a dye-marking zap trap and a killing zap trap. In the former, moths which approached the lure were shocked upon contacting the grid, and fell into the bowl, being marked by the dye and subsequently escaping. In the latter, the shocked moths drowned in water. Since the marking dye was water-soluble it was important to keep the marking traps dry. Large plastic garbage bins were placed upside down over the traps during the day when they were not in use, and when an overhead irrigation event was scheduled at night. Zap traps and batteries were tested for voltage output each day and zap units and/or grids were replaced if they failed to spark or produced a weak spark. Batteries were recharged once their voltage dropped below 11.0 V.

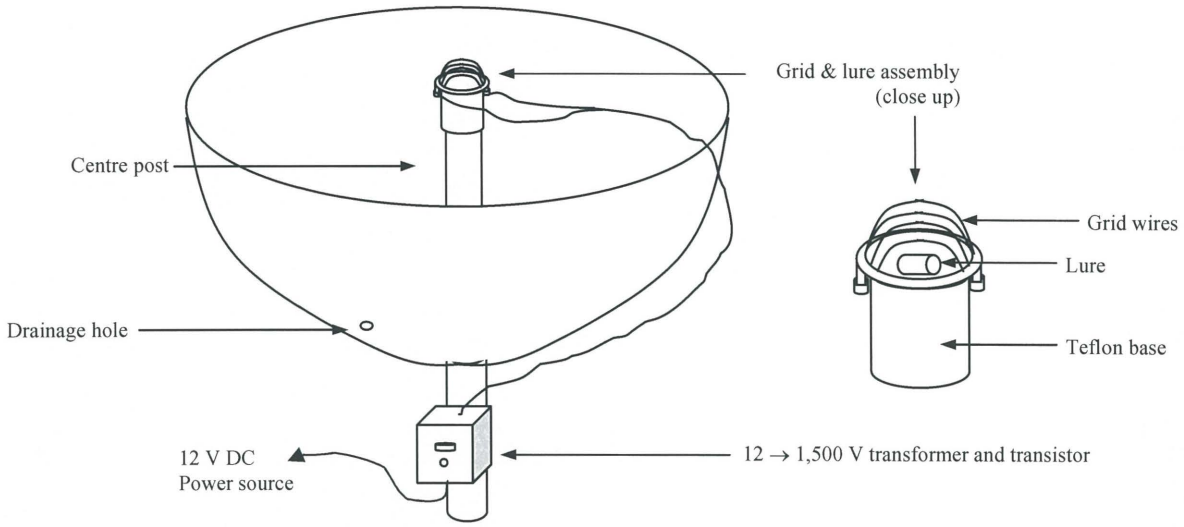

Figure 8.3 Diagram of zap trap, right image shows detail of grid.

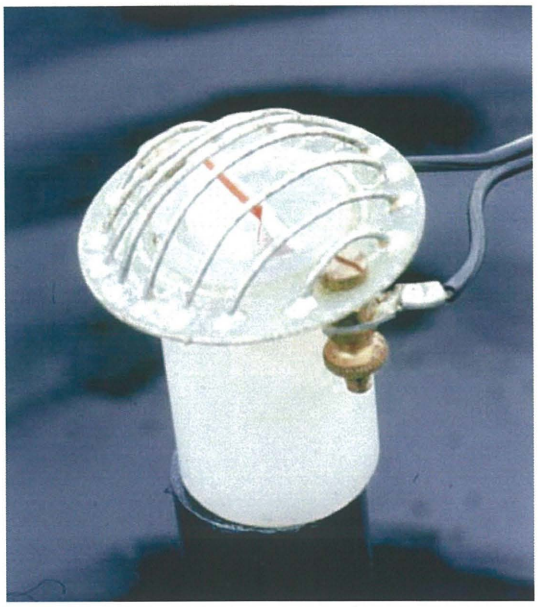

Figure 8.4 Photo of grid with commercial laminate lure in Teflon holder.

Figure 8.5 Marking zap trap with red dye. Note that dye has spread all over the inner surface of the trap.

Figure 8.6 Killing zap trap with soapy water in base. Dead moths are floating on the surface of the water.

Effects of shocking male moths - Does negative reinforcement occur?

Early observations on moths in zap traps indicated that moths were not killed by the shock, and could fly in a coordinated manner within 2 s of the shock. Fecundity, mating frequency and life-span of laboratory females were not affected by a single shock, and male moths still successfully mated with females after receiving a shock (O'Keeffe 2001). Electric shocks have been used for conditioning and negative reinforcement of insect behaviours (Alloway 1972), and it was of some concern that male moths which had received a shock after following a pheromone trail to the grid might be deterred from re-visiting pheromone sources. A wind tunnel experiment was carried out to determine if there was a significant deterrent effect following a shock.

Male moths often failed to respond to synthetic pheromone sources whilst in the wind tunnel (Chapter 2.87). Since the main requirement of wind tunnel observations in this instance was to determine if there was negative reinforcement of lure location following electric shock a combination of female moths and synthetic lures (rubber septa lure, Chapter 2.6) was used to overcome the lack of attraction of synthetic lures by themselves. The grid and lure was placed about 1 cm in front of a transparent plastic cage (dimensions) which contained three 2-3 day old

virgin female moths. Both the grid and cage were on a pedestal which raised the assembly 15 cm above the floor of the wind tunnel.

Observations of reverse-cycle laboratory reared moths were made during the peak female calling period which is approximately four to six hours from the start of scotophase (Hou & Sheng 2000). The behaviour of each 3 day old male moth placed in the downwind end of the wind tunnel was observed for 4 min. The number of contacts with cage with female moths and the number of contacts with grid (referred to as zaps) were recorded. Every second male was flown as a control to an inactive grid, so that when it contacted the grid it did not receive a shock. Test males flown to the activated grid were flown again after 1 h to test the short term effects of being shocked, and then again after 24 h to determine if there was a longer term effect of the shock.

Observations were recorded directly into The Observer (Noldus 1995) and the extracted data on the frequency and duration of behaviours analysed in S-Plus 2000 (MathSoft 1999) using χ^2 tests and ANOVA.

Marking efficacy of zap traps

Early observations of marking zap traps have shown that moths did not need to contact the electric grid in order to acquire dye markings. Dyes were spread over the inner surface of the trap bowl by wind, and by moth activity, so that any moth coming in contact with the sides of the bowl could be potentially marked, thus increasing the number of moths marked compared to those coming in contact with the grid only, then falling down into the bowl. Dye was initially placed in the base of the bowl, but after placement in the field either moth or wind activity distributes dye over the inner surfaces of the bowl. Figure 8.5 shows an example of a zap trap coated on the inside with red dye.

An additional concern was that the visual stimulus of different coloured dyes might also affect the number of moths coming near and contacting the grid on the trap. To determine if this was so observations of marking traps with the three different coloured dyes were made in a field of green French beans at Bowen, Qld between the 24th and 26th of April 2002 using the binocular night vision goggles (see Chapter 2 for details on observation techniques and analysis and locality). Moths were recorded as "approaching" (within general field of view and showing directed flight towards the lure) and "near" (within the diameter of the bowl), with an additional two behaviours, "dye contact" (touching the dye on the sides or base of the bowl), and "zap contact" (touching the

grid). A zap contact always resulted in dye contact, but the two behaviours were recorded separately for these observations.

Field layout:

A mark-recapture experiment was run between the 7th and 21st of January 2002 in sorghum, corn and fallow fields near Nangwee (Chapter 2.1). The overall layout of the experiment, distances

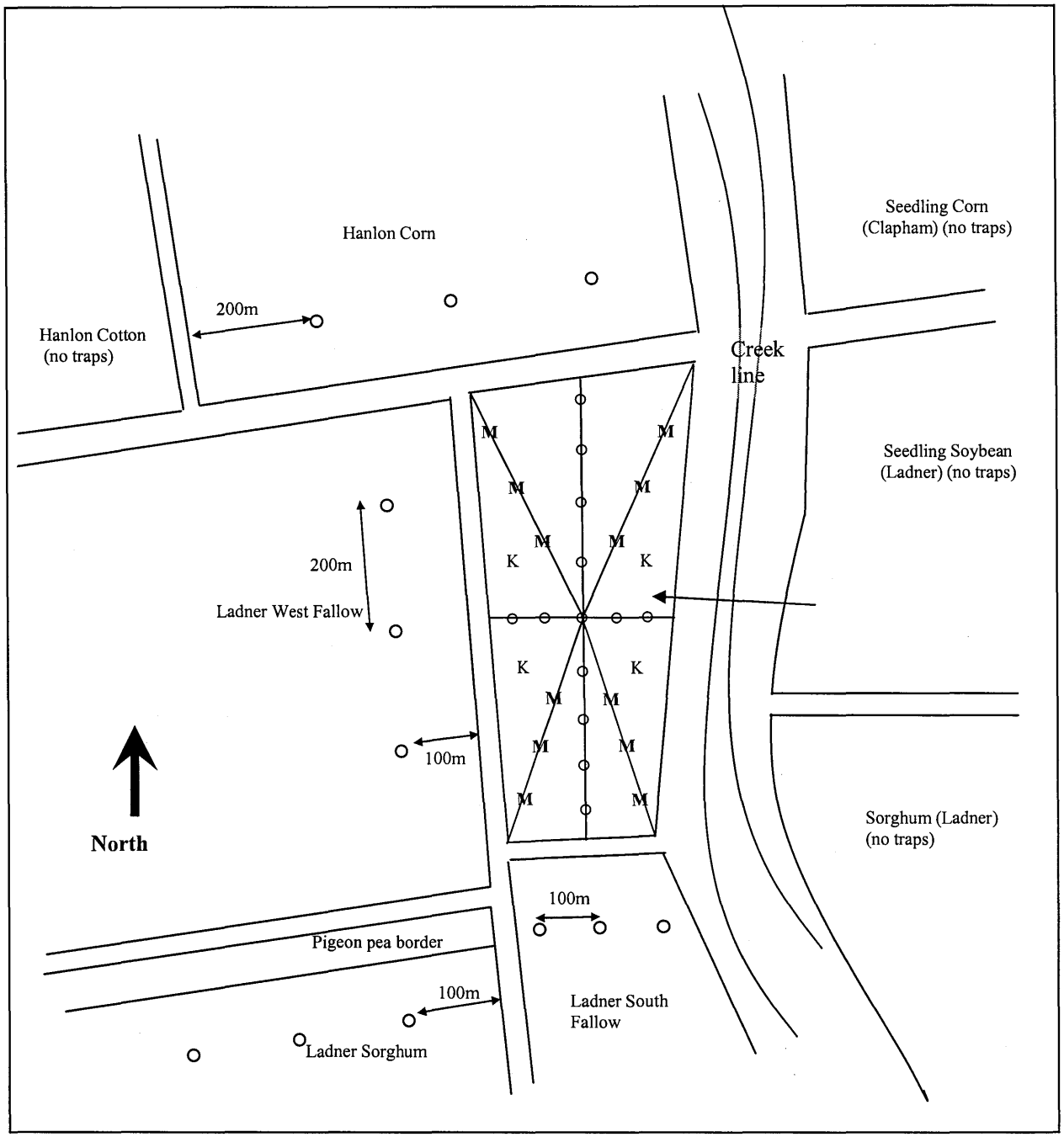

Figure 8.7 Diagram of the field layout in the mark recapture trial, showing crops, approximate distances between crops and other features, and trap locations. AgriSense recapture pheromone traps are indicated by "o", killing zap traps by "K", and marking zap traps by "M".

between traps and the position of recapture (AgriSense) traps are shown in Figure 8.7. The main experimental plot was a 21 ha field of early to pre-flowering sorghum that was moderately isolated from other "attractive" crops around it. The dimensions and the layout of the marking, killing and recapture traps within the main experimental plot are shown in Figure 8.8. An aerial photograph of the trial area with the trap layout superimposed is shown in Figure 8.9.

The marking zap traps were laid out along the diagonal axes of the plot with the distances between traps calculated in such a way as to ensure that the central traps were not too close together. Recapture AgriSense traps were laid out along the orthogonal axes in the same manner as the marking traps. The killing zap traps were placed in the interstitial spaces between the east-west trap line of Agrisense traps and the diagonal marking zap trap lines. Distances were between 40 to 50 m between traps to avoid possible competition between pheromone sources in the traps. The design used marking traps with three dye colours (red, orange and yellow) in an attempt to detect localised movements within the field of sorghum. Satellite AgriSense traps were placed in fallow fields, sorghum and corn crops around the perimeter of the main experimental field to measure male movement beyond the boundaries of the main field.

Analysis of results from field-collected data

The analysis method used for this experiment is essentially a Petersen/Lincoln method (Krebs, 1994). Of a nightly population of N male moths present in the field each night M males are marked. On the same night a total of n males are captured in AgriSense traps, of which m are marked. M can be estimated from the mean number of males per night found in the killing zap traps, allowing the calculation of N.

$$N = Mn/m$$

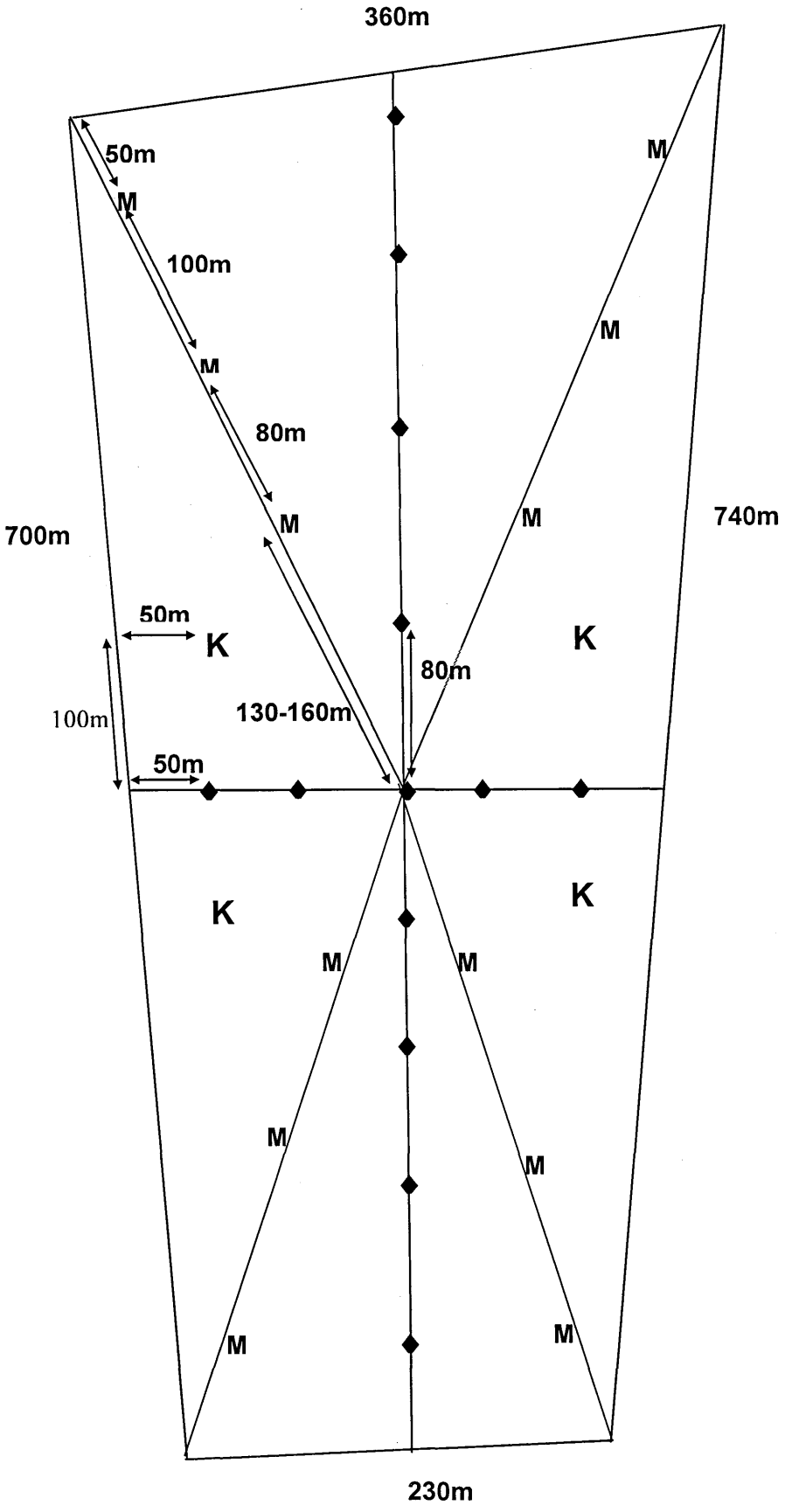

Figure 8.8 Detail of the trap arrangement in the main field of sorghum used for the mark recapture trial showing the dimensions of the field and the distances between traps. AgriSense recapture pheromone traps are indicated by "♦", killing zap traps by "K", and marking zap traps by "M".

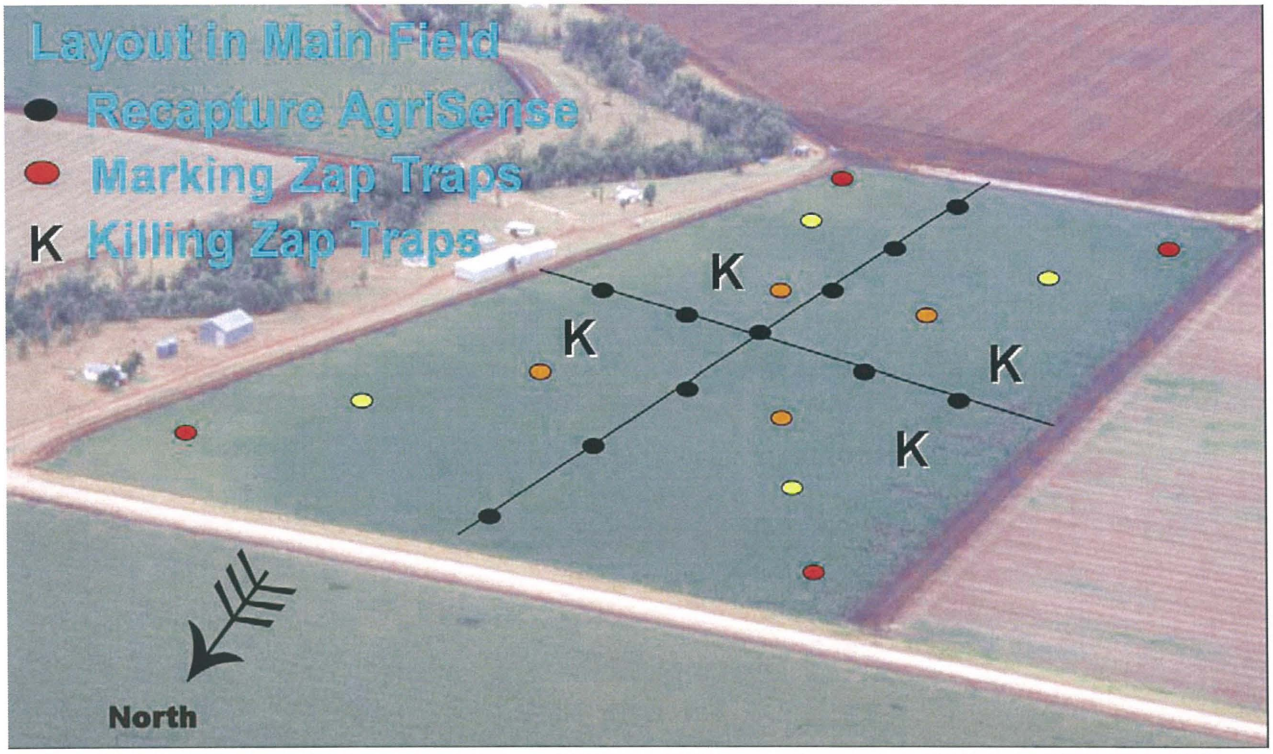

Figure 8.9 Aerial photograph of the main study area showing location of trap within the sorghum (satellite traps are not indicated). The fallow fields are the dark areas to the west and the south, the north is bounded by corn which has just passed the silking stage, directly east is seedling soybean, to the southeast and southwest is more sorghum. Coloured circles are marking zap traps, killing traps are indicated by "K", AgriSense recapture traps by black circles.

8.3 Results

Marking fidelity experiment

The expected result if marked moths could reliably be identified from unmarked moths would be three marked moths per trap. Although a substantial number of moths with traces of dye were recognized (63 of 177 males), these could still be distinguished from the 9 marked moths placed in the three traps. Table 8.1 lists the data collected from two nights of the marking fidelity experiment. The average marking error as calculated with the formula in the methods was 5.9 ± 1.8% for the two nights; all of this error was generated by the second night of data collection. The total number of moths per trap on the first night was lower than that of the second night, implying that activity levels may have a bearing on marking fidelity.

Table 8.1 Data collected from marking fidelity experiment over two nights (mean number per trap).

Date	Moths	Marked	2° Marked	Trace	Unmarked	% Error
8 Nov 2001*	11.5 ± 2.3	2.7 ± 0.2	0.3 ± 0.2	3.7 ± 1.6	4.8 ± 2.7	0
9 Nov 2001	18.0 ± 3.4	4.7 ± 0.2	1.5 ± 0.6	6.8 ± 1.3	5.0 ± 3.1	9.8 ± 1.3
Both Nights	14.8 ± 2.2	3.7 ± 0.3	0.9 ± 0.3	5.3 ± 1.1	4.9 ± 1.9	5.9 ± 1.8

Does negative reinforcement occur with zapped males?

A total of 23 males were flown in these wind tunnel trials. Two males that were trapped on the grid and repeatedly shocked were excluded from the analysis. There was a short term effect of being shocked, with shocked males being significantly less likely to contact the grid or the cage with the female moths within 1-2 h. This difference disappeared after 24 h and shocked males contacted the grid as freely as males which had not received a shock, suggesting that there is a short term physiological response caused by the shock rather than a long lasting negative reinforcement. The exact duration of this physiological response is not known, but it is assumed that the males would be able to fly to pheromone normally in less than 24 h. The severity of the response is likely to be linked to the duration and intensity of the initial shock.

Estimates of marking rates compared with killing rate in zap traps

Two nights of observations were made on zap traps in French beans in Bowen, Qld. A total of eleven 10 min observations were made for each of the three dye colours, red, yellow and orange. Table 8.2 summarizes the results of these observations. There was a significant reduction in the proportion of approaching males that were marked in the trap with a red dye compared to traps with yellow and orange dyes, although this difference was not observed when the proportion of zapped males was compared across dye colours.

Table 8.2 Marking rates and efficiency of zap traps for three colours. Means bearing the same letters are not significantly different.

Dye Colour	% Approaching males that got near (± 95% C.I.)	% Approaching males marked by dye (± 95% C.I.)	% Approaching males zapped (± 95% C.I.)
Orange	65.30 ± 5.70a	8.20 ± 3.29a	3.36 ± 2.16a
Red	55.25 ± 5.67a	3.05 ± 1.96b	1.02 ± 1.14a
Yellow	65.26 ± 5.32a	9.74 ± 3.31a	3.25 ± 1.98a

The overall proportion of males zapped was considerably lower than what would normally be expected; this may have been due to reduced flight activity near the lures. Weather during these observations was characterised by still, humid nights. Chapter 3, Section 3.3.5, discusses variability of moth behaviour with climatic variability. Of the climate factors relative humidity has the strongest effect on male behaviour, with a reduction of males approaching and getting near the lure. It was not clear whether this factor may have reduced the number of males zapped although it was observed that in general, the zap traps did not work as well during humid nights due to condensation on the electrical grids.

Estimate of the number of moths marked in the field per night.

The night observations at Bowen (see above) indicated that there was a ratio of 2.77 males marked for every male moth that was zapped. This conversion factor was calculated from the total number of moths marked (61) divided by the total number of males zapped (22) for all observations of all three dye colours. The average number of moths collected per killing zap trap in the sorghum was multiplied by 12 (the number of marking zap traps), then multiplied by the conversion factor of 2.77 to get the total number of moths marked per night.

Mark-recapture trial in sorghum

Figure 8.10 shows the mean number of males for each trap position within the main field of sorghum over the entire study period. There is considerable variation within the mean value for each trap due to the influx of greater numbers of males over several nights, but there were no significant positional biases between traps throughout the field (ANOVA, df = 12, F = 0.71, p = 0.74). A similar analysis of the ratio between marked and unmarked moths caught in each trap position also revealed no significant differences in the ability of traps in different positions to catch marked males (ANOVA, df = 12, F = 1.00, p = 0.45).

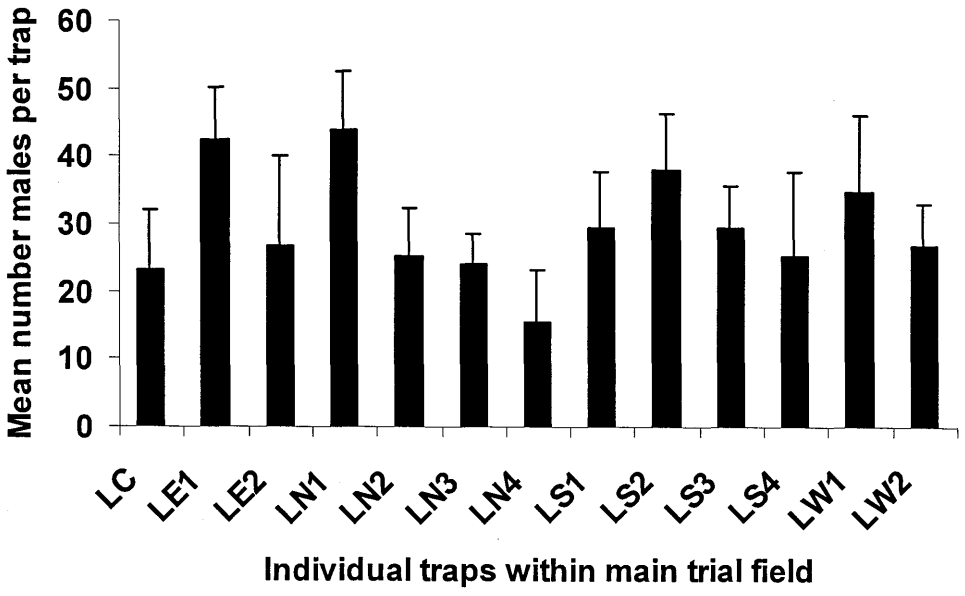

Figure 8.10 Mean numbers of males per AgriSense recapture trap over the entire ten night trial period for individual traps within the main trial field.

Figure 8.11 shows the estimated number of males marked per night and the estimated total number of males per hectare present in the sorghum crop. The maximum estimated number of males marked in one night was 2,121, whilst the minimum was 216. The maximum estimated number of males per hectare was 4,008 on night five.

This variability in population size, and the number of marked moths recovered suggested that the population was very fluid within the study area, with very few resident males present in the study area and almost complete replacement of the male moth population overnight. The initial design of this trial used three colours of fluorescent dye in an attempt to measure small scale local movements within the sorghum. It was evident that males were not resident within the entire 21 ha of sorghum so this approach was ignored in the analysis, and all dye colours were treated as the same. This could be shown by removing the marking zap traps. Recapture AgriSense traps captured only four marked males for the four nights after marking zap traps had been removed, indicating that most marked moths left the study area soon after the initial marking event.

There was an increase in the number of males per hectare peaking on the fifth night and dropping to 98 males per hectare by the eighth night. Figure 8.12 shows the relationship between the percentage of marked males and the mean number of males per trap. There was a weak inverse relationship between mean number of males and the percentage of marked males (Figure 8.13).

Figure 8.14 is a plot of the average percentage of marked males and the mean number of moths in the satellite traps per night. The absolute number of males captured in the satellite traps varied greatly, with traps in the fallow fields capturing relatively few males compared to traps in flowering sorghum to the southwest, and traps in corn to the north. As a result it is not possible to estimate absolute populations as was done within the main sorghum field. The percentage of marked males in satellite traps exhibited a similar pattern to that seen in the main field of sorghum, but peaked on the seventh night rather than the eighth. As with the main field there appeared to be a weak inverse relationship between the mean number of males in the traps and the percentage of marked males in the traps (see Figure 8.15).

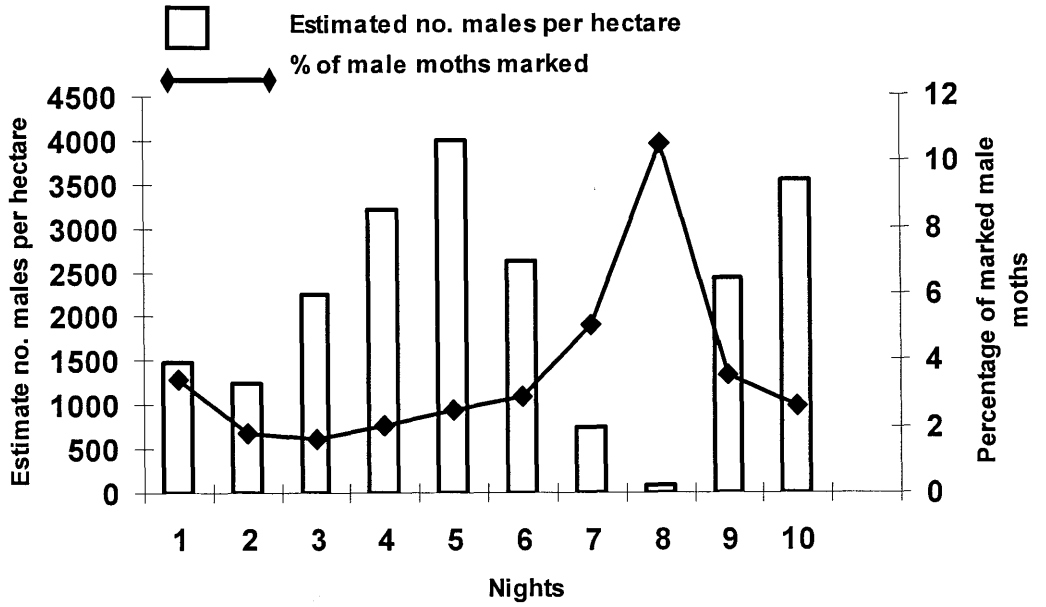

Figure 8.11 Estimated total numbers of male moths per hectare in the 21 ha field of sorghum and percentages of these males marked per night.

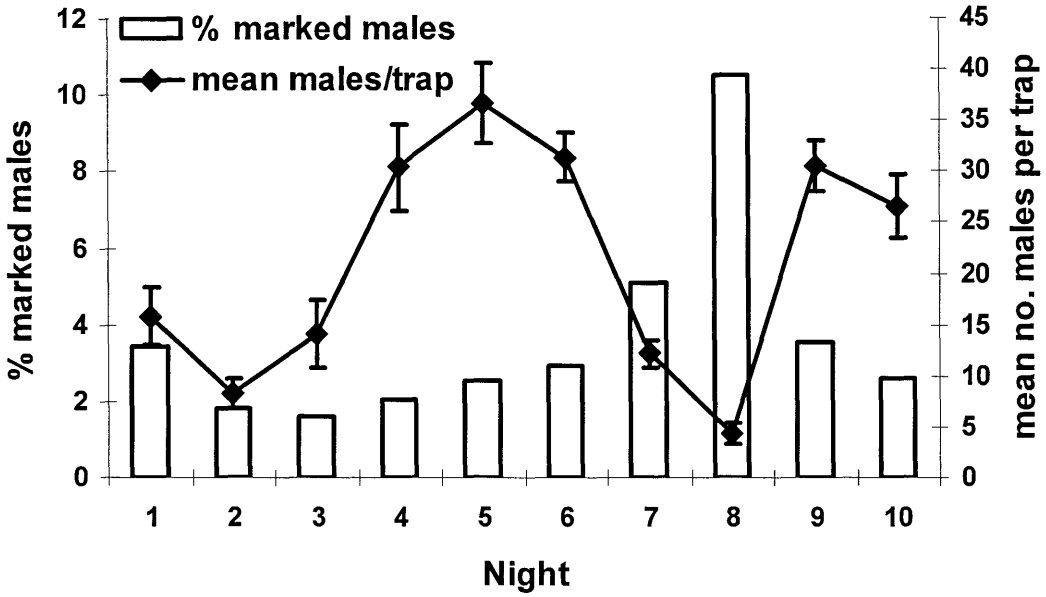

Figure 8.12 Percentages of marked males per night and the mean total numbers of males per trap per night ± SE for AgriSense recapture traps within the main field.

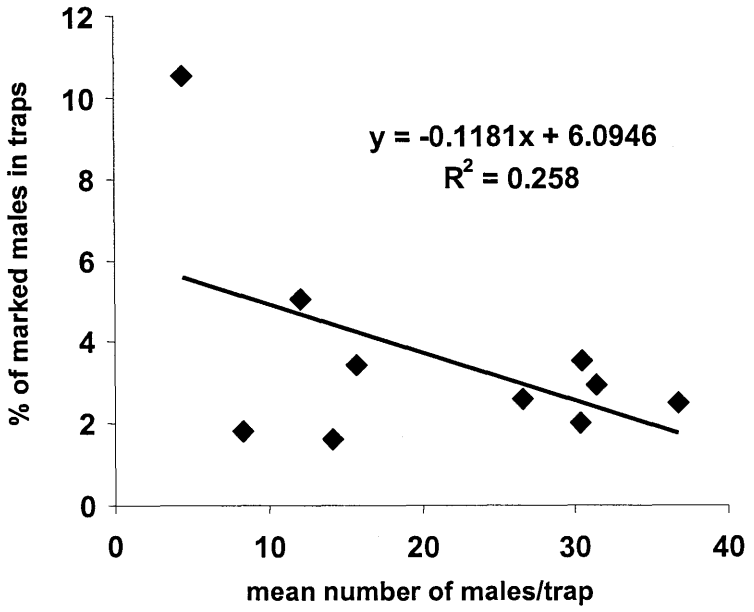

Figure 8.13 Percentages of marked males plotted against mean numbers of males per recapture trap for each night in the main trial field.

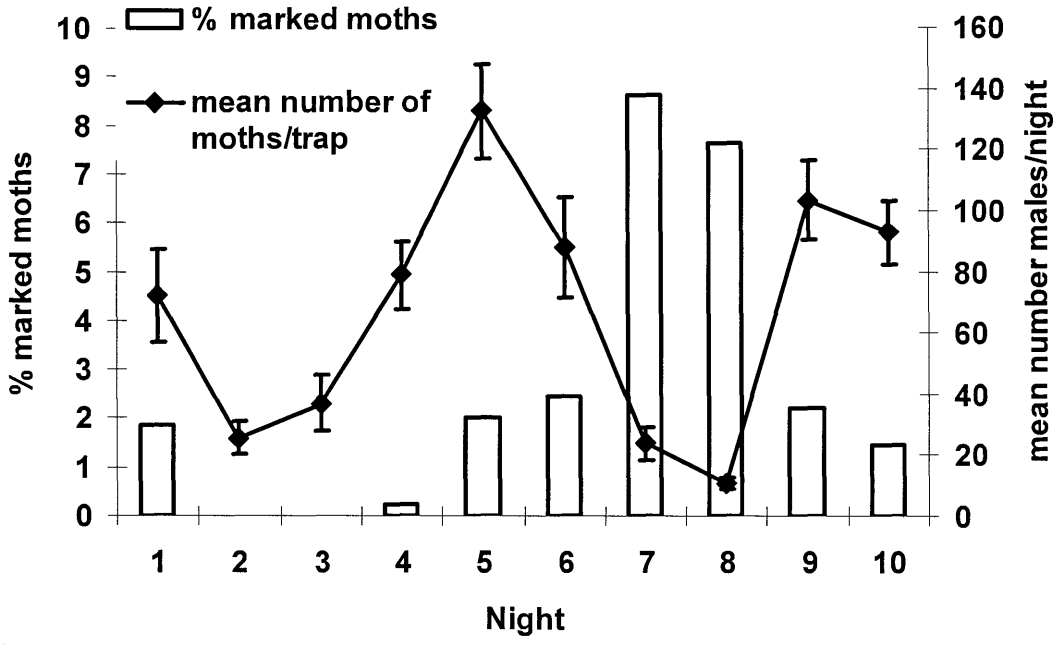

Figure 8.14 Percentages of marked males per night and mean total numbers of males per trap per night ± SE for satellite AgriSense recapture traps outside of the main trial field.

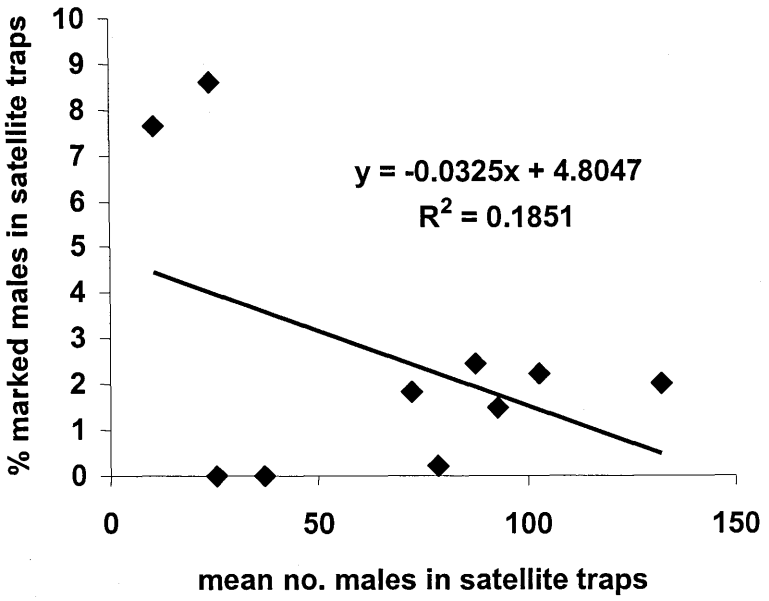

Figure 8.15 Percentages of marked males plotted against mean numbers of males per recapture trap for satellite traps outside of the main trial field.

8.4 Discussion

Basic assumptions

Mark-recapture experiments rely on making many assumptions which in turn increase the complexity of both the methodology and analysis, and in some cases, the reliability and accuracy of the results. The basic assumptions of mark-recapture are as follows (Krebs 1994):

1) Marked and unmarked animals are captured randomly.
2) Marked animals are subject to the same mortality rate as unmarked animals.
3) Marks are not lost or overlooked.

The first of these assumptions equates to assuming that the main field of sorghum had uniform numbers of males distributed throughout the field, and that males in any part of the field had just as much chance of being caught and marked as males somewhere else. There was no significant bias due to trap position as tested by comparing number of males and the proportion of marked to unmarked males captured in pheromone traps in different positions within the sorghum crop.

It is difficult to assess whether marking males in the field differentially increases mortality by (for example) making the moths more conspicuous to predators, or less able to evade predators. It was

unlikely that moths were adversely affected by coating with the dye powder, or by shocks based on the laboratory observations reported above.

Once moths had been marked it was unlikely that they would lose enough dye to be missed when being assessed under UV light. The short term nature of the experiment, and the ability of the dye to coat almost anything which came in contact with the powder would make it unlikely that dye marks would disappear easily. The dyes are water soluble, but there was no rain during the experimental period which would have affected the marks on the moths.

Other assumptions

Figure 8.16 shows a hypothetical ideal field in which the experimenter can measure all of the likely population parameters which would affect the results of the mark-recapture trial. Note that the "birth" parameter is replaced by emergence of males from local pupae in the ground. The effect of each population parameter on the proportion of marked to unmarked moths is indicated by the arrows on the right of the diagram. A flat line indicates that the number of marked moths should be equally affected by the parameter as unmarked moths, whilst n/a refers to parameters that are not relevant.

For this experiment, it was assumed that there were no resident males in the field, so the proportion of marked males represents an "instantaneous" measure of the total population per night. There would be minimal or no males emerging from within the field, as the field would have been tilled prior to planting with sorghum which would destroy virtually all pupae. Sorghum does not readily support *H. armigera* until flowering. The sorghum used here had just commenced flowering, so the only representatives of the next generation of moths would have been eggs or small larvae. The effect of emerging males on the proportion of marked males can therefore be ignored.

The final factor which may affect the proportion of marked males is the immigration of unmarked males. Since there is assumed to be very few males which remain resident over more than one night, the entire population of males can be considered as being in a state of constant flux, with rates of immigration and emigration, which are dependent on nightly overall variation in moth populations and flight activity. The dilution of marked males with unmarked immigrants is therefore proportional and matched by the emigration of both marked and unmarked individuals. The numbers reported here as the total population of *H. armigera* males in the field of sorghum

per night are also a direct measure of the immigration rate of males into the field. As indicated in Figure 8.16 this immigration could result in a potential dilution of the overall number of marked moths in respect to the total population, which could result in an overall underestimate of the total population of male moths. However, this underestimate should be consistent across nights, and permits a conservative estimate of the total number of male moths present in the field.

An additional source of error was the possibility of marked moths entering the recapture Agrisense traps and marking unmarked moths within the trap. The marking fidelity experiment results presented here indicate that this could be a problem. Even with the refined scoring method developed there could have been some additional wild moths scored as "marked" from the recapture traps. The marking fidelity experiment may have exaggerated the potential for this contamination to occur, as the load present on the test-marked moths was much higher than field-marked moths caught in the later trial in sorghum. The test-marked moths also had little

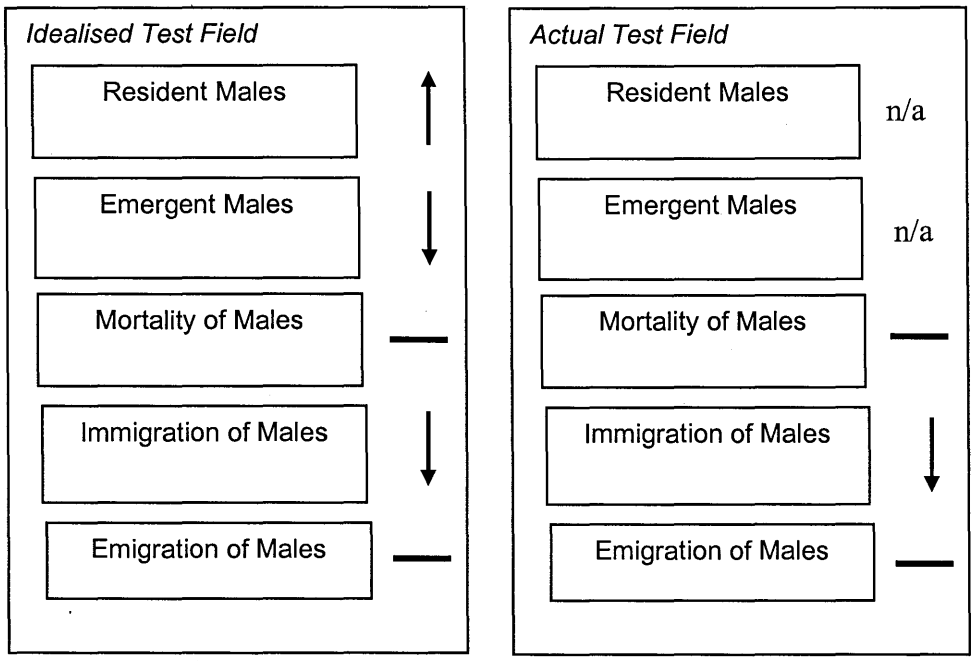

Figure 8.16 Hypothetical or idealised mark-recapture trial, and the assumed properties of the mark recapture trial in Darling Downs sorghum. The arrows indicate the potential effect of each component of the population of male *H. armigera* on the proportion of marked males in the field population.

opportunity to shed excess dye powder prior to being placed in the trap as they would if they were actively flying in the field. This form of contamination would rarely occur in the field trials of mark-recapture, as it would require a heavily marked moth to fly almost immediately into a funnel trap after the marking event in order to introduce sufficient excess dye powder for a "false" marking event within the trap. Field-marked moths collected during the mark-recapture trials rarely carried as much dye as seen in the test-marked moths (see Figure 8.2 for a typical range of

intensities of marking), so the errors measured in these marking fidelity trials may be well in excess of what might actually occur in the field.

Insights into the ecology of *H. armigera* gained by mark-recapture.

Estimates of site-specific nightly population turnover are lacking for heliothine moths, largely because suitable techniques allowing mark, release and recapture on a nightly basis are not available (Farrow & Daly 1987). The method used here may help solve this problem when applied to large field populations of *H. armigera*. For trials in crops such as sorghum and maize which do not support immature *H. armigera* until they flower, it is possible to discount the possibility of emergent males from within the cropping area. Estimates of population sizes from these crops using this method should give accurate information about population turnover.

A suggested modification to the methodology which may allow greater resolution of population parameters would be to use only one dye colour per night, and to change the dye colour each subsequent night. With three dye colours it might be possible to better estimate immigration and emigration rates, although very few males remained resident in the sorghum crop used for these observations.

Extrapolation to the practical use of attracticides for *H. armigera*

If the assumption is made that each marking event is equivalent to contact with an attracticide droplet it can be seen that the percentage of moths that could have been killed varied between nights. If the population density was low (<100 males per hectare), then even with the relatively low number of 12 attracticide sources in the 21 ha field it would be possible to kill up to 10% of males per night. However, at the highest population density measured (4,008 males/ha) it would have been possible to kill only 2.5%. The estimated population densities (100-4,000 males per ha per night) are comparable to those estimated in cotton by Del Socorro *et al.* (2003) using a plant volatile-based attract and kill system, and by Del Socorro and Gregg (unpublished data 2004) using moth flush counts.

Equation 4.1 detailed in Chapter 4 of this thesis was a simple model which could be used to predict the appropriate number of attracticide droplets required to kill a known number of moths. Using the population densities measured here it is possible to compare this model with the efficacy measured by mark-recapture. The results from the mark-recapture study indicated that

the best efficacy obtained for the 12 pheromone sources in 21 ha was 10.53% of males removed from the population (when population density was low at 97 males per hectare). When these figures are put into Equation 4.1 the efficacy of the 12 pheromone sources is predicted to be 5.7%, indicating that Equation 4.1 is a conservative estimate of the efficacy of the attracticide formulations.

The low number of marking sources in this trial is partially a function of the logistic limitations in running more than twelve marking traps per night on a regular basis. In practical application of attract and kill there would be many more attracticide sources per hectare, and the proportion of moths killed could be increased much above 2-10 %. Again, using Equation 4.1 with a high population density (4,500 males per hectare) the model predicts that 44 droplets per hectare will kill 99% of the males present. It is not likely that droplets will work with the full 10% efficacy, especially after weathering of pheromone components occurs, so a more conservative application rate would be 75 and 100 droplets per hectare to control large populations of males in the field. This application density is considerably lower than that reported in the literature for other pest species, which ranges between 250 to 3,000 sources per hectare (eg. Evenden & McLaughlin, 2004a). There is a need for field studies to investigate whether this predicted control can be achieved in practice.

There are likely to be functional limits to the number of attracticide droplets that can be placed in the field for control of this species. If the density of droplets is too high, a mating disruption effect might prevent males from locating individual droplets, leaving a situation which may not be either good attract and kill or adequate mating disruption. This limit is not well defined at present, with more work required to find out when mating disruption becomes evident. The results of this mark recapture experiment are therefore encouraging for the potential of attract and kill for males using pheromones.

9 Mating disruption in an isolated cropping region

A desirable progression from the previous chapters would have been a full-scale trial of attract and kill in the field. Unfortunately funding was not available for a trial of this scope for a largely novel and untested technique. However, an opportunity arose when a large horticultural company (SP Exports Pty. Ltd.) and Horticulture Australia Limited provided funding to undertake a full-scale commercial mating disruption trial for *H. armigera*. This chapter outlines the findings from this trial. Chapter 1.4 reviews literature and compares mating disruption and attract and kill; the following review sections discuss the practical considerations of setting up, running and interpreting the results of a large-scale mating disruption trial.

9.1 Introduction:

9.1.1 Practical considerations for mating disruption in the field: Dispenser selection, design and placement

Dispenser selection, design and placement are critical components of a mating disruption program. Synthetic pheromones are volatile and many are unstable, breaking down in the presence of atmospheric oxygen and UV light. Considerable effort has gone into development and formulation of slow-release pheromone lures and dispensers which contain stabilizers and UV filters to prevent premature degradation of the formulations. Weatherston (1990) summarizes the desirable characteristics for lure, dispenser and attract and kill formulations used for sex pheromone-related applications. Table 9.1 summarizes the main factors involved in design and application.

Traditional application of mating disruption has relied on placing varying numbers of dispensers in the field, typically 250 or more per hectare (Takai & Wakamura 1995, Kehat *et al.* 1998, 1999, Park *et al.* 1999, Suckling *et al.* 1999, Chamberlain *et al.* 2000, Polavarapu *et al.* 2001, Mitchell & Mayer 2001, Ohtani *et al.* 2001, Toyoshima *et al.* 2001, Zhang *et al.* 2001, Albajes *et al.* 2002). These dispensers can be constructed from various polymerized compounds with the pheromone impregnated in the compound, or sandwiched between layers of plastic in a laminate design, or sealed in low permeability polyethylene tubes (sometimes called "ropes"). They can be then manually tied onto plants, placed on stakes, looped over branches or attached by other means.

Some of the more modern mating disruption methods use low-density (eg. 25 dispensers/hectare) high-release devices. These may be electronically controlled microsprayers that emit a fine spray of pheromone at timed intervals from an internal reservoir (Mafra-Neto & Baker 1996, Baker *et al.* 1997, Ryne *et al.* 2001), or polyethylene bags which are manufactured so that the pheromone blend is released at a certain rate (Baker 2004). The reduction in application costs with low-density high-release dispensers has to be balanced by potential cost of dispenser technology, problems with reliability (Alway 1998), reduction in the efficacy due to potential gaps in the spatial coverage (Baker 2004), and the cost of pheromone components in the case of the polyethylene bag dispensers. These technologies are still being tested in field conditions, and are not yet used in commercial crops in Australia.

At the other end of the spectrum is the use of sprayable formulations where the pheromone is contained in microspheres which can be applied with or without a sticker onto the crop through conventional spray nozzles. The main disadvantage of this application method is that the pheromones are rapidly volatilized, and the disruption effect may only last for a short period (Betts & Gregg, unpublished data, Polavarapu *et al.* 2001, Albajes *et al.* 2002). The advantage of this technique is that application of the formulation can be achieved quickly with low labour costs. Even coverage of the crop with a sprayable formulation may also ensure efficacy of the mating disruption effect for the short term. This technique has been tested for *H. armigera*, providing good levels of mating disruption, but over a greatly reduced time compared to polymer and laminate formulations (Betts & Gregg, unpublished data).

A recent advance in pheromone formulations has been electrostatic delivery ("EntoStat" technology, Exosect Ltd., UK) for mating disruption ("ExoSex"). This uses a method dubbed "autoconfusion" where male moths are attracted to a pheromone source which also holds an electrostatic powder which contains pheromone. The charged pheromone-laden powder sticks to the male moths. After leaving the pheromone dispenser the contaminated male moths are unable to locate and mate with female moths, and become targets for other uncontaminated males which perceive these contaminated males as females. Trials with this method with codling moth, *Cydia pomonella,* have shown that it uses up to 1,000 times less pheromone than the normal mating disruption dispenser system as well as reducing labour costs by requiring much fewer stations/dispensers per hectare (Chandler 2003a, Chandler 2003b). This technology is yet to be tested for large mobile moths such as *H. armigera*.

9.1.2 Is it working? Monitoring mating disruption trials

Monitoring is a crucial element for the success of mating disruption (Cardé & Minks 1995). Monitoring establishes if mating is prevented in the treated areas and whether egg lay is occurring within treated areas. It is essential for establishing reasons why mating disruption may be failing, such as egg lay occurring due the movement of mated females into treated areas, or local increase in the population density. It can also assist in optimising dispenser density and formulation, especially when active ingredients are expensive and unstable. A key requirement for successful monitoring is the comparison of a "control" untreated area with the treated area. As most mating disruption trials either treat an entire field or a large area within a cropping region, the untreated areas cannot be considered controls in the strict sense, as they will always be subject to different field conditions compared to the treated area. This means that cautious and conservative interpretations of treatment and control data should be made, and that a number of different monitoring techniques should be implemented to compensate for naturally occurring differences between treatment and control areas.

There are several methods of monitoring the effect of mating disruption in a treated field. The most common method is the placement of pheromone traps with either synthetic lures, or live females, on treated and untreated areas. Failure to catch male moths in traps in the treated areas compared to those in the untreated areas indicates that pheromone-based communication is disrupted, ie. the pheromone released from the dispensers is preventing males from locating the lures/females. This result is often referred to as "trap shutdown" and if present, indicates that the basis for mating disruption has been established. Unless other modes of sexual communication such as visual or auditory signals are available, no mating will take place.

This technique is easy to set up and maintain, but there are several factors which must be taken into account. In the case of synthetic lures, the source must be at least as attractive as a calling female moth. Using female moths as pheromone sources in traps is labour-intensive, and is unreliable as female moths may not call whilst they are in the traps. Another problem is that traps are usually quite inefficient at catching moths, even if the correct lure is used. In the case of the AgriSense funnel traps used for *Helicoverpa* spp., fewer than 1% of approaching males are caught in the traps (personal observations, GP. Fitt pers.comm.). If moths are present in low numbers in the field this makes detection of differences between treated and untreated cropping areas difficult.

Table 9.1 Variables in dispenser design and application and their relative benefits and disadvantages. Superscript numbers refer to reference citations (beneath table)

Factor	Examples	Advantages	Disadvantages
Increased protection for pheromone components, controlled release rate	More elaborate dispenser technology, such as electronic dispensers (MSTRS™)[1,2]	More efficient use of pheromone, potential reduction in labour costs	Increased costs of dispenser devices, potential breakdown of disruption due to fewer sources in field
Increased dispenser loading	Increasing the size of dispensers and the amount of pheromone per dispenser[3]	Longer life span, reduced application/labour costs, reduced density of dispensers in the field, better disruption	Increased cost of pheromone components, potential breakdown of disruption due to fewer sources in field
Increased/decreased dispenser density	Changing the density of dispensers with corresponding changes in dispenser loading[4]	Potential improvement of disruption, better disruption in a range of weather conditions vs reduced cost of dispensers & application labour	Increased cost of dispensers and application/labour costs vs increased risk of disruption breakdown
Autoconfusion methods	Electrostatic "autoconfusion"(ExoSex)[14,15,16]	Reduced cost of pheromone components, reduced labour	Decreased life-span of treatments?
Application methods	Use of sprayable formulations[5, 6, 7, 11],	Reduced labour costs, ease of application	Decreased life-span of treatments
Reduced component purity, simplified blends, analogues and antagonists	Using major pheromone component(s) instead of a full blend[13], using analogues which mimic action of pheromones[8, 9], using antagonists that repel males[12]	Reduced cost of formulation, increased life span of lures	Decreased efficacy?

Citations in Table 9.1

1. Baker *et al.* (1997)
2. Mafra-Neto & Baker (1996)
3. Shorey *et al.* (1972)
4. Farkas *et al.* (1974)
5. Polavarapu *et al.* (2001)
6. Weatherston & Miller (1989)
7. Albajes *et al.* (2002)
8. Grant *et al.* (1989)
9. Wu *et al.* (1991)
10. Kaae *et al.* (1974)
11. Kehat & Dunkelblum (1993)
12. Witzgall *et al.* (1996)
13. Ohtani *et al.* (2001)
14. Chandler (2003a)
15. Chandler (2003b)
16. Exosect Limited (2003)

Another method of monitoring is light trapping, which can provide useful information on insect activity, especially in cases where, despite pheromone trap shutdown, eggs are still being laid in the treated areas. Light traps potentially catch both sexes, but catches depend on the mobility of the moths, and their responses to light, and these factors may vary between the sexes of particular moth species, resulting in biased sex ratios in the traps. The efficiency of light traps is affected by the weather (Morton *et al.* 1981, Bowden 1982) and nocturnal light (Bowden & Morris 1975). Light trap catches can assist in interpretation of pheromone trap catches. For example, smaller numbers of males in the light traps in treated compared to untreated areas may indicate that activity of males is reduced by pheromone treatment. Reduction of male catches in pheromone traps may also be due to competition from calling females (Hendricks *et al.* 1973, Kvedaras 2002); light traps may allow insight into the relative importance of these two mechanisms.

The more important role of light traps in monitoring of mating disruption is to provide samples of the wild female moths present in the treated and untreated areas. Comparison of the percentage of mated females in the untreated and treated areas can provide evidence of the efficacy of mating disruption. It can also provide information on the movement of mated females into treated areas. Female moths can be dissected to determine their reproductive status as indicated by the presence or absence of spermatophores in the bursa copulatrix. *H. armigera* adults will usually mate more than once over their life-span. The number of spermatophores stored in the bursa copulatrix represents the number of times that particular female has mated.

Monitoring of male activity in treated and untreated areas can also be carried out by putting sentinel tethered (Oyama 1977, McVeigh *et al.* 1983) or wing-clipped (Shaver & Brown 1993, Kehat *et al.* 1998) virgin female moths in the field. These are moths that have been reared under laboratory conditions so that their age and reproductive status are known. The females are placed on mating tables or trays, or directly on the plants in the field when they are reproductively active ie. producing pheromone and capable of mating. Each female is either tethered or wing-clipped so that it cannot escape from a mating tray, but is freely able to attract and mate with wild males. Females are usually exposed to mating opportunities for one night, then are collected and dissected to determine whether they have been mated. Results using this technique should also be treated with caution, as tethered or wing-clipped females in mating trays might be less able to undergo reproductive activities compared to wild females. The proportion of mated sentinel females present in mating trays may therefore be a conservative estimate of mating activity within the field. In cases where there are not many wild males present, there may be little or no mating among the sentinel females. Catches from pheromone and light traps in control areas can help

differentiate this situation from one in which mating disruption is working. Kvedaras *et al.* (2000) described suitable methods for sentinel females of *H. armigera*.

A final method of monitoring is by means of behavioural observations of moths in the field. Nocturnal observations of moths are done using either night-vision goggles or white light torches. Observations can include counting male searching flights which indicate that males are searching and attempting to locate calling females. In successfully treated areas these flights are less likely to be observed (Betts *et al.* 1993). The number of mating pairs of moths on the vegetation can also be counted. Night-vision goggles supplemented with infrared-filtered torch light are particularly useful in that the light does not usually disturb or alter moth behaviour, as the wavelengths of light used are invisible to moths. Night-vision goggles do not allow good perception of depth of field, so for capture of wild moths with a net, white-light torches are superior. Females can then be collected from around flowering crops where they are either feeding or laying eggs. Lingren *et al.* (1986) review the usage of night-vision equipment in relation to the reproductive biology and nocturnal behaviour of insects.

9.1.3 Limitations of mating disruption

Mating disruption as a pest management technique has many limitations, and the success rate of trials is low. The number of failed trials is probably greater than a literature search would indicate, as many negative results are not reported in the reviewed literature. Cardé and Minks (1995) in their detailed review of mating disruption successes and constraints highlight some of the success stories of mating disruption whilst discussing why mating disruption may sometimes fail.

Table 9.2 lists some of the critical factors which relate to success or failure of mating disruption, based on the review by Cardé and Minks (1995). In general successful trials have been associated with smaller, less mobile moth species which produce <300 eggs/female, and which have a well-characterized pheromone blend. Some of the species successfully controlled by mating disruption include pink bollworm, *Pectinophora gossypiella* in cotton, Oriental fruit moth *Grapholita molesta* in stone fruits, tomato pinworm *Keiferia lycopersicella* on tomatoes and lightbrown apple moth *Epiphyas postvittana* on apple (see Cardé & Minks (1995) for a review of these cases). All of these have wingspans less than 2 cm, are poor dispersers, and tend to be difficult to control with conventional insecticides as their larvae feed in a concealed manner.

Table 9.2 List of factors which may be critical for the success or failure of a mating disruption program (after Cardé & Minks (1995)

Category	Factor	Positive for Disruption	Negative for Disruption
Species-specific	Mobility of moth species	Low mobility	High mobility
	Fecundity of moth species	Low fecundity	High fecundity
	Pheromone blend used in dispensers	Components match one or more in natural blend	Components do not match any of those in natural blend
Site-specific	Area treated	Large/discrete areas	Small areas adjacent to untreated areas
	Shape of treated area	Square or circular	Long thin strips
	Exposure of treated area	Subject to gentle wind from one direction	Subject to gusty wind from a variety of directions
	Crop structure	Similar-aged with uniform height	Variety of ages, differing in height of plants, presence of attractive vegetative and reproductive parts.
Other	Cost and practicality of treatment compared to other available treatments	Other methods of control may not be effective or mating disruption may achieve similar or better results for less cost	May be expensive compared to other treatments, control not as good or unreliable compared to other treatments

Perhaps one of the most frequently cited reasons for success and failure of mating disruption for highly mobile and/or polyphagous pests is the degree of isolation of the treated area from other sources of the target species. Examples with *H. armigera* include Betts *et al.* (1993) and Chamberlain *et al.* (2000). This isolation is dependent on the biology of the pest species, as well as the nature of the agricultural produce. For moths with low mobility, the isolation might be less than 100 m, but for highly mobile moths such as *Helicoverpa armigera*, it might be >10 km. Polyphagy may create a situation where it is extremely difficult to isolate the treated area from sources of immigrating mated female moths. Some cropping systems are naturally clumped around water or similar resources, and may represent an "island" of habitat for the target moth species. In these cases the ideal situation would be to treat the entire island for mating disruption. Depending on the scale of the situation the island may be an entire catchment area in a valley. Crop management on this scale is often termed "area-wide" management. Area-wide management in the context of IPM can be defined as IPM which operates over a broad region (including agricultural and non-agricultural areas) and attacks the pest when and where it is ecologically weakest, without regard to economic thresholds.

A successful example of this is the area-wide mating disruption treatment of pome and stone fruits for Oriental fruit moth *Grapholita molesta* in Victoria, Australia. Mating disruption is a well-established technique in this area for control of *G. molesta* in stone fruit such as peaches or nectarines. However, pome fruits such as pear and apple were not normally treated with mating disruption, and received conventional insecticide treatment. Farmers and consultants noticed that areas of stone fruit next to insecticide-treated pear were being damaged by immigrating mated female *G. molesta* from the pear. By encouraging farmers to adopt an area-wide mating

disruption program which included pome fruits the overall damage level across all crops dropped to very low levels, along with pest management costs (Il'ichev 2002).

A key assumption in treating all areas of host crop within a region is that the target moths will only mate in the presence of suitable host plants. This is an assumption which is probably dependent on the target moth species, and is rarely tested in trials of mating disruption.

The influence of crop maturity and structure may influence application methods. For example, in a mature apple orchard it may be necessary to have three or more dispensers at different heights within each apple tree (Witzgall *et al.* 1999) to ensure adequate coverage.

Many moths are highly attracted to the fruiting bodies of plants as feeding, oviposition and mating sites eg. Heliothinae (Fitt 1989, Matthews 1999), so a cropping area with a range of different-aged plants may cause localized "hot-spots" of activity which may promote the breakdown of mating disruption. This situation may occur in market gardens where plantings of brassicas and other similar fast-growing crops are staggered so as to provide a steady stream of farm produce into the market.

The economics of mating disruption have slowed the uptake of the technique in many cases (Cardé & Minks 1995). Pheromone components can be very expensive to produce, and the amount required to achieve disruption continuously over a growing season may be prohibitively expensive. Most mating disruption systems require manual labour to place dispensers, and labour is often the most expensive item in many production systems. This is exacerbated when disruption is attempted for large scale field crops such as cotton. Labour costs may determine whether mating disruption is adopted, such as in Egypt for pink bollworm on cotton (von Boguslawski & Basedow 2001).

9.2 Methodology

9.2.1 Site description

The mating disruption trial in this thesis was carried out in the cropping regions to the west of Cordalba, Qld. (25°10'S 152°13'E). There were three general areas, two untreated controls (Roma Tomato RF75, Church Block, 500 m W of Cordalba and Capsicum CF10, 11, 12, Rapley's Blocks, 2.6 km NNW of Cordalba) and the treated area at Promised Land. Figure 9.1 shows the

relationship and relative size of the treated area to the control blocks, and the location of the trial area in Queensland. The treated areas were located within a cropping region 2.5 km by 8 km known as "Promised Land", which contained numerous fields of tomatoes and capsicums separated by areas of non-host crops. The eastern-most tip of the Promised Land crops was 6.4 km from the nearest capsicum (CF12) which was the nearest *H. armigera* host crop. The intervening land was sclerophyll scrub and forest which did not contain any *H. armigera* host plants. Figure 9.2 is a map of the Promised Land area and control blocks with block codes (capsicum CF10 to CF 19, gourmet tomato GF 81 to GF89, roma or egg tomato RF71, 72 and 75).

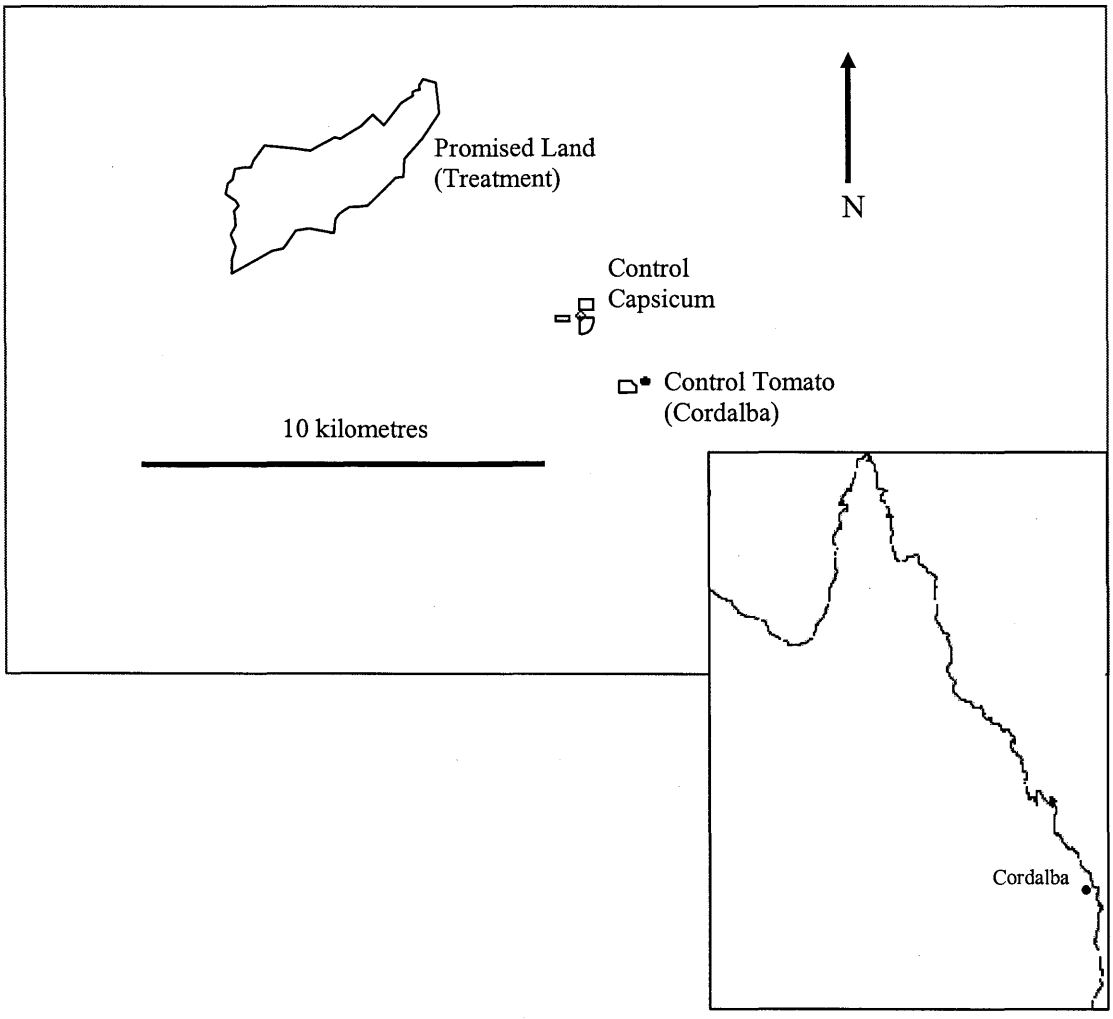

Figure 9.1 Location of the two control blocks and the treated block in relation to Cordalba, and the location of Cordalba in Queensland.

9.2.2 Planting and treatment application dates

Table 9.3 lists dates of planting for all relevant blocks and the subsequent treatment, reapplication and termination dates (to the nearest week). All areas of host crops within the Promised Land region were treated with pheromone dispensers.

Table 9.3 2003 planting, treatment, reapplication and termination dates for blocks used in the trial. "CF" are capsicum crops, "GF" are gourmet tomatoes, and "RF" are roma or egg tomatoes.

Block	Date planted	Date of Treatment	Date of Reapplication	Date Crop Terminated
CF10	7 Jan	Control	Control	30 April
CF11	7 Jan	Control	Control	29 April
CF12	6 Jan	Control	Control	27 April
RF75	5 March	Control	Control	30 June
CF13	14 Jan	7-14 Feb	8 Apr	1 June
CF14	30 Jan	14 Feb	15 Apr	2 June
CF15	29 Jan	14 Feb	15 Apr	25 June
CF16	12 Feb	14 Feb	15 Apr	25 June
CF17	13 Feb	26 Mar	25 May	25 June
CF18	20 Feb-7 March	26 Mar	25 May	27 July
CF19	19 Feb	26 Mar	25 May	28 July
RF71	28 Jan	15-20 Feb	16 Apr	2 June
RF72	11 Feb	15-20 Feb	16 Apr	27 June
GF81	25 Jan-19 Feb	4-15 Feb	5-8 Apr	4 June
GF82	29 Jan-19 Feb	4-15 Feb	9 Apr	23 June
GF83	10 Feb	3-7 Mar	1 May	27 June
GF84	14-17 Feb	20 Feb	21 Apr	18 July
GF85	20-24 Feb	20 Mar	19 May	late July
GF86	25 Feb	20 Mar	19 May	late July
GF87	3 Mar	7-20 Mar	6 May	late July
GF88	5 Mar	21 Mar	20 May	late July
GF89	6 Mar	21 Mar	20 May	late July
GF90	7 Mar	20 Mar	20 May	late July

9.2.3 Dispenser type

Selibate HA dispensers (AgriSense BCS Pty. Ltd., Pontypridd, South Wales, UK, Batch HA013A) were chosen for the trial on the basis of price and their proven ability to provide disruption in climatic conditions similar to that at Promised Land (Chamberlain *et al.* 2000). These dispensers consist of a ring of black extruded polymer made of a mixture of PVC/PVA (Cork *et al.* 1989) impregnated with 5% of a 10:1 blend of (*Z*)-11-hexadecenal and (*Z*)-9-hexadecenal, which is 160 ± 1 mg of active ingredient per dispenser. Figure 9.3 shows a dispenser placed on a tomato plant. Field trials in Pakistan showed that these dispensers had a life span of about 60 days (Chamberlain *et al.* 2000).

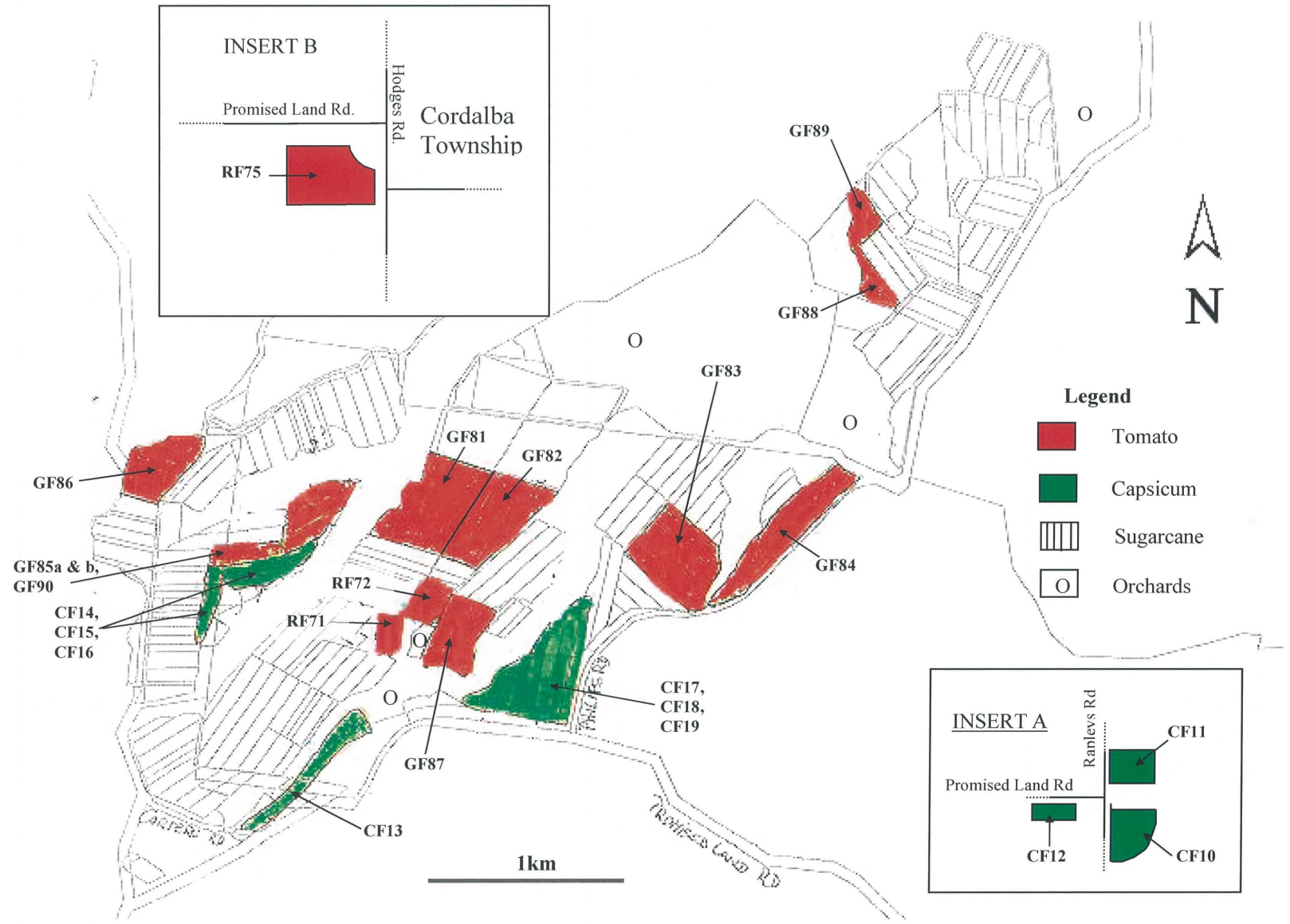

Figure 9.2 Position of the treated blocks in the Promised Land region (130 ha), the control capsicum 6.4km SE of Promised Land (INSERT A) , and the control tomato 9.1km NE by N of Promised Land (INSERT B).

9.2.4 Dispenser application and layout

Label data provided by AgriSense gave a recommended rate of application of Selibate HA dispensers in cotton of 250 per hectare, or 40 g a.i./ha. The spacing of dispensers in Promised Land tomato was based on the spacing of the wooden stakes. This resulted in an application rate of 240 dispensers per hectare, or 38.4 g a.i./ha. This corresponded to one dispenser every 5.2 m (or every stake) in a row on every fifth row. An equivalent application rate in capsicum was one dispenser every 7.3 m in a row for every seventh row. Spray tracks between bays of tomato or capsicum were counted as a single row so as to maintain an even concentration of released pheromone throughout the crop. A total of 130 ha were treated in the trial (all tomato and capsicum crops within the Promised Land region.

The dispensers were designed specifically for use in cotton where they are placed over the upper branches prior to flowering, which is the stage when *H. armigera* females commence laying eggs on the plant. Tomato and capsicum are vulnerable to attack from *H. armigera* as freshly planted seedlings. These seedlings are too small to carry the weight of individual dispensers, so in some cases alternative means of application were devised. Application techniques were as follows:

1) Direct application to plants – when seedlings were sufficiently tall and stout enough to hold the weight of a dispenser. This corresponds to greater than 3 weeks old for capsicum, and greater than 2 weeks old for tomato. Figures 9.3 and 9.4 shows the dispenser in place on a mature tomato plant.
2) Application with bamboo skewers (capsicum). A 25 cm bamboo skewer was pushed through the dispenser. When inserted into the ground the dispenser was 10-15 cm above the ground.
3) Fastening to plastic balloon sticks (capsicum only). A balloon stick (Paperware Distributors, Armidale, NSW) consisted of a 40 cm plastic tubing pushed into a circular plastic balloon holder. This balloon holder was slit so as to allow a dispenser to be held securely. Figures 9.5, 9.6 and 9.7 show the holder with and without the dispenser in place, and in the field.
4) Fastening to tomato stakes (tomato only). Stakes were placed in the tomato rows one to two weeks after planting. Several methods of attachment were used including nailing a 2.5 mm diameter flat-headed clout through the ring onto the top of the stake, stapling using either a hand stapler or a hammer tacker with 8 mm staples, and later in the season, placing the ring over the top of the stake. When the ring was nailed or stapled to the stake it was placed on the row side of the stake to avoid the dispenser being pinched by the top wire of the tomato trellis. Figures 9.8, 9.9 and 9.10 show the three attachment methods.

Figure 9.3 Selibate HA dispenser in place on mature tomato (applied when tomato was in seedling stage). White arrow indicates dispenser.

Figure 9.4 Selibate HA dispenser in place on mature tomato showing location in canopy (applied when tomato was in seedling stage). White arrow indicates dispenser.

Figure 9.5 Balloon holder with dispenser in place

Figure 9.6 Balloon holder with dispenser in capsicum block

Figure 9.7 Balloon holder in capsicum showing height relative to plant height

Figure 9.8 Dispenser stapled to side of tomato stake

Figure 9.9 Dispenser stapled to top of, and to the row-side of a tomato stake

Figure 9.10 Dispenser nailed to top of, and to one side of a tomato stake

Application timing was based on planting dates throughout the Promised Land region. Table 9.3 lists the planting dates, application dates, reapplication dates and crop termination dates. Because the varying types of application depended either on plants being large enough to support a dispenser or stakes being placed in the crop, the dispensers often were placed after the first *Helicoverpa* eggs were detected in the seedling crop. Reapplication dates were based on the 60 day active life span for the dispensers as used in Pakistan (Chamberlain *et al.* 2000), although this was delayed for several blocks by two to three weeks by lack of field workers on the ground.

9.2.5 Dispenser analysis

The loss of active ingredients from dispensers in the field was determined by gas chromatographic analysis performed by AgriSense BCS Pty. Ltd. The details of the analytical technique are contained in the Appendix 12.3. Forty-two dispensers were placed on tomato stakes on the 7th of February 2003 when the first tomato crop was treated. An equivalent number of dispensers from the same batch were wrapped in aluminium foil and stored at –18 °C for comparison with the weathered dispensers in the field. Six weathered dispensers were collected for analysis on each of the following dates: 7th of March, 11th of April and 6th of May 2003.

9.2.6 Monitoring

The experimental design used in this trial was based on the Before/After Control/Impact design (BACI) which is often used in environmental impact studies (Green 1979). This design uses measurements of both control and treated areas prior to the treatment to determine if any pre-existing differences between the control and treated areas are present. Monitoring of moth populations commenced two weeks prior to the first dispensers being placed in the treated crops to collect information on any pre-existing trends. The location of monitoring was based on obtaining representative data for regions within the treated (Promised Land) and control areas. Figure 9.11 shows the approximate divisions of the Promised Land region and the number and types of traps used. Note that this changed throughout the first half of 2003 in response to planting/removal of crops. Activity of moths in residual crops (melon and capsicum) was monitored from January to March. Intensive monitoring (using light traps, mating trays, daily checking of pheromone traps) was carried out for ten days/month for the whole duration of the trial, with weekly counts of pheromone traps recorded between these ten day intensive monitoring periods.

9.2.6.1 Pheromone traps

AgriSense traps were deployed to detect trap shutdown in treated areas and to monitor male moth populations in untreated areas (Chapter 2.7). The traps were suspended on steel curtain rods or PVC electrical conduit so that they were approximately 10 cm above the canopy of the crops. When crops were in the seedling stage the traps were set about 40-50 cm above the ground. Traps were spaced more than 70 m apart. Rubber septa lures were used for *H. armigera* (Chapter 2.6). Commercial laminate lures (AgriSense BCS Pty. Ltd., Pontypridd, South Wales, UK) were used for *H. punctigera*. Lures and pest strips were replaced each month.

Pheromone traps were cleared daily during the ten day intensive monitoring each month, and once a week outside these periods. Moths caught in traps were sexed and identified to species, as *Chrysodeixis argentifera*, (Noctuidae, Plusiinae), as well as *H. armigera* females were occasionally found to stray into the funnel traps.

9.2.6.2 Light traps

Light traps were based around dual 8 W black light tubes (NEC, FL8BL) in a 12 V DC batten suspended vertically on a wire frame above a fibreglass cone (airport runway marker) 48 cm deep, 79 cm wide with a 7 cm opening. The cones were seated with the small opening facing downwards on a plastic garbage bin 48 cm wide x 52 cm deep. Figure 9.12 shows this type of trap in the field. The traps were powered by a 12 V small car battery which was in turn charged by a 30 W self-regulating solar panel. The lights were automatically turned on and off by a light sensitive switch incorporating a 30 min delay after dusk to avoid catching large numbers of beetles and crickets which are usually present at dusk. Insects attracted to the light were collected in a 4 litre plastic jar containing 1 litre of 70% ethanol placed in the garbage bin. These jars were collected each morning, cleared, and put back into the bins an hour before dusk. The ethanol was replenished every time after nocturnal rainfall or every two nights in hot weather to compensate for evaporation. The light traps were reasonably reliable, although several gaps in data collection occurred during intensive monitoring due to equipment failure. *Helicoverpa* catches in the light traps were sorted to species level (*H. armigera* or *H. punctigera*), sexed, and females frozen until dissection for determination of mating status.

9.2.6.3 Mating trays with wing-clipped females

Laboratory-reared pupae of *H. armigera* were obtained from cultures maintained in the insectaries of Bidstrup Biologicals Pty. Ltd., Warra, Qld. Pupae were sexed, males discarded, and females placed in groups of 20 in plastic takeaway food containers (173 x 119 x 58 mm) with moist vermiculite. Pupae were kept under a light and temperature regime similar to that in the field until emergence (usually 2-7 days after sexing). Adult females were removed daily as they emerged, and held in groups of three in 160 ml plastic cups and provided with dental wicks soaked in 5-10% sucrose solution for food.

Two day old females were used in mating trays in the field. Females were first chilled at 5 °C for 8-10 min to temporarily immoblise them, then removed three at a time and wing-clipped as described by Kvedaras *et al.* (2000). The aim of wing-clipping is to prevent females from flying out of the mating trays. Wing-clipping involved cutting off one pair of wings at the base using dissecting scissors. This process was carried out as quickly as possible with minimum handling of moths to minimise damage and trauma to the moths. Figure 9.13 shows a wing-clipped female and mating tray.

The mating trays were described by Kvedaras *et al.* (2000). They consisted of 20 x 20 x 7 cm light galvanised metal sheeting spot-welded together with a metal gauze base and an open top. The vertical sides of the mating tables were coated in fluon (Dupont, Sydney, Australia) and plastic lips were attached on the edges to ensure that moths could not escape by crawling up. Each tray had a screw clamp so that the tray could be clamped onto a metal post (15 mm square x 160 cm tall) at an adjustable height so that the base was clear of any vegetation. A 5 cm barrier of white petroleum jelly was smeared around each metal rod just below each tray to prevent arthropod predators accessing the females.

Mating trays were spaced about 10 m or more apart. Three females were placed in each tray at dusk along with a dental wick soaked in 5-10% sucrose solution. The females were collected at first light the following morning to avoid bird predation. They were frozen then dissected in 70% ethanol to determine mating status.

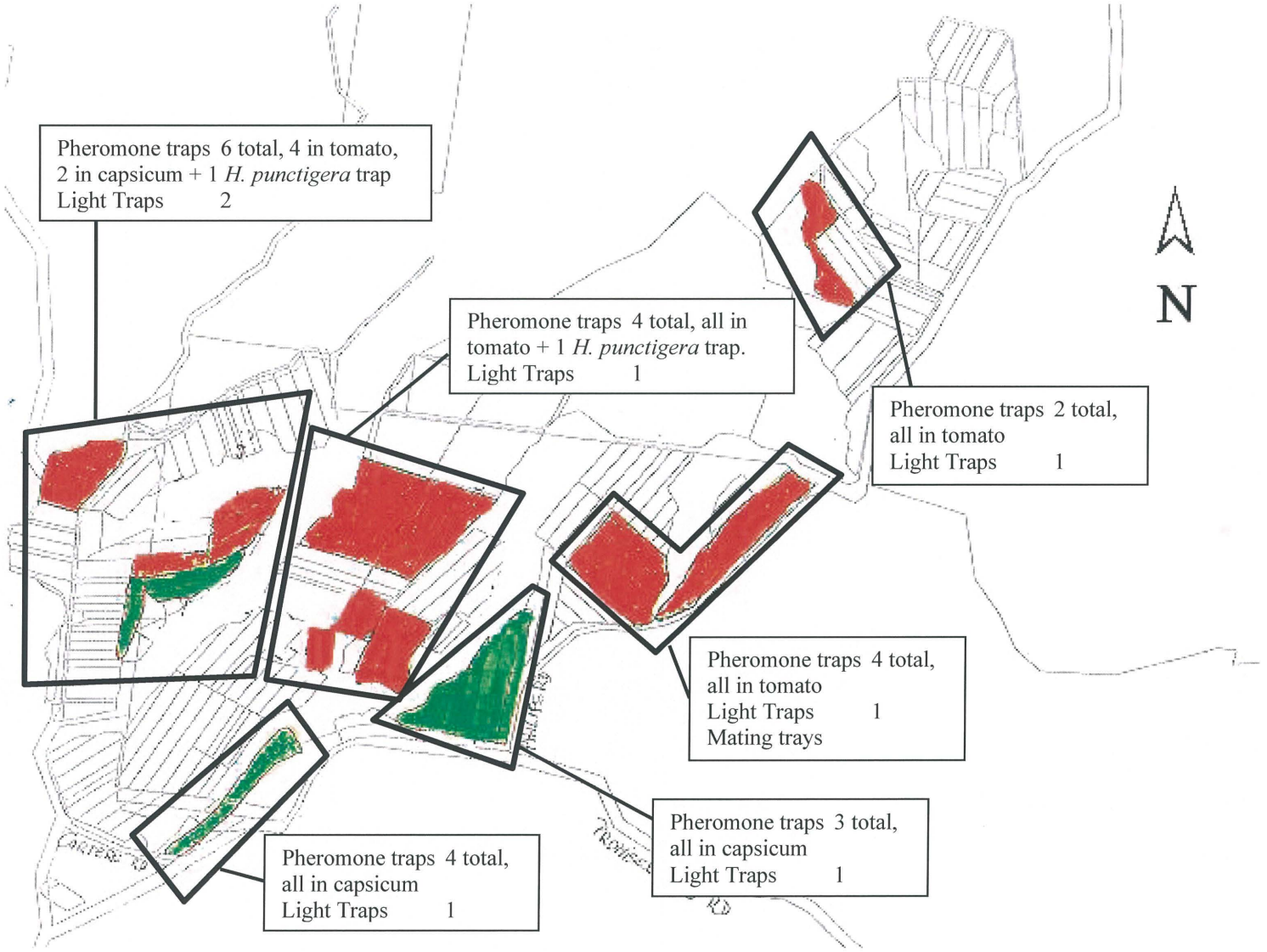

Figure 9.11 Location of monitoring traps in the Promised Land region, and the localised grouping used to provide representative monitoring data.

Figure 9.12 Light trap in capsicum. The full setup used in monitoring also had a 30W solar panel to recharge the 12V batteries.

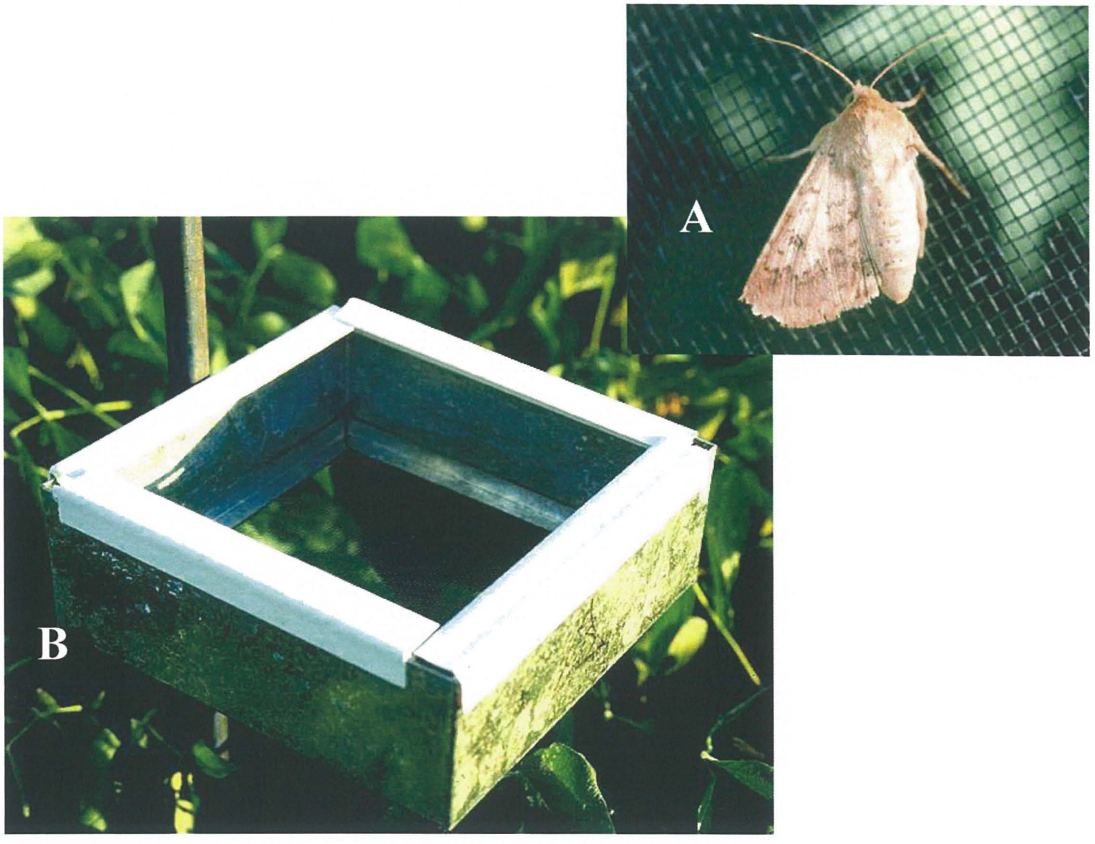

Figure 9.13 Wing-clipped female moth on base of mating tray (A) and mating tray (B). Photographs: P.C. Gregg

Table 9.4 Dates, locations and numbers of females used in mating tray comparisons in tomato and capsicum.

Date	Location	No. Females
30th Jan	Untreated Melon Residue, Promised Land, West	30
	Control Capsicum CF11	60
	Untreated Capsicum CF13	30
	Untreated Tomato GF81	60
1st Mar	Control Capsicum CF11	21
	Untreated Tomato GF83	21
	Treated Tomato GF84	21
2nd Mar	Control Capsicum CF11	24
	Untreated Tomato GF83	18
	Treated Capsicum CF13	18
3rd Mar	Control Capsicum CF11	24
	Untreated Tomato GF83	24
	Treated Tomato GF84	24
8th Apr	Treated Tomato GF83	12
	Control Tomato RF75	11
9th Apr	Treated Tomato GF83	12
	Control Tomato RF75	11

Mating tray comparisons were conducted on 14 nights throughout the trial, with a total of 840 females placed in the field. Table 9.4 lists the dates, locations and number of females used for these nights. Not all females were recovered the following morning; some were either missing or dead. Data on the proportion of these females that were mated presented in the results section of this chapter excluded missing or dead moths.

9.2.6.4 Egg and larval counts

Egg and larval counts on capsicum and tomato blocks were made by Emma Smith (agronomist for SP Exports Pty. Ltd.) every 5 to 10 days as part of routine crop monitoring and checking. Each check was done on 10 sites randomly selected from within a block. Each site usually consisted of an individual plant, but when plants were in the seedling stage more than one plant was checked to obtain sufficient numbers of flowers, terminals and leaves. Five flowers, 3 terminals, 5 leaves spaced from the top to the bottom of the plant were examined for each site. In tomato, 3 leaves touching the plastic or soil at the base of the plant were also checked. Data from tomato and capsicum were analysed separately because of this, and because of the perceived differences in attractiveness of the two crops to both female and male *Helicoverpa* spp. adults. The counts were only available as an average per site per block, and were treated as counts per check in the results section below. Eggs were recorded as "white" (freshly laid) or "brown" (older eggs near hatching), and the larvae were recorded as "small" (including neonates) and "large". The two types of eggs, and the two sizes of larvae were pooled together when considering data on a weekly basis.

Figure 9.14 Mating tray in sugarcane

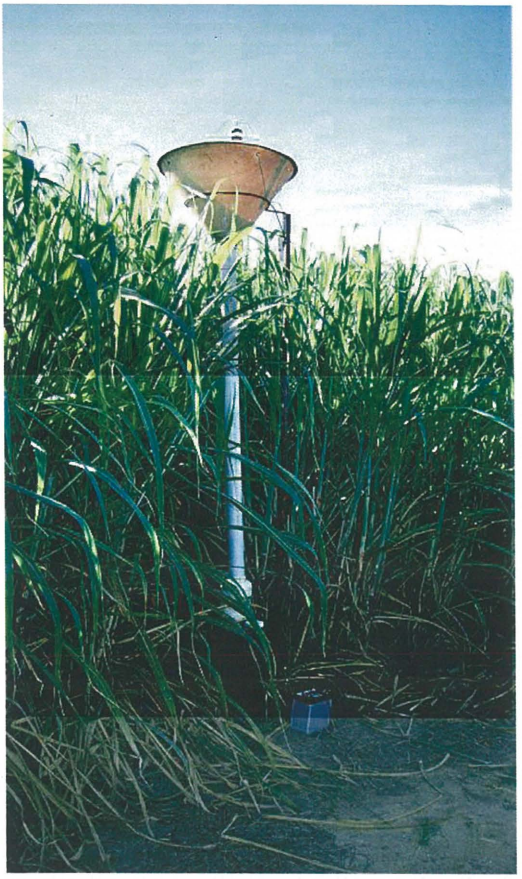

Figure 9.15 Modified light trap in sugarcane. Collecting jar is indicated by white arrow.

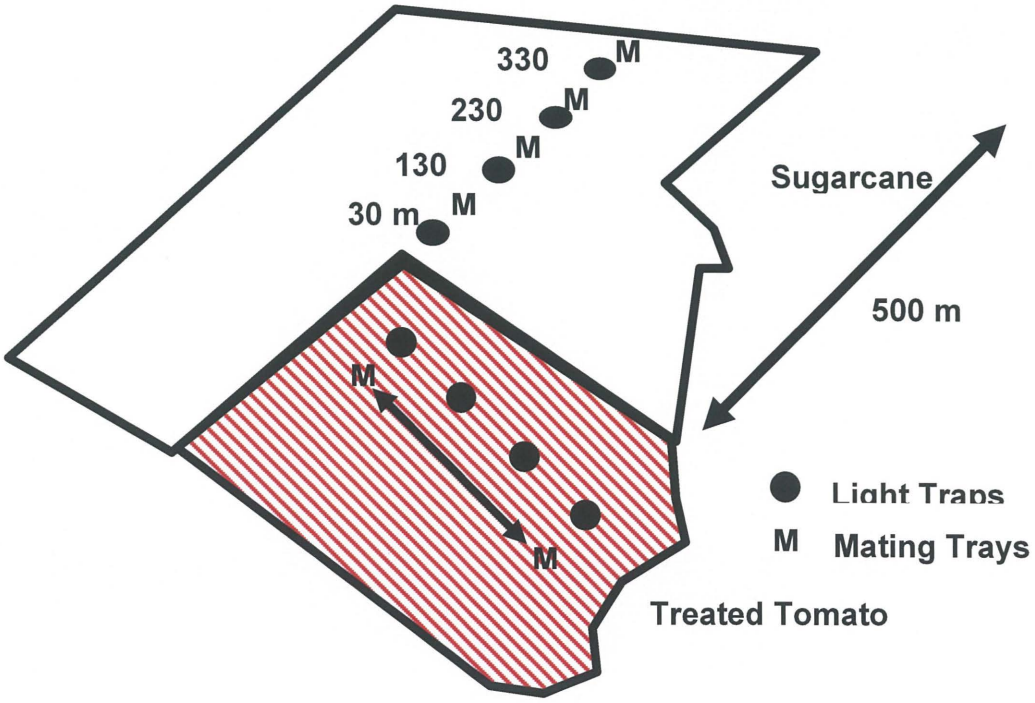

Figure 9.16 Layout for comparison between traps and mating trays along a transect in sugarcane and in treated tomato (GF83)

9.2.6.5 Comparison of Helicoverpa reproductive behaviour in sugarcane and in treated tomato

Two experiments were designed to determine if females were being mated near treated fields in a non-host crop (sugarcane) adjacent to the treated fields. Table 9.5 shows the dates, location and number of females used. The first experiment compared the reproductive status of wing-clipped virgin females in mating trays in untreated sugarcane 100 m north of a treated tomato block (GF83) with those placed 100 m into the treated tomato (GF83) (Figures 9.1 & 9.16). The methodology followed was similar to that described for previous mating tray experiments but with the trays in the sugarcane elevated on an additional 2 m stake (total height of 3.16 m) so that the trays were level with the top of the sugarcane. Figure 9.14 shows an example of the raised mating tray. This experiment was conducted on April 10 and April 11 2003 with a total of 28 females in the sugarcane and 26 females in the tomato.

Table 9.5 Dates, locations and numbers of females used in mating tray comparisons in tomato and sugarcane.

Date	Location	No. Females
10th Apr	Treated Tomato GF83	15
	Untreated Sugarcane 100 m Nth. GF83	14
11th Apr	Treated Tomato GF83	19
	Untreated Sugarcane 100 m Nth. GF83	15
30th Apr	Untreated Sugarcane 100 m Nth. GF83	3
	Untreated Sugarcane 200 m Nth. GF83	3
	Untreated Sugarcane 300 m Nth. GF83	6
	Untreated Sugarcane 400 m Nth. GF83	6
	Treated Tomato GF83	15
1st May	Untreated Sugarcane 100 m Nth. GF83	12
	Untreated Sugarcane 200 m Nth. GF83	15
	Untreated Sugarcane 300 m Nth. GF83	9
	Untreated Sugarcane 400 m Nth. GF83	9
	Treated Tomato GF83	45
2nd May	Untreated Sugarcane 100 m Nth. GF83	12
	Untreated Sugarcane 200 m Nth. GF83	15
	Untreated Sugarcane 300 m Nth. GF83	9
	Untreated Sugarcane 400 m Nth. GF83	9
	Treated Tomato GF83	44
3rd May	Untreated Sugarcane 100 m Nth. GF83	5
	Untreated Sugarcane 200 m Nth. GF83	6
	Untreated Sugarcane 300 m Nth. GF83	9
	Untreated Sugarcane 400 m Nth. GF83	9
	Treated Tomato GF83	33
4th May	Untreated Sugarcane 100 m Nth. GF83	6
	Untreated Sugarcane 200 m Nth. GF83	6
	Untreated Sugarcane 300 m Nth. GF83	6
	Untreated Sugarcane 400 m Nth. GF83	6
	Treated Tomato GF83	23
5th May	Untreated Sugarcane 100 m Nth. GF83	6
	Untreated Sugarcane 200 m Nth. GF83	0
	Untreated Sugarcane 300 m Nth. GF83	6
	Untreated Sugarcane 400 m Nth. GF83	6
	Treated Tomato GF83	17

The second experiment compared wild light-trapped females in a treated tomato block (GF83) with those captured in light traps in adjacent sugarcane along a transect running north of GF83, whilst simultaneously running mating trays as described above. Figure 9.16 shows the layout of light traps and mating trays in the sugarcane and the treated tomato. Four light traps were placed in the sugarcane at 30 m, 130 m, 230 m and 330 m from the edge of the tomato block GF83. Due to the height of the sugarcane plants the light traps in the sugarcane were extended so that they projected above the upper canopy of the sugarcane. Figure 9.15 shows a modified light trap in the sugarcane block. Four light traps were placed in treated tomato GF83 along the third bay in from the northern edge of GF83. These traps were separated from each other by at least 80 m and were at least 80 m inside the tomato crop.

The mating trays in the sugarcane were raised on tall stakes as for the previous experiment and placed in groups between light traps along the transect. The mating trays were sufficiently distant from the light traps that it would have been unlikely that the light would have interfered with mating in the trays. Mating trays were placed in an adjacent bay to the light traps in the treated tomato block. A total of 179 females were placed in the sugarcane trays, and 133 in the treated tomato block. This experiment was done from April 29 to May 5, 2003.

9.2.6.6 Other monitoring methods

Adults were collected at night on capsicum and tomato plants using a butterfly net and white light source. This procedure was not done on a systematic basis throughout the trial, but was concentrated around several nights during February and March. This method was discontinued due to the very low numbers of moths caught.

Potential host plants for *Helicoverpa* spp. around the trial area were also sampled for larvae by sweep netting. This was carried out on an opportunistic basis to determine presence/absence of larvae. A sweep net (diameter 380 mm) sample consisted of 20 sweeps.

9.2.7 Weather data

Weather data were obtained from the Bureau of Meteorology station based at Bundaberg Airport (24°54'S 152°19'E) 31.3 km NNE of Cordalba. Weather stations based in the Promised Land region provided incomplete data for the study period; comparison with the Bundaberg weather

station data indicated that the weather patterns were similar and this was used for comparison with moth activity in the Promised Land region.

9.2.8 Statistical analysis

An estimate of the efficacy of communication disruption was calculated based on trap shutdown:

$$\%TrapShutdown = \frac{ControlPlotCatch - TreatedPlotCatch}{ControlPlotCatch} \times 100$$

This formula was also used to calculate mating disruption based on mating tray data:

$$\%MatingDisruption = \frac{\%ControlMated - \%TreatmentMated}{\%ControlMated} \times 100$$

Proportional data were compared using χ^2-tests. Comparison of pairs of means between treated and untreated areas used Wilcoxon Signed Rank tests for non-normal and/or uneven sample size data, and with t-tests/ANOVA for normal data (MathSoft 1999). Differences were considered significant at $p < 0.05$. Variability in data is represented by the mean standard error for means and by 95% confidence intervals for proportional data.

9.3 Results

9.3.1 Dispenser placement – labour times, reliability

9.3.1.1 Tomato

Stapling the dispensers onto the tomato stakes (Figures 9.8 and 9.9) was the most reliable method of attaching dispensers, and was also the most rapid at 2.4 minutes per 100 m of row. The only drawback to this method was the reliance on staking of the tomatoes which meant that the seedling crop remained untreated until stakes were placed in the field. In most cases the tomatoes were staked less than two weeks after planting, although heavy rains in early March delayed staking, and hence mating disruption treatment for up to three weeks after planting in some fields. The other attachment methods took longer to deploy, were less reliable, or did not give adequate

coverage of the crop. Nailing dispensers to stakes (Figure 9.10) was much slower than stapling, required greater precision from the field worker, and was unreliable, as nails often caused the formulation to split and fall to the ground. In some cases larger nails were used (> 2.5 mm in diameter) which exacerbated this problem, requiring that large areas of tomato be re-treated. Direct application (Figures 9.3 and 9.4) was reliable when plants were at least three weeks old, providing good retention rates of dispensers. However, due to the growth habit of tomato where branching nodes remain relatively static during the growth of the plant, the dispensers often remained close to the ground, thus possibly restricting pheromone release to the lower parts of the plants. Direct application as a method of reapplication in tomato was not suitable as tomato plants were "hedged" to the top level of the stakes by a machine which prunes the upper growing tips. This could result in dispensers being trimmed from the tops of the plants. It was easier and faster to reapply the dispensers directly to the stakes. Table 9.6 summarises the advantages and disadvantages of the different methods.

Table 9.6 The results of dispenser application methods in tomato. "Reliability" refers to dispenser remaining where it was placed, "Coverage" refers to the amount of treated crop/dispenser; dispensers which remained close to the base of the plant were assumed to have poor coverage of the actively growing upper parts of the crop

Method	Time per 100 m row (minutes)	Reliability	Coverage	Comments
Direct Application	3.5	Good	Poor	Dispensers remain very low on the plant, dispensers cannot be placed until seedling is at least three weeks old
Nailing to Stake	4.6	Poor	Good	Nails frequently split the dispenser so that it falls off post, slow, requires staking
Stapling to Stake	2.4	Good	Good	Best method, requires staking
Over top of Stake	2.4	Good	Good	Can only be used for reapplication when top wire is on row, which is late in growing season

9.3.1.2 Capsicum

The best method for dispenser application in seedling capsicum up to mature plants was using plastic balloon sticks. This method was the slowest, but proved to be the only reliable method for this crop. Direct application from the seedling stage was not reliable for a number of reasons. The growth pattern of capsicum is similar to that of tomato in that dispenser rings placed around the first or second branch nodes stay at that level rather than grow into the canopy. Weed control practices in capsicum include using a cultivator which banks earth from the furrow up onto each row to smother weeds at the base of the capsicum plants. This banked earth tended to cover the dispensers on the seedlings. The rings were also prone to falling off when the lower seedling leaves fall off as the plant matures, thus removing the support for the rings. Direct application

Table 9.7 The results of dispenser application methods in capsicum. "Reliability" refers to dispenser remaining where it was placed, "Coverage" refers to the amount of treated crop/dispenser; dispensers which remained close to the base of the plant were assumed to have poor coverage of the actively growing upper parts of the crop

Method	Time per 100 m row (minutes)	Reliability	Coverage	Comments
Direct Application	3.5	Poor/Good	Poor/Good	Poor for seedling stages, but good for mature capsicum
Balloon Holders	4.9 (assembly time) + 3.5 (placement)	Good	Good	Best method from planting onwards, takes more time than other application methods
Bamboo Skewers	1.4 (assembly time) + 2.5 (placement)	Poor	Poor	Skewers split the dispenser, did not survive field conditions (fell over easily)

when the capsicum had reached a height close to the maximum was quick, effective and reliable. Bamboo skewers pierced through the rings caused dispensers to split in much the same way as nails did in tomato (see above). Table 9.7 lists the methods and their advantages and disadvantages.

9.3.2 Monitoring

9.3.2.1 General comments

Helicoverpa punctigera moths were present only in very low numbers for the duration of the trial. Adults are readily identifiable, but eggs can only be separated by using an antibody-based test (LepTon™ Test Kit, Abbott Laboratories, Chicago) or by rearing to the adult stage. Eggs were not identified to species during this trial. Due to the very low numbers of adults present in pheromone and light traps from both control and treated blocks it was decided to omit *H. punctigera* from any further analysis in the results. This assumes that *H. armigera* females were responsible for laying the majority of eggs counted during monitoring of the crops. Even if this assumption is incorrect it is likely that *H. punctigera* would be affected by the pheromone treatment in similar fashion to *H. armigera*, since Betts et al. (1993) found that *H. armigera* pheromone could result in trap shutdown for both species in treated cotton.

The large number of zero trap catches over the first two weeks of monitoring meant that no patterns could be observed in either the control or treatment areas. This meant that the BACI design could not be used to account for any pre-existing differences between the control and treatment areas. In addition to this control tomato fields were not available for comparison to the treated fields until four weeks after the initial treatment in the tomatoes (GF81) at Promised Land

region. Early trap catches from Promised Land tomato could only be compared to the control capsicum (CF10, 11, 12). The opposite situation occurred late in the trial, when the control capsicum crops (CF10, 11, 12) had been terminated whilst the treated capsicum fields were still extant in the Promised Land region. In both these cases it was assumed that the other crop was representative of the general activity in the region, but could not be used in a direct comparison.

The timing of dispenser placement meant that early in the season in the Promised Land region there were often older blocks which had been treated with dispensers as well as seedling blocks which were yet to be treated. For simplification of the analysis the treatment of tomato in the Promised Land region is considered to have commenced from the 4th of February 2003, and for the capsicum, from the 7th of February 2003.

9.3.2.2 Pheromone trap catches

Pheromone trap catches throughout the trial period between January and June were generally very low, averaging 0.83 ± 0.09 moths/trap/night in control capsicum, and 2.90 ± 0.30 moths/trap/night in control tomato. Trap catches in capsicum were always lower than in tomato. The timing of planting meant that no control tomato crop was available for the first 6 weeks of the trial, and 4 weeks after the first tomato in Promised Land (GF81) was treated, but traps in the control capsicum crop meant that some monitoring of activity around host crops outside of the Promised Land region was possible. The pheromone traps placed in residues left from late 2002 capsicum and melon crops caught very low numbers of males, with a maximum catch of 3 males/trap/night in the residual capsicum crop.

Figures 9.17 and 9.18 show the average weekly catch in controls compared to that of the treated areas and the percentage trap shutdown per week for capsicum and tomato respectively. The date when disruption treatments commenced is marked on each graph with an arrow, and dates where there was a significant difference between the mean number of males per trap per week between treated and control areas are indicated by an asterisk above control data points.

January and February catches prior to, and just after, the initial treatment were low in both control and treatment areas. Trap shutdown after the first treatment of tomato (early February) was observed when pheromone trap catches in treated tomato were compared to those in untreated capsicum. Figure 9.19 is a plot combining the control capsicum and the treated tomato for the initial treatment period up until early March for nightly pheromone trap catches. The first

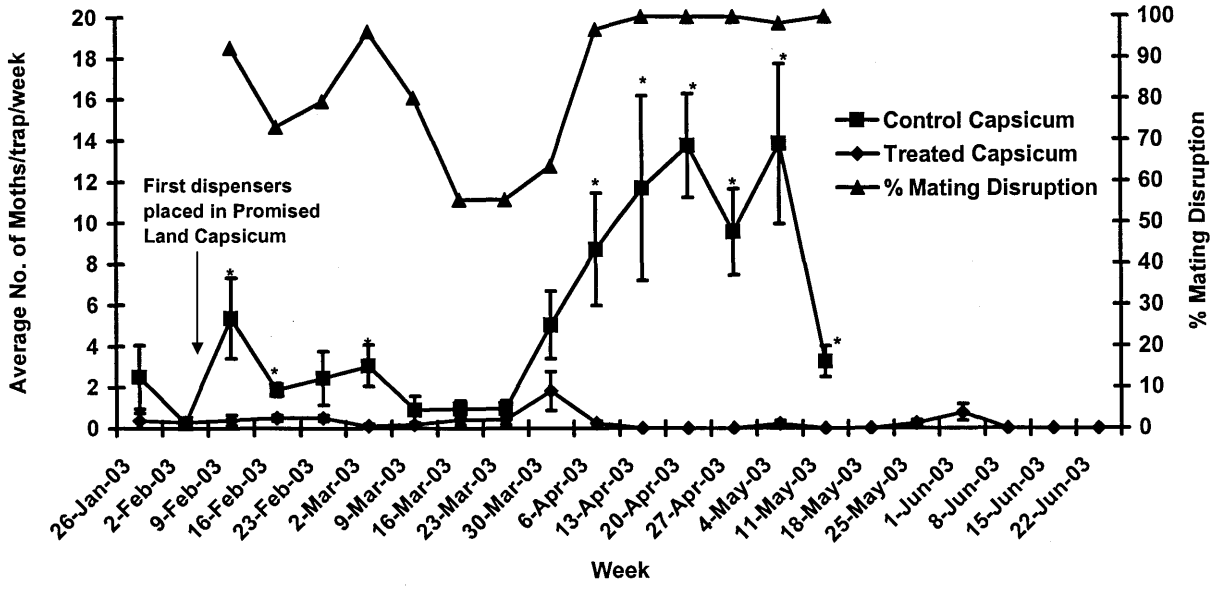

Figure 9.17 Mean weekly pheromone catches per trap in treated and control capsicum, and the percentage mating disruption each week. Asterisks indicate significant weekly differences between means in treated and control ($p<0.05$).

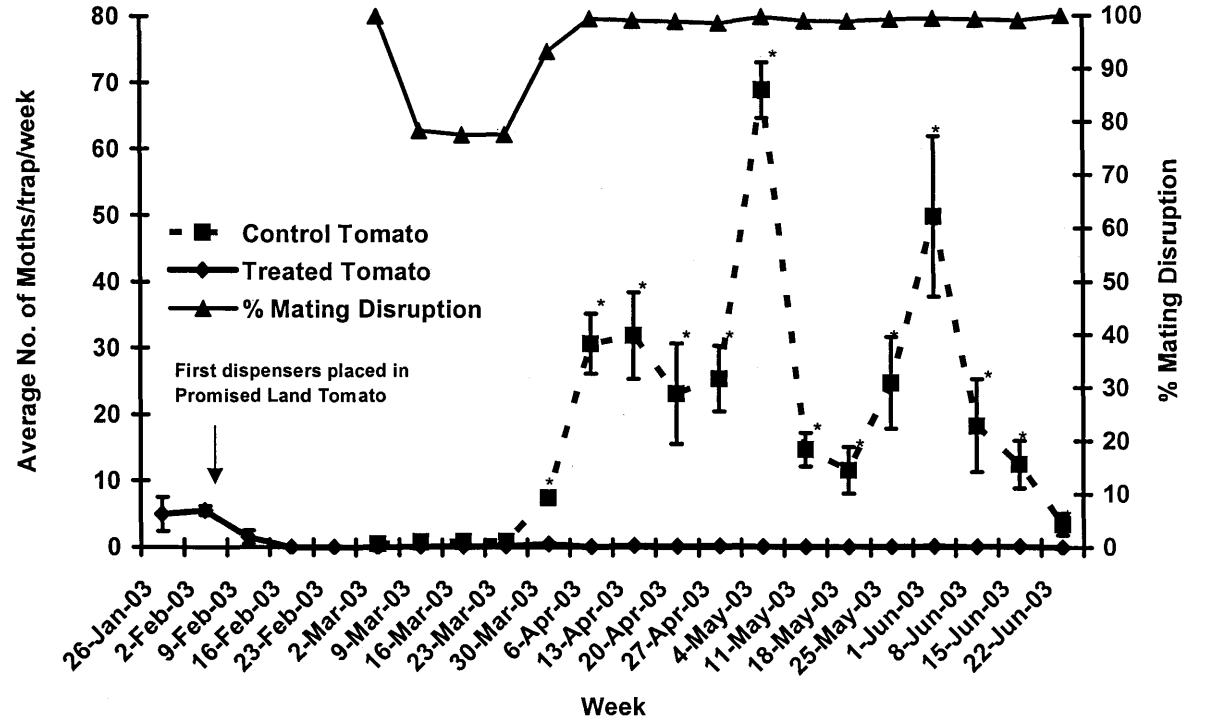

Figure 9.18 Mean weekly pheromone catches of *H. armigera* in treated and control tomato, and the percentage mating disruption each week. Asterisks indicate significant weekly differences between means in treated and control ($p<0.05$).

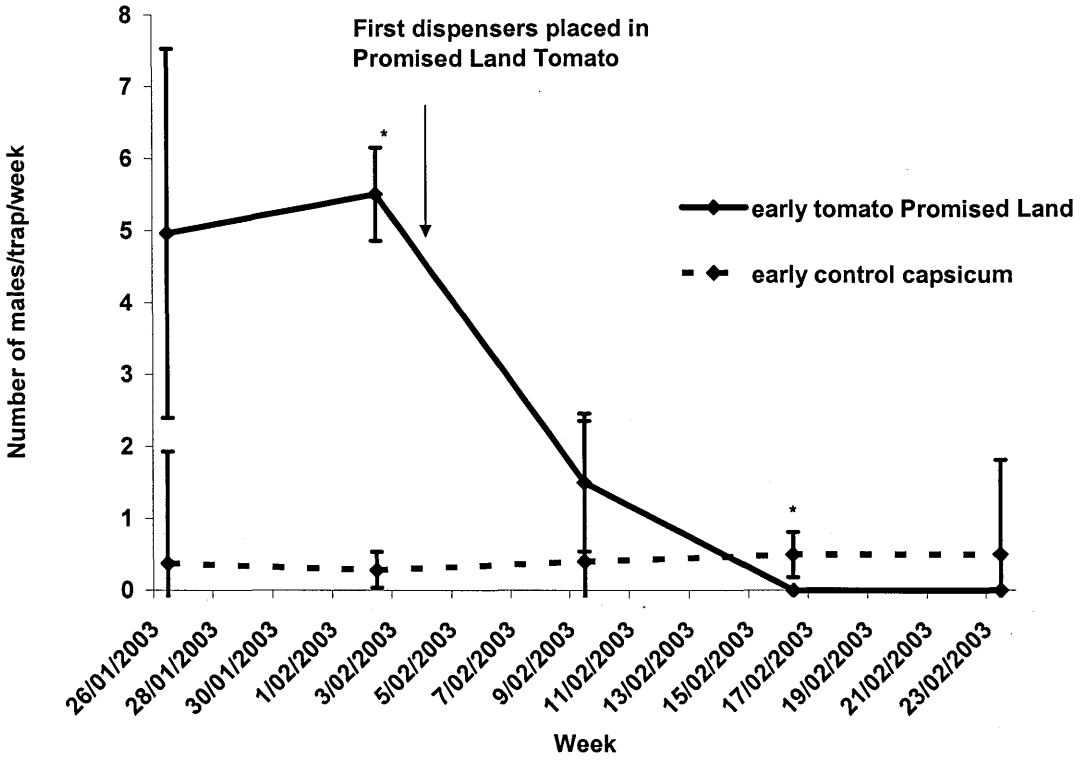

Figure 9.19 Comparison between mean weekly pheromone trap catches of male *H. armigera* in tomato and capsicum for the first five weeks of the trial before a control tomato crop became available. Asterisks indicate significant weekly differences between means in treated and control ($p<0.05$).

dispensers were placed out on the 4th of February, with a small section of tomato treated. Almost complete trap shutdown (absence of males in pheromone traps) was achieved after the 9th of February when the rest of the tomato was treated. This shutdown was not always evident when compared to the control crops due to very low moth numbers over some nights (eg. between the 15th and 20th of February, see Figure 9.19).

9.3.2.3 Light trap catches

Light trap catches were generally low throughout most of the trial period. Figure 9.20 shows the mean number of females captured per night in treated and untreated areas of tomato and capsicum. There were no significant differences in the number of moths for either crop (ANOVA, df = 1, $F = 1.07$, $p = 0.31$ and ANOVA, df = 1, $F = 3.45$, $p = 0.07$ respectively). However, the number of male moths present was significantly reduced in treated tomato compared to control tomato (ANOVA, df = 1, $F = 10.25$, $p < 0.01$) as well as in treated capsicum compared to control capsicum (ANOVA, df = 1, $F = 7.58$, $p < 0.01$).

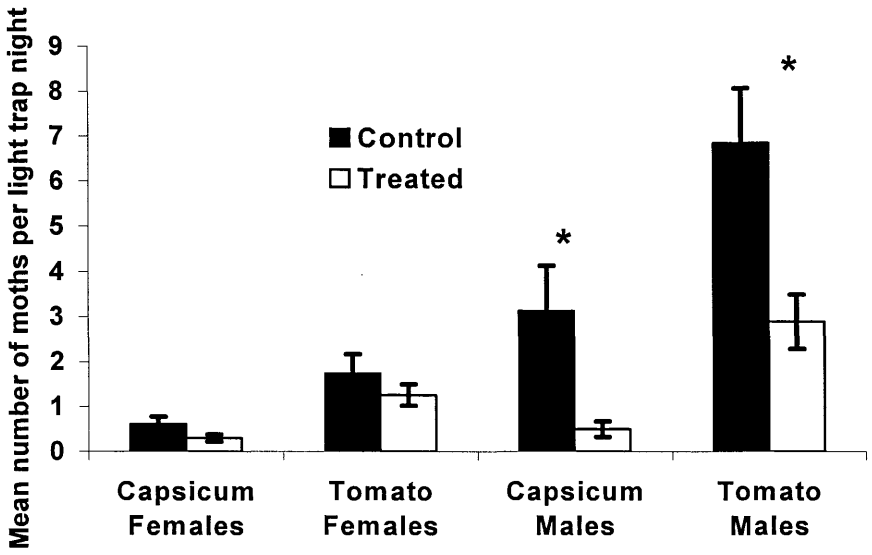

Figure 9.20 Comparisons between the mean numbers of male and females captured in light traps in control and treated areas. Asterisks indicate pairs of means that are significantly different.

There were no significant differences between the proportion of mated females caught in treated and untreated blocks in either capsicum ($\chi^2 = 0.04$, P = 0.84) or tomato ($\chi^2 = 0.002$, P = 0.96). Table 9.8 shows the percentage of females mated. Note that the proportion obtained for the capsicum control plot is based on very few females (n=4).

Table 9.8 The percentage ± 95% confidence interval of mated female *H. armigera* recovered from light trap catches from control and treated capsicum and tomato.

Crop	Number of Females	% Mated
Capsicum Control	4	50 ± 49
Capsicum Treated	11	81.82 ± 22.79
Tomato Control	26	88.46 ± 12.28
Tomato Treated	57	89.47 ± 7.97

The proportion of females with more than one spermatophore in the control tomato (38.46 ± 18.7) was significantly higher than that in the treated tomato (19.3 ± 10.25) ($\chi^2 = 5.88$, P< 0.05). The small number of females available for comparison between control and treated capsicum restricted any further statistical analysis for this crop.

9.3.2.4 Mating trays

A very small proportion of females in mating trays were mated over the entire trial period. Of the 14 nights listed in Table 9.4 only seven nights resulted in one or more females being mated.

Table 9.9 lists the nights when mating was recorded, the localities, and the number and proportion of females that were mated. A high recovery rate was achieved in most cases, with very few females missing or dead the morning after they were placed in the field. On two nights (11th April 2003 GF83, 3rd May 2003, GF83) a large proportion of females were killed in treated tomato by insecticide spraying; these locations and dates were not included in the analysis.

Table 9.9 List of dates, localities, number of female moths, and the percentage mated for those nights when mating was recorded in mating trays.

Date	Locality	Number Mated (Number Unmated)	Percentage Mated ± 95% confidence interval
2nd March	Control Capsicum CF11	1 (23)	4.2 ± 8.0
3rd March	Control Capsicum CF11	2 (22)	8.3 ± 11.1
	Partially Treated Tomato GF83	1 (23)	4.2 ± 8.0
8th April	Control Tomato RF75	1 (10)	9.1 ± 17.0
9th April	Control Tomato RF75	4 (6)	40.0 ± 30.1
10th April	Untreated Sugarcane 100 m Nth. GF83	4 (10)	28.6 ± 23.7
11th April	Untreated Sugarcane 100 m Nth. GF83	5 (9)	35.7 ± 25.1
2nd May	Untreated Sugarcane 400 m Nth. GF83	1 (8)	11.1 ± 20.5

No mated females were ever found from total of 233 females placed in trays in the treated areas, except for one female in a tomato block in which the application of dispensers had not been completed. The greatest contrast between treated and untreated areas was in the peak male activity periods in early April with up to 37% of females mated in one night in untreated tomato compared to zero in treated tomato. Over the entire trial period there was a significant difference ($\chi^2 = 54.66$, $P < 0.001$). Using the modified version of the mortality formula from Abbott (1925) 93.0% mating disruption in treated areas was achieved over the entire treatment period. The low numbers of females mated in untreated areas over the entire data set gives undue weight to the single mated female in the treated crops, and given that this female was in a partially treated field it is likely that the real level of mating disruption as judged by the mating trays was higher than the 93.0% measured.

9.3.2.5 Egg and larval counts

A feature of all of the egg and larval count data was the large variability within blocks in both treated and untreated areas. Figures 9.21 and 9.22 show the mean numbers of eggs (both white and brown) for each weekly check from March 3rd to June 8th 2003 in the treated and control blocks of tomato and capsicum respectively. Oviposition activity was low at the beginning of March, but by the end of the month there was a large increase in the number of eggs being laid in both capsicum and tomato. Overall, almost ten times more eggs were recorded in tomato than in capsicum. Larvae were rarely recorded in either crop in all blocks due to the extensive use of

insecticides (on average >2 per week per block). Because data was supplied as mean values per block it was not possible to compare individual weeks. However, comparing the data for treated vs control across all weeks post-treatment found no significant differences in tomato (ANOVA, df =1, F = 0.0694, p = 0.7928), but a significant reduction in egg counts in capsicum (ANOVA, df =1, F = 11.904, p < 0.001).

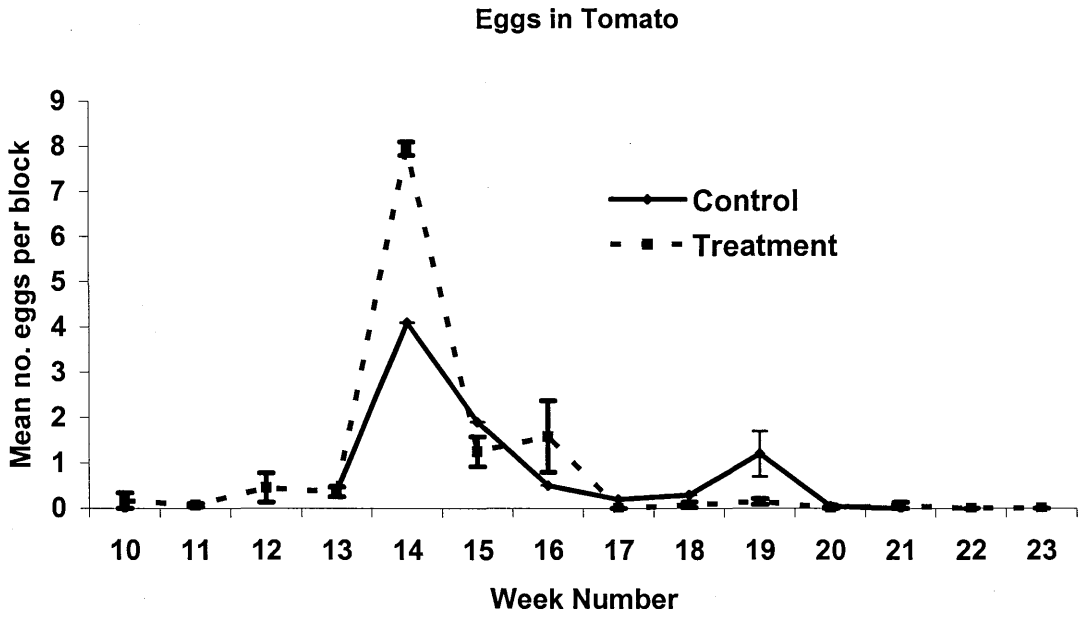

Figure 9.21 Mean ± standard error of eggs per check per week in tomato from Week 10 (Mar 3rd) to Week 23 (June 8th)

Although the lack of raw data made weekly statistical comparisons difficult there were some notable data present in the egg counts. In week 14 in tomato there was a reversal of the expected result, with many more eggs per plant counted in two treatment blocks (mean eggs per plant of 8.1 for RF71 and 7.8 for RF 72) compared to the control (mean eggs per plant of 4.3 for RF74/75). This contrasted strongly with capsicum for the same week where there was a mean of 0.32 ± 0.07 eggs per plant for five treated blocks and 0.5 ± 0.15 eggs per plant for three control blocks.

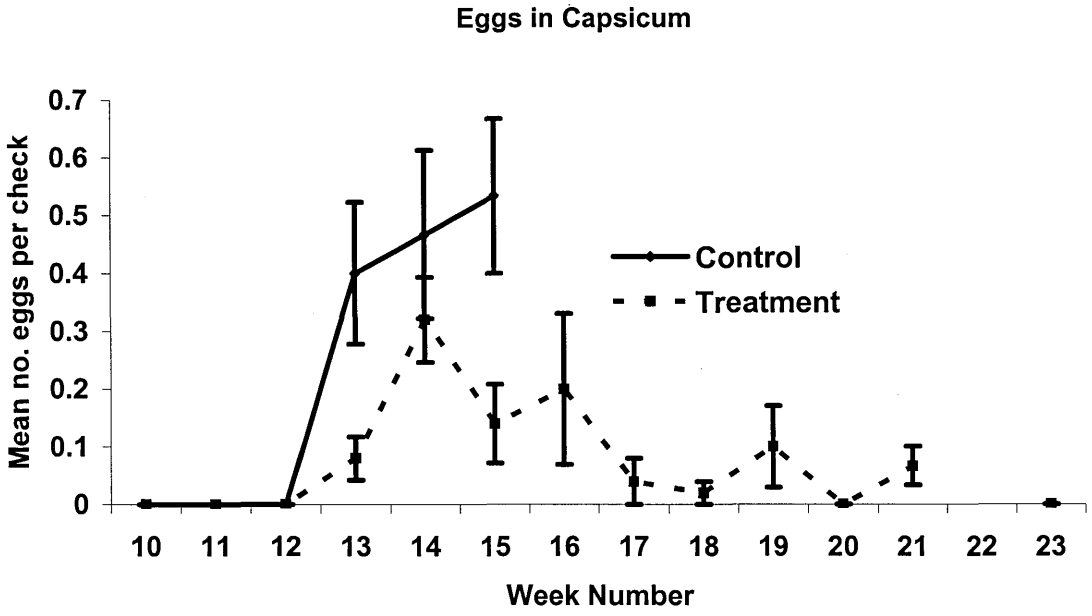

Figure 9.22 Mean ± standard error of eggs per check in capsicum per week from Week 10 (Mar 3rd) to Week 23 (June 8th)

9.3.3 Moth numbers and activity in sugarcane

Comparison of pheromone trap shutdown and mating tray data with egg counts and dissections of light-trapped females indicated that mated females were present in treated crops despite apparent disruption of mating behaviour. Results from sugarcane provided insights into this phenomenon.

An initial mating tray experiment was run over two nights (10th and 11th April) comparing wing-clipped females in trays located in sugarcane 100 m north of the treated tomato field GF83 (28 females) to trays located within the treated tomato block (26 females). The proportion of mated females in sugarcane over the two nights (32.14 ± 17.3%) was significantly greater ($\chi^2 = 23.84$, $P < 0.001$) than the zero mating in the adjacent treated tomato, suggesting that calling females in sugarcane could attract and mate with males even a short distance away from a pheromone-treated crop area.

This observation led to a more detailed experiment which aimed to determine whether wild females in sugarcane adjacent to treated tomato were being mated (as well as virgin wing-clipped females placed in mating trays in the sugarcane). Four light traps along a 400 m transect in sugarcane were run from 29th of April to the 5th of May and compared to four light traps in the treated tomato (GF83). Figure 9.16 shows the layout of these traps.

Females were caught in sugarcane light traps up to 330 m away from the treated tomato. The light trap at position 130 m was inoperable over three consecutive nights out of the seven trapping nights due to rain-damaged electronics. In contrast, there were significantly more males per night caught in light traps in the treated tomato compared to the sugarcane. Male moths were caught at all locations in the sugarcane, although the light traps at locations 230 m and 330 m caught only one male each. In general, the mean number of males caught per night was similar to the number of females caught in sugarcane light traps. Figure 9.23 shows the mean number of moths per trap per night for each sugarcane trap location and for the traps in the treated tomato.

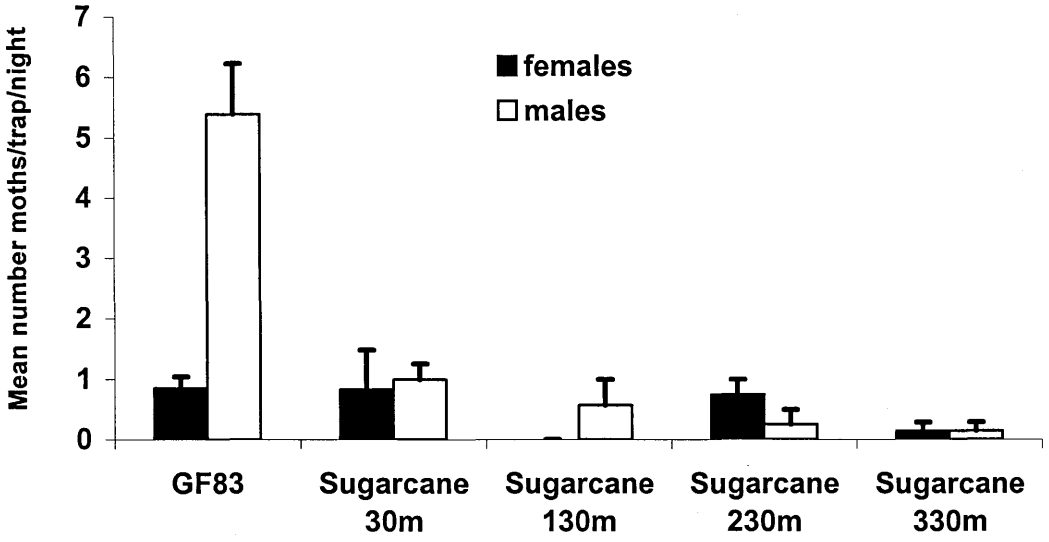

Figure 9.23 The mean numbers of *H. armigera* per night ± standard error for light trap catches in treated tomato (GF83) and along a transect in untreated sugarcane.

Table 9.10 lists details of the mating status of females caught in the light traps for each locality over all seven trapping nights. Only females caught in the sugarcane light trap 30 m from the treated tomato were mated, and only females caught in the treated tomato had more than one spermatophore present indicating multiple matings.

Table 9.10 Percentages of female *H. armigera* mated and numbers of multiple matings in light trap catches along a transect in sugarcane north of the treated tomato crop GF83

Locality	Total No. Females	Mated	% Mated	>1 Spermatophore
GF83	24	16	66.7 ± 18.9	4
Sugarcane 30 m	5	3	60.0 ± 42.9	0
Sugarcane 130 m	0	0	0	0
Sugarcane 230 m	3	0	0	0
Sugarcane 330 m	1	0	0	0

Results from the mating tray experiments in conjunction with light trapping showed that little mating occurred. Only one female out of a total of 134 wing-clipped virgin females placed in trays along the transect in the sugarcane was mated (400 m from the treated tomato) over the course of the experiment. None of the 133 females placed in the treated tomato were mated. This contrasts with the initial mating tray results described above when trays were placed 100 m into the sugarcane where 32% of the females were mated in the sugarcane compared to zero in the treated tomato.

9.3.4 Additional observations

Very few adult moths of either sex were collected during night observations (2nd, 7th, 8th April 2003, 28th March 2003). All of the females collected were mated (singletons from GF84, 2 April 2003, CF13, 7 April 2003, GF86 28 March 2003). Male searching flights were not observed above the canopy of treated crops, but these observations were not systematically compared to those in untreated tomato and capsicum due to time limitations in the field.

Searches of potential host plants for eggs and sweep net sampling for larvae on these plants indicated very few potential sources of adult *Helicoverpa* moths were present in and around the Promised Land region. The majority of the Promised Land region was planted to sugarcane prior to planting of the first tomato and capsicum crops in late January, 2003. Capsicum crops from late 2002 were present as undestroyed residue in two blocks (approx. 20 ha). This residue had been ploughed in by late February so that any remaining *Helicoverpa* pupae in the ground would have been destroyed. About 7 ha in the northwest of Promised Land were planted to watermelon, rock melon and honeydew melon, with sparse volunteer tomato plants present between the melon plants. These volunteer tomatoes would have been hosts to *Helicoverpa* larvae until early March, 2003 when these crops were ploughed in.

The sugarcane and the majority of the orchard area (Figure 9.1) were free of *Helicoverpa* host plants, although a newly planted citrus orchard at the northern tip (10 ha) had volunteer tomato and black nightshade *Solanum nigrum* (Solanaceae) from April 2003 onwards. *S. nigrum* is not a good host for *Helicoverpa* spp., and a concerted search (about 150 sweeps of a sweep net) of plants growing in this citrus orchard found only three larvae, whereas every volunteer tomato plant in this area had larvae, or showed signs of larval damage. A single larva was found feeding on new growth on a mandarin tree (*Citrus reticulata*). It was possible that this larva might have come from the surrounding *S. nigrum* plants, as *Citrus reticulata* is not a normal host plant for

Helicoverpa spp. Additional volunteer tomato was found growing between a green manure crop (sorghum). Forage sorghum (*Sorghum* sp.) is also a host plant for *Helicoverpa* spp., with eggs and larvae found during the pre-flowering and flowering stages of the crop. The crop was slashed and ploughed in prior to development of large larval populations. Searches of other weedy untreated areas throughout the Promised Land region failed to locate significant numbers of *Helicoverpa* eggs or larvae.

The forested areas surrounding the Promised Land region were also surveyed. This forested area is largely devoid of native *Helicoverpa* host plants with the exception of isolated and very low numbers of weedy hosts such as milkweed *Silybum marianum* (Asteraceae), and native daisies along the regularly maintained State Forest access roads. Several careful searches of the few potential host plants in the forest and roads failed to locate any immature stages of *Helicoverpa* spp.

9.3.5 Impact of weather on mating disruption

Weather parameters such as maximum, minimum and average temperatures, humidity and wind speed could not be consistently correlated with light and pheromone trap catches in the Promised Land region. Moths are affected by temperature, humidity and wind speed (Chapter 3); these parameters are not independent of each other, and this makes comparison of moth behaviour to weather variables problematic. Although low temperatures can inhibit female calling, and even lower temperatures can result in reduced catches at synthetic pheromone lures (Chapter 3.3.5) temperatures in the Promised Land region rarely dropped below thresholds for calling or response to sex pheromone.

9.3.6 Weathering of dispensers

Dispensers placed in the field on 7th of February 2003 had lost about 40% total weight of pheromone components after 28 days, and by 63 days they had lost 65% of the total weight of components, after which there was little further loss of pheromone components. Figure 9.24 shows the percentage loss of each pheromone component over the trial period. The minor component (*Z*)-9-hexadecenal was released at a slightly greater rate compared to the major component (*Z*)-11-hexadecenal. If the reduction in weight loss after 60 days reflects release rates the dispenser life-span would be no longer than this.

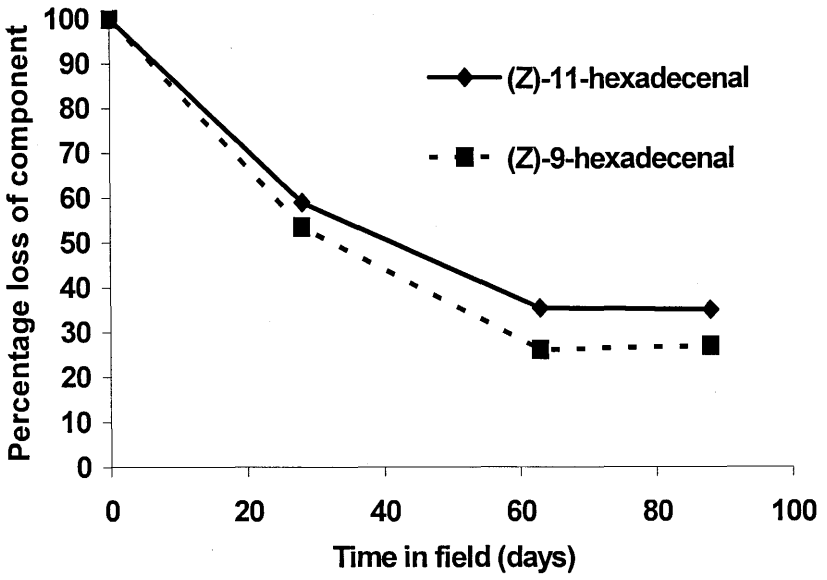

Figure 9.24 The percentage loss of pheromone components in field-weathered dispensers over an 88 day period.

9.4 Discussion

9.4.1 Data quality control issues

One of the main issues arising from monitoring of this mating disruption trial is that when there was less than one moth per trap per night it was difficult to ascertain how well the mating disruption treatment was working. This was particularly evident early on in the trial when there was only partial dispenser coverage of the Promised Land region. At this stage the weekly catches in pheromone traps in the control crops, particularly in control capsicum were very low. This meant that even singletons caught in traps in treated areas could greatly bias the percentage mating disruption so that it would appear that only 50% of disruption was occurring.

Females in mating trays often show low proportions of mating even when large numbers of male moths are present in the field (Kehat *et al.* 1998, Kvedaras 2002). It was not surprising that when male numbers were low in the field at Promised Land few or none of the females in mating trays were mated. Mating tray data obtained when wild males were abundant in the field can be assumed to reflect the real levels of mating disruption in the field. Additional problems occasionally arose due to confusion between farm staff and myself as to which field was being treated with insecticide. This resulted in some of the mating tray trials being sprayed with insecticide. Data from trials affected by insecticide were not included in the analyses, and as this

only occurred on two nights it can be assumed that insecticide usage did not change the overall conclusions obtained from analyses.

Another possible problem in interpreting the results of monitoring arises from variability due to the attractiveness of plants of differing phenology in different blocks. Plants of different ages are likely to vary in their attractiveness to both male and female moths due to the presence/absence of flowers and fruits which act both as a nectar resource (in the case of flowers) and as oviposition sites. The planting dates for both capsicum and tomato varied up to almost a month and a half apart between the first and last plantings. This variability within each crop may have been further exaggerated by the mixture of treated and untreated cropping areas within Promised Land. As it was not always possible to apply the dispensers immediately after planting there was often a delay of up to four weeks after planting before the crop was treated. This resulted in a mixture of treated, partially treated and untreated crops being present during February and March. However, despite this variability it was still clear that there was a strong mating disruption effect present within treated cropping areas within Promised Land.

The other critical issue relating to the interpretation of the results of this trial is the use of untreated blocks outside of the Promised Land region as control or "replicate" blocks. It might be possible to ascribe differences between the Promised Land data and the data collected from these untreated blocks to the geographic separation of the control blocks from Promised Land rather than to the treatments. The inability to provide a rigorous and independent control for comparison with the treated area is a common problem when attempting mating disruption and other area-wide trials in agricultural systems. The attempted BACI design was not fully implemented due to low moth numbers at the start of the trial. BACI designs still require that control areas are as similar as possible to the areas to be treated; in this respect the control areas for this trial were suitable for a BACI design. An additional weakness of the trial was that there was only one control area available for each crop. Ideally there should have been an additional one or two other separate control blocks for each crop (Underwood 1992). Even if these crops were available the logistics of monitoring additional control blocks with the available resources would have been difficult. Even with this problem, the trends observed in data from monitoring still show that a strong mating disruption effect was evident in treated areas.

The interpretation of the results from this trial was largely based on evident repeated differences between the treated and the control areas throughout the duration of the trial. Pheromone trap catches, light trap catches and mating tray data showed similar strong patterns of disruption in the

treated fields. However, more subtle differences such as the reduction of egg lay in capsicum and the reduction of multiple-mated females in the treated blocks may need to be treated with more caution.

A further method of interpretation of area-wide or large-scale trials is to repeat the same trial over several seasons. If the same patterns persist in the treated versus untreated areas it strengthens the argument that the treatment is generating a response. This method using repetition can be further strengthened by doing the same experiment in different geographic regions, or on different host crops. This requires much more time and resources than were available within the current trial framework.

9.4.2 Feasibility of mating disruption for Helicoverpa armigera

The results observed here highlight the potential difficulties in using mating disruption to control this highly mobile and fecund species. There was no reduction in oviposition in tomatoes, and the slight reduction in egg lay which was achieved in capsicum still left egg densities well above the spray threshold used for that crop.

It is likely that movement of females into the treated areas was the major reason for this failure. Influx of mated females is the usual rationale for the failure of mating disruption with mobile moth species such as *H. armigera*, and is thought to be the reason why mating disruption trials such as those in cotton in Pakistan (Chamberlain *et al.* 2000), Australia (Betts *et al.* 1992, 1993), and Israel (Kehat & Dunkelblum 1993) have failed to achieve reduction in infestation of the crop. It was hoped that if there were any females immigrating into the Promised Land region from distant sources, they would be unmated, and males would then be unable to locate them in the treated fields. Long-range migratory behaviour in *H. armigera* is seen as a facultative response to changes in host plant availability, and is generally considered to be pre-reproductive (Riley *et al.* 1992, Colvin & Gatehouse 1993a,b, Gatehouse & Zhang 1995). The Promised Land region is unusual among Australian agricultural areas in that it is relatively isolated from other sources of *H. armigera.* Whether this isolation requires moths to undergo long-range migratory behaviour in order to access the crops there is unknown. Normal short-range flights (including those by mated females) recorded in Australian agroecosystems tend to be over distances of up to 6 km per night (Del Socorro & Gregg 2001). The distance between Promised Land and the surrounding cropping areas is greater than 6 km. It is however possible that the forested regions may be attractive to adult moths as nectar sources. Many of the eucalypts flower for much of the year within the

region; and these could be attractive to *Helicoverpa* adults for feeding as shown by the presence of large numbers of eucalypt pollen in moth proboscis (Gregg 1993) and the attractiveness of eucalypt bouquets to moths in olfactometer studies (Del Socorro & Gregg 2002). Hence, the absence of larval host plants may not necessarily mean that the forested regions around Promised Land are unattractive to adult *Helicoverpa*.

Future work to determine the origin of the female moths collected in the treated crops at Promised Land and from surrounding cropping areas could use carbon/nitrogen isotope analysis which can provide clues as to the origin of the moth population. This analysis can reveal if larvae have developed on C3 (eg. tomato, capsicum, legumes) or C4 (eg. sorghum, corn) crops (Gould *et al.* 2002). Microsatellite DNA analysis of adult moths can also be used to determine the origin of migratory individuals. By determining genetic markers unique to certain geographic populations of *H. armigera* it may be possible to identify whether moths are local, or have flown in from another region. Scott *et al.* (2003, 2004) have demonstrated that it is possible to trace the origins of *H. armigera* and *H. punctigera* populations in south-western Queensland using microsatellites.

It was also assumed that females would not mate away from the treated crop. In general, animals often focus reproductive activities around resources which are required for their growth and development, and in the case of plant-feeding insects such as *Helicoverpa* these are the host plants suitable for larval development. In this respect there would seem to be an obvious association between reproductive behaviour and host plants. This assumption is supported by previous work with the closely related *Helicoverpa zea* where females produce more pheromone when associated with larval host plant species and the plant volatiles produced by larval host plants (Raina *et al.* 1992, Light *et al.* 1993). Raina *et al.* (1992) noted that *H. zea* required the volatile chemical signals from corn silk to trigger production of sex pheromone. A similar result was obtained by Raina *et al.* (1997) for another related species, *Heliothis virescens*. The related North American species *H. phloxiphaga* was even more specialised, and required the presence of the host plant *Castilleja indivisa* (Texas paintbrush) for pheromone production (Raina 1988). Kvedaras (2002) found that while pheromone production by *H. armigera* was increased in the presence of plant volatiles, the effects were not as strong as those recorded in the above mentioned species.

There is also evidence that the response of males to pheromone is affected by the presence of host plants. Light *et al.* (1993) found that *H. zea* males were more responsive to traps baited with both pheromone and host plant volatiles compared to traps baited with pheromone alone. Dickens *et al.* (1993) found a similar response when adding green leaf volatiles to pheromone blends for *Hs.*

virescens, and Meagher and Mitchell (1998) found that addition of the floral volatile phenylacetaldehyde increased upwind flight towards pheromone sources in wind tunnels. However, stimulation of male reproductive behaviour by plant volatiles may not be universal (Meagher 2001b) and *H. armigera* may be one species where the link is weak (Kvedaras 2002). These studies suggest that the association between host plants and the initiation and success of reproductive behaviour might not be as strongly expressed in *H. armigera* as it appears to be in other *Helicoverpa* and *Heliothis* species.

The assumption in the Promised Land study that females would mate only in the presence of host plants (tomato and capsicum) would appear to be flawed. The data obtained from both light traps and mating trays placed in sugarcane strongly suggest that virgin females were either moving from the treated crop areas into non-host plants around the treated crops where they then attract and mate with males, or were flying in (unmated) from regions outside of the Promised Land region, mating in sugarcane, then moving into the treated crops. Published results that show that mating disruption in a mosaic of host and non-host crops might require that the non-host crops be treated with mating disruption dispensers as well as the host crops appear to be scarce. An exception is the European Corn Borer *Ostrinia nubilalis* which favours grassy borders around corn fields for mating in preference to within the crop itself (Showers *et al.* 1976, Showers *et al.* 1980). Mating disruption trials for this species have exploited this behaviour by placing dispensers in the border vegetation instead of within the crop (Baker *et al.* 1997). The results presented in this chapter suggest that a re-evaluation of the dominant paradigm for area-wide mating disruption may be appropriate.

In previous studies the success of mating disruption in *H. armigera* in terms of reduced oviposition in the treated areas has been limited. Chamberlain *et al.* (2000) treated an approximately 2 x 2 km square area which contained cotton and cotton inter-planted with mango and citrus in Pakistan. They used the same type of dispensers as for the Promised Land trial and monitored adults and sampled immature stages along transects throughout the treated area. They did not report a significant reduction in the number of eggs laid or damage levels within the treated area. A second trial was proposed which would have used a 10 x 10 km square treated area to ensure a significant reduction in egg lay in crops situated in the centre of the treated area. This was not considered to be economically viable and the trial has not taken place (D. Chamberlain pers. comm.). An earlier mating disruption trial conducted in Australia (Betts *et al.* 1992) used a smaller area (30 ha) than either the Pakistani trial or the Promised Land trial, and obtained similar negative results in relation to egg lay in treated areas.

The one exception to the rule of commercial failure in trials of mating disruption for *H. armigera* is a trial in Japanese lettuce crops (Toyoshima *et al.* 2001). Diamolure dispensers which have a 125 mg loading of 36.0% (*Z*)-11-hexadecenal, 41.0% (*Z*)-11-hexadecenyl acetate and 23% stabilizer were used to treat both small (3 ha) and large (20 ha) lettuce fields. As with other mating disruption trials for *H. armigera* they readily obtained pheromone trap shutdown and reduced mating rates for tethered females in the treated areas, but they also demonstrated lower damage levels in treated lettuce in the 20 ha field. The authors did not mention what vegetation type surrounded the treated areas, but the control lettuce fields (600 ha) were only 500 m from the treated fields. Note that in this trial the dispensers contained the component (*Z*)-11-hexadecenyl acetate, which is not a major component of *H. armigera* pheromone and was not present in the dispensers used in the Promised Land trial or those previously reported in the literature. Traces of (*Z*)-11-hexadecenyl acetate have been recorded from *H. armigera* females in a Russian study (Konyukhov *et al.* 1984) but the biological activity in respect to *H. armigera* is unclear. It is similarly unclear whether the reported success of the Japanese trial is due to the presence of this compound or to the ecological characteristics of the trial site in Japan.

Despite the negative results associated with this trial some positives were established. It was clearly evident that the Selibate™ HA dispensers were effective at disrupting mating within treated areas, and that this disruption was maintained during high adult moth populations in both tomato and capsicum. This disruption gave significantly reduced egg lay in capsicum for part of the trial, but was not sufficient to result in reduced levels of conventional insecticide applications.

There were some notable results present in the egg count data. On week 14 of the trial there was considerably more egg lay in the treated tomato compared to control tomato, yet at the same time there was a reduction in the number of eggs laid in treated capsicum compared to the control blocks. The results obtained from observations in the sugarcane suggest that moths of both sexes were leaving areas treated with mating disruption dispensers, then mating on non-hosts. It is possible that mated females were then associating with the preferred host tomato rather than capsicum, causing a local increase in the population density of mated females in tomato, whilst reducing the number of eggs laid in capsicum. There is clearly a need for more work on responses of moths in and around areas treated with mating disruption, as this behavior could potentially be exploited by using a push/pull strategy by utilising trap crops or female attract and kill.

The majority of females collected from treated areas had only mated once, compared to females from untreated regions, most of which had mated more than once. This result is similar to that obtained for the trial with *H. armigera* and Selibate™ HA in Pakistan (Chamberlain *et al.* 2000). Although this reduction in mating frequency did not appear to give significant reductions in egg laying in this trial, it might assist in reducing the overall egg load on crops if mating disruption is used in conjunction with other pest management tactics.

For the two crops in this trial the life-span of dispensers (60 days) should require that two applications be made over the growing period. Labour costs would be an important consideration if the same type of dispensers were to be continued, but the relatively quick and reliable methods developed during this trial for placing the dispensers in the field should reduce the overall labour costs. In addition, the formulation could be altered to create dispensers that are designed for specific use in capsicum and tomato. An example of this might be a tubular dispenser which fits snugly over the top of a small wooden or plastic stake that could be pushed into the ground (Nick Brown, Business Manager, AgriSense BCS Pty. Ltd., pers. comm.).

The alternative electrostatic pheromone technology mentioned earlier (Exosect Limited 2003) may also offer benefits over conventional mating disruption. This method relies on "autoconfusion" where males visit pheromone stations, are coated with pheromone-laden electrostatic powders, and are reproductively neutralized as well as possibly contributing to mating disruption. This would mean that even when these contaminated moths leave the immediate treated region they would still be prevented from mating. Such an approach might reduce the frequency of mating in adjacent non-host crops (such as sugarcane in the Promised Land area).

10 General discussion – Is there a future for sex pheromones in IPM of *H. armigera*?

This thesis addresses the potential application of sex pheromone in the control of a mobile polyphagous pest, *Helicoverpa armigera*. In this chapter the major findings are summarised and a synthesis of the overall findings is made. Novel results are highlighted, and their implications for future research are discussed.

10.1 Major findings

- **Chapter 3:** The behaviour of male *H. armigera* at synthetic pheromone lures was observed in the field. Male activity at the lures was influenced by various factors such as diel periodicity, type of and stage of crop present, season, % relative humidity, wind run and overall climatic conditions. Of these factors the type of crop and seasonal factors were associated with the largest changes in number of moths flying to lures, and the percentage of moths contacting the lures. Despite the expectation that temperature would affect both pheromone release and moth activity, temperature by itself did not have a significant influence on male behaviour.
- **Chapter 4:** The effect on male *H. armigera* behaviour of lure formulation, appearance and presentation method was evaluated in the field. The performance of the synthetic lure was compared with captive female *H. armigera* to determine if there were components missing from the synthetic blend which might influence male behaviour. Observations indicated that the synthetic blend of 10:1 ratio of (*Z*)-11-hexadecenal and (*Z*)-9-hexedecanal was as effective or better than calling females and other tested blends The percentage of males contacting the lure could be increased by placing a dead decoy female next to the lure, by placing the lure on a natural substrate and by increasing the active surface of the lure by smearing it or placing it as many small droplets. The addition of a synthetic pyrethroid (bifenthrin) did not significantly repel males from sex pheromone lures.
- **Chapter 5:** The laboratory toxicology of bifenthrin in Sirene was evaluated. A concentration of 6% bifenthrin gave close to 100% mortality over 4 h. Concentrations less than 1.5% were also effective, but took over 14 h to achieve > 90% mortality. Males treated with sublethal concentrations of > 0.01% were 50% less likely to mate than control males, but other reproductive parameters such as success of mating as measured by production of fertile eggs were not affected.

- **Chapter 6:** The toxicity of formulations was compared in field conditions using a passive field wind tunnel. The 6% bifenthrin and 1% pheromone formulation provided the best result when presented with a dead decoy female moth. Mortality in the field wind tunnels was much lower than expected. This appeared to be due to the experimental conditions of the field wind tunnel altering the male behaviour.
- **Chapter 7:** Weathering of the Sirene-based pheromone formulations was studied under field conditions. The estimated life-span of 200 mg droplets was between 4-6 weeks. The rate of pheromone release increased by more than double when the formulation was smeared over the substrate.
- **Chapter 8:** A mark-recapture study of male behavior in 21 ha field of flowering sorghum found that populations of male *H. armigera* were non-resident, with large fluctuations between consecutive nights. The number of males present varied from 97 to 4,008 per ha per night within the field over the course of ten nights. Extrapolation from population estimates obtained in this study indicated that with only twelve attracticide sources in a 21 ha field it was still possible to remove 10% of males present when moth numbers were very low. At higher densities the number of lures would have to be increased greatly, as only 2% of males would be killed at the highest population density measured. The technique used in this study allows for an estimate of turnover on a nightly basis, and could be useful in other studies of insect movement.
- **Chapter 9:** A full-scale mating disruption trial of *H. armigera* on tomato and capsicum was carried out in an isolated cropping area. Mating was reduced to virtually zero in treated areas, and there was a significant reduction in the number of eggs laid in capsicum crops, and in the number of spermatophores per female in tomato crops. However, there was no sufficient decrease in egg lays in any of the treated areas that would allow any decrease in the frequency or application of conventional insecticidal sprays. Mating was occurring on non-host plants adjacent to the treated areas, and mated females were flying back into treated areas to lay eggs. This contrasts with other findings that heliothine and other noctuid moths prefer to mate on host plants, and places yet another constraint on employing sex pheromone to control *H. armigera*.

10.2 Synthesis and recommendations

10.2.1 Potential for attract and kill in cotton and associated field crops

The recommended attract and kill formulation is the protective base of Sirene, with 1% synthetic blend of *H. armigera* pheromone [10:1 (Z)-11-hexadecenal:(Z)-9-hexadecenal] and 6% bifenthrin (all weight/volume). This formulation did not demonstrate any repellent effects of bifenthrin to moths. The appearance and presentation of the lure formulation could be manipulated to increase the rate of contact of moths with the formulation. Increasing the active surface area of the lure could be practical in real agricultural systems, but manipulating the complex visual stimuli would not appear to be a realistic option. The interacting factors of season, weather and the type of crop treated impact upon the effectiveness of formulations, as all three factors influenced the number of males attracted to the formulations and the percentages of those males that contacted the lures.

When placed in the field as 200 mg droplets this formulation would kill approximately 10% of males which approached the lures. The males which contacted, but did not receive lethal doses would be incapacitated in regards to mating. Males contacting formulations with concentrations as low as 0.01% bifenthrin were prevented from mating over 12-48 hrs. However, higher concentrations, such as the recommended 6% bifenthrin, resulted in rapid incapacitation and death of males within 1-2 hrs. The number of adult males in the field as estimated in an attractive crop (flowering sorghum) ranged between 97 and 4,008 males per hectare per night. Using a simplistic model (Equation 4.1) with a population density of 4,500 moths per hectare, 99% control could be obtained with 44 droplets per hectare. It is not likely that droplets will work with the full 10% efficacy, especially after weathering of the formulation occurs, so a recommended application rate would be 75 and 100 droplets per hectare.

The functional life-span of the formulations in field conditions would be one month, after which the efficacy would decrease markedly. Crops such as cotton and tomato which are vulnerable to *Helicoverpa* attack for up to twelve weeks would require one or more additional treatments throughout the growing season.

10.2.2 Potential for mating disruption in cotton and associated field crops

The field conditions at Promised Land where the mating disruption trial was conducted were close to ideal for successful control using this technique against *H. armigera*. The isolation of the cropping region, the lack of alternative host plants for the target pest, and the ability to cover all of the vulnerable crop species with pheromone dispensers would have been highly favourable for

successful mating disruption of *H. armigera*. However, as reported in this thesis, there was significant mating occurring outside of the treated areas on non-host plants. If the results of mating disruption were to be improved, it would be necessary to treat all of the non-host plants surrounding the treated host crops. In this study, these non-host plants would potentially include sugar cane, native vegetation and orchards. The economics and logistics of this exercise would prevent the adoption of this approach at this time. Another suggested approach is to put all of the crops together to form a single mating disruption unit, but in the context of Promised Land this is not feasible because of increased risk of significant hail damage to crops.

The problem of mating occurring outside of the treated crop could also occur in an attract and kill program, but there are two factors which might mitigate in favour of attract and kill. The first is that males from within the treated crops are removed from the population, so they do not have the opportunity to mate on the periphery of the field. The other is that attract and kill lends itself to application on widely different plant species. As seen with the mating disruption trial here it is not always easy to place the large dispensers on the crop. The required densities, and the time needed to place the dispensers also act against mating disruption techniques. A liquid formulation like Sirene can be placed on trees, herbaceous plants, and even on large grasses. Attract and kill using formulations as suggested here would almost certainly be superior to mating disruption in this situation, but as discussed in the next section there is potential to combine mating disruption, attract and kill and other strategies to get even better results.

10.2.3 Attract and kill and mating disruption in IPM

As discussed above, attract and kill techniques can be used instead of mating disruption. A better method might be to use a combination of mating disruption dispensers within the attractive host crops to prevent mating, and an attract and kill formulation around the perimeter of the treated crops. This might be the Sirene-based formulation developed here, which would remove males searching for females on non-host crops on the perimeter of the treated areas. An attract and kill formulation based on plant volatiles (Magnet®) which kills both sexes of *H. armigera* and other noctuid pest species, might also be useful (Del Socorro *et al.* 2003). Large scale field trials in cotton and other crops have shown that relatively small amounts of Magnet® can significantly reduce populations of female moths over a large area. Plant volatile-based attractants such as this could be sprayed onto bordering non-host vegetation on the perimeter of the mating disruption treated crop to kill moths of both sexes that venture out of the treated crop to find mates. In addition to this, any influx of mated females from external sources into the area surrounding the treated crop might also be prevented from entering the mating disruption treated area. The current

plant volatile formulations have not been tried on horticultural crops such as tomato or capsicum. However, an application for a research permit which would allow such uses has recently been approved by the Australian Pesticides and Veterinary Medicines Authority.

The results of monitoring in the mating disruption trial suggest that another effect of mating disruption might be to "push" unmated females and males out of the treated areas. This might also concentrate mated females into certain other areas. This effect has been inferred from egg counts from control and treated areas in week 14 of the mating disruption trial at Promised Land, where the number of eggs increased in treated tomato blocks compared to control, whilst the opposite occurred in capsicum. It is possible that moths which had been in the treated blocks of both crops were being pushed into surrounding untreated areas to find mates. Mated females were then concentrated in treated tomato due to oviposition preferences, whilst the capsicum was left with fewer mated females. By better understanding these changes in local population density induced by mating disruption it may be possible to design applications of attract and kill around areas protected by mating disruption so as to maximise the number of both male and female moths removed from the population. It may be possible, for instance, to use an attractive trap crop to concentrate the moths which leave the treated areas into a management area where they can be killed by insecticide or other means.

The apparent utility of sex pheromones for management of mobile, fecund pests such as *H. armigera* may seem somewhat limited due to the many constraints which the ecology and behaviour of these pests place upon techniques such as mating disruption and attract and kill. However, there does seem to be great potential for inclusion of sex pheromone-based control into a more complex and refined IPM plan along with other semiochemicals, selective insecticides, pathogen dispersal systems, improved cultural methods and better integration of monitoring techniques. Area-wide management is becoming more common in agricultural systems (Lingren *et al.* 1998, Fitt 2000), and this greatly increases the possibility of including sex pheromone in IPM programs in a variety of guises from monitoring through to attract and kill. Although there will never be a "one-shot" miracle sex pheromone-based product to control *H. armigera*, it is almost certain that sex pheromones will become even more important as tools for managing this pest.

11 References

Abbott, WS. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**, 265-267.

Adamson D, Thomas G & Davis E. 1997. *An economic estimate of Helicoverpa's effect on Australian agricultural production*. CRC for Tropical Pest Management, Brisbane.

Agee HR 1971. Ultrasound produced by the wings of adults of *Heliothis zea*. *Journal of Insect Physiology* **17**,1267-1273.

Albajes, R, Konstantopoulu, M, Etchepare, O, Eizaguirre, M, Frerot, B, Sans, A, Krokos, F, Ameline, A & Mazomenos, B. 2002. Mating disruption of the corn borer *Sesamia nonagrioides* (Lepidoptera : Noctuidae) using sprayable formulations of pheromone. *Crop Protection.* **21**, 217-225.

Alloway, TM. 1972. Learning and memory in insects. *Annual Review of Entomology* **17**, 43-56.

Alway, T. 1998. Codling moth mating disruption and its role in Washington orchard pest management programs. Presented at the 41st Annual IDFTA Conference, February 21-25, 1998, Pasco, Washington, http://www.idfta.org/cft/1998/vol31no3/alway/alway.pdf, accessed July 2002.

Angeli, G & Ioriatti, C. 2000. Evaluation of an attract and kill formulation to control *Cydia pomonella* L. (Lepidoptera: Tortricidae). Proceedings of IOBC wprs Working Group Meeting, Samos, http://www.phero.net/iobc/samos/abstr/angeli.html, accessed 17 January 2001.

Arn, H, Tóth, M & Priesner, E. 2000. The Pherolist: List of Sex Pheromones of Lepidoptera, http://phero.net/index.html, accessed 6 March 2005.

Arredondojimenez, JI, Rodriguez, MH, Loyola, EG & Bown, DN. 1997. Behaviour of *Anopheles albimanus* in relation to pyrethroid-treated bednets. *Medical & Veterinary Entomology* **11**, 87-94.

Asaro, D & Berisford, CW. 2001. Seasonal changes in adult longevity and pupal weight of the nantucket pine tip moth (Lepidoptera: Tortricidae) with implications for interpreting pheromone trap catch. *Environmental Entomology* **30**, 999-1005.

Australian Pesticides and Veterinary Medicines Authority. 2004. Record of approved active constituents for chemical products, http://www.apvma.gov.au/actives/aa_A-K.pdf, http://www.apvma.gov.au/actives/aa_L-Z.pdf, accessed 27 January 2005.

Australian Pesticides and Veterinary Medicines Authority. Updated nightly. PUBCRIS - Registered Products Database, http://www.apvma.gov.au/pubcris/subpage_pubcris.shtml, accessed 27 January 2005.

Baker, TC. 1983. Variations in male oriental fruit moth courtship patterns due to male competition. *Experientia* **39**, 112-114.

Baker TC. 2004. Mating disruption of several lepidopterous pests using high-emission-rate widely spaced dispensers. Proceedings of 22nd International Congress of Entomology, 15-21 August 2004. CD-ROM, Brisbane, Queensland, Australia.

Baker, TC, Dittl, T & Mafra-Neto, A. 1997. Disruption of sex pheromone communication in the blackheaded fireworm in Wisconsin cranberry marshes by using MSTRS™ devices. *Journal of Agricultural Entomology* **14**, 449-457.

Baker, TC, Mafra-Neto, A, Dittl, T, & Rice, ME. 1997. A novel controlled-release device for disrupting sex pheromone communication in moths. *Technology Transfer in Mating Disruption, IOBC Working Papers Bulletin, Montpellier*, **20**, 141-149.

Baker TC, Haynes KF. 1989. Field and laboratory electroantennographic measurements of pheromone plume structure correlated with oriental fruit moth behavior. *Physiological Entomology* **14**,1-12.

Bartell, RJ. 1982. Mechanisms of communication disruption by pheromone in the control of Lepidoptera: A review. *Physiological Entomology* **7**, 353-364.

Bartlett, C. 1985. An olfactometer for measuring the repellent effect of chemicals on the stable fly *Stomoxys calcitrans* (L.). *Pesticide Science* **16**, 479-487.

Batiste, WC. 1970. A timing sex-pheromone trap with special reference to codling moth collections. *Journal of Economic Entomology* **63**, 915-918.

Betts, MD, Gregg, PC, Fitt, GP, and McQuillan, MJ. 1992. Mating disruption for *Heliothis*: a feasible option? Australian Cotton Growers Research Association. Brisbane, pp. 283-288.

Betts MD, Gregg PC, Fitt GP & MacQuillan MJ. 1993. A field trial of mating disruption for *Helicoverpa* spp. in cotton. In: *Pest Control and Sustainable Agriculture*, (eds. SA Corey, DJ Dall, & A Milne), pp. 298-300. CSIRO, Melbourne.

Birch, MC & Hefetz, A. 1987. Extrusible organs in male moths and their role in courtship behavior. *Bulletin of the Entomological Society of America* **33**, 222-229.

Bowden, J. 1982. An analysis of factors affecting catches of insects in light-traps. *Bulletin of Entomological Research* **72**, 535-556.

Bowden, J & Johnson, CG. 1976. *Migrating and other terrestrial insects at sea.* North-Holland Publishing Co. Amsterdam.

Bowden, J & Morris, MG. 1975. The influence of moonlight on catches of insects in light traps in Africa. III. The effective radius of a mercury-vapour light-trap and the analysis of catches using effective radius. *Bulletin of Entomological Research* **65**, 303-348.

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

Buleza, VV, Myttus, ER, Kovaleva, AS, Kagan, MZ, Kravchenko, VD & Apasov, SG. 1983. Flight of the corn earworm *Helicoverpa armigera* to components of the sex pheromone. *Doklady Akademii Nauk SSSR* **272**, 244-247.

Butenandt, A, Beckmann, R, Stamm, D & Hecker, E. 1959. Über den sexuallockstoff des seidenspinners *Bombyx mori*. Reindarstellung und konstitution. *Zeitschrift für Naturforschung* **14b**, 283-284.

Byford, RL, Lockwood, JA, Smith, SM & Franke, DE. 1987. Redistribution of behaviorally resistant horn flies (Diptera: Muscidae) on cattle treated with pyrethroid-impregnated ear tags. *Environmental Entomology* **16**, 467-470.

Cameron, PJ, Walker, GP & Herman, TJB. 1995. Status of resistance to fenvalerate in tomato fruitworm, *Helicoverpa armigera*. *New Zealand Journal of Crop and Horticultural Science* **23**, 429-436.

Campanhola, C & Plapp, FWJ. 1989. Pyrethroid resistance in the tobacco budworm (Lepidoptera: Noctuidae): insecticide bioassays and field monitoring. *Journal of Economic Entomology* **82**, 22-28.

Cardé RT. 1990. Principles of mating disruption. In: *Behaviour-modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants* (eds RL Ridgway, RM Silverstein & MN Inscoe) pp. 47-71. Marcel Dekker Inc., New York.

Cardé, RT, Charlton, RE, Wallner, WE & Baranchikov, YN. 1996. Pheromone-mediated diel activity rhythms of male Asian gypsy moth (Lepidoptera: Lymantriidae) in relation to female eclosion and temperature. *Annals of the Entomological Society of America* **89**, 745-753.

Cardé, RT & Hagaman, TE. 1979. Behavioral responses of the gypsy moth in a wind tunnel to air-borne enantiomers of disparlure. *Environmental Entomology* **8**, 475-484.

Cardé RT & Mafra-Neto A. 1996. Mechanisms of flight of male moths to pheromone. In: *Insect pheromone research: new directions* (eds RT Cardé &AK Minks) pp. 275-290. Chapman & Hall, New York.

Cardé, RT & Minks, AK. 1995. Control of moth pests by mating disruption: successes and constraints. *Annual Review of Entomology* **40**, 559-585.

Cardé, RT & Roelofs, WL. 1973. Temperature modification of male sex pheromone response and factors affecting female calling in *Holomelina immaculata* (Lepidoptera: Arctiidae). *Canadian Entomologist* **105**, 1505-1512.

Cardé, RT, Staten, RT & Mafra-Neto, A. 1998. Behaviour of pink bollworm males near high-dose, point sources of pheromone in field wind tunnels: insights into mechanisms of mating disruption. *Entomologia Experimentalis et Applicata* **89**, 35-46.

Carpenter, JE & Sparks, AN. 1982. Effects of vision on mating behavior of the male corn earworm moth. *Journal of Economic Entomology* **75**, 248-250.

Carson R. 1962. *Silent Spring*. Hamish Hamilton, London.

Castrovillo, PJ & Cardé, RT. 1980. Male codling moth (*Laspeyresia pomonella*) orientation to visual cues in the presence of pheromone and sequences of courtship behaviors. *Annals of the Entomological Society of America* **73**, 100-105.

Centner M 1983. The analysis of chemical communication in *Heliothis armiger* (Lepidoptera: Phalaenidae). In: *Proceedings of the Fourth Entomological Congress*. pp. 39-40, Pretoria, South Africa.

Chamberlain, DJ, Brown, NJ, Jones, OT & Casagrande, E. 2000. Field evaluation of a slow release pheromone formulation to control the American bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. *Bulletin of Entomological Research* **90**, 183-190.

Chandler, J. 2003a. A call for common sense. *Organic Farming* 24-25.

Chandler, J. 2003b. Pheromones fall foul of EU pesticide directives. *Pesticides News* **59**, 10-11.

Charlton, RE & Cardé, RT. 1990. Orientation of male gypsy moths, *Lymantria dispar* (L.) to pheromone sources: The role of olfactory and visual cues. *Journal of Insect Behavior* **3**, 443-469.

Charlton, RE, Kanno, H, Collins, RD & Cardé, RT. 1993. Influence of pheromone concentration and ambient temperature on flight of the gypsy moth *Lymantria dispar* (L.), in a sustained flight wind tunnel. *Physiological Entomology* **18** , 349-362.

Charmillot, P. J. and Hofer, D. 1997. Control of codling moth, *Cydia pomonella* L., by an attract and kill formulation. *Technology Transfer in Mating Disruption, IOBC Working Papers Bulletin, Montpellier*, **20**, 139-140.

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

Charmillot, PJ, Pasquier, D & Hofer, D. 2000b. Control of codling moth *Cydia pomonella* by autosterilisation, Proceedings of IOBC wprs Working Group Meeting, Samos, http://www.phero.net/iobc/samos/abstr/charmillot.html, accessed 17 January 2001.

Charmillot, PJ, Pasquier, D, Scalco, A & Hofer, D. 1996. Essais de lutte contre le carpocapse *Cydia pomonella* L. par un procédé attracticide. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* **69**, 431-439.

Colvin, J, Cooter, RJ & Patel, S. 1994. Laboratory mating behavior and compatibility of *Helicoverpa armigera* (Lepidoptera: Noctuidae) originating from different geographical regions. *Journal of Economic Entomology* **87**, 1502-1506.

Colvin, J & Gatehouse, AG. 1993a. Migration and genetic variation of the pre-reproductive period in the cotton-bollworm moth, *Helicoverpa armigera*. *Heredity* **70**, 407-412.

Colvin, J & Gatehouse, AG. 1993b. The reproduction-flight syndrome and the inheritance of tehtered-flight activity in the cotton-bollworm moth, *Heliothis armigera*. *Physiological Entomology* **18**, 16-22.

Common, IFB. 1953. The Australian species of *Heliothis* (Lepidoptera: Noctuidae) and their pest status. *Australian Journal of Zoology* **1**, 319-344.

Common, IFB. 1954. A study of the ecology of the adult Bogong moth, *Agrotis infusa* (Boisd.) (Lepidoptera: Noctuidae), with special reference to its behaviour during migration and aestivation. *Australian Journal of Zoology* **2**, 223-263.

Common IFB. 1990. *Moths of Australia*. Melbourne University Press, Carlton, Victoria.

Coombs, M. 1992. Diel feeding behaviour of *Helicoverpa punctigera* (Wallengren) adults (Lepidoptera: Noctuidae). *Journal of the Australian Entomological Society* **31**, 193-197.

Coombs, M. 1993. Endothermy and flight thresholds for *Helicoverpa punctigera* and *H. armigera* (Lepidoptera: Noctuidae). *Australian Journal of Zoology* **41**, 577-587.

Coombs, M, Del Socorro, AP, Fitt, GP & Gregg, PC. 1993. The reproductive maturity and mating status of *Helicoverpa armigera*, *H. punctigera* and *Mythimna convecta* (Lepidoptera: Noctuidae) collected in tower-mounted light traps in northern New South Wales, Australia. *Bulletin of Entomological Research* **83**, 529-534.

Cork, A, Hall, DR, Mullings, JL & Jones, OT. 1989. A new PVC resin formulation for controlled release of insect pheromones. In: *Proceedings of the 16th International symposium on controlled release of bioactive materials.* Chicago, USA.

Cork, A & Lobos, EA. 2003. Female sex pheromone components of *Helicoverpa gelotopoeon*: first heliothine pheromone without (Z)-11-hexadecenal. *Entomologia. Experimentalis et Applicata*, **107**, 201-206.

Cox, PG & Forrester, NW. 1992. Economics of insecticide resistance management in *Heliothis armigera* (Lepidoptera: Noctuidae) in Australia. *Journal of Economic Entomology* **85**, 1539-1550.

Cunningham, JP, Jallow, MFA, Wright, DJ & Zalucki, MP. 1998. Learning in host selection in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Animal Behaviour* **55**, 227-234.

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.

Czokajlo, D., Daterman, G., Eglitis, A., Sack, C., and Kirsch, P. A. Session J: Chemical Ecology in Forest Ecosystem. Attract and kill technology for management of european pine shoot moth, *Rhyacionia buoliana*, and western pine shoot borer, *Eucosma sonomana. Second Asian-Pacific Conference on Chemical Ecology: Current Scenarios and Trends in Chemical Ecology,* Penang, Malaysia.

Daly, JC. 1992. Decline in survival with age of resistant adult *Helicoverpa armigera* (Lepidoptera: Noctuidae) at a discriminating dose of the pyrethroid fenvalerate. *Journal of Economic Entomology* **85**, 1583-1586.

Daly, JC & Fitt, GP. 1990. Monitoring for pyrethroid resistance in relation to body weight in adult *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Journal of Economic Entomology* **83**, 705-709.

Daly, JC & Gregg, PC. 1985. Genetic variation in *Heliothis* in Australia: Species identification and gene flow in the two pest species *H. armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* **75**, 169-184.

Daly, KC & Figueredo, AJ. 2000. Habituation of sexual response in male *Heliothis* moths. *Physiological Entomology.* **25**, 180-190.

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.

Del Socorro, AP & Gregg, PC. 1997. Calling behaviour in *M. convecta* females under different temperature and photoperiodic conditions. *Physiological Entomology* **22**, 20-28.

Del Socorro, AP & Gregg, PC. 2001. Sunflower (*Helianthus annuus* L.) pollen as a marker for studies of local movement in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Australian Journal of Entomology* **40**, 257-263.

Del Socorro, AP, Gregg, PC, Tennant, R & Moore, C. 2003. Attract-and-kill heliothis for low pressure every season. *The Australian Cotton Grower* **24**, 14-19.

Delisle, J & McNeil, JN. 1986. The effect of photoperiod on the calling behaviour of virgin females of the true armyworm, *Pseudaletia unipuncta* (Haw.) (Lepidoptera: Noctuidae). *Journal of Insect Physiology* **32**, 199-206.

Delisle, J & McNeil, JN. 1987. The combined effect of photoperiod and temperature on the calling behaviour of the true armyworm, *Pseudaletia unipuncta*. *Physiological Entomology* **12**, 157-164.

Dethier VG. 1947. *Chemical Insect Attractants and Repellents*. The Blackiston Company, Philadelphia.

Dickens, JC, Smith, JW & Light, DM. 1993. Green leaf volatiles enhance sex attractant pheromone of the tobacco budworm, *Heliothis virescens* (Lep.: Noctuidae). *Chemoecology* **4**, 175-177.

Ditman, LP. 1937. Observations on poison baits for corn earworm control. *Journal of Economic Entomology* **30**, 116-118.

Doane, CC. 1968. Aspects of mating behavior of the gypsy moth. *Annals of the Entomological Society of America* **61**, 768-773.

dos Santos, WJ & 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*, pp. 712-715.

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.

Drake, VA & Farrow, RA. 1985. A radar and aerial trapping study of an early spring migration of moths (Lepidoptera) in inland New South Wales. *Australian Journal of Ecology* **10**, 223-235.

Dunkelblum, E, Gothilf, S & Kehat, M. 1980. Identification of the sex pheromone of the cotton bollworm *Heliothis armigera* in Israel. *Phytoparasitica* **8** , 209-212.

El-Sayed, A, Bengtsson, M, Rauscher, S, Löfqvist, J & Witzgall, P. 1999. Multicomponent sex pheromone in codling moth (Lepidoptera : Tortricidae). *Environmental Entomology* **28**, 775-779.

Evenden, ML & McLaughlin, JR. 2004a. Initial development of an attracticide formulation against the Oriental fruit moth, *Grapholita molesta* (Lepidoptera: Tortricidae). *Environmental Entomology* **33**, 213-220.

Evenden, ML & McLaughlin, JR. 2004b. Factors influencing the effectiveness of an attracticide formulation against the Oriental fruit moth, *Grapholita molesta*. *Entomologia Experimentalis et Applicata* **112**, 89-97.

Exosect Limited. 2003. Exosect® Advanced Pest Control Science. Technological Solutions for Effective No-dose or Low-dose Insect Pest Control. http://www.exosect.com/resources/exosect_brochure.pdf, accessed July 2003.

Extoxnet. 2005. http://extoxnet.orst.edu/ghindex.html The Extension Toxicology Network, A Pesticide Information Project of Cooperative Extension Offices of Cornell University, Michigan State University, Oregon State University, and University of California at Davis. accessed 13 October 2005.

Farkas, SR, Shorey, HH & Gaston, LK. 1974. Sex pheromones of Lepidoptera. The use of widely separated evaporators of looplure for the disruption of pheromone communication in *Trichoplusia ni*. *Environmental Entomology* **3**, 876-877.

Farrow, RA & Daly, JC. 1987. Long-range movements as an adaptive strategy in the genus *Heliothis* (Lepidoptera: Noctuidae): a review of its occurrence and detection in four pest species. *Australian Journal of Zoology* **35**, 1-24.

Farrow, RA & McDonald, G. 1987. Migration strategies and outbreaks of noctuid pests in Australia. *Insect Science and its Applications* **8**, 531-542.

Fitt GP. 1989. The ecology of *Heliothis* species in relation to agroecosystems. *Annual Review of Entomology* **34**, 17-52.

Fitt GP. 2000. An Australian approach to IPM in cotton: integrating new technologies to minimise insecticide dependence. *Crop Protection*, **19**, 793-800.

Fitt, GP, Dillon, ML & Hamilton, JG. 1995. Spatial dynamics of *Helicoverpa* populations in Australia - simulation modelling and empirical studies of adult movement. *Computers and Electronics in Agriculture* **13**, 177-192.

Fitzpatrick, SM, McNeil, JN & Dumont, S. 1988. Does male pheromone effectively inhibit competition among courting true armyworm males (Lepidoptera: Noctuidae). *Animal Behaviour* **36**, 1831-1835.

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.

Forrester, NW, Cahill, M, Bird, LJ & Layland, JK. 1993. Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Bulletin of Entomological Research Supplement Series*, **1**, 1-132.

Foster, SP, Muggleston, SJ & Ball, RD. 1991. Behavioral responses of male *Epiphyas postvittana* to sex pheromone-baited delta trap in a wind tunnel. *Journal of Chemical Ecology.* **17**, 1449-1468.

Furlong, MJ & Pell, JK. 2001. Horizontal transmission of entomopathogenic fungi by the diamondback moth. *Biological Control.* **22**, 288-299.

Gatehouse AG & Zhang XX. 1995. Migratory potential in insects: Variation in an uncertain environment. In: *Insect Migration: Tracking resources through space and time* (eds VA Drake & AG Gatehouse) pp. 193-242. Cambridge University Press, Cambridge.

Gemeno, C & Haynes, KF. 1998. Chemical and behavioral evidence for a third pheromone component in a North American population of the black cutworm moth, *Agrotis ipsilon*. *Journal of Chemical Ecology* **24**, 999-1011.

Gemeno, C & Haynes, KF. 2000. Periodical and age-related variation in chemical communication system of black cutworm moth, *Agrotis ipsilon*. *Journal of Chemical Ecology.* **26**, 329-342.

Gemeno, C & Haynes, KF. 2001. Impact of photoperiod on the sexual behavior of the black cutworm moth (Lepidoptera : Noctuidae). *Environmental Entomology.* **30**, 189-195.

Gentry, CR & Blythe, JL. 1978. Lesser peachtree borers and peachtree borers: a device for trapping, collecting, and marking native moths. *Environmental Entomology* **7**, 783-784.

Gothilf, S, Kehat, M, Jacobson, M & Galun, R. 1978a. Screening pheromone analogues by EAG technique for biological activity on males of *Earias insulana*, *Heliothis armigera*, and *Spodoptera littoralis*. *Environmental Entomology* **7**, 31-35.

Gothilf, S, Kehat, M, Jacobson, M & Galun, R. 1978b. Sex attractants for male *Heliothis armigera* (Hbn.). *Experientia* **34**, 853-854.

Gould, F, Blair, N, Reid, M, Rennie, TL, Lopez, J & Micinski, S. 2002. *Bacillus thuringiensis*-toxin resistance management: Stable isotope assessment of alternate host use by *Helicoverpa zea*. *Proceedings of the National Academy of Science* **99**, 16581-16586.

Grant, AJ, Mayer, MS & Mankin, RW. 1989. Responses from sensilla on antennae of male *Heliothis zea* to its major pheromone component and two analogs. *Journal of Chemical Ecology* **15**, 2625-2634.

Grant, GG. 1970. Evidence for a male sex pheromone in the noctuid, *Trichoplusia ni*. *Nature (Lond.)* **227**, 1345-1346.

Grant, GG, Brady, UE & Brand, JM. 1972. Male armyworm scent brush secretion: Identification and electroantennogram study of major components. *Annals of the Entomological Society of America* **65**, 1224-1227.

Green, RH. 1979. *Sampling design and statistical methods for environmental biologists*. Wiley, New York.

Gregg, PC. 1993. Pollen as a marker for migration of *Helicoverpa armigera* and *H. punctigera* (Lepidoptera: Noctuidae) from western Queensland. *Australian Journal of Ecology* **18,**: 209-219.

Gregg, PC. 1995. Migration of cotton pests: Patterns and implications for management. In: *Challenging the future,* (eds GA Constable & NW Forrester) CSIRO Publishing, Brisbane, Australia, 423-433.

Gregg, PC & Del Socorro, AP. 2002. Attractants for moths. *International Patent Number WO 02/089577, World Intellectual Property Organisation* .

Gregg PC, Fitt GP, Zalucki MP & Murray DH. 1995. Insect migration in an arid continent II. *Helicoverpa* spp. in eastern Australia. In: *Insect migration: tracking resources through time and space* (eds VA Drake &AG Gatehouse) pp. 151-172. Cambridge University Press, London.

Gregg, PC and Wilson, AGL. 1991. Trapping methods for adults. In: *Heliothis: Research Methods and Prospects* (ed. MP. Zalucki) pp.30-48, Springer-Verlag. New York..

Gross, HR Jr, Carpenter, JE & Sparks, AN. 1983. Visual acuity of Heliothis zea (Lepidoptera: Noctuidae) males as a factor influencing the efficiency of pheromone traps. *Environmental Entomology* **12**, 844-847.

Guillet, P, N'Guessan, R, Darriet, F, Traore-Lamizana, M, Chandre, F & Carnevale, P. 2001. Combined pyrethroid and carbamate 'two-in-one' treated mosquito nets: field efficacy against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus*. *Medical & Veterinary Entomology*. **15** , 105-112.

Gunning, RV. 1996. Bioassay for detecting pyrethroid nerve insensitivity in Australian *Helicoverpa armigera* (Lepidoptera, Noctuidae). *Journal of Economic Entomology* **89**, 816-819.

Gunning, RV, Devonshire, AL & Moores, GD. 1995. Metabolism of esfenvalerate by pyrethroid-susceptible and -resistant Australian *Helicoverpa armigera* (Lepidoptera, Noctuidae). *Pesticide Biochemistry and Physiology* **51**, 205-213.

Gunning, RV, Easton, CS, Balfe, ME & Ferris, IG. 1991. Pyrethroid resistance mechanisms in Australian *Helicoverpa armigera*. *Pesticide Science* **33**, 473-490.

Gunning, RV, Easton, CS, Greenup, LR & Edge, VE. 1984. Pyrethroid resistance in *Heliothis armiger* (Hübner) (Lepidoptera:Noctuidae) in Australia. *Journal of Economic Entomology* **77**, 1283-1287.

Gunning, RV, Moores, GD & Devonshire, AL. 1996a. Esterases and esfenvalerate resistance in Australian *Helicoverpa armigera* (Hübner) (Lepidoptera : Noctuidae). *Pesticide Biochemistry and Physiology* **54**, 12-23.

Gunning, RV, Moores, GD & Devonshire, AL. 1996b. Insensitive acetylcholinesterase and resistance to thiodicarb in Australian *Helicoverpa armigera* Hübner (Lepidoptera, Noctuidae). *Pesticide Biochemistry and Physiology* **55**, 21-28.

Gunning, RV, Moores, GD & Devonshire, AL. 1998a. Insensitive acetylcholinesterase and resistance to organophosphates in Australian *Helicoverpa armigera*. *Pesticide Biochemistry and Physiology* **62**, 147-151.

Gunning, RV, Moores, GD & Devonshire, AL. 1998b. Insensitive acetylcholinesterase causes resistance to organophosphates in Australian *Helicoverpa armigera* (Hübner) (Lepidoptera, Noctuidae). *Pesticide Science* **54**, 319-320.

Gunning, RV, Moores, GD & Devonshire, AL. 1999. Esterase inhibitors synergise the toxicity of pyrethroids in Australian *Helicoverpa armigera* (Hübner) (Lepidoptera : Noctuidae). *Pesticide Biochemistry and Physiology* **63**, 50-62.

Hackett, DS & Gatehouse, AG. 1982. Diapause in *Heliothis armigera* (Hübner) and *H. fletcheri* (Hardwick) (Lepidoptera: Noctuidae) in the Sudan Gezira. *Bulletin of Entomological Research* **72**, 409-422.

Hagler, JR & Jackson, CG. 2001. Methods for marking insects: current techniques and future prospects. *Annual Review of Entomology* **46**, 511-543.

Hardwick, DF. 1965. The corn earworm complex. *Memoirs of the entomological Society of Canada* **40**, 1-247.

Harlan, DP & Roberts, RH. 1976. Tabanidae: use of a self-marking device to determine populations in the Mississippi-Yazoo river delta. *Environmental Entomology* **5**, 210-212.

Hassan, ST & Wilson, LT. 1993. Simulated larval feeding damage patterns of *Heliothis armigera* (Hübner) and *Heliothis punctigera* (Wallengren) (Lepidoptera: Noctuidae) on cotton in Australia. *International Journal of Pest Management* **39**, 239-245.

Haynes, KF & Baker, TC. 1985. Sublethal effects of permethrin on the chemical communications system of the pink bollworm moth, *Pectinophora gossypiella*. *Archives of Insect Biochemistry and Physiology* **2**, 283-293.

Haynes, KF & Birch, MC. 1984. The periodicity of pheromone release and male responsiveness in the artichoke plume moth, *Platyptilia carduidactyla*. *Physiological Entomology* **9**, 287-295.

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, Graham, HM, Guerra, RJ & Perez, CT. 1973. Comparison of the numbers of tobacco budworms and bollworms caught in sex pheromone traps vs. blacklight traps in Lower Rio Grande Valley, Texas. *Environmental entomology* **2**, 911-914.

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

Henneberry, TJ & Butler, GD. 1986. Effects of high temperature on tobacco budworm (Lepidoptera: Noctuidae) reproduction, diapause and spermatocyst development. *Journal of Economic Entomology* **79**, 410-413.

Herman, TJB, Cameron, PJ & Walker, GP. 1995. Effect of pheromone trap position and colour on tomato fruitworm moths and bumblebees. *Proceedings of the New Zealand Plant Protection Society*, http://www.hortnet.co.nz/publications/nzpps/proceedings/94/94_154.htm, accessed 6 March 2005.

Hodge, S & Longley, M. 2000. The irritant and repellent effects of organophosphates on the Tasmanian lacewing, *Micromus tasmaniae* (Neuroptera : Hemerobiidae). *Pest Management Science* **56**, 916-920.

Hofer, D. 1994. Sirene®: A new approach to pink bollworm control in cotton. *Insect Control Newsletter* **3**, 2-5.

Hofer, D. 1996. "Attract and kill": a novel approach for boll weevil control in Brazil. *Insect Control Newsletter* **1**, 6-7.

Hofer, D. 1997. Attract and kill: another step forward - first sales of Sirene® CM. *Insecticides Newsletter* **1**, 8-9.

Hofer, D & Angst, M. 1995. Control of pink boll worm in cotton with SIRENE, a novel sprayable attract & kill formulation. *Proceedings of the Beltwide Cotton Conferences* **2**, 949-952.

Hofer, D., Charmillot, P.-J., and Angst, M. 1996. Control of the codling moth (*Cydia pomonella*) with Sirene® CM, a novel attract & kill formulation. *XX International Congress of Entomology, Firenze 1996, Papers contributed by Ciba,* 36-42.

Hogsette, JA. 1983. An attractant self-marking device for marking field populations of stable flies with fluorescent dusts. *Journal of Economic Entomology* **76**, 510-514.

Hou, ML & Sheng, CF. 2000. Calling behaviour of adult female *Helicoverpa armigera* (Hübner) (Lep., Noctuidae) of overwintering generation and effects of mating. *Zeitschrift für angewandte Entomologie* **124**, 71-75.

Huang, Y., Xu, S., Tang, X., and Du J. 1996a. Male orientation inhibitor of *Helicoverpa armigera* (Lepidoptera: Noctuidae). *The 1996 International Symposium of Insect Pest Control with Pheromones,* Suwon, Korea.

Huang, Y, Xu, S, Tang, X, Zhangwu, Z & Du, J. 1996b. Male orientation inhibitor of cotton bollworm : identification of compounds produced by male hairpencil glands. *Entomologia Sinica* **3**, 172-182.

Huang, Y, Xu, S, Tang, X, Zhao, Z & Du J. 1997. Male orientation inhibitor of cotton bollworm: inhibitory effects of alcohols in wind tunnel and in the field. *Entomologia Sinica* **4**, 173-181.

Hummel HE & Miller TA. 1984. *Techniques in Pheromone Research*. Springer-Verlag, New York.

Hutt, RB & White, LD. 1977. Mating response to visual stimulus in the male codling moth. *Environmental Entomology* **6**, 567-568.

Il'ichev, AL. 2002. Area-wide mating disruption for improved control of Oriental fruit moth Grapholita molesta in Victoria, Australia. *Use of pheromones and other semiochemicals in integrated production, IOBC wprs Bulletin* **25**, 1-13.

Ioriatti C & Angeli G. 2002. Control of codling moth by attract and kill. In: *Use of pheromones and other semiochemicals in integrated production.* Proceedings of IOBC wprs Working Group Meeting, Samos, http://phero.net/iobc/samos/bulletin/ioriatti.pdf

Ioriatti, C, Charmillot, PJ & Bloesch, B. 1987. Étude des principaux facteurs influençant l'émission d'attractifs sexuels synthétiques à partir de diffuseurs en caoutchouc et en plastique. *Entomologia Experimentalis et Applicata* **44**, 123-130.

Izquierdo, JI. 1996. *Helicoverpa armigera* (Hübner) (Lep, Noctuidae) - relationship between captures in pheromone traps and egg counts in tomato and carnation crops. *Zeitschrift für angewandte Entomologie* **120**, 281-290.

Jallow, MFA & Zalucki, MP. 1995. A technique for measuring intraspecific variation in oviposition preference in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). *Journal of the Australian Entomological Society* **34**, 281-288.

Jallow, MFA, Zalucki, MP & Rogers, J. 1999. Host selection and management of *Helicoverpa* (Lepidoptera: Noctuidae) on cotton. *CRDC Occasional Papers: Insects* 1-36.

Johnson A & Farrell T. 2004. *2004-2005 Cotton pest management guide.* NSW Department of Primary Industries, NSW.

Johnson, CG. 1969 *Migration and dispersal of insects by flight* Methuen. London.

Johnson, MW, Stinner, RE & Rabb, RL. 1975. Ovipositional response of *Heliothis zea* to its major hosts in North Carolina. *Environmental Entomology* **4**, 291-297.

Jones OT. 1998a. Lure and kill. In: *Insect Pheromones and their Use in Pest Management* (eds PE Howse, IDR Stevens & OT Jones) pp. 300-313. Chapman and Hall, London.

Jones OT. 1998b. Mass trapping. In: *Insect Pheromones and their Use in Pest Management* (eds PE Howse, IDR Stevens & OT Jones) pp. 280-299. Chapman and Hall, London.

Jones, D. 1984. Use, misuse, and role of multiple-comparison procedures in ecological and agricultural entomology. *Environmental Entomology* **13**, 635-649.

Kaae, RS, Shorey, HH, Gaston, LK & Hummel, HH. 1974. Sex pheromones of Lepidoptera: Disruption of pheromone communication in *Trichoplusia ni* and *Pectinophora gossypiella* by permeation of the air with nonpheromone chemicals. *Environmental Entomology* **3**, 87-89.

Kanno, H. 1981a. Mating behaviour of the rice stem borer moth, *Chilo suppresalis* Walker (Lepidoptera: Pyralidae) V. Critical illumination intensity for female calling and male sexual response under various temperatures. *Applied Entomology and Zoology* **16**, 179-185.

Kanno, H. 1981b. Mating behaviour of the rice stem borer moth, *Chilo suppresalis* Walker (Lepidoptera: Pyralidae) VI. Effects of photoperiod on the diel rhythms of mating behaviours. *Applied Entomology and Zoology* **16**, 406-411.

Kanno, H & Sato, A. 1979. Mating behaviour of the rice stem borer moth, *Chilo suppresalis* Walker (Lepidoptera: Pyralidae) II. Effects of temperature and relative humidity on mating activity. *Applied Entomology and Zoology* **14**, 419-427.

Kanno, H & Sato, A. 1980. Mating behaviour of the rice stem borer moth, *Chilo suppresalis* Walker (Lepidoptera: Pyralidae) III. Joint action of temperature and relative humidity on mating activity. *Applied Entomology and Zoology* **15**, 111-112.

Karlson, P & Lüscher, M. 1959. "Pheromones" a new term for a class of biologically active substances. *Nature* **183**, 55-56.

Kaster, LV, Showers, WB & Mulder, PG. 1989. Seasonality in the hourly reproductive behavior of adult *Agrotis ipsilon* (Lepidoptera: Noctuidae) in Iowa. *Environmental entomology* **18**, 674-677.

Kehat, M, Anshelevich, L, Gordon, D, Harel, M & Dunkelblum, E. 1998. Evaluation of Shin-Etsu twist-tie rope dispensers by the mating table technique for disrupting mating of the cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae), and the pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). *Bulletin of entomological research* **88**, 141-148.

Kehat, M, Anshelevich, L, Gordon, D, Harel, M, Zilberg, L & Dunkelblum, E. 1999. Effect of density of pheromone sources, pheromone dosage and population pressure on mating of pink bollworm, *Pectinophora gossypiella* (Lepidoptera : Gelechiidae). *Bulletin of Entomological Research.* **89**, 339-345.

Kehat, M & Dunkelblum, E. 1990. Behavioral responses of male *Heliothis armigera* (Lepidoptera: Noctuidae) moths in a flight tunnel to combinations of components identified from female sex pheromone glands. *Journal of Insect Behavior* **3**, 75-83.

Kehat, M & Dunkelblum, E. 1993. Sex pheromones: achievements in monitoring and mating disruption of cotton pests in Israel. *Archives of insect biochemistry and physiology* **22**, 425-431.

Kehat, M, Gothilf, E, Dunkelblum, E & Greenberg, S. 1980. Field evaluation of female sex pheromone components of the cotton bollworm, *Heliothis armigera*. *Entomologia Experimentalis et Applicata* **27**, 188-193.

Kehat, M, Gothilf, E, Dunkelblum, E & Greenberg, S. 1982. Sex pheromone traps as a means of improving control programs for the cotton bollworm, *Heliothis armigera* (Lepidoptera: Noctuidae). *Environmental Entomology* **11**, 727-729.

Kelber, A, Balkenius, A & Warrant, EJ. 2002. Scotopic colour vision in nocturnal hawkmoths. *Nature* **419**, 922-925.

Kimura, T & Honda, H. 1999. Identification and possible functions of the hairpencil scent of the yellow peach moth, *Conogethes punctiferalis* (Guenée) (Lepidoptera : Pyralidae). *Applied Entomology & Zoology* **34**, 147-153.

King, ABS, Armes, NJ & Pedgley, DE. 1990. A mark-capture study of *Helicoverpa armigera* dispersal from pigeonpea in southern India. *Entomologia Experimentalis et Applicata* **55**, 257-266.

Kirsch, PA. 1997a. Amazing daze: confusion brings change. *Technology Transfer in Mating Disruption, IOBC Working Papers Bulletin, Montpellier*, **20**, 249-256.

Kirsch, PA. 1997b. Sirene® CM adds a new dimension in the United States. *Insecticides Newsletter* **1**, 12-13.

Kirsch, PA. 2001. Session H: Chemical Ecology in Agroecosystem. Attract and kill technology: A "Knock-out" in application of pheromones for pest management. *Second Asian-Pacific Conference on Chemical Ecology: Current Scenarios and Trends in Chemical Ecology,* Penang, Malaysia.

Kirsch, PA, Booysen, G, and Czokajlo, D. 2001. Session H: Chemical Ecology in Agroecosystem. Controlling tree fruit pests with Last Call: Innovative attract and kill technology. *Second Asian-Pacific Conference on Chemical Ecology: Current Scenarios and Trends in Chemical Ecology,* Penang, Malaysia.

Kirsch, PA, Booysen, G, Czokajlo, D, and McLaughlin, J. 2003. Pheromone dose response of attract and kill formulations targeting leafrollers. *Proceedings of the 77th Annual Western Orchard Pest & Disease Management Conference*, Portland, Oregon.

Kirsch, PA, Shukin, K, and Gries, G. 2003. Last Call CM: Effective codling moth control in apple orchards in British Columbia. *Proceedings of the 77th Annual Western Orchard Pest & Disease Management Conference*, Portland, Oregon.

Konyukhov, VP, Kovalev, BG, Minyailo, VA, Stan, VV & Oprunenko, YuF. 1978. The synthesis of cis-9-tetradecenal and cis-11-hexadecenal and the determination of their effect on the cotton bollworm using the electroantennogram method. *Khemoretseptsiya Nasekomykh* **3**, 37-40.

Konyukhov, VP, Kovalev, BG & Sammar-Zade, NR. 1984. Isolation and identification of the components of the sex pheromone of the corn earworm *Heliothis armigera* Hb. *Soviet Journal of Bioorganic Chemistry (English translation of Bioorganicheskaia khimiia)* **9**, 782-787.

Kou, R & Chow, Y-S. 1987. Calling behavior of the cotton bollworm, *Heliothis armigera* (Lepidoptera: Noctuidae). *Annals of the Entomological Society of America* **80**, 490-493.

Krebs, CJ. 1994. *Ecology: The experimental analysis of distribution and abundance.* 4th ed. Harper Collins. New York.

Krupke, CH, Roitberg, BD & Judd, GJR. 2002. Field and laboratory responses of male codling moth (Lepidoptera: Tortricidae) to a pheromone-based attract-and-kill strategy. *Environmental Entomology* **31**, 189-197.

Kuenen, LPS & Baker, TC. 1981. Habituation versus sensory adaptation as the cause of reduced attraction following pulsed and constant sex pheromone pre-exposure in *Trichoplusia ni*. *Journal of Insect Physiology* **27**, 721-726.

Kumar, K & Chapman, RB. 1984. Sublethal effects of insecticides on the diamondback moth, *Plutella xylostella* (L.). *Pesticide Science* **15**, 344-352.

Kvedaras, OL. 2002. The influence of host plants on the mating behaviour of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Ph.D. dissertation, University of New England, NSW, Australia.

Kvedaras, OL, Gregg, PC & Del Socorro, AP. 2000. Techniques used to determine the mating behaviour of Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae) in relation to host plants. *Australian Journal of Entomology* **39**, 188-194.

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.

Lanier GN. 1990. Principles of attraction-annihilation: Mass trapping and other means. In: *Behavior-modifying chemicals for insect management: applications of pheromones and other attractants* (eds RL Ridgway, RM Silverstein & MN Inscoe) pp. 25-43. M. Dekker, New York.

Latheef, MA, Lopez, JD & Witz, JA. 1993. Capture of corn earworm (Lepidoptera: Noctuidae) in pheromone traps and hand nets: Relationship to egg and adult densities in field corn, Texas Brazos River Valley. *Journal of Economic Entomology* **86**, 407-415.

Lewis, T & Macaulay, EDM. 1976. Design and elevation of sex attractant traps for pea moth *Cydia nigricana* (Steph) and the effect of plume shape on catches. *Ecological Entomology* **1**, 175-187.

Light, DM, Flath, RA, Buttery, RG, Zalom, FG, Rice, RE, Dickens, JC & Jang, EB. 1993. Host-plant green-leaf volatiles synergise the synthetic sex pheromones of the corn earworm and codling moth (Lepidoptera). *Chemoecology* **4**, 145-152.

Lincoln, C, Dean, G, Phillips, JR, Matthews, EJ & Nelson, GS. 1966. Molasses-insecticide sprays for control of bollworm. *Arkansas Farm Research* **15**, 4.

Lingren PD, Raulston JR, Henneberry TJ & Sparks AN. 1986. Night-vision equipment, reproductive biology, and nocturnal behavior: Importance to studies of insect flight, dispersal, and migration. In: *Insect flight dispersal and migration* (ed W Danthanarayana) pp. 253-264. Springer-Verlag, Berlin/Heidelberg.

Lingren, PD, Raulston, JR, Sparks, AN & Wolf, WW. 1982. Insect monitoring technology for evaluation of suppression via pheromone systems. In: *Insect suppression with controlled release pheromone systems* (eds F Kydonieus &M Beroza) pp. 171-193. CRC Press, Boca Raton, Florida.

Lingren, PD, Raulston, JR & Shaver, TN. 1998. Introduction. *Southwestern Entomologist, Supplement*, **21**, 1-7.

Lindgren, PD, Sparks, AN, Raulston, JR & Wolf, WW. 1978. Applications for nocturnal studies of insects. *Bulletin of the Entomological Society of America.* **24**, 206-212.

Linn, LE & Roelofs, WL. 1984. Sublethal effects of neuroactive compounds on pheromone response thresholds in male oriental fruit moths. *Archives of Insect Biochemistry and Physiology* **1**, 331-344.

Lockwood, JASTC & Storey, RN. 1984. Evolution of insecticide resistance to insecticides: a re-evalution of the roles of physiology and behavior. *Bulletin of the Entomological Society of America* **30**, 41-51.

Longley, M & Jepson, PC. 1996. The influence of insecticide residues on primary parasitoid and hyperparasitoid foraging behaviour in the laboratory. *Entomologia Experimentalis et Applicata* **81**, 259-269.

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.

Mafra-Neto, A & Baker, TC. 1996. Timed, metered sprays of pheromone disrupt mating of *Cadra cautella* (Lepidoptera, Pyralidae). *Journal of Agricultural Entomology* **13**, 149-168.

Mafra-Neto, A & Cardé, RT. 1994. Fine-scale structure of pheromone plumes modulates upwind orientation of flying moths. *Nature* **369**, 142-144.

Mafra-Neto, A & Cardé, RT. 1995a. Effect of the fine-scale structure of pheromone plumes: pulse frequency modulates activation and upwind flight of almond moth males. *Physiological Entomology* **20**, 229-242.

Mafra-Neto, A & Cardé, RT. 1995b. Influence of plume structure and pheromone concentration on upwind flight of *Caudra cautella* males. *Physiological Entomology* **20**, 117-133.

Mallett, J, Korman, A, Heckel, DG & King, P. 1993. Biochemical genetics of *Heliothis* and *Helicoverpa* (Lepidoptera: Noctuidae) and evidence for a founder effect in *Helicoverpa zea*. *Annals of the Entomological Society of America* **86**, 189-197.

Mansour, M, McLaughlin, J, Kirsch, P. 2003 Last Call in Syria: Effective codling moth control under extreme pressure. *Proceedings of the 77th Annual Western Orchard Pest & Disease Management Conference*, Portland, Oregon.

MathSoft Inc. 1999. S-Plus 2000 Professional, Release 1.

Matthews, M. 1987. The African species of *Heliocheilus* Grote (Lepidoptera: Noctuidae). *Systematic Entomology* **12**, 459-473.

Matthews M. 1999. *Heliothine moths of Australia: A guide to pest bollworms and related noctuid groups*. CSIRO Publishing, Melbourne.

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

Mazomenos, BE, Pantazi-Mazomenou, A & Stefanou, D. 2000. Attract and kill of the olive fruit fly *Bactrocera oleae* (Gmel.) in Greece as a part of an integrated control system. Proceedings of IOBC wprs Working Group Meeting, Samos, http://www.phero.net/iobc/samos/abstr/mazomenos.html, accessed 17 January 2001.

McLaughlin, JR., Czokajlo, D, Carroll, J, and Kirsch, PA. 2001. Session K: Chemical Ecology in Urban Entomology. Attract and kill technology to control ticks. *Second Asian-Pacific Conference on Chemical Ecology: Current Scenarios and Trends in Chemical Ecology,* Penang, Malaysia.

McLaughlin, JR & Mitchell, ER. 1982. Practical development of pheromones in *Heliothis* management, In: *Proceedings of the International Workshop on Heliothis Management*, (eds. W Reed & V Kumble) pp. 309-318, ICRISAT, Patancheru, A. P., India.

McVeigh, EM, McVeigh, LJ & Cavanagh, CG. 1983. A technique for tethering females of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) to evaluate pheromone control methods. *Bulletin of Entomological Research* **73**, 441-446.

Meagher, RL. 2001a. Collection of fall armyworm (Lepidoptera : Noctuidae) adults and nontarget Hymenoptera in different colored unitraps. *Florida Entomologist.* **84**, 77-82.

Meagher, RL. 2001b. Trapping fall armyworm (Lepidoptera : Noctuidae) adults in traps baited with pheromone and a synthetic floral volatile compound. *Florida Entomologist.* **84**, 288-292.

Meagher, RL & Mitchell, ER. 1998. Phenylacetaldehyde enhances upwind flight of male fall armyworm (Lepidoptera : Noctuidae) to its sex pheromone. *Florida Entomologist* **81**, 556-559.

Michael PJ & Woods WM. 1980. *An entomological review of cotton growing in the Ord River area of Western Australia.* Department of Agriculture of Western Australia, Perth, WA.

Micinski, S. 2001. Relationship between bollworm (Lepidoptera : Noctuidae) pheromone trap catches and yield differences in sprayed and nonsprayed BT cotton. *Southwestern Entomologist.* **26**, 137-142.

Mitchell, ER. 2002. Promising new technology for managing diamondback moth (Lepidoptera : Plutellidae) in cabbage with pheromone. *Journal of Environmental Science & Health - Part B: Pesticides, Food Contaminants, & Agricultural Wastes.* **37**, 277-290.

Mitchell, ER, Agee, HR & Heath, RR. 1989. Influence of pheromone trap color and design on capture of male velvetbean caterpillar and fall armyworm moths (Lepidoptera: Noctuidae). *Journal of Chemical Ecology* **15**, 1775-1784.

Mitchell, ER & Mayer, MS. 2001. *Spodoptera exigua*: Mating disruption, measurement of airborne concentration of pheromone, and use of specialist receptor cell responses for comparison to female pheromone emission. *Journal of Environmental Science & Health - Part B: Pesticides, Food Contaminants, & Agricultural Wastes.* **36**, 467-488.

Moore, RF. 1980. Behavioral and biological effects of NRDC-161 as factors in the control of the boll weevil. *Journal of Economic Entomology* **73**, 265-267.

Moore, RF. 1988. Inhibition of chemical communication between male and female bollworms (Lepidoptera: Noctuidae) by sublethal amounts of permethrin. *Journal of Economic Entomology* **81**, 78-82.

Morton, R, Tuart, LD & Wardhaugh, KG. 1981. The analysis and standardization of light-trap catches of *Heliothis armigera* (Hübner) and *H. punctiger* Wallengren (Lepidoptera:Noctuidae). *Bulletin of Entomological Research* **71**, 207-225.

Murlis, J & Bettany, BW. 1977. The night-flight towards a sex pheromone source by male *Spodoptera littoralis* (Boisd.) (Lepidoptera, Noctuidae). *Nature* **268**, 433-434.

Murlis, J, Bettany, BW, Kelly, J & Martin, L. 1982. The analysis of flight paths of male Egyptian cotton leafworm moths, *Spodoptera littoralis*, to a sex pheromone source in the field. *Physiological Entomology* **7**, 435-441.

Nakasuji, F & Fujita, K. 1980. A population model to assess the effect of sex pheromones on population suppression. *Applied Entomology & Zoology* **15**, 27-35.

Nesbitt, BF, Beevor, PS, Hall, DR & Lester, R. 1979. Female sex pheromone components of the cotton bollworm, *Heliothis armigera*. *Journal of Insect Physiology* **25**, 535-541.

Nesbitt, BF, Beevor, PS, Hall, DR & Lester, R. 1980. (Z)-9-hexadenenal: a minor component of the female sex pheromone of *Heliothis armigera* (Hübner) (Lepidoptera, Noctuidae). *Entomologia Experimentalis et Applicata* **27**, 306-308.

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

Nyambo, BT. 1989. Assessment of pheromone traps for monitoring and early warning of *Heliothis armigera* Hübner (Lepidoptera, Noctuidae) in the western cotton-growing areas of Tanzania. *Crop Protection* **8**, 188-192.

Ochieng, SA, Park, KC & Baker, TC. 2002. Host plant volatiles synergize responses of sex pheromone-specific olfactory receptor neurons in male *Helicoverpa zea*. *Journal of Comparative Physiology A-Sensory Neural & Behavioral Physiology.* **188**, 325-333.

Ohtani, K, Witjaksono, Fukumoto, T, Mochizuki, F, Yamamoto, M & Ando, T. 2001. Mating disruption of the Japanese giant looper in tea gardens permeated with synthetic pheromone and related compounds. *Entomologia Experimentalis et Applicata.* **100**, 203-209.

O'Keeffe, S. 2001. The ZapTrap: an analysis of efficacy and utility in the trapping of *Helicoverpa armigera*. B.Sc. (Hons.) Rur.Sci. Thesis, University of New England, Armidale, NSW, Australia.

Oyama, M. 1977. Mating suppression of *Spodoptera littoralis* (F.) by (Z,E)-9, 12-tetradecadienyl acetate. *Applied Entomology and Zoology* **12**, 281-286.

Park, KC, Boo, KS, Cork, A & Hall, DR. 1999. Monitoring *Helicoverpa assulta* (Guenee) (Lepidoptera : Noctuidae) with synthetic sex pheromone and small scale disruption trial with controlled-release PVC resin formulation. *Applied Entomology and Zoology* **34**, 19-22.

Parsons, FS. 1939. Investigations on the cotton bollworm,*Heliothis armigera* Hubn. (obsoleta Fabr.).Part I. The annual march of bollworm incidence and related factors. *Bulletin of Entomological Research* **30**, 321-328.

Parsons, FS. 1940. Investigations on the cotton bollworm, *Heliothis armigera* Hubn. Part III. Relationships between oviposition and the flowering curves of food plants. *Bulletin of Entomological Research* **31**, 147-177.

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.

Phillips, JR & Lincoln, C. 1968. Improved bollworm control with molasses-insecticide sprays. *Arkansas Farm Research* **17**, 12.

Piccardi, P, Capizzi, A, Cassani, G, Spinelli, P, Arsura, E & Massardo, P. 1977. A sex pheromone component of the old world bollworm *Heliothis armigera*. *Journal of Insect Physiology* **23**, 1443-1445.

Plapp, FWJ, Jackman, JA, Campanhola, C, Frisbie, RE, Graves, JB, Luttrell, RG, Kitten, WF & Wall, M. 1990. Monitoring and management of pyrethroid resistance in the tobacco budworm (Lepidoptera: Noctuidae) in Texas, Mississippi, Louisiana, Arkansas, and Oklahoma. *Journal of Economic Entomology* **83**, 335-341.

Plapp, FWJ & Vinson, SB. 1977. Comparative toxicities of some insecticides to the tobacco budworm and its ichneumonid parasite, *Campoletis sonorensis*. *Environmental Entomology* **6**, 381-384.

Polavarapu, S, Lonergan, G, Peng, H & Neilsen, K. 2001. Potential for mating disruption of *Sparganothis sulfureana* (Lepidoptera : Tortricidae) in cranberries. *Journal of Economic Entomology*. **94**, 658-665.

Poullot, D, Beslay, D, Bouvier, JC & Sauphanor, B. 2001. Is attract-and-kill technology potent against insecticide-resistant Lepidoptera? *Pest Management Science.* **57**, 729-736.

Prokopy, RJ, Wright, SE, Black, JL, Hu, XP & McGuire, MR. 2000. Attracticidal spheres for controlling apple maggot flies: commercial-orchard trials. *Entomologia Experimentalis et Applicata.* **97**, 293-299.

Raina, AK. 1988. Selected factors influencing neurohormonal regulation of sex pheromone production in *Heliothis* species. *Journal of Chemical Ecology* **14**, 2063-2069.

Raina, AK, Jackson, DM & Severson, RF. 1997. Increased pheromone production in wild tobacco budworm (Lepidoptera: Noctuidae) exposed to host plants and host chemicals. *Environmental Entomology* **26**, 101-105.

Raina, AK, Kingan, TG & Mattoo, AK. 1992. Chemical signals from host plant and sexual behaviour in a moth. *Science* **255**, 592-594.

Raulston, JR, Lingren, PD, Sparks, AN & Martin, DF. 1979. Mating interaction between native tobacco budworms and released backcross adults. *Environmental Entomology* **8**, 349-353.

Raulston, JR, Snow, JW, Graham, HM & Lingren, PD. 1975. Tobacco budworm: Effect of prior mating and sperm content on the mating behaviour of females. *Annals of the Entomological Society of America* **68**, 701-704.

Reddy, GVP & Manjunatha, M. 2000. Laboratory and field studies on the integrated pest management of *Helicoverpa armigera* (Hübner) in cotton, based on pheromone trap catch threshold level. *Zeitschrift für angewandte Entomologie* **124**, 213-221.

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

Riley, JR, Armes, NJ, Reynolds, DR & Smith, AD. 1992. Nocturnal observations on the emergence and flight behaviour of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in the post-rainy season in India. *Bulletin of Entomological Research* **82**, 243-256.

Robertson JL & Preisler HK. 1992. *Pesticide Bioassays with Arthropods*. CRC Press, Boca Raton.

Roelofs WL & Cardé RT. 1974. Sex pheromones in the reproductive isolation of lepidopterous species. In: *Frontiers of Biology, Vol.32, Pheromones.* ed. M. Birch, pp. 96-114. North-Holland Pub. Co. Amsterdam.

Roome, RE. 1975. Activity of adult *Heliothis armigera* (Hb.) (Lepidoptera, Noctuidae) with reference to the flowering of sorghum and maize in Botswana. *Bulletin of Entomological Research* **65**.

Rothschild, GHL. 1978. Attractants for *Heliothis armigera* and *H. punctigera. Journal of the Australian Entomological Society* **17**, 389-390.

Rotundo, G & Tremblay, E. 1985. Field evaluation of female sex pheromone of the artichoke moth, *Gortyna xanthenes. Entomologia Experimentalis et Applicata* **38**, 201-204.

Rumbo, ER. 1993. Interaction between male moths of the lightbrown apple moth, *Epiphyas postvittana* (Walker) (Lep.: Tort.), landing on synthetic sex pheromone sources in a wind-tunnel. *Physiological Entomology* **18**, 79-84.

Ryne, C, Svensson, GP & Lofstedt, C. 2001. Mating disruption of *Plodia interpunctella* in small-scale plots: Effects of pheromone blend, emission rates, and population density. *Journal of Chemical Ecology.* **27**, 2109-2124.

Saario, CA, Shorey, HH & Gaston, LK. 1970. Sex pheromones of noctuid moths. XIX. Effect of environmental and seasonal factors on captures of males of *Trichoplusia ni* in pheromone-baited traps. *Annals of the Entomological Society of America* **63**, 667-672.

Sack, C., McLaughlin, J. R., Kirsch, P. A., Booysen, G., and Czokajlo, D. 2001a. Session D: Application of Bioreactive Compounds. Novel new attractant and formulations for control and monitoring of tephritid fruit flies. *Second Asian-Pacific Conference on Chemical Ecology: Current Scenarios and Trends in Chemical Ecology,* Penang, Malaysia.

Sack, CV, McLaughlin, JR, Kirsch, PA, Booysen, G & Czokajlo, D. 2001b. Attract and kill formulations in South African citrus and subtropical fruit pest management. *The Entomological Society of America Annual Meeting - 2001: An Entomological Odyssey of ESA*, San Diego, California, USA

Sanders CJ. 1996. Mechanisms of mating disruption in moths. In: *Insect pheromone research: new directions* (eds RT Cardé & AK Minks) pp. 333-346. Chapman & Hall, New York.

Sanders, CJ. 2000. Can windtunnels help improve mating disruption? Proceedings of IOBC wprs Working Group Meeting, Samos, http://www.phero.net/iobc/samos/abstr/sanders.html, accessed 17 January 2001.

Schneider, D. 1999. Insect pheromone research: some history and 45 years of personal recollections . *Proceedings of IOBC wprs Symposium, Scents in Orchards - Plant and Insect Semiochemicals from Orchard Environments, Dachau*, **22**, 139-140.

Scott, KD, Lange, CL, Scott, LJ & Graham, GC. 2004. Isolation and characterization of microsatellite loci from *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). *Molecular Ecology Notes* **4**, 204-205.

Scott KD, Wilkinson KS, Merritt MA, Scott LJ, Lange CL, Schutze MK, Kent JK, Merritt DJ, Grundy PR, Graham GC. 2003. Genetic shifts in *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) over a year in the Dawson/Callide Valleys. *Australian Journal of Agricultural Research*, **54**:739-744

Shaver, TN & Brown, HE. 1993. Evaluation of pheromone to disrupt mating of *Eoreuma loftini* (Lepidoptera: Pyralidae) in sugarcane. *Journal of Economic Entomology* **86**, 377-381.

Shorey, HH. 1966. The biology of *Trichoplusia ni* (Lepidoptera: Noctuidae). IV. Environmental control of mating. *Annals of the Entomological Society of America* **59**, 502-506.

Shorey, HH & Gaston, LK. 1970. Sex pheromones of noctuid moths. XX. Short-range visual orientation by pheromone-stimulated males of *Trichoplusia ni*. *Annals of the Entomological Society of America* **63**, 829-832.

Shorey, HH, Kaae, RS, Gaston, LK & McLaughlin, JR. 1972. Sex pheromones of Lepidoptera. XXX. Disruption of sex pheromone communication in *Trichoplusia ni* as a possible means of mating control. *Environmental Entomology* **1**, 641-645.

Showers, WB, Reed, GL, Robinson, JF, & DeRozari, MB. 1976. Flight and sexual activity of the European corn borer. *Environmental Entomology* **5**, 1099-1104.

Showers, WB, Berry, EC, & Von Kaster, L. 1980. Management of 2nd-generation European corn borer by controlling moths outside the cornfield. *Journal of Economic Entomology* **73**, 88-91.

Slosser, JE, Phillips, JR & Herzog, GA. 1978. Bollworm damage and population development in relation to phenology of the cotton plant. *Environmental Entomology* **7**, 144-148.

Smith, R, Larsen, M, and Kirsch, P. 2003. Last Call CM: Effective codling moth control in Canadian Maritime apple orchards. *Proceedings of the 77th Annual Western Orchard Pest & Disease Management Conference*, Portland, Oregon.

Sower, LL, Shorey, HH & Gaston, LK. 1970. Sex pheromones of noctuid moths. XXI. Light:dark cycle regulation and light inhibition of sex pheromone release by females of *Trichoplusia ni*. *Annals of the Entomological Society of America* **63**, 1090-1092.

Sower, LL, Shorey, HH & Gaston, LK. 1971. Sex pheromones of noctuid moths. XXV. Effects of temperature and photoperiod on circadian rhythms of sex pheromone release by females of *Trichoplusia ni*. *Annals of the Entomological Society of America* **64**, 488-492.

Sparks, AN, Raulston, JR, Lingren, PD, Carpenter, JE, Klun, JA & Mullinix, BG. 1979. Field response of male *Heliothis virescens* to pheromonal stimuli and traps. *Bulletin of the Entomological Society of America* **25**, 268-274.

Spessa, AC. 1991. The oviposition dynamics of *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) (Lepidoptera: Noctuidae): An experimental study of some factors affecting reproductive performance and a simulation study within different cropping systems. Master of Rural Science thesis, University of New England, 293 pp.

Srivastava, CP, Pimbert, MP & Reed, W. 1992. Monitoring of *Helicoverpa* (=*Heliothis*) *armigera* (Hübner) moths with light and pheromone traps in India. *Insect science and its applications* **13**, 205-210.

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, Karg, G & Bradley, SJ. 1996. Apple foliage enhances mating disruption of light-brown apple moth. *Journal of Chemical Ecology* **22**, 325-341.

Suckling, DM, Karg, G, Green, S & Gibb, AR. 1999. The effect of atmospheric pheromone concentrations on behavior of lightbrown apple moth in an apple orchard. *Journal of Chemical Ecology.* **25**, 2011-2025.

Takai, M & Wakamura, S. 1995. Control of the beet armyworm, *Spodoptera exigua* (Hübner), with synthetic sex pheromone. *Food & Fertilizer Technology Center Technical Bulletin* 1-8.

Tamhankar, AJ, Rajendran, TP, Hariprasad Rao, N, Lavekar, RC, Jeyakumar, P, Monga, D & Bambawale, OM. 2003. Variability in response of *Helicoverpa armigera* males from different locations in India to varying blends of female sex pheromone suggests male sex pheromone response polymorphism. *Current Science* **84**, 448-450.

Taylor LR. 1986. The four kinds of migration. In: *Insect Flight*, (ed W Danthanarayana), pp. 265-280. Springer-Verlag. Berlin.

Teakle RE & Jensen JM. 1991. *Heliothis punctiger.* In: *Handbook of Insect Rearing Vol. II* (eds P Singh &RF Moore) pp. 313-322. Elsevier, Oxford.

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.

Tomlin CDS. 1997. *The Pesticide manual : a world compendium.* British Crop Protection Council, Farnham, Surrey.

Topper, CP. 1987. Nocturnal behaviour of adults of *Heliothis armigera* (Hübner) (Lepidoptera: Noctuidae) in the Sudan Gezira and pest control implications. *Bulletin of Entomological Research* **77**, 541-554.

Toyoshima, G, Kobayashi, S & Yoshihama, T. 2001. Control of *Helicoverpa armigera* (Hübner) by mating disruption using diamolure in lettuce fields. *Japanese Journal of Applied Entomology & Zoology.* **45**, 183-188.

Traynier, RMM. 1968. Sex attraction in the Mediterranean flour moth, *Anagasta kuhniella*: location of the female by the male. *The Canadian Entomologist* **100**, 5-10.

Traynier, RMM. 1970. Sexual behaviour of the Mediterranean flour moth, *Anagasta kuhniella*: some influences of age, photoperiod and light intensity. *Canadian Entomologist* **102**, 534-540.

Trematerra, P & Capizzi, A. 1991. Attracticide method in the control of *Ephestia kuehniella* Zeller: studies on effectiveness. *Zeitschrift für angewandte Entomologie* **111**, 451-456.

Tunstall, JP. 1968. Preliminary trial of the addition of molasses to insecticides. *Cotton Growing Review* **45**, 198-206.

Uk, S & Dittrich, V. 1986. The behaviour-modifying effect of chlordimeform and endosulfan on the adult whitefly *Bemisia tabaci* (Genn.) which attacks cotton in the Sudan. *Crop Protection* **5**, 341-347.

Underwood, AJ. 1992. Beyond BACI: The detection of environmental impacts on populations in the real, but variable, world. *Journal of Experimental Marine Biology and Ecology* **161**, 145-178.

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

Vickers, NJ & Baker, TC. 1992. Male *Heliothis virescens* maintain upwind flight in response to experimentally pulsed filaments of their sex pheromone (Lepidoptera: Noctuidae). *Journal of Insect Behavior* **5**, 669-687.

Vickers, NJ & Baker, TC. 1994. Reiterative responses to single strands of odor promote sustained upwind flight and odor source location by moths. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 5756-5760.

Vickers, NJ & Baker, TC. 1997. Flight of *Heliothis virescens* males in the field in response to sex pheromone. *Physiological Entomology* **22**, 277-285.

Vickers, RA., Jones, P. & Rumbo, E. 2000. Behaviour modification of the diamondback moth. In: *CSIRO Entomology – Report of Research 1997-99* ed. M. Robertson, CSIRO Black Mountain, ACT.

von Boguslawski, C & Basedow, T. 2001. Studies in cotton fields in Egypt on the effects of pheromone mating disruption on *Pectinophora gossypiella* (Saund.) (Lep., Gelechiidae), on the occurrence of other arthropods, and on yields. *Zeitschrift für angewandte Entomologie* **125**, 327-331.

Wall, C & Perry, JN. 1983. Further observations on the responses of male pea moth, *Cydia nigricana*, to vegetation previously exposed to sex-attractant. *Entomologia Experimentalis et Applicata* **33**, 112-116.

Wall, C, Sturgeon, DM, Greenway, AR & Perry, JN. 1981. Contamination of vegetation with synthetic sex-attractant released from traps for the pea moth, *Cydia nigricana* (F.). *Entomologia Experimentalis et Applicata* **30**, 111-115.

Wardhaugh, KG, Room, PM & Greenup, LR. 1980. The incidence of *Heliothis armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae) on cotton and other host plants in the Namoi valley of New South Wales. *Bulletin of Entomological Research* **70**, 113-131.

Ware GW. 1999. An introduction to insecticides. In: *Radcliffe's IPM World* (eds EB Radcliffe &WD Hutchison) 3rd Edition, University of Minnesota, St. Paul, Minnesota. http://ipmworld.umn.edu/chapters/ware.htm, accessed 2 January 2001

Ware GW. 2000. *The Pesticide Book.* Thomson Publications, Fresno, California.

Weatherston I. 1990. Principles of design of controlled-release formulations. In: *Behaviour-modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants* (eds RL Ridgway, RM Silverstein & MN Inscoe) pp. 93-111. Marcel Dekker Inc., New York.

Weatherston, I & Miller, D. 1989. Release kinetics of liquid flowable formulations of gossyplure, sex pheromone of the pink bollworm moth Pectinophora gossypiella Saunders (Lepidoptera: Gelechiidae). *Journal of Chemical Ecology.* **15**, 1767-1773.

Webster, RP. 1988. Modulation of the expression of calling by temperature in the omnivorous leafroller moth, *Platynota stultana* (Lepidoptera: Tortricidae) and other moths: a hypothesis. *Annals of the Entomological Society of America* **81**, 138-151.

Weissling, TJ & Meinke, LJ. 1991. Potential of starch encapsulated semiochemical-insecticide formulations for adult corn rootworm (Coleoptera: Chrysomelidae) control. *Journal of Economic Entomology.* **84**, 601-609.

Willis, MA, David, CT, Murlis, DJ & Cardé, RT. 1994. Effects of pheromone plume structure and visual stimuli on the pheromone-modulated flight of male gypsy moths (*Lymantria dispar* L.) in a forest. *Journal of Insect Behavior* **7**, 385-409.

Wilson, AGL, Lewis, T & Cunningham, RB. 1978. Overwintering and spring emergence of *Heliothis armigera* (Hübner) (Lepidoptera:Noctuidae) in the Namoi valley, New South Wales. *Bulletin of Entomological Research,* **69**, 97-109.

Wilson, AGL & Morton, R. 1989. Some factors affecting the reliability of pheromone traps for measurement of the relative abundance of *Helicoverpa punctigera* (Wallengren) and *H. armigera* (Hübner) (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* **79**, 265-273.

Witzgall, P, Backman, AC, Svensson, M, Koch, UT, Rama, F, El-Sayed, A, Brauchli, J, Arn, H, Bengtsson, M & Löfqvist, J. 1999. Behavioral observations of codling moth, *Cydia pomonella*, in orchards permeated with synthetic pheromone. *BioControl* **44**, 211-237.

Witzgall, P, Bengtsson, M, Karg, G, Bäckman, A-C, Streinz, L, Kirsch, PA, Blum, Z & Löfqvist, J. 1996. Behavioral observations and measurements of aerial pheromone in a mating disruption trial against pea moth *Cydia nigricana* F. (Lepidoptera, Tortricidae). *Journal of Chemical Ecology* 191-206.

Wu, W-Q, Tang, X-H, Xu, SF & Du, J-W. 1991. The diel rhythm of calling behavior and sex pheromone production of *Helicoverpa armigera* (Hübner)(Lepidoptera: Noctuidae). *Contributions from Shanghai Institute of Entomology* **10**, 57-62.

Zalucki, MP, Daglish, G, Firempong, S & Twine, P. 1986. The biology and ecology of *Heliothis armigera* (Hübner) and *Heliothis punctigera* Wallengren (Lepidoptera:Noctuidae) in Australia: what do we know? *Australian Journal of Zoology* **34**, 779-814.

Zalucki, MP, Murray, DAH, Gregg, PC, Fitt, GP, Twine, PH & Jones, C. 1994. Ecology of *Helicoverpa armigera* (Hübner) and *H. punctigera* (Wallengren) in the inland of Australia: Larval sampling and host plant relationships during winter and spring. *Australian Journal of Zoology* **42**, 329-346.

Zhang, GF, Yan, XH, Sheng, CF & Meng, XZ. 2001. Mating disruption of the Chinese tortrix *Cydia trasias* (Lepidoptera : Olethreutidae) by atmospheric permeation with synthetic sex pheromone. *Environmental Entomology.* **30**, 7-11.

12 Appendices

12.1 Table of Taxonomic authorities associated with flora and fauna cited in this thesis

Acrobasis nuxvorella Neunzig
Agrotis infusa (Boisduval)
Agrotis ipsilon (Hufnagel)
Agrotis segetum (Denis & Schiffermüller)
Amblyomma americanum (Linneaus)
Anthonomis grandis Boheman
Bactrocera cucurbitae (Coquillett)
Bactrocera dorsalis (Hendel)
Bombyx mori (Linneaus)
Brassica Linneaus
Capsicum Linneaus
Castilleja indivisa Englemann
Ceratitis capitata (Weidemann)
Ceratitis cosyra (Walker)
Ceratitis rosa Karsch
Citrus reticulata Linneaus
Chrysodeixis argentifera (Guenée)
Contarinia oregonensis Foote
Cryptophlebia leucotreta Meyrick
Cydia nigricana (Fabricius)
Cydia pomonella (Linneaus)
Dermacentor variabilis (Say)
Diabrotica Chevrolat
Ephestia kuehniella Zeller
Epiphyas postvittana (Walker)
Eucosma sonomana Kearfott
Fragaria x *ananassa* Duchesne
Gossypium hirsutum Linneaus
Grapholita molesta (Busck)
Helicoverpa (Hardwick)
Helicoverpa armigera (Hübner)
Helicoverpa assulta (Guenée)
Helicoverpa gelotopoeon (Dyar)
Helicoverpa punctigera (Wallengren)
Helicoverpa zea Boddie
Heliocheilus Grote
Heliothis maritima Graslin
Heliothis obsoleta (Fabricius)
Heliothis Ochsenheimer
Heliothis ononis (Denis & Schiffermüller)
Heliothis peltigera (Denis & Schiffermüller)
Heliothis phloxiphaga Grote & Robinson
Heliothis subflexa Guenée
Heliothis virescens (Fabricius)
Holomelina immaculata (Reakirt)
Ixodes scapularis Say
Keiferia lycopersicella (Walshingham)
Lycopersicon esculentum Miller
Lymantria dispar (Linneaus)
Ostrinia nubilalis (Hübner)
Pectinophora gossypiella (Saunders)
Phaseolus vulgaris Linneaus
Phthorimaea operculella (Zeller)
Pisum sativum Linneaus
Platyptilia carduidactyla (Riley)
Plutella xylostella (Linneaus)
Protoschinia scutosa (Denis & Schiffermüller)
Pseudaletia unipuncta (Haworth)
Pyrrhia umbra (Hufnagel)
Rhyacionia buoliana (Denis & Schiffermüller)
Rhyacionia frustrana (Comstock)
Schinia bina (Guenée)
Schinia meadi (Grote)
Silybum marianum (Linneaus) Gaertner
Solanum nigrum Linneaus
Sorghum Linneaus
Spodoptera frugiperda (Smith)
Spodoptera Guenée
Stomoxys calcitrans (Linneaus)
Synanthedon pictipes (Grote & Robinson)
Trichoplusia ni (Hübner)
Zea mays Linneaus

12.2 Totals and means for data from in Chapter 4

Table 1 Comparisons between male *H. armigera* flying to synthetic and natural pheromone sources. Different letters after the numbers in each column indicate that there are significant differences between treatments (χ^2 test, $p < 0.05$ for percentage data, paired t-test, $p < 0.05$ for mean data).

	Total number of males for each behaviour (number in parentheses is the percentage of the total number of males approaching the lure)	
	Approaching	Near
standard lure	271	230 (84.87)a
standard lure in blind cage	119	91 (76.47)a
3 females in blind cage	118	82 (69.49)a
	Mean no. moths per second (± standard error)	
	Approaching	Near
standard lure	0.0568 (0.0044)a	0.0482 (0.0043)a
standard lure in blind cage	0.0250 (0.0060)b	0.0191 (0.0042)b
3 females in blind cage	0.0176 (0.0046)b	0.0124 (0.0035)b
	Mean time spent approaching and near the lure (± standard error)	
	Approaching	Near
standard lure	3.37 (0.91)	7.05 (0.85)
standard lure in blind cage	2.02 (0.39)	10.37 (2.03)
3 females in blind cage	2.56 (0.40)	13.59 (2.05)

Table 2 Comparisons between lures with different component ratios and composition.

	Total number males for each behaviour (number in parentheses is the percentage of the total number of males approaching the lure)		
Approaching Near		Contact	
standard two component 10:1 ratio	115	105 (91.30)	6 (5.22)
standard two component 97:3	86	74 (86.05)	8 (9.30)
standard blend with (n)-hexadecanal	72	66 (91.67)	9 (12.50)
	Mean no. moths per second (± standard error)		
	Approaching	Near	Contact
standard two component 10:1 ratio	0.0322 (0.0078)	0.0294 (0.0070)	0.0017 (0.0004)
standard two component 97:3	0.0239 (0.0056)	0.0206 (0.0055)	0.0022 (0.0008)
standard blend with (n)-hexadecanal	0.0199 (0.0033)	0.0183 (0.0035)	0.0025 (0.0007)
	Mean time spent approaching and near the lure (± standard error)		
	Approaching	Near	
standard two component 10:1 ratio	2.19 (0.16)	6.73 (0.60)	
standard two component 97:3	2.78 (0.17)	6.67 (0.65)	
standard blend with (n)-hexadecanal	1.96 (0.21)	7.13 (0.86)	

Table 3 Comparisons between lures with three presentation treatments. Different letters after the numbers in each column indicate that there are significant differences between treatments (χ^2 test, $p < 0.05$ for percentage data, paired t-test, $p < 0.05$ for mean data).

	Total number of males for each behaviour (number in parentheses is the percentage of the total number of males approaching the lure)		
	Approaching	Near	Contact
1ml single droplet	1020	943 (92.45)a	50 (4.90)a
5 x 4 small droplets (total of 1ml)	876	819 (93.49)a	167 (19.06)b
1ml smeared	797	746 (93.60)a	199 (24.97)c

Mean no. moths per second (± standard error)

	Approaching	Near	Contact
1ml single droplet	0.2809 (0.0484)a	0.2597 (0.0436)a	0.0138 (0.0040)a
5 x 4 small droplets (total of 1ml)	0.2418 (0.0424)a	0.2260 (0.0400)a	0.0460 (0.0139)b
1ml smeared	0.2206 (0.0338)a	0.2064 (0.0321)a	0.0551 (0.0051)b

Mean time spent approaching and near the lure (± standard error)

	Approaching	Near
1ml single droplet	1.09 (0.16)	3.88 (0.52)
5 x 4 small droplets (total of 1ml)	0.84 (0.07)	3.28 (0.39)
1ml smeared	0.88 (0.18)	3.58 (0.40)

Table 4 Effects of placing a decoy female *H. armigera* on the Sirene lure, and placing the lure on plastic versus a natural substrate (sunflower). Different letters after the numbers in each column indicate that there are significant differences between treatments ($p<0.01$) with paired proportions tests using $\chi 2$ for count data, Kruskall-Wallis Rank Sum Test for males per second data.

	Total number of males for each behaviour (number in parentheses is the percentage of the total number of males approaching the lure)		
	Approaching	Near	Contact
Plastic	687	560 (81.5)a	31 (4.5)a
Plastic + female	531	425 (80.0)a	125 (23.5)b
Sunflower	442	372 (84.2)a	44 (10.0)c
Sunflower + female	536	446 (83.2)a	105 (19.6)b

Mean no. moths per second (± standard error)

	Approaching	Near	Contact
Plastic	0.2183 (0.0757)a	0.1778 (0.0700)a	0.0098 (0.0031)a
Plastic + female	0.1616 (0.0320)a	0.1289 (0.0295)a	0.0377 (0.0112)b
Sunflower	0.1479 (0.0304)a	0.1244 (0.0292)a	0.0149 (0.0069)a
Sunflower + female	0.1858 (0.0604)a	0.1503 (0.0568)a	0.0352 (0.0138)a

Mean time (seconds) spent approaching and near the lure (± standard error)

	Approaching	Near
Plastic	1.77 (0.24)a	2.269 (0.26)a
Plastic + female	2.13 (0.61)ab	2.70 (0.69)ab
Sunflower	3.12 (0.40)b	3.78 (0.36)b
Sunflower + female	2.30 (0.48)ab	3.57 (0.59)ab

Table 5 Comparison of five different visual treatments. Different letters after the numbers in each column indicate that there are significant differences between treatments ($p<0.01$) with paired proportions tests using χ^2.

	Total number of males for each behaviour (number in parentheses is the percentage of the total number of males approaching the lure)		
	Approaching	**Near**	**Contact**
Plastic	555	391 (70.45)a	29 (5.23)cde
Four Wings	660	535 (81.06)b	61 (9.24)c
Fore Wings	792	640 (80.81)b	55 (6.94)c
Hind Wings	738	632 (85.64)b	45 (6.10)cd
Black Line	474	351 (74.05)a	13 (2.74)de

	Mean no. moths per second (± standard error)		
	Approaching	**Near**	**Contact**
Plastic	0.2097 (0.0508)	0.1484 (0.0391)	0.0111 (0.0041)
Four Wings	0.2577 (0.0404)	0.2093 (0.0522)	0.0238 (0.0055)
Fore Wings	0.3070 (0.1134)	0.2500 (0.1308)	0.0216 (0.0085)
Hind Wings	0.2987 (0.0966)	0.2573 (0.1132)	0.0181 (0.0063)
Black Line	0.2757 (0.0631)	0.2261 (0.0728)	0.0129 (0.0082)

	Mean time (seconds) spent approaching and near the lure (± standard error)	
	Approaching	**Near**
Plastic	2.75 (1.29)	3.53 (1.35)
Four Wings	3.52 (1.94)	4.09 (1.80)
Fore Wings	2.67 (0.91)	3.47 (0.84)
Hind Wings	2.48 (0.74)	2.87 (0.62)
Black Line	1.86 (0.38)	0.31)

Table 6 Evaluation of the effect of including bifenthrin in Sirene with synthetic pheromone.

	Total number of males for each behaviour (number in parentheses is the percentage of the total number of males approaching the lure)		
	Approaching	**Near**	**Contact**
No insecticide	1541	1306 (84.8)	155 (10.1)
6% bifenthrin	1519	1237 (81.4)	114 (7.5)

	Mean no. moths per second (± standard error)		
	Approaching	**Near**	**Contact**
No insecticide	0.0726 (0.0115)	0.0616 (0.0109)	0.0073 (0.0012)
6% bifenthrin	0.0697 (0.0088)	0.0565 (0.0076)	0.0051 (0.0006)

	Mean time (seconds) spent approaching and near the lure (± standard error)	
	Approaching	**Near**
No insecticide	4.24 (0.58)	4.52 (0.74)
6% bifenthrin	4.93 (0.62)	5.17 (0.80)

12.3 Analysis of extruded polymer formulations by gas chromatography

(Source: AgriSense BCS Pty. Ltd./Enzo Casagrande)

1. SCOPE

1.1 This method can be used to analyse for the active ingredients in extruded polymer formulations.

2. FIELD OF APPLICATION

2.1 This method can be applied to extruded polymer formulations, including Selibate CS, Selibate HA, Selibate PBW, Frustrate PBW.

3. REFERENCES

None

4. PRINCIPLE

4.1 Portions of the extruded polymer formulations are extracted with solvent for a period of time and analysed by gas chromatography (GC) using a flame ionisation detector (FID).

4.2 Quantitative analysis is carried out by means of an internal standard.

5. HEALTH, SAFETY & ENVIRONMENTAL PROTECTION

5.1 Safety glasses, gloves and a properly fitting, fastened lab coat must be worn during this analysis. All work must be carried out in a fume cupboard. Any waste chemicals must be collected for proper disposal according to legislation.

5.2 All laboratory work should be carried out by competent, suitably trained personnel.

5.3 Acetone – highly flammable

5.4 Hexane – highly flammable, harmful

5.5 Methyl myristate – harmful

5.6 Refer to individual safety data sheets for information on the pheromone(s) under test.

5.7 Compressed gas cylinders should only be used by competent, suitably trained personnel. It is essential the correct regulators, piping and fittings be used in the installation of GC gas supplies.

5.8 Helium gas – asphyxiant at high concentrations, high pressure container.

5.9 Hydrogen gas – highly flammable gas, high pressure container.

5.10 Compressed air – high pressure.

6. REAGENTS

6.1 Acetone, analytical grade.

6.2 Hexane, analytical grade.

6.3 Extraction Solvent

6.3.1 Mix acetone (250 ml) and hexane (750 ml). Store in a suitable bottle.

6.4 Helium gas, GC grade.

6.5 Hydrogen gas, GC grade.

6.6 Compressed air, GC grade.

6.7 Methyl myristate, 99% or better.

6.8 Internal Standard Solution (1 mg/ml)

6.8.1 Using an analytical balance, accurately weigh out 100 mg of methyl myristate (6.7) into a 100ml volumetric flask.

6.8.2 Make up to the mark with extraction solvent (6.3) and mix thoroughly. Store tightly sealed in a brown bottle.

6.9 Standard Solutions

6.9.1 Accurately weigh out 10 mg of each of the active ingredients in the formulation into separate 10 ml volumetric flasks and make up to the mark with Internal Standard solution (6.8). The components are present at approximately 1mg/ml.

7. APPARATUS

7.1 Analytical balance capable of measuring to 4 decimal places (i.e.0.0000g) or better.
7.2 Glass pipettes, grade B or better (10ml).
7.3 Volumetric flasks, grade B or better (10ml)
7.4 20 ml (approx) screw top vial.
7.5 Ultrasonic bath (optional)
7.6 Microlitre syringes
7.7 Gas chromatograph with split injection system and flame ionisation detector (FID)
7.8 Fused silica capillary column, BP-1, 25m, 0.22 mm I.D., 0.1 µm film thickness or equivalent. Alternatively a BPX-70, 25m, 0.22 mm I.D., 0.25µm film thickness or equivalent is also suitable.

8. PROCEDURE

8.1 Accurately weigh approximately 100 mg of the extruded polymer formulation into a screw top vial and, using a 10ml glass pipette, transfer 10 ml of Internal Standard solution (6.8) to the vial. Ensure the sample is completely covered by solvent.
8.2 Tightly cap the vial and leave to stand for 24 hours in a refrigerator. Alternatively, an ultrasonic bath can be used to accelerate the extraction.
8.3 After extraction is complete, using a microlitre syringe, inject an aliquot of sample solution (8.2) into the injection port of the GC. Suggested operating parameters for the GC are included in later in appendix. Make replicate injections as required.
8.4 With a microlitre syringe inject an aliquot of standard (6.9) into the injection port of the GC. Make replicate injections as required.

9. RESULTS

9.1 Calculate the average weight of each analyte in the formulation using the following formula for each component:

$$\text{Weight (mg)} = \frac{C_{std} \times \text{Purity} \times R_a}{R_s}$$

where:

C_{std} = weight of analyte, in mg, in standard solution (6.13.4)

R_a = ratio of peak area of analyte to internal standard in the assay sample

R_s = ratio of peak area of analyte to internal standard in the standard sample

Purity = % purity of each analyte

10. NOTES

10.1 The purity of individual components can be found by analysis of a solution of the pheromone made to approximately 1mg/ml in extraction solvent (6.3)

Suggested Operating Conditions

BP1 Column

25 m, 0.22mm i.d., 0.1μm film thickness (available from SGE)

Program:

100° C held for 2 mins,
ramp at 20° C/min to 130° C,
ramp at 3° C/min to 180° C,
ramp at 30° C/min to 250° C, hold for 30s,
ramp at 30° C/min to 270° C, hold for 30s.

Injector Temp: 290° C

Detector: FID

Detector Temp: 290° C

Carrier Pressure: 15 psig

Split: 50 : 1 (approximately)

BPX 70

25 m, 0.22 mm i.d., 0.25μm film thickness (available from SGE)

Program: 100° C held for 2 mins,
Ramp at 20° C/min to 120° C
Ramp at 4° C/min to 170° C
Ramp at 20° C/min to 240° C, held for 1 min,
Ramp at 30° C/min to 255° C, held for 1 min.

Injector Temp: 290° C

Detector: FID

Detector Temp: 290° C

Carrier Pressure: 15 psig

Split: 50 : 1 (approximately)