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Feasibility of mating disruption for heliothis species in tomatoes and capsicums

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Mating Disruption Trials for *Helicoverpa armigera* in Tomato and Capsicum for SP Exports, Childers, Qld.

Report

by

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1. Plain English Summary

A mating disruption trial for *Helicoverpa armigera* (Heliothis or Cotton Bollworm) was carried out between February and July, 2003 in the Promised Land region, Childers, Queensland. Field-grown tomato and capsicum crops (total area 125 ha) in the Promised Land area were treated with AgriSense BCS Selibate HA pheromone dispensers at a rate of 240 dispensers per hectare. The nearest untreated areas of capsicum, tomato or other heliothis-susceptible plants were more than 6km to the east of the Promised Land area. The activity of *Helicoverpa* spp. in these untreated tomato and capsicum crops was compared to that of the treated areas to determine efficacy of the treatment within the Promised Land area.

Mating disruption works by infusing the treated area with high concentrations of sex pheromone of the target species (in this case *H. armigera*). This high concentration prevents males from finding and mating with females in the crop, and if effective, subsequently prevents fertile eggs from being laid in the crop.

A number of methods were used to determine if the mating disruption effect occurred in the treated areas:

1) Funnel traps baited with synthetic sex pheromone were placed in the crops to mimic female moths, with a reduced or zero catch indicating that males were unable to find females in the treated areas.
   - Pheromone trap catches indicated between 95 and 100% disruption in the treated tomato and capsicum.
2) Unmated wing-clipped laboratory-reared female moths were placed overnight into treated and untreated crops as well as sugarcane adjacent to the treated tomato where wild male moths could access them.
   - Treatment reduced mating of these captive females to almost zero compared to untreated crops and untreated sugarcane near treated tomato where up to 30% of the females were mated overnight.
3) Light traps were operated within the treated and untreated crops as well as sugar cane to monitor both male and female moth activity. Female moths were dissected to determine mating status.
   - Treatment reduced the number of males caught in light traps, suggesting that males were less active in the treated areas. There was no difference between treated and untreated areas in the percentage of mated females, indicating that mated females were moving

freely into treated areas. Female moths collected in untreated areas were more likely to have been mated more than once.

4) Egg and larval counts from treated and untreated crops were monitored by SP Exports.
   - There was a significant reduction in eggs in treated capsicum, but not in the treated tomato. Egg counts in the capsicum remained above the spray threshold despite the overall reduction in numbers.

Despite a marked reduction in the number of females being mated in treated areas, the trial did not lead to an agronomically significant reduction in egg counts in treated areas. This appears to be due to immigration of mated females into treated areas.

The source of these mated females has not been determined, but there is good evidence that *H. armigera* females present in the treated areas were moving into and being mated in the untreated sugarcane adjacent to the treated areas. These mated females then moved into the crops where they laid similar numbers of eggs as for females in untreated crop areas. Sugarcane is not normally a larval host plant of *H. armigera*, and it had been assumed that mating would not occur in this crop.

The failure of mating disruption to prevent female *H. armigera* from laying eggs in treated areas suggests that this pest management strategy might be useful when used in conjunction with future IPM tactics in field-grown tomato and capsicum, but not as a stand-alone tactic.

2. Acknowledgements

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Jeff and Marilyn Bidstrup of Bidstrup Biologicals Pty. Ltd., Warra supplied *H. armigera* pupae at no cost.

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3. Introduction:

3.1 *Helicoverpa* spp.: biology, ecology and importance in agronomic systems.

Helicoverpa (Heliothis) armigera (Cotton bollworm, tomato fruitworm, corn earworm, Old World bollworm) (Lepidoptera: Noctuidae) is a destructive pest of many crops including tomato and capsicum. The adults moths are highly mobile, and females lay numerous eggs on many different crop and non-crop food plant species. Matthews (1999) lists over 130 species of food plants for *H. armigera* within Australia. The larvae usually feed on flowers, fruits and developing shoots of the plants, often damaging the most economically critical stages of crops. In addition to these life-history characteristics which favour pest status *H. armigera* has developed resistance to most of the commonly available insecticides, including pyrethroids, carbamates and organophosphates. In some cases insecticide resistance has led to greatly increased usage of insecticides, pushing crop production costs above economic thresholds (Wilson 1974). *H. armigera* is also a serious pest in other parts of its extensive range including Oceania, Asia, Europe and Africa.

H. punctigera is native to Australia and is also a pest of many crops including tomato and capsicum. *H. punctigera* has not developed significant resistance to insecticides, and also exhibits strong, seasonally-driven migratory patterns which are lacking in *H. armigera*. Further details of the ecology of *Helicoverpa* spp. in relation to agronomic practices can be found in Fitt (1989).

3.2 Sex pheromones and their uses in pest management.

Many moths use sex pheromones as a method of mate location. In most cases a female moth releases minute amounts of species-specific blends of volatile chemicals into the environment. When a male moth of the same species detects these chemicals it flies upwind following the airborne trail of chemicals until it locates the female and mating takes place. These highly specific chemicals emitted by the females are called sex pheromones. The chemical components

of sex pheromones produced by moths are typically simple straight chain carbon-based molecules between seven and twenty-one carbons long, usually with a functional group such as an aldehyde, alcohol, or acetate group attached to one end of the chain. Further specificity in the components is determined by the presence and placement of double bonds in the carbon chain. These chemical components are specific not only in terms of the number of carbons, the attached functional groups, and the presence, number and placement of double bonds, but also the correct *cis*- or *trans*- isomer (represented by *Z* or *E* in chemical shorthand). This is sometimes a problem when producing synthetic chemical mixtures to mimic the blend produced by the female moth, as most chemical syntheses produce a blend of the two isomers, which are sometimes difficult (and often expensive) to separate. The presence of the incorrect isomer may have no effect, or may have a repellent effect on the male moth, so purity of synthetic pheromone mixtures is a major issue when attempting to produce lures (Howse *et al.* 1998). In *H. armigera* the major pheromone component is (Z)-11-hexadecenal (Piccardi *et al.* 1977; Rothschild 1978), a molecule which has a sixteen carbon chain, an aldehyde functional group, a double bond on the eleventh carbon, and is present as the *cis*- (or *Z*) isomer.

In most cases a moth species will have a blend of two or more specific components. The ratio of these components within the blend is often just as important as the identity of the components. For example, the currently used blend for *H. armigera* in Australia is a 10:1 mixture of two aldehydes, (Z)-11-hexadecenal and (Z)-9-hexadecenal (Gregg and Wilson 1991). In Asia the ratio is 97:3. Excessive amounts of (Z)-9-hexadecenal will reduce the attractiveness of a test lure, but both components have to be present in order for the lure to be maximally attractive (Kehat *et al.* 1980; Kehat and Dunkelblum 1990). In summary, in order to elicit the proper response of the male moth to a pheromone source there must be the right components present, in the correct configuration, in the correct blend, and in the correct ratio. Despite these exacting requirements it may be possible to disrupt male responses to natural pheromone sources by using a synthetic blend which is quite different to the normal blend produced by the female. Diamolure dispensers used by Toyoshima *et al.* (2001) in lettuce have a 125mg loading of 36.0% (Z)-11-hexadecenal and 41.0% (Z)-11-hexadecenyl acetate which successfully disrupted male *H. armigera* responses to natural pheromone sources.

Because sex pheromones are naturally produced in such minute amounts the early research into pheromones relied on extracting the pheromone components from literally thousands of female moths (Butenandt *et al.* 1959). Identification of these sex pheromones and advances in organic chemistry allowed for production of the first synthetic pheromone blends (Berger 1966). Early

synthetic blends often consisted of only one pheromone component, as the extraction and characterisation methods used were often not sensitive enough to detect other minor components. As larger amounts of synthetic pheromone became available it became possible to experiment with various airborne concentrations of pheromone and record the changes in the responsiveness of males when exposed to these varying concentrations. As concentrations of pheromone increased rather than increasing the responsiveness of males, the responsiveness decreased to negligible levels and males could no longer locate calling females in these conditions (Shorey *et al.* 1967). The mechanisms behind this decrease (which has been called mating disruption) in responsiveness are complex, and are still the subject of ongoing research (Valeur 1998). The practical applications of this phenomenon, however, were immediately evident (Shorey *et al.* 1967; Jacobson and Beroza 1964). Mating disruption has been attempted with a large number of moth species, predominately in orchard systems, but also in other horticultural crops and in some cases, larger areas of field crops such as cotton. There are several detailed reviews on the mechanisms and application of mating disruption including Cardé and Minks (1995) examining the successes and constraints of applied mating disruption, and that of Valeur (1998) which looks at the mechanisms resulting in mating disruption.

Other uses of sex pheromone have been devised, the most widespread of which is monitoring of pest species. Pheromone traps can be used to detect the presence or absence of pests in a given area, or to establish a threshold above which intervention to control the pest is required.

Attract and kill and mass trapping methods use pheromone lures to attract the pest species to a trap or dispenser where it can be killed or rendered infertile. Mass trapping is only effective in special cases, as most traps are highly inefficient when it comes to catching insects, and require frequent servicing. Attract and kill is a developing field in applied entomology, and with improvements in formulations and delivery mechanisms may become a viable tactic within integrated pest management strategies.

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

An additional practical problem emerges when using synthetic pheromones in the field. Most are highly volatile and many are unstable, breaking down in the presence of atmospheric oxygen and UV light and becoming ineffective. 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. These factors also come into consideration when designing the application of sex pheromone for mating disruption depending on the pest species involved, the cost of pheromones and a number of other factors. Table 1 summarizes the main factors involved in design and application.

Traditional application of mating disruption has relied on large numbers of dispensers placed in the field (eg. 250 or more per hectare). These dispensers can be constructed from various polymer and rubber 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"). These dispenser 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, or polyethylene bags which are manufactured so that the pheromone blend is released at a certain rate. The reduction in application costs with low-density high-release dispensers has to be balanced by potential costs of the dispensers, reduction in the efficacy due to potential gaps in the spatial coverage, 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 currently used in Australian field and horticultural crops.

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 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. 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 *et al.* 1992).

A further development in pheromone formulations has been the development of 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 dispenser holding the electrostatic powder which contains the pheromone. The charged pheromone-laden powder sticks to the male moths. After leaving the pheromone dispenser the contaminated male moths then become mobile mating disruption units, as not only are they unable to locate and mate with female moths, but they also become targets for other uncontaminated males who see 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*.

3.4 Is it working? Monitoring mating disruption trials

Monitoring is a crucial component of any mating disruption trial. Monitoring establishes if mating is prevented in the treated areas, whether egg lay is occurring within treated areas, and if so suggest possible reasons, such as the movement of mated females into treated areas. 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 a discrete spatial unit of crop, or treat 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

Table 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 dispenser density	Increasing the density of dispensers whilst decreasing dispenser loading[4]	Potential improvement of disruption, better disruption in a range of weather conditions	Increased cost of dispensers and application/labour costs
Decreased dispenser density	Decreasing the density of dispensers whilst increasing the dispenser loading[4]	Reduced cost of dispensers and application labour	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 efficacy, 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 1

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

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 traps with either synthetic lures, or live females as pheromone sources. Failure to catch male moths in treated areas compared to untreated areas indicates that the pheromone released from the dispensers is preventing males from locating the lures/females. This result is often referred to as "trap shutdown". 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 nearly as attractive as a calling female moth. Using female moths as pheromone sources within a trap is much more labour-intensive, and is unreliable as female moths may not call whilst they are in the trap. 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 (pers. obs.). If moths are present in low numbers in the field this makes detection of differences between treated and untreated cropping areas difficult.

Another method of monitoring is light trapping, which can provide useful information on general moth activity, especially in cases where, despite pheromone trap shutdown, eggs are still being laid in the treated areas. Depending on the species of moth under investigation light traps usually catch both sexes. Light trap catches depend on the responses of moths to the light, and on the mobility of the moths, 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, being lower in cool, windy conditions, and by nocturnal light, with bright nights often reducing catches. It can be assumed that these factors are similar in both treated and untreated areas, the number of males present allows for comparison between the pheromone traps and the actual population of male moths present in both treated and untreated areas. 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. The more important role of light traps is to provide samples of the wild female moths present in the treated and untreated areas. The percentage of mated females in the untreated and treated areas can 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. A spermatophore is produced by the male moth, and contains a mixture of sperm and nutrients. *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 or wing-clipped 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 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 the 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 from these trials should be treated with caution, as females in mating trays are usually less able to undergo reproductive activity compared to wild females. The proportion of mated sentinel females present in mating trays is therefore 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.

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. The number of mating pairs of moths on the vegetation can be 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 in the infra-red region which is 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 be collected from around flowering crops where they are either feeding or laying eggs.

3.5 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 2 lists some of the critical factors which relate to success or failure of mating disruption. Note that some of these factors may not be as critical in some agronomic systems compared to others.

The species-specific factors that influence mating disruption are difficult to ascertain *a priori*. In general successful trials have been associated with smaller (wingspan < 1 cm), less mobile moth species which produce <300 eggs/female, and which have a well-characterized pheromone blend.

Table 2 List of factors which may be critical for the success or failure of a mating disruption program

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 of treatment compared to other available treatments	Similar levels of control achieved for similar or less cost	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 is the degree of isolation of the treated area from other sources of the target pest species. 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, it might be >10 km. Polyphagy (where larvae that feed on a diverse range of food plants) 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. The approach taken for moth pests in pome fruit orchards has been to treat each separate orchard as an "island" of host plant. This situation requires that your neighbour's orchard is also treated, and that there are no alternative hosts or feral pome fruit trees growing in untreated areas. This assumes that moths will only mate in the presence of suitable host plants, an assumption which is probably dependent on the target moth species.

A mating disruption program in an orchard system requires quite a different approach to that in broad-acre crops such as cotton. The influence of crop structure is critical. For example, in a mature apple orchard it may be necessary to have three or more dispensers at different heights within each apple tree. Many moths are highly attracted to the fruiting bodies of plants as feeding, oviposition and mating sites, 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 often occurs 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. 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 costs are often the most expensive item in many production systems. This is exacerbated when disruption is attempted for large scale field crops such as cotton. The cheap manual labour available in some parts of the world has enabled mating disruption and associated semiochemical management methods to be used on high value field crops such as cotton (eg. for control of pink bollworm in Egypt). The economics are often tightly linked to the type of dispenser used, with the factors described in Table 1 determining whether adoption of a functional mating disruption system occurs. An additional component which may influence the economics of use of mating disruption is the risk of control failures occurring. When this risk is significantly higher than that of conventional insecticides the economic risk of significant damage occurring to the crop may prevent adoption.

4. Methodology

4.1 Site description

The trial 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, 500m W of Cordalba and Capsicum CF10, 11, 12, Rapley's Blocks, 2.6km NNW of Cordalba) and the treated area at Promised Land. Figure 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 2.5km by 8km known as "Promised Land". The eastern-most tip of the Promised Land crops was 9.1km NW of Cordalba, and 6.4km from the nearest capsicum (CF12), whilst the western-most end was 11.5km WNW of Cordalba and the northern-most 9.8km NW of Cordalba. Figure 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).

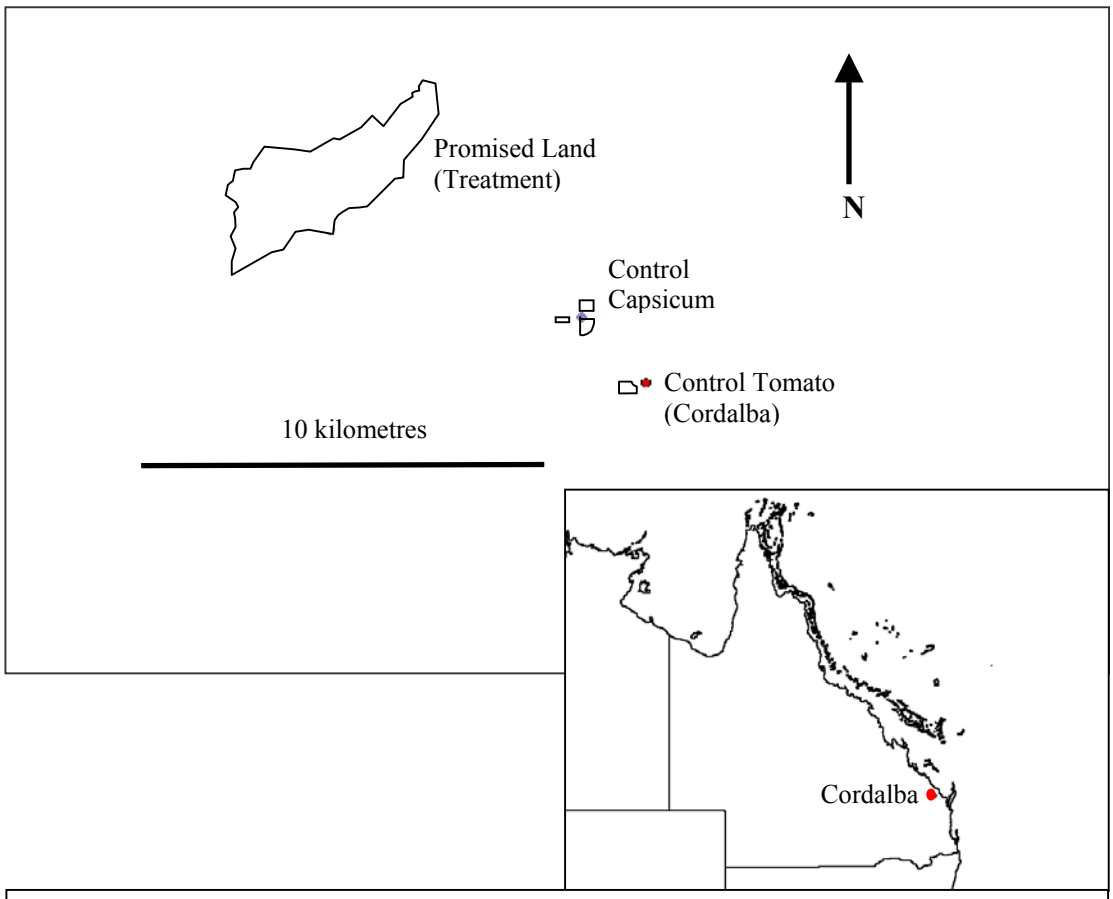

Figure 1 Location of the two control blocks and the treated block in relation to Cordalba, and the location of Cordalba in Queensland.

Promised Land is isolated from the nearest *H. armigera* host crop by 6km of sclerophyll scrub and forest. 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, and it can be safely assumed that very few adult *H. armigera* or *H. punctigera* would be produced within these forested areas. Several careful searches of the few potential host plants in the State Forest around the Promised Land region failed to locate any immature stages of *Helicoverpa* spp..

The majority of the Promised Land region was planted to sugar cane prior to planting of the first solanaceous crops in late January. Late capsicum crops from the previous year were present as undestroyed residue in two blocks (approx. 20ha). This residue had been ploughed in by late February so that any remaining *Helicoverpa* pupae in the ground would have been destroyed. About 7ha in the north west of Promised Land were planted to watermelon, rock melon and honeydew melon, with some volunteer tomato plants present between the melon plants. Both these cucurbits and the volunteer tomatoes would have been hosts to *Helicoverpa* larvae until early March when these crops were ploughed. The sugar cane and the majority of the orchard area were free of *Helicoverpa* host plants, although a newly planted citrus orchard (10ha) at the northern tip had volunteer tomato and black nightshade *Solanum nigrum* (Solanaceae) from April 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 tomato plant had larvae, or showed signs of larval damage.

4.2 Planting and treatment application dates

Table 3 lists dates of planting for all relevant blocks and the subsequent treatment, reapplication and termination dates (to the nearest week). Note that weather conditions sometimes delayed staking in tomato, which then delayed application of dispensers. Availability of field labour also affected treatment dates.

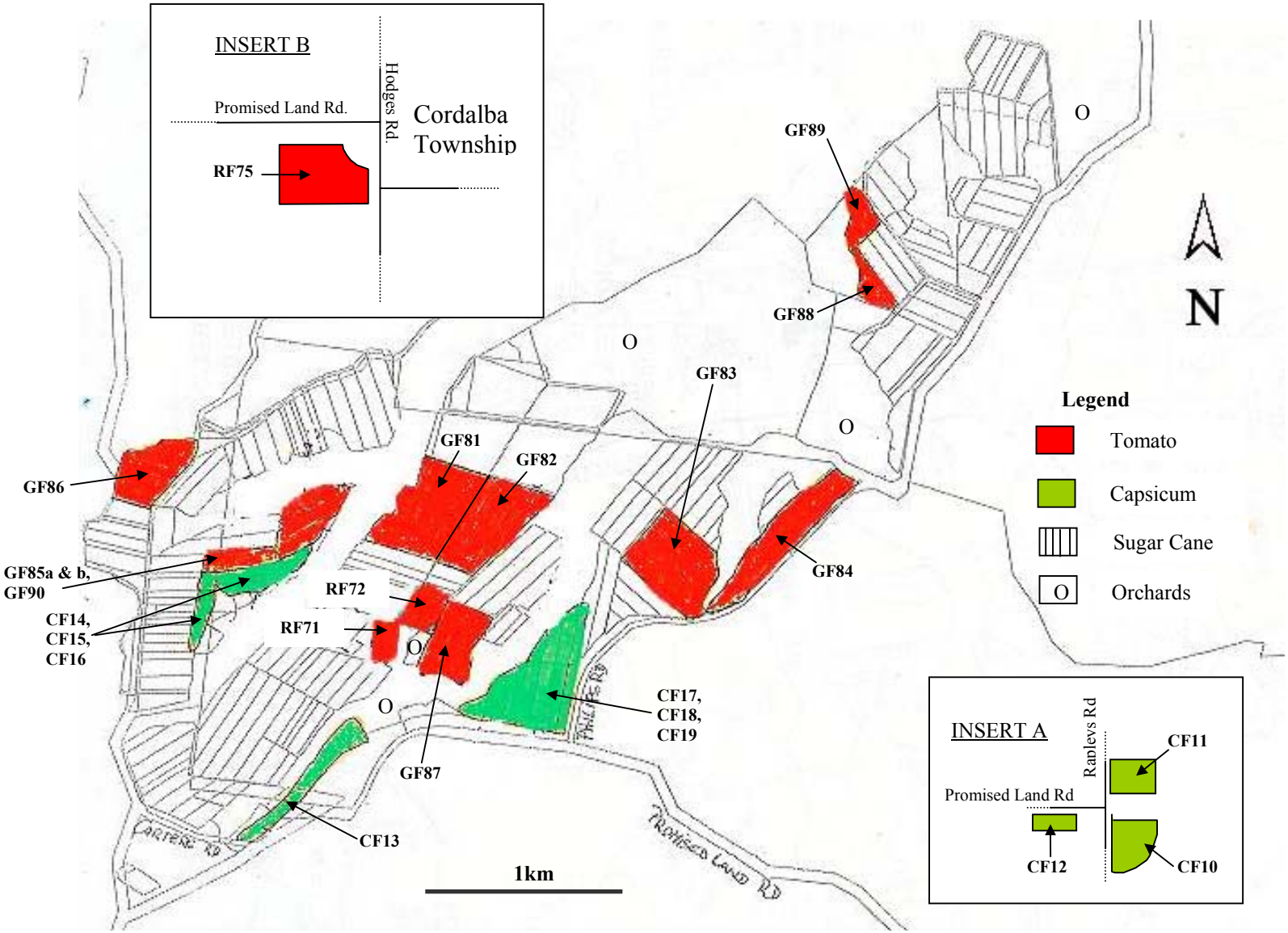

Figure 2 Position of blocks in the Promised Land region, the control capsicum 6.4km SE of Promised Land (INSERT A) , and the control tomato 9.1km NE by N of Promised Land (INSERT B).

Table 3 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
CF13	14 Jan	7-14 Feb	8 Apr	1 June
CF14	30 Jan	14 Feb		2 June
CF15	29 Jan	14 Feb (3 Apr in patches)		25 June
CF16	12 Feb	14 Feb		25 June
CF17	13 Feb	26 Mar		25 June
CF18	20 Feb-7 March	26 Mar		27 July
CF19	19 Feb	26 Mar		28 July
RF71	28 Jan	15-20 Feb		2 June
RF72	11 Feb	15-20 Feb		27 June
RF75	5 March	Control	Control	30 June
GF81	25Jan-19 Feb	4-15 Feb	5-8 Apr	4 June
GF82	29Jan-19 Feb	4-15 Feb	9 Apr	23 June
GF83	10 Feb	3-7 Mar		27 June
GF84	14-17 Feb	20 Feb (3-7 Mar repair)		18 July
GF85	20-24 Feb	20 Mar		
GF86	25 Feb	20 Mar		
GF87	3 Mar	7-20 Mar		
GF88	5 Mar	21 Mar		
GF89	6 Mar	21 Mar		
GF90	7 Mar	20 Mar		

4.3 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 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 3 shows a dispenser in placed on a tomato plant. Field trials in Pakistan showed that these dispensers had a life span of about 60 days after which the amount of released pheromone was insufficient to provide adequate levels of mating disruption (Chamberlain *et al.* 2000).

4.4 Dispenser application and layout

Label data provided by AgriSense gives the recommended rate of application of Selibate HA dispensers in cotton as 250 per hectare, or 40g a.i./ha. The existing 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 38.4g a.i./ha. This corresponded to one dispenser every 5.2m (or every stake) in a row on every fifth row. An equivalent application rate in capsicum was one dispenser every 7.3m 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.

The dispensers have been 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 capscium, and greater than 2 weeks old for tomato. Figures 3 and 4 shows the dispenser in place on a mature tomato plant.
2) Application with bamboo skewers (capsicum only). A 25cm bamboo skewer was pushed through the ring so that when inserted into the ground the dispenser was 10-15cm above the ground surface.
3) Fastening to plastic balloon sticks (capsicum only). A balloon stick (Paperware Distributors, Armidale, NSW) consisted of a 40cm 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 5, 6, 7, and 8 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 2.5mm diameter flat-headed clout through the ring onto the top of the stake, stapling using either a hand stapler or a hammer tacker with 8mm 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, 10 and 11 show the three attachment methods.

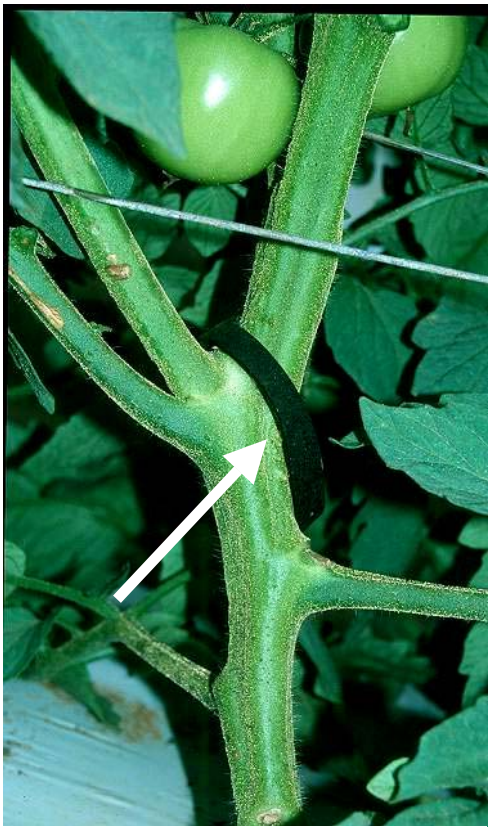

Figure 3 Selibate HA dispenser in place on mature tomato (applied when tomato was in seedling stage). White arrow indicates dispenser.

Figure 4 Selibate HA dispenser in place on mature tomato showing location in canopy (applied when tomato was in seedling stage). White arrow indicates dispenser.

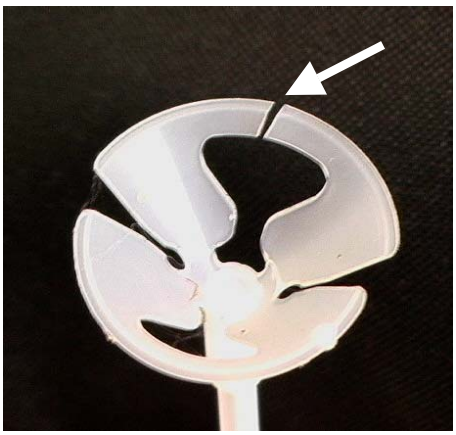

Figure 3 Balloon holder showing cut in plastic (white arrow) for dispenser attachment

Figure 4 Balloon holder with dispenser in place

Figure 7 Balloon holder with dispenser in capsicum block

Figure 8 Balloon holder in capsicum showing height relative to plant height

Figure 9 Dispenser stapled to side of tomato stake

Figure 10 Dispenser stapled to top of, and to the row-side of a tomato stake

Figure 11 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 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 in some cases this was delayed by lack of field workers to do the job.

4.5 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 (pers. comm. Enzo Casagrande). 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 bag were wrapped in aluminium foil and stored in –18 °C freezer for comparison with the weathered dispensers in the field. Six weathered dispensers were collected on the 7th of March, 11th of April and 6th of May 2003 for analysis.

4.6 Monitoring

In general monitoring attempted to record moth activity of both sexes from representative regions within the treated (Promised Land) and control areas. Figure 12 shows the approximate divisions of the Promised Land region and the number of different 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.

4.6.1 Pheromone traps

Standard universal funnel traps (AgriSense BCS Pty. Ltd.) were deployed to detect trap shutdown in treated areas and to monitor male moth populations in untreated areas. Figure 13 shows a version of this funnel trap with a clear base. Dichlovos-impregnated pest strips

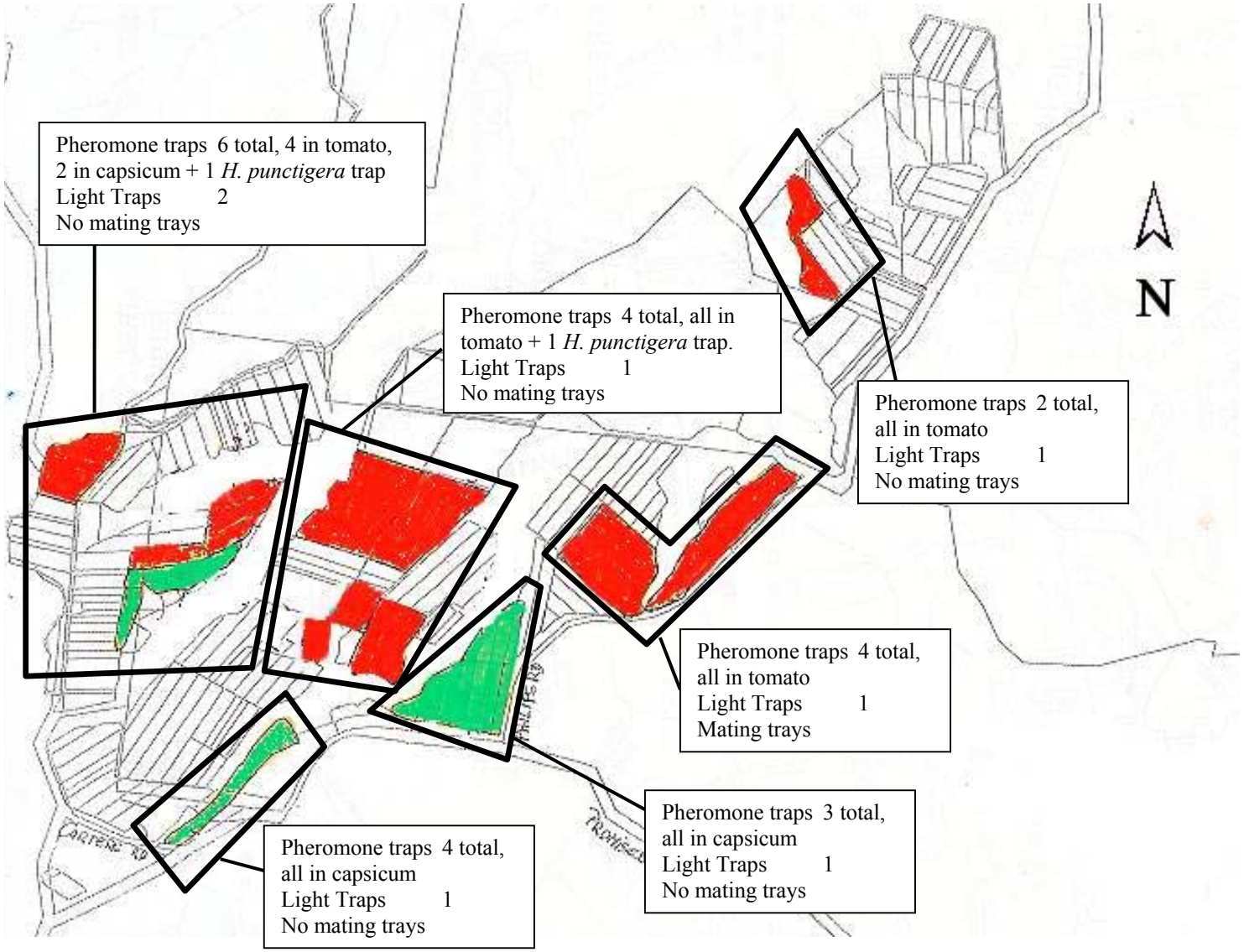

Figure 12 Location of monitoring devices in the Promised Land region, and the localised grouping used to divide up trapping effort.

(Sureguard Ministrips, Kiwi Brands Pty. Ltd., Clayton South, Victoria) were used as killing agents in the traps. The traps were suspended on steel curtain rods or PVC electrical conduit so that they were approximately 10cm above the canopy of the crops. When crops were in the seedling stage the traps were set about 40-50cm above the ground. Traps were spaced more than 70m apart.

Rubber septa lures for monitoring of *H. armigera* were formulated by placing 80 small standard septa (Pherobank, Plant Research International, Netherlands) in 40ml hexane (~99% GC purity, Fluka) with 130mg (Z)-11-hexadecenal, 15mg (Z)-9-hexadecenal (>95% purity, Pherobank, Plant Research International, Netherlands), and 7mg 2,6-di-tert-butyl-4-methyl phenol (butylated hydroxytoluene) (99%, Lancaster) as an antioxidant. This gave a loading of ~1.8mg/septum of a 10:1 ratio of the two pheromone components, which approximates the loading found in many commercially supplied *H. armigera* lures. A comparison of these lures with commercial *H. armigera* laminate lures (AgriSense-BCS Pty. Ltd.) showed comparable or better performance than the commercial lures for the first three weeks when used in universal funnel traps (AgriSense-BCS Ltd.) (P. Gregg and D. Britton unpublished data). Commercial laminate lures supplied by AgriSense BCS Pty. Ltd. were used for *H. punctigera*. Lures for both species 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 several other moths superficially resembling *Helicoverpa* spp., as well as *H. armigera* females were occasionally found to stray into the funnel traps.

4.6.2 Light traps

Light traps were based around dual 8W black light tubes (NEC, FL8BL) in a 12V DC batten suspended vertically on a wire frame above a fibreglass cone 48cm deep, 79cm wide with a 7cm opening. The cones were seated with the small opening facing downwards on a plastic garbage bin 48cm wide x 52cm deep. Figure 14 shows this type of trap in the field. The traps were powered by a 12V small car battery which was in turn charged by a 30W self-regulating solar panel. The lights were automatically turned on and off by a light sensitive switch incorporating a 30min delay after dusk designed to avoid the extremely large numbers of beetles and crickets which are usually caught when light traps are run at dusk (P. Gregg, pers. obs.). Insects

Figure 13 Agrisense pheromone funnel trap. Unlike this trap the traps used in this trial had an opaque green plastic base.

Figure 14 Light trap in capsicum. The full setup used in monitoring also has a 30W solar panel.

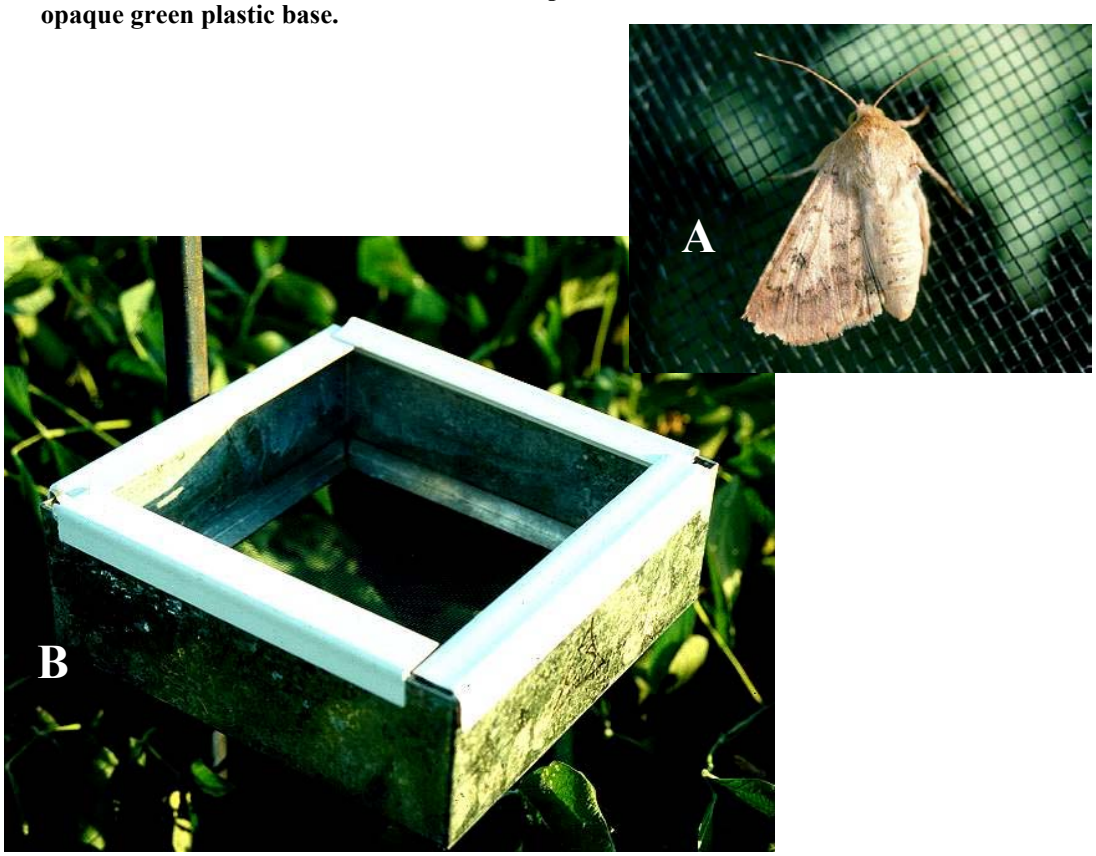

Figure 15 Wing-clipped female moth on base of mating tray (A) and mating tray (B).

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. Light trap specimens were sorted to species level (*H. armigera* or *H. punctigera*), sexed, and females dissected to determine mating status. *Helicoverpa* catches were frozen until dissection.

4.6.3 Mating trays with wing-clipped females

Laboratory-reared pupae of *H. armigera* were obtained from large cultures kept at Warra, Qld (Bidstrup Biologicals Pty. Ltd.). Pupae were sexed, males discarded, and females placed in groups of 20 in plastic takeaway food containers (173 x 119 x 58mm) with moist vermiculite. Air holes were drilled in the lids to prevent excessive buildup of water inside the containers. These pupae were kept under conditions 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 160ml plastic cups provided with dental wicks soaked in 5-10% sucrose solution for sustenance.

Two day old females were used in mating trays in the field. Females were first chilled at 5 °C for 8-10min to temporarily immoblise them, then removed three at a time and wing-clipped. 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 (Kvedaras *et al.* 2000). Figure 15 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 7cm 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 (15mm square x 160cm tall) at an adjustable height so that the base was clear of any vegetation. A 5cm barrier of white petroleum jelly was smeared around each metal rod just below each tray to prevent ants, spiders and other predators accessing the females.

In general mating trays were spaced about 10m 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 dawn the following morning to avoid bird predation. They were frozen then dissected in 70% ethanol to determine mating status.

Mating tray comparisons were conducted on 14 nights throughout the trial, with a total of 840 females placed in the field. Table 4 lists the dates, locations and number of females used for these nights. Note that not all females were recovered the following morning; some were either missing or dead. The proportion mated omits missing and dead moths.

Table 4 Dates, locations and numbers of females used in mating tray comparisons

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
10th Apr	Treated Tomato GF83	15
	Untreated Sugarcane 100m Nth. GF83	14
11th Apr	Treated Tomato GF83	19
	Untreated Sugarcane 100m Nth. GF83	15
30th Apr	Untreated Sugarcane 100m Nth. GF83	3
	Untreated Sugarcane 200m Nth. GF83	3
	Untreated Sugarcane 300m Nth. GF83	6
	Untreated Sugarcane 400m Nth. GF83	6
	Treated Tomato GF83	15
1st May	Untreated Sugarcane 100m Nth. GF83	12
	Untreated Sugarcane 200m Nth. GF83	15
	Untreated Sugarcane 300m Nth. GF83	9
	Untreated Sugarcane 400m Nth. GF83	9
	Treated Tomato GF83	45
2nd May	Untreated Sugarcane 100m Nth. GF83	12
	Untreated Sugarcane 200m Nth. GF83	15
	Untreated Sugarcane 300m Nth. GF83	9
	Untreated Sugarcane 400m Nth. GF83	9
	Treated Tomato GF83	44
3rd May	Untreated Sugarcane 100m Nth. GF83	5
	Untreated Sugarcane 200m Nth. GF83	6
	Untreated Sugarcane 300m Nth. GF83	9
	Untreated Sugarcane 400m Nth. GF83	9
	Treated Tomato GF83	33

4th May	Untreated Sugarcane 100m Nth. GF83	6
	Untreated Sugarcane 200m Nth. GF83	6
	Untreated Sugarcane 300m Nth. GF83	6
	Untreated Sugarcane 400m Nth. GF83	6
	Treated Tomato GF83	23
5th May	Untreated Sugarcane 100m Nth. GF83	6
	Untreated Sugarcane 200m Nth. GF83	0
	Untreated Sugarcane 300m Nth. GF83	6
	Untreated Sugarcane 400m Nth. GF83	6
	Treated Tomato GF83	17

4.6.4 Egg and larval counts

Egg and larval counts on capsicum and tomato blocks were made by Emma Smith (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 check 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 are presented as an average per site per block, and in the results section are treated as counts per check. 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 are lumped together when considering data on a weekly basis.

4.6.5 Comparison of *Helicoverpa* reproductive behaviour in sugarcane and in treated tomato

Observations from egg counts and from light-trapped females indicated that mated females were immigrating into pheromone-treated fields. Two experiments were designed to try and determine if females were being mated near treated fields in non-host crop (sugarcane) adjacent to the treated fields.

The first experiment compared the reproductive status of wing-clipped virgin females in mating trays in untreated sugarcane 100m north of a treated tomato block (GF83) with those placed 100m into the treated tomato (GF83). The methodology followed was similar to that described for previous mating tray experiments but with the trays in the sugarcane elevated on an additional 2m stake (total height of 3.16m) so that the trays were level with the top of the sugarcane. Figure 16

Figure 16 Mating tray in sugarcane

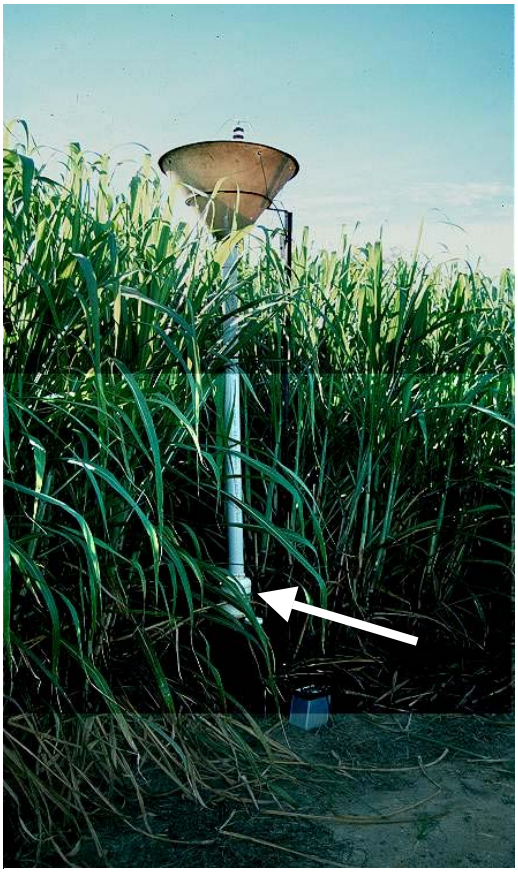

Figure 17 Modified light trap in sugarcane. Collecting jar is indicated by white arrow.

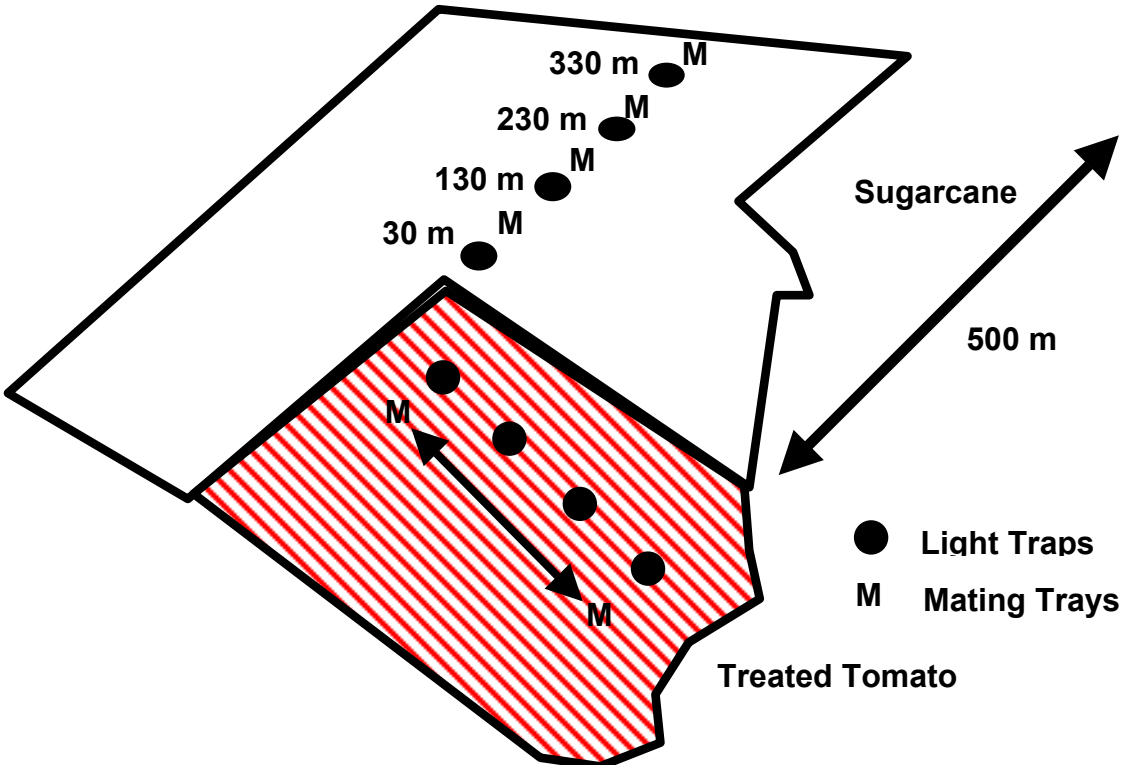

Figure 18 Layout for comparison between traps and mating trays along a transect in sugarcane and in treated tomato (GF83)

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.

The second experiment compared wild light-trapped females in a treated tomato block (GF83) with those captured in adjacent sugar cane along a transect running north of GF83, whilst simultaneously running mating trays as described above. Figure 18 shows the layout of light traps and mating trays in the sugarcane and GF83. Four light traps were placed 30m, 130m, 230m and 330m from the edge of 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 17 shows a modified light trap. Four light traps were placed in GF83 along the third bay in from the northern edge of GF83 so that they were at least 80m from the edge of the tomato. These traps were separated from each other by at least 80m.

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 within GF83. This experiment was done from April 29 to May 5, 2003.

4.6.6 Other monitoring methods

A 3V head torch and butterfly net (diameter 456mm, handle length 119cm) were used to sample adults at night on capsicum and tomato plants. This procedure was not done on a systematic basis each trip, but was concentrated around several nights during the February-March intensive sampling trip. 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 380mm) sample consisted of 20 sweeps.

4.7 Weather data

Weather data was obtained from the Bureau of Meteorology station based at Bundaberg Airport (24°54'S 152°19'E) 31.3km 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.

4.8 Statistical analysis

An estimate of the efficacy of the mating disruption was calculated using Abbott (1925)'s formula for calculating insect mortality with the following substitutions:

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

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 was with 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 the $p < 0.05$. Variability in data is represented by the mean standard error for means and by 95% confidence intervals for proportional data.

5. Results

5.1 Dispenser Placement – labour times, reliability

5.1.1 Tomato

Stapling the dispensers onto the tomato stakes (Figure 9) was the most reliable method of attaching dispensers, and was also the most rapid at 2.4 minutes per 100m of row. The only drawback to this method was the reliance of staking of the tomatoes which means 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. 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 11) was much slower than stapling, required greater precision from the labourer, and was unreliable, as nails often caused the formulation to split and fall to the ground. In some cases larger nails were used (> 2.5mm in diameter) which exacerbated this problem, requiring that large areas of tomato be re-treated. Direct application (Figures 3 and 4) was reliable when plants were three weeks or more older, 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 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 5 summarises the advantages and disadvantages of the different methods.

5.1.2 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 capsicum. 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 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 6 lists the methods and their advantages and disadvantages.

5.2 Monitoring

5.2.1 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, Agricultural Research, Cotton Incorporated) 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.

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.

In addition to this, 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.

Table 5 The results of dispenser application methods in tomato

Method	Time (minutes) per 100m row	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

Table 6 The results of dispenser application methods in capsicum

Method	Time (minutes) per 100m row	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) estimate only– not measured	Poor	Poor	Skewers split the dispenser, did not survive field conditions (fell over easily)

5.2.2 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 the maximum catch being 3 males/trap/night in the residual capsicum crop. This suggests that these residues were not major sources of moths during the trial.

Figures 19 and 20 show the average weekly catch in controls compared to that of the treated areas and the percentage mating disruption 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 21 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 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 21).

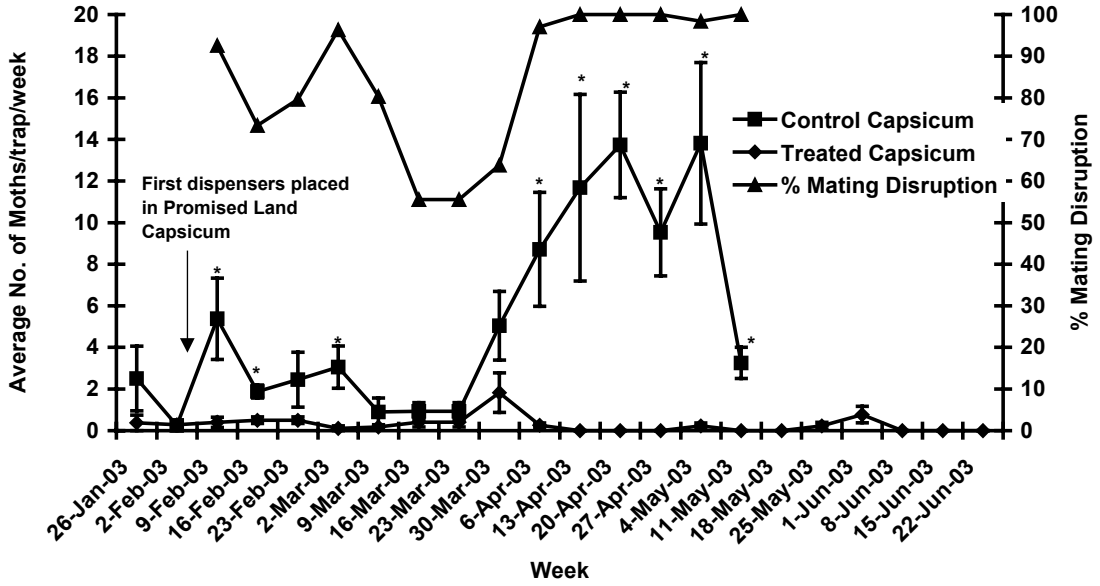

Figure 19 Mean weekly pheromone catches per trap in treated and control capsicum, and the percentage mating disruption each week. Asterisks indicate significant weekly differences between means in treated and control.

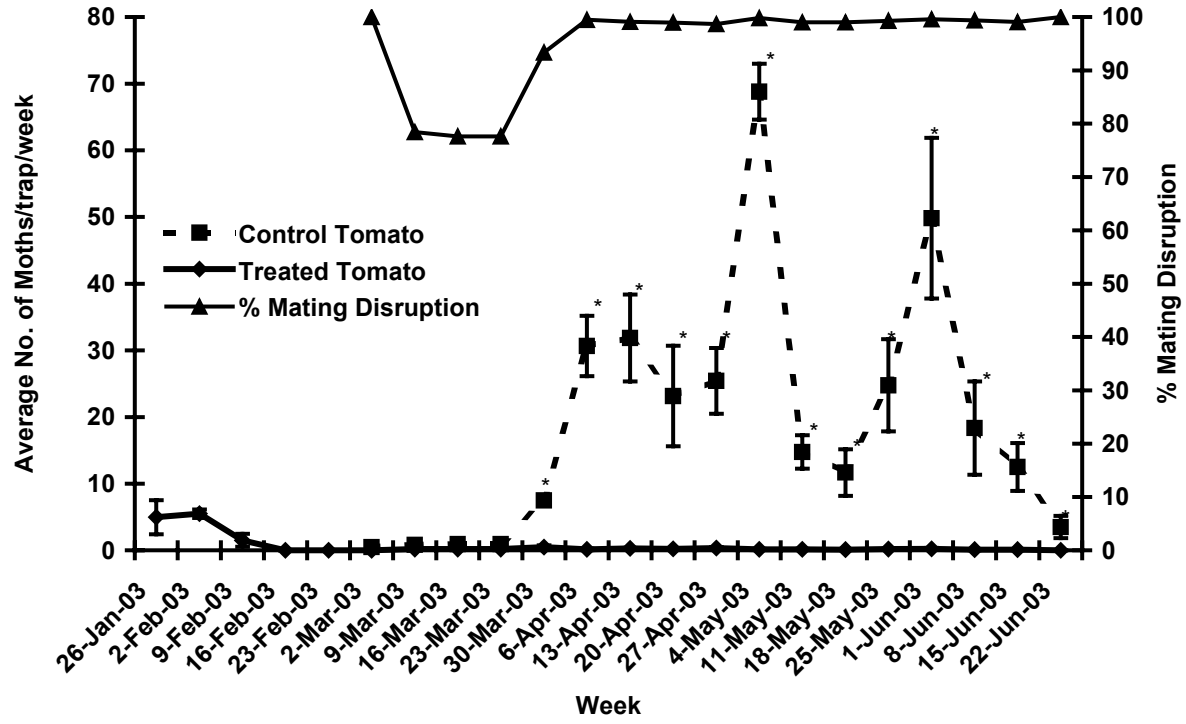

Figure 20 Mean weekly pheromone catches of *H. armigera* in treated and control tomato, and the percentage mating disruption each week. Asterisks indicate significant weekly differences between means in treated and control.

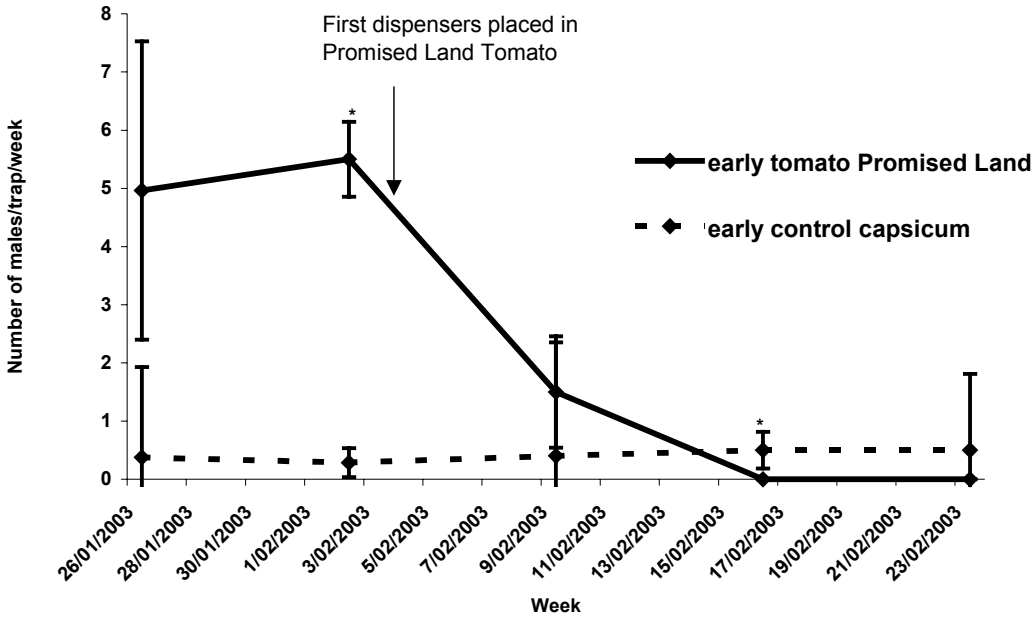

Figure 21 Comparison between mean weekly pheromone trap catches of male *H. armigera* in tomato and capsicum for the first five weeks of the trial before a control tomato crop became available.

5.2.3 Light trap catches

Light trap catches were generally low throughout most of the trial period. Table 7 lists the average nightly catches for male and females in both capsicum and tomato for the entire trial period.

Table 7 Average ± standard error for nightly catches of *H. armigera* per light trap over the entire trial period

Crop	Male	Female
Capsicum Control	3.12 ± 1.00	0.62 ± 0.16
Capsicum Treated	0.68 ± 0.17	0.28 ± 0.07
Tomato Control	6.84 ± 1.21	1.74 ± 0.43
Tomato Treated	2.11 ± 0.47	0.98 ± 0.19

Figure 22 shows that there were no significant differences in the mean number of females captured per night between the treated and untreated areas in tomato and capsicum(ANOVA, df = 1, F = 1.07, p = 0.31 and ANOVA, df = 1, F = 3.45, p = 0.07 respectively). However, male

activity 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).

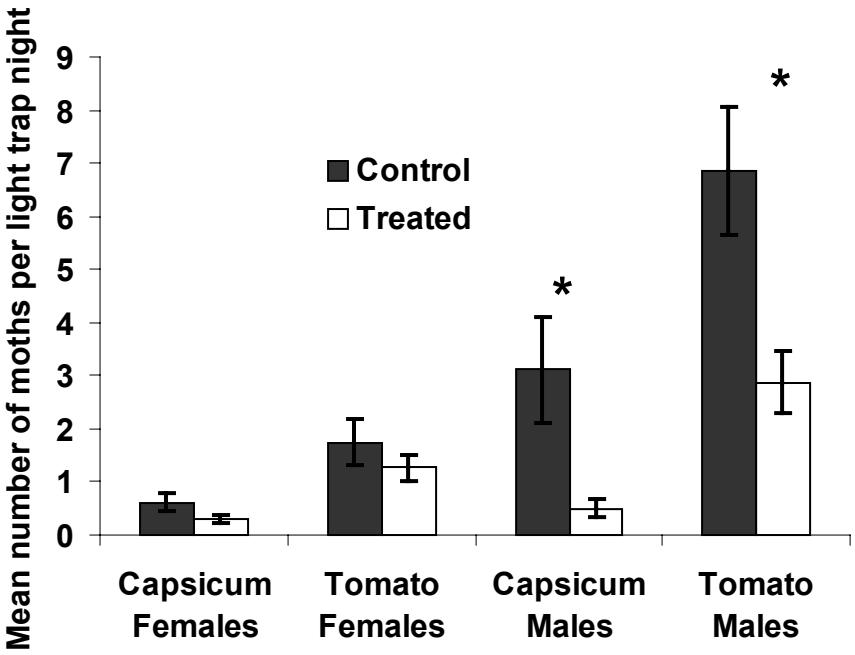

Figure 22 Comparisons between the mean number of male and females captured in light traps in control and treated areas. Asterisks indicate pairs of means that are significantly different.

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 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 8 The percentage ± 95% confidence interval of female *H. armigera* mated in 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.

5.2.4 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 4 only seven nights resulted in one or more females being mated. Table 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 List of dates, localities, number of female moths, and the proportion mated for those nights when mating was recorded in mating trays.

Date	Locality	Number Mated (Number Unmated)	Proportion 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 100m Nth. GF83	4 (10)	28.6 ± 23.7
11th April	Untreated Sugarcane 100m Nth. GF83	5 (9)	35.7 ± 25.1
2nd May	Untreated Sugarcane 400m Nth. GF83	1 (8)	11.1 ± 20.5

No mated females were ever recovered from treated fields, except for one female in a tomato field in which the application of dispensers had not been completed. Trials conducted during the peak male activity periods in early April gave the clearest results, 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 ($\chi^2 = 54.66$, $P < 0.001$) in the number of mated females in untreated compared to treated blocks, with only one female out of 233 mated in the treated blocks and 17 out of 278 females mated in the untreated blocks (0.4 ± 0.8% mated in treated blocks compared with 6.1 ± 2.8% in untreated blocks). Using the modified version of Abbott (1925)'s formula, 93.0% mating disruption in treated areas was achieved over the 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 would be safe to say that 100% mating disruption was achieved for the trial.

5.2.5 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 23 and 24 show the mean number 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 due to the extensive use of insecticides. Small larvae have been included in the remainder of the analysis to highlight the significance of

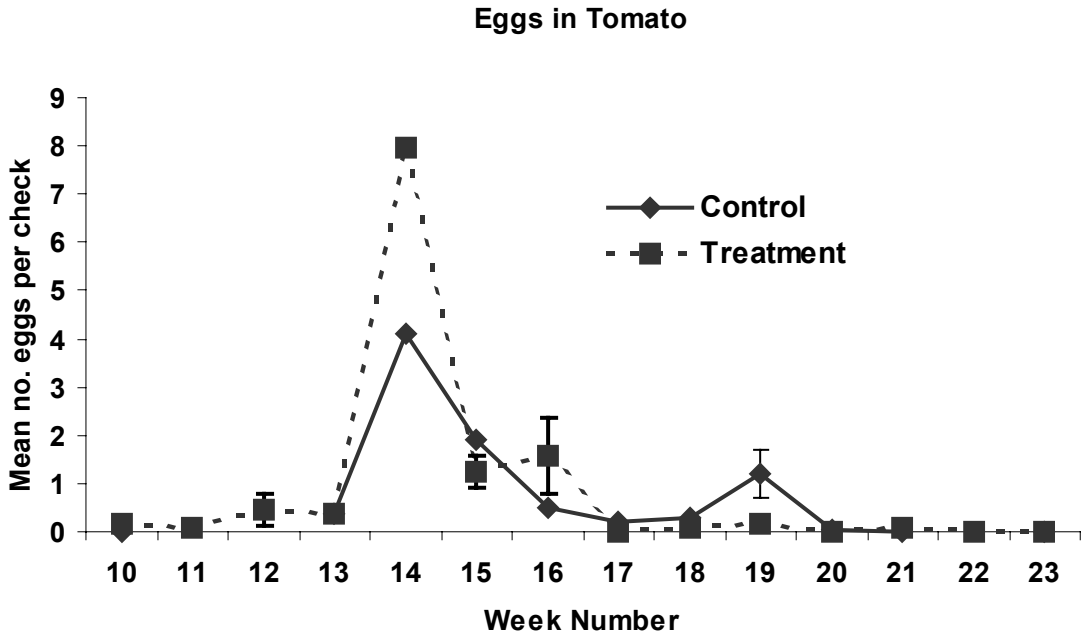

Figure 23 Mean ± standard error of eggs per check per week in tomato from Week 10 (March 3rd) to Week 23 (June 8th)

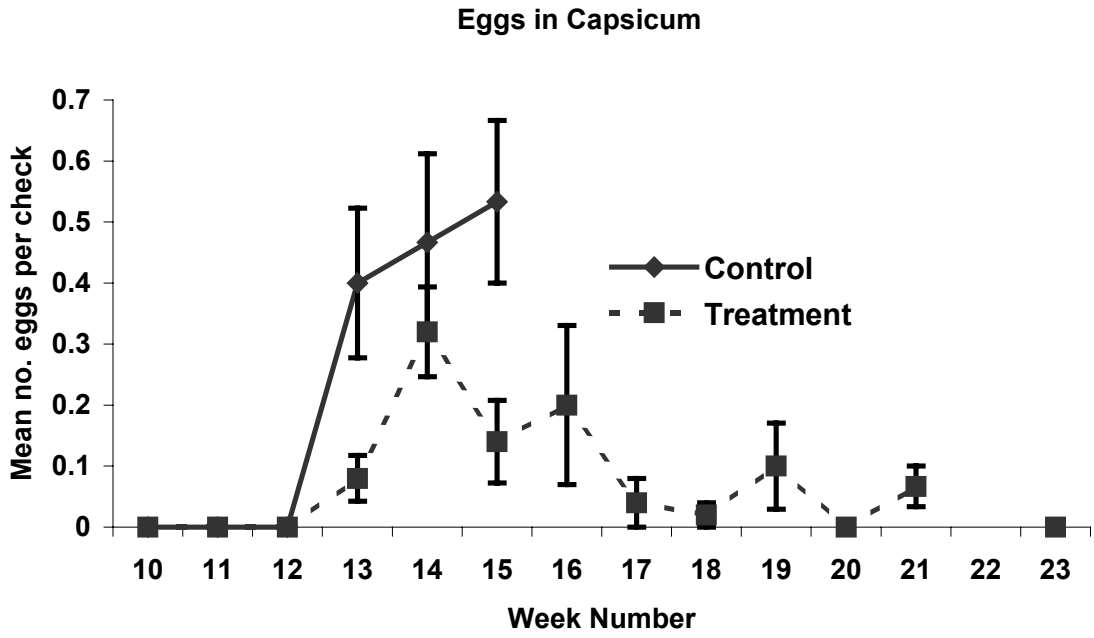

Figure 24 Mean ± standard error of eggs per check in capsicum per week from Week 10 (March 3rd) to Week 23 (June 8th)

Figures 25 and 26 graph the mean number of white and brown eggs, and small larvae per check for treated and untreated blocks over the period from March 3rd to June 8th for tomato and capsicum respectively. There were no significant differences in tomato counts, but a significant difference between the mean number of white and brown eggs in control capsicum compared to treated capsicum. This was also reflected in the mean number of small larvae per check.

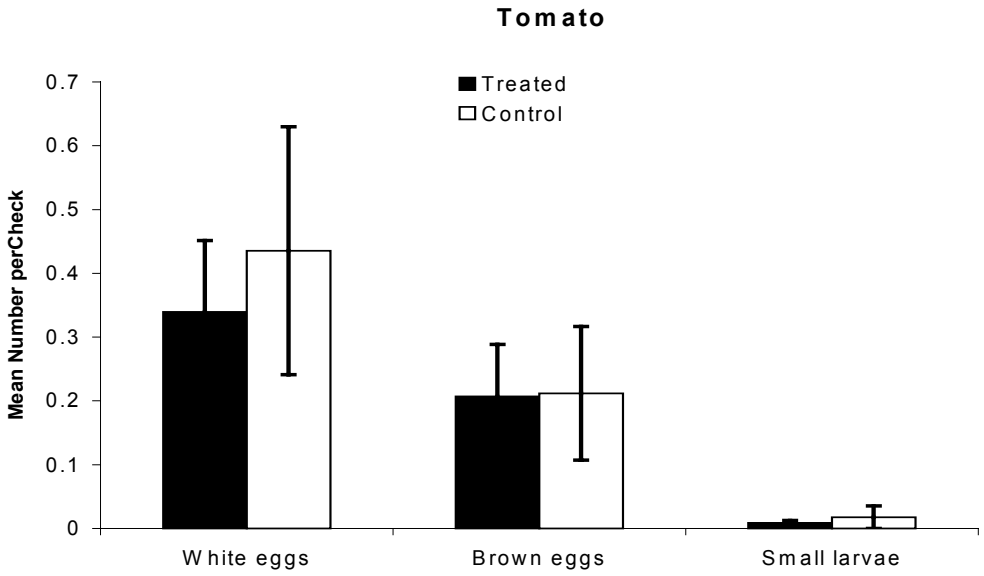

Figure 25 The mean ± standard error of eggs per check in tomato over the period from March 3rd to June 8th

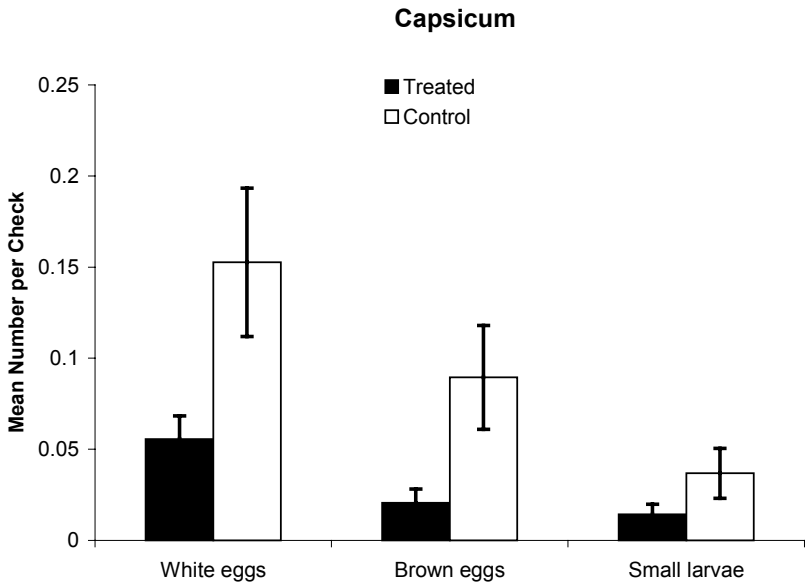

Figure 26 The mean ± standard error of eggs per check in capsicum over the period from March 3rd to June 8th

5.2.6 Movement in sugarcane

When it became evident from egg counts and dissections of light-trapped females that immigration of mated females into treated crops was occurring a variation in the monitoring program was devised in an attempt to locate where mating was occurring. An initial mating tray experiment was run over two nights (10th and 11th April) comparing wing-clipped females in trays located in sugarcane 100m north of the treated tomato field GF83 (28 females) to trays located within GF83 (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 400m 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).

Similar numbers of females per trap per night were caught in the sugarcane, particularly in the light traps 30m and 230m away from the treated tomato (GF83). Females were caught in sugarcane light traps up to 330m away from the treated tomato. The light trap at position 130m was inoperable over three consecutive nights out of the seven trapping nights due to rain-damaged electronics, which may explain the absence of females from this location on the transect. 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 230m and 330m 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 27 shows the mean number of moths per trap per night for each sugarcane trap location and for the traps in the treated tomato.

Table 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 30m from the treated tomato were mated, and only females caught in the treated tomato had more than one spermatophore present indicating multiple matings.

Table 10 Percentage of female *H. armigera* mated and number 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 30m	5	3	60.0 ± 42.9	0
Sugarcane 130m	0	0	0	0
Sugarcane 230m	3	0	0	0
Sugarcane 330m	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 (400m from the treated tomato) over the course of the experiment. None of the 133 females placed in the treated tomato were mated.

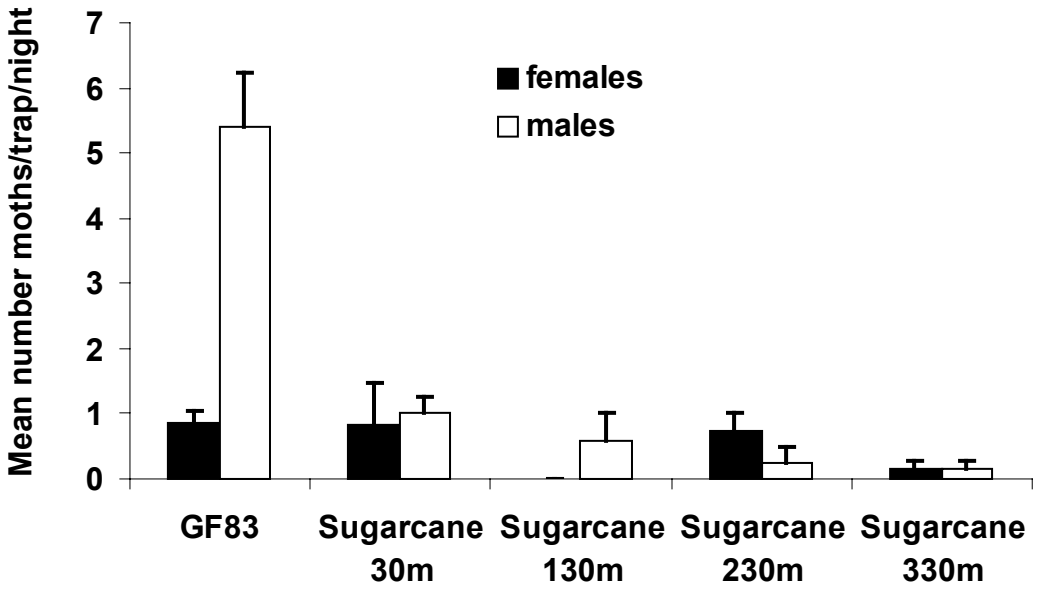

Figure 27 The mean number of *H. armigera* per night ± standard error for light trap catches in treated tomato (GF83) and along a transect in untreated sugarcane.

5.2.7 Additional observations

Very few adult moths of either sex were observed at night in treated blocks in four nights of observation (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 actively or systematically compared to that 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 detected some weedy plant hosts outside of the treated blocks, but within the Promised Land region. Volunteer tomato plants were present in low numbers in untreated blocks in the northern section of Promised Land. Volunteer tomatoes at flowering and fruiting stages nearly always had larvae and eggs. These included plants that were growing between a green manure crop (sorghum), and plants growing in newly planted citrus orchard. 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. Black nightshade (*Solanum nigrum*) is a common weed throughout both the tomato and capsicum blocks at Promised Land and was also present in the newly planted citrus orchard to the north of Promised Land. *S. nigrum* is not a good host for *Helicoverpa* spp.. Three larvae were found in over five sweep net samples from these plants within the orchard area. A single larva was found feeding on new growth on a mandarin tree (*Citrus reticulata*). It was possible that this larva might have also come from the surrounding *S. nigrum* plants. Searches of other weedy untreated areas throughout the Promised Land region failed to locate significant numbers of *Helicoverpa* eggs or larvae.

5.3 Impact of climate on mating disruption

Weather parameters such as maximum, minimum and average temperature, humidity and wind speed did not show significant correlation with data on moth activity in the Promised Land region. Moths are likely to be affected by temperature, humidity and wind speed; these parameters are not independent of each other, and this makes comparison of moth behaviour to climatic variables somewhat difficult. Low and high numbers of moths in pheromone traps did not appear to have any strong relationship with hot or cool daytime and nighttime temperatures or with humid wet days compared to dry. *H. armigera* females are unlikely to attempt to call and mate with males once nighttime temperatures drop below 15°C, but males are often attracted to synthetic lures at temperatures lower than this (D. Britton, pers. obs.). This temperature threshold makes meaningful comparison of pheromone trap catches to the actual reproductive behaviour in the field difficult.

5.4 Weathering of dispensers

Dispensers placed in the field on 7th of February 2003 had lost over 40% total weight of pheromone components after 28 days, and by 63 days they had lost 35% of the total weight of

components, after which minimal pheromone was released by the dispenser. Figure 28 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. These data indicate that dispenser life-span would be about 60 days.

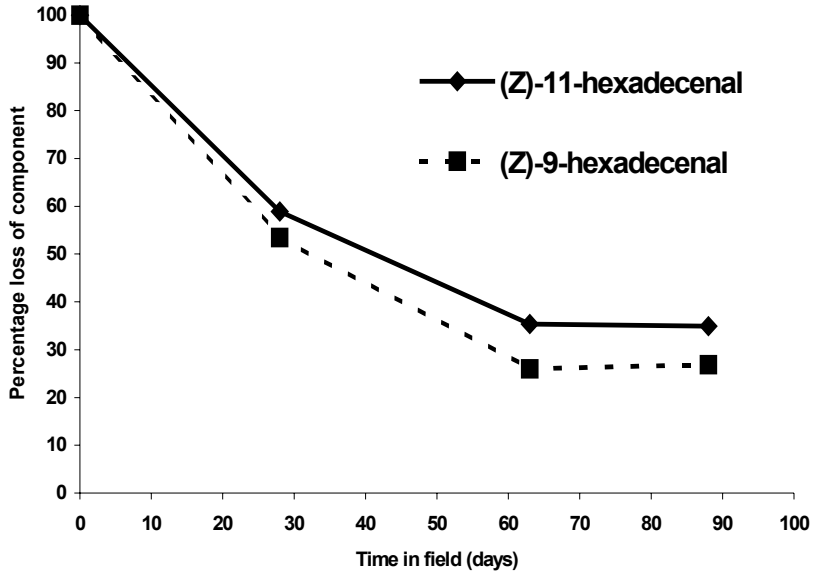

Figure 28 The percentage loss of pheromone components in field-weathered dispensers over an 88 day period.

6. Discussion

6.1 Mating disruption for *Helicoverpa armigera*

This study highlights one of the main problems with mating disruption, that is, the migration of mated females into treated areas. Although the Promised Land region is isolated from other *Helicoverpa* host crops there was no evidence of a significant reduction of mated females within treated areas. It was hoped that females immigrating into the Promised Land region would be unmated, that males would have been unable to locate them in the treated fields, and that females would not mate away from the treated crop. These assumptions were based on previous work, with the closely related *Helicoverpa zea*, the New World corn earworm, 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). A similar result was obtained by

(Raina *et al.* 1997) for another related species *Heliothis virescens*. Raina *et al.* (1992) noted that *H. zea* required the volatile chemical signals from corn silk to trigger production of sex pheromone. The related North American species *H. phloxiphaga* was even more specialised, and required the presence of the host plant *Castilleja indivisa* (Texas paintbrush) pheromone production (Raina 1988).

A similar relationship emerges when the response of males to pheromone in the presence or absence of host plants is considered. 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*. Meagher and Mitchell (1998) found that addition of the floral volatile phenylacetaldehyde increased upwind flight towards pheromone sources in wind tunnels.

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 host plants suitable for larval development. In this respect there would seem to be an obvious association between reproductive behaviour and host plants. However, it does not seem to be the case for *H. armigera*. Kvedaras (2003) has shown that the addition of plant volatiles (phenylacetaldehyde or (Z)-3-hexenyl acetate) to the pheromone blends for *H. armigera* did not significantly increase trap catches of males, and in some cases decreased trap catches. Similarly, (Meagher 2001) found that addition of phenylacetaldehyde to pheromone blends did not significantly increase trap catches of the North American fall armyworm *Spodoptera frugiperda*. Kvedaras (2003) did not detect significant increases in calling behaviour or pheromone production in either laboratory-reared or wild *H. armigera* females. These studies suggest that the link between host plants and the initiation and success of reproductive behaviour might not be as strongly expressed in *H. armigera* as it is in other *Helicoverpa* and *Heliothis* spp.

The assumption that females attempting to mate would only do so in host plants (in this case tomato and capsicum) would appear to be flawed in the case of *H. armigera.* 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 from regions outside of the Promised Land region, mating in sugarcane, then moving into the treated crops. To our knowledge this study is the first to 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.

Another possible source of mated females in the treated fields might be the migration of females which have been mated outside of the Promised Land region. This 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 and Dunkelblum 1993)have failed to achieve reduction in infestation of the crop. However, long-range migratory behaviour in *H. armigera* is seen as a facultative response to changes in host plant availability, and is generally considered to occur between emergence from the pupal stage and the commencement of reproductive behaviour (Riley *et al.* 1992). The Promised Land region is somewhat unique in terms of Australian agricultural areas in that it is relatively isolated from other sources of *H. armigera*, but whether this isolation is such that it requires moths to undergo long-range migratory behaviour in order to access the crops there is unknown. Normal short-range flights recorded in Australian agroecosystems tend to be over distances of 2 to 4km per night (Del Socorro and Gregg 2001), although distances of up to 10km have been recorded on some instances (eg. in the Sudan Gezira between crops (Topper 1987)). The distance between Promised Land and the surrounding cropping areas is considerably greater than 4km. It is 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 are highly attractive to *Helicoverpa* adults as nectar sources. Large numbers of eucalypt pollen grains have been found on the mouthparts of *H. armigera* (Del Socorro & Gregg, pers. comm.). The absence of larval food plants may not mean that the forested regions around Promised Land are unattractive to adult *Helicoverpa*.

In previous trials 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 2km 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 used a 10 x 10km 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 (1992) used a smaller area (30ha) 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 the trial in Japanese lettuce crops (Toyoshima *et al.* 2001). Diamolure dispensers which have a 125mg loading of 36.0% (Z)-11-hexadecenal and 41.0% (Z)-11-hexadecenyl acetate were used to treat both small (3ha) and large (20ha) 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 20ha field. The authors did not mention what vegetation type surrounded the treated areas, but the control lettuce fields (600ha) were only 500m 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 similar unclear whether the reported success of the Japanese trial is due to the presence of this compound or to the ecological characteristics of the moths or the trial site in Japan.

Moths collected at Promised Land and from surrounding cropping areas have been stored for 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. A further version of this analysis is currently being tested to determine if larvae have fed on nitrogen-fixing plants (eg. legumes such as soybean, lablab). These analyses may help clarify the contribution of immigration of mated females from outside the Promised Land area to the failure of mating disruption in this trial.

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 *Helicoverpa armigera* it may be possible to identify whether moths are local, or have flown in from another region. Graham (2000) has demonstrated that it is possible to trace the origins of *Helicoverpa armigera* and *H. punctigera* populations in south-western Queensland using microsatellites. This technology is still being developed, and is currently hampered by the lack of laboratories with suitable expertise.

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 or reduced damage to flowers and fruit in either tomato or capsicum.

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.

With the life-span of dispensers used in this trial (60 days), mating disruption should work with two applications over the growing period from seedling to final picking. 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 (pers.comm. Nick Brown, Business Manager, AgriSense).

Although the overall results from this mating disruption trial with *H. armigera* suggest that the technique is limited even in areas isolated from other host crops, other semiochemical techniques might work in conjunction with mating disruption and isolation factor. Attract and kill techniques can be used instead of mating disruption. This involves using sex pheromone or other attractants to lure the moths to a formulation which delivers a lethal dose of insecticide, or some other incapacitating compounds. If sex pheromone is used as the attractant attract and kill systems work in a somewhat similar way to mating disruption, in that it prevents mating. However, unlike mating disruption, these males are permanently removed from the field population. These sex pheromone-based attract and kill systems use much less sex pheromone compared to conventional mating disruption. For example, attract and kill for pink bollworm in Egyptian cotton is estimated to use about $1/80^{th}$ the pheromone of mating disruption methods (Hofer 1994). A system of attract and kill is being investigated for *H. armigera* in cotton, but is still in the developmental

stage (Britton *et al*. 2002). It is possible that an attract and kill system used in place of the mating disruption system at Promised Land may have removed the males that were mating with females on the edges of the treated blocks of tomato and capsicum.

The alternative electrostatic mating disruption 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 in effect become their own mating disruption dispensers. 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 prevent the use of adjacent non-host crops (such as sugarcane in the Promised Land area) as temporary mating locations for females which then move back into the treated crops.

Another method of using sex pheromones to control *H. armigera* may be to use mating disruption dispensers in the cropping region as in this trial, but to also include an additional attract and kill formulation around the perimeter of the treated crops. Del Socorro *et al.* (2003) have developed plant volatile-based attract and kill formulations which kill both sexes of *H. armigera* and other noctuid pest species. Large scale field trials in cotton with these formulations have shown that relatively small amounts can significantly reduce populations of female moths over a large area. Plant-based attractants could be sprayed on the sugarcane or other vegetation on the perimeter of the tomato or capsicum to kill moths of both sexes that venture out of the treated crop and into the sugarcane. In addition to this, mated females migrating into the treated area might also be killed. The current plant volatile formulations have not been tried on horticultural crops such as tomato or capsicum. An application for a research permit which would allow such uses is currently before the Australian Pesticides and Veterinary Chemicals Authority.

6.2 Data quality control issues

One of the main issues arising from monitoring these trials 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.

For the experiment comparing light traps and mating trays in sugarcane and treated tomato the low numbers of both sexes of *H. armigera* present in light traps prevented any firm conclusions in regards to the behaviour of male and female moths in the vicinity of the treated crop. The experiment did strongly suggest that female moths left the treated host crops and moved into non-hosts (in this case sugarcane) to be mated.

Mating trays typically achieve low proportions of mating even when large numbers of male moths are present in the field (Kvedaras 2003; Kehat *et al.* 1998). It was not surprising then that when male numbers were low in the field at Promised Land few or none of the females in mating trays were mated. Additional problems occasionally arose due to confusion between the agronomist, the spray operators and researchers 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 which were suspected to be affected by insecticide were not included in the analyses, and as this occurred infrequently it can be assumed that insecticide usage did not change the conclusions obtained from the analyses. Mating tray data obtained when wild males were abundant in the field can be assumed to reflect the real situation in the field.

An additional problem in interpreting the results of our monitoring arises from variability in data. In many respects this is not surprising. 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 crops to the latest crops. 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 the crop plant 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 is possible to ascribe differences between the Promised Land data and the data collected from these untreated blocks as arising from the geographic separation of the control blocks from Promised

Land. For example, the isolation of Promised Land may act to reduce numbers in pheromone traps compared to the control blocks regardless of mating disruption treatment. This is sometimes referred to as "psuedoreplication", and is a common problem when designing field experiments (Hurlbert 1984).

The inability to provide a rigorous and independent control for comparison with the treated area is a common problem when attempting area-wide trials in agricultural systems. There are several ways to ensure that the data measure an effect of the treatment rather than a difference that would have occurred anyway. One method is to make measurements prior to the treatment, so that any pre-existing differences between the control and treated areas can be observed. This type of experimental design is sometimes referred to as a Before/After Control/Impact (BACI) design (Green 1979). The design of the current trial allowed for two weeks of "before" measurements in both the control and Promised Land region, but because of low moth numbers at the start of the trial means that it was difficult to ascertain whether the control and Promised Land regions had similar moth numbers of similar reproductive status, or if there was an initial difference between them. 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 available. 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.

Our 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 was available within the current trial framework, and given the results of this trial, is unlikely to be supported by industry sources.

7. Recommendations

It can be assumed that *H. armigera* will become increasingly resistant to the conventional insecticide usage, potentially leading to unsustainable economic losses in crops such as tomato and capsicum. Although this trial did not give the desired control outcomes, it does point the way to future research areas which may assist in management of *H. armigera* in the Promised Land region so that the reliance on insecticides is reduced. The following recommendations are derived from observations made during this study to assist in directing further research with semiochemicals for control of *H. armigera* in the Promised Land region.

- Determine sources of *Helicoverpa* adults in the Promised Land region (whether local or immigrant) using microsatellite DNA analysis or isotope (C & N) analysis. Better understanding of the sources of incoming *Helicoverpa* may allow for better management of moth populations within the Promised Land region.
- Investigate the use of female attractants such as those under development by (Del Socorro *et al.* 2003) in conjunction with sex pheromones.
- Investigate incorporation of semiochemicals into a broader IPM strategy rather than a single stand-alone tactic.
- Investigate and cost attract and kill using sex pheromones.
- Investigate and cost alternative mating disruption techniques such as the ExoSex/autoconfusion method.

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9. Appendix

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)