



Cotton Research & Development Corporation

***A REVIEW OF BEHAVIOUR MODIFYING CHEMICALS
IN RELATION TO PEST HOST SELECTION AND
MANAGEMENT ON AUSTRALIAN COTTON***

(DAN 142 C) (June-August 1999)

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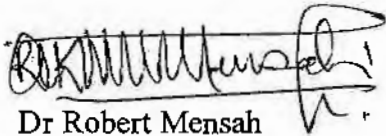
" Commissioned Report prepared for the Cotton Research and Development Corporation"

DECLARATION

This review was commissioned by the Australian Cotton Research and Development Corporation (CRDC) under CRDC Project DAN 142C and prepared by Dr Robert Mensah (NSW Agriculture) and Dr Chris Moore (QDPI, Chemical Ecology Unit) under the following terms of reference:

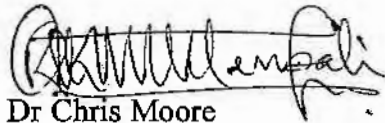
1. A Review of relevant literature and a research in progress in the area of behaviour modifying chemicals (semiochemicals) in relation to host pest selection; and
2. An appraisal of the most promising areas for further study in relation to exploiting the semiochemicals for the management of the key pests of Australian cotton.

The report has been refereed by peers and considered adequate.



Dr Robert Mensah
(Senior Research Entomologist, NSW Agriculture)

Date: 20-8-99



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Date: 20-8-99



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**APPROVAL FOR SUBMISSION OF COMMISSION REVIEW REPORT ON
BEHAVIOUR MODIFYING COMPOUNDS (SEMIOCHEMICALS) AND PEST
MANAGEMENT (DAN 142 C) - Dr Robert Mensah**

Dr Robert Mensah has been granted approval to submit to Cotton Research and Development Corporation (CRDC) the attached Commissioned report on the "Review of Behaviour modifying chemicals in relation to pest host selection and management on Australian cotton" (DAN 142C).

The report has been refereed by peers and considered to be very adequate.

Mr Dallas Gibb (Program Leader, Plant Fibres)
20 August 1999

Date: 20/8/99.

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EXECUTIVE SUMMARY

The review examines semiochemicals, substances or mixtures of substances emitted by one species that can be used to modify the behaviour of receptor organisms, and the role of these semiochemicals, particularly those derived from the inner tissues and organ surfaces of cotton plants, in the sustainable management of *Helicoverpa* spp. in cotton.

In the review, we examine the behavioural sequence leading to oviposition and how it is influenced by semiochemicals. The importance of leaf surface chemicals as cues in host plant selection will become evident in the review. For ovipositing moths such as *Helicoverpa* spp. that do not feed on the same plant, and thus do not contact the inner tissues, recognition and selection of the host plant for oviposition after landing, is determined by small quantities of many types of chemical substances. These include free amino acids, organic acids, sugars, secondary metabolites, vitamins, minerals, growth regulators etc. that come from the inner tissues of the plant onto the plant surface, as well as surface wax components. Detection of these substances on the leaf or organ surfaces of the plant provides specific information to the female moth on plant health, physiology and nutrition. Such information guides the moth to either accept or reject the plant for oviposition. The review then examines the evidence for learning in adult moths' host recognition and selection behaviour and concludes that while learning or prior experience of the moth with the plant, either during the development of the larval or adult stages, may be an important factor under laboratory or glasshouse conditions, it may not be important under large scale commercial field conditions. One weakness in the literature that we noted about insect learning and host selection was that all the information available comes from either laboratory or glasshouse studies and may not hold true in the field.

Behaviour manipulation methods for pest management are reviewed. There are three principal elements of a behavioural manipulation method: a behaviour of the pest, a means by which the behaviour is manipulated appropriately, and a method that utilises the behavioural manipulation method for the protection of the resource from the pest. Stimuli that act over a long distance such as chemical stimuli (e.g. pheromones), visual stimuli, attractants and repellents enable the insect to encounter the plant. After the insect has landed, short range stimuli such as stimulants and deterrents, which occur on the organ or leaf surfaces of the plant, guide the insect to either accept or reject the plant for oviposition or feeding. Most stimulants and deterrents are non-volatile chemicals and are perceived by the insect (particularly moths) on the leaf or organ surface of the plant. It is clear in the review that short-range stimuli (stimulants and deterrents) are considered more likely to be useful for pest management than long-range stimuli (mating disruption, moth attractants). This is because successful manipulation of the pestilential behaviour (e.g. feeding on the resource) or a behaviour related to the resource (e.g. finding the resource) (i.e. short range stimuli) will ensure the protection of the resource, but successful manipulation of behaviours unrelated to the resource (e.g. mating disruption for moths,

moth attractants) may reduce the local population but still not protect the resource because of immigration of outside populations into the area being protected.

The review then examines the role of semiochemical research in *Helicoverpa* behaviour and management in Australian cotton. The pest management strategies being used in the cotton industry include beneficial insects, trap cropping, intercropping, transgenic cotton, biological pesticides, host plant resistance and synthetic insecticides. It was clear in the review that virtually all the methods of pest management currently used in the cotton industry involve some changes in pest behaviour, whether intentional or not. Ultimately, the success of any particular strategy to manage *Helicoverpa* in cotton will depend on the feeding and oviposition behaviour of *Helicoverpa* spp. in relation to the host plant. The use of stimulants in conjunction with biological pesticides can increase contact that may be suppressed if the pest responds to the toxin by ceasing to feed to avoid a lethal dose. Trap crop strategy is being utilised to reduce the size of *Helicoverpa* populations by diverting *Helicoverpa* eggs from cotton to the trap crops. It was clear in our review that most potential trap crops are only briefly more attractive than cotton. *H. armigera* female oviposition and orientation on pigeon pea is elicited by different optimum stimulus concentrations. The same kairomone concentration that attracts *H. armigera* females is active as an oviposition deterrent, whereas a lower kairomone concentration stimulates egg laying. This selective response reveals that each plant-released stimulus concentration has to be determined on the plant surfaces to enable effective use of these crops as either a trap or refuge crop in pest management.

We then examine the role of semiochemicals on the survival of *Helicoverpa* spp. larvae on transgenic cotton plants and find that there is a significant gap in our understanding of the behavioural events that lead *Helicoverpa* larvae to feed and survive on the transgenic crops. For example, it is not clear whether any chemicals on the leaf surface of transgenic plants are different from those on non-transgenic plants and if they are whether these chemicals are detectable by the larvae by contact, serving as a cue to the insect to alter its feeding behaviour on transgenic cotton plants so as to avoid a lethal dose of the toxin similar to the feeding behaviour of *H. virescens* on cotton with gossypol.

Then we examine the research capabilities of NSW Agriculture and Queensland Department of Primary Industry's Chemical Ecology Unit in Brisbane. We conclude that the two State organisations are well placed to undertake complementary semiochemical research should CRDC decide to invest in that area to exploit plant surface chemicals to manage key pests in Australian cotton.

Finally, we make a series of recommendations based on this review or background information. These are:

Recommendation 1: that the CRDC commission research to assess and compare chemical components of the organ surfaces and inner tissues of cotton plants, other hosts

and non-host plants, and also potential trap crops in relation to genotype, plant growth stage, conditions and physiology to determine how these chemicals influence acceptance or rejection of hosts, as a step towards development of better management strategies for *Helicoverpa* spp. in cotton.

Recommendation 2: that the research initiated in recommendation 1, as part of the objective identify those plant surface chemicals that can be better exploited as stimulants, deterrents, attractants and repellents in conventional spray programs, either alone or in combinations with biopesticides or synthetic insecticides to enhance the management of *Helicoverpa* spp. in cotton. The project application on semiochemicals to CRDC in 1999 entitled "Semiochemicals of organ surfaces of cotton plants and pest management" under which this review was commissioned seek to address recommendations 1 and 2. In the light of this review and given that a new project application could not occur until the year 2000/2001, we recommend that the preliminary project as per 1999/2000 application be revised as a joint NSW Agriculture and QDPI Chemical Ecology Unit project and continue this season to allow preliminary investigations in line with recommendations 1 and 2.

Recommendation 3: that the research initiated in recommendations 1 and 2, as part of their objectives, assess the opportunities for creative management of crop insect pests through genetic modification of the host's chemical profile or in novel plant breeding programs to produce cotton plants which are a less suitable host and trap crops which are a more attractive host to *Helicoverpa* spp.

Recommendation 4: that the CRDC commission research to assess differences in leaf surface chemistry of Bt and non-Bt plants in response to stress, water-logging, cloud cover, pest damage, humidity etc., and determine whether there is a linkage between changes in organ surface chemistry of Bt plants and the expression of Bt toxins and how all these relate to the feeding behaviour and survival of *Helicoverpa* spp. larvae on Bt plants, as a step towards development of improved resistance management strategy for Bt plants. CRDC has approved NSW Agriculture research to examine the expression of Bt plants under different agronomic conditions. These experiments could be used for initial investigation into semiochemicals on Bt plants this season or for future semiochemical research on Bt plants.

Recommendation 5: that the CRDC approve joint research collaboration between NSW Agriculture and QDPI's Chemical Ecology Unit in Brisbane in the establishment of a "Semiochemical Research Unit". This unit which would develop, lead and co-ordinate research into both long and short range chemical stimuli to identify behaviour modifying compounds from organ surfaces or inner tissues of plants to manage *Helicoverpa* spp.

1.0 Introduction

Cotton crops in Australia, as in the rest of the world, are attacked by a wide range of pests. Many insect species have been recorded in Australian cotton but only 6 are regarded as major pests, with another 17 considered to be minor pests (Hearn and Fitt, 1992; Fitt, 1994). The key pests in decreasing order of importance are *Helicoverpa* spp. (*Helicoverpa armigera* and *H. punctigera*); two-spotted mites (*Tetranychus urticae*); green mirid (*Creontiades dilutus*); thrips (*Thrips tabaci*) and aphids (*Aphis gossypii*). *Helicoverpa* spp. occur in all regions and are considered the most important economic insect pests of cotton and other field crops (Fitt, 1989, 1994). Both species are polyphagous, highly mobile and feed preferentially on young growing tips or reproductive structures of cotton plants. The estimated cotton crop losses due to *Helicoverpa* spp. in Australia in the 1996-97 season were valued at A\$161.7 million despite the expenditure of A\$87.2 million on control (Adamson *et al.* 1997). During 1998-99 season, it has been estimated that A\$200 million was spent on pest control (Dallas Gibb, pers comm.). The control of these pests relies exclusively on the use of synthetic insecticides. Over-reliance on synthetic insecticides, together with the associated problems of insecticide resistance, disruption of beneficial insects and environmental pollution has cast doubt on the long-term classical synthetic insecticide approach. The focus of the Australian cotton industry, therefore, is to reduce its dependence on synthetic insecticides. To achieve this, there has been a strong push by the industry towards an integrated pest management (IPM) system and research into alternative methods of pest control such as transgenic cotton crops, food sprays, biological pesticides, strip and trap-cropping, etc. The use of behaviour-modifying compounds such as feeding deterrents (or antifeedants), oviposition deterrents, attractants, repellents, mating disruptants, etc. that reduce insect feeding or egg laying without killing pests has intuitive appeal because such compounds should be safer to non-target organisms and can reduce the use of synthetic insecticides.

The objective of this review is to provide background information on the current and potential use of semiochemicals or behaviour modifying compounds for insect management, particularly of *Helicoverpa* (and *Heliothis*) spp. in cotton and other field crops. The roles of semiochemicals in host selection and in influencing the behavioural sequence leading to feeding and/or oviposition are discussed. We will review what is known about the leaf surface chemicals and other relevant leaf chemical constituents and how these can affect the oviposition behaviour of adult insects such as *Helicoverpa* or *Heliothis* which do not feed on the host plant, but nevertheless can determine its quality before depositing their eggs. While a discussion of volatile chemicals forms a minor part of this review, reflecting our focus on on-plant behaviour, such chemicals will also be discussed as appropriate, since they play an important role in influencing insect behaviour, and show considerable promise as tools of behaviour modification. In addition, we will review the feeding behaviour of *Helicoverpa* larvae in relation to the leaf surface chemistry of the host plants, particularly cotton, and the implication of this feeding

behaviour for the survival of *Helicoverpa* larvae. Particular reference will be made to survival on transgenic cotton. We will end the review with recommendations for future research directions and the most promising areas for further study in relation to exploiting behaviour modifying compounds to manage key pests of Australian cotton. In Section 8, we will outline the research capabilities and equipment that already exists in the industry and in relevant research organisations, and in Section 9 will identify any additional expertise or equipment we may require should the industry decide to proceed with studies into the research area of semiochemicals.

2.0 What are semiochemicals?

Semiochemicals (literally, "signalling" chemicals) are chemical compounds emitted by one organism that modify the behaviour of an organism receiving the signal. (Tinsworth, 1990). Pheromones represent a sub-class in which the signals are transmitted between organisms of the same species. In addition to the pheromones, semiochemicals also include: (1) chemicals emitted by one species that modify the behaviour of a different species to the benefit of the receptor species (kairomones), e.g., insect secretions detected by a parasite; (2) chemicals emitted by one species that modify the behaviour of a different species to the benefit of the emitting species (allomones), e.g., defensive secretions; and (3) chemicals emitted by one species that modify the behaviour of a different species to the benefit of both the emitting and receptor species (synomones), e.g., floral scents which lead to nectar feeding by an insect together with pollination of the flower. In 1979, the United States Environmental Protection Agency (EPA), Office of Pesticide Programs (OPP), recognised that semiochemicals were inherently different from synthetic insecticides and so made a policy statement encouraging the development and registration of semiochemicals as safer alternatives to conventional pesticide products (Tinsworth, 1990).

3.0 Role of Semiochemicals in the behavioural sequence leading to oviposition

Oviposition is an important step in an insect's reproductive process. This step ensures the continuity of the species generation and any mistakes committed by the adult female in selecting an oviposition site will affect the offspring dearly. For lepidopteran insects such as *Helicoverpa* spp. that do not feed on the host plant, the oviposition step is particularly crucial, because the hatching larvae are often not very mobile (neonate stage) and thus depend on the judicious choice of food plant by the adult female (Chew and Robbins, 1984; Feeny *et al.*, 1983; Renwick, 1989). To achieve this, the adult females have to rely on a wide variety of behavioural events to determine or obtain information about the quality or health of the host plant before ovipositing a large proportion of their eggs on the plant.

Searching, orientation, encounter, landing, surface evaluation and acceptance or rejection are the sequence of behavioural events leading to oviposition by lepidopterans (Kogan,

1977; Renwick and Chew, 1994). Searching, orientation and encounter events are very difficult to differentiate (Jones, 1992) and therefore will be considered in this review as the first stage of the behavioural sequence. This is followed by landing, contact evaluation and acceptance or rejection. All stages of the host finding and acceptance sequence depends on a wide variety of cues both sensory (Fitt, 1991; Renwick and Chew, 1994) and chemical cues (Renwick, 1989). However, experiments to differentiate between the mechanisms involved in searching, orientation and encounter are difficult to perform (Morris and Kareiva, 1991). Most studies in this area have focussed on visual factors such as colour, shape and size of the host plants (Prokopy and Owens, 1983; Stanton, 1983). However, Mitchell *et al.* (1991) reported that many lepidopteran insects use airborne volatiles emitted from plants to locate their host in contrast to visual cues such as colour, shape and size of the host plant. Since *Helicoverpa* spp. adults are known to migrate and also lay most of their eggs during night time (Fitt, 1989), it is possible that they may utilise airborne volatiles to locate their host plants rather than visual cues such as colour, shape and size which are of potentially greater importance during daylight.

After the insect alights on a plant, contact perception of both physical and chemical characteristics of the leaf or other organ surface becomes the most important factor in determining the suitability of the host for oviposition. The behaviour of many herbivorous insects immediately after arriving on the plant surface indicates that they are evaluating the plant as a potential food source or oviposition site (Blaney, 1970).

3.1 Searching, orientation and encounter of the host plant by the insect

Searching behaviour of moths and butterflies have been recently reviewed (Morris and Kareiva, 1991; Jallow *et al.* 1998). Their conclusions suggest that the predominant sensory cue for host finding are visual cues, with shape and colour playing a major role (Prokopy and Owens, 1983). However, Ramaswamy *et al.* (1987) reported that *H. virescens* females can choose between cotton and groundcherry even when olfaction and vision are prevented, indicating the importance of plant volatiles in host location. The important role of plant volatiles in the orientation of various moths to their host plants has also been reported (Renwick and Chew, 1994; Hartlieb and Rembold, 1996). Volatiles emanating from flowering cotton and maize may act as long range attractants for *H. armigera* females allowing the moths to concentrate within areas of flowering hosts (Hedin, 1976; Hopper, 1981). Similar suggestion has been made for *H. punctigera* on lucerne and peas (Cullen, 1969). Substantial research has been done on the effect of plant volatiles acting as oviposition stimulants or deterrents for moths (Renwick, 1989; Renwick and Chew, 1994), but little is known about long range perceptible host plant signals. Hartlieb and Rembold (1996) suggested that ovipositing *H. armigera* females are strongly attracted by volatiles from a pigeon pea steam distillate which stimulates two behavioural reactions important for host finding: orientation to the odour source from a distance, and landing. They identified several sesquiterpenes in the distillate. Knowledge of long range perceptible plant signals will benefit the management of these moths because

of the potential to attract these insects to non-hosts, or to assist in the breeding of crop varieties that may prevent moths locating them as host plants. The contrast between a moth's orientation toward preferred and non preferred plants has been used to identify attractants involved in pre-oviposition behaviour. For example, the cabbage looper oriented towards volatiles from a susceptible variety of soybean, but was repelled by volatiles from a resistant soybean line (Khan *et al.* 1987). The attraction of moths to plant volatiles is not always related to the finding of a suitable host for oviposition because the insects have to undertake contact evaluation of the plant before deciding to accept or reject it. In pigeon pea/cotton interplants more *Helicoverpa* spp. moths were found on the pigeon pea when it was in flower compared to cotton, however, more eggs were laid on the cotton crop than the pigeon (Mensah and Singleton, unpublished data). The responses of moths to floral volatiles do not necessarily indicate orientation to an oviposition site, because many insects alternate bouts of nectaring with bouts of oviposition (Stanton, 1983).

3.2 Landing of the insect on the host plant

The final step in the orientation process is the landing of the moth on the plant. According to Renwick and Chew (1994), landing may be triggered by either physical or chemical cues or a combination of both. The role of vision is well documented (Prokopy and Owens, 1983; Mensah and Madden, 1992; Mensah, 1996, 1997), and colour and shape of the leaves are particularly important (Singer, 1993; Stanton, 1984). The host abundance or quality during a particular season may change the landing frequencies of the orientating moths (Rausher, 1979). However, in night-flying *Helicoverpa* spp., volatile chemicals may play a more important role than colour in promoting landing on a host plant such as cotton. The role of plant volatiles in eliciting landing has been suggested in several reviews (Jackson *et al.*, 1984), although the observed effects may often be attributed to attraction (Saxena and Goyal, 1978). According to Saxena and Goyal (1978), citrus volatiles cause a higher frequency of visits by *Papilio demoleus*. Stimuli that prevent or discourage landing on non-hosts or unsuitable hosts play an important role in the selection of oviposition sites. Such stimuli can also be utilised as repellents to manage these pests. Several attractants, arrestants and repellents that may be involved in landing or avoidance behaviour have been identified (Norris, 1990; Waage and Hedin, 1990).

3.3 Leaf surface evaluation of the host plant by the insect

This step in the oviposition process is very crucial in the life cycle of the insect because it is the final decision process for the female to accept or reject the plant for oviposition. Therefore a mistake at this stage in gathering leaf surface information by the female, may lead to oviposition on an unsuitable plant which may affect the survival of the offspring and the continuity of the generation. Most phytophagous insects, immediately after landing on a plant, commence the evaluation of array of sensory or chemical information on the plant surface (Renwick and Chew, 1994; Eigenbrode and Espelie, 1995). The

chemical subset of these stimuli or "chemical search image" plays an important role in host plant recognition (Stadler, 1986). For ovipositing insects such as *Helicoverpa* spp. that do not contact the inner tissues of the plant, but test only the leaf surface, recognition and selection of the host plant after landing could be determined by small quantities of many types of chemical substances that come from the inner tissues of the plant and that are present on the plant surface; for example, free amino acids, organic acids, sugars, secondary metabolites, vitamins, minerals and growth regulators (Tukey, 1971; Fokkema, 1981; Stadler, 1986; Derridj *et al.* 1989, 1992, 1996; Fiala *et al.*, 1990; Stadler and Roessingh, 1990; Jackson *et al.*, 1991; Roessingh *et al.*, 1992). These substances, especially secondary metabolites, could give species-specific information to a phytophagous insect (Soldaat *et al.*, 1996). Many secondary metabolites, however, are large molecules that do not diffuse through the membranes and cell walls easily, and thus are unlikely to be present on the leaf surfaces of all plants. In contrast, primary metabolites are present on the leaf surface of all plants (Tukey, 1971). Recent developments in chemotaxonomy have shown that plants can be discriminated against on the basis of their internal proportion of primary metabolites (Soldaat *et al.*, 1996). Yeoh *et al.* (1984) described species-specific amino acid proportions in leguminous plants. It has also been shown that proportions of free amino acids in the leaf surfaces of maize and sunflower are very stable and specific and affect host selection of these plants by the moth *Ostrinia nubilalis* (Derridj, 1989). Plant surface chemicals that stimulate females to oviposit have been isolated for *H. zea* on corn (Wiseman *et al.*, 1988), *Heliothis suflexa* on groundcherry (Mitchell and Heath, 1987), and *Heliothis virescens* on tobacco (Jackson *et al.*, 1984; Mitchell *et al.*, 1990). Mitchell *et al.*, (1990) also isolated oviposition stimulant compounds for *H. virescens* from leaves and squares of cotton plants. Rembold and Tober (1985) showed that *H. armigera* females responded differentially in oviposition trials to odours obtained by pulling air over the leaf surfaces of seedlings of two cultivars of pigeon pea. Tingle *et al.* (1989) also found that *H. subflexa* females displayed positive flight responses to odours emanating from washings from leave surfaces of its host, groundcherry. It is also known that *H. virescens* can choose between cotton and groundcherry even when olfaction and vision are prevented indicating that chemoreception and mechanoreception alone allow discrimination of host plants in this species (Ramaswamy *et al.*, 1987). These findings clearly support the suggestion that leaf or organ surfaces of plants contain chemicals which provide information to the insect by contact regarding the suitability of the host plant for oviposition. The female moths obtain this information from the leaf surface by fluttering, wing fanning, walking and ovipositor dragging after initial contact with the leaf surface before oviposition. The fact that the plant surface cues on the leaf that discourage biting or oviposition before any damage occurs should benefit the plant (Chapman, 1977). Similarly, female moths should benefit from rapid assessment of host quality on the basis of host surface cues (Bernays and Chapman, 1975).

3.4 Acceptance or rejection of plants by the insect

For ovipositing moths that do not feed on the plant, surface cues of the plant play a major role in the final decision to oviposit or not (Schultz, 1988). Rothschild and Schoonhoven (1977) demonstrated that *Pieris brassicae* assesses egg load of plants by means of leaf surface cues. Since this study, chemical cues mediating egg laying and distribution or avoidance of occupied foliage has been reported for moths (Poirier and Borden, 1991). Hartlieb and Rembold (1996) demonstrated that a sesquiterpene mixture from a pigeon pea distillate in a 1:50 dilution, stimulated egg laying on the pigeon pea. However, increasing concentration of the sesquiterpenes resulted in the rejection of the plant for oviposition. This means that orientation and oviposition on pigeon pea are elicited by different optimum stimulus concentrations. The same kairomone concentration that attracts *H. armigera* females to pigeon pea is, at a higher concentration, active as an oviposition deterrent, whereas a lower concentration of the same kairomone can stimulate egg laying. At what growth stage of the pigeon pea does it produce oviposition deterrent or stimulant kairomones? What other factors affect the production of the oviposition deterrent and stimulant kairomones? An understanding of these issues will enhance effective utilisation of pigeon pea as trap or refuge crops in cotton. Sesquiterpenes have rarely been found as insect attractants or ovipositional stimulants, whereas aliphatic short chain alcohols and monoterpenes are more common semiochemicals (Metcalf, 1988). Some sesquiterpenes (I-humulene, I-bulnesene and J-caryophyllene have been identified in cotton plants (Elzen *et al.*, 1985). Methyl esters of fatty acids have been reported as oviposition deterrent compounds for *O. nubilalis* (Thiery and Quere (1991). Mitchell *et al.* (1990) washed the leaf surfaces of susceptible tobacco (NC2326) and resistant tobacco (TI 1112) and 1 ml of each extract was pipetted onto the centre of a piece of white broadcloth, as an oviposition substrate. They reported that *H. virescens* laid more eggs on cloths treated with susceptible extract and very few eggs on cloths treated with resistant tobacco extract indicating that the moth can differentiate between the susceptible and resistant plants by leaf surface contact. The result was consistent with those of Jackson *et al.* (1983), who conducted similar competitive tests outdoors in small field cages. The positive ovipositional response recorded from *H. virescens* from the leaf wash of susceptible tobacco was due to the presence of divane diterpenes and conversely, the ovipositional nonpreference displayed by the moth towards the resistant tobacco was due to the lack of or reduced level of divane diterpenes (Jackson *et al.*, 1986).

It is also known among moths that chemicals extracted from nonhost plants and sprayed onto known acceptable host plants can exhibit varying levels of deterrence by ovipositing females (Tingle and Mitchell, 1984; Williams *et al.*, 1986). This could mean that identification of the chemicals stimulating oviposition or feeding presents opportunities for characterisation of the behavioural and physiological factors regulating this essential process in the life cycle of the pest. Further, it may prove possible to mask such chemicals, thereby deterring oviposition, using other products, or greatly reduce or

eliminate them from otherwise desirable cultivars through genetic manipulations, so imparting a degree of resistance to pest attack.

Epicuticular waxes and leaf surface lipids have also been suggested as potential cues used by ovipositing females to reject or accept a plant for oviposition (Eigenbrode and Espelie, 1995). Major classes of these plant epicuticular lipids have been reported (see review by Eigenbrode and Espelie, 1995). The epicuticular lipids of a plant can vary with plant part, age and environmental conditions (Baker, 1982). The epicuticular lipid composition of the abaxial leaf surface may differ dramatically from that of the adaxial surface (Bokovac *et al.*, 1979) and this may affect egg distribution on the host plant.

Fiala *et al.* (1990) studied the relationships between the plant oviposition preference of the European corn borer (the lepidopteran *O. nubilalis*) and the biochemical composition of the host plant, particularly maize. The European corn borer is a night flying insect similar to *Helicoverpa* spp. in Australia. The insect prefers plants, especially at twilight, which are richest in soluble carbohydrate contents and, further, selects those having the richest leaves, without any feeding (Fiala *et al.*, 1985; Derridj *et al.*, 1986). To accept or reject a plant for oviposition without feeding, the insect needs to detect the quality of the plant on the leaf surface. Plants have pores or micropores in the cuticle and in the cell wall of epidermal cells, which could promote the leaching of water soluble compounds from the inside of the leaf surface (Charmel, 1986; Miller, 1986). These may allow the moths or insects, which do not feed on the plant before making an oviposition decision, to determine the quality of the plant from the surface. The molecules leaching onto the plant surface are diverse and include soluble carbohydrates, free amino acids, organic and phenolic acids, terpenes, and alkaloids (Fokema, 1981; Merall, 1981). Derridj and Fiala (1983) observed a positive correlation between oviposition preference of European corn borer female moths and low molecular weight carbohydrates on the leaf surfaces of two corn hybrids. The method for collecting soluble substances on the leaf surface has been described in detail (Fiala *et al.* 1990).

In addition to the plant compounds involved in species recognition, other chemicals, particularly those containing nitrogen, can provide information to ovipositing females on the relative quality of a particular species or individual (Thompson and Pellmar, 1991). Changes in nitrogen levels are often accompanied by changes in other chemical constituents on the leaf surface of plants particular sugars, secondary carbohydrates (Thompson and Pellmar, 1991). Meijden *et al.* (1989) sampled neighbouring pairs of ragwort plants: one of which had an egg mass, the other did not and found that cinnabar moths selected plants with high concentration of both nitrogen and sugars on the leaf surface. Plants poor in nitrogen and sugars on the leaf surfaces were less likely to receive eggs (Thompson and Pellmar, 1991). The effects of nitrogen-containing alkaloids which are positively correlated with total-nitrogen and negatively correlated with soluble carbohydrate levels, have been shown to have no effect on oviposition (Thompson and Pellmar, 1991).

Though ovipositing female moths are capable of detecting suitable host plants just through contact with the leaf surface, ovipositional "mistakes" i.e. ovipositions onto plant species outside the normal range of acceptable hosts, are common (Singer, 1984). Such mistakes may be the raw material for host shifts. They may mark the broadening of the number of plant species used by an insect population, favouring females that save time in searching for hosts by adding this species to those they use. Alternatively, these mistakes may mark the beginnings of a complete shift onto a new plant species. On the other hand, they may simply serve to select against females that are less specific than others in their choice of host plants (Futuyma, 1983). *Helicoverpa* spp. particularly *H. armigera*, undertake "distress" laying similar to ovipositional mistakes when given oviposition substrates or plants they do not prefer. Such a behaviour usually complicates host preference trials in the laboratory and makes it impossible to translate these results to the field.

4.0 Oviposition preference and larval performance

A major working hypothesis on the evolution of oviposition behaviour is that females will select plant species that will maximise the survival of the larvae (Rausher, 1982; Thompson and Pellmyr, 1991). Studies so far reviewed suggest that ovipositing female moths, after landing on a plant, can detect the quality and therefore suitability of the plant for oviposition on the leaf surface. An important question is whether the biochemical composition of the leaf is detectable by the larvae by contact. Hedin *et al.* (1988), reported that the first stage larvae of *H. virescens* can detect and avoid feeding on glands that contain gossypol when they hatch from the eggs. When the larvae leave the cotton terminal where the eggs hatched, they move onto small squares and then prefer to feed along the calyx crown until they moult into the second stage when they are unaffected by gossypol and then consume the glands (Parrott *et al.*, 1983). Further studies have shown that the young *H. virescens* larvae feed less on squares of high gossypol plants than those with low densities of gossypol (Parrott *et al.*, 1989). It has been suggested that anthocyanin-containing cells surrounding the gossypol glands deter the neonate larvae from feeding, and thus tissues containing toxins are avoided (Bernays and Chapman, 1994).

Studies conducted in Australia have shown that *Helicoverpa* spp. females lay the same number of eggs on transgenic and non-transgenic crops (Mensah *et al.* unpublished data, Fitt and Wilson, unpublished data) suggesting that the adult moth cannot detect the Bt toxin by using surface chemical cues to discriminate against the transgenic plants as oviposition sites. The question is, can the larvae use surface chemicals as cues, even before their first bite, to discriminate against or change their feeding behaviour on a plant? If this is so, then such a discrimination, particularly on transgenic cotton, may partially explain changes in larval movement and feeding behaviour which lead to higher than expected survival on transgenic plants. If the Bt toxin is not expressed uniformly in the

whole plant tissues, then the larvae may avoid feeding on tissues high in Bt until they have grown to sufficient size that they are no longer strongly affected by the Bt protein. This will ensure the survival of the larvae because adult female moths are sometimes known to make "ovipositional mistakes" to expand their host range. In a situation, it is up to the larvae to test the host plant and correct the situation by changing their feeding behaviour to survive on the plant. A plant commonly chosen for oviposition, but poor for larval survival or growth, may be a recent addition to a habitat, and selection may not have had sufficient time to favour females that avoid that plant species (Thompson and Pellmyr, 1991). For example transgenic cotton plants have only recently been introduced into the Australian cotton system and therefore selection may not have had sufficient time to favour females. It is possible that with time, *Helicoverpa* spp. females may discriminate against these plants as oviposition sites.

5.0 Behaviour manipulation methods for pest management

The manipulation of a pest's behaviour to protect a crop is not a new concept. According to Foster and Harris (1997), there are three principal elements of a behavioural manipulation method. They are (1) a behaviour of the pest (2) a means by which the behaviour is appropriately manipulated and (3) a method that utilises the behaviour manipulation to protect the crop. The manipulation of the pest feeding on the crop or the finding of the crop or host plant is more likely to be useful for pest management than manipulation of insect or pest behaviours unrelated to the crop (for example, mating disruption) (Foster and Harris, 1997). If the the feeding behaviour of the pest is manipulated successfully, it will ensure that the crop or resource is protected. However, successful manipulation of an unrelated behaviour may reduce the local population but still not protect the resource because of immigration of outside populations which may be already mated into the protected area, as can occur in moths (Carde and Minks, 1995).

In an attempt to avoid the pitfalls of characterising semiochemicals in terms of unanalysed behavioural effects, Kennedy (1978) classified chemical stimuli that act over a long distance (finding-type behaviours) as attractants and repellents and those that act at a short distance (acceptance-type of behaviours) as stimulants and deterrents. Nevertheless, it appears that an earlier clasification (Dethier *et al.*, 1960), has stood the test of time. This definition invokes oriented movement towards (attractant) or away (repellent) from the source of stimulus, and the eliciting (stimulant) or inhibition (deterrent) of feeding or oviposition (Bernays and Chapman, 1994). We apply this classification to the discussion which follows.

5.1 Attractants

Sex pheromones are the mostly widely used chemical stimuli world wide. Among lepidoptera, the female-produced sex pheromones are used for mating disruption ("confusion control") or for monitoring (attraction of males to a trap). There is also the

potential to pursue an attract-and-kill or mass-trapping strategy. However, the removal of a large proportion of males from the population is unlikely to have a large impact on the size of the subsequent generations compared to removal of females (Lannier, 1990). Nevertheless, there are a few examples of effective control by mass trapping based on sex pheromones including citrus flower moth on lemons in Israel (Sternlicht *et al.*, 1990) and gypsy moth in the United States (Kolodny-Hirsch and Swalbe, 1990). Sex pheromones have also been used as attractants to facilitate dispersal of pathogens in pest populations (Pell *et al.*, 1993). Most work on the use of attractants to disrupt a finding behaviour has focussed on mate location, particularly moths in the mating disruption method (Minks and Carde, 1988). A mating disruption method using synthetic sex pheromones has been used successfully for control of pests such as pink bollworm, *Pectinophora gossypiella*, on cotton; the Oriental fruit moth, *Grapholita molesta* on stone fruits; the tomato pinworm, *Keiferia lycopersicella* on tomatoes and the currant clearwing, *Synanthedon tipuliformis* on blackcurrents (Carde and Minks, 1995).

In the Australian cotton system, synthetic sex pheromones have been used for several years in mating disruption and monitoring of *Helicoverpa* spp. movements in cotton. However, to date mating disruption to control *Helicoverpa* spp. in Australia has been unsuccessful. The use of sex pheromones in an attract-and-kill method in the Australian cotton agroecosystem is non-existent. Most of the strategies have failed because of the potential for immigration of mated females from outside the treated area to attack the protected crops.

A wide variety of volatile plant secondary metabolites have been implicated in the food-finding behaviour of plant-feeding insects. By far the most common class of chemical compounds represented are the terpenoid hydrocarbons and related oxygenated derivatives (Hsiao, 1985). Green leaf volatiles, the six-carbon products of fatty acid oxidation, have also been shown to demonstrate attractive properties. Insects with a narrow host range are apparently finely tuned to a few specific chemicals characteristic of their hosts, the isothiocyanates of crucifers and their attraction for the diamondback moth being just one example.

Exploitation of such volatiles as agents of behaviour modification has proved to be slow. Undoubtedly, the complexity of many plant-derived volatile mixtures, and the consequent sophistication of the analytical-chemical research required to unravel the signal sensed by the insect, has contributed to this. Further, appropriate bioassays are more difficult to design than is the case with pheromones, partly due to less specific responses at the behavioural level, but also to the need to formulate multi-component mixtures to optimise response (Hsiao, 1985). Despite such difficulties, there is research currently investigating the potential of volatile chemicals derived from plants to attract adult *H. armigera*, particularly females in Australian cotton (Del Socorro *et al.*, 1999). This is complementing similar research conducted in recent years in the USA, Germany, the UK, India and China.

5.2 Repellents

The repellent strategy is to stop the pest from finding the protected crop. Most work on the practical use of volatile repellents has been to protect humans from insect bites, but repellents to protect crops have received little attention. This contrasts with studies of the chemical basis of plant resistance against insects (Renwick, 1990). Useful repellents can be derived from natural sources such as insects and plants. An alarm pheromone secreted by aphids, (E)-J-farnesene, is a repellent that has been used to protect a plant resource from aphid infestations. The same compound has been shown in laboratory and field trials to increase contact with toxic chemicals and fungal pathogens by *Myzus persicae* (Griffiths and Pickett, 1987). Plants that are known to be avoided by a pest may contain some chemicals which might be used as repellents against the particular insect. The agricultural practice of intercropping relies to some extent on the masking effect of the volatiles from one plant species on the volatiles of another, as sensed by an insect. Unequivocal demonstration of the phenomenon in lepidoptera has not been achieved, however. Studies into practical use of chemical repellents to prevent oviposition of *Helicoverpa spp.* on cotton in Australia has so far received little attention compared to attractants and deterrents.

5.3 Stimulants

After the insect has landed on the plant, it performs a contact evaluation of the plant surface to make a decision as to accept or reject the plant for oviposition or feeding. The stimuli detected by the insect may also effect the numbers of eggs the female deposits on the plant.

Potential uses of stimulant chemicals include increasing an insect's ingestion of toxins and pathogens etc., and stimulating egg lay by a pest on a refuge or trap crop in order to reduce the size of the population developing in the primary crop or resource. Feeding stimulants are especially useful in conjunction with toxins (Ave, 1995), because they can increase the pest's contact with the toxin so that a lethal dose of the toxin is picked up by the pest. Oviposition stimulants have the potential to be used in combination with moth attractants to ensure that the moths lay their eggs on a less preferred host plant, where both moths and larvae will be destroyed. Thus, oviposition stimulants could be used to divert oviposition from a protected resource to an alternative crop to reduce the size of the population. Stimulants would be one priority for future semiochemical research conducted by the industry. With the advent of trap-cropping and the use of biological pesticides there is the need for the cotton industry to develop stimulants to complement the performance of these technologies.

It is likely that the most important feeding stimulants for the majority of insects on most plants are simple sugars, such as sucrose and fructose, and that the effect is

concentration- dependent (Bernays and Chapman, 1994). However, many plant secondary metabolites have been implicated in this phenomenon, such as the flavonoid glycoside rutin, which stimulates feeding in polyphagous species, including the larvae of *H. zea*. Other glucoside conjugates of secondary metabolites have been shown to function as both feeding and oviposition stimulants.

With respect to oviposition stimulants, there is evidence that sesquiterpenes which are detected by moths on the leaf surface of pigeon pea, which is now being used as trap and refuge crop for *Helicoverpa* spp. in both normal and transgenic cotton, influence the moths' acceptance or rejection of the crop as an oviposition site in a concentration-dependent manner. Higher concentrations of sesquiterpenes can act as oviposition deterrent and lower concentrations as oviposition stimulants. Such compounds are sufficiently volatile to function as attractants as well. Aliphatic short chain alcohols and monoterpenes are more common ovipositional stimulants whereas sesquiterpenes have rarely been found as ovipositional stimulants (Metcalf, 1988). Soluble carbohydrates leaching onto the plant surface have also been identified as oviposition stimulant for moths (Derridj and Fiala, 1983). In *H. virescens*, high levels of duvane triterpenes were found to stimulate the insect's egg lay on tobacco plants (Jackson *et al.* 1984). In the case of *H. zea*, sesquiterpene carboxylic acids stimulate oviposition (Coates, 1988) and *H. subflexa* oviposited in response to methanolic extracts of groundcherry (Mitchell and Heath, 1987).

The identification of semiochemicals on organ surfaces of trap or refuge crops planted within or around cotton is likely to lead to the identification of stimulants or deterrents that can be used to divert oviposition from cotton onto the alternative crops.

5.4 Deterrents

A deterrent is a chemical that inhibits feeding or oviposition behaviour when applied to a site where the behaviour normally occurs (Bernays and Chapman, 1994). In pest management, a deterrent is applied directly to reduce a pest's feeding or oviposition behaviour. The presence of deterrents at the surface of leaves plays a major role in discriminatory behaviour of ovipositing moths. However, in the case of a polyphagous insect it is likely that tolerance to deterrents is comparatively high. Very few compounds responsible have been identified, but research in this area is accelerating. Perhaps the best known feeding deterrent is the triterpenoid secondary metabolite azadirachtin, from the neem tree *Azadirachta indica*. Other triterpenoids, such as limonin, together with a variety of alkaloids, show potent feeding-deterrent activity. Recently, triterpenoid derivatives from bitter-gourd leaves were identified and shown to deter feeding in the false armyworm, *Spodoptera litura*, a polyphagous insect often used to evaluate plant-derived feeding deterrents (Yasui *et al.* 1998).

Differences between the susceptibility of cabbage varieties to larval feeding by the diamondback moth have been related to the deterrent effects of certain leaf surface wax components (long-chain primary alcohols and triterpenols). The complexity of the phenomenon is illustrated by the fact that similar long-chain alcohols stimulate feeding on an appropriate substrate by newly hatched silkworms.

Feeding deterrence towards *Spodoptera exigua*, as well as growth-inhibitory and insecticidal effects, have been demonstrated by compounds isolated from avocado plants (Rodriguez-Saona *et al.*, 1998). The compounds were isolated from unusual idioblast oil cells. Such cells occur in leaves and other organs of dicotyledonous species in several plant families, and constitute a potential source of natural chemicals involved in defense against herbivores.

Many plant extracts have been tested for deterrent activity on a variety of insects (Renwick, 1990), mainly with the view of using deterrents in pest control programs (Renwick, 1988). Polar extracts are known to be the most effective compounds that deter oviposition of lepidopterans (Renwick, 1990). Though nonpolar extracts are known to be less effective as oviposition deterrents, it is possible that the lipid material making up the bulk of such extracts may form a layer over polar stimulants at the leaf surface, thus preventing the insects from detecting a stimulant or a deterrent compound. If a nonpolar extract masks the effect of an oviposition stimulant, then it will act as a deterrent because it will prevent the insect from recognising the resource as a host. For example, applying Envirofeast or petroleum spray oils to cotton may mask the effect of a stimulant on the leaf surface, thereby suppressing oviposition of *Helicoverpa* spp. on these plants (Mensah *et al.* 1994; Mensah, 1996). Because deterrents suppress feeding and oviposition behaviours of insect pests, they are often found by studying the chemistry of non-host plants of a particular pest species (Bernays, 1983). Deterrents are seen by most researchers as more important than stimulants (Bernays and Chapman, 1994). Several studies have focussed on organ surface chemicals affecting oviposition of *Helicoverpa* spp. Duvane diterpenes are found on leaf surfaces of tobacco plants. Reduced levels of these compounds have been found to deter oviposition of *H. virescens* on tobacco (Jack *et al.*, 1986). A higher concentration of sesquiterpenes in pigeon pea is also known to act as an oviposition deterrent. However, in a low concentration mixture of 1:50 dilution, these compounds stimulate egg laying of *Helicoverpa* spp., suggesting that the oviposition-stimulating activity of the synthetic mixture is concentration dependent (Hartlieb and Rembold, 1996).

There is the need within the cotton industry to understand changes in concentration of sesquiterpenes on the leaf surfaces of pigeon pea in relation to the growth stage of the plant to enable the effective use of pigeon pea as a trap or refuge crop in cotton. Such compounds can be considered volatile enough to exhibit attractant or repellent activity as well. This illustrates the fact that deterrents, or for that matter stimulants, need not be involatile compounds. Rembold *et al.* (1989, 1991) suggested that the development of

attractant that is selectively attractive to mated *H. armigera* females (much less for nonlaying females and not attractive for females) and larvae may be important for use in attract-and-kill method in cotton and other crops attacked by this pest.

There is a general view that the efficacy of a deterrent based method may be increased if used in combination with another method that attracts the pest to a nonvalued resource in a stimulo-deterrent diversion (Miller and Cowles, 1990) or push-pull (Pyke *et al.* 1987) strategy. The stimulo-deterrent strategy was suggested for insect herbivores but is applicable to many pests and any resource type (Foster and Harris, 1997). The onion fly, *D. antiqua* has been deterred from laying eggs on seedling onions by cinnamaldehyde and stimulated to lay eggs on nonvalued cull onion bulbs that were planted in the same field (Cowles and Miller, 1992). In large scale trials, Miller and Cowles (1992) used encapsulated cinnamaldehyde as a deterrent and cull onions as a stimulant to control *D. antiqua*. *Helicoverpa* spp. moths were deterred from ovipositing on cotton by azadirachtin and stimulated to oviposit on pigeon pea or maize crops (Pyke *et al.* 1987). Smart *et al.* (1994) have shown in separate field trials that the adult pea and bean weevil, *Sitona lineatus*, can be deterred from feeding on a leguminous crop by neem oil and attracted into other crop fields using aggregating pheromones. In the cotton industry, it seems likely that such combined behavioural manipulation methods may reduce the size of *Helicoverpa* spp. population in cotton, provided that suitable deterrents or stimulants can be identified.

6.0 *Helicoverpa* spp. Behaviour and Management Strategies on Cotton: Role of Semiochemical research

Pest management in Australian cotton for some years had relied exclusively on repeated application of synthetic insecticides. Currently there is an increased desire to reduce the reliance on broad-spectrum insecticides and also increased interest in behavioural manipulation for pest management. The pest management strategies being used include biological pesticides, beneficial insects, transgenic cotton, trap cropping, intercropping, behavioural manipulation methods of both pest and beneficial insects such as mating disruption and food sprays, host plant resistance and synthetic insecticides. Virtually all methods of pest management involve some changes in pest behaviour, whether intentional or not (Gould, 1991). Ultimately, the success of any particular strategy to manage *Helicoverpa* spp. in cotton will depend on the feeding and oviposition behaviour of the pest in relation to the host plant.

6.1 Use of Biological Pesticides in pest management

Biological pesticides, particularly foliar Bt and NPV virus (Gemstar), are the only biopesticides used to manage *Helicoverpa* spp. in cotton in Australia. These pesticides are specific to lepidopteran larvae. The toxins have to be ingested by the larvae on the plant to achieve mortality. Therefore, it is important that the larvae consume a lethal dose

when feeding on the plant. To achieve this, persistence of the product on the plant should be maximised, and the larvae should ingest the toxin during feeding. Thus, the efficacy of the biological pesticides is affected by UV light, spray coverage and the ability of the insect to ingest a lethal dose of the toxin during feeding. The UV light destroys the product, thus affecting the persistence and efficacy of the product. The pest can also respond to the toxin by ceasing to feed or feeding less thus avoiding a lethal dose. Mensah and Harris (1996) increased the efficacy of NPV virus (Gemstar) by mixing the virus with Envirofeast (food) product and applying it to the cotton plant. The efficacy of the virus was also increased when it was mixed with a UV protected petroleum spray oil (Mensah and Singleton, 1999).

Feeding stimulants are especially useful in conjunction with toxins because they can increase contact that may be suppressed if the pest responds to the toxin by ceasing to feed. These stimulants are mostly plant extracts or chemicals and are identified by studying plant surface chemistry in relation to the target insect's behavioural response.

Currently NSW Agriculture, in collaboration with Swiss Federal Institute of Technology, has in stock about 55 crude extracts containing secondary plant compounds with potential semiochemical properties, of which 20 have been screened against *Helicoverpa* spp. on cotton. Some of these secondary products have been shown to act as phagostimulants, repellents, deterrents and attractants of *Helicoverpa* spp.

6.2 Use of beneficial insects in cotton pest management

The use of food sprays and strip-cropping with lucerne helps to conserve and increase the efficacy of beneficial insects in cotton (Mensah, 1997; Mensah and Khan, 1997). Semiochemical research is essential to identify beneficial insect attractants or aggregation pheromones that can be used to attract and augment beneficial insects in cotton. Identification and isolation of such attractants will enhance the efficacy of the beneficial insects and improve biological control in cotton farms. As an example, a two-spotted ladybird beetle attractant is being developed by the Chemical Ecology Group at Rothamsted Experimental Station (Pickett, pers comm.).

6.3 Use of Trap Cropping in cotton pest management

The Australian cotton industry is focussing on the use of trap crops to manage *Helicoverpa* spp. in cotton. The idea of trap cropping is to divert *Helicoverpa* spp. eggs from cotton to the trap crops, thus reducing the size of *Helicoverpa* population on cotton. Crops used to trap *Helicoverpa* spp. ovipositing females need to be more attractive and acceptable to the moths as oviposition sites than the crop to be protected. However, studies have shown that most potential trap crops are only briefly more attractive than the crops they are intended to protect (Fitt, 1989). It was therefore suggested that for successful utilisation of a trap crop strategy, sequential plantings of

trap crops are necessary (Fitt, 1989). Nevertheless, despite sequential planting of trap crops, the strategy may not be successful if the behavioural events that lead an ovipositing moth to accept or reject a plant for oviposition are not identified and utilised to stimulate the moths to lay on the trap crops.

As indicated in this review, the cues that lead insects to oviposit or feed on a host plant are associated with leaf or organ surfaces of the host plant. A simple contact of the insect with the leaf or organ surface may provide it with information about the plant's health, physiology and nutrition. Unless the leaf surface cues are studied and identified in relation to the insect's behaviour and also the plant's growth conditions or stages, the effective use of trap crops within the cotton industry will be a failure. For example, pigeon pea has been reported as a good host for ovipositing *Helicoverpa* moths. However, there have been several reported cases within the industry where pigeon pea has been less attractive to *Helicoverpa* spp. oviposition than cotton. In a trial where two-30 rows of pigeon pea crops were planted adjacent to 15 rows of cotton at Norwood and Yarral during the 1997/98 and 1998/99 seasons, the results showed that the cotton crops received about 3 times more eggs per metre than the pigeon pea crops, despite the smaller size occupied by cotton compared to the pigeon pea (1:4). Also, more moths were found on the pigeon pea crops than the cotton during moth observation. However, the moth numbers did not reflect the egg lay on the pigeon pea crops.

This raises several questions, e.g.: Were the moths using the pigeon pea for nectaring rather than oviposition bouts? Were there measurable differences between levels of potentially oviposition-stimulating sesquiterpenes or other stimulants between the leaf surfaces of both pigeon pea and cotton during the moth activity? A higher concentration of sesquiterpenes on the leaf surface will deter oviposition and a lower concentration will act as an oviposition stimulant (Hartlieb and Rembold, 1996). If this is so, what are the growth conditions/stages necessary for the trap crop to produce optimum levels of sesquiterpenes to stimulate oviposition on the pigeon pea crop? Generally, which leaf surface chemicals in crops being used as traps for *Helicoverpa* spp. would stimulate *Helicoverpa* spp. to oviposit on these crops? The semiochemicals of the leaf should be studied and understood to enable the effective use of trap cropping in pest management strategies in cotton.

Jallow (1998) explained an unsuccessful use of trap crops to control *Helicoverpa* spp. as due to learning which might result in the females by-passing the trap crop and ovipositing on the protected crop the moth might have had experience with. Though learning may help some insects to select their host, the learning process will actually help them to locate a plant. However, after landing on the plant, the insect will have to test the plant surface for cues before accepting or rejecting the plant for oviposition. The learning process is not a guarantee that the insect will automatically select a plant it might have had experience with. The learning process reported by Jallow (1998) might be regarded as an "ovipositional mistake" (Singer, 1984) which results in host shifts or the expansion of

host range. Learning by insects in host selection may be more important in laboratory conditions than in large scale field conditions. Nearly all the work that forms the basis of our understanding of learning and host selection behaviour of *Helicoverpa* spp. and other moths has been carried out in the laboratory and glasshouse with no field studies whatsoever. The interpretation of these results under field conditions, following studies of the plant's organ surface chemistry, will help to unravel some of the mysteries of host selection and also strengthen our understanding of the host selection behaviour of these species.

6.4 Use of inter/strip cropping with lucerne to manage pests

Lucerne crops planted as strips within cotton serve as refuge for beneficial insects and a trap crop for green mirids, *Creontiades dilutus* (Mensah and Khan, 1997; Mensah, 1999).

The green mirid prefers lucerne to cotton and in commercial cotton crops interplanted with lucerne, numbers of green mirids and their damage per metre were lower in the cotton than in cotton crops without lucerne strips. Nevertheless, in the absence of interplanted lucerne, the mirids will oviposit and survive on cotton (Mensah and Khan, 1997). Why does green mirid prefer lucerne to cotton? There are other plants around the cotton growing areas that the green mirids cannot survive on (Khan pers. comm) and therefore are a non-preferred host for the green mirid. Studies into the chemical composition of the host and non-host plants of the green mirids will help to isolate and identify either attractants, stimulants, repellents or deterrents which can be used to manipulate the green mirid's behaviour and manage it in cotton.

Jallow et al. (1998) suggested in their review of host selection of *Helicoverpa* spp. that the planting of lucerne as strip crops will amplify the *Helicoverpa* problem because of the learning of these insects to oviposit on lucerne. The increase in the size of lucerne will increase *Helicoverpa* numbers in cotton. Though a lucerne crop in flower can attract a higher number of *Helicoverpa* spp. moths onto the crop, sampling of *Helicoverpa* pupae throughout the season in lucerne has failed to find pupae under the crop - suggesting that none of the larvae could make it into pupae in lucerne. The mortality of the larvae in the lucerne, thus, is very high. The reason for the high mortality is that the lucerne crop is known to have a very high virus load and also the crop is slashed alternatively every 28 days to encourage fresh regrowths which are preferred by the green mirids. These factors have made lucerne unable to support generations of *Helicoverpa* spp. The planting of lucerne itself could be a suicidal crop for *Helicoverpa* spp.

6.5 Use of behaviour manipulation methods to manage *Helicoverpa* spp. in cotton

6.5.1 Beneficial insect attractants (food sprays)

Application of food supplements (Envirofeast (food) sprays) on cotton plants can attract, augment and conserve beneficial insects in cotton (Mensah, 1997). The product can also deter oviposition of *Helicoverpa* spp. on cotton (Mensah, 1996) and *Ostrinia nubilalis* on maize crop (Mensah *et al.*, in press). The mechanism involved might be the masking of the stimulant on the leaf surface preventing the female moth from detecting the stimulant, thus deterring oviposition. Similarly, Petroleum spray oils applied to cotton plants can also deter *Helicoverpa* spp. oviposition (Mensah *et al.*, 1994). Studies into the leaf surface chemistry of cotton plants will lead to the identification of any stimulant present which is being masked by Envirofeast and the petroleum spray oils, or uncover an alternative mechanism of action. Such stimulant compounds might then be used in combination with moth attractants in a push-pull strategy (Pyke *et al.*, 1987) to manage these pests.

6.5.2 Use of mating disruption and moth attractants

Mating disruption of *Helicoverpa* spp. and adult moth attractants is being studied and developed for use in the management of *Helicoverpa* in cotton. In virtually all cases where these methods have been attempted, disruption has been only partially achieved in the treated area (Carde and Minks, 1995; Foster and Harris, 1997). This is because the success of this method for management of a given pest depends to a large extent on its biology and particularly on the potential for immigration of mated females from outside the treated area (Carde and Minks, 1995). According to Foster and Harris (1997), manipulation of a pestilential behaviour (e.g. feeding on a resource) or a behaviour closely related to the pestilential behaviour (e.g. finding the resource) is more likely to be useful for pest management than manipulation of behaviours unrelated to the resource (e.g. mating disruption). Therefore, successful manipulation using attractants, stimulants and deterrents (acceptance type of behaviour) is more likely to be useful in pest management. Moth attractants can be more effective when used in combination with deterrents and stimulants in a stimulo-deterrent diversion (Miller, 1986) or push-pull (Pyke *et al.*, 1987) strategy. Stimulants and deterrents usually occur on the leaf surfaces of plants and are detected by moths during the contact evaluation of the leaf surface after landing on the plant. Therefore studies of the chemical composition of the organ surfaces of plants may lead to the identification of these compounds and their subsequent use to manage *Helicoverpa* spp. in cotton.

6.6 Use of transgenic crops

Transgenic cotton crops are currently one of the major IPM tools in Australian cotton farms. However, there is a very high risk of *Helicoverpa* spp. developing resistance to the

transgenic crops and negating any benefit this technology may bring to the industry in the short term. Most research is focussed on gene expression, efficacy, protein dynamics etc. and techniques to control *Helicoverpa* spp. larvae before they have had a chance to feed on the Bt plants. What is lacking is studies into the behavioural events that lead to the survival of *Helicoverpa* spp. larvae on the Bt plants. Most of the behavioural events that lead *Helicoverpa* spp. to oviposit or feed on host plants are known to be associated with leaf surface cues (Derridj *et al.*, 1996). The question is whether the biochemical composition of organ surfaces of Bt and non-Bt plants are different and whether such differences are detectable by the larvae. If they are detectable, do the larvae change their feeding behaviour (i.e. feed less if they detect toxin) to avoid a lethal dose to enable them to survive on the plant? The Bt toxin molecules are known to be too large to leak onto the leaf surface to be detected by the insect (Fitt, pers. comm.). However, is there any chemical compound on the leaf or organ surface of Bt plants that serves as a cue to the larvae to enable them to be aware of the presence of a toxin in the leaf even before they have had the chance to take the first bite? If so, it may elicit changes in their feeding behaviour similar to the reduced feeding of *H. virescens* larvae on gossypol-containing plants (Parrott *et al.*, 1983). Another question is, does the chemical composition of organ surfaces of stressed, water logged, etc. Bt plants differ from that of healthy plants, and does this relate to the expression and survival of larvae on these plants? All these questions can only be answered through studies into semiochemicals of organ surfaces of cotton plants. The knowledge gained thereby is likely to lead to better management of the resistance phenomenon in Bt plants.

6.7 Use of synthetic insecticides, entomopathogens

H. armigera has developed a high level of resistance to most of the synthetic insecticides used in the cotton industry, especially the synthetic pyrethroids. For most of the contact/stomach poisons where the pest has to ingest a lethal dose to effect mortality, efficacy of the insecticide will increase if it is mixed with phagostimulants to enhance the larval feeding. Feeding stimulants can increase contact and exposure to the insecticides that may be suppressed if the larvae responds to the insecticide by ceasing to feed thus avoiding the lethal dose. They have the potential also to increase the uptake and dispersal of highly specific microorganisms and viruses pathogenic towards pest insects. The review suggests that most of the stimulants that act as a cue to feeding or oviposition occur on the leaf or organ surface of the plant, hence plant chemistry is the most important source of information.

7.0 Suggestion on Future Directions of Research in Semiochemicals for a Sustainable Management of *Helicoverpa* spp. in cotton

In this section, we will provide an appraisal of the most promising areas for further research in relation to the exploitation of semiochemicals in the management of key pests in Australian cotton. In light of the evidence provided in the literature, most

phytophagous insects, especially lepidopterans, use long and short range stimuli to encounter their host. Jallow *et al.* (1998), in their review of host selection, reported that moths are more likely to orient to and lay on plants they have had prior experience with (learning). This is in contrast with Rausher (1979) who reported that the host abundance or quality (physiological state), not the learning state during a particular season, may change the landing frequencies of the orientating moth. This is particularly true for lepidopteran adult females that do not feed on the plant before accepting or rejecting a host plant for oviposition. Therefore, in *Helicoverpa* spp. ovipositing female adults, learning about, or prior experience of, a particular plant through larval feeding may not change subsequent landing frequencies and host plant acceptance or rejection. *Helicoverpa* spp. larvae, after completing development on a host plant, may not have any special preference whatsoever for any particular plant they are faced with after turning into adult moths and having to make a choice under large scale commercial farm situations. Learning therefore may be important under laboratory, but not field conditions, in relation to selection and encounter of the host plant.

Most phytophagous insects, immediately after landing on a plant, commence the evaluation of array of sensory or chemical information on the plant surface (Renwick and Chew, 1994; Eigenbrode and Espelie, 1995; Derridj *et al.*, 1996). The chemical subset of these stimuli, or "chemical search image", plays an important role in host plant recognition (Stadler, 1986). For ovipositing insects such as *Helicoverpa* spp. that do not contact the inner tissues of the plant (i.e. feed on the plant), recognition and selection of the host plant after landing could be determined by small quantities of many types of chemical substances that come from the inner tissues of the plant and that are present on the plant surface (Stadler, 1986; Derridj *et al.*, 1989, 1992, 1996; Fiala *et al.*, 1990). Thus, plant surface cues may play a major role in host selection and acceptance for oviposition and feeding of *Helicoverpa* spp. If these plant surface chemicals are identified and synthesized, or otherwise produced in sufficient quantities, they have the potential to manipulate the behaviour of the pest to protect the resource.

The first area of research suggested by this review, therefore, stems from the fact that chemicals on the plant surface and inner tissues can be used to manipulate the behaviour of *Helicoverpa* spp. to protect a resource (i.e. cotton). Such chemicals can include volatile and semi-volatile components, as well as non-volatile (contact) semiochemicals. There are three principal elements of a behavioural manipulation method: a behaviour of the pest, a means by which the behaviour is manipulated appropriately, and a method that utilises the behavioural manipulation for protection of a resource from the pest.

The manipulation of pestilential behaviour (e.g. feeding on the resource) or a behaviour closely related to the pestilential behaviour (e.g. finding the resource) is more likely to be useful for pest management than manipulation of behaviours unrelated to the resource (e.g. mating disruption). Successful manipulation of the pestilential behaviour will ensure protection of the resource. In contrast, successful manipulation of unrelated behaviour

may reduce the local population but still not protect the resource because of immigration of outside populations into the area being protected, as can occur in the mating disruption method for moths and attractants for moths. Thus, stimuli that act at close distance (stimulants and deterrents or combination of attractants/stimulants/ deterrents) could be more useful in pest management than stimuli that act over long distances (attractants, repellents, visual and chemical stimuli (pheromones)).

The stimuli that act at a close distance are perceived by the insect after it arrives on the plant. These stimuli can either stimulate a behaviour by keeping the insect at the resource/plant (host acceptance) or inhibit that behaviour resulting in the rejection of, and possibly movement away from, the resource or plant. Virtually all the short-range stimuli used in behaviour manipulation are chemicals found in the leaf surface or inner tissues of the plant or are applied to the surface of the resource or plant to mask the effect of the short-range chemicals.

Recommendation 1: that the CRDC commission research to assess and compare chemical components of the organ surfaces and inner tissues of cotton plants, other hosts and non-host plants, and also potential trap crops in relation to genotype, plant growth stage, conditions and physiology to determine how these chemicals influence acceptance or rejection of hosts, as a step towards development of better management strategies for *Helicoverpa* spp. in cotton.

Recommendation 2: that the research initiated in recommendation 1, as part of the objective identify those plant surface chemicals that can be better exploited as stimulants, deterrents, attractants and repellents in conventional spray programs, either alone or in combinations with biopesticides or synthetic insecticides to enhance the management of *Helicoverpa* spp. in cotton. The project application on semiochemicals to CRDC in 1999 entitled "Semiochemicals of organ surfaces of cotton plants and pest management" under which this review was commissioned seek to address recommendations 1 and 2. In the light of this review and given that a new project application could not occur until the year 2000/2001, we recommend that the preliminary project as per 1999/2000 application be revised as a joint NSW Agriculture and QDPI Chemical Ecology Unit project and should commence this season.

Recommendation 3: that the research initiated in recommendations 1 and 2, as part of their objectives, assess the opportunities for creative management of crop insect pests through genetic modification of the host's chemical profile or in novel plant breeding programs to produce cotton plants which are a less suitable host and trap crops which are a more attractive host to *Helicoverpa* spp.

Another area of research suggested by this review is to gain a better understanding of organ surface chemicals in relation to the behavioural events that lead *Helicoverpa* spp. larvae to feed and survive on transgenic crops. The question is whether the biochemical

composition of organ surfaces of Bt and non-Bt plants are different and whether any differences are detectable by the larvae. If they are detectable, do the larvae change their feeding behaviour (i.e. feed less if they detect toxin) to avoid a lethal dose to enable it survive on the plant? Are there any chemical compounds on the leaf or organ surface of Bt plants that serve as a cue to the larvae indicating the presence of a toxin in the leaf of the plants? Do the larvae change their feeding habit if they detect that there is a toxin in the plant? Another question is, is there a change in the chemical composition of organ surfaces of stressed, water logged etc., Bt plants when compared with the same plants that are not expressing the toxin, and how do any such changes relate to the feeding behaviour change and survival of larvae on these plants? Is it possible to apply stimulants to leaves of Bt plants to enable larvae to consume more so as to ingest a lethal dose, or to apply a chemical that may mask the effect of any surface compounds that enable the larvae to distinguish between Bt and non-Bt plants?

Recommendation 4: that the CRDC commission research to assess differences in leaf surface chemistry of Bt and non-Bt plants in response to stress, water-logging, cloud cover, pest damage, humidity etc., and determine whether there is a linkage between changes in organ surface chemistry of Bt plants and the expression of Bt toxins and how all these relate to the feeding behaviour and survival of *Helicoverpa* spp. larvae on Bt plants, as a step towards development of improved resistance management strategy for Bt plants. CRDC has approved NSW Agriculture research to examine the expression of Bt plants under different agronomic conditions. These experiments could be used for initial investigation into semiochemicals on Bt plants this season or for future semiochemical research on Bt plants.

The final area of suggestion emerging from this review is that semiochemical research to identify chemical compounds that can be used in behaviour manipulation and management of *Helicoverpa* spp. requires access to sophisticated analytical and synthetic chemical facilities and technical support, and the participation of highly skilled organic chemists. The Queensland Government has recently committed substantial financial resources to the support of the Combating Heliothis initiative. One aspect of this initiative is the formation of a Chemical Ecology Unit in Brisbane under Dr Chris Moore, of the Farming Systems Institute. A range of high-capital-cost analytical instruments is now available for dedication to this research. Further analytical chemical capacity will be required, and it is possible that NSW Agriculture's Analytical Laboratory at Wollongbar under Dr Ian Southwell will be able to provide complementary skills and instrumentation. A collaborative research agreement between these two State Departments of Agriculture can effectively undertake the semiochemical research in this area, though some supplementation of instrumental capacity, research personnel, and operating budgets is likely to be required.

For future research into semiochemicals to be successful and cost effective, appropriate co-ordination of research activities would seem essential. A collaborative arrangement

between NSW Agriculture and QDPI Chemical Ecology Unit, to establish a "Semiochemical Research Unit" would provide the needs to lead and co-ordinate future semiochemical research. As both organizations are core partners in the Australian Cotton CRC, the establishment of such a research unit would only strengthen the core activities of the CRC.

Recommendation 5: that the CRDC approve joint research collaboration between NSW Agriculture and QDPI's Chemical Ecology Unit in Brisbane in the establishment of a "Semiochemical Research Unit". This unit which would develop, lead and co-ordinate research into both long and short range chemical stimuli to identify behaviour modifying compounds from organ surfaces or inner tissues of plants to manage *Helicoverpa* spp.

8.0 Research Capability

NSW Agriculture, through the establishment of an Insect Management Team within the Department's Centre of Excellence for Cotton, Pulses and Oilseeds at the Australian Cotton Research Institute in Narrabri, has a wide range of specialist Entomologists and a network of extension staff undertaking research and extension into cotton pests ecology, dynamics, integrated pest management (IPM), Insecticide and Transgenic Cotton Resistance Management strategies, strip cropping and refugia strategies, food sprays, UV protected petroleum spray oils, biopesticides and Area Wide Management to manage cotton pests. These staff are all employed by NSW Agriculture, supervised by a Program Leader (Plant Fibres) based in Narrabri, ensuring effective collaboration between members, state organisations, universities, and CSIRO to achieve research outcome.

The Queensland Department of Primary Industries, through the project Combating *Heliothis* (*Helicoverpa*), offers close integration of three new R&D units, the Biopesticides Unit (BPU), the Chemical Ecology Unit (CEU) and the Novel Options Unit (NOU). These units operate in concert with an existing team of field entomologists and a network of extension staff who are researching and developing a wide-area approach to manage *Heliothis* at regional level and who are using modern extension methods (including action learning) to ensure understanding and adoption of complex technology and processes by producers.

The core strengths of these two organisations include a capacity for field assessment of behaviour modifying compounds such as stimulants, attractants, deterrents etc, biopesticides such as viruses, Bt, entomopathogenic fungus and other novel options such as food sprays, petroleum oils, neem extract etc. In addition, the two organisations bring to bear on this specific problem a suite of high-technology analytical instrumentation, including Gas Chromatography (GC), Gas Chromatography-Mass Spectrometry (GCMS) and automated thermal desorption equipment, together with the skills of an experienced organic chemists with considerable published research output in the area of insect chemical ecology. A Gas Chromatography with electroantennographic

detection will also be available in the University of Queensland to precisely target a variety of chemical compounds which stimulate *Helicoverpa* spp. antennae. This will facilitate the identification of attractants, the study of masking phenomena, and the investigation of repellence/deterrence phenomena.

9.0 Immediate and Essential Needs

Collaborative arrangements between QDPI Chemical Ecology Unit in Brisbane and NSW Agriculture Wollongbar Chemical Ecology Laboratories could identify compounds that modify the behaviour of *Helicoverpa* spp. moths and neonate caterpillars. Chemical studies will focus on original material shown to influence the behaviour of larvae on plants and/or the behaviour of moths in selecting/rejecting plants for feeding and egg laying. Such studies involve the fractionation of the biologically active material to make fractions progressively richer in active principles, until finally they are obtained pure. At this point the analysis of chemical structure can begin.

The process of refining and identifying active principles which are volatile or semi-volatile can be reasonably straightforward in the hands of an expert. Essential instrumentation (e.g. GC and GCMS) and skills appropriate to such studies already exist. On the other hand, plant-surface compounds which are sensed by contact (stimulants, deterrents) are likely to require alternative methods of refinement based upon liquid chromatography (LC) methodology, and identification often based upon Liquid Chromatography-Mass Spectrometry (LCMS). Other instrumentation of molecular structure analysis complementing the electron-impact mass spectrometry incorporated in the GCMS will need to be accessed (e.g. electrospray MS, NMR).

Immediate minimum needs include the essential liquid chromatography equipment (HPLC) and an additional organic chemist with skills in this technique and in LCMS structure determination. Sufficient funding to access services from external providers of LCMS and NMR analyses is also required, together with some supplementation of the existing operating budget for the Analytical Chemists.

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