

A Pheromone Manual

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The publication is an output from a research project funded by the United Kingdom Department for International Development (DFID) for the benefit of developing countries. However, DFID can accept no responsibility for any information provided or views expressed.

R8304 Crop Protection Research Programme DFID

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Cork, A. (2004). Pheromone Manual, Natural Resources Institute, Chatham Maritime ME4 4TB, UK.

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Abbreviations

ANOVA	Analysis of Variance
AWM	Area-Wide Management
BCPC	British Crop Protection Congress
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BWACT	Boll Weevil Attract and Control Tubes
CC	Catch Concentration
%CD	Percentage Communication Disruption
CPC	Cumulative Proportional Catch
CPP	Crop Protection Programme of the DFID
DAE	1,2-Dianilinoethane
DFID	UK, Department for International Development
ESA	Effective Sampling Area
ETL	Economic Threshold Limit
FFS	Farmer Field School
GC	Gas Chromatograph
ICRISAT	International Centre for Research Semi-Arid Tropics
IPM	Integrated Pest Management
IPMT	International Pheromone McPhail Trap
IS	Internal standard
LDPE	Low density polyethylene
LO	<i>Leucinodes orbonalis</i>
ND	Not detected
NRI	Natural Resources Institute, University of Greenwich, UK
NPV	Nucleopolyhydrosis virus
NWSW	New World Screwworm
OILB (IOBC)	International Organisation for Biological Control
PVA	Polyvinyl acetate
PVC	Polyvinyl chloride
R	Gas constant
RP	Reverse phase
SGM	Sweet Gourd Mash
SME	Small and Medium Enterprises
SSR	Seasonal Sampling Range
$t_{1/2}$	Half life
ZETA	(<i>Z,E</i>)-9,12-Tetradecadienyl acetate

Chapter 1: Introduction

From the time of their discovery in the late 19th century sex pheromones were recognised as potent agents for control of insect pests of economic importance. Nevertheless, it was almost a century later that the first Lepidopterous female sex pheromone, Bombykol, was chemically characterised by Butenandt and Hecker (1961). This led to an explosion of activity in the identification and synthesis of pheromones and the recognition that pheromones were only one class of a family of chemical signals known collectively as semiochemicals.

Many semiochemicals have subsequently been exploited for use in monitoring and control of insect populations. Control has been achieved by a range of techniques that result either in target pests being trapped and killed or confusing them so that they can not locate potential mates.

The utilisation of pheromones for control of insects in developing countries was recognised soon after Butenandt and Hecker's monumental work. This provided the impetus for the identification of pheromones of a number of major pests of economic importance in the mid-1970's. Notably, the female sex pheromones of the Egyptian leafworm, *Spodoptera littoralis* (Nesbitt *et al.*, 1973), pod borer, *Helicoverpa armigera* (Nesbitt *et al.*, 1979, 1980), potato tuberworm moth *Phthorimaea operculella* (Persoons *et al.*, 1976) and striped rice borer *Chilo suppressalis* (Nesbitt *et al.*, 1975).

This research endeavour persisted throughout the 1980's with the identification of numerous pheromones of pests of economic importance in South Asia (Cork & Hall 1995). During this same period considerable efforts were also made to develop ways of utilising these new tools to the benefit of farmers and ultimately consumers, the intention being to replace toxic synthetic insecticides with benign natural products, pheromones, for control of insect pests (Cork, *et al.*, 2003, Srivastava & Satpathy, 2001).

At the OILB Working Group meeting on the 'Use of Pheromones and other Semiochemicals in Integrated Control' at the Natural Resources Institute, UK, in 1993 Wightman and Rango Rao (1993) presented a paper on 'pheromone trapping in South Asia: Review and requirements'. This paper contained a table that summarised the status of 'pheromone activities' associated with a number of economically important insect pests in South Asia. The table was presented in a form that showed progress of research from the confirmation of a pheromone, identification and synthesis to use in monitoring, forecasting, regional network and control. On reviewing the contents of the list it became apparent that no significant progress had been made in the intervening 11 years since the list was compiled. The only exception being that of the pheromone of *Maruca vitrata*, where an effective pheromone system has been developed and used, but only in west Africa (Downham *et al.*, 2004).

More worryingly none of the pheromones listed were developed to the point where they had been shown to have an effect on control and only two, *Helicoverpa armigera*

and *Spodoptera litura*, were considered of sufficient value to be used for monitoring and applied in regional networks. Indeed Wightman and Ranga Rao (1993) stated that 'a *Helicoverpa* network was run from ICRISAT for over a decade at 30 locations'. This network provided extremely valuable information on the timing of moth emergence their movement and the influence of weather on populations. However, this information does not appear to have been fully exploited for the benefit of farmers.

Fortunately, the list presented by Wightman and Ranga Rao (1993) was by no means exhaustive and considerable advances have been made in the development of pheromone technologies since 1993 with other pest species.

Work conducted on the yellow stem borer, *Scirpophaga incertulas* was perhaps more successful leading to the development of a technology for controlling the pest by mating disruption (Cork & Basu, 1996; Cork *et al.*, 1996, 1998) and later by the more cost-effective method of mass trapping (Krishnaiah *et al.*, 2000; Katti *et al.*, 2001). Around the same time promising developments were observed with the pheromones of the early shoot borer, *Chilo infuscatellus*, and internode borer, *Chilo sacchariphagus indicus* in sugarcane (Narasimhan, 1995). Yet despite the optimism recorded in the proceedings of various workshops such as the 'Innovative Pest and Disease Management in Horticultural and Plantation Crops' workshop conducted in 2000 none of these pheromone products have been commercially developed into viable products that have been widely adopted by farmers in South Asia.

More recently considerable efforts have been made to develop a pheromone-based system for the control of the brinjal fruit and shoot borer, *Leucinodes orbonalis* (Cork *et al.*, 2002, 2003). This work has culminated in the development of a highly cost-effective mass trapping technology acceptable to farmers as part of an integrated pest management (IPM) package. The two pheromone components are stable, and relatively high molecular weight C₁₆ aliphatic carbon chain compounds that would have a long field life. The compounds are relatively simple to prepare because they each have a single carbon-carbon double bond, although they are commercially available on the international market in kg quantities at reasonable cost. Given the regional significance of brinjal in the diets of rich and poor alike and the extraordinary levels of insecticides being applied to control the pest in some locations (Rashid *et al.*, *in press*) there is every incentive for this technology to reach and be accepted by farmers across the region.

Survey of SMEs and the Pheromone Manual

The Crop Protection Programme (CPP), one of the implementing bodies of the UK, Department for International Development's Renewable Natural Resources Knowledge Strategy has been in the forefront of research to develop pheromone-based technologies for a wide range of economically important insect pests in South Asia. Much of the technology was developed in partnership with farmers on the assumption that the products would ultimately be commercialised to provide much-needed environmentally acceptable alternatives to currently used insecticides. However, despite much of the pioneering

fieldwork being undertaken in the 1980's and 90's the level of impact of these technologies at the farmer level has been lower than achieved in developed countries with similar needs.

There are undoubtedly many reasons for the lack of impact of pheromone products at the farmer level in South Asia. Nevertheless, the CPP took the view that small and medium enterprises (SMEs) involved in the production and commercialisation of pheromone products held the key to delivering these products into the marketplace. Unless and until pheromone products are made accessible in the marketplace they can never achieve widespread acceptance or adoption by farmers.

So why hadn't SMEs in South Asia succeeded in developing a large market base for these products? In order to understand the nature of the constraints that prevented large-scale adoption the CPP commissioned a short project entitled, 'Technical support for SMEs supplying pheromone-based pest control technologies in South Asia'. The project envisaged that constraints could be identified through a survey of SMEs. The survey was conducted in two parts, ten companies were visited by researchers and 30 more sent postal surveys.

The project was not only designed to identify constraints but also to provide solutions. Thus, results from the survey were expected to inform researchers of needs and it was anticipated that those needs would be addressed both through a workshop for SME's and the production of a 'pheromone manual'. The latter was to be designed to provide reference material for SME's, examples of technical and commercial success and entry points to further information.

This manual has been written with the express intention of providing commercial companies with information that will enable them to better understand the nature of the products that they are producing and how, through their own endeavours, they could improve both their efficacy and cost-effectiveness.

It is anticipated that by providing farmers with better quality products companies will assist the process of creating a sustainable market for the technology which will ultimately result in higher profits both for the farmers but importantly for the SME's who have invested time and effort to promote the technology.

It is assumed that most companies would not wish to synthesise pheromone components themselves and so the emphasis of the manual has been placed on formulation and use. However, to better understand why some formulations are more effective than others the influence of environmental parameters such as heat, sunlight, oxygen and the polymer material used to prepare lures are discussed. Finally, an extensive list of source material is provided so that issues raised can be probed in greater depth.

The full results of the survey will be published separately but the technical issues highlighted by the survey provided the framework for the manual.

The survey confirmed that the 'market' for pheromone products in South Asia was characterised by a small

number of products, sold in relatively high volume at low price, with the exception perhaps of attractants for palm weevils. The sale-price closely reflected the cost and availability of active material and not demand. The major products were pheromone trap systems for *S. litura* and *H. armigera*. Much of the production in India was driven by the need to satisfy state procurement quotas. A procedure that some SMEs believed focused on quantity at the expense of quality, with the result that farmers were said to receive inferior products that did not meet their needs.

When asked why the State procurement system required so many lures for *H. armigera* and *S. litura* the answers suggested that it was the relative economic importance of the insects and a desire to assist farmers rather than an understanding of the utility of the products that drove the intervention. Given the significance of these two products to producers it would have been anticipated that their utility would have been well understood. However, the absolute number of insects trapped by the pheromone was almost universally used as a measure of the success of a product and not whether that attribute translated into a useful management tool for farmers.

For example, some SME's surveyed suggested that the *S. litura* pheromone was more 'effective' than the *H. armigera* lure. This conclusion was based simply on the number of insects caught without reference to the relative size of populations of each species or, perhaps more importantly, the effect that removing adult males from a population had on crop damage. Nevertheless, the issue of the effectiveness of the *H. armigera* lure continues to dominate the thinking of researchers and SMEs alike. The recent assertion that *H. armigera* is pheromone polymorphic (Kumar and Shivakumara, 2003; Tamhankar *et al.*, 2003) looks set to complicate the issue still further. If there really are different populations of *H. armigera* and each responds to a different pheromone blend, as has been suggested for other species (Guerin *et al.*, 1984; Cork *et al.*, 1992; Picimbon *et al.*, 1997) production of lures will be complicated and farmer confusion exacerbated.

In order for SMEs to successfully promote technologies to farmers they need to fully understand what it is that they are selling. Only then will farmers appreciate the value of the products and utilise them in a way that enables them to benefit most from the technology.

A cursory look at the literature reveals that researchers too are obsessed with developing systems that maximise catch but rarely investigate the effect of trap catches on subsequent damage levels or whether the number of insects caught over time relates in any way to the population dynamics of the species. Where such studies have been conducted even fewer researchers have developed simple rules that will enable farmers to relate insect catch to 'action thresholds' that would enable farmers to more accurately time interventions.

Farmers need to understand whether the system they are using is meant to contribute to control of a pest or whether it is purely for use as a monitoring tool. If the latter is the case then farmers need to know how and where to place traps and what actions they should take on the basis of trap catch, these issues are addressed in Chapter 7 of the Pheromone Manual. Importantly, most successful

monitoring systems that have been developed involve the trapping of small numbers of insects. Farmers will not invest time to count large numbers of insects on a daily basis and traps that are easily saturated, such as sticky delta traps, are inefficient when they catch large numbers of insects.

Pheromones have been successfully adopted worldwide for use in control of insect pests. Chapters 8 and 9 provide examples of their utility in mass trapping and mating disruption respectively. Included in these chapters are discussions on attempts to utilise the pheromone of *H. armigera* and to some extent *S. litura* for control and the reasons why they have not been successful to-date.

Pheromones are by their nature composed of organic chemicals. In order to understand how these chemicals behave in the environment and in controlled release systems these issues are addressed in Chapters 4 and 5 respectively. Importantly physicochemical data is presented in Chapter 5 on the relative rates of release of typical pheromone components from standard septa under a range of temperatures. This data was published by McDonough *et al.* (1989) and will enable manufacturers to provide farmers with information on field longevity of lures at different times of the year. The efficacy of lures may well be dose dependent and research should always be undertaken to assess the relative attractiveness of lures as they age, i.e. changing release rates.

Trap design is of course important for the effectiveness of a pheromone monitoring or mass trapping system. The parameters that make one design more effective than another are poorly understood. Nevertheless, a review of the designs available, their uses, strengths and limitations is provided in Chapter 5.

For the curious, Chapter 2 includes a detailed summary of the definitions of semiochemicals. Chapters 10 and 11 provide sources for further reading and contact details of pheromone companies in South Asia and selected companies outside the region that can provide services to support pheromone producers, from bulk chemicals or pheromone blends to finished lures and trap systems.

Acknowledgement

The publication is an output from research project R8304 funded by the UK's Department for International Development (DFID). However, DFID can accept no responsibility for any information provided or views expressed.

Chapter 2: Semiochemicals

A compound or a specific group of compounds that can elicit a specific behavioural response from an organism are considered to be **semiochemicals** from the Greek *semeion* or sign (Law & Regnier, 1971). Semiochemicals were subsequently divided into two groups, pheromones and allelochemicals.

Pheromones were originally defined by Karlson and Lüscher (1959) as 'substances secreted to the outside by an individual and received by a second individual of the same species in which they release a specific reaction, for instance a definite behaviour or developmental process'. The word pheromone is derived from the Greek *pherein*, to transfer, and *hormōn*, to excite or stimulate. Pheromones are further divided into groups based on their mode of action.

Sex pheromones are individual compounds or specific blends of compounds that are produced and released by one gender of a species that initiate a sequence of behavioural activities which ultimately results in mating with another member of the same species. The female sex pheromones of Lepidoptera that can attract male conspecifics over long distances are perhaps the best known examples. Many thousands of moth sex pheromones have been identified (Arn *et al.*, 2000) but only a relatively small number have been successfully exploited for pest control purposes.

However, some Lepidoptera, notably the diurnal butterflies, are attracted to potential mates by visual cues although males often produce pheromones or aphrodisiacs when in close proximity to a potential mate that predispose them to copulate (Vane-Wright & Boppré, 1993). Other species such as Sunn pest, *Eurygaster integriceps*, a Heteropterous pest of wheat throughout much of Asia, is thought to produce a male sex pheromone. However, it is possibly the vibrational signals produced when males and females are in close proximity that enable the insects to establish the identity of species, sex and perhaps a readiness to copulate that ultimately leads to successful mating.

Aggregation pheromones are compounds or specific blends of compounds that are produced and released by one gender of a species that attract both sexes of the same species. The purpose of the attraction is to enable members of the same species to exploit a food resource or provide assistance with overcoming the defences of a potential food resource such as the resin produced by pine trees. Because they bring together both sexes of a species aggregation pheromones also assist the process of reproduction. Aggregation pheromones are typically produced by Coleopterous species and are particularly well known and exploited in pest management strategies for bark beetles in northern Europe and America and palm weevils in Asia and Central America.

Alarm pheromones are well defined in the social insects, notably members of the Hymenoptera and other insects that live communally such as the Aphididae and Thripidae.

Their function is to alert conspecifics of potential threats. In the case of aphids and thrips the pheromones result in a dispersion of conspecifics. In the case of ants, bees and termites the result may be to raise a colony's preparedness for defensive action against intruders.

To assist members of some social groups to locate food sources insects returning from successful foraging expeditions lay **trail pheromones**. More frequently associated with walking insects, such as ants, the trails are formed from very volatile compounds. This means that when a food source is exhausted unsuccessful returning insects do not re-enforce the message and the trail rapidly dissipates.

Host marking pheromones are designed to reduce competition between individuals. Parasitoids mark a host on which they have laid an egg thereby alerting conspecifics to the potential competition for resources should they also lay eggs on the host (Rosi *et al.*, 2001). Similarly, female Bruchidae deposit **epideictic pheromones** (Prokopy, 1981) before egg laying which deter conspecific females (Messina & Renwick, 1985) and result in evenly spaced oviposition that ensures the highest fitness for their progeny.

Allelochemicals are those semiochemicals produced by one species that elicit specific behavioural responses from members of another species and these are grouped according to whether the emitter or receiver benefits from perceiving and acting on the signal or message.

Nordlund (1981) defined **kairomones** as substances produced or acquired by an organism that, when it contacts an individual of another species in the natural context, evokes in the receiver a behavioural or physiological response that is adaptively favourable to the receiver, but not the emitter. Similarly, **allomones** are defined as substances that are favourable to the emitter alone and **synomones** are defined as beneficial to both the emitting and receiving species, though the benefits may not be equally shared.

Allelochemicals have been further grouped by Ruther *et al.* (2002) according to the function of the benefiting organism, by introducing terms such as *foraging kairomone*, *enemy avoidance kairomone*, *sexual kairomone* and *aggregation kairomone*. They further suggested defining groups according to the effect they elicited from the benefiting species leading to terms such as primer and releaser kairomones. Where **primer kairomones** are those which induce physiological responses in the benefiting organism whereas **releaser kairomones** elicit an immediate behavioural response from the benefiting organism such as the anemotactic up-wind flight of female mosquitoes elicited by host odours.

Foraging kairomones are used by the benefiting organism for the location of food for itself and/or its progeny. Examples, include chemical cues exploited by parasitoids, predators and most importantly disease vectors such as mosquitoes, *Glossina*, *Triatominae*, and agents of myiasis such as screwworms and *Lucilia* spp. .

Enemy avoidance kairomones are used by benefiting species to reduce the impact of natural enemies. Such kairomones have been identified in a wide range of

animals including mammals (Dicke & Grostal, 2001). For example several plant species are thought to respond to grazing by herbivores by releasing volatile compounds that attract the natural enemies of herbivores (Dicke, 1999).

Sexual kairomones are host odours that are used by the benefiting species to locate potential mates. For many species host-derived chemicals may synergise female produced sex pheromones. Thus, synthetic pheromone traps will often fail to trap male moths in fallow fields but are effective in fields containing host plants provided the plants are in a physiological stage that is conducive for oviposition. Similarly, male *Cochliomyia hominivorax* are attracted by host wound fluids (A. Cork & M. Hall unpublished) but were not been observed to land. The assumption is that they then patrol around wounded mammals for potential mates since the host odours are probably attractive to unmated naive as well as mated females.

The term **aggregation kairomones** was introduced by Loughrin *et al.* (1995) to describe the effect of plant volatiles that attract both sexes of the Japanese beetle, *Popillia japonica* Newman. Aggregation can benefit species by enabling them to exploit food resources, locate mates or defend a territory more effectively. Thus, aggregation could be more precisely defined by one of the other terms depending on the outcome of the aggregation.

Chapter 3: Pheromone chemistry

Chemical structure

Pheromones are composed of a wide range of organic compounds that are either volatile or involatile. Diptera, for example, utilise a wide range of contact pheromones that assist male flies identify the sex and species of other flies they encounter in flight, such compounds are essentially involatile. Nevertheless, almost all pheromones that have been commercially exploited are volatile in nature.

In order for insects and other terrestrial organisms to exploit volatile compounds for communication there is a limitation on the molecular weights they can utilise. Wilson & Bossert (1963) suggested that molecules containing between 5 and 20 carbon atoms (molecular weight 80 to 300) would represent the limitations of molecular diversity. Molecules above 300 were not sufficiently volatile, while molecules with less than 5 carbon atoms provided too small a molecular diversity. Similarly, sex pheromones might be expected to contain higher molecular weight compounds than alarm pheromones because the latter signal is needed to disperse quickly and the former is required to persist for longer time periods. Thus, alarm pheromones of ants have molecular weights of between 100 and 200 (Hölldobler & Wilson, 1990) and Lepidopterous sex pheromones have molecular weights typically between 200 and 300 (Arn *et al.*, 1997).

Lepidoptera utilise straight-chained aliphatic compounds derived from fatty acids. The compounds have typical chain lengths of between 10 and 23 carbon atoms. The most common compounds have 12 to 16 carbon atoms in length. A typical Lepidopterous sex pheromone will consist of two or three structurally related compounds often containing one oxygenated functional group. These can be terminal groups such as acetates, alcohols or aldehydes or they may be functional groups positioned at specific locations on the chain such as epoxides or ketones. Unsaturation is common in the sex pheromones of Lepidoptera usually comprising between one and three double bonds with specific positional and geometric stereochemistry.

Pheromones of taxonomically related species

Sex pheromones can act as powerful pre-mating isolating mechanisms for new species that are evolving. Thus, while males and females of closely related species could physically mate if coupled, differences in the chemical composition of the female sex pheromone and the males' response to that pheromone can act to ensure species isolation. This is particularly important where species are sympatric. Nevertheless, closely related species often utilise similar blends of chemical components in their pheromones. It is the way that the responding gender perceives and responds to the different blends of compounds that is crucial to the success of a new species. Thus, male moths will often only respond to a narrow range of pheromone blends related to that produced by the conspecific female moth.

In South Asia there are two related noctuid cotton bollworms, *Earias insulana* and *E. vittella*. The sex pheromone of *E. insulana* was studied using insects from Africa (Hall *et al.*, 1980) and Israel (Kehat *et al.*, 1981) and *E. vittella* using insects from Pakistan. (10*E*,12*E*)-10,12-Hexadecadienal (EE10,12-16:Ald) was isolated from *E. insulana* pheromone glands and found to be attractive to male moths at all three locations. There was also some evidence to suggest that addition of Z11-16:Ald increased catch. Pheromone gland extracts from female *E. vittella* were found to contain a 1 : 2 : 10 : 2 : 4 : 1 blend of 16:Ald, Z11-16:Ald, EE10,12-16:Ald, octadecanal (18:Ald), (Z)-11-octadecenal (Z11-18:Ald) and (10*E*,12*E*)-10,12-hexadecadien-1-ol (EE10,12-16:OH). Field tests in cotton fields in Pakistan showed that a 2 : 10 : 2 mixture of Z11-16:Ald, EE10,12-16:Ald and Z11-18:Ald was as attractive to male *E. vittella* as the 6-component mixture and equal in attractiveness to a virgin female. However, omitting Z11-16:Ald or Z11-18:Ald significantly reduced this attractiveness (Cork *et al.*, 1988).

The above data would suggest that the difference in the pheromone compositions between *E. insulana* and *E. vittella* was due to a single compound Z11-18:Ald since this was the only compound found in the pheromone of *E. vittella* and not *E. insulana*. Nevertheless, this was not the case. In early field work conducted in Pakistan Derek Campion (personal communication) demonstrated that traps baited with the pheromone of *E. vittella* only remained attractive provided the lures were removed from the field during daylight hours. It had been known for some time that the main pheromone component EE10,12-16:Ald was very susceptible to isomerisation in sunlight (Chapter 4) and would eventually form a racemic mixture of the ZE, EZ, ZZ and EE-isomers in a 15 : 15 : 5 : 65 blend of compounds.

By adding known amounts of each of the isomers of 10,12-16:Ald to a 3-component blend of EE10,12-16:Ald, Z11-16:Ald and Z11-18:Ald and protecting the pheromone lures from sunlight by covering them with aluminium foil it was possible to demonstrate that the attractiveness of the lures to male *E. vittella* was significantly reduced by addition of (10*E*,12*Z*)-10,12-hexadecadienal (EZ10,12-16:Ald). However, addition of the latter compound also increased the attractiveness of the lure to male *E. insulana*. Indeed during the course of this work a 10 : 1 blend of EE10,12-16:Ald and Z11-16:Ald was shown to be as attractive to male *E. insulana* as a virgin female, and the attractiveness of this mixture was further increased by addition of EZ10,12-16:Ald.

The sex pheromones of closely related Lepidoptera that feed on the same plant might be expected to be chemically very different. This was found to be the case with the pheromones of *Chilo* spp. that attacked different stages of sugarcane, *C. auricilius* (Nesbitt *et al.*, 1986), *C. infuscatellus* (Wu *et al.*, 1984) and *C. sacchariphagus indicus* (Nesbitt *et al.*, 1980; David *et al.*, 1985) (Table 3.1). The sex pheromones of *C. partellus* (Nesbitt *et al.*, 1979) and *C. suppressalis* (Nesbitt *et al.*, 1975; Ohta *et al.*, 1976; Tatsuki *et al.*, 1983; Mochida *et al.*, 1984) are chemically similar but they are found primarily on different host plants, maize and rice respectively. Interestingly Z11-16:OH was identified in the sex pheromone glands of the related rice striped stalk borer,

C. suppressalis but found to be inhibitory to male moths (Tatsuki *et al.*, 1983).

A 1 : 3 blend of Z9-16:Ald and Z11-16:Ald is commonly used in South Asia for attracting the yellow rice stem borer, *Scirpophaga incertulas* but (Z)-11-hexadecen-1-ol was found to reduce trap catch of male *S. incertulas* and (Z)-9-octadecenal has not apparent effect on catch (Cork *et*

compounds in pheromone gland extracts and effluvia collected from calling female moths. These were tentatively identified as Z9-16:Ald, Z11-16:Ald and (Z)-11-Octadecenal. However, in field trials the hexadecenal isomers were found to reduce trap catch, even when added at the 0.1% level, while Z11-18:Ald alone was as attractive

Table 3.1 Pheromones identified from *Chilo* spp.

Common name	Species name	Pheromone	Ratio
Sugarcane stalk borer	<i>Chilo auricilius</i>	(Z)-7-Dodecenyl acetate	4
		(Z)-8-Tridecenyl acetate	8
		(Z)-9-Tetradecenyl acetate	4
		(Z)-10-Pentadecenyl acetate	1
Early shoot borer	<i>Chilo infuscatellus</i>	(Z)-11-Hexadecen-1-ol	
Internode borer	<i>Chilo sacchariphagus indicus</i>	(Z)-13-Octadecenyl acetate	4.9
		(Z)-13-Octadecen-1-ol	0.45
Striped stalk borer	<i>Chilo suppressalis</i>	Hexadecanal	26
		(Z)-9-Hexadecenal	5
		(Z)-11-Hexadecenal	48
		(Z)-11-Hexadecen-1-ol	5
		Octadecan-1-ol	1
		(Z)-13-Octadecenal	6
Maize stalk borer	<i>Chilo partellus</i>	(Z)-11-Hexadecenal	20
		(Z)-11-Hexadecen-1-ol	3

¹Compounds written in *italics* were found to reduce male moth catches

al., 1985). However, in many areas of Southeast Asia *S. suppressalis* and *S. incertulas* occur in almost equal numbers on rice. The pheromone blend commonly used for *S. suppressalis* is a 1 : 10 : 1 blend of Z9-16:Ald, Z11- 16:Ald and (Z)-13-octadecenal (Z13-18:Ald) and it is only the presence of Z13-18:Ald that deters attraction of male *S. incertulas*. Should there be a need to develop an attractant that could catch male moths of both species no doubt a blend of Z9-16:Ald and Z11-16:Ald would suffice, but this would need to be optimised in field trials.

The white rice stem borer, *S. innotata*, is a pest of rice in central and South East Asia. Beevor *et al.* (1994) identified three electrophysiologically-active

as virgin female moths. While the chemical composition of the sex pheromone of *S. innotata* is superficially similar to that of *S. incertulas* and *C. suppressalis* it is again the response of male moths to the octadecenal isomer that provides the basis for the pre-mating isolation mechanism.

Aliphatic unsaturated alcohols

As mentioned earlier alcohol analogues of aldehydic components of pheromones are often good inhibitors of male attraction, but not always as in the case of *Chilo sacchariphagus indicus* where it is needed for attracting male moths. Pheromone components are often stored in pheromone glands as

Table 3.2 Pheromones identified from *Scirpophaga* spp.

Common name	Species name	Pheromone ¹	Ratio
Top shoot borer	<i>Scirpophaga excerptalis</i>	E11-16:Ald	78
		Z11-16:Ald	22
Yellow stem borer	<i>Scirpophaga incertulas</i>	Hexadecanal	10
		(Z)-9-Hexadecenal	15
		(Z)-11-Hexadecenal	60
		(Z)-11-Hexadecen-1-ol	10
		(Z)-9-Octadecenal	6
White rice borer	<i>Scirpophaga innotata</i>	(Z)-9-Hexadecenal	trace
		(Z)-11-Hexadecenal	trace
		(Z)-11-Octadecenal	1

¹Compounds written in *italics* were found to reduce male moth catches

alcohols and in the case of *Heliothis* spp. Teal *et al.* (1986) established that the final oxidative step to form the aldehyde is undertaken on the cuticular surface of the gland. In the case of the sugarcane stalk borer, *Chilo auricilius* the pheromone glands were found to contain four electrophysiologically-active compounds (*Z*)-7-dodecenyl acetate (Z7-12:Ac), (*Z*)-8-tridecenyl acetate Z8-13:Ac, (*Z*)-9-tetradecenyl acetate (Z9-14:Ac) and (*Z*)-10-pentadecenyl acetate (Z10-15:Ac) in a 2 : 8 : 4 : 1 ratio. However, blends tested that contained Z7-12:Ac were found to catch fewer male moths than those without suggesting the compound was inhibitory (Nesbitt *et al.*, 1986). The biological role of Z7-12:Ac in the sex pheromone of *C. auricilius* has not been determined.

The sex pheromone of *H. armigera* contains the same compounds used to attract male *S. incertulas*, Z9-16:Ald and Z11-16:Ald, but in a 3 : 97 ratio. In order to achieve species isolation the pheromones of related Heliothine are chemically distinct from that of *H. armigera*. Thus, in Table 3.3 are listed the range of compounds that have been identified in species of Heliothine to-date. The majority have Z11-16:Ald as the major component except for *H. assulta* in which Z9-16:Ald is the major component (Cork *et al.*, 1992) and *H. gelotopoeon* where Z11-16:Ald was not identified at all and

indeed when added to a synthetic pheromone lure resulted in a reduced trap catch (Cork & Lobos, 2003).

Saturated aliphatic compounds

The role of saturated compounds in Lepidopterous sex pheromones is not well understood. Hexadecanal (16:Ald) was found to significantly increase trap catch of *H. gelotopoeon* baited with Z9-16:Ald and Arn *et al.* (1985) demonstrated that dodecan-1-ol enhanced the attractiveness of synthetic blends of the pheromone of codling moth, *Cydia pomonella*. An observation subsequently confirmed in wind tunnel studies conducted by Witzgall *et al.* (2001). In contrast, 16:Ald was not found to affect the trap catches of the cotton caterpillar, *Diaphania indica* when added at the natural 2% level of the major pheromone component (*E*)-11-hexadecenal (Wakamura *et al.*, 1998). Similarly, 16:Ald and related saturated aliphatic compounds were not found to evoke behavioural responses in studies conducted on the cotton leaf roller, *Notarcha derogata* (Himeno & Honda, 1992), fulvous clover moth, *Heliothis maritima* (Szocs *et al.*, 1993), pandora moth, *Coloradia pandora pandora* (McElfresh, *et al.* 2000) and buck moth, *Hemileuca maia* (McElfresh, *et al.*, 2001).

Table 3.3 Chemicals identified in ovipositor washings of *Helicoverpa* and *Heliothis* species

Compound	Species ^a									
	a	b	c	D	e	f	g	h	i	j
14:Ald	0	0	0	0.8	0	0		0	2	0
Z9-14:Ald	0	0	0	14.6	0	0		0	3.9	0
Z9-14:OH	0	0	0	6.5	0	0		0	0	0
Z9-14:Ac	0	0	0	2	0	0		0	0	0
16:Ald	49	353	100	3.7	8.2	9		18	11.7	4.8
Z7-16:Ald	0	0	0	1.1	0	tr		0	1.6	1.2
Z9-16:Ald	7	1333	100	1.5	0.5	tr		66	1.2	18.4
Z11-16:Ald	100	100	0	100	100	100		100	100	100
Z9-16:OH	0	22.7	0	0	0	0		48	0	0
Z11-16:OH	21	tr	0	24.3	7.2	75		40.6	3.9	0
16:Ac	0	53.7	0	0	0	0		0	0	0
Z7-16:Ac	0	0	0	0	0	0		5.5	0	0
Z9-16:Ac	0	421.3	0	0	0	0		14.3	0	0
Z11-16:Ac	0	18.4	0	4.8	4.8	50		41	0	0

^a	Species	Origin	Reference
a	<i>Helicoverpa armigera</i>	Malawi	Nesbitt <i>et al.</i> , 1979, 1980
b	<i>Helicoverpa assulta</i>	Korea	Cork <i>et al.</i> , 1992
c	<i>Helicoverpa gelotopoeon</i>	Argentina	Cork & Lobos, 2003
d	<i>Heliothis peltigera</i>	Israel	Dunkelblum & Kehat, 1989
e	<i>Heliothis phloxiphaga</i>	United States	Raina <i>et al.</i> , 1986
f	<i>Heliothis punctiger</i>	Australia	Rothschild <i>et al.</i> , 1982
g	<i>Heliothis maritima</i>	Hungary	Szocs, <i>et al.</i> , 1993
h	<i>Heliothis subflexa</i>	United States	Teal <i>et al.</i> , 1981
i	<i>Heliothis virescens</i>	United States	Klun <i>et al.</i> , 1980a
j	<i>Heliothis zea</i>	United States	Klun <i>et al.</i> , 1980b

Adapted from Cork *et al.*, 1992

Geographical variation

While not all the chemicals isolated from female gland extracts necessarily contribute significantly to the attraction of male moths, as determined by field trapping experiments they might still be considered to be components of the sex pheromone. In the case of *H. assulta* nine compounds were identified (Table 3) (Cork *et al.*, 1992) and of these only two, Z9-16:Ald and Z11-16:Ald, were required to achieve a high level of attraction as measured by trap catches. The function, if any, of the other compounds identified has yet to be established.

The best attractant blend for *H. assulta* was found to vary with the location of the trials. A 7.5 : 1 blend of Z9-16:Ald and Z11-16:Ald was found to be the most attractive in Thailand while a 20 : 1 blend of the same compounds was most attractive in Korea. In China both blends were equally attractive. This data was interpreted as suggesting that there were at least two populations of *H. assulta*. Whether these populations represent sub-species or should be classed as different species was not clarified.

There have been a number of studies that have identified variation in the pheromone blend produced by species of Lepidoptera in different regions of the world. Perhaps one of the earliest was conducted by NRI on *Spodoptera littoralis* (Campion *et al.*, 1980) where variation in the pheromone composition and response of male moths was observed on different islands (Cyprus and Crete) and the Mediterranean mainland (Egypt). The most comprehensive study was conducted on *Agrotis segetum*. Toth *et al.* (1992) studied male responses at 11 sites in Europe, Asia and Africa. They found that variation was more or less continuous in Eurasia and North Africa while a clearly distinct pheromone type was present in areas south of the Sahara desert.

A similar situation was found with the white rice stem borer, *Maliarpha separatella*. Working in West Africa Cork *et al.* (1991) showed that three compounds were present in pheromone gland extracts, Z9-14:OH, ZE9,12-14:OH and EE10,12-14:OH. Field trials established that the monoene, Z9-14:OH reduced trap catches but that ZE9,12-14:OH and EE10,12-14:OH in ratios from 9 : 1 to 39 : 1 were attractive. In contrast Drs H. Arn and P. Guerin working with insects from Madagascar showed that the pheromone was composed of four compounds, Z9-14:OH, ZE9,12-14:OH and the related aldehydes, Z9-14:Ald and ZE9-12-14:Ald. This blend was not attractive in West Africa but a 5 : 1 blend of ZE9,12-14:Ald and ZE9,12-14:OH was attractive in Madagascar (H. Arn, pers. comm.). Insects were provided to the Natural History Museum, UK and based on taxonomic characters the insects were reclassified as two sub-species renamed *Maliarpha separatella separatella* and *Maliarpha separatella vectiferella* for insects from Madagascar and West Africa respectively (Cook, 1997). It later transpired that the sub-species from Madagascar was present in East and parts of West Africa and that the sub-species found in Sierra Leone was quite restricted in its range. Such information is particularly important where IPM strategies are being developed for control of these insect pests since there may well be significant differences in the way the two species respond to management options for example the use of biopesticides for control.

More recently Kawazu *et al.* (2000) examined the female sex pheromone of the Japanese rice leafroller moth, *Cnaphalocrocis medinalis* Guenée (Lepidoptera: Pyralidae). They identified (Z)-11-octadecenal, (Z)-13-octadecenal, (Z)-11-octadecen-1-ol and (Z)-13-octadecen-1-ol at a ratio of 11 : 100 : 24 : 36 from ovipositor extracts of virgin females. Field bioassays in Kagoshima, Japan, showed that the two aldehydes were essential for male attraction and the alcohols may have a synergistic effect on the aldehydes. However, the four component blend was quite different from the sex pheromone components reported previously for the same species of either Philippine or Indian origin where the components were shown to be (Z)-11-hexadecenyl acetate and (Z)-13-octadecenyl acetate at a ratio of 98 : 2 and 1 : 10 respectively (Ganeswara Rao *et al.*, 1994). Furthermore, in field tests in Japan, neither the Philippine blend nor the Indian blend showed any attractiveness, while the Japanese blend attracted significant numbers of male moths. These results suggest either that there are remarkable geographical variations in the sex pheromone composition of this species or there are several morphologically similar species in the region that utilise different sex pheromone blends.

Consequences of blend variation for commercialisation of pheromones

The fact that not all compounds identified in pheromone glands are components of pheromones or indeed play no measurable role in the behaviour elicited by synthetic pheromones is a complication for commercial companies because they can not then decide which blend to incorporate into their lures. For many companies in India buying pre-mixed blends of pheromones with a guaranteed composition, such as those supplied by Margo Biocontrol Ltd. has solved that problem. When lures prepared from these blends are efficacious then even if the blend composition is not fully disclosed both lure producer and end-user are nevertheless satisfied with performance. However, where 'apparent' problems have arisen with the performance of attractants in the field, as has been observed with pheromone lures for *H. armigera* and *Diaphania indicus* then the company formulating and distributing the lures has no means of resolving the problem. Under such circumstances there is a need for the company or researchers to undertake appropriate laboratory and field studies to determine the best pheromone blend for use under local field conditions.

We have seen above that there can be geographical variation in pheromone blends or species thought to be present may well have been mis-identified. In either event it is often preferable to check the pheromone composition of the indigenous moths to ensure that commercial blends are suitable for field use in the locality it is marketed. Commercial companies and academics rarely undertake such activities, primarily because of the cost and uncertainty of obtaining a satisfactory outcome from such a study. Nevertheless, a significant amount has already been undertaken and researchers are well advised to study earlier works on the subject. Piccardi *et al.* (1977) identified the major component of the female sex pheromone of *H. armigera* as Z11-16:Ald but in subsequent publications by Nesbitt *et al.* (1979, 1980) a further four compounds were described, Z11-16:OH, Z9-16:Ald, 16:Ald and 16:OH based on analysis of insect

material from Malawi, Sudan and India. Importantly the ratio of Z9-16:Ald to Z11-16:Ald was not found to be fixed but the amount of Z9-16:Ald varied from 1.7 to 7.9% of Z11-16:Ald with an average of 3%. However, the authors do not record whether the ratio of the two compounds varied more within or between the countries of origin. Indeed recent work by Kumar *et al.* (2003) suggested that there is variation in the response of male *H. armigera* to pheromone blends in different regions of India. Given the mobility of the species (King *et al.*, 1990; Armes and Cooter, 1991) this would appear to be an unexpected result (see Chapter 7 for more detailed discussion). It remains to be seen whether the wide range of ratios of Z9-16:Ald to Z11-16:Ald produced by female moths and that male *H. armigera* are attracted to are the result of natural plasticity in the population or whether there are well defined populations of the species that produce and respond to different blends of the pheromone.

It is important that researchers and commercial companies jointly develop with end-users research programmes that identify where problems exist and how best to tackle them. Ideally such initiatives should be conducted with the active participation of statutory authorities who are in a position to provide funding and guidance to ensure that the work is correctly focused and end results provide meaningful recommendations for pheromone companies that will ultimately benefit farmers.

Chapter 4

Pheromone stability

Influence of chemical structure on stability of pheromone components

Many organic chemicals are quite stable if kept in an appropriate environment as evidenced by oil and gas deposits that are millions of years old. Nevertheless, the vast majority of organic compounds have the capability to react with either themselves or other chemicals to form new compounds or rearrange into more stable structures. The nature of the chemical transformation that occurs is dependent on the structure of the compounds present, the influence of energy sources, such as light and heat, catalysts and reactive species such as oxygen. Sex pheromone components are composed of specific blends of organic chemicals and their relative stability is dependent on the number and type of functional groups each compound contains and the medium in which they are stored. A **functional group** is formed by replacement of hydrogen or addition of another atom, molecule or change in carbon-carbon bond structure in a straight-chained hydrocarbon.

Saturated straight-chained hydrocarbons are the most stable organic compounds with no functional groups. Addition of oxygen (oxidation) to one of the terminal carbon atoms (-CH₃) to form either a primary alcohol (-CH₂OH) or aldehydic (-CH=O) functional group is common in Lepidopterous sex pheromones. Aldehydes are readily oxidised in air to the more stable acid (-CO₂H). Primary alcohols are more stable than aldehydes although they can react with aldehydes to form hemi-acetals, in the presence of traces of acid, and with acids to form esters.

Most Lepidopterous sex pheromone components contain unsaturated, straight chain aliphatic compounds. **Unsaturation** results from the removal of two or four hydrogen atoms from adjacent carbon atoms to form either carbon-carbon double (alkenes) or triple bonds (alkynes) respectively. Alkenes can exist in two structural forms or geometrical **isomers**, *cis* (*Z*) or *trans* (*E*) depending on the configuration of the substituents around the double bonds. Alkenes are relatively stable and are not easily changed (a process known as **isomerisation**) between the *Z* and *E* isomers once formed.

However, Lepidopterous pheromone components often contain two or more carbon-carbon double bonds. When two double bonds are conjugated, that is present on adjacent carbon atoms (e.g. 10,12-hexadecadiene), the compounds, conjugated **dienes**, can exist in four isomeric forms, *ZE*, *EZ*, *ZZ* and *EE*. Conjugated dienes are susceptible to isomerisation and oxidation. Isomerisation can be induced by sunlight, as process known as **photoisomerisation**. Thus, if a hexane solution of a compound containing a conjugated diene functionality is placed in a glass tube in sunlight and a trace of iodine added as a catalyst the compound will isomerise to form the thermodynamically most stable mixture which is a 15 : 15 : 5 : 65 blend of *ZE*, *EZ*, *ZZ*, *EE* isomers (Ideses & Shani, 1988). Oxidation of conjugated dienes occurs *via*

the allylic hydroperoxide to the corresponding furan (Brown and McDonough, 1986; Shani *et al.*, 1981) (see antioxidants below).

Conjugated dienes are inherently more unstable than non-conjugated dienes that in turn as chemically stable as monoenes. Isomers such as the 3,13-octadecadienyl acetate and the corresponding alcohols commonly found in the female sex pheromones of the clearwing moths, Sesiidae (Arn *et al.*, 2000) as relatively stable compounds. However, methylene interrupted dienes, such as components of the sex pheromones of species of *Spodoptera*, notably the African armyworm, *Spodoptera exempta*, (*Z,E*)-9,12-tetradecadienyl acetate (Cork *et al.*, 1989) are a special case. The methylene group (CH₂) between the two carbon-carbon double bonds is particularly prone to oxidation *via* allylic hydroperoxides (Gunstone, 1984).

The reactivity of Lepidopterous sex pheromone components has been a major challenge for synthesis and formulation chemists to overcome. Synthesis chemists need to produce the compounds in a pure enough form so that they are suitable for use in synthetic pheromone blends. On the other hand formulation chemists have to blend the chemicals and incorporate them into controlled release formulations that can release the pheromone at an appropriate rate over a predetermined time interval while maintaining their structural integrity often under intense natural sunlight and high temperatures.

Antioxidants

Oxidation of dienes and aldehydes are undesirable reactions that have to be controlled if pheromone components are to be protected in controlled release formulations. **Autoxidation** is a chain process initiated by radicals such as iron and copper. This process can be controlled by antioxidants. However, many of the synergists added to antioxidants such as citric, ascorbic, phosphoric and ethylene diamine-tetraacetic acid can enhance the efficiency of metal ions by forming chelates with them and so should be avoided. Antioxidants are frequently phenolic compounds of natural (tocopherol) or synthetic origin, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate and tertiary butylhydroquinone. BHT is most often used nowadays in a 1 : 1 ratio with the pheromone. It should be remembered that many antioxidants are also volatile and are sometimes released faster than the pheromone depending on the formulation employed.

Photooxygenation requires singlet oxygen and this is formed from normal triplet oxygen by interaction with light in the presence of a sensitizer such as chlorophyll, rose bengal or methylene blue. Oxidation of a carbon-carbon double bond then occurs by an ene reaction in which the singlet oxygen adds to an olefinic carbon atom and the double bond migrates one position. This reaction is unaffected by antioxidants but can be stopped by 'singlet oxygen quenchers' such as carotene. Photooxygenation is much faster than autoxidation and Gunstone (1984) points out that autoxidation could be initiated by hydroperoxides formed by photooxygenation promoted by traces of

pigments. Thus, care should be taken when selecting the colour pigments used for rubber of synthetic polymer controlled release dispensers.

Photo-isomerisation of conjugated diene aldehydes

From earlier work we have seen that radicals produced by the action of sunlight on such molecules as iodine and thiophenol can promote isomerisation. Antioxidants such as BHT and BHA have no influence on isomerisation of conjugated double bond systems. In cases where the other diene isomers have no effect on trap catch (Campion *et al.*, 1980) isomerisation is not a problem, and will at worst simply reduce the amount of active compound present. Nevertheless, this is not always the case and the isomers formed can be inhibitory to male moths in small quantities. Thus, in work conducted in Pakistan with the spotted bollworm, *Earias vittella*, the main pheromone component a conjugated diene aldehyde EE10,12-16:Ald was found to be susceptible to isomerisation in rubber septa under the influence of strong sunlight (Cork *et al.*, 1988). At least one of the isomers was inhibitory to male *E. vittella* although the same compound appeared to be attractive to the related male *E. insulana* and it was postulated that the compound EZ10,12-16:Ald acted as a key to speciation between the sympatric species. In this instance isomerisation was terminated by covering the lures with aluminium foil.

The major component of the sex pheromone of the cabbage head-borer, *Hellula undalis* was identified by Aari *et al.* (1982) as (11E,13E)-11,13-hexadecadienal (EE11,13-16:Ald) a closely related chemical to the main component of the pheromones of *Earias* spp. EE10,12-16:Ald. However, the compound is chemically unstable and in order to develop an effective controlled release formulation to enable it to be used as a synthetic bait for *H. undalis* there is a need to protect it from oxidation, trimerisation and photo-isomerisation induced by sunlight (Dunkelblum *et al.*, 1984). Oxidation was controlled by the addition of an antioxidant, BHT and trimerisation is not usually a problem once the compound has been incorporated into the matrix of a controlled release formulation. However, isomerisation of conjugated dienes is more difficult to control.

A starting sample of EE11,13-16:Ald was assayed by GC and GC-MS and found to contain 98.9% of the EE isomer with trace amounts of the ZE and EZ isomers (Table 20). If allowed to isomerise in sunlight the thermodynamically most stable mixture would be produced containing a 15 : 15 : 5 : 65 ratio of ZE : EZ : ZZ : EE isomers respectively. By assaying lures for the quantity of each isomer over time it is possible to provide a measure of the degree of protection offered by different techniques.

Lures prepared with rubber septa were variously protected in the field by a trap with foil, a trap without foil, no trap with foil and no trap without foil. The trial was conducted for 10 days with the lures collected daily from the field and stored at -20°C before dispatch to NRI for analysis of total 11,13-16:Ald content and isomeric composition.

In the absence of protection from sunlight the isomeric composition of the 11,13-16:Ald changed significantly with the EE isomer accounting for only 68.8% of the total compound (Table 4.1). It was anticipated that by protecting the lures inside the pheromone trap or more

effectively with aluminium foil the level of isomerisation would reduce to a negligible level. However, even with the maximum level of protection, inside the trap and with aluminium foil the EE isomer only accounted for 88.7% of the compound remaining. Surprisingly the Day 1 sample gave almost the same result as the Day 10 sample suggesting that the level of isomerisation was negligible during the 10 day period when the lures were in the field. This result indicates that isomerisation had occurred prior to the samples being placed in the field. Lures containing EE11,13-16:Ald were not found to be attractive even on the first night. This result could be explained by the high level of isomerisation observed in new lures, or it might be due to other compounds identified in the female sex pheromone glands (Cork and Sivapragasam, unpublished) being important for attraction of male moths.

Lures were prepared at NRI in rubber septa and polyethylene vials then exposed at room temperature (20°C) in the laboratory for different periods of time. Collected and stored at -20°C before solvent extraction and analysis by GC. The initial solution was assayed as having a mixture of ZE and EE isomers in a 2.5 : 97.5 ratio. However, even in the time it took for the solvent to evaporate from preparing the rubber septa the ratio of isomers of 11,13-16:Ald had changed to a 3.7 : 6.2 : 0 : 90.1 mixture of the ZE : EZ : ZZ : EE isomers respectively (Table 4.2). On further exposure at room temperature in rubber septa there was a gradual change in the ratio of isomers which appeared to favour the EZ isomers resulting in a 5.8 : 18.5 : 0 : 75.7 ratio of the ZE : EZ : ZZ : EE isomers (Table 4.2). This result confirmed that even before the lures had reached their field destination the 11,13-16:Ald had isomerised and if one or more of the unnatural isomers was repellent or inhibitory to the male moths then the lures would have lost their attractiveness before being bioassayed.

Isomerisation was not found to occur in the polyethylene vials where the initial ratio of approximately 2.1 : 2.1 : 0 : 95.8 of the ZE : EZ : ZZ : EE isomers respectively was maintained throughout the period of exposure. However, because this mixture of isomers was different from the starting blend it must be assumed that some isomerisation had occurred during formulation of the dispenser.

The finding that a conjugated diene aldehyde is isomerised in a standard natural rubber septa is of significance for a wide range of Lepidopterous pheromones. The ability to arrest the isomerisation by using polyethylene vials augers well for the future development of a stabilised controlled release system for such pheromone components. While the unnatural isomers of EE11,13-16:Ald apparently inhibited attraction of male moths this could be exploited for controlling the moth by use in mating disruption. Given that *H. undalis* is present in relatively low population densities the value of such a technique for control could be considerable.

Table 4.1 Change in composition of isomers of 11,13-16:Ald impregnated on rubber septa and exposed to different levels of sunlight

Foil & Trap¹										
Date	Day	11,13-16:Ald (mg)					11,13-16:Ald (Percentage)			
		ZE	EZ	ZZ	EE	Total	ZE	EZ	ZZ	EE
03-Mar	1	0.020	0.022	0.029	0.644	0.715	2.76	3.01	4.10	90.13
04-Mar	2	0.021	0.075	0.044	0.585	0.725	2.96	10.33	6.01	80.70
05-Mar	3	0.020	0.018	0.024	0.588	0.651	3.15	2.83	3.62	90.40
06-Mar	6	0.028	0.021	0.022	0.557	0.628	4.46	3.37	3.54	88.63
09-Mar	8	0.021	0.020	0.024	0.506	0.571	3.59	3.46	4.28	88.67
11-Mar	9	0.019	0.017	0.019	0.485	0.540	3.48	3.14	3.56	89.82
12-Mar	10	0.023	0.018	0.019	0.470	0.530	4.35	3.40	3.59	88.66
Foil, no trap										
Date	Day	11,13-16:Ald (mg)					11,13-16:Ald (Percentage)			
		ZE	EZ	ZZ	EE	Total	ZE	EZ	ZZ	EE
03-Mar	1	0.025	0.021	0.028	0.612	0.686	3.58	3.07	4.03	89.32
04-Mar	2	0.020	0.021	0.023	0.548	0.613	3.30	3.45	3.83	89.42
05-Mar	3	0.021	0.020	0.025	0.572	0.637	3.24	3.07	3.90	89.79
06-Mar	6	0.021	0.019	0.021	0.504	0.565	3.70	3.39	3.71	89.19
09-Mar	8	0.022	0.022	0.023	0.474	0.541	4.13	4.05	4.21	87.61
11-Mar	9	0.020	0.021	0.023	0.429	0.493	4.12	4.35	4.61	86.92
12-Mar	10	0.020	0.020	0.021	0.364	0.425	4.68	4.70	4.97	85.65
Trap, no foil										
Date	Day	11,13-16:Ald (mg)					11,13-16:Ald (Percentage)			
		ZE	EZ	ZZ	EE	Total	ZE	EZ	ZZ	EE
03-Mar	1	0.020	0.018	0.023	0.594	0.654	3.10	2.69	3.46	90.75
04-Mar	2	0.020	0.023	0.026	0.587	0.655	3.05	3.52	3.91	89.53
05-Mar	3	0.022	0.024	0.026	0.572	0.644	3.38	3.72	4.07	88.83
06-Mar	6	0.020	0.025	0.027	0.485	0.557	3.53	4.47	4.81	87.18
09-Mar	8	0.022	0.028	0.031	0.460	0.540	4.00	5.27	5.65	85.08
11-Mar	9	0.016	0.029	0.031	0.385	0.461	3.53	6.28	6.72	83.46
12-Mar	10	0.020	0.023	0.025	0.404	0.472	4.26	4.87	5.26	85.61
No foil, no trap										
Date	Day	11,13-16:Ald (mg)					11,13-16:Ald (Percentage)			
		ZE	EZ	ZZ	EE	Total	ZE	EZ	ZZ	EE
03-Mar	1	0.019	0.018	0.024	0.640	0.701	2.71	2.63	3.38	91.27
04-Mar	2	0.018	0.035	0.036	0.459	0.547	3.31	6.31	6.55	83.83
05-Mar	3	0.020	0.035	0.035	0.389	0.479	4.27	7.31	7.27	81.15
06-Mar	6	0.018	0.036	0.035	0.303	0.392	4.55	9.29	8.89	77.27
09-Mar	8	0.022	0.038	0.034	0.211	0.306	7.29	12.42	11.12	69.17
11-Mar	9	0.022	0.032	0.029	0.183	0.267	8.37	12.03	10.82	68.79
12-Mar	10	0.020	0.036	0.033	0.196	0.285	7.12	12.61	11.50	68.77
Starting compound assayed by GC-MS							0.43	0.58	0	98.99
Starting compound assayed by GC							0.36	0.56	0.23	98.85

¹ Lure protected from direct sunlight by combination of aluminium foil and/or white plastic delta trap.

Table 4.2 Change in composition of isomers of 11,13-16:Ald impregnated into controlled release formulations stored at room temperature (20°C) in a laboratory.

Dispenser	Days exposed	Rep	11,13-16:Ald isomers			
			ZE	EZ	ZZ	EE
Solution	0	1	2.51	0.00	0.00	97.49
		2	2.43	0.00	0.00	97.57
Rubber septa	0	1	3.32	5.78	0.00	90.90
		2	4.01	6.64	0.00	89.35
	1	1	4.23	7.42	0.00	88.34
		2	4.42	10.35	0.00	85.23
	3	1	5.66	12.05	0.00	82.29
		2	4.20	10.34	0.00	85.46
	8	1	5.48	19.38	0.00	75.15
		2	6.13	17.67	0.00	76.20
Polyethylene vials	0	1	2.47	2.73	0.00	94.79
		2	1.52	2.82	0.00	95.66
	1	1	2.31	2.50	0.00	95.18
		2	1.89	1.82	0.00	96.29
	2	1	2.16	2.19	0.00	95.65
		2	2.34	2.31	0.00	95.35
	6	1	2.23	2.12	0.00	95.65
		2	2.02	2.02	0.00	95.96

Reaction of pheromone components with chemicals in the controlled release formulation

Most formulations used for controlled release of pheromones, particularly in developing countries, utilise synthetic polymers that were intended for other uses. Polymers based on natural rubber often contain compounds that could react with pheromone components. Steck *et al.* (1979) found that pheromone blends containing aldehydes made from some batches of red rubber septa either failed to catch insects or caught the wrong species. They were able to demonstrate that extracting the septa with hot alcohol solved the problem and that adding the extract back to 'good' lures resulted in a loss of activity. Chemical analysis of the active fraction obtained after passing the extract through a chromatographic column showed that 1,2-dianilinoethane (DAE) (Wanzlick & Löchel, 1953) was present. DAE is known for its fast reaction with aldehydes. DAE was thought to have been added to the rubber as an antiozonant. Solvent extraction of polymers is not

recommended before use, because of cost, but it is important to ensure that the polymers do not adversely react with the pheromone. Ideally polymers should be sourced from a reputable company where the chemical composition is well defined and any problems with incompatibility of polymer and pheromone can be quickly resolved.

Storage of chemicals

Given that Lepidopterous pheromone components, in common with most organic chemicals are susceptible to chemical change or degradation, as shown above, the method of storage is crucial for ensuring a long 'shelf life'.

Ideally chemicals should be stored at low temperature (-20°C) in sealed glass vessels under nitrogen. Plastic containers should be avoided although aluminium canisters are becoming increasingly common. It should be remembered that glass is inherently acidic in nature and can contain traces of metals. For particularly labile (unstable) compounds the glass should be silylated or at

A. Cork

least washed with base and thoroughly rinsed and dried afterwards.

Acetates are more stable than alcohols, which in turn are more stable than aldehydes. We have seen above that conjugated dienes are more stable than monoenes. Conjugated diene aldehydes, such as the major component of the pheromone of *Earias* spp., EE10,12-16:Ald is particularly unstable. Even in storage at low temperature EE10,12-16:Ald can polymerise to a trimer. The trimer is very difficult to identify because it is chemically similar to the parent compound. This compound is best stored as a dilute solution in a neutral solvent such as hexane. Ideally it should be prepared and stored as the corresponding alcohol and converted to the aldehyde as required, pretty much as female *Helicoverpa* do with their pheromone compounds, where the aldehydes are formed by enzymatic oxidation near the cuticular surface of the pheromone gland prior to release (Teal & Tumlinson 1986).

The extent to which a sample of EE10,12-16:Ald has trimerised or indeed any compound has degraded can be assessed by gas chromatographic analysis. In the case of



Figure 4.1 Storage of pheromone samples in sealed brown glass bottles at -20°C ensures chemical integrity of pheromone active ingredients is maintained. Courtesy of A.G. Bio Systems Pvt Ltd.

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EE10,12-16:Ald a comparison of the peak area of a sample of the compound should be compared with that of a known quantity of an appropriate standard such as hexadecanal.

Lepidopterous pheromone components and blends are conveniently stored for long periods as standard solutions in hydrocarbon solvents. Ideally they should be prepared with an equivalent weight of BHT added. Such solutions can be stored between -20°C and $+5^{\circ}\text{C}$.

It is important to remember that if a particular volume of solution is required for a job then the solution should be removed from the cold store and allowed to warm up to room temperature before use because solutions reduce in volume when cooled. Hexane solutions are ideal for dosing dispensers such as rubber septa or polyethylene vials because of their relatively low boiling point. Chlorinated solvents should be avoided because they form hydrochloric acid and phosgene over time which can catalyse decomposition of pheromone compounds. However, if chlorinated solvents can not be avoided they should be freshly distilled before use.



Figure 4.2 Quantitative GC analysis of compounds ensures high quality products. Note filters to purify carrier gases. Courtesy of SunAgro Pvt Ltd.

Chapter 5: Controlled release

In order to release pheromones into the environment in a manner that approximates to the natural situation there is a need for a device that can store, protect and moderate release. In the simplest form these carriers can be sealed bags such as **polyethylene sachets** used for releasing very volatile compounds at high rates (Torr *et al.*, 1997). Thicker walled **polyethylene vials**, hollow cylinders with pressure sealed caps, have been successfully adapted for controlled release dispensers of less volatile compounds associated with Lepidopterous sex pheromones. However, the most commonly used polymer for controlled release of Lepidopterous sex pheromone is the natural **rubber septum** (Reolofs *et al.*, 1972). In this case the pheromone is absorbed through the surface of the substrate and the dispenser is not sealed. In South Asia the septa is commonly replaced by rubber tubing that is cut into pieces to give the required loading after adding the pheromone as a solution into the tube.

All these formulations require the pheromone to be added to the pre-formed polymer however some dispensers are prepared by mixing the compounds with a pre-polymer and then curing the mixture to entrap the pheromone in the polymer, as has been achieved with polyvinyl chloride (**PVC**) and polyvinyl acetate (**PVA**) based monolithic polymers (Cork *et al.*, 1989). In order to moderate release rates some monolithic polymers have been prepared as sandwiches, such as the **plastic laminate** dispenser (Bierl *et al.*, 1976) where release is thought to be essentially restricted to the exposed edges of the central polymer.

Polyethylene sachets

Sachets are prepared from lay-flat plastic tubing and are characterised by a zero order release rate. This means that release is independent of loading. A characteristic that is particularly useful for maintaining release rates over long time periods. Release rate however can be moderated in a controlled manner by changing the thickness of the polyethylene walls and the surface area of the sachet. Release rate is also affected by temperature change in a predictable manner although this parameter can not necessarily be controlled in the field.

Release rates of individual compounds can be determined by loading the sachet with chemical and measuring weight loss over time. The high release rates from sachets mean that changes in weight are measurable using a weighing machine with an accuracy of 0.1 mg.

Release rates of individual compounds vary both as a function of molecular weight but also with functionality. Polyethylene is non-polar by nature and so apolar compounds release more quickly through the substrate than polar compounds.

Sachets are widely used for tsetse kairomones where the release characteristics are well defined (Torr *et al.*, 1997).

Polyethylene vials

Polyethylene vials as described earlier are thick walled polyethylene containers manufactured from low density polyethylene with a typical wall thickness of 1.5 mm, 8 mm diameter and 25 mm length. The pheromone is dispensed into the vial in typically 0.1 ml of hexane and the solvent allowed to evaporate off before the cap is closed under hand pressure. Release is assumed to be through the polyethylene walls. However, with very polar compounds, such as aliphatic carboxylic acids, release may well be through the gap between the cap and the vial wall because when the cap and wall are heat-sealed release is essentially stopped (A. Cork and D. I. Farman, unpublished data).

Polyethylene vials exhibit similar first order release rate characteristics to rubber septa (see below) although typical Lepidopterous sex pheromone components release more quickly from polyethylene vials than they do from rubber septa. This is particularly important during the winter months where temperatures can drop to 0°C at night in northern India. Under such circumstances the release of aliphatic compounds with carbon chain lengths of C₁₆ or greater will be negligible. Figures 5.1a and 5.1b show the relative residence times of E11-16:Ac and E11-16:OH, components of the brinjal fruit and shoot borer, *Leucinodes orbonalis* pheromone (Attygalle *et al.*, 1988; Cork *et al.*, 2001; Zhu *et al.*, 1987) in white rubber septa and polyethylene vials in a wind tunnel (27°C, wind speed 5 km/h) with an initial loading of 100 µg per compound. Where residence times represent the amount of pheromone not released per unit time. Assuming that degradation in the formulation is negligible then the pheromone lost to the dispenser is assumed to have been released. Such data can be produced by aerial entrainment of pheromone from dispensers onto solid absorbents.

The data shown in Figures 5.1a and 5.1b clearly demonstrate that the alcohol, E11-16:OH is released more quickly than the acetate, E11-16:Ac meaning that the ratio of alcohol to acetate will be higher 1 : 100 blend added to the polymer in the first instance but as the lure ages so the ratio will change until the alcohol is completely exhausted. Under the conditions in the wind tunnel the half lives of the alcohol and acetate in the polyethylene vial were approximately 22 and 52 days respectively, thus giving a field life of approximately one month for the blend. In contrast the rubber septa 76 and 92% of the pheromone remained after 40 days exposure. In related studies white rubber septa loaded with 100, 300 and 1000 µg of pheromone had 86, 89 and approximately 100% remaining after 90 days exposure in West Bengal during the winter months (Cork *et al.*, 2001). Under such conditions trap catches were negligible and polyethylene vials offer a useful alternative. However, in more tropical conditions rubber septa may be more appropriate to use although McDonough *et al.* (1989) calculated the half lives of hexadecenyl acetates of 2,900 and 200 days between 15 and 35°C (Table 5.1).

The field longevity of polyethylene vials containing the pheromone of *L. orbonalis* was determined in Bangladesh in January 2003 by placing lures either in the shade or in direct sunlight, where the former simulated conditions in pheromone traps (Cork *et al.* 2001). The results (Figures 5.2a and 5.2b) confirmed that the major

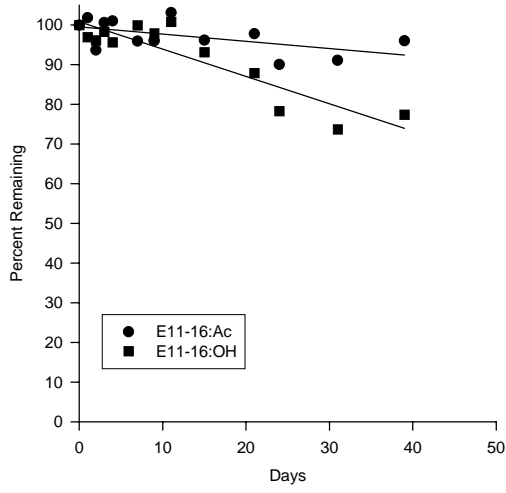


Figure 5.1a Residence times of *L. orbonalis* pheromone from white rubber septa in a wind tunnel at 27°C.

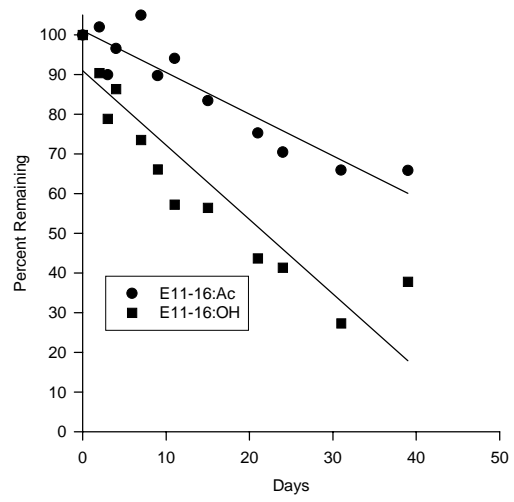


Figure 5.1b Residence times of *L. orbonalis* pheromone from polyethylene vials in a wind tunnel at 27°C.

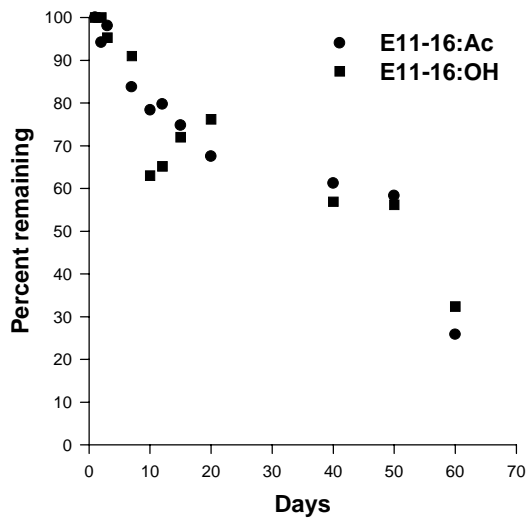


Figure 5.2a Release of *L. orbonalis* pheromone in direct sunlight

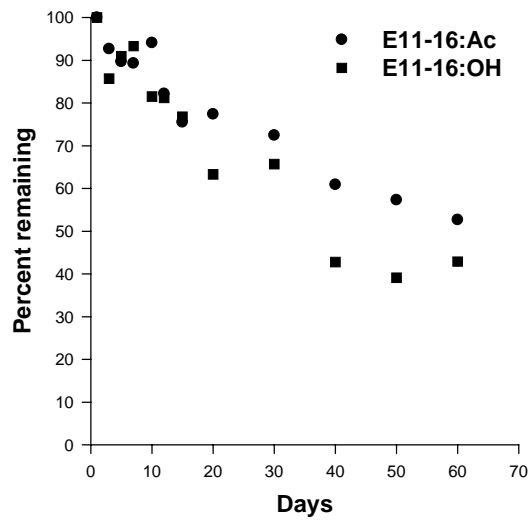


Figure 5.2b Release of *L. orbonalis* pheromone in shade

component, E11-16:Ac had half lives of 42 and in excess of 60 days under exposed and shaded conditions and that the minor component, E11-16:OH released at approximately the same rate of release. The latter result was unexpected since earlier data had suggested it would release at a higher rate although earlier data was generated under controlled laboratory conditions at a higher temperature.

Rubber septa

Compounds are usually applied to rubber septa as dilute solutions in hexane (typically 0.1 ml of a 10mg/ml solution) into the 'cup' of the septum. The solvent is then allowed to evaporate off at room temperature ideally in a fume hood or under a carefully controlled stream of nitrogen. It is important that the volume of solvent used is not less than 100 μ l otherwise there is insufficient to ensure that the pheromone penetrates and is retained below the surface of the rubber. If 50 μ l or less solvent are used considerable amounts of the pheromone are left on the surface of the septum and this results in a high release rate on the first day of field exposure (Golub *et al.*, 1983; McDonough, 1991).

The release rates of pheromones from septa have been determined for a number of pheromones notably pink bollworm, *Pectinophora gossypiella*, (Flint *et al.*, 1978), pea moth *Cydia nigricana* (Greenway *et al.*, 1981), *L. orbonalis* (Cork *et al.*, 2001).

Release rates of a range of Lepidopterous sex pheromone components with acetate and primary alcohol functional groups, as mentioned earlier, was published by McDonough *et al.* (1989). They collected compounds released from lures onto an adsorbent at a fixed temperature and analysed the amount recovered in a unit of time to determine release rates. The release rates from septa are first order, which means that release rate is dependent on the amount remaining. The half life, $t_{1/2}$, of lures (the time it takes half of a compound to release from a controlled release formulation at a given temperature) can be calculated from the equation 1.

$$\text{Equation 1} \quad t_{1/2} = \frac{P \ln 2}{R}$$

Where P is the amount of pheromone remaining after collection, R is the amount of pheromone released in the time of the experiment (rate of evaporation). In each case, septa were aged for up to 3 days to ensure that residues on the surface of the septa were removed before testing release rates. Both pheromone released from the septa and collected on the adsorbent (RP-18 column, Sep Pak liquid chromatographic cartridge, Waters Associates) and residual pheromone in the lure were collected and analysed.

McDonough *et al.* (1989) eluted the RP-18 cartridge with a dichloromethane-methanol (9 : 1) mixture (20 ml) in portions and forced the solvent through the cartridge under pressure. They then added

heptane (6 ml) and concentrated down the resulting solution to approximately 5 ml using a rotary evaporator. The extract was analysed by GC to quantify the amount of pheromone present. Residual pheromone remaining in the septa was extracted with dichloromethane (50 ml) for 4 h and then re-extracted overnight with a further aliquot of dichloromethane (50 ml). The extracts were combined and 30 ml of heptane added. The combined extract was concentrated down to approximately 25 ml with a rotary evaporator ready for analysis by gas chromatography.

In our experience extraction with dichloromethane overnight, at room temperature, is sufficient to ensure complete extraction of pheromone. Ideally the dichloromethane should be made up as a solution containing an **internal standard** (IS) (e.g. 0.2 mg/ml of tetradecyl acetate (14:Ac)) and a septum extracted with 5 ml of the solution (1 mg of IS). The quantity of pheromone can then be determined by comparing peak areas with that of the IS. As the ratio of the two peak areas is independent of the quantity of sample analysed there is no need to record the volume of sample analysed.

For example:

If the peak area of the standard is 100 that is equivalent to 1 mg (5 x 0.2 mg) of 14:Ac. If the peak area of the pheromone component is 15 then the quantity of compound present in the septa was ((1*15)/100) = 0.15 mg. This assumes that the response factor for the GC detector is the same for both compounds. **Response factors** can be determined by comparing the peak areas of GC peaks for known quantities of standards and test compounds. Notably alcohols often give peak areas that are 90% those of the related acetate for a given weight of compound. Thus, if an acetate was used as an IS for analysing an alcohol then the quantity of alcohol actually present will be 10% greater than identified by the comparison with the IS.

McDonough *et al.* (1989) estimated the release rates of a number of *n*-alkyl and *n*-alkenyl acetates that are utilised by Lepidoptera as components of their sex pheromones, these are given in Table 5.1. The rates of release (loss by evaporation) were found to be correlated closely with Equation 2.

$$\text{Equation 2} \quad t_{1/2} = \frac{\Delta H}{RT} + \gamma_o$$

Where ΔH is the heat of evaporation, R is the gas constant, T absolute temperature and γ_o is a constant. The data clearly show that temperature and molecular weight (which is related to the rate of evaporation) have a profound and quantifiable effect on release rate. Thus, for a given temperature decreasing the molecular weight by one methylene group (CH₂) almost triples the release rate. While increasing the temperature by 5°C can almost double release rate. The monoene acetates had shorter half-lives than the corresponding saturated compounds and changes in the position of the double bond also affected release rate although

these differences were small compared to the effect of temperature (Table 4.1).

Where the molecular weight and structure of compounds in a pheromone are similar, such as blends of Z9-16:Ald and Z11-16:Ald used for *H. armigera* (Nesbitt *et al.*, 1980) and *Scirpophaga incertulas* (Cork *et al.*, 1985) the release rates of each compound will be essentially constant and hence the ratio of compounds released will remain a function of that applied to the dispenser. However, where a pheromone is composed of a blend of compounds with significantly different molecular weights such as the pheromone of *Chilo auricilius* that is composed of a 8 : 4 : 1 blend of Z8-13:Ac, Z9-14:Ac and Z10-15:Ac (Nesbitt, *et al.*, 1986) the relative ratio of compounds released will change over time as the more volatile compounds become disproportionately depleted due to the faster release from a septum. Nevertheless, the work by McDonough *et al.* (1989) and others has demonstrated that this process is predictable. Thus by assessing the range of ratio's over which insects such as male *C. auricilius* respond, the initial blend could be modified to produce a lure that maintains its attractiveness in the field for the longest period of time.

McDonough (1991) warns against the determination of release rates from rubber septa for aldehydes because 'aldehydes are not entirely chemically stable in rubber septa, half-lives for evaporative loss can not be determined by the residue method'. This

suggests that while loss is measured much of that loss is due to degradation and not evaporation. Thus, the use of a residual method of assessing release will inevitably produce an over estimate of release. McDonough (1991) goes on to suggest that the only acceptable means of assessing release of aldehydes from septa is to collect the material that evaporates from the surface. However, here again it is important to ensure that the type of absorbent used for collection does not degrade the compounds released. For example, activated charcoal is a common absorbent but this substrate can degrade aldehydes and recovery of alcohols is particularly poor. McDonough (1991) ascribes the longer half-lives for aldehydes published by Heath *et al.* (1986) to their use of activated charcoal as an absorbent.

The half-lives of aldehydes were found to be very similar to those of acetates containing two fewer methylene groups. Thus, the half-lives of 14:Ac and 16:Ald at 30°C were 84.2 and 86.9 days respectively. This result was to be expected because the molecular weights and polarity of the compounds are similar which would result in the compounds having similar ΔH values. Thus, data determined for acetates e.g. Table 5.1 can be utilised to determine the approximate release rates of aldehydes from rubber septa.

Table 5.1. Half-lives of *n*-alkyl and *n*-alkenyl acetates released from rubber septa at various temperatures

Compound	$t_{1/2}$ (days)				
	15°C	20°C	25°C	30°C	35°C
10:Ac	7.96	4.91	3.07	1.96	1.26
11:Ac	22.6	13.4	8.14	5.01	3.13
12:Ac	64.0	36.8	21.5	12.8	7.78
13:Ac	181	101	57.0	32.9	19.3
14:Ac	515	276	151	84.2	47.9
15:Ac	1,460	755	399	216	119
16:Ac	4,140	2,070	1,060	553	295
Z7-12:Ac	44.9	26.1	15.5	9.33	5.71
Z9-14:Ac	361	196	108	61.2	35.2
Z11-16:Ac	2,900	1,470	760	402	217
Z7-10:Ac		4.44		1.78	
Z9-12:Ac	57.7	33.3	19.6	11.7	7.11
Z11-14:Ac	464	249	137	76.7	43.8
Z13-16:Ac	3,730	1,870	960	504	270

Table 5.2 Half-lives of *n*-alkyl and *n*-alkenyl aldehydes released from rubber septa at 20 and 30°C

Compound	<i>t</i> _{1/2} (days)		Compound	<i>t</i> _{1/2} (days)	
	20°C	30°C		20°C	30°C
12:Ald	4.42	1.73	Z7-14:Ald	27.6	9.71
13:Ald	12.5	4.61	Z9-16:Ald	222	68.8
14:Ald	35.2	12.3	Z11-18:Ald	1,784	488
15:Ald	101	32.7	Z9-14:Ald	28.8	ND
16:Ald	285	86.9	Z11-16:Ald	231	ND
17:Ald	808	232	Z13-18:Ald	1,854	ND
18:Ald	2,289	616			

¹ Data from McDonough 1991. ND Not determined

Table 5.3 Half-lives of *n*-alkyl and *n*-alkenyl primary alcohols released from rubber septa at 20 and 30°C¹

Compound	<i>t</i> _{1/2} (days)		Compound	<i>t</i> _{1/2} (days)	
	20°C	30°C		20°C	30°C
12:OH	14.8	ND	Z7-12:OH	13.5	ND
13: OH	ND	ND	Z11-14:OH	117	ND
14: OH	130	ND	Z11-16:OH	432	ND
15: OH	269	ND			
16: OH	399	ND			
17: OH	1117	ND			
18: OH	609	ND			

¹ Data from Butler and McDonough 1981. ND Not determined

The half-lives of long-chained aliphatic alcohols (Table 5.3) were on average half those of the corresponding acetates (Table 5.1). Thus, at 20°C the half-lives of 14:Ac and 14:OH were 276 and 130 days respectively. A result that is similar to the release rates of acetates and alcohols from polyethylene vials (Figure 5.1b) although the overall rates of release were faster from polyethylene vials than septa. Surprisingly, 18:OH released much quicker than 16:OH. Butler and McDonough (1981) attribute this effect to the inability of the 18:OH to penetrate the cross-linked lattice of the rubber septa. Thus, the release rate reflects release from the surface of the septa rather than from inside the polymer matrix.

Packaging

Apart from storage of pheromones and their components before formulation it is particularly important for SMEs to know the effective shelf life of packaged products. There is very little data on this subject in the literature, although it is assumed that companies have their own arrangements for addressing such issues. At a recent workshop held in Bangalore for SME's Dr M C Sharma (2004) suggested that lures used in India were packaged in LDPE, paper or trilaminated aluminium foil sachets and in subsequent discussions the aluminium sachet was recommended as a standard. In addition it was also recommended that lures were individually

packaged. Nevertheless, manufacturers probably need to add additional quantities of pheromone to ensure that by the time it is sold the quantity of pheromone remaining is within tolerance levels for their product.

Preliminary work conducted by A. Cork and D. I. Farman (unpublished) suggests that standard *H. armigera* lures sealed in trilaminated aluminium sachets do lose active ingredient depending on storage time and crucially temperature (Table 5.4). At -20°C the loss of pheromone over a six month period was negligible but at 'room' temperature the lures lost 33.8% of the pheromone. Research was not undertaken to determine the fate of the pheromone lost but nevertheless the end-user would receive lures that had already lost a third of the active ingredient.

Table 5.4 Effect of temperature on residual *Helicoverpa armigera* in rubber septa lures stored in metalised sachets for six months

Temperature	Storage unit	Pheromone (mg)
-20°C	Freezer	1.98
+5°C	Fridge	1.75
+20°C	Room	1.31

Chapter 6: Trap Design

Good trap design is central to the effective utilisation of pheromone-based systems for monitoring and control of insect pests. An effective pheromone lure can attract responsive adult insects to the vicinity of a trap but entry and retention of insects is dependent on the trap design. Over the years numerous trap designs have been developed and each has advantages and disadvantages over others for catching members of different insect families, working in different environments and meeting the needs of different end-users. Thus, like so many technical fixes trap design is inevitably a compromise between the need to develop the most efficient trap and traps that are most appropriate for use in a particular market place.

Catching insects in traps is characterised by two elements, entry and retention. The latter can be divided into capture and killing. Ideally traps should provide the easiest entry for target insects and cause the minimum disturbance to the odour plume. Trap designs that do not affect plume structure irrespective of wind direction are known as omni-directional traps.

Once insects have entered the trap they need to be captured and killed. In order for them to be retained there is often a second chamber built into the trap in which entry is easy but escape more difficult. Nevertheless, escape is always possible to some degree from a second chamber and so a killing mechanism is needed. Killing insects can either be passive or active. The use of poisons such as insecticides or mineral oils and water-surfactant mixtures are active, the latter resulting in the insects being asphyxiated. Trap designs such as sleeve traps are passive because insects die through heat stress and dehydration resulting from them being denied access to food and water.

The need for a second chamber in a trap design reduces efficiency because the insect must cross two barriers. Crop pest insects that primarily locate semiochemical sources by flight are normally reluctant to enter traps because they represent unnatural conditions for them. If the semiochemical bait is sufficiently attractive then the 'trap entry response' is overcome and the insect enters the trap. This is the reason why unbaited traps very rarely catch moths, even if they contain a source of water, although there are exceptions such as *Ephestia* spp. which has been shown to be attracted to water alone (Ryne *et al.*, 2002) and irrigating cotton fields has been observed to result in increased catches of *Spodoptera littoralis* in pheromone baited traps (A. Cork unpublished).

Traps that contain sticky surfaces to retain insects overcome the need for a second chamber and can be

as simple as a flat disc. The ability of the 'sticky material' to hold insects is central to their efficiency and the subject of much research by commercial companies. Specific adhesives are available for different 'types' of insect. Thus, adhesives for Diptera are different from those for Lepidoptera mainly because scales surround the tarsi of Lepidoptera but Diptera do not have scales on their bodies. Such adhesives need to be resistant to sunlight, non-setting and water repellent. 'Sticky traps' are most effective where populations are low because they become 'saturated' with insects at high population pressures unlike 'non-saturating' traps that are more effective at high population pressures.

In a laboratory study conducted by Sanders (1986) with eastern spruce budworm, *Choristoneura fumiferana* the efficiency of a range of traps was assessed in a wind tunnel by measuring the time of first entry, percentage of insects entering a trap and percentage of total moths retained. In each case 50 insects were tested per trap. The results demonstrated that the highest entry rates were attained with traps that provided unrestricted entry, omni-directional traps, with up to 90% of insects entering within 5 min. and the highest levels of retention were attained with 'sticky' traps and traps with funnel shaped baffles that prevented escape.

Ideally trap design experiments should be undertaken in controlled conditions where the behaviour of the insects can be observed, such as wind tunnels (Quartey & Coaker, 1992; Foster & Muggleston, 1993) but nevertheless simple manipulation of trap design can be successfully addressed in field experiments.

In one such field experiment Cork *et al.* (2001) concluded that with low population densities sticky traps were more efficient than funnel traps for trapping *L. orbonalis* (Table 6.1) and that increasing the area for entry increased trap efficiency of the sticky trap (delta to open-delta traps).

A similar result was obtained with *S. incertulas* where an open-delta trap was more efficient than the sleeve or standard delta trap in low density populations. Similar results were obtained with the white rice stem borer, *Maliarpha separatella separatella* in West Africa where catches with delta and sticky disc traps were not significantly different (Table 6.3) (Cork *et al.*, 1991).

In contrast the delta trap was found to be ineffective compared to a sleeve trap for catches of the African armyworm, *Spodoptera exempta* (Table 6.4) although catches with an open sticky disc trap were not significantly different from those of the sleeve trap.

Table 6.1 Effect of trap design on catch of male *L. orbonalis*

	Trap design	Pheromone trap catches		
		Mean catch/trap/night	(±) SE	Newman - Keuls ³
November 2000 ¹	Delta	0.89	0.21	b
	Wing	0.82	0.143	b
	Uni-	0.08	0.036	a
	Spodoptera	0.03	0.019	a
June 2001 ²	Delta	0.44	0.50	a
	Open-Delta	2.34	0.32	b
	Water	0.60	0.42	a
	Funnel	0.52	0.04	a

¹ Jessore District, 3 replicates, 24 nights.

² Jessore District, 5 replicates, 10 nights

³ Means followed by the same letter in a group are not significantly different $P < 0.01$ by Newman-Keuls multiple range test on $\log(x + 1)$ transformed data.

Table 6.2 Optimisation of pheromone trap design for *S. incertulas*¹

Trap design	Pheromone dose (μg)	Mean catch per trap per night ²	S.E.
Delta	1,000	0.41	± 0.16 ab
Sleeve	1,000	0.72	± 0.17 ab
Sticky plate	1,000	0.85	± 0.28 ab
Open-delta	1,000	1.15	± 0.29 b
Funnel - Plastic cylinder	1,000	0.47	± 0.11 ab
Plastic pot	1,000	0.22	± 0.07 a

¹ Doulatpur, Laksam, Comilla District, T. Aman 2001, 4 Replicates, 42 nights.

² Means followed by the same letter in a group are not significantly different $P < 0.05$ by Newman-Keuls multiple range test on $\log(x + 1)$ transformed data.

Table 6.3 Effect of trap design on catches of *M. s. separatella*

Trap design	Total catch/treatment ¹	DMRT ²
Bottle (water)	1	b
Delta	56	a
Sticky disc	29	a

¹ Total trap catches; 4 replicates, 12 nights

² Means followed by the same letter are not significantly different at the 5% level by Duncan's Multiple Choice Test using $\log(x + 1)$ transformed data.

Table 6.4 Effect of trap design on catches of *S. exempta*

Trap design	Mean catch/trap/night ¹	DMRT ²
Page (water)	5.7	b
Delta	1.3	c
Funnel	37.7	a
Sticky disc	26.4	a

¹ Actual mean catches/trap/night;

² Means followed by the same letter are not significantly different at the 5% level by Duncan's Multiple Range Test using $\log(x + 1)$ transformed data.

Table 6.5 Effect of size of sticky disc on catch of male *L. orbonalis*

Radius (cm)	Ratio of Catch/radius	Pheromone trap catches ¹		
		Mean catch/trap/night	(±) Standard Error	Newman - Keuls ²
2.5	0.00	0.00	0	a
5	0.09	0.44	0.058	b
10	0.07	0.72	0.085	c
20	0.09	1.72	0.10	d

¹ Jessore District, 5 replicates, 10 nights.

² Means followed by the same letter in a group are not significantly different
 $P < 0.01$ by Newman-Keuls multiple range test on $\log(x + 1)$ transformed data.

Removing the entry barrier completely from a delta trap would result in an open sticky plate where trap efficiency would be determined only by the ability of the adhesive to retain insects and might be expected to be dependent on the area of the plate. This concept was tested using a range of sticky discs with different diameters (Table 6.5)

The results showed that increasing the area of the trap resulted in a higher catch. However, the trap catch was found to be proportional to the radius and not the area of the trap over the range of sizes tested. This can be interpreted as suggesting that the longer an insect is in the proximity of a sticky surface, i.e. when it is flying upwind to an attractant the higher the chance of it being caught. Rothschild and Minks (1977) came to a similar conclusion in their study on male oriental fruit moth, *Cydia molesta* Busck (Lepidoptera: Tortricidae) where traps with larger surface areas resulted in higher trap catches. Foster and Muggleston (1993) studied the influence of trap design on catch of light-brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) and found that field catch of male *E. postvittana* was linearly dependent upon the length of a delta trap. In their case the length of the delta trap was equivalent to the diameter of the sticky disc trap.

When comparing trap designs trap catch data should be collected at regular intervals. The interval between sampling depends in part on the size of trap catches. Saturating trap designs in particular need to be monitored regularly. In Table 6.6 the cumulative trap catch in delta traps is shown in a trial comparing different pheromone blends. On day 5 there are clear differences between the total number of moths caught by each lure. However, by day 19 LO99/16 and 17 have caught essentially the same number of moths. From Day 24 to 34 trap catch hardly increases suggesting that the traps were essentially full or saturated.

Trap saturation is only one of a range of factors that affect trap efficiency. We will see later that trap position, height of trap, trap spacing and age of lure all affect the performance of individual traps.

For all field trials whether they are comparing pheromone blends, doses, dispensers or trap designs each treatment

should be replicated (a minimum of three times) and trap positions should be changed so that each treatment occupies each trap site an equal number of times and over as short a time period as possible. This would ensure that the insect population sampled remained relatively constant for the duration of the trial. Ideally traps should be moved daily to randomise their positions.

Adult populations of moths such as *L. orbonalis* remain relatively constant for long time periods while others, such as rice stem borers and armyworms, have distinct population peaks associated with the emergence or migration of successive generations. Where lures have only finite field longevity it is impractical to place the traps in fields during periods when adults are unavailable. Under such conditions the trial can be laid out without lures. Separate traps can then be installed to monitor the population (pheromone or light traps) and then when the population begins to increase the lures can be placed out in the trial for a pre-determined time.

The time period used for trials should be a multiple of the number of treatments. Thus, if five trap designs (or lures) are to be compared ideally there should be five replicates and each trap should be sampled for a multiple of five times. If successive samples are taken twice weekly then the trial will take a minimum of two and a half weeks (five data points). Thus, the longer the interval between sampling the greater the number of replicates that should be employed to generate better quality data, given that the data may require averaging and transformation (e.g. $\log(x+1)$) before differences in treatments can be defined as statistically significant.

Trap height

For many moth species catches are higher when traps are placed in a crop canopy than above. Mason *et al.*, 1997 for example caught more European corn borer, *Ostrinia nubilalis*, when traps were located 0.1 m below the top of the canopy than when located 0.5 m above. Similarly, tree crop pests are most frequently trapped within a canopy (Bartelt *et al.*, 1994), although David and Horsburgh (1989) found the highest number of the leafroller, *Platynota flavedana*, were caught outside the apple tree canopy while the sibling species *P. idaeusalis* were caught inside the canopy. Such findings suggest that optimal trap

location will vary from species to species and perhaps even between generations of a single species (David and Horsburgh, 1989).

Unlike many cereal crops for example eggplant has a relatively open canopy that would allow sustained flight inside the crop thus trap location to maximise catch for the brinjal borer was uncertain. In order to determine the optimal trap height for *L. orbonalis* the catches from traps placed 1.0, 0.5, and 0 m below canopy height were compared with those caught in traps placed 0.5 m above canopy height. The results (Table 6.7) clearly showed that traps placed at or just above crop height (1.5 m) caught significantly more moths than those located 0.5 m above or below the crop canopy (ANOVA, $F = 19.2$, $P < 0.01$) (Cork *et al.*, 2003).

Nevertheless, it is unclear from the results whether male *L. orbonalis* normally search for female moths at canopy height or whether the extended pheromone plume produced by placing the trap at that height attracted more male moths.

Trap selection

From the point of view of commercialising pheromone traps the criteria for selecting a trap design may not accord with those of scientists developing pheromone products although they should be broadly similar to the needs of the end-user, the farmer. Thus, while a scientist may judge a trap that catches and retains the highest number of insects as the 'best' trap this may not correspond with farmers' needs. Table 6.8 attempts to show how the criteria for researchers, companies and end-users may vary and the scores will depend on the target insect pest.

The following section provides a list of trap designs used to catch adult Lepidoptera, Coleoptera and Diptera with semiochemical baits. The list is not exhaustive but meant to illustrate the range of designs that have been employed by researchers and later adopted by commercial companies for use in population monitoring and control.

Table 6.6 Cumulative catch of *L. orbonalis* in delta traps baited with different lures

Date	Day	LO99/14 Catch	LO99/15 Catch	LO99/16 Catch	LO99/17 Catch	Total
14-Apr-99	2	1	0	0	2	3
17-Apr-99	5	1	0	3	8	12
21-Apr-99	9	7	0	9	14	30
24-Apr-99	12	7	0	12	17	36
28-Apr-99	16	7	0	16	18	41
01-May-99	19	8	0	30	35	73
05-May-99	24	23	5	35	44	107
08-May-99	27	23	7	35	44	109
12-May-99	31	23	7	35	44	109
15-May-99	34	24	7	36	45	112

Table 6.7 Effect of trap height on catch of male *L. orbonalis*

	Trap height	Trap height with	Pheromone trap catches ¹		
	Above ground level (m)	respect to crop canopy (m)	Mean catch/trap/night	(±) Standard Error	Newman - Keuls ²
November 2000 ¹	0.5	- 1.0	1.23	0.26	a
	1.0	- 0.5	0.92	0.23	a
	1.5	0	2.17	0.59	b
	2.0	0.5	0.92	0.16	a

¹ Jessore District, Wing trap, 4 replicates, 24 nights.

² Means followed by the same letter in a group are not significantly different $P = 0.015$ by Newman-Keuls multiple range test on $\log(x + 1)$ transformed data.

Table 6.8 Criteria for selection of trap design for use in mass trapping

Criteria	Researcher	Farmer	Commercial producer
Catches most insects	5	5	5
Low production costs	1	1	5
Made from locally available parts	1	1	5
Compact and easy to transport	2	2	5
Maintenance costs (time and effort)	1	5	3
Strong and lasts many seasons	4	5	2
Quality finish	2	4	5
Retail price	3	2	3

1 = lowest importance, 5 = highest importance

Table 6.9. Criteria for selection of trap designs for use in mass trapping

Criteria	Delta traps	Water traps	Sleeve trap
Catches most insects	?	?	?
Production costs	1	3	1
Made from locally available parts	4	1	1
Compact and easy to transport	5	3	5
Maintenance costs (time and effort)	3	3	1
Strong and lasts many seasons	2	4	3
Quality finish	3	3	3
Retail price	3	3	2

1 = low attribute, 5 = highest attribute

In the same way the criteria can be adopted to compare trap designs. Thus, in Table 6.9 three trap designs delta (sticky), water and sleeve (funnel) trap are compared. Plastic sleeve traps are very popular in India because they are easily fabricated from locally available materials, they last more than one season, are easily transported and are low maintenance for farmers. Water traps are likely to

be less popular because they require more maintenance, have higher production costs and although they are stronger they are also more attractive to other potential users! Sticky delta traps have low production costs are easy to transport but require a considerable amount of maintenance and the adhesive is not readily available.

Pheromone trap designs***Traps for Lepidoptera****Funnel and sleeve traps*

Figure 6.1 Funnel trap. Made in plastic from inverted white plate (lid) yellow funnel, and opaque orange container for retaining insects. The funnel is held in place on the lid of the plastic container by a ring clip or rubber band. Widely used by NRI in Pakistan, Africa and south east Asia in 1980s and 90s, produced from locally available materials.



Figure 6.2 Bucket trap. This funnel trap has incorporated the funnel into the lid of the second chamber of the trap. Traps are available in a variety of coloured plastics and the bottom chamber can be produced in a clear plastic for ease of use. A highly successful trap design building on earlier models used for cotton pests in Pakistan. Sometimes known as the Uni-trap because it was thought to be universally effective for all moth species. However, early trials in Bangladesh showed that it was not effective for *L. orbonalis*. Cost limits its potential for use in South Asia although the inherent strength of the design would make it a good long-term investment for appropriate species. (Trap courtesy Agrisense-BCS Ltd., UK)



Figure 6.3 Funnel trap This funnel trap is the most popular in India. Cheap to produce often from recycled plastic. It is a hybrid trap design combining the funnel from the 'funnel' trap but utilising a long thin plastic bag from the 'sleeve' trap as the second chamber for retaining and killing insects. In Israel the funnel was dispensed with altogether to produce the open-sleeve trap (Kehat *et al.*, 1981). The advantages of sleeve traps are their ease of assembly low maintenance costs and visibility of trap catch for the end-user. Disadvantages of the trap are the long bag, that discourage farmers putting the trap at crop height when crops are young which reduces their efficiency. Plastic bags have been banned in some countries such as Bangladesh where they may not be perceived as environmentally acceptable.



Figure 6.4 Double funnel trap. Made from inverted metal plate (lid), yellow funnel with slits to allow pheromone out from a lure. The yellow and white funnels act to direct the insects into the retaining plastic bag. Early versions of the trap design used widely in Egypt had problems with the plastic bags that split under intense sunlight and wind action. ICRISAT Design (*Heliothis* trap).

Sticky traps



Figure 6.5 Delta trap. The traditional delta trap design provides shelter for the pheromone lure from sunlight and protects the adhesive from water. Any water that enters the trap can exit through slits near the bottom corners. Fixed orientation means that moth entry is restricted to two sides and this interferes with the pheromone plume but the cover prevents the build up of non-target insects and dust associated with open sticky disc traps.
(Trap courtesy of Agrisense-BCS, UK)



Figure 6.6 Open-delta trap. This modified Delta trap was found to catch Brinjal borer, *L. orbonalis* and rice stem borer, *S. incertulas* more efficiently than the conventional Delta trap but in common with the conventional Delta trap has a limited capacity that reduces its usefulness for field applications. Adhesives such as 'Tangle Foot' are unaffected by sunlight and are rain-fast. The adhesive is not available in South Asia although is widely available, at low cost, on the international market.
(NRI, University of Greenwich, UK)



Figure 6.7 Sticky plate trap. Prototype trap developed by Syngenta Bangladesh Limited for use with yellow rice stem borer. Cost-effective trap design produced from local materials. Required only single support and lures were well protected. (Trap courtesy of Syngenta Bangladesh Limited)



Figure 6.8 Sticky plate trap. The figure shows the effect of ageing on the glue surface when exposed to sunlight and trap efficiency compromised due to build up of dust, plant debris and non-target insects. (Trap courtesy of Syngenta Bangladesh Limited)



Figure 6.9 Sticky disc trap. Highly efficient but saturating design limits trap catch and open surface susceptible to build-up of debris. Lure protection relied on black paint applied to base of pheromone lure. (NRI, University of Greenwich, UK)



Figure 6.10 Sticky disc trap. Similar to NRI design but with 'roof' to protect pheromone lure. Centre fold meant it could be readily transported and erected at field site.
(Trap courtesy of Agrisense-BCS, UK)

Water traps



Figure 6.11 Champion Water Trap, with lid. Trap surrounded by pheromone lures to assess whether trap catch can be suppressed by close proximity of lures. Experimental set-up used to assess likely impact of mating disruption.
(NRI, University of Greenwich, UK)



Figure 6.12 Champion Water Trap, without lid. Solid construction, high capacity, ideal for research purposes but in common with all water traps requires high level of maintenance and insect identification can be problematic if oil is added to water. Alternatives include surfactants such as washing detergents. (NRI, University of Greenwich, UK)



Figure 6.13 Alam water trap. Highly practical and cost effective water trap prepared from locally available materials in Bangladesh. Because these can be purchased in the local market transport costs are not important but in common with other water traps they require considerable maintenance.
(Trap courtesy Dr N. S. Alam, BARI, Bangladesh)



Figure 6.14 Alam water trap. Care should be taken over the choice of container because some locally produced plastics are not stable under intense sunlight, the loss of plasticiser can cause the fabric of the traps to become brittle, as seen in this prototype.
(Trap courtesy Dr N. S. Alam, BARI, Bangladesh)



Figure 6.15 Lobos trap. Constructed from local materials using light engine oil as a killing agent. The Lobos trap although small was very effective for trapping *Helicoverpa gelatopoeon*. (Courtesy of Dr E. Lobos, Santiago del Estero, Argentina)



Figure 6.16 Page Trap. The Page trap was used for trapping African armyworm, *S. exempta*. The design incorporates a self-levelling device so that excess rainwater is released through the edges of the trap thereby retaining the upper oil layer. NRI, University of Greenwich, UK



Figure 6.17 WOTA-trap. Innovative patented design. Well engineered from high quality plastic. Utilises a single support. Designed for use with sugarcane borers.
(Trap courtesy of Dr P. K. Jayanth, BCRL, India)

Traps for Coleoptera



Figure 6.19 Open-bucket trap. Has found application in South Asia, Middle East and Central America for control of palm weevils when baited with the aggregation pheromones and / or food baits. The traps were effective at reducing adult weevil populations in Costa Rica where removal of infested trees alone was ineffective (Oehlschlager, *et al.*, 2002).
(Slide courtesy of Oehlschlager, ChemTica Ltd.)



Figure 6.20 Bucket trap. Applied for control of red palm weevil *Rhynchophorus ferrugineus* in coconut palms.
(Courtesy of Serendib Natural Products limited, Sri Lanka)



Figure 6.21 Boll weevil attract and control tubes (BWACT). Over 1 million produced per annum for control of boll weevil in south and central America
(Courtesy Plato Industries Ltd.).



Figure 6.22 Boll weevil monitoring trap. Weevils land on the yellow cone and walk up into the netted collection chamber.



Figure 6.23 Vane trap. Females of insects such as the coffee white stem borer, *Xylotrechus qradripes* are attracted by male produced pheromones but are known to alight some distance from the male. It is then assumed that the male moves to the female and initiates courtship. With this in mind it was assumed a trap with a large surface area was needed to catch the females. However, the sticky vane trap proved to be too 'sticky' to handle!"

(Trap courtesy of Agrisense-BCS Ltd., UK)



Figure 6.24 Vertical sticky plate trap. The vertical sticky plate proved to be more durable for coffee white stem borer. Note the aluminium shield to protect the pheromone lure. Even under a tree canopy sunlight can degrade sensitive pheromone components.

(NRI, University of Greenwich, UK)

Traps for Diptera

Figure 6.25 Insecticide-impregnated target for tsetse fly. Primarily used for control because insects landing on the target surface are killed by exposure to the insecticide but not retained in the vicinity of the target. The blue colour is highly attractive to the diurnal flying insects, while they preferred to land on black. The target is pivoted because tsetse are attracted by moving objects and coated with insecticide as a killing agent. The pyrethroid used for killing the insects is suitably formulated to withstand photo-degradation and rain. (NRI, University of Greenwich, UK)



Figure 6.26 Tsetse fly entry trap for assessing population densities and species composition of insects attracted by the olfactory and visual cues presented by the trap. Flies entering the trap are attracted by light from above and move upward and are retained in the transparent collecting vessel. (NRI, University of Greenwich, UK)



Figure 6.27 Odour-baited sticky target. Black sticky plates used for catching New World Screwworm (NWSW) in Mexico. Experimental lures were applied to simple cotton wicks. A 0.5 x 0.5 m square could catch up to 1000 insects. (NRI, University of Greenwich, UK)



Figure 6.28 Close-up of catch of NWSW on odour-baited target. Insects landed in close proximity to those already trapped leading to the concept of decays to increase capture. The adhesive used did not adhere to human skin. (NRI, University of Greenwich, UK)



Figure 6.29 Sweet gourd mash (SGM) trap Highly attractive to female fruitflies. Dr Md. Nasiruddin, BARI, Bangladesh



Figure 6.30 Sweet gourd mash (SGM) trap Close up of fruitflies feeding on surface of attractant. Dr Md. Nasiruddin, BARI, Bangladesh

Chapter 7: Monitoring

Integrated pest management (IPM) is widely recognised as the method of choice for controlling insect, disease, weed or other members of a crop pest complex. This applies whether the 'pest' is in a field, sheltered crop, storage facility, domestic or commercial food production unit, human or animal disease vector. IPM is knowledge and labour intensive, requiring appropriate training and frequent scouting to monitor target pests. Many IPM strategies rely on stakeholders, such as farmers, taking action only at predetermined action thresholds. Pheromones and related semiochemicals can provide stakeholders with a potentially sensitive, selective and labour saving method of monitoring insect pests and are universally used in the food and tobacco industries (Rangaswamy 1985, Mueller *et al.* 1990) to monitor insect pest populations.

This chapter will primarily deal with insect pests of field crops although examples of other insect pests will be provided to give a more comprehensive overview. Examples have been taken from work conducted in South Asia or on insect pests that are of economic importance in South Asia.

Components of a monitoring system

In order that a monitoring system based on semiochemicals, such as pheromones, can operate a number of parameters have to be standardised such as the attractant, dispenser, trap design and trap location (Table 7.1). Invariably the pheromone trap catches the adult of a species while it is the larva that is the injurious stage. For quarantine and detection purposes it is enough to catch insects and so these trapping systems tend to use the most efficient trap designs and lures. However, where the information is to be used in a predictive manner, such as assessing the timing of the next generation of larvae a good understanding of the biology of the pest and the effect of weather and crop stage on development is important. This is particularly true where the trap is baited with a Lepidopterous sex pheromone since they catch only male moths which are not necessarily a good indicator of the likely level of oviposition expected in a crop and has no bearing on the survivability of their progeny. Nevertheless, there are many examples of male trap catches being successfully related to subsequent pest incidence. The degree of accuracy of these monitoring systems can be increased taking into account actual or predicted weather conditions but such refinements are not necessarily appropriate where farmers do not have access to such information or not able to utilise it.

When developing a pheromone trapping system it is important to decide whether the traps are to be used for monitoring or control. Often the most attractive pheromone baits are not the most appropriate to use in a population monitoring system. If the trap design chosen is easily saturated by high numbers of insects then a less attractive bait should be employed. For monitoring purposes farmers have no desire or need to count large numbers of insects and so thresholds based on low insect

catches are preferable. Such lures are also cheaper to produce (and sell) because they may involve the use of less active ingredient.

Pheromones by definition are more selective than light or food-baited traps because they attract only the target species. Nevertheless, there are examples of pheromone baits being used for more than one species in a single trap. This has clear advantages where trap costs are high, but it is important to test out the system thoroughly before it can be recommended. The pheromones of some species do not affect the attraction of other species, for example, *S. litura* and *H. armigera*, while the pheromones of other closely related species are mutually incompatible such as *Earias insulana* and *E. vittella*. Other species that affect a single crop sometimes utilise similar compounds in their pheromones, such as the rice pests *Chilo suppressalis* and *Scirpophaga incertulas*. In such instances a case could be made for developing an off-blend that was equally attractive to both species for monitoring purposes. This idea was attempted with the pheromones of *S. incertulas* and *Sesamia inferens*. However, the resulting lure whether presented as a combined attractant or dispensed in separate lures in the same trap resulted in a significant reduction in the catch of *S. incertulas* (A. Cork and J. C. Saha Choudhury, unpublished).

Food baits have long been used to attract insects from the traditional 'sugaring' of trees to attract moths by amateur entomologists to palm extracts for attracting palm weevils (Vidyasagar & Subaharan, 2000). Food baits are particularly useful where a potential host is subject to attack from a complex of species, such as crops in storage Collins *et al.* (2004).

More recently there have been increasing efforts to combine pheromone and host odour-attractants to enhance the catch of target species. Thus for example, Varma *et al.* (2002) reported significantly increased trap catches of *S. incertulas* through the addition of basmati rice extracts to pheromone baited traps. However, those efforts were primarily directed at the development of trapping systems for control rather than monitoring. The currently available pheromone blend is more than sufficient for use in monitoring without the complication of adding non-pheromone components that could give rise to registration and quality assurance problems for commercial producers.

The composition of the pheromone dispenser is crucial for successful operation of a trapping system since it must protect and release the pheromone in a sustained and controlled manner. Numerous controlled release dispensers have been devised but perhaps the best known for sex pheromones are based on polyethylene vials and rubber tubes and septa. Both materials provide an environment from which typical Lepidopterous pheromones can be released over a period of up to two months. However, the release rates such monolithic polymers are first order, which means that the release rate is proportional to the amount present. Thus, the relative attractiveness of lures will diminish with time (Ganeswar Rao and Krishnaiah, 1995). Ideally trap catch data should be corrected for the age of the lure but this is seldom done. Nevertheless, it becomes very important if trap catch data is compared from lures of different ages (see Chapter 5).

Trap design significantly affects the efficiency of capturing and retaining insects attracted to a semiochemical bait. They also contribute significantly to the cost of the trapping system and so the choice of trap design is usually a trade-off between trapping efficiency and cost. Numerous trap designs have been developed and they have been reviewed in a very readable book by Muirhead-Thompson (1991). Traps rely on water containing oil, water / surfactant, sticky surfaces, volatile insecticide, electrocution, or simply heat exhaustion to kill the insects. The choice of killing agent usually depends on the insects' susceptibility and cost of alternatives (see Chapter 6).

Most insects are not equally available for trapping in all locations instead they tend to spend much of their time in close association with potential or actual hosts. This is particularly true of crop pests, although even there populations are not homogenous. Similarly, the presence of competing cues may reduce the number of insects available for attraction to pheromone traps, most importantly virgin females (Witz *et al.*, 1992). Thus, most traps used for field crops should be placed away from the edge of a field and at least 50 m from trees and low shrubs. Artificial light sources should also be avoided. Trap height is important and it is generally recognised that crop height or just below is optimal for most species. However, for orchard and forest pests an understanding of the insects' natural preference becomes important, and trials to ascertain the most effective trapping height should be undertaken. For storage pests trap designs and deployment vary markedly depending on whether the insects walk or fly into the trap. Thus, probe traps might be deployed in grain storage facilities for walking insects while flight traps would be more appropriate for such species as *Ephesia cautella*.

Applications of monitoring systems

Thus, the type of monitoring system to be developed depends on the purpose for what it is to be used (Table 6.1). In the case of quarantine and eradication the concern is that immigrants will enter a previously uninfested or controlled environment respectively. Under those conditions baited traps need only show the presence or absence of the target pest.

In yet other species the timing and magnitude in fluctuations in catch may be the critical factors that determine whether action is taken or not. In such cases the ability of pheromone traps to provide an early warning of pest incidence is to some extent dependent on whether the pest occurs in discrete generations throughout a crop cycle, such as rice stem borers. This is particularly important to farmers who do not need to undertake remedial action unless the pest exceeds a known damage or economic action threshold. For many pest species pheromone trap monitoring has been linked to weather data so that predictions on the severity and likely timing of attack can be more accurately assessed. Examples include, monitoring for the pea moth, *Cydia nigricana*, in the United Kingdom and the African armyworm, *Spodoptera eximpta*, in East and North East Africa.

There has always been a perceived need to know the **range** of a pheromone lure, which is the distance from which a lure can attract insects. Such knowledge is rarely determined because the experimentation required is time

consuming and not always conclusive. Nevertheless, studies have been undertaken most notably a recent study on male European pine sawflies *Neodiprion sertifer* by Östrand and Anderbrant (2002). The authors marked and released insects 50, 100, 200, 400 and 800 m along the four cardinal directions from a centrally placed pheromone trap. Based on a linear regression of transformed trap catch data they calculated the **Seasonal Sampling Range** (r_s) to be 1,040 m and were able to provide an estimate of the **Effective Sampling Area** (r_{eff}) of 4.9 ha. However, this data assumed that the insects were evenly distributed around the trap and that they are attracted from a circular area around it. The authors went on to define the **Cumulative Proportional Catch** (CPC) that gives the proportion of trap catch that originated from an area within a distance r of the trap. At $r = r_s$ CPC = 1, and in their study 50% of the trapped insects were thought to have originated up to 450 m from the trap. They also defined the **Catch Concentration** (CC), which is the ratio of the radius of the effective sampling area (r_{eff}) to r_s . When r_{eff} is close to r_s , the catch adequately mirrored the population within most of its sampling range. The authors went on to express the view that through the application of the two new concepts it will be possible to better understand why monitoring traps sometimes mirror the local population but not in others.

Vegetable crop pests

Pea moth, *Cydia nigricana*

Much of the pea production in the UK is linked to the production of high value frozen produce. In order to ensure the produce is pest free control of the pea moth, *C. nigricana* is essential. This process is assisted by use of a pheromone monitoring system developed by Rothamsted Experimental Station, UK and commercialised by Oecos, Kimpton, UK. Each system is composed of two delta traps placed 100 m apart and at least 5 m into the crop on adjacent headlands on the side of the prevailing wind. Traps are mounted on stands and the height is adjusted to two-thirds of crop height. Lures are prepared from rubber septa containing 3 mg of an analogue of the pheromone, (*E*)-10-dodecen-1-yl acetate (E10-12:Ac) (Greenway and Wall, 1981). The system is usually set up before the flight season (mid-May) and normally continued until mid-August or harvest whichever is the sooner. The two traps are examined daily and records kept of catches. Insecticide applications to control *C. nigricana* are only applied if the catch threshold is exceeded. The current threshold is reached if ten or more moths are caught in either trap on two consecutive 2-day periods.

The **economic threshold** for insect pest control is dependent on the cost of insecticides and the value of a crop. Nevertheless, for some crops consumer tolerance for insect damage is very low. This is the case with the frozen pea market in the UK. Thus, while it may not be economically justified to apply insecticides the **action threshold** is accepted to ensure very low damage levels.

The timing of insecticide sprays is based on the date that the newly hatched larvae are anticipated to be entering the pods. The date pheromone trap catches exceed the threshold is used as the starting point for calculating the spray date applying a developmental model based on maximum and minimum daily temperatures.

The system has been successfully run for many years and farmers have been able to ground truth the system based on their own experience. Importantly the system does not use the pheromone blend because that would attract too many moths, which would lead in turn to the traps becoming saturated requiring excessive amounts of time to empty them (Macauley *et al.* 1985).

Pea midge, *Contarinia pisi*

Delta traps baited with lures containing three components of the female sex attractant of the pea midge, *Contarinia pisi*, 2-acetoxytridecane, (2*S*,11*S*)-diacetoxytridecane, and (2*S*,12*S*)-diacetoxytridecane were found to be effective at attracting newly emerged male midges at a range of sites previously cropped with vining peas during 1999-2001 in the UK. A commercial version of the system was made available for the 2002 season and found to provide effective early warning for the protection of nearby susceptible vining pea crops (Biddle *et al.*, 2003).

Diamondback Moth, *Plutella xylostella*

Reddy and Urs (1996) demonstrated that *P. xylostella* pheromone baited sticky delta traps caught most moths when placed 30 cm above the crop canopy, and that catch was significantly reduced after 28 days field exposure. Importantly they also found that synthetic lures attracted similar numbers of adult male moths as 10 virgin female moths suggesting that the synthetic pheromone could have considerable potential for use in monitoring and/or control. Reddy and Guerrero (2000) subsequently established an economic threshold based on pheromone trap catch of eight moths per trap per night.

Walker *et al.*, (2003) monitored *P. xylostella* larval and adult populations in brassica crops at five locations in New Zealand by sampling between 50 and 100 plants at each site and using pheromone traps. They found that increases in moth catches accurately predicted increases in larval infestations in three of four spring crops by two to three weeks and gave two weeks warning of larval damage in four of the five summer crops monitored. The authors suggested that the use of IPM action thresholds led to a 75% reduction in insecticide use although this result was influenced by a natural epizootic of *Zoophthora radicans* (*Erynia radicans*) that caused a rapid decline in larval populations in mid to late January at all sites.

Prasad and Guerrero (2001) conducted field trials to assess whether pheromone traps could be used to determine the optimum timing of insecticide applications against *P. xylostella* in cole crops, cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*) and knol khol (*B. oleracea gongylodes*). Their results demonstrated that applications of the insecticide cartap hydrochloride during a 12 to 24 h period after the pheromone traps had caught on average of 8, 12 and 16 males per trap per night in cabbage, cauliflower and knol khol, respectively, were significantly more effective than regular insecticide sprays at 7, 9, 12 or 15 days after transplantation. The effectiveness of the trials were assessed by estimating the mean number of eggs and larvae per plant, the percentage of holes produced, as well as the marketable yield of the three crops at each location tested. A good correlation was obtained between the immature stages, infestation level,

estimated crop yield and number of moths caught in pheromone traps indicating their usefulness for predicting population densities of the pest.

Beet armyworm, *Spodoptera exigua*

Kennedy *et al.* (1998) suggested that insecticide spraying could be reduced by 85% by using entomopathogenic viruses in combination with pheromone traps to control *Spodoptera exigua*, *Thrips tabaci* and *T. parvispinus* on shallots. Using an action threshold control approach, insecticide use they reduced insecticide applications by an average of 63% compared with twice weekly spraying.

Groundnut leaf-miner, *Aproaerema modicella*

Groundnut leaf-miner, *Aproaerema modicella* has become a serious pest of groundnut in Madhya Pradesh. Seasonal activity was studied by Das (1999) in West Nimer Valley, using pheromone trapping. The pest was found to be active during the cropping season (July to November), with peak catches recorded in August, which coincided with the reproductive stage of the crop. Significant positive correlations were observed between trap catches and morning and evening relative humidity, and number of rainy days. Das (1999) suggested that the results indicated that pheromone trapping may be a promising tool for monitoring *A. modicella* in groundnut fields.

Fruitfly, *Bactrocera dorsalis*, *B. cucurbitae*, *Ceratitis capitata*

Control of fruit flies has often been achieved by the sterile insect technique with semiochemicals being used to monitor the efficacy of the technique and location of re-infestations. Monitoring has involved a wide range of semiochemicals such as the feeding stimulant methyl eugenol (4-allyl-1,2-dimethoxybenzene-carboxylate) and para-pheromone cue-lure (4-(*p*-acetoxyphenyl)-2-butanone) which are highly attractive to the oriental fruit fly, *Bactrocera dorsalis* (Hendel), and melon fly, *B. cucurbitae* (Coquillett), respectively. In California and Florida, trimedlure is used to monitor for re-infestation by the Mediterranean fruit fly, *Ceratitis capitata* (Weidemann) (Landolt, 1999).

Early detection of fruit fly is important for control. Papadopoulos *et al.* (2001) compared the effectiveness of International Pheromone McPhail Traps (IPMT) baited with female targeted attractants, ammonium acetate, putrescine, and trimethylamine, and Jackson traps baited with the male specific parapheromone, trimedlure, for detection and monitoring of the Mediterranean fruit fly, *C. capitata* (Wiedemann) in a mixed-fruit orchard in northern Greece. Traps were suspended from various host trees, using trap grid densities of either 1.5 or 15 traps per ha. The IPMT detected the first adult females in apricot trees, which are among the earliest maturing hosts in the area and on peaches from the end of July followed by apricots in the fruit ripening sequence. IPMT traps captured predominately females (approximately 50 to 80% of the total catch) and out-performed the Jackson traps in early detection. Comparing the performance of two trap grid densities on apple trees (the common host in the two grids), the authors found that the high-density trap grid detected the first adults one-week earlier than the low-density trap grid.

Cereal crops

Krishnaiah (1995) listed three cereal crops of importance in India, rice, sorghum and maize, although wheat continues to increase in demand. The sex pheromones of many of the major insect pests of these crops have been identified (Cork and Hall, 1995). However, the value of these crops is such that the commercial viability of pheromone systems for control is questionable (Cork 1998) but have considerable potential for use in IPM systems for monitoring pest infestations.

African armyworm, *Spodoptera exempta*

As an example, the sex pheromone of the African armyworm, *Spodoptera exempta*, (Cork *et al.* 1989) has been used to provide an extensive monitoring trap network throughout much of East Africa. Data obtained from pheromone traps were posted every week to a regional centre where the data were collated and compared with historical data gathered from both pheromone and light traps. Changes in adult male populations over time and space were compared with meteorological data to predict the location and extent of larval outbreaks. When a high probability of an outbreak was forecast in an area the presence of larvae was 'ground truthed' by sending out trained technicians. If their findings confirmed an outbreak was imminent the larvae were controlled either by ground spraying with ultra-low volume sprayers or using aircraft provided by the Desert Locust Control Organisation for East Africa (Dewhurst, 1993). Broza *et al.* (1991) monitored secondary outbreaks of *S. exempta* in Kenya with pheromone traps as an effective method of locating and timing the application of *Bacillus thuringiensis* (*Bt*) for control. They reported a 95% reduction in neonate larvae as a result of the early detection of egg masses and timely application of *Bt*. A similar system could be developed for other migratory pest species such as rice hispa in Bangladesh.

Millet stem borer, *Coniesta ignefusalis*

Working in Ghana Tanzubil *et al.* (2003) demonstrated that pheromone trap data for the millet stem borer *Coniesta ignefusalis* provided significant positive correlations between trap catches and larval numbers three weeks later and also between trap catches and the number of bored plants three weeks later. The trap catches suggested that there were four generations per year, with peaks in adult population in June, July, September and October. Nevertheless, they did not find a significant relationship between larval infestation and crop damage, suggesting that larval count alone may not be a reliable indicator of *C. ignefusalis* damage to millet.

Rice striped stem borer, *Chilo suppressalis*

The rice stem borer (*Chilo suppressalis*) has gradually increased in importance as a rice insect pest in Taiwan since 1980, occasionally causing severe damage on rice. Monitoring of its population dynamics and forecasting the time and amount of adult emergence are important for assessing the level and nature of control strategies that should be adopted. Cheng (2000) reported that data collected with sex pheromone traps and suction light traps in paddy fields showed that pheromone traps were more efficient than suction light traps in monitoring the

populations. The data showed that there were five stem borer generations a year with three generations in the first cropping season and two generations in the second. The adult population in the first cropping season was markedly higher than in the second. Disruption of the habitat between cropping seasons, high temperatures and rainfall in the early growth stage of rice were thought to limit the population in the second cropping season. Several equations were developed by the authors for forecasting the time and amount of adult emergence from this study.

Yellow stem borer, *Scirpophaga incertulas*

The main emphasis of research on the pheromone of *S. incertulas* has been on the development of control strategies (Cork *et al.*, 1998; Katti *et al.*, 2001). Nevertheless, studies have been conducted on the relationship between trap catch and crop damage (Du *et al.*, 1987) and the relationship between the timing of pheromone trap catches and subsequent larval damage, as shown in Figure 6.1 (Cork *et al.*, unpublished data), is well understood.

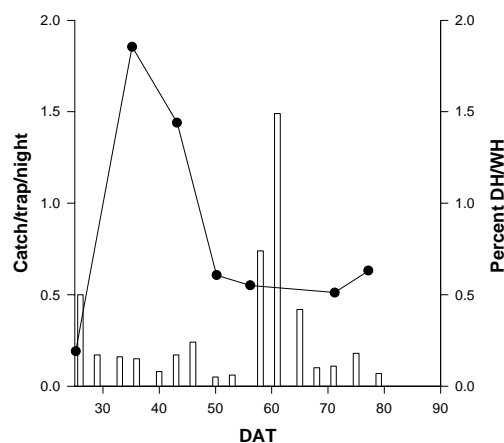


Figure 6.1 Pheromone trap catch (curve) and percentage larval damage (columns) in, Transplanted Aman (2002) rice crop, Comilla, Bangladesh

A particularly detailed study on the relationship between pheromone trap catches and larval damage was reported by Varma *et al.* (2000) working from the 1996 kharif season to the 1997 rabi season in Medchal, Andhra Pradesh. Three peak periods of moth activity were observed during the kharif season (July, August, September) and two during the rabi season (January and March/April). Late planted crops were found to be more affected by *S. incertulas* than early planted crops. The authors established that there was a temporal relationship between peak pheromone trap catches and field infestation. Thus, peak dead heart and white head infestations occurred two to three weeks after peaks in pheromone trap catches. From regression equations of trap catch and field infestation data, trap catches of 30 and 19 male moths/week/trap resulted in 10% dead heart and 5% white heads respectively. When catches exceeded 30 moths/trap/week insecticide application was considered to be economically justified. They estimated that insecticide applications should be applied 95 to 110 degree days after the peak in trap catches in order to synchronise application

with egg hatching. However, larval development and adult emergence were found to be affected if temperatures fell below 16 or above 35°C. Maximum temperatures of 29 to 30°C and a minimum of 20°C were considered to be the most favourable for development with development from egg to adult completed in 430 to 530 degree days. Under adverse weather conditions (below 16 or above 35°C) 630 to 670 degree days were required to complete development.

Maize stalk borer, *Chilo partellus*

On maize in Kenya Unnithan and Saxena (1990) found a positive correlation between male *Chilo partellus* trap catches and larvae, pupae, and percent infested plants, using traps baited with virgin females.

In related work Sharma (1996) assessed the efficiency of virgin female baited traps for monitoring and trapping male *Chilo partellus* on sorghum in Madhya Pradesh, India, between 1989 and 1990. Catches of male *C. partellus* with virgin female traps were recorded from July to late October with three peaks in catch observed in mid-July, late-August and mid-September. Among the seven parameters studied, only adult field population significantly positively influenced trap catches.

No related work has been conducted with synthetic pheromone baits in South Asia, even though components of the sex pheromone were identified by Nesbitt *et al.*, 1979 using insects from India. In the Republic of South Africa a pheromone trap network was established (B. Beck, pers. comm.) but results of this work have yet to be published.

Cotton

American bollworm, *Helicoverpa armigera*

Various studies have been conducted on *H. armigera* with pheromone and light traps in order to understand insect movement and in an attempt to produce a predictive model that would enable management decisions to be taken on the basis of trap catch. In early work, Dent and Pawar (1988) showed that night-time activity of *H. armigera* as measured by pheromone and light trap catches were not related. However, Srivastava and Srivastava (1989), and Srivastava *et al.* (1992) demonstrated a close relationship between pheromone and light trap catches in season-long monitoring of *H. armigera*. Trap catches with both devices were temperature dependent with 12°C being the minimum at which catches were recorded. Moonlight was found to have a profound effect on light trap catch. High illumination causing significant reductions in trap catches. In contrast, moon illumination was reported to have no effect on pheromone trap catches suggesting that moonlight had more effect on light trap catches than on male moth activity. Wind speed affected trap catches in different ways. Low wind speeds favoured the light trap with catches of both male and female moths decreasing above 4 km/h whereas pheromone trap catches were higher when wind speeds were between 5 and 12 km/h. The latter result was thought to reflect the 'active area' of the pheromone plume, which would increase with wind speed. A similar result was subsequently found by Patil and Kulkarni (1997).

In Australia Flint *et al.* (1989) found that pheromone trap efficiency was low and varied with moth population density as male moths attempted to drive one another away in high-density populations. Fitt and van den Elst (1988) decided that light trap catches provided more accurate predictions of egg laying and adult populations than pheromone traps. Witz *et al.* (1992) compared the number of tobacco budworm, *Heliothis virescens* trapped on a daily basis compared to male and female adult densities measured by nocturnal field sampling. They found that there was a decline in trap efficiency with increasing female density. However, they developed a simple relationship for estimating *H. virescens* male density from trap catch, $P\delta = (E_2/13)^2$. Where $P\delta$ is male density and E_2 is the two-day exponential average of number of male moths trapped. Thus, a trap catch of 10 represented a density of 0.4 male moths per ha and 100 moths trapped represented a density of 70 male moths per ha. However, the accuracy of the prediction was within 0.5 to 2.0 times the actual population. Thus, a prediction of 70 moths per ha could actually be anything from 35 to 140 moths per ha with 95% confidence. The work took no account of environmental effects and would only be valid for the particular trap system used by the authors. Changing trap efficiency or the attractiveness of the pheromone lure would adversely affect the accuracy of the predictions and should ideally be repeated for each trap and lure system.

Irrespective of the method of monitoring *H. armigera* in Australia, Zalucki *et al.* (1998) believed that IPM-based control strategies adopted were failing because local management of highly mobile, multivoltine, polyphagous species such as *Helicoverpa* were inappropriate. They proposed an area-wide management (AWM) strategy in which early season control of populations in low value crops such as sorghum and maize with soft options such as NPV was undertaken. The idea was to prevent population build-up in more valuable crops such as cotton later in the season. Such co-ordinated action is unlikely to be adopted in South Asia but does nonetheless question the value of control strategies as currently adopted.

Flight patterns of *H. armigera* were successfully monitored in Pakistan (Srivastava *et al.*, 1991) and India (Srivastava *et al.*, 1992) for over a decade using pheromone and light traps. It was anticipated that such data together with egg and larval population densities would enable a model to be developed (Wightman and Ranga Rao, 1991) that could be used to estimate crop damage in relatively small areas, such as 5 ha. That goal was not achieved, but Srivastava *et al.* (1992) were able to calculate regression equations based on larval counts and pheromone trap catches using data from larger areas, 1,300 ha. Wightman and Ranga Rao (1991) suggested that the inability to utilise pheromone traps to predict crop damage could be due to an inappropriate pheromone blend, trap design or the impact of olfactory competition from the environment. Their conclusions have gained credence by recent work reported by Kumar and Shivakumara (2003). Working at five locations in Karnataka they reported that density and pheromone polymorphism were important factors affecting male moth trap catches. As mentioned earlier trapping efficiency is well known to decrease with high population pressures (Fitt *et al.*, 1989) but the suggestion that *H. armigera* may consist of populations that responded to different pheromone blends is surprising given its mobility.

In mark-and-recapture trials with the related species *Heliothis virescens* Hartstack and Witz (1981) reported trapping male moths 8.3 km from the point of release after a single night. A similar conclusion was derived from a mark-and-recapture trial conducted in India using strontium chloride (King *et al.*, 1990) as the marker. Of the estimated 50,400 moths that emerged over a 20-day period from the treated pigeonpea field, 7% were recaptured, predominantly in traps up to 3 km and downwind of the source field. The authors concluded that taken together with flight mill and radar data *H. armigera* was capable of undertaking rapid dispersal from source fields, even when they remain attractive, but at low elevation (< 100m) constituting 'long range' non-migratory dispersion (Farrow and Daly, 1987).

Nevertheless, Prasad *et al.* (1993) working on cotton in India was able to calculate a damage threshold of 7 moths/trap/night, using sleeve traps baited with 2 mg of pheromone. Jhala and co-workers estimated the action threshold to be 5 moths/trap/night employing 5 to 10 traps/ha in cotton but found that this threshold did not hold true under all circumstances (R. C. Jhala personal communication). In related work in Pakistan Chamberlain and Ahmad (in press) demonstrated a correlation between male moth trap catches and the number of eggs laid during the middle and latter part of the cropping season when most crop losses due to larval infestation occurred.

Further work on trap design has been reported by Krishna Kant *et al.* (1999) who also studied the rhythmicity, and orientation on trapping of *H. armigera* with sex pheromone. They found that the peak of attraction was between 23.00 and 04.00 h, in agreement with data produced by Dent and Pawar (1988). This data is also in agreement with studies on the production of pheromone. Nesbitt *et al.* (1980) working with insects from Sudan, Malawi and India found that the maximum amount of pheromone was produced, and presumably released, by 2 to 3 day old virgin female *H. armigera* 5 to 8 h into the scotophase. Funnel, Texas and sticky traps suspended 1.8 m high were found to catch a greater number of moths than those at 1.2 and 0.6 m height. In earlier work Pawar and Dent (1988) placed traps at 2 m height although they do not mention why this height was chosen. Krishna Kant; *et al.* (1999) considered funnel and Texas traps to be more effective than sticky traps at catching and retaining *H. armigera* moths. In a separate study Nyambo (1988) compared white and yellow ICRISAT and Ukiriguru traps with the Mushi, Texas, and yellow funnel or El-Dieb trap (Critchley and El-Dieb, 1981). Overall, funnel trap designs caught more moths with the El-Dieb trap catching the highest number. Interestingly, the yellow traps caught more than the white traps. The Texas and ICRISAT traps caught similar numbers of moths but were less effective than the El-Dieb trap and more effective than the Mushi trap. Studies on the optimisation of trap design continue as evidenced by a recent publication by Nandagopal and Prasad (2004) indicate.

Loganathan *et al.* (1999) assessed the field longevity of *H. armigera* pheromone lures. On the basis of male responses to field exposed lures they estimated that septa with a 1 mg loading of pheromone had a field life of only 11 days but that this was increased to 13 days by using carbon free septa. Such lures would be unacceptable for use by farmers and clearly suggest a need for additional

work to develop lures that are efficacious for longer periods of time.

In Southern Spain, as in many other regions of the world, *H. armigera* is the main pest of cotton. Despite its resistance to insecticides they are still used for control. Sánchez, *et al.* (2000) reported that although the IPM programme was still based on field sampling of young larvae, pheromone traps had provided a good index of adult activity over the cotton area in Andalusia since the 1980's. Pheromone trap data showed three generations per season beginning in June, mid-July and August respectively. Every season the captures followed a similar pattern in the area monitored.

In an unusual study Ballal (1998) examined the effect of applying insecticides on pigeonpea fields on the ability of *H. armigera* pheromone traps to predict egg and total adult population. The results suggested that in insecticide free fields, trap catch data could be effectively used to predict egg and total population of adult *H. armigera*, while in fields where either cypermethrin or endosulfan had been applied only the total population of *H. armigera* could be predicted.

Overall, despite many promising results and the ready availability of pheromone traps for *H. armigera* in India few farmers are using them to monitor pest incidence and base their pest control actions on trap catches. Rather farmers' view them as a means of control by 'mass monitoring', a corruption of the term mass trapping. Extension workers and promoters of the technology have not discouraged this view because it is assumed that removal of any pest insects will contribute to the overall control of a species however small the effort but such attitudes leave farmers at risk of crop failure.

Pink bollworm, *Pectinophora gossypiella*

El-Zanan, and El-Hawary, (1999) examined the sensitivity of adult male *Spodoptera littoralis*, *Pectinophora gossypiella* and *Earias insulana* to pheromone and light traps, and the relationship between trap catches and subsequent larval damage. They concluded that all three species were more attracted to pheromone than to light traps and that pheromone traps were better at detecting pest populations particularly early in the growing season. Correlation values between percentage infestation of *E. insulana* and the number of trapped adults were higher in pheromone traps than light traps, but they only found a weak correlation between *S. littoralis* adult catch and egg deposition despite high adult moth catches. Correlation coefficient values (*r*) between percentage infestation and adult *P. gossypiella* trapped by light and pheromone traps were 0.41 and 0.91 respectively in the first season.

In a related study Nassef and Watson (2002) studied the relationship between the early appearance of *P. gossypiella* moths in sex pheromone traps caught six days prior to the first squaring and blooms, and larval infestations of bolls in early and late fruiting stages. The data showed that mean catches of male moths per trap per day were positively correlated with percentage larval infestations. Also, the larval population in blooms showed a positive correlation with that in bolls during early and late fruiting stages. The authors went on to suggest that the data were useful in identifying cotton fields and areas that had a

probability of developing economically important infestations during the season.

Hussein and Kostandy (2002) studied the relationship between early spring trapping of *P. gossypiella* moths and its population during the cotton season. They found that the number of *P. gossypiella* moths that emerged from diapausing larvae in the spring was correlated with the population in the flowering period and that this in turn was correlated with the number of moths caught during the boll formation period.

Ortiz *et al.* (1999) applied the principles of IPM to cotton in Costa Rica and Brazil, during 1997 and 1998. They set an economic threshold of 5% of plants attacked by colonies of three or more aphids, *Aphis gossypii*, 10-15% of plants attacked by *Heliothis virescens* and *Spodoptera* spp. They used pheromone traps to monitor *P. gossypiella* and established a control threshold of 10 adults/trap for 2 consecutive nights. In related studies in Sao Paulo, Brazil Fernandes, *et al.* (1992) used an economic threshold of 5 to 15 adults/trap/night for *P. gossypiella* on cotton. They observed a cyclic pattern in the population density that they related to changes in climatic conditions and food availability. They recorded the highest population density during the 4th and 5th phenological stages of the crop. They also observed high catches in between crop seasons, which they attributed to the so-called 'suicidal population', since the adults were unable to establish a new generation, as observed in other countries notably Egypt.

Leafworm, *Spodoptera litura*

Ranga Rao *et al.* (1991b) tested the efficacy of four types of pheromone traps (single- and double-funnel, small (5 cm dia.) and large (20 cm dia.) sleeve traps) for catching adult males of *S. litura* in groundnut fields. They conducted night observations and found that many moths escaped from sleeve traps. They found no significant difference in the performance of single- and double-funnel traps and because the single-funnel trap (20 cm dia.) captured more moths than any other type of trap they were adopted for use in a national monitoring network in India. In related work Ranga Rao *et al.* (1991a) showed that not only was trap catch affected by trap height but that the relative catch at different heights varied across a season. The authors interpreted this as a reflection of the migration of insects because the highest catches after the crop had been harvested were from traps placed at 4 m height.

Significantly Krishnaiah (1986) reported on the use of pheromone traps for both monitoring and control of *S. litura* on black gram, *Vigna mungo*, in rice fallows in the Gunter district of Andhra Pradesh, during the 1982-83 and 1983-84 rabi seasons. In this study he demonstrated that a catch of 160 males/trap per night in sleeve traps baited with pheromone was calculated to be the threshold level from which the pest would reach economically damaging levels within a week.

Sridhar *et al.* (1988) studied the relationship between pheromone trap catches and population of egg-masses in the groundnut crop and Syobu *et al.* (2003) studied the fluctuation in the number of soybean leaves newly injured by young *S. litura* larvae from 1999 to 2002 in Saga Prefecture, Japan. They found that pheromone trap catches were a good predictor of the timing of leaf damage by plus

or minus three days in both low populations in mid to late August and high populations in mid to late September. On the other hand, effective accumulative temperature was found to be ineffective as a means of predicting larval damage.

Mahalingam *et al.* (2003) studied the effects of total rainfall, maximum and minimum temperature, relative humidity, percentage and number of hours sunshine on pheromone trap catches of *S. litura* in groundnut. They found that rainfall had no effect on trap catches although maximum temperature had a positive association with trap catches during winter 2000-01 (99% probability) and during the 2000 rainy season (90% probability). Trap catches during both winter seasons were significantly positively correlated with the total number of hours of sunshine. Relative humidity affected catches positively during the rainy season and negatively during the winter season. Similarly, Senapati *et al.* (1990) showed that there was a significant positive relationship between pheromone trap catches of *S. litura* and the maximum temperature in studies conducted in West Bengal between 1986 and 1988.

Root Crops

Sweet potato weevil, *Cylas formicarus*

In order to develop a monitoring or mass trapping system for insect pests it is important to know the best locations, type and density of traps that are needed to provide the desired result. In a preliminary study on the sweet potato weevil, *Cylas formicarus* Chiranjeevi *et al.* (2002) studied the effect of trap placement, height and density on catch. They found that the further the traps were placed from an infested field (1 to 10 m) the lower the number of weevils caught and that traps placed 10 m apart caught more than those placed at 5, 15, 20 or 25 m apart. Importantly, they found that lures placed among sweet potato tubers attracted female weevils confirming that traps placed in stores could well encourage immigration into the store resulting in increased weevil populations and hence damage.

Potato tuber moth, *Phthorimaea operculella*

Chandel *et al.* (2001) used pheromone traps under field and storage conditions to monitor adult potato tuber moth, *Phthorimaea operculella* in the Kangra valley of Himachal Pradesh, India, during 1997 - 98. The traps indicated that adult activity started by the first week of February and peaked in mid-May under field conditions, which corresponded with harvest. Tuber infestation ranged from 2.1 - 4.0% at harvest and increased to between 43 and 60% in country stores. Mean temperature had a significant and positive correlation on adult population while relative humidity (RH) and rainfall had a negative association with pest population build-up. Temperature and RH contributed 67-76% of the moth catch during the 1997 and 1998 cropping seasons.

Soft fruits

Currant clearwing, *Synanthedon tipuliformis*

Karalius *et al.* (2003) monitored the currant clearwing, *Synanthedon tipuliformis* (Lepidoptera, Sesiidae) with delta traps baited with 0.5 mg pheromone (*E,Z*)-2,13-octadecadien-1-yl acetate) in Lithuania over a three-year

period and were able to show that there were significant correlations between the average number of male moths caught during the flight seasons and infestation levels in the spring of the same year ($r = 0.70$), the absolute number of male moths in a currant field ($r = 0.58$) and, most interestingly, the infestation level in the currant fields in the spring of the following year ($r = 0.78$) using the regression curve $y = 0.125x$, where y was the level of infestation and x the number of male moths caught based on a threshold of 10% damaged stems corresponding to 80 moths per trap per season.

Grape-berry moth, *Sparganothis pilleriana*

Samoilov (2003) reported the use of pheromone traps to establish the optimal time and rate of application of biological control agents for grape-berry moth, *Sparganothis pilleriana*, in vineyards in the Ukraine. The results confirmed the possibility of using combinations of microbial insecticides and fungicides for successful disease and pest control in a region, Odessa, where damage was typically 20 - 30% and can reach 50 - 60% during epidemics. The author reported the use of a complex of biological control measures for diseases and pests on grape including use of *Bacillus thuringiensis* against *S. pilleriana* and spider mites (*Tetranychidae*) and *Pseudomonas* (Gaupsin) against other pests and diseases.

Tree crops

Coffee berry borer, *Hypothenemus hampei*

Coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) is a major introduced pest of coffee in India. Cárdenas (2000) reported an evaluation of traps baited with methanol and ethanol mixtures for monitoring *H. hampei* on coffee in several regions of Colombia. Baits composed of a 3 : 1 blend of methanol and ethanol together with processed coffee placed in funnel traps was found to be the most effective for detecting the foci of major infestations or monitoring the impact of control procedures on populations.

Codling moth, *Cydia pomonella*

Cydia pomonella is a world-wide pest of apple and this has resulted in a wide range of pheromone monitoring systems and associated guidelines being developed for interpreting trap data (Riedl, 1980a). Nevertheless, at least lures usually consist of 1 mg of the major component of the sex pheromone (*E,E*)-10,12-dodecadien-1-ol (Reolofs *et al.*, 1971) impregnated on rubber septa. Interpretation of trap catch data is dependent on the locality because of differences in orchard type, topography, climate, cultural practises, host species, density and use (Riedl, 1980a). Thus for example, in many countries there are two to three overlapping generations of moths, whereas in England there is one generation and South Africa there are three generations. In England a simple threshold of five moths per trap per week is used to determine whether insecticide should be used against the moths' progeny (Alford *et al.*, 1979). The insecticide is then applied 140 day-degrees after threshold (Glen & Brain, 1982), and a second application 100 day-degrees later. Similar strategies were adopted in other countries but with a range of thresholds dependent on local conditions and experience (Wall, 1989).

Macadamia borer, *Ecdytopha torticornis*

In the late 1980's a new pest was observed in macadamia nut tree plantations in Costa Rica. Field studies conducted by Blanco *et al.* (1993) concluded that a single generation of the pest was completed in 36 days and available field data indicated that there may be have been up to four generations a year, with the development of larvae

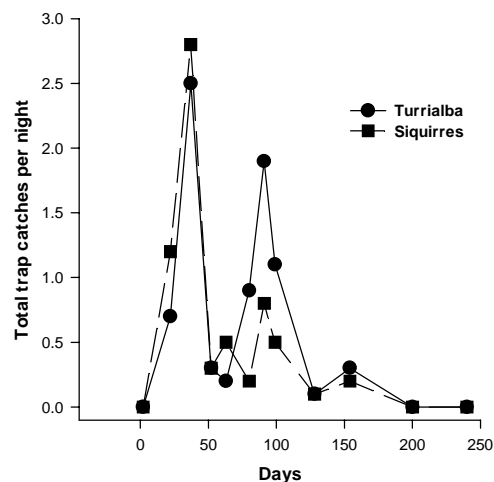


Figure 6.2 Pheromone trap catches of *Ecdytopha torticornis* in macadamia plantations, Costa Rica, 1988

correlated to nut development. Chamberlain *et al.* (2003) established that the moth was *Ecdytopha torticornis* (Meyrick) (Lepidoptera: Olethreutidae) and identified the pheromone as (*E*)-8-dodecenyl acetate (E8-12:Ac). Employing delta traps baited with 1 mg of E8-12:Ac significant numbers of male moths were caught at two locations between May and October 1988 (Figure 6.2). Two peaks in catch were observed, the first occurring in the middle of June with a second smaller peak in August. No moths were trapped between the months of November through to the following May. Pheromone trap catch data obtained were consistent with macadamia nut development suggesting that pheromone traps could play a significant role in understanding the population dynamics of *E. torticornis*. In particular, the traps could provide the basis for an effective population monitoring system to assess the impact of control options implemented in macadamia orchards in Costa Rica.

Macadamia borer, *Cryptophlebia ombrodelta*

In a related study Vickers *et al.* (1998) evaluated pheromone traps for monitoring *Cryptophlebia ombrodelta* in macadamia orchards in New South Wales and Queensland during October 1996 and April 1997. Pheromone trap catches were found to either precede or coincide with a rise in oviposition. When data on the number of eggs recovered was adjusted using an empirical formula a correlation between trap catch and oviposition was established. Thus, the authors recommended that when the first record of six or more moths per eight traps per day was observed egg sampling should begin.

Orgyia thyellina

Hosking *et al.* (2003) reported the first successful eradication of a forest pest established in an urban area. The eradication programme was undertaken between 1996 and 1998 in Auckland's eastern suburbs against a newly established Lymantriid moth, *Orgyia thyellina*. Key components of the programme were: accurate definition of the infested area, quarantine action to prevent further spread, aerial and ground application of *Btk*, intensive monitoring using live female moths and a synthetic pheromone, strong science input to every facet of the programme coupled with a major communications initiative.

Red palm weevil, *Rhynchophorus ferrugineus*

The red palm weevil (RPW), *Rhynchophorus ferrugineus*, is a key pest of palms in Asia, Africa and Europe. It is widely distributed in over 20 countries and attacks more than a dozen palm species of which coconut and date palm are of economic importance. Hallet *et al.* (1993) identified 4-methyl-5-nonanone and 4-methyl-5-nonanol as the major components of the aggregation pheromone. It was later found that 4-methyl-5-nonanol (ferrugineol) was more attractive than 4-methyl-5-nonanone and host plant volatiles such as coconut bark steam distillate. Vidyasagar and Subaharan (2000) recommend a release rate of 3 mg per day for operational trapping programmes in traps placed 2 m above ground level. They also suggest that vane (sticky) traps are more effective than bucket traps (Chapter 6) and that insecticide-free funnel traps are as effective as traps with insecticide.

Pheromone traps have been used to determine the flight periods of adult weevils, 1800 - 2000 and 0600 - 0800 h, and to assess the impact of IPM programmes. However, they are mainly used for controlling weevils by mass trapping rather than monitoring and so will be discussed in more detail in Chapter 8.

Parasitoids

Concern has been expressed about whether insect parasitoids could be adversely affected by attraction to pheromone traps. The finding by Colazza *et al.* (1997) that the oligophagous egg parasitoid species *Telenomus busseolae* is attracted by the pheromone of the corn stalk borer *Sesamia nonagrioides* would seem to confirm this. Nevertheless, the literature provides many positive examples of how host pheromone traps have been used to provide an insight into the movement and prevalence of host parasites, parasitoids as well as the target pest species.

However, although parasitoids might be trapped in pheromone traps it is not always due to the pheromone bait. Schöller and Prozell (2003) examined the responses of three stored-product moth parasitoids, *Habrobracon hebetor*, *Trichogramma evanescens* and *Venturia canescens* (Hymenoptera: Braconidae, Trichogrammatidae, Ichneumonidae), towards funnel traps with three different colours (dark green; green top, yellow funnel and white cup; green top, green funnel and transparent cup). The traps were baited with the synthetic main component of the female sex pheromone, (*Z,E*)-9,12-tetradecadienyl acetate (ZETA). The study confirmed that the colour of the traps was responsible for attracting the parasitoids. Indeed they went on to suggest that the colour of trap could be selected depending on whether the

operator wanted to monitor only the moth species or both the moth and parasitoids thus enabling mass releases of parasitoids to be monitored. To minimise the by-catch of parasitoids and other small non-target organisms small exit holes can be incorporated in trap designs to affect easy escape without adversely affecting catches of the target species.

Table 7.1 Uses of insect semiochemicals in population surveillance

Detection	Quarantine inspection - absence	Khapra beetle, <i>Trogoderma</i> spp. Cotton boll weevil, <i>Anthonomus grandis</i> Old World Screwworm, <i>Chrysomya bezziana</i>	USA USA, South America Australia from PNG
	Early warning of pest - presence	African armyworm, <i>Spodoptera exempta</i> American bollworm, <i>Helicoverpa armigera</i>	East Africa India
	Assess population density & distribution	Larger grain borer, <i>Prostephanus truncatus</i> Spruce budworm, <i>Choristoneura fumiferana</i> Gypsy moth, <i>Lymantria dispar</i> Culicidae (Ovitrap)	East & West Africa Canada North America USA
Thresholds	Timing of control procedures	Pea moth, <i>Cydia nigricana</i> Codling moth, <i>Cydia pomonella</i> Summer tortrix, <i>Adoxophyes orana</i> Cotton leafworm, <i>Spodoptera litura</i>	UK Cosmopolitan Europe India
	Efficacy of control procedures	Pink bollworm, <i>Pectinophora gossypiella</i> Tomato pinworm, <i>Kiereria lycopersicella</i> Grape berry moth, <i>Eupoecilia ambiguella</i> Spruce budworm, <i>Choristoneura fumiferana</i> Pine shoot moth, <i>Eucosma sonomana</i> Oriental fruit moth, <i>Grapholitha fumiferana</i> Striped rice stem borer, <i>Chilo suppressalis</i> Tsetse fly, <i>Glossina</i> spp.	USA, Egypt USA, Mexico USA Canada Canada Australia, USA Spain Sub-Saharan Africa

Table 7.2 Components of pheromone monitoring systems

Component	Function	Comments	
Attractant	Specificity Sensitivity	Species specific or blend Need to avoid trap saturation	Trap one or more species Bigger trap, weaker lure.
Dispenser	Protect semiochemical Uniform release	Sunlight, high temperature, oxygen Ideally zero order, independent of loading	
Trap Design	Maximise trap entry Minimise escape (retention) Ideally use non-saturating trap Maintenance	Depends on size of insect and behaviour Ease of maintenance important for farmer acceptance	Colour, shape, killing agent and cost are important
Trap Deployment	Orchard & forestry pests	Geographical variation in population Locate in tree canopy	Hot spots, place traps in matrix
	Field crops	Avoid artificial light sources Avoid trees or shrubs Usually place at height of crop canopy	
	Storage	Uneven population distribution Flying vs walking entry Sample inside or outside commodity	Aggregations can be temporal Trapped insects may be immigrants
Interpretation of trap catch data		Interpretation of catch depends on sound knowledge of pest biology often in relation to developmental stage of crop and weather conditions.	

Chapter 8: Attract and Kill

Population control with insect semiochemicals

Several techniques have been developed to control insect pests with semiochemicals. These techniques depend on using semiochemicals to attract a pest and then killing it or disorienting the insect in such a way that it is unable to utilise the natural olfactory cues produced by its potential host(s) or mate. In this chapter we will deal with control by attract and kill techniques. Techniques that control by the use of attractants or repellents to cause disorientation will be discussed in Chapter 9.

Attract and kill, as the name suggests, are control technologies that rely on semiochemicals to attract members of a target species to a killing device. The technique depends on the ability of the attractant and killing agent to remove a sufficient proportion of a pest population during the susceptible stages of a crop, for example, in order to reduce the impact of their progeny below the economic threshold.

To be effective there are a number of parameters in attract and kill technologies that need to be fully optimised and include:

- Pheromone blend and formulation
- Dose per point source
- Spacing between point sources
- Efficiency of killing agent
- Method and timing of application

Pheromone blend & formulation

Unlike insect monitoring where a small but representative proportion of a population needs to be sampled in order to decide whether control is required the use of attractant baits for control requires the use of the most attractive bait possible. Normally the natural blend of compounds present in a pheromone, for example, is the most attractive bait available for attracting the target organism. However, the level of attraction can be modified by changing the release rate of the chemicals per unit time and perhaps by adding other chemicals such as host-derived chemicals. Such tactics have been successfully demonstrated for a number of species notably, yellow stem borer, *S. incertulas* (Varma *et al.*, 2002), plum curculios, *Conotrachelus nenuphar* (Pinerio & Prokopy, 2003, Prokopy *et al.*, 2003) and codling moth, *Cydia pomonella* Yang *et al.* (2004). There are cost implications to increasing the number and purity of compounds, especially more volatile compounds employed in an attractant bait. These costs have to be balanced against the value of the crop, economic importance of the pest and market interest.

Dose per point source

Dose of semiochemical is indirectly related to release rate. Most formulations, such as rubber septa release

semiochemicals using first order kinetics and so release rate is dependent on the amount of material in the formulation (Chapter 5). For mass trapping the use of high doses can result in the need for fewer sources therefore cutting down on the number and hence cost of traps needed to achieve control per unit area. However, if dose is too high attraction can be impaired resulting in a drop in trap catch or inhibition of male moths for example alighting on an insecticide treated point source.

Spacing between point sources

As suggested above the spacing between point sources, such as traps for example, is dependent on a balance between the cost of each point source and the number of insects caught by each trap.

Ranga Rao *et al.* (1991b) investigated the effect of trap density on catch of *S. litura* using rubber septa baited with 1 mg of pheromone in funnel traps. They found that four traps per ha was optimal with more traps resulting in no additional catch and fewer than four traps resulting in lower catches.

Lure and kill systems that incorporate insecticide into the point sources rather than using traps tend to be applied in much higher numbers of point sources in a crop and may control as much by false trial following as killing by insecticide poisoning.

Efficiency of killing agent

If insecticides are to be used in combination with point sources as in lure-and-kill or attracticides then the controlled release formulation used to protect and release the semiochemical has to be sufficiently versatile to protect the killing agent and provide a means of allowing the insecticide to make contact with the target pest. This is normally achieved by employing a 'sticky' substrate so that the formulation adheres to the insect when it alights on the pheromone source. Source contact and transfer of insecticide is more easily achieved with Diptera than Lepidoptera because Diptera do not have the scales often associated with Lepidoptera. Equally the insecticide has to achieve a fast knockdown without producing a deterrent effect.

Method and timing of application

Hand application of point sources, as used in attracticides, can be time-consuming and hence expensive to apply in the field, although farmers save the cost and maintenance associated with traps used in mass trapping.

The timing of application can be critical for success. This could be based on pheromone trap monitoring or alternatively it could be applied prophylactically but the method of choice will be dependent on the value of the crop, the nature of the damage caused by the pest and the damage threshold tolerated by consumers.

Ideally the timing of application of mass trapping using pheromones would be just before the emergence of the first adult generation or the flowering period of a crop when pests such as *H. armigera* are known to arrive. However, the timing of application may not be convenient for the farmer and so attract and kill systems need to be developed in partnership with farmers to ensure that the technology meets their needs as well as solving the pest problem otherwise they will not be adopted.

Lure-and-kill

Lure-and-kill is a name applied to a particular form of population suppression technique in which male moths are lured to an attractant bait and killed after alighting on the bait and picking up a lethal dose of insecticide. However, the killing agent could be an insecticide, trap, or electrocution device. Where traps are used the technique is commonly known as mass trapping. When baited with Lepidopterous sex pheromones the traps catch only male moths, but since most male insects can mate more than once a high proportion of the male population must be removed before this can have any impact on population levels. The technique suits moths that are weak fliers, such as the cocoa pod borer, *Conopomorpha cramerella*, and the groundnut leaf-miner, *Aproaerema modicella*. Early attempts made to control pests such as *S. littoralis* by mass trapping in Egypt were unsuccessful (D. G. Campion personal communication). The technique is not thought to be suitable for use with strong fliers that are known to disperse over large areas, such as *H. armigera*, (see Chapter 7) although is apparently promoted by commercial companies in India who advocate employing 30 to 40 traps/ha (R. C. Jhala personal communication).

The use of food baits, such as floral odour cues, can provide an alternative or complementary strategy that could be more efficacious since it would attract both male and female moths and so have a greater impact on population levels. Kairomone baited targets impregnated with insecticide have found particular application for the control of tsetse fly, *Glossina* spp. in southern Africa. Target densities of as little as two per square km have been found to reduce population densities by 99.99% in less than one year (Colvin and Gibson, 1992; Jordan, 1990).

Brinjal shoot and fruit borer, *Leucinodes orbonalis*

Brinjal (*Solanum melongena* L.) is an economically important vegetable grown throughout much of South and South East Asia. Highly regarded by rich and poor alike South Asia possesses a wide range of varieties that have been developed and adapted to local preferences. The crop is long-duration (up to eight months) and because mature crops can be harvested weekly it is popular with resource poor farmers providing an assured income. Nevertheless, it is susceptible to a number of insect and disease pests. The main insect pest within the complex is the brinjal shoot and fruit borer, *Leucinodes orbonalis*. Larval damage in the fruit commonly results in losses of 50% or more in intensively grown areas. Insecticides appear to be largely ineffective for control of *L. orbonalis* (K. Srinivasan, personal communication) in part because of the protection offered by the fruit itself but also because overuse has undoubtedly led to insecticide tolerance if not resistance. Nevertheless, in the absence of alternatives

farmers apply insecticides in ever increasing doses which has led to concern over farmer and consumer health.

The main component of the female sex pheromone of *L. orbonalis* was identified by Zhu *et al.* (1987) as (*E*)-11-hexadecenyl acetate (E11-16:Ac) however Attygalle *et al.* (1988) subsequently identified a minor component of the pheromone (*E*)-11-hexadecen-1-ol (E11-16:OH) which was subsequently shown by Cork *et al.* (2001) to be important for attraction at the between 1 and 10% of E11-16:Ac. The authors also found that trap catch was positively correlated with pheromone release rate, with the highest dose tested, 3000 µg catching significantly more male moths than lower doses.

Trap catch was found to be influenced by trap height, with catches being maximized at or just below crop height. Trap design had a profound effect on catch. Of those tested the Alam water trap was found to be the most effective (Chapter 6) (Cork *et al.*, 2003).

Initially small scale replicated mass trapping trials (three 0.5 ha plots per treatment) were conducted in farmers fields in Bangladesh with young and mature brinjal crops. Farmers were allowed to continue applying insecticides at least three times a week in both check and mass trapped plots. In the mass trapped plots open-delta traps (Chapter 6) were introduced, 50 per plot, and infested shoots were removed manually once a week by the researchers. In the young crop pheromone trap catches were reduced significantly from 2.0 to 0.4 moths per trap per night in check and IPM plots respectively and in the mature crop 1.1 to 0.3 moths per trap per night in check and mass trapped plots respectively. Over the course of the trial fruit damage was significantly reduced from an average of 41.8% and 51.2% in young and mature check plots respectively to 22% and 26.4% respectively in the associated mass trapped plots. The relative impact of removing infested shoots and mass trapping on *L. orbonalis* larval populations was not established in these trials.

In both trials there was an estimated 50% increase in marketable fruit obtained by the combination of control techniques compared to insecticide treatment alone (Cork *et al.* 2002). Subsequent mass trapping trials without the use of insecticides resulted in even higher levels of control. This was attributed to the combined effects of mass trapping and natural enemies, the latter were previously suppressed by the application of insecticides. In addition, secondary pests such as mites and whitefly were reduced in the IPM plots compared to the insecticide treated checks (S. N. Alam *et al.*, unpublished data).

Yellow rice stem borer, *Scirpophaga incertulas*

One third of all food grain produced in India comes from rice, approximately 74 million tonnes in 1990 from 42 million ha (Kanth, 1993). Yield losses associated with pre-harvest insect damage in India have been estimated at between 25 and 30% percent in unprotected rice (Sasmal *et al.*, 1986). In India the major pre-harvest loss is associated with the feeding activities of the larvae of the rice stem borers, and the yellow rice stem borer, *Scirpophaga incertulas* (Walker) (Lepidoptera: Pyralidae) (Chelliah *et al.*, 1989; Kalode & Krishnaiah, 1991) in particular.

In southern India, *S. incertulas* completes between 4 and 5 generations a year, depending on prevailing environmental conditions. On average the life cycle is completed in 31 to 40 days. Oviposition of the first generation on a crop occurs during the nursery stage and, if unchecked, can lead to significant damage during the vegetative and reproductive growth stages.

The sex pheromone of *S. incertulas* was identified by Cork *et al.* (1985) as a 1 : 3 blend of, (Z)-9-hexadecenal (Z9-16:Ald) and (Z)-11-hexadecenal (Z11-16:Ald). However, it was not until the late 1980s that formulations became available that allowed the pheromone to be exploited for control by mating disruption. Nevertheless, even though this was found to be efficacious (Chapter 9) it was not cost-effective given the relatively low value of the rice crop or acceptable to the rice farmer (Cork, 1998).

Krishnaiah *et al.* (2000)
Katti *et al.*, 2001.

In order to try to reduce the cost of pheromone-based technologies for control of *S. incertulas* workers at the Directorate of Rice Research, Hyderabad, India, embarked on a programme of research to develop an effective mass trapping system. This proved to be highly effective using indigenous traps and lures at a density of 20 traps per ha (Cork & Krishnaiah, 2000).

Farmer participatory evaluation trials were conducted to assess the significance of the technology for rice cultivators. The trials were co-ordinated by non-governmental development organisations and monitored by socio-anthropologists and entomologists. Results suggested that rice cultivators were interested to participate by providing land for trials, applying the lures and attending meetings arranged to learn more about their social, economic, cultural and institutional constraints to adopting the technology. Nevertheless, there was no evidence to suggest that they were prepared to modify their normal pest control practices when using mating disruption, a situation re-enforced by a natural aversion to risk. The project highlighted deficiencies in communication linkages and problems with introducing individual technologies at variance with established pest control procedures (Cork 1998).

To address these issues a new strategy was proposed that would endeavour to incorporate the pheromone-based control technology into an existing Farmer Field School programme developed by Syngenta Bangladesh Limited in collaboration with the Bangladesh Rice Research Institute (BRRI). In a two-year project researchers from Syngenta, BRRI and NRI were able to develop a highly sensitive pheromone trapping system. Larval field data together with pheromone trap catch data in two consecutive Boro seasons demonstrated that intervention with insecticides was not required, because populations remained below the damage threshold, and that mass trapping provided an effective means of controlling *S. incertulas* in the T. Aman season.

Efforts have also been made by Varma *et al.* (2002) to increase the attractiveness of pheromone baits for *S. incertulas* by adding vitamin E and or an extract of the rice cv. Pusa Basmati. The authors found that addition of both Pusa Basmati and vitamin E to the synthetic pheromone resulted in a doubling of catch compared to pheromone alone.

Palm and cotton weevils

Rhynchophorus palmarum is the primary pest of oil, coconut and palmetto palm in central and south America. It is also the vector of red ring nematode, *Bursaphelenchus cocophilis*. Adult male *R. palmarum* weevils produce an aggregation pheromone that attracts both male and female weevils. The pheromone is used to bait bucket traps along with food volatiles and insecticide. In order to control *R. palmarum* in Coto District, Costa Rica in 1989 4,500 trees were removed from 5,500 ha of palm oil, but by 1992 that number had increased to 22,000. A clear indication that 'sanitation' felling of trees was failing to control the weevil. Mass trapping started in 1992 with one trap per ha using lures produced by Chem Tica Limited (Costa Rica). By 1994 the number of trees needing to be felled was reduced to less than 3,000 per annum demonstrating that mass trapping can be highly effective in controlling palm weevil populations (Alpizar *et al.*, 2002; Hallett *et al.*, 1999; Oehlschlager *et al.* 2002).

In South Asia the related species *Rhynchophorus ferrugineus*, is a pest of date, oil and coconut palm. *R. ferrugineus* also produces an aggregation pheromone and this is used to bait bucket traps with food volatiles and insecticide. The market for *R. ferrugineus* lures is of the order of 200,000 per annum in the Middle East (Arab Emirates, Saudi Arabia, Oman and Bahrain). The traps are also used in Egypt, India and Sri Lanka.

Pheromone traps for the adult weevils could provide effective means for monitoring and, more importantly, control of *R. ferrugineus*, as has been reported for the related *R. palmarum* in Central and South America by Oehlschlager *et al.* (1993, 1995), and large-scale trials are being carried out in Sri Lanka. The aggregation pheromone of another major pest of coconut palms, the coconut rhinoceros beetle, *Oryctes rhinoceros* has been identified by Hallett *et al.* (1995) and is also being evaluated by the CRI in Sri Lanka.

The cotton weevil, *Anthonomus grandis*, is a major pest of cotton in the Americas. Male *A. grandis* produce an aggregation pheromone, Grandlure, that has been successfully incorporated into a 'bait stick' known as Boll Weevil Attract and Control Tubes (BWACT). Over 1,000,000 tubes are produced by Plato Industries in the USA per year. Having successfully controlled the boll weevil in the USA current control efforts are focused in South America. The Paraguay Ministry of Agriculture, "Plan de Reactivacion del Cultivo del Algodonero, 1997-2002" employed 225,000 BWACT in 14 provinces at the rate of one per ha (total cotton area). This work resulted in a >85% reduction in weevil populations, delayed insecticide applications by up to 6 weeks, required eight fewer sprays per season and reduced damage from 40% to minimal. The BWACT is also used in, Colombia (100,000 ha), Brazil (150,000 ha), Argentina (50,000 ha)

and Bolivia (20,000 ha). (Tom Plato, Plato Industries Ltd., Texas USA. personal communication).

Spodoptera litura

Significantly Krishnaiah (1986) reported on the use of pheromone traps for both monitoring and control *S. litura* on black gram, *Vigna mungo*, in rice fallows in the Gunter district of Andhra Pradesh, during the rabi seasons of 1982-83 and 1983-84. In this study he demonstrated that a catch of 160 males/trap per night in sleeve traps baited with pheromone was calculated to be the threshold level from which the pest would reach economically damaging levels within a week.

The synthetic pheromone lure was evaluated for use in monitoring and mass-trapping males of *S. litura*. Based on the relationship between captures and larval incidence/damage, Mass-trapping of males over a 240-ha area at a rate of 5 traps/ha was found to be promising in the suppression of the pest and in reducing insecticide usage. In a trial on the effect of pheromone trap density on moth captures, 10 traps/ha were significantly more effective than the other lower trap densities evaluated, indicating the possibility of improving the efficiency of the technique by increasing trap density.

Tsetse fly, *Glossinidae* spp.

'Sleeping sickness' in humans and "nagana" in cattle are diseases transmitted by the obligate blood-feeding tsetse fly. Until the mid-1980's tsetse were controlled by ground and aerial application of insecticide. However, international donors were not prepared to continue funding widespread use of residual insecticides because of their effect on non-target organisms. This provided the impetus for the development of alternative control technologies.

Early work by Vale and colleagues successfully demonstrated that tsetse were attracted to potential hosts by a number of cues, visual (colour, movement) and host-related odour. These findings led to an intensive search for host odours that could be utilised as baits for controlling tsetse.

Bursell *et al.* (1988) reported the identification of a number of host odour attractants for tsetse that led directly to the development of odour-baited traps and targets. Currently available odour-baited targets utilise polyethylene sachets (Torr, 1997) and 'blood bottles' to release the kairomones have a field life of one year. When used at a density of 4 per km² they have been found to be very effective for strategic control of tsetse populations (Hargrove 1993, Green 1994). Nevertheless, farmers prefer to use insecticide-based 'pour-ons'. Cattle treated with a pour-on become, in effect, a lethal target for tsetse but because of the clustering of cattle herds such an approach is not effective for area-wide control. In 1984 when odour-baited targets were beginning to be used in endemic areas of north east Zimbabwe to control tsetse on a large scale 10,000 cases of nagana were reported but by 1997 this had been reduced to zero. The work not only demonstrated the efficacy of odour-baited trap technology for control of tsetse but more importantly it had controlled disease transmission (S. J. Torr, personal communication). The technique is now used as a practical and cost-effective alternative to insecticide spraying in areas in excess of

5,000 sq. km and currently provides a barrier in North Eastern Zimbabwe to reinvasion from Mozambique. In this instance the low reproductive potential of tsetse and its susceptibility to the insecticide used both significantly contribute to the efficacy of the technique. Visit the website tsetse.org for the latest updates on the technology and technical assistance for implementation.

Fruit fly

Fruit flies are familiar causes of economic damage in tropical fruits throughout much of the world and South Asia in particular, notably in mango and papaya. However, they are equally important in vegetable fruits and in particular gourds. In fact the family Tephritidae includes species that infest stems, galls and flowers as well as fruit. The species range from monophagous to polyphagous in habit and can generally be divided between Old World, *Bactrocera* species and New World, *Anastrepha* species.

Considerable research has been conducted on the pheromones of fruit flies with varying degrees of success. This work has been reviewed by various authors over the years. Early reviews of note include those by Nation (1977) and Chambers (1977). Sivinski and Calkins (1986) reviewed the use of pheromones and parapheromones for their use in control and eradication of fruit flies. This review was updated by Fletcher and Kitching (1995) and Landolt and Averill (1999). Despite the research conducted on fruitfly pheromones they are not widely used for fruit fly control. The general situation was summarised by Landolt and Averill's (1999) comment on the pheromones of the Caribbean fruit fly, *Anastrepha suspensa*, where none of the chemicals identified had been demonstrated to be attractive to Caribbean fruit flies in field trials and that progress in developing lures was hampered, in part, by a lack of chemicals and methods for formulating blends comprised of chemicals of differing volatilities. The situation is further complicated by the realisation that for many species sound and visual cues are also important.

Most male fruit flies produce pheromones that attract females. However, the female-produced pheromone of the olive fly, *Bactrocera oleae*, is perhaps the most widely used pheromone for control. The major component of the pheromone was identified by Baker *et al.* (1980) as (1,7)-dioxaspiro-[5,5]-undane (olean), and three other components, α -pinene, *n*-nonanal and ethyl dodecanoate were subsequently identified by Mazomenos and Haniotakis (1985). *B. oleae* is now widely controlled by a lure-and-kill technology that incorporates the use of an attractant blend comprised of a food attractant (ammonium bicarbonate), sex pheromone (olean), feeding stimulant (sugar) visual cue (yellow board) and insecticide (Haniotakis *et al.*, 1991).

The parapheromones methyl eugenol and cuelure are most widely used for attraction and control of oriental fruit fly, *Bactrocera dorsalis* (Hendel), and melon fly, *Bactrocera cucurbitae* (Coquillett) respectively and so there has been little incentive for research on the sex pheromones of these species.

Table 8.1 Application of lure-and-kill for control of insect pests

Technique	Killing agent	Bait	Examples	Country used
Lure-and-kill	Traps (mass trapping)	Pheromone-baited traps	Cocoa pod borer, <i>Conopomorpha cramerella</i> Spruce bark beetle, <i>Ips typographus</i> Fir beetles, <i>Dendroctonus</i> spp. Boll weevil, <i>Anthonomus grandis</i> Palm weevil, <i>Rhynchophorus palmarum</i> Olive fly, <i>Dacus oleae</i>	Malaysia Scandinavia Canada, USA USA Costa Rica, Spain
		Food-baited traps	House fly, <i>Musca domestica</i> Sheep blowfly, <i>Lucillia cuprina</i> New World Screwworm	USA, Europe Australia Central America
	Insecticides	Pheromone-baited (attracticide)	Egyptian cotton leafworm, <i>Spodoptera littoralis</i> Pink bollworm, <i>Pectinophora gossypiella</i>	Egypt USA
		Kairomone-baited targets	Tsetse fly, <i>Glossina</i> spp.	Africa
		Food-baited	German Cockroach, <i>Blatella germanica</i> , American cockroach, <i>Periplaneta americana</i>	USA, Europe USA, Europe
	Electrocution	Pheromone-baited	House fly, <i>Musca domestica</i> German Cockroach, <i>Blatella germanica</i> , American cockroach, <i>Periplaneta americana</i>	USA, Europe USA, Europe USA, Europe

Chapter 9: Mating Disruption

Given the importance of semiochemicals to insects for the identification and location of potential mates, the ability to interrupt these messages could provide a powerful means of controlling individual pest species. Indeed the most commonly utilised application of semiochemicals to affect population control of noxious insect species has been by 'mating disruption'. As the name suggests application of the technique results in the disruption of semiochemical mediated communication. Bartell (1982) proposed five mechanisms by which this could be achieved:

- 1) Sensory adaptation of pheromone receptors and central nervous habituation;
- 2) False trail following caused by a multiplicity of trials produced by synthetic odour sources;
- 3) Inability of insects to discriminate odour trials from background;
- 4) Imbalance of sensory input caused by the release of an unnatural ratio of pheromone components;
- 5) Peripheral sensilla blocked by pheromone analogues.

These definitions were later refined by other authors, notably Cardé and Minks (1995) and Sanders (1997). Nevertheless, despite widespread application of mating disruption the mechanism or mechanisms by which it operates are poorly understood. This lack of knowledge is important because, as Cardé and Minks (1995) have already pointed out, if we understood the underlying mechanism(s) that cause or result in the behavioural response we call 'mating disruption' we would be better placed to understand why some formulations are successful and others not. In fact a more thorough understanding of the underlying mechanism(s) of mating disruption would enable the design of more effective and predictable products. This in turn would deliver long term cost-savings for manufacturers and through increased end-user confidence, higher levels of adoption and hence market share.

Wyatt (2003) pointed out that mating disruption 'has often come to the rescue when pests have become resistant to conventional pesticides'. Such statements provide credence to what researchers have felt for many years, that mating disruption is not the method of choice but rather adopted as a last resort. Why should this be so? One theory put forward for the lack of adoption of mating disruption by Cork (1998) while working on control of *S. incertulas* in rice in India was farmer risk aversion but in reality this is only part of the story. Farmers are risk averse and frequently overestimate their losses due to insects and other pests resulting in an over-application of pesticides (Heong & Escalada 1998, Cork & Iles unpublished). While this overuse might be expected to occur in high value crops, it is not restricted to such crops. Huan *et al.* (1999) reported the success of a media and **farmer field school** (FFS) campaign to influence pest control strategies used by 2.3 million rice farmers in the Mekong delta, Vietnam. Over a five-year period they

were able to demonstrate that the average use of insecticides was reduced from 3.4 to 1.0 spray per season. Interestingly, the authors felt that the FFS training method that reached 4.3% of the farmers and the media campaign were complimentary and contributed in different ways to change farmers' perceptions of the need to control early season leaffolder populations. For many rice farmers in India the market value of the crop is too low for farmers to afford to use high application rates of pesticides, except in regions where the crop is specifically grown as a cash crop or a speciality, aromatic rice that commands a high value. Frequently, where farmers are generally content with the control options they use there is little incentive for change. This makes the introduction of newer technologies such as mating disruption difficult. Nevertheless, when the level of economic loss increases and insecticides do not control the main pests then farmers begin to be more receptive to alternatives. An example of this is the current success of introducing mass trapping for control of the brinjal fruit and shoot borer, *L. orbonalis* in South Asia.

Even if insecticides do not fully meet the needs of farmers they are well understood and their impact is immediately apparent by the presence of dead larvae. In contrast, mating disruption has no apparent immediate impact on the target pest and it has to be taken on trust that the crop will benefit in the longer term. For these reasons control by mating disruption has not yet been accepted on any crop in South Asia. Despite these drawbacks if farmers are exposed to mating disruption and provided with sufficient training then adoption can be achieved. There are numerous examples of area-wide adoption in a range of crops and some notable examples of success will be provided later in the Chapter.

In early work on mating disruption it had been assumed that a very high density of point sources was required to produce an effective fog of pheromone to disrupt male moths locating and orientating along a pheromone trail or plume. Thus, formulations such as aqueous suspensions of micro-capsules (Pectone[®]) (Hall & Marrs 1989) and hollow fibres were developed. The former having the advantage that it could be applied using conventional spray equipment. However, with the advent of the ShinEstu twist tie formulation and the realisation that fewer point sources releasing higher quantities of pheromone could achieve the same result, though presumably by generating false trails rather than the camouflage of natural plumes with high concentrations of synthetic pheromone.

Unlike mass trapping the natural pheromone of the species being controlled does not need to be employed for mating disruption to be effective. Thus, the use of so-called 'off-blends' can result in considerable cost savings both in manufacture and to the end-user. Cost savings were achieved by using blends of chemicals with lower technical specification, incomplete blends, or blends where the ratio of components is changed to reduce the amount of the more expensive compounds. This approach was successfully adopted by Cork *et al.* (1996) for control of *S. incertulas* in rice where cost was an

important factor in the development of a pheromone-based control strategy. The use of an off-blend of pheromone components would be expected to lead to an imbalance of sensory input (Flint & Mertle, 1983).

Pheromone analogues have been developed in place of natural compounds. This work was stimulated by the desire to develop either more stable analogues of pheromones, such as formates to replace the less stable aldehydes (Beevor *et al.*, 1981) or to reduce the cost of natural compounds. While some have been shown to be efficacious they are invariably less active than the natural compounds and so any cost savings are lost through the need for greater application rates.

Antagonists have also been developed and although they would be expected to find application through the blockage of the natural function of peripheral sensilla they have either not been found to be efficacious or remained as research tools for probing the underlying mechanism of transduction in insect sensilla.

It is generally accepted that the natural blend will produce the best levels of mating disruption (Minks and Cardé, 1988). However, this conclusion has been questioned by Suckling and Shaw (1992) and Cork *et al.* (1996) found no difference in the efficacy of natural and unnatural blends of the *S. incertulas* pheromone for use in control by mating disruption. Nevertheless, Mochizuki *et al.* (2002) reported resistance to mating disruption of the smaller tea tortrix moth, *Adoxophyes honmai* after large scale commercial application for 16 years. The level of control with a single component of the pheromone, (*Z*)-11-tetradecenyl acetate (Trade name Hamak-con) was found to be reduced from 99% to less than 50% between 1996 and 1998. However, by changing to the natural pheromone blend (63 : 31 : 4 : 2 ratio blend of (*Z*)-9-tetradecenyl acetate, (*Z*)-11-tetradecenyl acetate, (*E*)-11-tetradecenyl acetate and 10-methyldodecyl acetate) the percentage of mating disruption obtained was returned to its former level of 99%.

Formulations

As mentioned earlier a wide range of controlled release formulations have been developed for use in mating disruption. These have been reviewed (Howse *et al.*, 1998) and so will only be briefly discussed here. As with all controlled release formulations developed for pheromones they need to both protect and release pheromone components in a predictable manner.

Early work in the USA resulted in the commercialisation of hollow fibres developed by Albany International (subsequently Scentry, then Yellowstone International) and laminate flakes developed by Hercon Division of Health-Chem Corporation and sold under licence by BASF in Germany. The flakes consisted of layers of plastic laminate with a porous layer impregnated with pheromone in between. Varying the size and thickness of the laminate flakes changed the release rate characteristics (Quisumbing & Kydonius, 1982). The hollow fibres were composed of polyacetate capillary tubes (15 mm length, 0.2 mm diameter) sealed at one end and containing approximately 250 µg of pheromone (Golub & Weatherstone, 1984) that were applied by specialist equipment and held onto plant substrates by mixing with

a, none setting, polybutene glue. Being capillary tubes they had a finite field life, and for crops that were susceptible to insect damage over long periods, such as cotton there was a need to reapply the dispenser several times in a season. This situation was also true of micro-encapsulated pheromones such as PECTONE®.

Twist-tie dispensers were developed by Shin Etsu Company, Japan and marketed by Mitsubishi Corporation under the trade name of PBW Rope. The twist-ties consisted of 100-200 mm long polyethylene tubes containing a soft-wire stiffener and vitamin E as an antioxidant. They could be seen as a compromise between simple polyethylene sachets (Torr *et al.* 1997) and polyethylene vials used for lures in pheromone traps. In common with polyethylene sachets twist-ties have long field lives and presumably by modifying the polymeric composition, wall thickness and surface area of the dispensers release rates could be optimised for different pheromones. More recently, PVC based monolithic polymers have been employed as substrates for releasing Lepidopterous pheromones (Cork *et al.*, 1989). PVC polymer dispensers are prepared by mixing the pheromone with a pre-polymer and heating in the absence of air to 180°C. The resulting dispensers can be prepared in any shape or colour and release rate is dependent on the ratio of surface area to volume. Thus, cubes and spheres would provide dispensers with the field lives of up to one year (Cork *et al.*, unpublished data).

Optimising parameters for mating disruption

Optimisation of parameters for mating disruption is important not only to achieve a high level of communication disruption but also to achieve the most cost-effective technology. However, it should be appreciated that such parameters as dose and spacing between dispensers may well be affected by the size of the area treated, so that, in general, the larger the area treated the greater the dispenser spacing that can be used and the lower the dose required to achieve mating disruption.

As a general guide application rates of between 10 and 100 g per ha per season are required to achieve communication disruption and hence mating disruption, with typical spacings of 4 to 10 m between point sources.

Timing and need for application of mating disruption can be determined by the use of pheromone traps to monitor crops pests. Where a pest is a perennial problem mating disruption can be used prophylactically, timing would then depend on knowledge of the growth stages of the crop that are susceptible to the pest.

In order to determine the most efficacious pheromone blend and test out different controlled release formulations it is advisable to initially conduct replicated small scale trials of between 0.1 and 1 ha.

Only when satisfactory levels of communication disruption had been demonstrated is it worth scaling up the trials to between 1 and 10 ha per plot so that more extensive assessments, such as insect damage and yield data can be taken to assess the level of control achieved by mating disruption.

Measuring the efficacy of mating disruption

Pheromone traps have often been used to assess the efficacy of mating disruption. Where mating disruption has been achieved pheromone trap catch would be expected to fall to zero in treated plots but remain high in untreated plots. The difference is expressed as a percentage communication disruption (%CD):

$$\%CD = T_{MD} \times 100 / (T_C - T_{MD})$$

Where T_{MD} is trap catch in a mating disruption plot and T_C is trap catch in the control plot. To be effective the %CD has to be above 95% throughout the period when the crop is at risk. Particularly as %CD gives at best only an indirect measure of actual communication disruption. This can however be measured directly by assessing the level of mating achieved by tethered virgin female moths in both treated and non-treated plots (McVeigh *et al.*, 1983). However, the technique is rarely used because it involves considerably more work and access to an insect culture to provide a continuous supply of insects in the correct physiological state for the research.

In order for the %CD to provide a good estimate of mating disruption the pheromone lure chosen for the work needs to be essentially as attractive as a virgin female moth. In principle this can be measured by comparing the catch from traps baited with virgin female moths and a range of synthetic lures. However, most studies take no account of the fact that the female moth only calls for a fixed time period while a synthetic lure is attractive throughout the day and night. Where male attraction is synchronised to the time when female moths produce their pheromone then the comparison between synthetic and natural lures is valid. Nevertheless, in the few cases where this has been studied male moths usually have a longer period of time in the scotophase when they are receptive to pheromones than conspecific female moths release their pheromone.

The inability of synthetic baits to compete effectively with natural baits during the critical time when the females are 'calling' can undermine the outcome of a mating disruption trial and this will only become apparent when the density of egg masses, larvae or crop damage is assessed.

Damage assessments

For most insect pests damage assessments are routine work irrespective of whether the control technology employed was an insecticide or another technology. Nevertheless, because pheromones are airborne signals and hence subject to movement away from the source the location of control or check plots is critical for a realistic comparison between treatments.

Mating disruption is more efficacious over large areas. This is in part because large areas reduce the impact of gravid females immigrating into treated plots but also because where a low density of point sources are employed the larger the area treated, the greater the chance of overlap and mixing between the plumes. Indeed where mating disruption has been commercially adopted the density and placement of dispensers has often

been reduced as the area treated has increased, resulting in considerable cost savings to farmers.

Because early trials are invariably conducted in large areas (typically 1 - 10 ha) there is a temptation to use a single replicate of each treatment or to describe a single contiguous area divided into sub-plots as replicates. Neither practice is fully acceptable and can invalidate the data being generated.

Ideally trials should be undertaken in partnership with farmers in their fields. This will ensure that the technology being developed was applicable to their practises and that any data generated on efficacy was valid in the agricultural context in which it will ultimately be used.

Yield data

Farmers assess the value of a pest control technology on the difference in yield between what they would have expected to get and the observed yield. They also take account of their neighbours' crop in their assessment. Nevertheless, mating disruption in and of itself may not lead to a higher yield because yield is dependent on a wide range of parameters. In particular, the choice of variety, fertiliser, and control of other constraints such as weeds can have a significant impact on yield beyond that of insect pests depending on the severity and timing of attack.

Benefits of the technology

In the majority of cases where mating disruption has been adopted it has invariably been found to be more effective and less expensive to the end-user than conventional insecticides. Yields are at least comparable with those obtained from use of insecticides and in some cases pest incidence has declined year on year with the result that control is not always subsequently required. Control by mating disruption is fully compatible with the use of other IPM component technologies. Thus, allowing the adoption of a wide range of options to control other pest species as required. However, experience has shown that where mating disruption is adopted for key pests the absence of insecticide inputs has led to a decrease in the incidence of secondary pests such as mites, jassids and whitefly.

Many of the benefits of using mating disruption may not be immediately apparent to either farmer or indeed researchers. The increase in honey production as a result of using mating disruption for control of *P. gossypiella* in Egypt was an unexpected bonus for farmers (Khidr *et al.*, 1986) as indeed was the increase in boll size (Moawad *et al.*, 1991). Rice farmers in Spain benefited indirectly from a use of mating disruption for control of the rice stem borer, *Chilo suppressalis* (Casagrande, 1993) through the higher prices paid for their produce, which could then be classed as 'organic'. Given the current political drive to make India an 'Organic Hub' the use of eco-friendly technologies such as mating disruption can provide an entry point into organic production where pest pressures are above economic levels.

Other benefits are less tangible such as the conservation of natural enemies and other non-target organisms but are

nonetheless important to the development of sustainable management strategies that minimise the impact of agricultural activity on the environment.

Pink bollworm, *Pectinophora gossypiella*

The major insect pest on cotton in the early 1980's was the pink bollworm *Pectinophora gossypiella*, primarily a pest of cotton bolls but entering the crop at the pin-square stage resulting in the formation of 'rosetted' flowers. The female sex pheromone was identified by Bierl *et al.* (1974) as a 50 : 50 mixture of (Z,E)- and (E,E)-7,11-headecadienyl acetate. Because the double bonds are not conjugated and the terminal functions of both compounds are acetates the pheromone components are relatively stable at high temperatures and unaffected by oxidation or isomerisation in sunlight. The chemical stability of the pheromone, economic importance of the pest and the large acreage of cotton grown worldwide encouraged researchers to develop control options based on mating disruption. Later when the technology was established a novel metathesis reaction developed by Phillips Petroleum in the USA provided a means of producing the compounds in bulk at low cost, thus paving the way for adoption of the technology on a large scale.

Pioneering work on *P. gossypiella* was conducted in the USA, Israel and Egypt. A micro-encapsulated pheromone formulation developed jointly by the Natural Resources Institute (NRI) with Syngenta, UK (Pectone[®]) was initially used on a small scale in Egypt. This work confirmed that the technique was effective both in terms of reduced mating and crop damage. Since the efficiency of mating disruption was expected to increase with increased area of application the research was rapidly scaled up to plots of 50 ha. The micro-encapsulated pheromone formulation was aqueous based and so ideal for spraying with conventional spray equipment and because the Egyptians routinely applied pesticides and fertiliser by air the same equipment was used for applying *P. gossypiella* pheromone. Both sprayable and commercially available non-sprayable formulations were tested and shown to give high levels of control (Critchley *et al.* 1983, 1985).

Despite being shown to be highly efficacious and cost-effective for control of *P. gossypiella*, it was not until the early 1990's that mating disruption began to be used in Egypt on any significant commercial scale. By 1996 almost the entire 400,000 ha of cotton in Egypt was treated with pheromone for control of *P. gossypiella*. The reasons for the delay in implementation are complex. It depended in part on the development of long-lived controlled release formulations that were easy to apply, cost-competitive with insecticides, socio-economic constraints and in particular a political willingness to move away from conventional insecticides. However, by the early 1990's formulations had been developed by NRI and other workers in Europe and the USA that released pheromone continuously throughout a complete cotton-growing season. This allowed farmers to make single applications that lasted throughout the period when the crop was susceptible to infestation by *P. gossypiella*. Development in the USA was more rapid with registration procedures streamlined to assist the development of suitable technology and assist their adoption resulting in 4 to 500,000 ha of cotton being

treated with pheromone for control of *P. gossypiella* by mating disruption (Staten *et al.*, 1997). This technology has now been superseded by the development of transgenic cotton varieties that have the ability to control a whole range of cotton bollworms and *Heliothis* spp. in particular, which increased in economic importance throughout the 1990's as a result of increasing insecticide resistance. Nevertheless, mating disruption is once again being considered alongside transgenic cotton in a bid to eradicate *P. gossypiella* from large areas of the USA.

Tomato pinworm, *Keiferia lycosicella*

Mexico had a 1 billion US\$ tomato industry that was under threat from a pest complex becoming increasingly resistant to insecticides. The farmers responded by applying more insecticide but this only led to their crops being rejected because of high insecticide residues. The main pest was the tomato pinworm, *K. lycosicella* the sex pheromone of which had been identified as (E)-4-tridecen-1-yl acetate. Application of a hollow fibre formulation of the pheromone resulted in high levels of control when applied at the rate of 1,000 point sources per ha, equivalent to a dose rate of 10 g per ha. However, hollow fibres have to be replaced between three and four times in a season, yet despite the high cost of pheromone and labour the technique was cost-competitive with conventional insecticide regimes. Other moth species are controlled by applications of *Bacillus thuringiensis*, abamectin and the egg parasitoid, *Trichogramma* (Trumble, 1997).

Three tomato crops were produced per year in Mexico. *K. lycosicella* populations are low in the autumn crop and although the damage levels were similar in IPM and conventional crops the IPM treatment was cheaper than the insecticide treatments. In spring and summer crops fruit damage is higher in fields treated with insecticides (70 to 90%) than IPM plots (33 to 35%). It was estimated that over a year, growers using the IPM schedule would earn an additional US\$3,500 per ha to those using conventional insecticide based regimes and their crops met the stringent USDA standards for pesticide residues on crops.

Codling moth, *Cydia pomonella*

C. pomonella is a worldwide pest of apples, pears and occasionally stone fruits such as walnuts. The larvae have been controlled for many years with organophosphate insecticides. However, with the development of resistance to insecticides and the implementation of the Food Quality Protection Act in the USA leading to restrictions on the use of organophosphates farmers are looking more to IPM options for control. This has led to the widespread adoption of pheromone monitoring for pest incidence and mating disruption and *Trichogramma* egg parasitoids for control (Quarles, 2000). Nevertheless, research continues to find methods of improving the efficacy and cost-effectiveness of the technology by incorporating host odours (Yang *et al.*, 2004).

In the western United States a regional program was implemented in 1994 to assess an integrated strategy for suppression of *C. pomonella* in fruit orchards. The program was designed to alleviate the impact of

insecticides on natural enemies while continuing to control insect pest populations. Five pilot test sites were selected, to demonstrate the value of using mating disruption, biological control, and in one site, release of sterile moths to control codling moth and secondary pests with a minimum use of insecticides. After three years Calkins (1998) reported that the strategy was a success.

C. pomonella is currently controlled by mating disruption in 94,700 ha of apple orchards worldwide. The biggest users are USA (46,000 ha), South Africa (14,000 ha), Italy (12,000 ha), Argentina (6,400 ha), Chile (4,200 ha), The Netherlands (4,000 ha), France (3,500 ha), Australia (2,600 ha) and Canada (2,000 ha) (Sexton, 2004).

Oriental fruit moth, *Grapholita molesta*

Mating disruption has been successfully used to control *G. molesta* for many years in stone fruit orchards with 48,000 ha currently treated worldwide (Sexton, 2004). Recent research in Australia has focused on improving the technology to reduce damage to peach adjacent to pear crops where insect damage in the pear crop is controlled by insecticides (Il'ichev *et al.* 1998). Il'ichev *et al.* (2004) found that barrier treatments of 50 to 60 m into the neighbouring pear crops successfully reduced peach damage due to *G. molesta* in the first season and almost eliminated the damage in the second season.

European grape moth pests

The two main insect pests of grape in Europe, European grape moth, *Eupoecilia ambiguella* and Grape vine moth, *Lobesia botrana*, are successfully controlled by mating disruption on almost 25,000 ha, Germany 20,000 ha, France 10,000 ha and Spain 4,500 ha.

Yellow rice stem borer, *Scirpophaga incertulas*

The first successful mating disruption trials to control *S. incertulas* were conducted in West Bengal in 1992 in trials conducted in collaboration between NRI and the Hindustan Fertiliser Corporation (Cork & Basu, 1996). The trials utilised a new PVC resin monolithic polymer formulation jointly developed by researchers at NRI and Agrisense BCS Ltd. (Cork *et al.*, 1989) and formed the basis of the formulation subsequently commercialised by Agrisense, Selibate[®]YSB. The initial trials demonstrated that mating disruption was feasible but it was not until subsequent trials conducted by the Directorate of Rice Research (DRR), Hyderabad, in collaboration with NRI that large and small scale researcher-led, on-farm development trials were conducted to optimise the application rate, pheromone blend and timing of application.

Large-scale, on-farm trials conducted in a range of agro-climatic zones confirmed the efficacy of the technique. In each case the results were similar, mating disruption with the natural blend of pheromone components, a 1 : 3 ratio of (*Z*)-9-hexadecenal and (*Z*)-11-hexadecenal, at an application rate of 40 g AI per ha per season provided a level of control that was comparable to that obtained with conventional insecticides applied by farmers (Cork *et al.* 1998). Control of *S. incertulas* by mating disruption resulted in a 10% increase in yield compared to areas that had been treated with insecticides and in excess of 25% in

areas where farmers did not apply insecticides (Cork *et al.* 1998). Small-scale trials confirmed that the technology was efficacious when applied at doses of greater than 20 g AI per ha per season, although even at 10 g AI per ha per season there was a measurable effect on damage levels (Cork, 1998).

Mating disruption was not cost-competitive with insecticides for control of *S. incertulas* in India except perhaps in areas where rice was grown as a cash crop (e.g. in West Godavari District, Andhra Pradesh) and so not commercially developed. In contrast, the technology has been commercialised for use in Spain (Casagrande *et al.* 1993) with up to 10,000 ha of rice being treated for control of the related species, *Chilo suppressalis* per year. Related mating disruption trials on 1,000 ha of rice are on-going in Iran using controlled release dispensers developed by Agrisense-BCS Ltd., UK (Nick Brown, personal communication).

American bollworm, *Helicoverpa armigera*

Given the success of the PVC resin formulation Selibate[®]YSB for control of *S. incertulas* and the economic importance of *H. armigera* a related formulation containing a 5% loading of the pheromone, Z9-16:Ald : Z11-16:Ald in the ratio of 3 : 97 was manufactured in an attempt to demonstrate that mating disruption could control the pest. A single trial was conducted in Pakistan by Chamberlain *et al.* (2000) on an area of 394 ha of cotton, predominately *Gossypium hirsutum* var. CIM 240. The data were compared with four farmers' practise plots located at least 1 km from the pheromone-treated plots. A high degree of trap 'shut-down' was observed in the pheromone treated plot suggesting that communication disruption had been achieved. Night observations confirmed that mating disruption occurred as a smaller percentage of mated female moths were collected in the pheromone-treated plot compared to the checks. In addition most of the mated females in the pheromone-treated plots had only mated once, whereas those in the checks had undergone multiple matings. A greater percentage of tethered females mated in check plots than the pheromone-treated plot, confirming that mating disruption had occurred. Nevertheless, the number of eggs, larvae, infested flowers and infested bolls were higher in the pheromone-treated plot than the insecticide-treated check plots. These data suggest that although mating disruption had been achieved the highly dispersive pre-oviposition flight behaviour of female moths migrating in from surrounding areas had resulted in significant levels of oviposition that negated the effect of mating disruption. The authors went on to point out that the flight mill work conducted by Armes and Cooter (1991) showed that male and unmated female *H. armigera* undertake dispersive flights of up to 30 km whereas mated females made a large number of short flights with a total median distance of 2.3 km. Given that the mating disruption plot was approximately 4 km² it was too small to stop immigrating mated female moths ovipositing on the crop. However, they speculated that control could be achieved with a plot of 1,000 ha (10 km²), although the 2 km border would be susceptible to egg laying by gravid female moths and would need to be treated with other control treatments to ensure population suppression was maintained throughout the plot.

Chapter 10: Further Reading

Pheromones & Chemical Ecology

Mammalian Semiochemistry: The Investigation of Chemical Signals Between Mammals

Authors: Eric S. Albone, Stephen G. Shirley
 Publisher: John Wiley and Sons Ltd
 ISBN: 0471102539
 Binding: Hardcover
 Publication Date: 9 May, 1984
 Number of Pages: 372

Book Description

The first chemically oriented text on the subject of signals between mammals. It considers what and how chemistry can contribute to this field, the key chemical terms, and the current state of knowledge in mammalian semiochemistry, with an emphasis on major sources of semiochemicals, such as skin, specialized scent glands, and secretions of the reproductive tract.

Pest Management with Insect Sex Attractants (ACS Symposium Series)

Edited by Morton Beroza
 Publisher: American Chemical Society
 ISBN: 0841203083
 Binding: Hardcover
 Number of pages: 192
 Publication date: August 1976

Insect Behaviour

Editors: Ring T. Cardé, Tom Baker
 Publisher: Kluwer Academic Publishers
 ISBN: 0412277409
 Publication Date: 1 September 1995
 Number of Pages: 350
 Binding: Paperback

Book Description

This text deals with the proximate and ultimate causes of insect behaviour. There is heavy emphasis on asking the related questions of what are the mechanisms that allow a particular behaviour to occur and why is this behaviour pattern followed? Topics such as sexual selection, foraging theory and other evolutionary perspectives are considered and emphasis will also be given to the analysis of behaviour patterns and considerations of what constitutes good behaviour studies. This book should be of interest to senior undergraduates and postgraduates in entomology and animal behaviour, and researchers in related fields such as pest control and agricultural science.

Chemical Ecology of Insects 2

Edited by Ring T. Cardé & William J. Bell
 Publisher: Kluwer Academic Publishers, Boston
 ISBN 0-412-03951-6
 Publication Date: July 1995
 Binding: Hardbound
 Number of Pages: 435
 Price: EUR 204.00 / USD 224.00 / GBP 141.00
 ISBN 0-412-03961-3
 Publication Date: July 1995
 Binding: Paperback,

Number of Pages: 435

Price: EUR 110.50 / USD 122.00 / GBP 76.00

During the past decade, the study of the chemical structures used by insects has advanced from a subject that could be reviewed in a single volume to a vastly more advanced level. This important new volume brings together a focused group of reviews that offer perspective on the most interesting advances in insect chemical ecology.

Chemical Ecology of Insects 2 brings together an internationally respected group of experts covering such topics as chemoreception and integration, orientation mechanisms, plant–insect interactions and insect–insect interactions. An important benefit of these reviews lies in the identification of the boundaries of our current knowledge and the most profitable areas in which we should expect these areas to develop.

Insect Pheromone Research New Directions

Edited by Ring T. Cardé and Albert K. Minks
 Publisher: Kluwer Academic Publishers, Boston
 ISBN: 0-412-99611-1
 Publication Date: February 1997
 Binding: Hardbound
 Number of Pages: 712
 Price: EUR 198.00 / USD 218.00 / GBP 137.00

Synopsis

This edited volume assembles contributions from most of the leading researchers on pheromone biochemistry and biology worldwide. Included are reviews of the control of pheromone production, the sensory processing of pheromone signals, the neuroethology of pheromone-mediated responses, the use of pheromones in direct control of behavior, and the evolution of pheromone communication. Researchers and graduate students in chemical ecology, entomology, natural products and neurobiology will find this up-to-date volume an essential resource.

Pheromones of Non-lepidopterous Insects Associated with Agricultural Plants

Edited by: Jim Hardie & Albert K. Minks
 Publisher: CABI Publishing
 ISBN 0 85199 345 1
 Publication Date: September 1999
 Number of Pages: 480
 Binding: Hardback
 Price: GBP 85.00

Methods in Chemical Ecology: Biological Assays

Author: Kenneth F. Haynes
 Publisher: Kluwer Academic Publishers
 ISBN: 0412080419
 Publication Date: June 1998
 Binding: Hardcover
 Number of Pages: 432
 Price: GBP 78.25

Synopsis

This second volume of a two-volume set describes special techniques in chemical ecology, both field and laboratory including: wind tunnels, GC-EAD techniques and other electrophysiological techniques, trapping studies, olfactometers and feeding bioassays. The second section

in this volume is taxonomically organized, discussing intraspecific (pheromones) and interspecific (kairomones) communication as applied to plant-plant, insect-plant, insect-animal, and animal-animal interactions.

Insect pheromones and their use in pest management

Howse, P. E., Stevens, I. D. R. & Jones, O. T.
 Publisher: Chapman and Hall. London
 Publication Date: 1998

Insect Pheromones in Plant Protection

Edited by: A.R. Jutsum and R.F.S. Gordon
 Publisher: John Wiley and Sons Ltd;
 ISBN: 0471920193
 Publication Date: 29 March 1989
 Binding: Hardcover
 Number of Pages: 386

Synopsis

The use of pheromones is a highly topical theme given the increasing concern for environmental safety and the need for integrated pest management and this publication provides a techno-commercial review of the use of pheromones in the control of insect pests in agriculture. It addresses the current scientific background to the subject: practical experience in the field at a scientific and commercial level, the chemical manufacture of pheromones and the socio-economic and commercial factors in the introduction of such novel systems. The recent progress in pest management contained in this book has been achieved by the determination of an international band of pure and applied scientists, academics and industrial researchers.

CRC Handbook of Insect Pheromones and Sex Attraction

Mayer, McLaughlin
 Publisher: CRC Press
 ISBN: 0849329345
 Publication Date: 31 December 1990
 Number of Pages: 1083
 Binding: Hardcover

Synopsis

Encyclopedic treatment (in the form of mini-reviews) of the chemical systems used by over 1,600 insects in pre-mating communications. Each review, in cases where the chemicals are identified, documents the analytical procedures used and the behavioural responses that establish the validity of the identifications.

Methods in Chemical Ecology: Chemical Assays

Edited by Jocelyn Millar & K. F. Haynes
 Publisher: Kluwer Academic Publishers
 ISBN: 0412080710
 Publication Date: October 1997
 Binding: Hardcover
 Number of Page: 384
 Price: GBP 78.00

Synopsis

This volume consists of chemical and biological assays applicable to all five parts of the biological world. This first volume of a two-volume set details the applicability of chemical ecological techniques to investigate problems in the interactions between and among species and points to present limitations. The book is practical, with emphasis on planning, design, and execution of experiments. It points out the advantages and limitations of various techniques, the types and amounts of

information to be gained from particular techniques, as well as practical experimental details as to how to prepare and execute various bioassays and procedures. This book should be of interest to graduate students and professional ecologists interested in the natural product-based chemical interactions between plants and animals and between animals and other animals.

Management of Insect Pests with Semiochemicals: Concepts and Practice

Mitchell, E. R.
 Publisher: Kluwer Academic Pub
 ISBN: 0306406306
 Publication Date: 1 March 1981
 Binding: Hardcover

Handbook of Natural Pesticides: Insect Attract Repellents, Volume VI

E. David Morgan & N. Bhushan Mandava
 ISBN: 0849336627
 Publication Date: 3 May 1990
 Number of Pages: 264
 Price USD 220

Synopsis

Information on the search for new and environmentally acceptable pesticides. First coverage of this information in one series. Chapters written by experts in the field. Extensive tables of chemicals, their activities and insect species. This volume addresses chemical interactions between insects and plants, such as feeding and ovipositional attractants and deterrents. It begins with a general introduction to insects in a chemical world. Included is a discussion of molecular biology and genetics in insect control, with respect to potentially inserting the genes for the synthesis of a protective substance into a crop plant. Also covered is the detoxification of plant substances by insects. This volume is especially helpful for chemists and biologists in the field of pesticide research.

Trap Responses of Flying Insects: The Influence of Trap Design on Capture Efficiency

Author: R.C. Muirhead-Thomson
 Publisher: Academic Press
 ISBN: 0125097557
 Publication Date: 3 April 1991
 Number of Pages: 304
 Binding: Hardcover

Synopsis

Insect trapping is a basic field research tool for many biologists, whether they are studying insect pests, disease vectors or insect ecology for its own sake. Any field entomologist contemplating a new insect trapping programme or looking to improve or develop an existing scheme will benefit from this broad review of flying insect traps, in which the author draws on a wide variety of methods used by different research projects from all over the world. Over the years a great many traps have been developed and endlessly modified to suit particular species, habitats, and research requirements. In virtually every case the design of the trap interacts with the specific behaviour of the insects involved to bias trap efficiency. In addition, the limited dialogue between workers in different subject disciplines and habitats has caused a shortage of new information available to field entomologists as a whole.

Semiochemicals: Their Role in Pest Control

Edited by Donald A. Nordlund, Richard L. Jones, W. Joe Lewis

Publisher: John Wiley & Sons Inc.

ISBN: 0471058033

Date of Publication: 8 July 1981

Binding: Hardcover

Number of Pages: 306

Biological and Biotechnical Control of insect Pests.

Jack E. Rechcigl and Nancy A. Rechcigl

Publisher: CRC Press, Andover, UK

ISBN 1-56670-479-0

Price: GBP 52.99

Behavior-modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants

Edited by Richard L. Ridgway

Publisher: Marcel Dekker

ISBN: 0824781562

Publication Date: 1 April 1990

Number of Pages: 761

Binding: Hardcover

Synopsis

Presenting an authoritative overview of current findings on pheromone applications, this reference reviews the principles involved in employing these compounds, their chemistry, and delivery systems for efficient use. In addition, it provides case studies of current and potential practical applications.

Insect Pheromones and Other Behaviour-modifying Chemicals

Edited by R L. Ridgway

Publisher: British Crop Protection Council

ISBN: 0948404620

Publication Date: 1992

Binding: Paperback

Area-wide control of fruit flies and other insect pests.

Edited by Keng-Hong Tan

Publisher: Penerbit Universiti Sans Malaysia

ISBN: 983-861-195-6.

Publication Date: 1998

Binding: Hardback

Pheromones and Animal Behaviour

Communication by Smell and Taste

Author: Tristram D. Wyatt

Publisher: Cambridge University Press

ISBN: 0521485266

Publication Date: February 2003

Binding: Paperback

Number of Pages: 408

Price: GBP 27.95

Synopsis

Gives an introduction to the rapid progress in our understanding of olfaction at the molecular and neurological level. In addition, it offers chemists, molecular and neurobiologists an insight into the ecological, evolutionary and behavioural context of olfactory communication.

We are entering one of the most exciting periods in the study of chemical communication since the first pheromones were identified some 40 years ago. This rapid progress is reflected in this book, the first to cover

the whole animal kingdom at this level for 25 years. The importance of chemical communication is illustrated with examples from a diverse range of animals including humans, marine copepods, *Drosophila*, *Caenorhabditis elegans*, moths, snakes, goldfish, elephants and mice. It is designed to be advanced and up-to-date, but at the same time accessible to readers whatever their scientific background. For students of ecology, evolution and behaviour, this book

Contents

1. Animals in a chemical world; 2. Discovering pheromones; 3. Sex pheromones: finding and choosing mates; 4. Coming together and keeping apart: aggregation and host-marking pheromones; 5. Scent marking and territorial behaviour; 6. Pheromones and social organization; 7. Pheromones and recruitment communication; 8. Fight-or-flight: alarm pheromones; 9. Perception and action of pheromones: from receptor molecules to brains and behaviour; 10. Finding the source: pheromones and orientation behaviour; 11. Breaking the code: illicit signallers and receivers of semiochemical signals; 12. Using pheromones: applications; 13. On the scent of human attraction: human pheromones?; Appendix A: An introduction to pheromones for non-chemists; References

Chemical analysis**Capillary Gas Adsorption Chromatography**

Author: VG Berezkin

Publisher: Wiley-VCH

ISBN: 3527296751

Publication Date: 1 November 1996

Binding: Hardcover

Price: GBP 49

Gas Chromatographic Environmental Analysis - Principles, Techniques, Instrumentation: Principles, Techniques, Instrumentation

Author: F Bruner

Publisher: John Wiley & Sons Inc

ISBN: 047118778X

Publication date: 16 August 1993

Binding: Hardcover

Number of Pages: 234

Price: GBP 84.00

Synopsis

Gas Chromatographic Environmental Analysis is the first, truly complete, up-to-date, homogeneous coverage in book form, of the analytical techniques applied to the chemical study of environment. The book describes the use of gas chromatography in environmental analysis. After an introduction in the fu

Handbook of GC/MS

Author: Hans-joachim Hubschmann

Publisher: Wiley-VCH

ISBN: 3527301704

Publication Date: 9 March 2001

Binding: Hardcover

Number of Pages: 608

Price: GBP 107.00

Synopsis

This is the first comprehensive reference work for GC/MS. It offers broad coverage, from sample

preparation to the evaluation of MS-Data, including library searches. Fundamentals, techniques, and applications are described. A large part of the book is devoted to numerous examples for GC/MS-applications in environmental, food, pharmaceutical and clinical analysis. These proven examples come from the daily practice of various laboratories. The book also features a glossary of terms and a substance index that helps the reader to find information for his particular analytical problem. The author presents in a consistent and clear style his experience from numerous user workshops which he has organized. This is a thoroughly revised and updated English edition based on an edition which was highly successful in Germany.

Gas Chromatographic Techniques and Applications

Author: James N. Martin

Publisher: CRC Press

ISBN: 0849305217

Publication Date: 15 February, 2001

Binding: Hardcover

Number of Pages: 320

Synopsis

Presents recent advances in the procedures and equipment involved with gas chromatography (GC) analysis. The nine contributions describe developments in sample preparation, sample injection systems, column technology, the flame ionization detector, miniaturization, mass spectrometry in combination w

GC/MS: A Practical User's Guide

Marvin McMaster, Christopher McMaster

Publisher: John Wiley & Sons Inc

ISBN: 0471248266

Publication: 1 August 1998

Binding: Hardcover

Number of pages: 184

Price: GBP 30.00

Synopsis

Gas chromatography; mass spectrometry (GC;MS) is an analytical system used by organic chemists and analytical chemists in research and in professional analytical labs, including environmental labs. This text is designed to provide information on using, maintaining, and troubleshooting analytical instrument systems in operations laboratories. It offers practical tips based on actual experience, and enough theory to show the operational variables and how to control them. The text also: offers comparisons between GC;MS and competitive instrumentation; provides an overview of components of the generic GC;MS system; guides the reader through setting up a system and performing an analysis; gives specific guidelines for optimizing a system; describes specific applications in research labs and clinical, environmental, and forensic analysis labs; and covers real-world terminology and technology.

Chemical Analysis

The Basic Gas Chromatography (Techniques in Analytical Chemistry)

Edited by Harold McNair, Jim Miller

Publisher: John Wiley & Sons Inc

ISBN: 0471172618

Publication Date: 24 November 1997

Binding: Paperback

Number of Pages: 224

Price: GBP 25.50

Book Description

Based on Harold McNair's best-selling industry guide, *Basic Chromatography*, this updated and enlarged book blends coverage of principles with practical information on the instrumentation, operation, applications and limitations of gas chromatography--one of the most popular techniques for analyzing substances. It provides thorough information on practical operator principles and describes theories without resorting to complicated equations and derivations.

Principles and practice of chromatography

Ravindranath, B. Ellis Howood

ISBN 0745802966.

Publication Date: 1989

A Practical Guide to the Care, Maintenance and Troubleshooting of Capillary Gas Chromatographic Systems: 3rd Edition

Dean Rood

Publisher: Wiley-VCH

ISBN: 3527297502

Publication Date: 11 January 1999

Binding: Hardcover

Number of Pages: 343

Price: GBP 55.00

Synopsis

The field of gas chromatography continues evolving, demonstrated by the continuous series of developments in columns, equipment, apparatus, techniques, and applications that have occurred since the publication of the first edition of this book. Taking the same approach as in the previous edition, the author illustrates the various problems experienced and the different approaches to trouble-shooting and problem solving. This book is intended for the average GC user, both novice and expert. The topics covered are based on the most common problems, questions, and misconceptions about capillary gas chromatography and are presented in a practical, and concise format suitable for even the most inexperienced user.

Integrated Pest Management

Dictionary of Biological Control and Integrated Pest Management

Edited by J. Coombs, K. Hall

Publisher: CPL Press

ISBN: 1872691765

Publication Date: November 1998

Binding: Paperback

Number of Pages: 200 pages

Book Description

This book is a completely revised second edition that contains more than 5500 definitions relating to biological control and integrated pest management based on the glossary section of the *Worldwide Directory of Agrobiologicals* on CD-ROM. Includes descriptions and definitions for: Latin and Common Names, Beneficial and Pest Organisms, Insects and Mites, Nematodes, Micro-organisms, Plant and Animal Diseases, Bacteria, Fungi and Viruses, Crops and Weed Plants, Biopesticides, Bioherbicides, and Other Agrobiologicals, Pheromones and Other Lures.

Methods in Ecological and Agricultural Entomology

Edited by D. R. Dent, & M. P. Walton,

Publisher: CABI Publishing

ISBN: 0851991327

Publication Date: March 1997

Number of Pages: 400

Binding: Paperback

Price: GBP 30.00

Description

Entomology as a branch of biological science has undergone rapid expansion and development in recent decades. There have been major advances in the technologies associated with pest management and the ecological studies that underpin much of this work. Greater emphasis is now placed on topics such as modelling and biochemical techniques, with new approaches to the study of insect behaviour and insecticide efficacy making inroads into traditional approaches. This book aims to integrate the new approaches and technologies with traditional and well-proven methods. It provides a critical analysis and evaluation of methods available, through reference to general principles, but emphasis is also placed on providing detailed descriptions of methods and their application. Written by leading authorities from the UK, USA and Australia, the book is aimed at advanced undergraduate and postgraduate students in entomology and pest management.

Insect Pest Management

David Dent

ISBN: 0851993419

Publication Date: June 2000

Number of Pages: 432

Binding: Paperback

Price: GBP 27.50

Synopsis

This is a major textbook for advanced undergraduate and graduate students taking courses in applied entomology or crop protection. It provides in-depth coverage of the principles, research methodologies and practice for each component of the subject. The first two chapters describe how insect populations and their pest status can be assessed. Chapters then follow covering the main methods of pest management, using chemicals, host-plant resistance and biological control. Chapter 7 covers cultural control, that is manipulation of the environment to render it unfavourable to the host, such as hermetic storage system for crops post-harvest and intercropping for crops in the field. Chapter 8 covers interference methods, such as semiochemicals, the sterile insect technique and genetic engineering. The final two chapters then consider issues such as quarantine, legislation, modelling and systems analysis, decision making, social issues, farming systems and research management. Throughout, emphasis is placed on the need for socio-economic evaluation of integrated pest management techniques, and detailed examples are drawn from both temperate and tropical regions. Most relate to crop protection, but some examples from medical and veterinary entomology are also included.

The first edition of this book, published in 1991, was well-received as an upper-level undergraduate textbook for courses in agricultural entomology and pest management. Since the publication of the first edition,

many new advances have taken place in the subject, and these have been incorporated into the new version. The content has been updated throughout to provide balanced, comprehensive coverage.

**Integrated Vegetable Pest Management
Safe and sustainable protection of small-scale
brassicac and tomatoes**

Authors: H. Dobson, J. Cooper, W. Manyangairirwa, J. Karuma, W. Chiimba

Publisher: Natural Resources Institute, Chatham Maritime, UK

ISBN: 0-85954-536-9

Publication Date: 2002

Number of pages: 179

Binding: Softback

Description

A handbook for extension staff and trainers in Zimbabwe. It contains photographs and information to help identify the major pests and diseases of brassicas and tomatoes and gives advice on the safe and effective use of cultural, biological and chemical technologies within integrated pest management systems. Two posters which adapt the key information for use by farmers are also available.

Although developed in Zimbabwe, much of the content of the publication will be useful for expanding small-scale horticultural sectors in many developing countries in Africa.

Microbials in Insect Pest Management

Edited by S. Ignacimuthu, Alok Sen, S. Janarthanan

Publisher: Science Publishers, Inc

ISBN: 1578081718

Publication Date: August 2001

Binding: Hardcover

Number of Pages: 188

Price: GBP 46.00

Synopsis

This collection of 24 papers discusses the success of different entomopathogens in various cropping systems, their effect on natural enemies, compatibility of different microbes as well as with pesticides, and their mass production. Improvement in field performance through molecular techniques and s

**A Synopsis of Integrated Pest Management in
Developing Countries in the Tropics**

Author: M. Iles

Publisher: Natural Resources Institute

ISBN: 0859542963

Publication Date: 1992

Binding: Paperback

Number of Pages: 20

Price: GBP 7.50

**Constraints on the Adoption of IPM in Developing
Countries: A Survey**

Authors: M. Iles, A. Sweetmore

Publisher: Natural Resources Institute

ISBN: 0859542998

Publication Date: November 1991

Binding: Paperback

Number of Pages: 37

Price: GBP 2.50

Integrated Pest Management: Potential, Constraints and Challenges (Cabi Publishing)

Edited by: O. Koul, G. W. Cuperus, G. S. Dhaliwal
 Publisher: CABI Publishing Publication
 ISBN: 0851996868
 Date: 1 December 2003
 Binding: Hardcover
 Number of Pages: 350

Integrated Pest Management in the Tropics: Current Status and Future Prospects

Edited by Annalee N. Mengech, Kailash N. Saxena,
 Hiremagalur Gopalan
 Publisher: John Wiley and Sons Ltd
 ISBN: 0471960764
 Publication Date: 25 October, 1995
 Number of Page: 186

Synopsis

Integrated pest management (IPM) has been relatively successful in North America, yet its role in tropical agriculture is less well known. For this reason, a global review of IPM to assess the impact of related activities in tropical regions of Asia, Africa and South America was undertaken by UNEP and the International Centre for Insect Physiology and Ecology (ICIPE). This review examines the current status and prospects for IPM in tropical regions. It aims to outline the new paradigms and directions that IPM must take if it is to be adopted by farmers and governments on the scale necessary to change the current reliance on chemical pesticides.

Integrated Pest Management: Ideals and Realities in Developing Countries

Stephen Morse, William Buhler
 Publisher: Lynne Rienner Publishers
 ISBN: 1555876854
 Publication Date: 30 June 1997
 Binding: Hardcover
 Number of Pages: 192 pages
 Price: GBP 15.50

Synopsis

Since its inception in the 1960s, integrated pest management (IPM) has become the dominant paradigm in crop protection. Its ecological approach - involving a minimum use of pesticides - has accounted for much of its popularity, and it has been widely adopted by a range of development agencies. This study outlines some of the classic IPM success stories (primarily from North America) and contrasts them with the results obtained in developing countries. Conventional explanations for IPM's failure in developing countries focus on problems with extension, farmer co-operation, funding, government direction, or even conspiracy in the pesticide industry. In contrast, Morse and Buhler demonstrate that the main reason for the poor performance of IPM has more to do with the nature of IPM itself. A product of agricultural industrialization, IPM may be effective in the context of large-scale industrial farming, argue the authors, but it is not suitable for resource-poor farmers operating on a relatively small scale.

Concepts in Integrated Pest Management

Norris
 Publisher: Prentice Hall
 ISBN: 0130870161
 Publication Date: 14 August 2002
 Binding: Hardcover

Number of Pages: 586

Price: GBP 54.00

Synopsis

Designed for courses in pest management, this text presents the basic principles of integrated pest management as they apply to plant pathogens, weeds, nematodes, molluscs, arthropods, and vertebrates.

General**Pest and Disease Management Handbook**

Edited by David Alford
 Publisher: BCPC
 ISBN 0 632055 03 0
 Publication Date: November 2000
 Binding: Hard cover
 Number of Pages: 624
 Price: GBP 110.00

This book updates the 3rd edition of the Pest and Disease Control Handbook. It commences with a new introductory chapter covering the principles of pest and disease management with following chapters, each written by acknowledged experts in their field, covering a range of major temperate northern hemisphere crops. As well as comprehensive details of pest and disease management strategies, each chapter also includes a classification scheme for the cited pests and diseases.

**The BioPesticide Manual
A World Compendium**

Edited by Leonard G Copping
 Publisher: BCPC
 ISBN: 1901396266
 Publication Date: November 2001
 Binding: Hard cover
 Number of Pages: 528
 Price: GBP 105.00

The BioPesticide Manual is the authoritative world compendium of commercial biopesticide products. This new, revised and updated second edition contains comprehensive entries on 273 biocontrol agents used in the production of over 1,000 commercial products. The following biocontrol types are covered:

- 96 micro-organisms
- 54 macro-organisms
- 53 semiochemicals
- 51 natural products
- 19 genes

Each entry includes:

- nomenclature & source
- production
- target pests & target crops
- mode of action
- biological activity
- commercialisation
- application & compatibility
- toxicity and environmental impact

In addition there are extensive sections covering:

- organic farming
- Latin/English glossary
- directory of biocontrol companies

This book is complementary to *The Pesticide Manual* with entries easily cross-referenced. This is an invaluable

reference book for all those concerned with biotechnology, crop and environmental protection, integrated crop management, horticulture, food production, processing and marketing.

The Neem Tree: Source of Unique Natural Products for Integrated Pest Management, and Medicinal, Industrial and Other Purposes

Author: H. Schmutterer
 Publisher: Wiley-VCH
 ISBN: 3527300546
 Publication Date: 11 July 1995
 Binding: Hardcover
 Number of Pages: 696

Synopsis

This is a monograph on the tropical Neem tree (*Azadirachta indica*), which can be taken as an example to demonstrate the broad range of applications of a renewable resource. The text details the sources of natural plant products for use in pest management, medicine and industry. It is intended for scientists concerned with the development of biological pesticides or the uses of plant metabolites for the production of pharmaceuticals. Integrated Pest Management in Developing Countries: Experience and Prospects (PSTC)

The Pesticide Manual

Edited by Clive Tomlin
 Publisher: BCPC
 ISBN 1 901396 12 6
 Publication Date: November 2000
 Binding: Hard cover
 Number of Pages: 1,250
 Price: GBP 165.00

This world compendium is an essential reference book for anyone with a professional interest in pesticides. The extensively revised and updated twelfth edition contains 812 detailed main entries as well as abbreviated details covering 598 superseded products. Entries cover herbicides, fungicides, insecticides, acaricides, nematicides, plant growth regulators, herbicide safeners, repellents, synergists, pheromones, beneficial microbial and invertebrate agents, rodenticides and animal ectoparasiticides.

All 812 main entries include, as appropriate:

- chemical structure, discipline and class
- nomenclature, including common, IUPAC and Chemical Abstracts names, CAS RN, EEC number and development code
- full physical chemistry details
- commercialisation information including patent, history and manufacturer
- mode of action, biochemistry, uses, formulation type, trade names and mixtures
- mammalian toxicology profiles
- ecotoxicity data etc
- environmental fate information

The 6th edition of the CPL Scientific study Biopesticides: Markets, Technology, Legislation and Companies

CPL Scientific
 CAB International, Wallingford, Oxfordshire, OX10 8DE, UK
www.cplsis.com

Price: GBP 6,000

Synopsis

The study focuses on products used as insecticides, herbicides or fungicides, in which the active principle is based on or derived from a micro-organism. The study is in 4 volumes. It has 640,000 words on 1,167 pages.

Volume 1 - Markets for Biopesticides
 With 105,000 words on 263 pages with 6 sections -
 Section 1 - Market summary and overview;
 Section 2 - Markets by market sector;
 Section 3 - Markets by geographic region;
 Section 4 - Factors impacting the market;
 Section 5 - Going into biopesticides?
 Section 6 - 42 success factors.

Volume 2 - Technology of Biopesticides
 With: 314,000 words on 441 pages in 7 sections -
 Section 7 - R&D and production;
 Section 8 - Bacterial biopesticides;
 Section 9 - Fungal biopesticides;
 Section 10 - Viral biopesticides;
 Section 11 - Nematodes and protozoa;
 Section 12 - Microbially-derived products;
 Section 13 - The future.

Volume 3 - Legislation about Biopesticides
 With 138,000 words on 264 pages in 5 sections -
 Section 14 - Summary and overview;
 Section 15 - The United States;
 Section 16 - The European Union;
 Section 17 - Other countries;
 Section 18 - IPR, QC etc.

Volume 4 - Companies
 With 83,000 words in 199 pages -
 This volume discusses and analyses 124 companies: 71 companies presently in the biopesticides business and 53 historical entries.

Websites

Biosignal

Safe insect control with pheromones and kairomones
<http://www.biosignal.org/>

International Society of Chemical Ecology

Website: <http://www.chemecol.org/>

IOBC Working Group

<http://phero.net/iobc/>

Pherobase

<http://www.pherobase.com/>

Pherolist

<http://nysaes.cornell.edu/pheronet>
 Cornell University, Geneva NY (USA)
<http://www-pherolist.slu.se>
 SLU, Swedish University of Agricultural Sciences, Alnarp (Sweden)

Pheronet

<http://www.phero.net>

Chapter 11: Pheromone suppliers

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36, Prince Industrial Estate
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Note: Margo Biocontrol act as exclusive agents for
Agrisense products in South Asia.

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Email: info@biotech-int.com

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10 Willowbrook Technical Units
St. Mellons
Cardiff
UNITED KINGDOM
Phone: 02920-779612
Email: "chandra pant" <c.pant@ntlworld.com>

Contact: Mr T. V. Rappai
Chem Exports Pte Ltd.
Erinjeri Angadi, Post Box No. 72
Thrurur - 680 001
Kerala, India
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Fax: 0487 2442005
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A. Cork

Pheromone Manual

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Kimpton
Hertfordshire SG4 8RA
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Fax: 44 1438 832157
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Website: www.oecos.co.uk

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E-mail: raffarrd@yahoo.co.in

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